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Diversity analysis and genetic potency identification of local rice cultivars in Penajam Paser Utara and Paser Districts, East Kalimantan

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Abstract. *Nurhasanah, Sadaruddin, Sunaryo W. 2016. Diversity analysis and genetic potency identification of local rice cultivars in Penajam Paser Utara and Paser Districts, East Kalimantan. Biodiversitas 17: 401-408.* Local rice cultivars provide genetic diversity in rice genepool that is very useful for rice breeding programs. Less is known about local rice genetic diversity in East Kalimantan, because their existence only depends on traditional cultivation and conservation by local farmers based on needs and tendencies towards certain varieties. According to the current exploration study conducted in Penajam Paser Utara (PPU) and Paser, the smallest districts in East Kalimantan, there were high genetic diversities of rice existed in that two districts. As many as 71 local rice cultivars were collected, consisted of 53 rice and 18 glutinous rice. Traits characterization showed that there were large variation of plant height (66 to 209.33 cm), culm number (1 to 41.67), culm diameter (0.23 to 1.03 cm), leaf length (39 to 108.33 cm), leaf width (0.83 to 2.67 cm), leaf angle (10 to 50 degree), ligule length (11 to 55 mm) and weight of ten seeds (0.13 to 0.40 gram) in the local rice population showing high phenotypic variations of agro-morphological traits in the population. A strong negative correlation between culm number (tiller) and culm diameter, leaf length as well as leaf width was observed, which indicated that culm number is few if the culm diameter is big, and the leaves are longer and wider. Genetic diversity analysis based on Agro-morphological characters clustered the cultivars in nine and four classes for rice populations in PPU and Paser districts, respectively. Pre-identification of the local rice genetic potencies showed some superior and potential traits which will be very useful for rice breeding programs to develop new superior rice varieties.

Keywords: Diversity, East Kalimantan, genetic potency, local rice, traits characterization

INTRODUCTION

Rice is a staple food for most of Asian country. It is widely cultivated all over the world, not only in Asia, but also in America, Europe, Australia and Africa (Longtau 2000; FAO 2016). Even though it is wide world cultivated, but more than 90% of this rice is consumed in Asia (IRRI 2016). Population growth is one of the main factors leads to the increase demand for rice. To fulfil the need for the food, various efforts have been done to enhance rice productivity, including the use of superior rice variety.

Nowadays, plant breeder is not only focused on the development of new superior variety having high yield and quality, but also tolerance and adaptable to environmental stress factors towards resilient and sustainable agricultural system (Brummer et al. 2011; Meybeck et al. 2012). The existence of rice genetic diversity is very important in supporting this purpose, since the genetic diversity is the raw material for the assembly of new superior varieties.

As a center of biodiversity spot in Indonesia, East Kalimantan is a home of various plant species, including rice. Hundred local rice varieties were reportedly existed in East Kalimantan, included lowland and upland rice varieties. Local rice cultivars have high genetic variability due to their adaptation to a wide range of agro-ecological conditions (Yawen et al. 2003; Sarawgi and Bine 2007). They may provide genetic diversity to diversify rice genepool that is very useful for new superior rice varieties development. Unfortunately, most of local species diversity is not well recorded and characterized. Therefore less information is also available about their characteristic.

Traits characterization has been one of the important step of crop improvement. Each species possess a specific functional trait, such as genetic, phenological, dispersal, physiological, etc (Lebrija-Trejos et al. 2010). Almost all morphological traits were associated with macro habitats. Morphological traits are directly related to the interaction between a species and its environment (Westoby and Wright 2006). Morphology includes some of the most accessible and functionally important traits, with the potential to be measured for each species (Fukuoka et al. 2006).

Assessment of genetic diversity is important in plant breeding. Genetic diversity is commonly measured by genetic distance or genetic similarity implying either differences or similarity at the genetic level showing relationship among genotypes in germplasm is very useful for plant breeding programs. It will support the selection decision from large genotypes population for crossing combinations in plant development. Genotype relationship is mainly based on information about plant characteristic. Agronomic and morphological characters are usually used as an initial tool to distinguish between varieties (Li et al. 2010).

The aim of the present study was to collect local rice germplasm in PPU and Paser Districts for genetic diversity

analysis, and to characterize their agro-morphological characters, identify their genetic potency and analyze the genetic distance among cultivars.

MATERIALS AND METHODS

Exploration study

Exploration study was carried out in Penajam Paser Utara (PPU) and Paser Districts in East Kalimantan Province. In Paser District, the exploration was conducted in Kayongo Sari, Muara Pias, Munggu, Olung, Papara, Putang, Riwang, Sekuan Makmur and Sungai Tuak Villages. The exploration sites in PPU District were in Api-api, Babulu Laut, Labangka, Riko, Rintik and Sumber Sari Villages (Figure 1).

The exploration was conducted in 2014 at two different times, along rice growing period and at or after harvest time. Rice genetic diversities were collected directly from local farmers. Information about rice varieties and the genetic potencies (interesting traits based on local community observation) were collected from informal interviews and dialogues through direct participatory technique using both directed and open-ending questioning with community members and the farmer.

Field experiment

The rice cultivars collected from exploration study were grown in green house for phenotypic traits observation. The seeds were grown in plastic pots containing 10 kg of soil based on their cultivation types, upland or lowland. The pots were arranged in completely randomized designs with three replications, each pot was considered as one replication. The upland rice cultivars were grown in perforated pot, while the lowland were in the unperforated. Prior to planting, the lowland rice seeds were germinated, after the seedlings were 21 days old, they were transferred to the growing pots. For the upland rice cultivars, the seeds were directly sown in the soil. Each pot contained only one plant. The plants were treated according to general rice cultivation procedure.

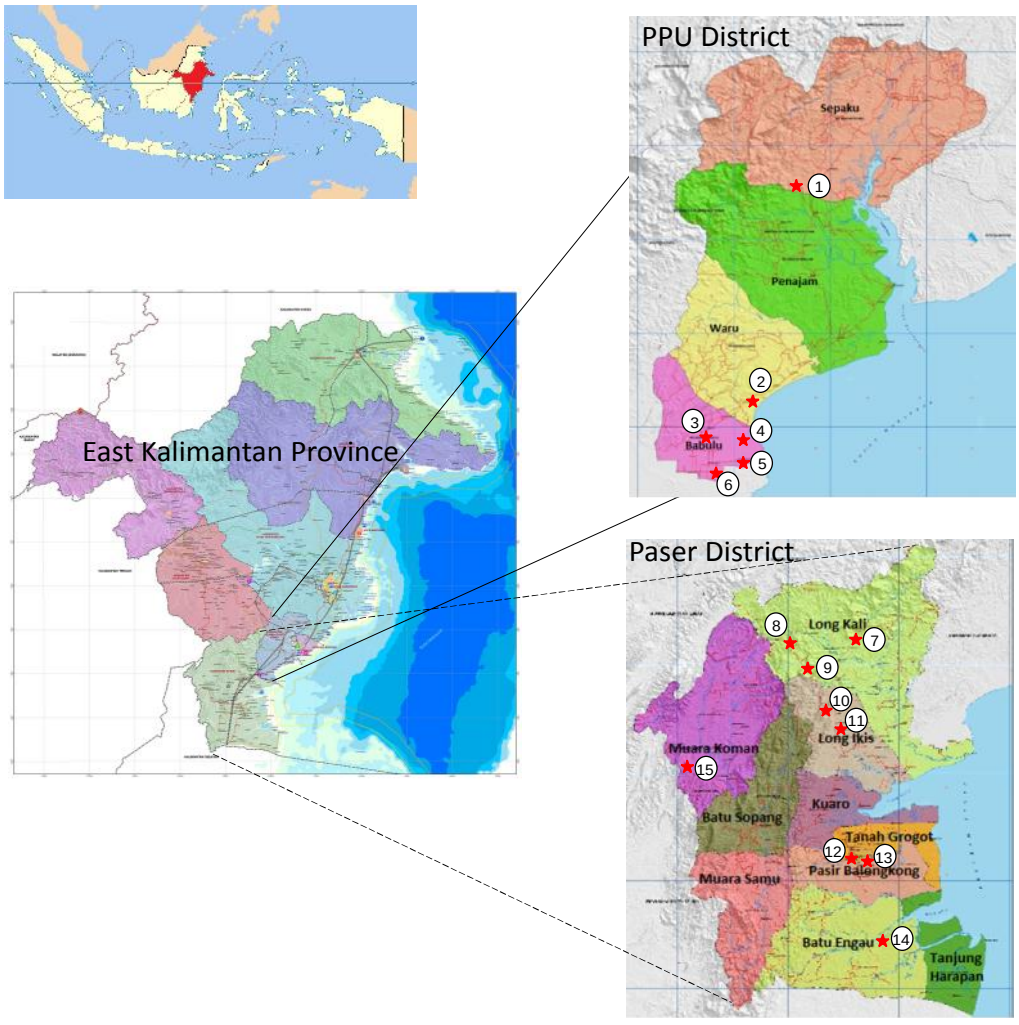

Figure 1. Rice diversity exploration sites in Penajam Paser Utara (PPU) and Paser Districts in East Kalimantan. Stars (*) indicating the exploration sites: 1. Riko, 2. Api-api, 3. Labangka, 4. Babulu Laut, 5. Sumber Sari, 6. Rintik (PPU District); 7. Munggu, 8. Muara Pias, 9. Putang, 10. Olung, 11. Kayongo Sari, 12. Sungai Tuak, 13. Papara, 14. Riwang, 15. Sekuan Makmur (Paser District).

Agro-morphological trait observation and data analysis

Several Agro-morphological traits, i.e: plant height, culm (number, diameter, angle, internode color), leaf (length, width, angle, blade pubescence, basal leaf sheath color, blade color), ligule (length, color, shape, auricle color), auricle color and grain (length, width, weight of 10 seeds, lemma and palea color, awning, apiculus color) were observed to characterize the cultivars phenotypically. The traits were characterized based on descriptors for rice procedure by IRRI (1980). The data were used to classify rice cultivars into different groups based on their similarity degree using cluster analysis. The cluster analysis was carried out using Euclidian Distance, the distances between clusters were determined using single linkage analysis, which were performed using SPSS Statistic version 20. The cluster analysis result was presented on dendrogram. Correlations between quantitative traits were carried out using Pearson correlation to analyze the relationship between the traits.

RESULTS AND DISCUSSION

Local rice biodiversity

There was a relatively high rice diversity observed in PPU and Paser Districts. The rice biodiversity is unequally dispersed in nine villages in Paser and six villages in PPU District (Table 1). Riwang and Kayongo Sari are the spots of local rice diversity in Paser, as Riko and Sumber Sari in PPU. Thirty and fourty one rice cultivars were collected in PPU and Paser Districts, respectively. The higher rice diversity found in Paser District than in PPU was not only due to the more villages were visited, but also the wider area. PPU is the smallest district in East Kalimantan, followed by Paser. The large of the regions are only 2.52% (PPU) and 8.79% (Paser) of East Kalimantan Province. The land use for agriculture is also higher in Paser than in PPU, that is 429.950 Ha and 93.125 Ha for the two regions, respectively (BPS-Statistics of East Kalimantan Province (2015).

Most of the rice are non-glutinous rice, and only about 25% of them are glutinous rice. Based on the cultivation method commonly applied by the local farmer, majority of the rice was cultivated as upland rice (Table 2), especially in Paser District. The higher upland rice diversity might be caused by plant domestication process and adaptation to the local cultivation procedure that has been applied by local community. Generally the local people practices the traditional, low effort and low technology of cultivation. Therefore, the upland rice are preffered rather than low land rice, which is lead to the increase of upland rice diversity. On the other hand a slightly higher number of low land than upland rice diversity was observed in PPU. PPU is placed near to Balikpapan and Samarinda City, and most of the areas are easily to attain. It is also a gate of Paser, it means that we have to enter PPU first before entering Paser. Therefore cultivation information and technology is easy to touch the local community. As a result, the local farmer has adapted several upland rice cultivars, such as Mayas, Sereh, Sasak Jalan, as well as glutinous rice cultivars which are normally cultivated as upland rice in other Districts, to be cultivated in low land method for higher yield purpose.

We observed several grain color variation in the local rice population. There are brown and black, beside white grain colour. The black grain is a glutinous rice, the brown is unglutinous rice, and the white grain color consist of unglutinous and glutinous rice. The black and brown grain rice can be used as functional food, which is not only as carbohydrate source but also contains active substance beneficial for health and special diets. Black and brown rice contains vitamin B complex, essential fatty acids, fiber and anthocyanins as well as a low glycemic index which are beneficial to health (Zhang et al. 2010). Brown rice is also a source of selenium, a mineral that can boost the natural killer cells of cancer cells, mobilizes the cells to fight the cancer cells and may act as an antioxidant (Smith and Charter 2010). Diet foods made from rice, could be expected to lower the risk of obesity, hepatic steatosis, hyper glycemic, diabetic (Jang et al. 2012), and prevent headaches, colon cancer, heart disease, Alzheimer's disease and reduce hypertension (Sutharut and Sudarat 2012).

Trait characterization

Traits variation were observed in agronomical and morphological characters of the local rice. A large range of plant height, culm number, culm diameter, leaf length, leaf width, leaf angle, ligule length and weight of ten seeds in local rice population originated from PPU (Figure 2) and Paser (Figure 3) showed a high genetic diversity. A range of 126.67 to 203.33 cm of plant height was noticed for the rice population in PPU, in which most of the cultivar‘s plant height is higher than 1.5 m (Table 3). Interestingly, about 55 % of the population was lowland rice cultivars, and none of them have plant height lower than 1 m, as a normal plant height of lowland rice variety. In addition, Menyan cultivar which has the highest plant height of 203.33 cm is also grown as lowland rice.

Table 1. Rice exploration result in PPU and Paser Districts in East Kalimantan

Districts	Villages	Number of rice cutivars
PPU	Api-api	1
	Babulu Laut	4
	Labangka	1
	Riko	9
	Rintik	5
	Sumber Sari	10
Paser	Kayongo Sari	8
	Muara Pias	5
	Munggu	3
	Olung	1
	Papara	2
	Putang	2
	Riwang	9
	Sekuan Makmur	7
	Sungai Tuak	4
Total		71

Large genotypic variation of plant height was observed in Paser rice population, range from 66 to 209.33 cm (Table 3). The lowest plant height of 60 cm is Siang Inul cultivar, and the highest is Sereh Kuning, which were grown in upland condition. Althought plant height is affected by many factors, such as plantation method, plant density and fertilizer application (Aide and Beighly 2006), but in this case all the environmental factors are similarly applied. Therefore, the character differences showed the genetic potency of the genotypes.

Plant height in rice is generally considered to be controlled by both qualitative and quantitative genes (Huang et al. 1996). The presence of dwarf plant like Siang Inul, and tall plant as Sereh Kuning cultivar can be used to further identify the genes controlling plant height in rice, and for assembling superior dwarf upland rice variety. Plant height has positive correlation to lodging, the displacement of culms from an upright position, which is often associated with yield loss (Navabi et al. 2006; Hui-Jie et al. 2000). The higher of plant hight leads to high risk of lodging. Therefore plant height reduction is a specific interesting in breeding programs to overcome the lodging problem. Although taller plants tend to be more susceptible to lodging, in this study several tall genotypes showed tolerance to lodging (data was not shown) indicating their genetic tolerance.

Variation was also observed for other agronomical characters as presented in Table 3, especially for the traits having significant correllation with plant height. Culm diameter, leaf length and leaf width have strong correlations with plant height (Table 4). Therefore, the traits widely varied following the large variation of plant height. Previous study conducted by Lasalita-Zapico et al. (2010) also observed a very significant positive correlations between plant height and culm diameter, leaf length and leaf width, supporting the current result. Significant positive correlations of the traits showed that, the higher of plant height, the bigger culm diameter, and the longer as well as the wider the leaves. In this study, it was observed that the tall plants also have big culms, length and wide leaves, and the shorter plants have small culms, short and narrow leaves.

There were strong negative correlations between culm number (tiller) and culm diameter, leaf length as well as leaf width (Table 4). It means that culms number are few if the culms diameter are big, the leaves are longer and wider. It was assumed that the plants that have fantastic vegetative growth, tall and big culm, long and wide leaf, have low ability to produce more tillers, eventhought variation was also observed in the population. The same indication was also observed by Wu et al. (2011), who stated that the large culm cultivars exhibited greater plant size, culm diameter, and flag leaf length and width, as well as lower tiller numbers, meaning that the large culm cultivars had markedly fewer tillers compared with the common culm width.

Tillering is one of the most important agronomic characters for grain production in rice (Smith and Dilday 2003). Tiller number per plant determines the panicle number, a key component of grain yield. High culm number is also one of the criteria for new superior rice variety. In the present study, most of the genotypes had culm number less than 15 in PPU (including into intermediate) and less than 10 (including into low) in Paser population. However, there were several local rice cultivars had high culm number. The highest culm number was produced by Ketan Pasero (41.67 culms), followed by Siam (33.33 culms), Ketan Botol (30.33 culms), Siam Mas (30 culms), Muncul (29 culms), Sereh2 (26.33 culms) in PPU's rice population, and Pance Puteh (35 culms), Pance Kuning (26.67 culms) in Paser's rice population. These genotypes can be used as parental candidate for the assembling of new superior upland rice variety. Although further investigation should be done for productive tiller and its relationship with high yield production.

Genetic variation for weight of 10 seeds was markedly large, varied from 0.16 to 0.38 and from 0.13 to 0.40 (Table 3) for PPU and Paser rice population, respectively. Nevertheless, most of the cultivars have weight of 10 seeds of more than 0.20 gram. Heavy seeds weight cultivars, having grain weight of more than 0.35 gram for ten seeds, was observed in Sasak jalan1 (0.38 gram) in PPU, and Sasak Jalan2 (0.36 gram) in Paser rice population. Most of the heavy seeds weight cultivars were dominated by glutinous rice variety, as Ketan Pasero (0.35 gram) in PPU, and Ketan Petion (0.36 gram), Ketan Kuatok (0.37 gram), Ketan Buyung Silong (0.38 gram), Ketan Belanda Krimpang (0.39 gram) and Ketan Tangkai Ngeno2 (0.40 gram) in Paser. One of phenotypic modifications of cereals from their wild progenitors is increaseing of seed sizes (Hancock 2004). This change, recognized as the domestication syndrome, is included into a basic requirement for effective seed harvest and planting and higher grain yield that made cultivation worthwhile.

Rice grain weight is considered to be a stable varietal character (Rabiei et al. 2004), with less than 5% coeffecient of variation (Cassman 1993). It means that genetic mainly influences its variation. Seeds weight is affected by seeds shape, i.e: long, width and thickness. According to Vanaja and Babu (2006), grain length and width atributes to rice yield. In this study, most of the cultivars included into very long grain and medium width (Table 5), and none of the genotype has short grain. Rice grain characteristics such as length, width, and shape have a direct effect on the marketability, and influence the commercial success of modern rice cultivars. Specific consumen prefer specific shape of rice grain, such as long and slim grain or short and big grain, which is also depends on the purpose. Therefore the availability of large genetic variation of rice grain is very important to provide all of the preference for the consumen.

Genetic diversity analysis

Cluster analysis was performed to find out the relationship among the cultivars. Based on several agro-morphological characters, the genetic relatedness among the cultivars could be figured out (Figure 4 and 5). The cultivars were grouped into nine and four classes for local rice population collected from PPU and Paser, respectively (Table 6). Most of the cultivars were grouped into the first

Table 2. Local rice genetic diversity in PPU and Paser Districts in East Kalimantan

Districts		PPU	Paser
Number of cultivars		**30**	**41**
Rice type	Rice	22	31
	Glutinous rice	8	10
Cultivation type	Lowland	16	5
	Upland	14	36
Grain color	Brown	2	0
	White	27	41
	Black	1	0

Table 3. Minimum (Min), maximum (Max) and mean value of quantitative traits of rice cultivars in PPU and Paser Districts, East Kalimantan

Characters	Min.	Max.	Mean	SD
PPU District				
Plant height (cm)	126.67	203.33	161.97	21.31
Culm number (culm)	4.33	41.67	13.80	10.48
Culm diameter (cm)	0.23	1.03	0.75	0.19
Leaf length (cm)	48.33	105.00	84.44	13.91
Leaf width (cm)	0.83	2.13	1.91	0.29
Leaf angle (degree)	10.00	50.00	26.67	12.25
Ligule length (mm)	13.00	55.00	22.29	7.98
Weight of 10 seeds (g)	0.16	0.38	0.28	0.05
Paser District				
Plant height (cm)	66.00	209.33	156.36	26.45
Culm number (culm)	1.00	35.00	7.85	8.45
Culm diameter (cm)	0.37	1.03	0.73	0.17
Leaf length (cm)	39.00	108.33	90.25	16.57
Leaf width (cm)	1.10	2.67	1.80	0.32
Leaf angle (degree)	16.67	50.00	34.04	9.41
Ligule length (mm)	11.00	26.00	19.85	5.09
Weight of 10 seeds (g)	0.13	0.40	0.23	0.4

Note: SD = Standart Deviation

Table 4. Correlations among several quantitative traits

Characters	Plant height	Culm number	Culm diameter	Leaf length	Leaf width	Leaf angle	Ligule length
Culm number	-0.28						
Culm diameter	0.60**	-0.50**					
Leaf length	0.62**	-0.61**	0.54**				
Leaf width	0.54**	-0.41**	0.73**	0.39**			
Leaf angle	0.38*	-0.38*	0.14	0.56**	0.06		
Ligule length	0.12	-0.23	0.15	0.29	0.06	0.11	
Weight of 10 seeds	0.20	-0.27	0.31*	0.20	0.35*	-0.09	-0.07

Note: *, **Significant at p=0.05 and p=0.01, respectively.

Table 5. Grain characteristics of local rice in PPU and Paser Districts, East Kalimantan

Character	Type	Number of genotypes	
		PPU	Paser
Grain length	Very long	26	38
	Long	4	3
Grain width	Big	6	2
	Medium	24	39

Table 6. Clustered class of PPU and Paser District local rice populations

Class	Genotype
PPU District	
I	Cilamaya, Dupa, Kemang Sungkai, Ketan Gunung, Ketan Hitam, Ketan Pasir, Ketan Tangkai Panjang, Lungku Dupa, Mayas, Mayas Merah, Muncul, Menyan, Putih (Siam), Sungkai, Sasak jalan1, Sasak jalan2, Sasak Jalan3, Sereh1, Sereh2, Tangkai mayang, Tihung
II	Siam Mas
III	Ketan Nunuk
IV	Ketan Botol
V	Ketan Pasero
VI	Siam
VII	Jambu
VIII	Jambu Jambu
IX	Ketan Merah
Paser District	
I	Geragai 1, Geragai 2, Ketan Kuning, Ketan Serang, Ketan Tagkai Ngeno, Lekatan Pelam, Lupa Pantai, Mayas Kuning, Mayas Putih, Benalu, Loreng, Pance Kuning, Pance Puteh, Raden Darat, Sasak Jalan1, Sasak Jalan2, Sereh Gunung, Sereh Kuning, Sereh Putih, Sebuyung Biasa, Sebuyung Harum, Siam Gunung, Tempu Maya
II	Prari
III	Siang Inul
IV	Rendah Kuning

Table 7. Genetic potency of the local rice varieties

Superior character*	Genotypes	Origin
Many tillers	Sereh Putih, Sereh Gunung, Pance Putih	Paser
Aromatic	Lupa Pantai, Jambu-jambu	Paser
	Ketan Botol, Ketan Pasir, Padi Menyan	PPU
Long panicle	Ace Cina, Sereh Kuning	Paser
Good taste	Ketan Kuning, Siam Gunung, Mayas Kuning, Pance Puteh, Elvi, Lekatan Pelam, Ketan Mayas, Tempu Maya, Padi Loreng, Mayas	Paser
	Jambu, Lungku Dupa, Padi Putih (Siam), Sasak Jalan, Ketan Gunung, Pare Kiongo, Ketan Hitam, Ketan Merah, Mayas Merah, Sungkai	PPU
Drier and firmer	Sasak Jalan	Paser
	Cilamaya, Cilamaya, Sasak Jalan, Sereh, Siam	PPU
Drier, firmer and aromatic	Pance Kuning	Paser
Uniform harvest time	Rendilo	Paser
Fluffy taste	Mayas Putih, Sebuyung Biasa, Raden Darat, Rendah Kuning	Paser
	Ketan Tangkai Panjang, Muncul, Sereh, Siam Mas, Tangkai Mayang, Tihung	PPU
Fluffy and aromatic	Sebuyung Harum, Padi Benalu	Paser
	Dupa, Kemang, Sungkai	PPU
Dwarf	Ketan Jenggot / Pulut Jangko'	Paser

Note: * The superior characters were based on the reason of cultivation by the local community

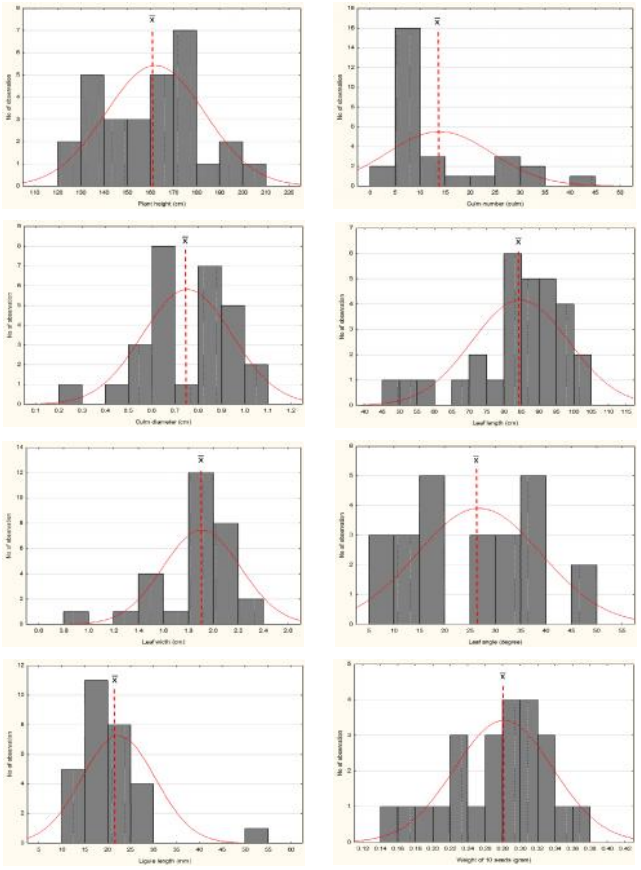

Figure 2. Quantitative traits distribution of local rice varieties in PPU District, East Kalimantan

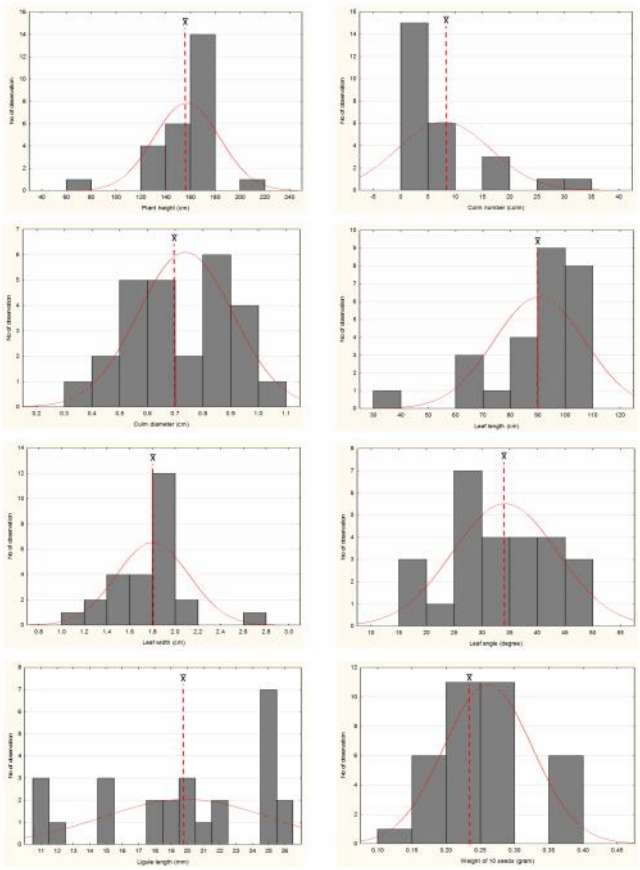

Figure 3. Quantitative traits distribution of local rice varieties in Paser District, East Kalimantan

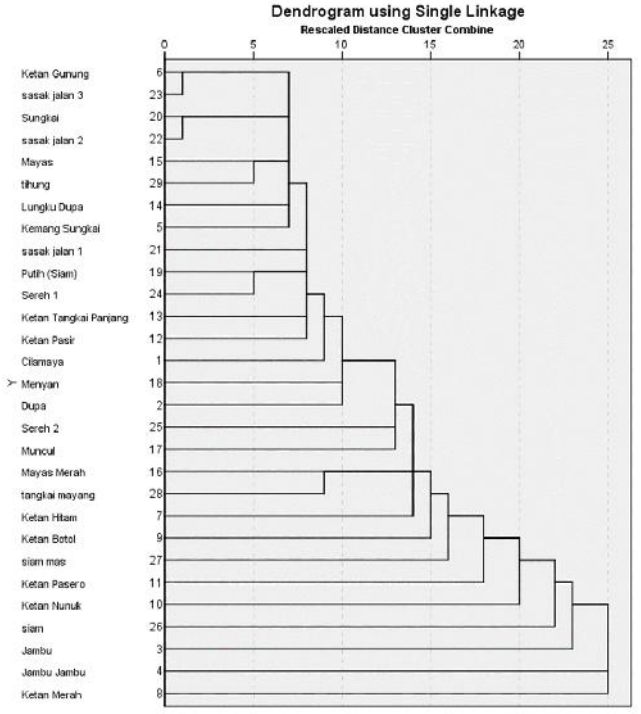

Figure 4. Cluster analysis of rice cultivars in PPU District, East Kalimantan

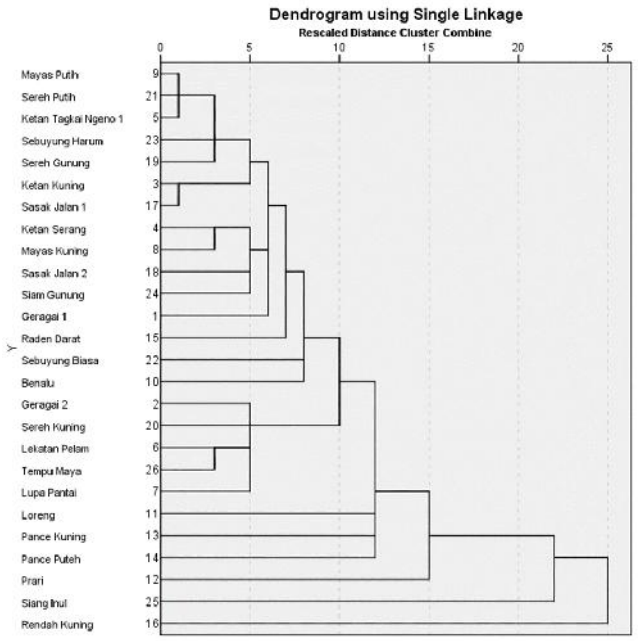

Figure 5. Cluster analysis of rice cultivars in Paser District, East Kalimantan

class for both of populations. Ketan Merah and Jambu-Jambu cultivars from PPU and Rendah Kuning from Paser District were the most distinct variety since they stood out as the most far apart cluster from the others (Figure 4 and 5).

The divergence for genetic diversity analysis was contributed by plant height, culm (number, diameter, angle, internode color), leaf (length, width, angle, blade pubescence, basal leaf sheath color, blade color), ligule (length, color), and grain (length, width, weight of 10 seeds, lemma and palea color, awning, apiculus color). Several morphological characters, i.e: auricle color (whitish), leaf color (green), leaf blade pubescence (intermediate), basal leaf sheath color (green), ligule shape (truncate), ligule color (whitish), had no variance among all cultivars.

Variance was observed in the same cultivars but collected from different location as in Sasak Jalan1, Sasak Jalan2, dan Sasak Jalan3 (having 68% similarity, with different ligule length, culm diameter and culm number); Sereh1 and Sereh2 (having 48% similarity, having different leaf angle, leaf length, culm diameter and culm number) which were collected from PPU District (Figure 4). Geragai1 and Geragai2 (60% similarity, with differerent culm angle, leaf angle, auricle length, seed length and awning color), Sasak Jalan1 and Sasak Jalan2 (having 76% simmilarity, with differerent culm number and auricle length) from Paser District were also grouped in the same class. Even thought there were variances, but they might genetically similar.

Genotypes having similar local name, such as Jambu and Jambu-Jambu, Siam and Siam Mas were clustered in different classes (Figure 4 and Table 6). In the study with aromatic rice landraces, Fukuoka et al. (2006) concluded that significant variation may be found among genotypes with the same name. According to Li et al. (2002) diversity analysis based on phenotypic values may not be the perfect representation of the natural groupings of cultivars, because the phenotypic characters are influenced by environmental. Therefore, further investigation using DNA based analysis should be conducted, to conclude whether the cultivars are genetically the same or not.

Genetic potency

Sustainability of local rice genetic diversity mostly depend on local community, because its cultivation and conservation mainly proceeded by the local farmer. Therefore, the availability of rice seed rely on the planting season and the tendency of the local community towards the certain genotypes. Generally, the farmer has a particular interest for certain variety. That specific interest is the reason for the farmers to grow the variety continuously and unconsciously preserve it.

Based on information collected from informal interviews with the local community, several genetic potencies of the local rice were recorded. The interesting trait of the variety is presented in Table 7. According to the local community observation, certain local rice cultivars carry specific superior characters showing their genetic potency. The superior characters were also the reason why the cultivars are cultivated by the local communities.

Most of the superior traits observed by the farmers are characters related to the taste quality, i.e: aromatic, good taste, fluffy, dried and firmer. Rice quality traits relate to the texture of the grain. Texture of the cooked rice is an important attribute of food acceptance by consumers. Waxy and non-waxy rice are usually classified according to their grain dimensions, amylose content, amylograph consistency, gelatinization properties of the extracted starches (Gonzales et al. 2004), which further determine the texture is fluffy or dried and firmer.

Good taste quality is a well known characteristic of East Kalimantan local rice (WWF 2013). It is also a reason for its high price, which is about 1.5-2 folds than that of the national rice variety in regional market. The price is even higher in international market of the neighborhood countries. In addition, the local rice cultivars also suggested to have tolerance to biotic and abiotic stress and carry other important alleles which have not been examined yet.

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Soil and leaf nutrient status on growth of *Macaranga gigantea* in secondary forest after shifting cultivation in East Kalimantan, Indonesia

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Abstract. *Susanto D, Ruchiyat D, Sutisna M, Amirta R. 2016. Soil and leaf nutrient status on growth of* Macaranga gigantea *in secondary forest after shifting cultivation in East Kalimantan, Indonesia. Biodiversitas 17: 409-416. Macaranga gigantea* is an important pioneer plant species in the tropical secondary forest of Kalimantan and as far the attractive wood species was not commercially cultivated. This study aims to determine the soil and nutrient status on growth of *M. gigantea* in the secondary forest particularly after shifting cultivation activity. For this purposes, the observation plots with 50m x 50m sizes were made and measured to collect the data of diameter, height, soil conditions and leaf nutrient concentrations (N, P and K) of *M. gigantea* in different ages of natural growth. A simple linear correlation analysis was used to determine the relationship of plant growth with the leaf and soil nutrient concentrations as well. The results showed that the soil condition on growth of *M. gigantea* has the average at pH 4.7, CEC 5.57 meq/100g, base saturation 30.22%, and the concentration of soil nutrients were 0.062±0.015% (N), 12.65±4.9 ppm (P), and 57.76±33 ppm (K). We also found that the leaf nutrient concentration was 1.94±0.13% (N), 0.22±0.08% (P) and 0.66±0.27% (K), respectively. Moreover, the highest growth of diameter was found from the 6 years old of plant (27.88 m). The annual yield of diameter and high were 4.65 cm year^{-1} and 2.96 year^{-1} and it was gradually decreased until the 10 years old of plant. The negative correlations was observed from the soil nutrient K and growth of diameter and high of *M. gigantea* ($r=0.95$, $p< 0.05$). The positive correlation was observed from the P and K content in the leaf of plant and growth of *M. gigantea* (diameter, height and volume increment, $p<0.1$). We suggested that phosphorus and kalium content was play an important roles on the growth of *M. gigantea* and this nutrient factor should be considered well when this species will be cultivated for the commercial purposes in the future.

Keywords: *Macaranga gigantea*, nutrient status, soil condition, secondary forest

INTRODUCTION

Shifting cultivation or slash-and-burn farming or swidden agriculture, is an age-old and prevailing subsistence farming practice in the tropical regions, and is one of the traditional practices of forest and land management (Imang et al. 2008; Inoue et al. 2010; Comte et al. 2012; Li et al. 2014). Generally, shifting cultivation involves three essential features: (i) the clearing of natural vegetation, (ii) a cropping period, usually one to three years, and (i) iia fallow period, 10-20 years, during which the land is abandoned to natural vegetation. Usually the fallow period must be longer the cropping period. Traditionally, in many areas, it is 10-20 years or more, but with increasing population density, the fallow period will decrease dramatically (Gauguin et al. 2002). Shifting cultivation system adopted by the Dayak Kenyah people in Samarinda, East Kalimantan, Indonesia an area of forest is cleared usually rather incompletely, the debris is burnt, and the land is cultivated for a few years-usually less than five years-then allowed reverts to forest or secondary vegetation (fallow period) before being cleared again. The average of fallow period in Pampang Village, Samarinda was 6-10 years and forest reopened limited to young secondary forest. The purpose of this shifting cultivation is to rest the land so that the land can be fertile again and replanted (Lahji et al. 1992; Imang et al. 2008). Imang et al. (2008) classified fallow periods among the Kenyah in four categories commonly used at field sites: shrub vegetation (0-2 years), young secondary forest (5-8 years), old secondary forest (more than 9 years), and primary forest. During the fallow period, soil fertility is regenerated through naturally occurring processes. The different mechanisms are partly related to build-up of nutrients in plant biomass, litter layer and soil organic matter during the succesional reforestation under the fallow period (Gauguin et al. 2002). The accumulation of nutrients in plant biomass is affected by soil fertility and crop species. When compared, relatively fertile Inceptisol soil accumulated considerably more nutrients than the less fertile Oxisol. A two year *Piper aduncum* fallow accumulated twice as much nitrogen (N), three times as much phosphorous (P), almost seven times as much potassium (K) and twice as much calcium (Ca) and magnesium (Mg) than did a two year *Imperata cilindrica* fallow (Noordwijk et al. 2008).

Macaranga gigantea is a fast-growing pioneer species in secondary tropical rain forests, and abundantly in the open *mixed dipterocarp* forests after shifting cultivation (Lawrence 2001; Lawrence 2005; Kiyono and Hastaniah 2005; Susanto et al. 2015). Imang et al. 2008 reported that

Kenyah farmers also recognize some dominant species in secondary forests (fallow period) that indicate the land is fertile or not fertile for rice cultivation (cropping period). *Macaranga* is important indicator species of trees in young and old secondary forests. When the species found are dominant and grow well in a certain area, it means that the land is fertile enough for cropping period. Information about flowering, fruiting and seed germination also has been reported by Susanto et al. (2016). On the other hand, *M. gigantea* has not yet been cultivated and information about soil, leaf nutrient status, growth and its correlation from naturally succession in various tread and age in secondary forests is not yet known.

One of approaches in determining fertilizer requirements on plants which can be applied properly is soil analysis. Plant tissue analysis is more practical to determine nutrient status on plant than other methods. Plant tissue generally analyzed is leaf. Nutrients in leaves not only have a role in photosynthesis but also represent plant nutrient status. Leaves also consist of tissues which always available for analysis of plant nutrient status. Leaf analysis has been used as indicator in nutrient diagnosis and as basic recommendation for fertilizer application on fruit crops at some countries (Smith 1962; Leece 1976; Shear and Faust 1980; Liferdi et al. 2008). Kim et al. (2015) reported the foliar N and P concentration could be used as a parameter to assess the nutrient environments of tree species restored in a fire-disturbed urban forest. Singh (2006) also reported that the growth rates (height, diameter and volume increment) were positively related to foliar N and P concentration.

Therefore, here in this research we focus our work on soil and leaf nutrient status of *M. gigantea* in fallow period of shifting cultivation areas. By obtaining information about soil and leaf nutrient status of this plant, expected to support if this species will be cultivated for the commercial purposes in the future.

MATERIALS AND METHODS

Study area

This research was conducted in Pampang, Sungai Siring (village of Dayak Kenyah), Samarinda, East Kalimantan, Indonesia. It was located between the coordinates of $00^0$20'15.4"-$00^0$21'58.8" South and $117^0$13'46.6"-$117^0$14'11.0" East (Figure 1). The average of annual rainfalls (2003-2013) was 2423 mm, the highest annual rainfall interception was 2757.5 mm in 2008. The highest monthly rainfall interception was in April (288.3) and the lowest was in August (115.3 mm). Wet period occurs between 9-12 months, while dry period occurs between 0-3 months. Average monthly temperature was 27.5^0 and average air humidity was 82% (Anon. 2012; Susanto et al. 2016). Soil and leaf nutrient analysis, and data analysis were conducted in the Soil Science Laboratory at the Faculty of Forestry and Plant Physology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Mulawarman, Samarinda, East Kalimantan, Indonesia. The research was conducted from July 2011 to January 2012.

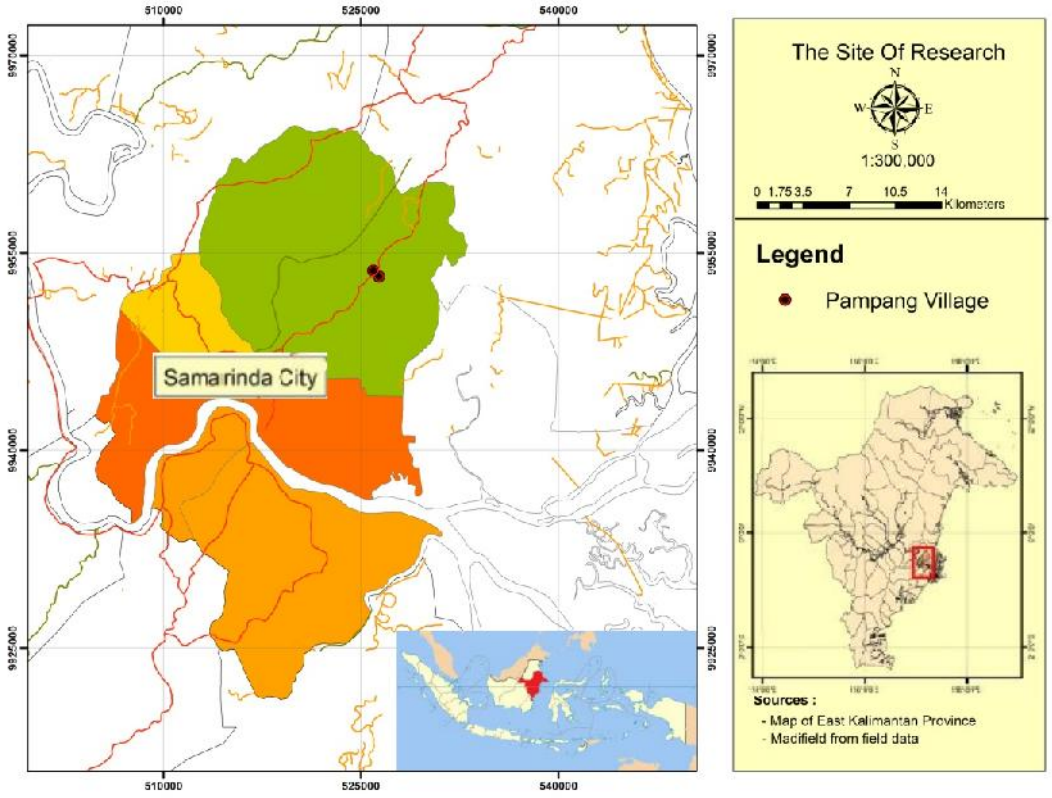

Figure 1. Map of study area in fallow period shifting cultivation at Pampang Village Samarinda, East Kalimantan

Procedures

Research in the area of fallow periods shifting cultivation begins by conducting interviews with land owners (farmer) to obtain information about shifting cultivation cycles, location and age of fallow lands. For each age fallow land made one plot size of 50 m x 50 m. Coordinate point is determined using GPS, then measuring the following: number of trees *M. gigantea*, diameter and height of trees, sampling the soil and leaf of *M. gigantea*.

Measurement of diameter and height M. gigantea

On each plot was measured *M. gigantea* plants, include: the number of trees; diameter at breast height (dbh); and height (h) is measured with clinometer.

Soil sampling

Soil sampling was conducted in all research plots with drill ground at a depth of 0-30 cm and 30-60 cm. On each plot was take 4 point drilling, soil samples composited for each plot. Composite soil samples were then taken 500 g of each class of depth, put into plastic bags, labeled and transported to the laboratory to be analyzed concentration nutrient elements (C, N, P, K).

Sampling leaf M. gigantea

Leaf sampling conducted in all research plots using a pole. In each plot, four trees set an example. The leaves of the tree each sample is then taken in such a way by observing the position of the individual components of the canopy leaf vertically (bottom, middle and top) and horizontal position of each of these components in the stem (the part closest, medium and farthest part). Further examples of composite leaf for each study plot, sampled 500 g, put in plastic bags and transported to the laboratory to be analyzed concentration nutrient elements (N, P, K).

Soil analysis

Soil sample was taken from the depth of 0-30 cm, 30-60 cm and the results were calculated after being dried in the oven with the temperature of 150^0C until constant weight was reached. Composite sample was air-dried and its pH, base saturation, cation exchange capacity, organic carbon content, total Nitrogen (Kjendal), available phosphor (Bray), available potassium were analyzed.

Analysis on leaf nutrient concentrations

The total N concentrate was measured using Kjeldahl method (extraction, distillation, and titration). To measure the element of P and K, the plant components were extracted using High Pressure Digestion method at the temperature of 180^0C for 10 hours with HNO_3 as a reductant. Phosphor was measured by using calorimetric technique and using nitrate-molybdate-vanadate acid as a coloring agent and was measured by using spectrophotometer at the wavelength of 470 nm. Potassium (K) was measured by using Atomic Absorption Spectrophotometer at the wavelength of 766,5 nm, 489,5 nm and 245,2 nm.

Data analysis

The data obtained from the observation in the field, soil and leaf concentration were analyzed descriptively. Correlation between soil N, P, K concentration (X) with leaf N, P, K concentration and growth of *M. gigantea* (Y) was explored through regression analysis, for significance through a two-tailed Student's t-test (p= 0.05-0.10).

RESULTS AND DISCUSSION

Soil chemical properties

Results of soil chemical analysis showed that the soil pH range between 4.4-4.7 with an average of 4.63 ± 0.11 that included the category of acid and very acid. Cation exchange capacity is very low (3.66 to 4.96 meq. $100g^{-1}$) to low (5.64 to 7.63 meq $100gr^{-1}$) with average 5.90±1.18 meq $100gr^{-1}$. Effective base saturation is medium and low (24.81 to 43.43%) at a depth of 0-30 cm and the status of low and very low (9.19 to 33.14%) at depth of 30-60 cm. Base saturation tends to decrease from the fallow period of 0.5 to 6 years and increased again in the fallow period 7 to 10 years (Table 1)**.**

Results of soil chemical analysis showed that the carbon concentrations ranged from 0.3 to 0.78% with the average of 0.53 ± 0.17 with a very low status. A carbon nutrient concentration in the 0-30 cm soil depth class is always greater than 30-60 cm soil depth class on all plots. The concentration of N status is very low in the range of 0.04 to 0.09% with the average value of 0.062 ± 0.015. Further away from the surface of the soil, the concentration of N decreased in all study plots. The concentration of P has the status of extremely low (5.33 ppm) to high (24.23 ppm) with the average of 12.65 ± 4.9 which is low. Phosphorus nutrient concentrations at a depth of 30-60 cm class are always better than for the class of 0-30 cm depth. The concentration of K nutrient status is very low (17.11 ppm) to low (122.56 ppm) with average 57.76 ± 33.8 were classified as very low. Kalium nutrient concentrations in the 0-30 cm soil depth class higher than the class of the depth of 30-60 cm, has a tendency to continue to decline until the fallow fields 6 years and increased again in the fallow period of 7 years and 10 years (Table 2).

Plant growth

The results of the field studies showed that *M. gigantea* found on fallow land age of 0.5 years to 10 years. Fallows old 0.5 years, the mean diameter is 0.73 cm, with 0.58 m height that still includes categories seedling. A 2 years, the mean diameter *M. gigantea* is 2.27 cm and 3.03 m heigh. *M. gigantea* in fallow land 3 years, the average diameter is 6.05 cm with a height of 5.69 m. Fallow land 6 years, the average diameter was 27.88 cm and a height of 17.74 m. Fallow land 7 years the average diameter *M. gigantea* is 22.02 cm and heigh is 15.37 m. *M. gigantea* in fallow land 10 years the average diameter is 26.79 cm and height 18.17 m. The study showed that highest mean diameter (27.88 cm) was obtained in *M. gigantea* from fallow 6 years old, then decreased. Diameter and height growth increment of the largest (4.65 $cm.yr^{-1}$ and 2.96 m. $year^{-1}$) obtained on the plant from fallow 6 years old and continued to decline until the age of 10 years (Table 3).

Table 1. Soil chemical properties in each age fallow period shifting cultivation in Pampang Village, Samarinda, East Kalimantan

Fallow lands old (yr)	Soil depth (cm)	Soil chemical properties		
		pH (H_2O)	CEC (meq.100 g^{-1})	BS (ppm)
0.5	0-30	4.7	6.10	37.31
	30-60	4.7	6.38	30.23
2	0-30	4.6	6.81	43.43
	30-60	4.7	7.63	28.57
3	0-30	4.7	6.59	32.86
	30-60	4.6	7.01	25.18
6	0-30	4.7	3.77	24.81
	30-60	4.8	3.87	9.19
7	0-30	4.4	5.36	41.11
	30-60	4.5	6.35	29.09
10	0-30	4.5	5.30	40.74
	30-60	4.6	5.64	33.14
Mean		0.53±0.17	4.63±0.11	31.30±93

Note: CEC= cation excange capasity, BS= base saturation

Table 2. Soil Nutrient concentration in each age fallow period shifting cultivation in Pampang Village, Samarinda, East Kalimantan

Fallow lands old (yr)	Soil depth (cm)	Soil nutrient concentrations			
		C (%)	N (%)	P (ppm)	K (ppm)
0.5	0-30	0.71	0.07	14.25	104.24
	30-60	0.39	0.05	17.82	122.56
2	0-30	0.77	0.08	5.70	90.87
	30-60	0.33	0.06	24.23	78.09
3	0-30	0.74	0.07	12.83	72.43
	30-60	0.37	0.04	15.68	27.84
6	0-30	0.78	0.08	9.76	31.45
	30-60	0.30	0.05	18.3	17.11
7	0-30	0.55	0.06	7.13	45.31
	30-60	0.32	0.04	10.69	19.64
10	0-30	0.76	0.09	5.33	50.37
	30-60	0.35	0.04	10.13	33.14
Mean		0.53±0.17	0.062±0.015	12.65±4.9	57.76±33.8

Leaves nutrient concentrate

Result of leaf analysis showed that *M. gigantea* leaves from fallow land shifting cultivation had different N, P, K concentrations. The concentration of N in the leaf *M. gigantea* in the range of 1.75 to 2.12%, with the average 1.94% ± 0: 13. Concentration of P has a range from 0.14 to 0.36%, and K has from 0.37 to 1.06%, by average, respectively 0.8% ± 0.22 (P) and 0.66% ± 0.27 (K). The concentration of N, P, K leaf *M. gigantea* increased until the age of 6 years and then declining age of 7 and 10 years old.

Correlation between plant growth with soil and leaf N, P, K concentration

The concentration of nitrogen and phosphorus in the soil does not correlate with the diameter and height of plants *M. gigantea*, whereas the concentration of potassium in the soil at a depth of 0-30 cm and 30-60 cm negatively correlated with stem diameter, height, diameter increment, height increment and volume increment. Results of t- test on the value of r shows that statistically significant ($p < 0.05$). (Table 5, and Figure 3).

The relationship between the concentration of nutrients P, K leaves with average stem volume and volume increment, statistically significant for the elements K, and diameter increment and height increment statistically significant for the elements P. (Table 6, Figure 4).

Table 4. Concentration of leaf nutrient *M. gigantea* in each age fallow period shifting cultivation in Pampang Village Samarinda.

Fallow land old (yr)	Leaf nutrient concentrations		
	N (%)	P (%)	K (%)
0.5	2.00	0.18	0.61
2	1.89	0.14	0.38
3	1.89	0.25	0.56
6	2.12	0.36	0.85
7	2.00	0.20	0.37
10	1.75	0.15	1.06
Mean	1.94±0.13	0.22±0.08	0.66±0.27

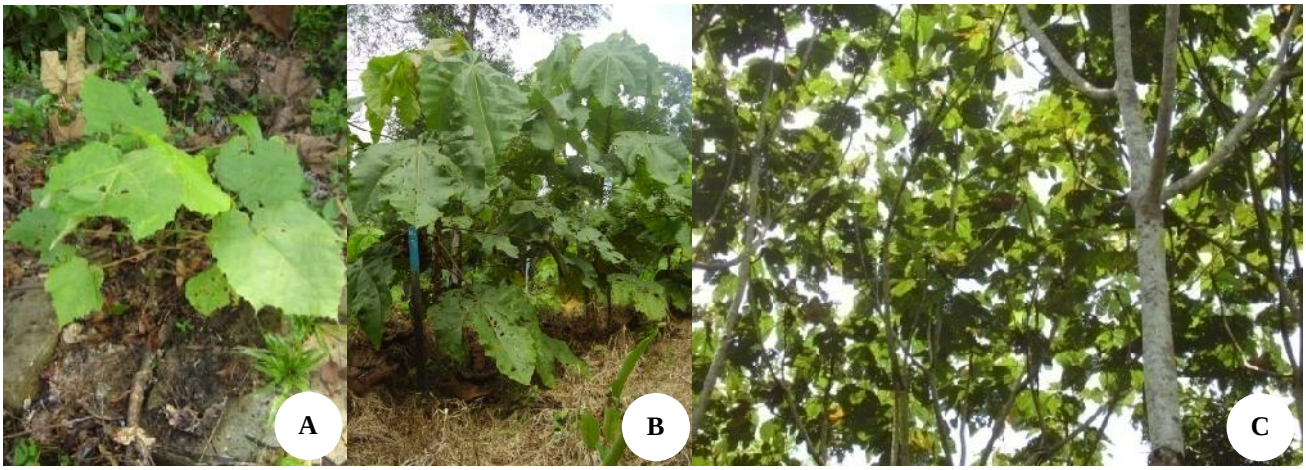

Figure 2. *Macaranga gigantea* plants: A. seedling, B. sapling, C. mature trees

Table 3. Data inventory vegetation of *M. gigantea* in each age fallow period shifting cultivation in Pampang Village, Samarinda

Fallow land old (yr)	N 2500m$^{2-1}$	N ha^{-1}	Diameter (cm)	Height (m)	Volume (m^3 ha^{-1})	Diameter increment (cm th^{-1})	Height increment (m th^{-1})	Vol increment (m^{3}ha^{-1}th^{-1})
0.5	9	36	0.73	0.58	0.0005	0.73	0.580	0.0005
2	38	152	2.27	3.03	0.13	1.1	1.135	0.065
3	12	48	6.05	5.69	0.44	2.0	2.017	0.147
6	5	20	27.88	17.74	11.06	4.65	2.96	1.843
7	9	36	22.02	15.37	8.77	3.2	2.196	1.253
10	12	48	26.79	18.17	25.88	2.7	1.870	2.588
Mean						2.4±1.4	1.8±0.8	0.9±1.1

Table 5. Correlation coefficient from results regression analysis and p-value for significance throught t-test between soil nutrient concentration and growth of *M. gigantea*

Soil concentration	Depth (cm)	D	t	H	t	V	t	DI	t	HI	t	VI	t
N (%)	0-30	0.29	0.585	0.28	0.596	0.566	0.223	0.10	0.850	0.06	0.911	0.505	0.305
	30-60	0.47	0.352	0.50	0.316	0.472	0.344	0.34	0.509	0.40	0.432	0.441	0.381
P (ppm)	0-30	0.48	0.339	0.52	0.289	0.881	0.232	0.25	0.627	0.22	0.672	0.818	0.265
	30-60	0.73	0.195	0.63	0.176	0.084	0.146	0.38	0.458	0.35	0.502	0.105	0.183
K (ppm)	0-30	0.95	**0.04**	0.96	**0.03**	0.666	0.149	0.97	**0.01**	0.94	**0.05**	0.823	**0.04**
	30-60	0.77	**0.07**	0.81	**0.05**	0.498	0.314	0.83	**0.04**	0.93	**0.007**	0.622	0.187

Note: Bold values denote significance at $p < 0.05$. D= stem diameter, H= stem height, V= volume of stem, DI= diameter increment, HI= height increment, VI= volume increment and t = t-test values.

Table 6. Correlation coefficient from results regression analysis and p-value for significance throught t-test between leaf nutrient concentration and growth of *M. gigantea*

Leaf concentration	D	t	H	t	V	t	DI	t	HI	t	VI	t
N (%)	0.09	0.863	0.03	0.952	0,388	0.445	0.42	0.413	0.32	0.539	0.15	0.773
P (ppm)	0.41	0.417	0.38	0.463	0,010	0.954	0.74	**0.094**	0.75	**0.082**	0.20	0.700
K (ppm)	0.60	0.212	0.56	0.234	0,789	**0.062**	0.41	0.420	0.32	0.542	0.76	**0.081**

Note: Bold values denote significance at $p < 0.10$. D= stem diameter, H= stem height, V= volume of stem, DI= diameter increment, HI= height increment, VI= volume increment and t= t-test values

Discussion

The soil chemical properties in this study area pH (H_20) is very acid to acid reaction, cation exchange capacity is very low to low and effective base saturation is low to medium at a depth of 0-30 cm and 30-60 cm. Base saturation tends to decrease from the fallow lands of 0.5 to 6 years and increased again in the fallow lands 7 to 10 years (Table 1)**.** Ruhiyat and Lahjie (1992) reported that the soils in fallow lands of shifting cultivation systems of Dayak Kenyah in Barong Tongkok, East Kalimantan, have very acid to acid reaction, with pH (H_2O) value of 4.0-5.2. Effendi et al. (2009) also reported that the soil properties in fallow lands under intensified shifting cultivation systems in Sarawak, Malaysia could be characterized by a strongly acidic nature with low levels of exchangeable bases. During the fallow, soil organic carbon, cation exchange capacity (CEC), nitrate, total phosphorous, and extractable basic cations all manifest positive associations with fallow length after 3 to 11 yr of fallow. No declines in soil parameters were detected between plots based on the frequency of past slash-and-burn activity (Kleinman et al.1996).

The soil carbon, nitrogen and phosporus concentrations in this study area a very low, phosphous has the status of extremely low (Table 2). Ruhiyat and Lahjie (1992) also reported that status carbon concentration ranges 2.6 to 5.9% in 1 month fallow periods, increases by 4.3-7.2% and 5.7-14% in the fallow periods of 2 and 3 years. At plots with a longer fallow period it is obvious that the carbon concentration decreases again. Nitrogen concentration in 1 month fallow periods shows the lowest value (0.34%), increase up to the fallow 3 years (0.64%), and N concentration to decrease to 0.58% in fallow periods 5 years. The decrease in N concentration continues until the fallow periods 8 years (0.33%), and fallow period of 35 year 0.43%. At plots with duration of up to 2 year for the fallow period the P concentration is lowest (0.3-0.9 ppm), increasing to 2.7-17.3 ppm at fallow period 3-5 year, P concentration decreases again with longer fallow period 8 and 35 years. K concentration at plot the plot with fallow

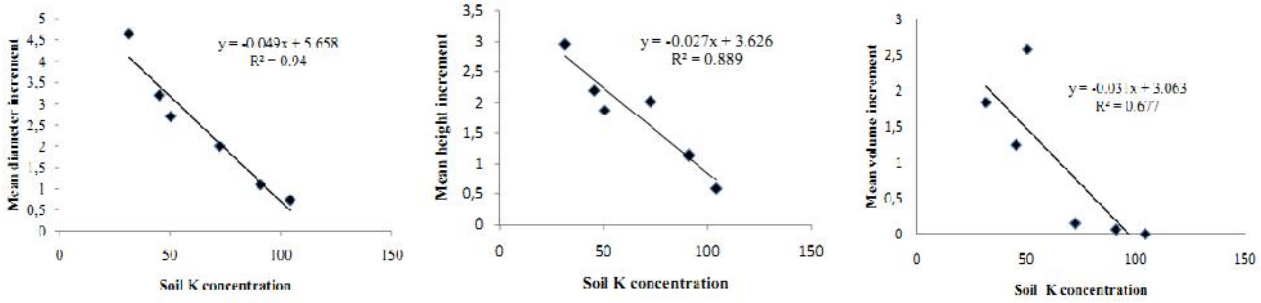

Figure 3. Relationships between soil K concentration and growth of trees *M. gigantea* at fallow periods shifting cultivation. (a) diameter increment Vs soil K concentration (0-30 cm) (b) height increment Vs soil K concentration (0-30 cm), and (c) volume increment Vs soil K concentration (0-30 cm).

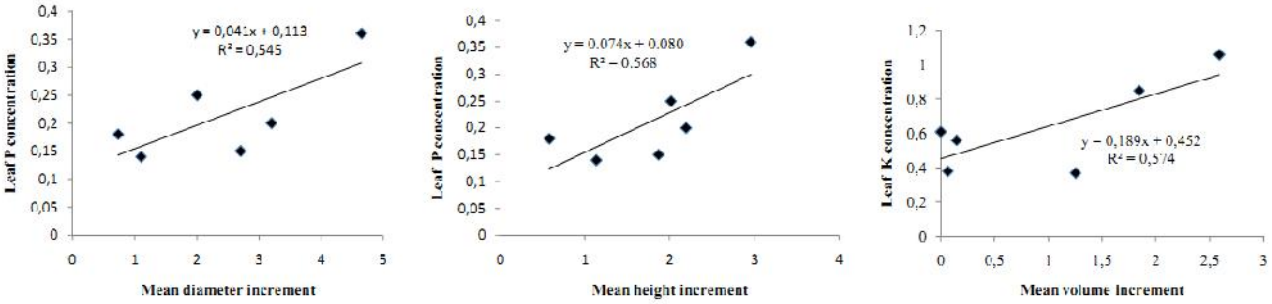

Figure 4. Relationships between leaf P and K concentration with growth of trees *M. gigantea* at fallow periods shifting cultivation. (a) diameter increment Vs leaf P concentration (0-30 cm) (b) height increment Vs leaf P concentration (0-30 cm), and (c) volume increment Vs leaf K concentration (0-30 cm).

period of 2-5 years show a relative increase, then its decrease again at plots with a fallow period of 8 to 35 years. Funakawa et al. (2010) reported that he soils in East Kalimantan were generally less fertile. The lower N availability could be ascribed to the loss of N from soil ecosystems and the insufficient recovery of soil N pools under poor vegetation regrowth due to intensive land use in this area (Effendi et al. 2009). Styger et al. (2007) recommended that upland agricultural intensification and diversification based on improved soil fertility through optimized organic and inorganic inputs and fire-less land management that encourages the re-establishment of nutrient stocks. The study showed that highest mean diameter (27.88 cm) was obtained in *M. gigantea* from fallow 6 years old, then decreased. Diameter and height growth increment of the largest (4.65 cm.yr^{-1} and 2.96 m. year^{-1}) obtained on the plant from fallow 6 years old and continued to decline until the age of 10 years (Table 3). Hiratsuka et al. (2006) reported that the average stem diameter of *M. gigantea* at the age of three years after forest fire in East Kalimantan, Indonesia was 4,6 cm (average diameter increment was 1,53 cm). On the other side, Davies et al. (1998) reported that the diameter growth of *M. gigantea* was 0.22 mm year^{-1}, at seedling or sapling stratum was 0.22 mm year^{-1}, 0.37 mm year^{-1} at reproductive stratum, 0.74 mm year^{-1} at reproductive stratum and 0.39 mm year^{-1} at mature stratum (Figure 2). Compared with this data, our results showed that the diameter increment and height of *M. gigantea* in fallow after shifting cultivation activity was higher than previously reported.

Imang et al. (2008) reported that in shifting cultivation, vegetation succession more real existence and simpler for farmers instead of using the number of years of fallow period. The condition of vegetation, especially of key species is a direct indication of the level of soil fertility. If farmers describe the plot of fallow land to describe the status of their real ecological succession. If the indicator species has grown and become the dominant vegetation, it means that the land was fertile and cleaned ready for replanting. Farmers use their experience and knowledge with a view vegetation species key to determiner that particular plot of a land enough fertile for farming use. In Pampang, *Blumea balsamifera* is a good indicator species of vegetation most fertile soil in the shrub vegetation, while *Macaranga triloba* is a tree species important indicator for young secondary forests. *Macaranga spp, Hibiscus macrophylus, Spatholobus oblongifolius* are important indicators for the old secondary forest. Tree species important indicator for primary forest is *Shorea* spp. If the species were discovered dominant and growing in a certain area, it means that the land is enough fertile for farming. Cultivators Kenyah also recognize some dominant species in secondary forest indicates that the soil is not fertile.

These species include *Dicranopteris linearis*, *Melastoma malabatricum*, *Imperata cylindrica*, *Oncosperma horridum* and *Tristaniopsis whiteana.*

The leaf nitrogen concentration in this study is 1.94% ± 0: 13 (1.75 to 2.12%), concentration of P has a range from 0.14 to 0.36%, and K has from 0.37 to 1.06%, by average, respectively 0.8% ± 0.22 (P) and 0.66 ± 0.27% (K) (Table 4). Ishida et al. (2004) reported the N concentrate ranged between 15-20 mg g^{-1}, while phosphor concentrate ranged between 1.7 to 2.7 mg g^{-1} in seedling canopy *M. gigantea*. On the other hands, it was also reported that the highest nitrogen concentrate of *M. gigantea* leaves which grow naturally was 2.5 mol kg^{-1} and 2.0 mol kg^{-1} when the plant was growth under sapling stratum; and the lowest nitrogen concentrate was found at seedling, sucker stratum and mature stratum, that only gave 1.5 mol kg^{-1}. Breulmann et al. (2002) stated that phosphorus concentrate on *Macaranga* growth in the natural forests in Malaysia was ranged between 0.06 to 0.09%, while potassium content was 0.71 to 0.82%, respectively. The largest size of *M. gigantea* leaves was found at sapling stadium, reaching 60 cm long and 50 cm wide (Okuda 1996; Silk et al. 2000). The same is expressed by Gauguin et al. (2002) that the biomass of leaves and nutrients stored in leaf increased rapidly in the first years of fallow shifting cultivation, where after it will reach its maximum level. Nutrients more concentrated on the delicate parts of the plant such as leaves, twigs and small branches rather than the more rugged components such as rods. Delicate parts only make up a small proportion of secondary forests growing older but often have a large proportion of total nutrients in above-ground biomass. The nitrogen content in the biomass secondary forest stands ex-shifting cultivation increases linearly only up to 5 years. After this initial period the leaf canopy is fully developed and the increase in weight of the stand increases in nitrogen poor bole material. For biomass of P on the same tendency that increased linearly only up to 7 years, being the elements K and Ca are very similar to N. During the first years the availability of nutrients K are relatively high but when taken out of many available nutrients taken with a strong and next at a lower rate. Likewise, the Mg nutrients are just higher at the beginning of growth

The correlation between growth of *M. gigantea* trees with soil nutrient concentration at a depth of 0-30 cm, 30-60 cm statistically significant only in the element of K (p=0.05), while for the other elements are not significant. Concentration of potassium in the soil at a depth of 0-30 cm and 30-60 cm negatively correlated with stem diameter, height, diameter increment, height increment and volume increment (Table 5, Figure 3). *M. gigantea* growth was negatively correlated with soil K concentration. Based on the r-value, soil K concentration had moderately correlated with *M. gigantea* growth. The r-value of 0.96 and 0.77 showed that 96% and 77% variance of *M. gigantea* growth change could be explained by soil K concentration variable, while the remain of 4% and 23% could be explained by other factors.

On the other hand, relationships between the concentration nutrients of foliage *M. Gigantea* and growth of *M. gigantea*, statistically significant (p=0.10) for nutrient P and K (Table 6, Figure 4). Liferdi et al. (2008) reported that calibration test gives meaning of leaf analysis value obtained from laboratory become interpreted data, whether nutrient concentration in leaf is very low, low, moderate high, and very high. Only plants having low nutrient concentration require fertilizer. Kim et al. (2015) reported that the foliar N and P concentration could be used as a parameter to assess the nutrient environments of tree species restored in a fire-disturbed urban forest.

In the tropical rain forests of East Kalimantan servings of nutrient base class (K, Ca and Mg) more accumulates on vegetation than in the soil, while the portion of nutrient N largely accumulates in the soil (Ruhiyat 1993), and in forest plantation reported that plant forests of *Eucaliptus deglupta* and *Paraseriantes falcataria* at the age of 5-10 years in East Kalimantan also accumulate nutrient element of K in a largest amount, followed by calcium, nitrogen and magnesium. Therefore, the availability nutrient element of K to fulfill the need of *Eucalyptus deglupta* stand should get a prioritized attention. In the other parts of natural forests in East Kalimantan show that 70 to 94% of base elements exist in the stand biomass (Ruhiyat 1993; Meckensen 1999; Meckensen et al. 2001).

In conclusion, soil condition of *M gigantea* in fallow periods has the average at pH 4.63±0.11, CEC 5.90±1.18 meq $100g^{-1}$, base saturation 31.30±0.93%, and the concentration of soil nutrients were 0.062±0,015% (N), 12.65±4.9 ppm (P), and 57.76±33.8 ppm (K). We also found that the leaf nutrient concentration was 1,94±0,13% (N), 0,22±0,08% (P) and 0,66±0,27% (K), Diameter and height increment of the largest (4.65 cm yr^{-1} and 2.96 m $year^{-1}$) obtained on the plant *M. gigantea* at fallow land 6 years old and continued to decline until the age of 10 years. The negative correlations was observed from the soil nutrient K and growth of diameter, high, diameter increment, high increment and volume increment of *M. gigantea* (p=0.05). The correlation between the concentration of N leaf with diameter and hight stem statistically not significant, but for P leaf nutrient statistically significant with diameter increment and height increment, and K leaf nutrient with stem volume and stem volume increment.

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Short Communication: Microscopic decay pattern of yellow meranti (*Shorea gibbosa*) wood caused by white-rot fungus *Phlebia brevispora*

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Abstract. *Erwin. 2016. Microscopic decay pattern of yellow meranti (*Shorea gibbosa*) wood caused by white-rot fungus* Phlebia brevispora. *Biodiversitas 17: 417-421.* The anatomical changes of wood decaying caused by white-rot fungus *Phlebia brevispora* could provide the basis for evaluating and analysis of decay on yellow meranti (*Shorea gibbosa*) heartwood. By using soil-block test procedure of JIS K-1571 and microscopic analysis, a progressive decay *in vitro* of *S. gibbosa* wood caused by *P. brevispora* was well characterized.The percentage of wood weight loss was ranged from 0.91% to 12.34%in 2-12 weeks' incubation. On the first 6 weeks of incubation of *S. gibbosa* infected with *P. brevispora*, the early stages decay, in which pit erosion and slight erosion of cell walls facilitated by hyphal spreading among cells. The intermediate decay features of numerous and conspicuous holes as well as erosion troughs in cell walls were found after 8 weeks' incubation. Furthermore, complete degradation of wood cell components, defined as the advanced stage of decay, was found in some areas of wood after 12 weeks' incubation. The pattern of wood decay was similar to those of the decayed xylem of *S. gibbosa* stem canker in field conditions.

Keywords: Cell degradation, microscopic, *Phlebia brevispora*, *Shorea gibbosa*, wood decay

INTRODUCTION

White rot basidiomycetes that cause the decay are especially important in wood decomposition because they are the only fungi capable of degrading all cell wall components (cellulose, lignin, hemicelluloses) of wood (Blanchette 1991; Schmidt 2006; Schwarze 2007). Micromorphological aspects of two main types of white rot, selective delignification and simultaneous rot, have been distinguished (Blanchette 1984; Otjen et al. 1987; Anagnost 1998; Schwarze 2007). In selective delignification, lignin in the secondary wall and middle lamella is almost entirely removed, whereas as large quantities of cellulose in the S2 layer of the cell wall are left intact and are separated from one another. Simultaneous rot is characterized by removal of both cellulose and lignin, leaving cells either riddled with bore holes and erosion troughs, or with extensively thinned secondary walls.

A white-rot fungus has been isolated from decayed xylem of *Shorea gibbosa* stem canker, namely *Phlebia brevispora* (Erwin et al. 2010) and was suspected to cause serious wood decay on this tree species (Erwin 2012).

Shorea gibbosa is known as a member of yellow meranti group (Ogata et al. 2008) which has been long managed for timber production and used for many wood products, therefore, the microbial decay processes go along with a loss of wood quality will affect the lumber value andthe wood products in use.

The present study is intended to the previous reports of Erwin (2010) and Erwin et al. (2012) with presented the anatomical features of *S. gibbosa* heartwood infected with *P. brevispora* under laboratory conditions (*in vitro*). Although microscopic observation techniques are not applicable in the field use, however, the decay pattern of the infected wood can clearly be characterized and very useful for providing valuable information and understanding the stages of wood degradation by the fungal attack.

The aim of this study was to (i) evaluate the ability of *P. brevispora* to degrade *S. gibbosa* heartwood, and (ii) confirm the decay pattern of the fungus in artificial laboratory conditions by microscopic observations.

MATERIALS AND METHODS

Fungal strain

The decay fungus isolated from decayed xylem of *S. gibbosa* stem canker, and designated as YM3, was genetically identified by their internal transcribed spacers (ITS) sequence as *Phlebia brevispora* (Erwin et al. 2010). The fungal strains were maintained at 4°C on PDA slants.

Decay test procedure

For this experiment, inoculation procedures followed the JIS K 1571 soil-block test procedure (JIS K 1571 2004). A medium of 250 g quartz sand and 80-85 ml of nutrient solution (4.0% glucose, 0.3% peptone, and 1.5% malt extract) used for culture media. Twelve sound wood-blocks (20 mm x 20 mm x 10 mm) in radial, tangential, and longitudinal directions, respectively, were obtained from uninfected heartwood of the stem disks of *S. gibbosa*. The

blocks were oven dried and weighed, then sterilized with gaseous ethylene oxide at 50 ^{o}C for 5 h. The blocks were introduced in four glass jars (each glass jar containing three blocks), and inoculated with the liquid fungal culture of the isolated fungus, then aseptically incubated at 26 ± 2 ^{o}C and 70-80% RH for each 2, 4, 6, 8,10 and 12 weeks. The blocks of each incubation period were brushed clean to remove superficial mycelia. Nine blocks were oven-dried at 70 ^{o}C until a constant dry weight was reached. Percent weight loss due to decay was then calculated; three blocks were reserved for microscopic observations.

Microscopic observation

The dried blocks were sectioned with razor blade then fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) at 4 ^{o}C overnight, washed four times in 0.1 M phosphate buffer at pH 7.2 for 15 minutes each, and rinsed three times in distilled water for 5 minutes each. The blocks were placed in an ethanol dehydration series of 50, 80, 95, 100% each for 20 minutes, then three times in 100% ethanol. The dehydrated blocks were freeze-dried, and mounted on SEM stubs, then coated with gold-palladium using Jeol JFC-1200 Fine Coater. The coated samples were observed under a JEOL Scanning Microscope (JSM-5310) and the EDAX application program used to obtain SEM images of the altered properties of wood.

RESULTS AND DISCUSSION

The weight loss of *S. gibbosa* wood after *P. brevispora* decay over 2-12 weeks is shown in Table1. Based on classification of natural durability of Indonesian woods (Seng 1990), the infected wood of *S. gibbosa* with 12.34% weight loss, were categorized non-resistant (class IV) against *P. brevispora* attack. The result indicated the fungus capable of attacking the heartwood of *S. gibbosa* under laboratory conditions, thus, it should be taken as a consideration for wood protection, and otherwise, the fungus can produce an extensive degradation into wood under favorable temperature and humidity. Meanwhile, microscopic observations of this decay showed various stages of decay, as shown in Figures1-4.

After 2-4 weeks' incubation, the wood blocks had lost 0.91-2.24% in weight. Abundant clamped hyphae colonizing the lumina of vessels were observed in transverse, radial and tangential views (Figures 1.A-D). However, in axial parenchyma cells-rays and fibers adjacent to heavily infected vessels-hyphae were not observed. In this case, hyphae propagated mainly in vessels where they could either grow parallel to the cell axis and diagonally across the lumina, supported at their points of attachment with the cell walls, or in the central part of the lumina, where they are held in place by hyphal branches extending from the main hyphae attached to the cell walls. Hyphae passing through the perforation plates were also detected (Figure 1.E). Despite hyphae being attached deep within the vessel walls, they did not severely damage cell walls (Figure 1.F).

Table 1. Weight loss in *S, gibbosa* wood infected with *P. brevispora* for periods of 2, 4, 6, 8, 10 and 12 weeks

Incubation period (weeks)	Weight loss percentage Mean ï SE
2	0.91 ± 0.10
4	2.24 ± 0.60
6	5.02 ± 1.03
8	8.23 ± 1.22
10	11.80 ± 5.15
12	12.34 ± 2.76

After 6 weeks' incubation, wood blocks had lost 5.02% of their weight. Fungal hyphae had extended from heavily infected vessels into rays, axial parenchyma cells and fibers mainly through pits, causing slight erosion of the cell wall (Figure 2). In vessels, rounded pit erosion wasseen (Figure 2.A). Hyphal penetration into rays, parenchyma cells and fibers could also be seen (Figures 2.B-D).

Initial colonization of vessels by fungal hyphae is the typical decay pattern of simultaneous rot in hardwood caused by white-rot fungi (Zabel and Morrell 1992). Such typical decay appeared in these *S. gibbosa* wood samples, where fungal hyphae became quickly established, first in vessels (in 2-4 weeks' incubation), then spreading from these vessels into adjacent rays, parenchyma cells and fibers until 6 weeks decay process was reached.

At the early colonization phase of decay, damage is limited, and any visible evidence is not easily observed on the lumen surfaces as termed. This is the incipient or hidden stage of decay (Zabel and Morrell 1992; Schwarze 2007). Nowadays, this decay stages can be detected within several days by FT-NIR (Fourier transform near-infrared) spectroscopy (Fackler et al. 2006, 2007a,b) and multiplex PCRs methods (Nicolotti et al 2009).

After 8 weeks' incubation, wood blocks had sustained an average weight loss of 8.23%. Rounded pit erosions of vessels were enlarged enzymatically and coalesced to form numerous and conspicuous holes (Figure 3.A). Numbers of fungal hyphae in rays and parenchyma cells increased, and hole formation and cell wall destruction became clear (Figure 3.B-D). Figure 3.E shows the lysis zones that developed around elongated holes and which were frequently observed in parenchyma cell walls. Portions of the secondary walls were removed as well as the compound middle lamella, resulting in erosion troughs within cell walls. Hyphae had also begun to colonize fibers intensively but did not damage cell walls (Figure 3F).

Early in the degradation process, depressions could be seen on the inner surfaces of the secondary walls, the S3 layer, under and in the neighborhood of the hyphae, as shown in Figures 1 and 2. In later stages of degradation (in 8 weeks' incubation), the hyphae caused wide and deep erosion troughs. In this decay stage, the lysis zones that developed around bore holes and axially elongated troughs showed clearly the effects of fungal enzymes on cell walls, which were gradually eroded. Anagnost (1998) and Schwarze (2007) expressed that numerous bore-holes appear between two neighboring cells, showed an intermediate stage of decay had occurred.

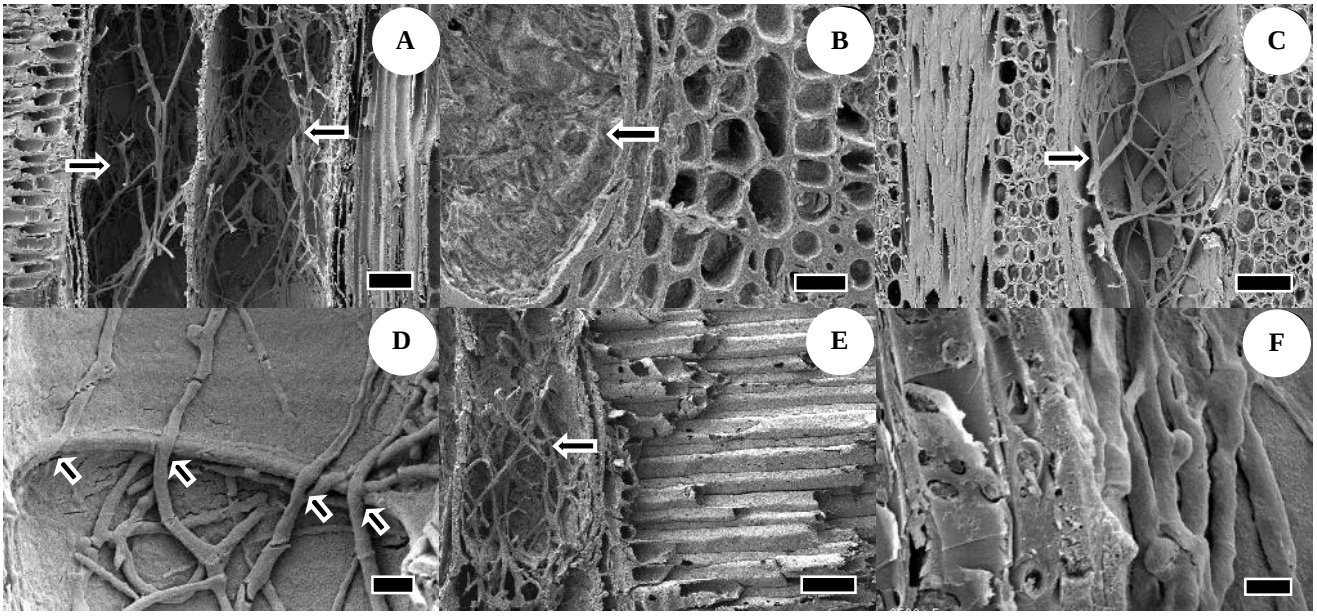

Figure 1. Decay in *S. gibbosa* wood blocks caused by fungus *P. brevispora* after 2-4 weeks'incubation. A. Hyphal colonization in the lumen of two neighboring vessels at after 2 weeks'incubation (arrows). *Bar* 50 μm; B. Transverse view of hyphal colonization (arrow) in vessels after 2 weeks' incubation. *Bar* 20 μm; C. Tangential view of hyphal colonization (arrow) in vessels after 2 weeks' incubation. *Bar* 40 μm; D. Radial view of hyphal colonization (arrow) in vessels after 2 weeks' incubation. *Bar* 40 μm; E. Fungal hyphae (arrows) passing through perforation plates of vessels after 4 weeks' incubation. *Bar* 10 μm; F. Hyphae attached deep within the vessel walls after 4 weeks' incubation. *Bar* 5 μm.

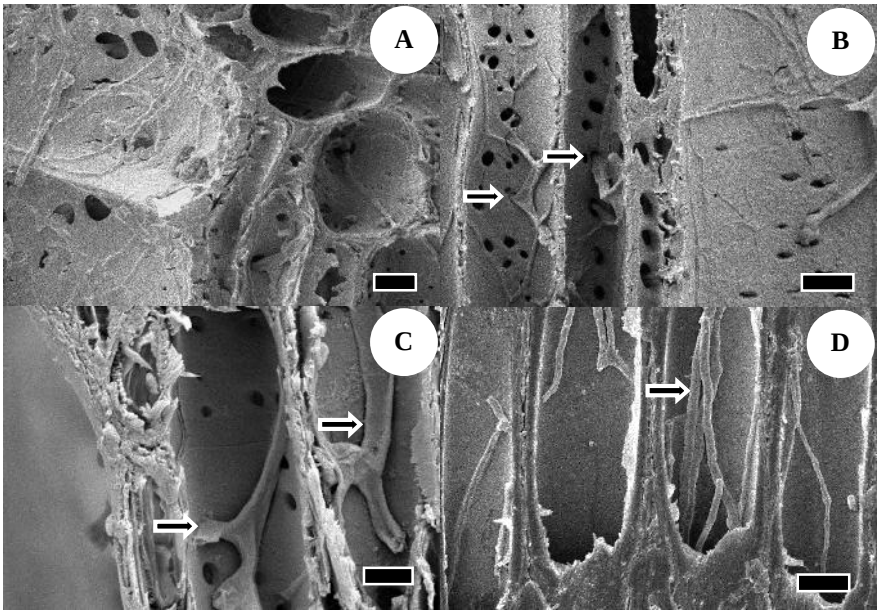

Figure 2. Decay in *S. gibbosa* wood blocks after 6 weeks' incubation. A. General view of hyphae colonizing vessels, rays and parenchyma cells. *Bar* 10 μm; B. Hyphae penetrating parenchyma cell through pits (arrows). *Bar* 10 μm; C. Hyphae begin penetrating ray cell walls (arrows). *Bar* 5 μm; D. Hyphae present in fibers (arrow). *Bar* 10 μm

Meanwhile, the area of decay in cell walls was found at an extended distance from the hyphae, in accordance to Takano et al. (2006), suggesting that extracellular enzymes of white-rot fungus can diffuse some distance from the fungal cell wall. The lysis zones indicated pre-delignification before the cell walls were completely removed. The extracellular enzymes of *P. brevispora* as reported by Arora and Rampal (2002) and Ponting et al. (2005) were known as laccase, then, Sharma and Arora (2011) identified xylanase and carboxymethyl cellulase

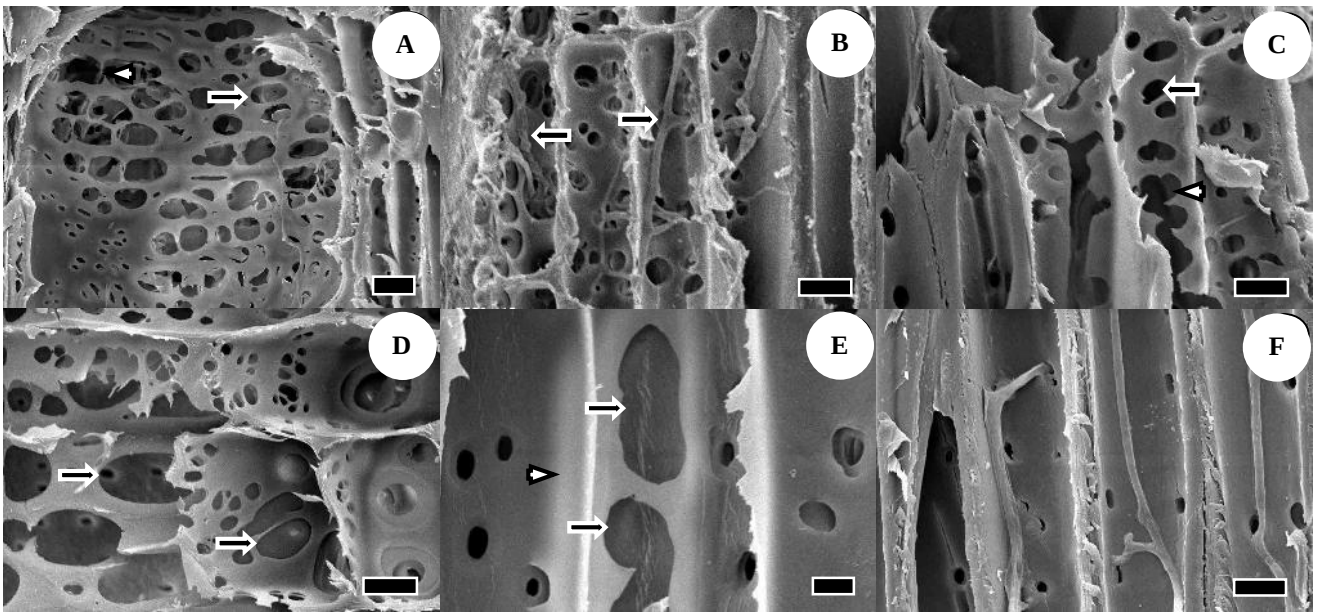

Figure 3. Decay in *S. gibbosa* wood blocks after 8 weeks' incubation. A. Rounded pit erosion (arrow) and coalesced holes (head arrow) in vessels. *Bar* 20 μm; B. Hyphae begin to heavily colonize parenchyma cells (arrows). *Bar* 10 μm; C. Rounded pit erosion (arrow) and coalesced holes (head arrow) in parenchyma cells. *Bar* 10 μm; D. Enlarged holes in rays (arrows). *Bar* 10 μm; E. Erosion troughs (arrows) and lyses zone (head arrow) in parenchyma cells. *Bar* 10 μm; F. Hyphae begin to heavily colonize fibers. *Bar* 10 μm

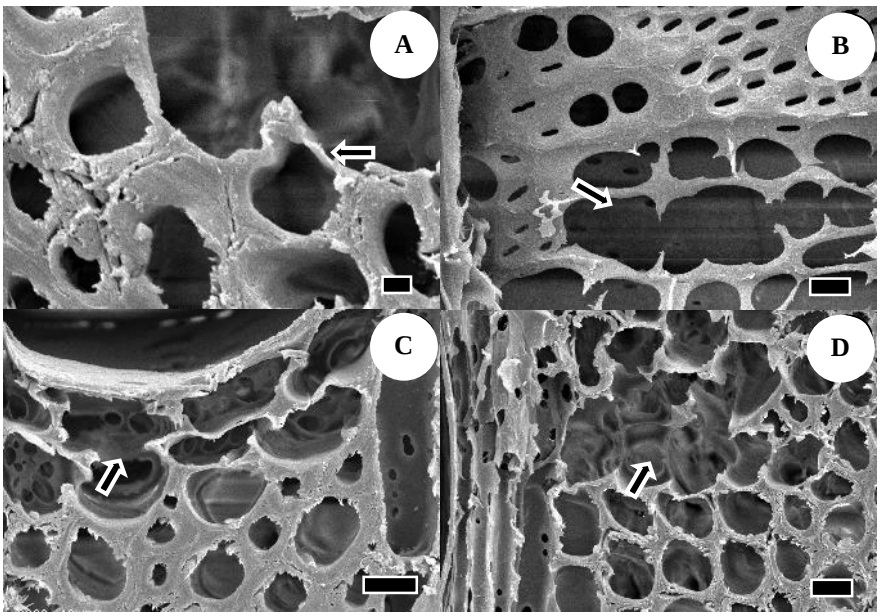

Figure 4. Decay in *S. gibbosa* wood blocks after 10-12 weeks' incubation. A. Partial thinning of fiber cell wall (arrow). *Bar* 2 μm; B. Coalesced holes appear enlarged (arrow). *Bar* 10 μm; C. Erosion channels in parenchyma cells adjacent to infected vessels (arrow). *Bar* 10 μm; D. Complete removal of wood cells (arrow). *Bar* 10 μm

could also be released. They were responsible for degradation of lignin and cellulosic materials of wood cell walls. Due to its ability to produce such extracellular enzymes, *P. brevispora* was classified as one of hydrolytic fungi (Mtui 2012).

After 10 and 12 weeks' incubation, wood block sustained an average weight loss of 11.80% and 12.34%, respectively. Partial thinning of fiber walls was frequently observed adjacent to the completely removed cells (Figure 4a). In some vessels, the rounded and coalesced holes

appeared to be enlarged, resulting in severe cell wall damage (Figure 4b). Parenchyma cell walls adjacent to infected vessels appear partially removed, forming a channel-like appearance (Figure 4c), and in some decay areas, due to advanced delignification, parenchyma cells have been completely removed. This decay process exhibited complete degradation of the compound middle lamella and cell corners that recognized as advanced stages of decay (Schwarze 2007). Meanwhile, complete degradation of cell wall components resulted in large voids that appeared in transverse sections of the decayed areas, as shown in Figure 4d. It seems to be a general sign of this decay stage that large holes appeared in transverse section, where all cell types had already been disintegrated, for instances *Populus* sp decayed by *Trametes trogii* (Levin and Castro 1998) and decaying of *Populus deltoides* by *Pycnoporus sanguineus* (Luna et al 2004).

In conclusion,*S. gibbosa* wood is susceptible to colonization and decay caused by *P. brevispora* under favorable temperature and humidity with a progressive decay pattern that has been well characterized here. The first 6 weeks of incubation was classified as the early stages decay, in which pit erosion and slight erosion of cell walls facilitated hyphal penetration among cells. Numerous and conspicuous holes as well as erosion troughs in cell walls, which were found at the end of 8 weeks' incubation, showed that an intermediate stage of decay had occurred. Furthermore, complete degradation of wood cell components, termed the advanced stage of decay, was found in some areas of wood blocks after 12 weeks' incubation.

The decay pattern *in vitro* that presented in this study was similar to those of the decayed xylem of *S. gibbosa* stem canker as reported in previous work of Erwin (2012). Therefore, a further inoculation experiment is necessary to confirm the pathogenicity of *P. brevispora* to *S. gibbosa* standing trees and also to clarify whether this fungus is one of causal agents of wood decay on the trees.

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Morphological, anatomical and isozyme variation among giant taro (*Alocasia macrorrhizos*) accessions from Central Java, Indonesia

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Abstract. *Suratman, Pitoyo A, Kurniasari S, Suranto. 2016. Morphological, anatomical and isozyme variation among giant taro (*Alocasia macrorrhizos*) accessions from Central Java, Indonesia. Biodiversitas 17: 422-429.* The objective of this study was to evaluate morphological, anatomical and isozyme variation among giant taro (*Alocasia macrorrhizos* (L.) G.Don) accessions from Central Java (Indonesia). A total of 20 giant taro accessions were collected from different collection sites in Central Java. Identification of morphological characters was done by direct observation of roots, leaves, stems, and corms. Anatomical characters were observed from both paradermal and transverse sections of leaf. Identification of biochemical markers was done by using peroxidase and esterase isozyme system. The genetic similarity among giant taro accessions was measured by using Group Average Clustering. The results of the analysis of variance revealed highly significant differences for majority of the tested morphological and anatomical characters suggesting that there was a high degree of diversity among the giant taro accessions. Isozyme polymorphism was observed in giant taro accessions using peroxidase (two banding patterns) and esterase (four banding patterns). Based on the dendogram, giant taro accessions were segregated into two major clusters. In Cluster I, the closest relationship were showed in KTN 2 and WNG 1 accessions from Klaten and Wonogiri that had 80.95% of similarity coefficient. The five accessions (SKA, SKH, WNG 4, KRA 3, KRA 4) from Surakarta, Sukoharjo, Wonogiri and some parts of Karanganyar were clustered separately as Cluster II with similarity coefficient of 50%.

Keywords: *Alocasia macrorrhizos*, anatomy, Central Java, giant taro, isozyme, morphology

INTRODUCTION

Alocasia is recorded as the largest genus in the family Araceae which comprises more than 100 species of herbaceous, laticiferous, diminutive to gigantic, usually robust herbs (Boyce 2008). This genus inhabits wet disturbed sites, areas of regrowth, large canopy gaps, and roadside ditches, but there are also forest undergrowth species (Hay 1990; Ivancic et al. 2009). Giant taro (*Alocasia macrorrhizos* (L.) G.Don) is a species of the genus *Alocasia* and may have originated from Sri Lanka or India (Purseglove 1979; Plucknett 1984; Ivancic and Lebot 2000). From this area, it has spread to almost all tropical and subtropical regions (Groen et al. 1996; Lebot 1999; Matthews 2004; Nauheimer et al. 2012). The corm of giant taro is very rich in carbohydrates, which is mainly starch at 77.9% and 1.4% crude fiber, on Dry Matter (DM) basis. The corm is edible and also a good source of dietary protein, thiamin, riboflavin, sodium, iron, magnesium, kalium, phosphorus, zinc and a very good source of vitamin B6, vitamin B12, vitamin C, vitamin E, niacin, potassium, copper and manganese (Soudy et al. 2010; Manner 2011). This edible corm has been served either as staple food or mixed with other vegetables, usually after cooking (Kumoro et al. 2014). The utilization of the corms as a staple food in many parts of the tropics and sub-tropics providing about a third of the food intake of more than 400 million people (Soudy et al. 2010). The corms, cormels, stems and leaves also can be used as vegetable and animal fodder. For medical puposes, the chopped roots and leaves are used as a rubefacient and juice from the petiole is used againts coughs (Groen et al. 1996). Giant taro is also cultivated and introduced as a tropical ornamental plant, which a number of varieties have been recognised (Furtado 1941).

The genetic diversity of giant taro accessions from Central Java (Indonesia) is poorly documented. In order to ascertain the level of genetic variation among and within species, populations or accessions, a variety of morphological, anatomical, biochemical and molecular markers are used. Morphological markers are routinely used for estimating genetic diversity of plants since they are inexpensive, simple and fast (Jingura and Kamusoko 2015). Anatomical characters are also valuable in taxonomy and identification of groups of plant (Rahayu et al. 2012; Chikmawati 2013).

Isozyme as the classical biochemical marker can be used to determine genetic variation of cultivars, natural populations and accessions in germplasm collections, if the morphological characters appear to overlap due to strong influence of environment (Suranto 2001; Fernandez de Souza and Primo 2001; Padmanaban et al 2013). Isozymes have several advantages over traditional markers such as morphological or anatomical traits to study polymorphism because they are not influenced by environmental factors making identification possible in early stages of development (Torres 1990). A range of enzyme loci also can be studied easily using a small quantity of material with minimum preparation and cost (Johnson et al. 2010; Kovacevic et al 2010).

Information on genetic diversity and relationship among and between individuals, accessions, populations, varieties, and species of plant are also important for plant breeders in guiding the improvement of plants (Dharmar and De Britto 2011). This information can provide predictive estimation of genetic variation within species thus facilitating breeding material selection (Qi et al. 2008).

The objective of this study was to evaluate morphological, anatomical and isozyme variation among giant taro accessions from Central Java (Indonesia). This is the first study to combine morphological, anatomical and isozyme markers to evaluate genetic variation in giant taro accessions from Java, especially in Central Java, Indonesia.

MATERIALS AND METHODS

Plant materials

A total of 20 giant taro accessions were collected from different collection sites in Central Java (Table 1, Figure 1). Plants were then transplanted into polybags and kept in screen house in Department of Biology, Universitas Sebelas Maret for 8 weeks before young corms were collected. The plantation site is situated at 126 m asl altitude, 28 ºC of temperature, 8200 lux of ligh intensity, 85% of air humidity and 50 % of soil humidity.The young corms of each accession were then used for isozymes extraction.

Morphological analysis

Identification of morphological characters (both quantitative and qualitative) was done by direct observation of vegetative structures such as roots, corms, stems and leaves of the giant taro plant. The observed of morphological characters were plant height, leaf length, leaf width, petiole length, petiole width, sheath length, sheath width, corm length: width ratio, root length: width ratio, abaxial secondary veins

Table 1. The geographic variation of giant taro (*A. macrorrhizos*) accessions originated from Central Java, Indonesia with climatic data for each collection site

No.	Acces-sions	Collection site	Alt. (m. asl.)	Temp. (ºC)	Light int. (x 1000 lux)	Air humid. (%)	Soil humid. (%)
1	BYL 1	Boyolali	548	27	17.2	84	28
2	BYL 2	Boyolali	607	26	13.5	82	10
3	BYL 3	Boyolali	802	23	2.4	80	18
4	BYL 4	Boyolali	970	22	7.4	83	30
5	KTN 1	Klaten	475	26	8.3	91	10
6	KTN 2	Klaten	457	28	9.8	69	30
7	KTN 3	Klaten	726	25	1.7	80	10
8	KTN 4	Klaten	802	23	1.7	100	15
9	WNG 1	Wonogiri	225	30	53.8	65	50
10	WNG 2	Wonogiri	381	28	5.4	84	80
11	WNG 3	Wonogiri	600	25	7.4	81	75
12	WNG 4	Wonogiri	677	23	6.9	78	82
13	KRA 1	Karanganyar	396	29	7.6	64	55
14	KRA 2	Karanganyar	641	28	9.6	66	10
15	KRA 3	Karanganyar	740	26	5.2	69	76
16	KRA 4	Karanganyar	905	23	77.8	32	60
17	SRG 1	Sragen	197	23	2.4	91	80
18	SRG 2	Sragen	304	23.3	3.2	100	75
19	SKH	Sukoharjo	119	28	50.8	54	30
20	SKA	Surakarta	119	29	3.9	80	50

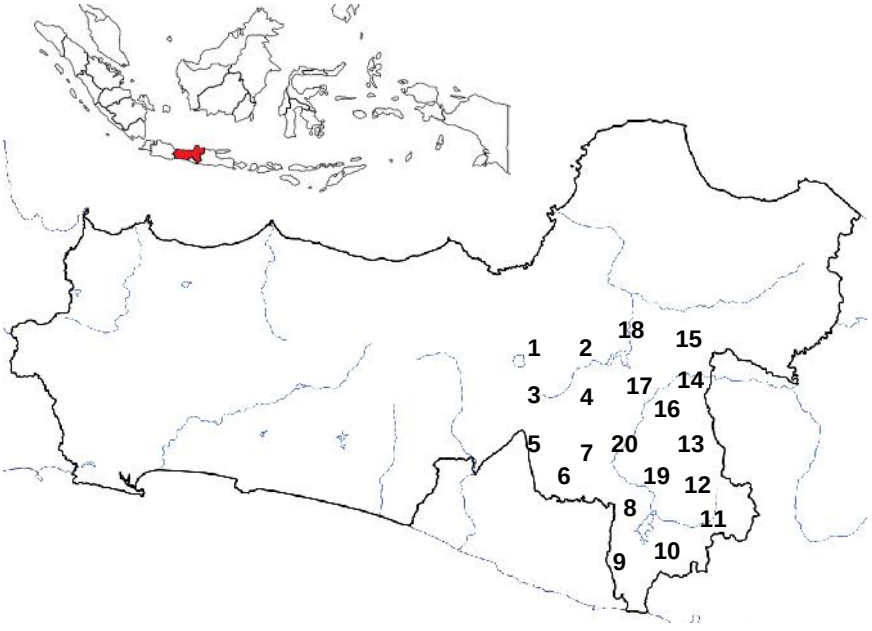

Figure 1. Map of the collection areas for giant taro (*A. macrorrhizos*) accessions studied in Central Java. The number (1 to 20) indicated location of each collected accession

Anatomical analysis

Leaf anatomy was observed from both paradermal and transverse sections. The leaf paradermal and transverse section were carried out as described by Chikmawati (2013). The section were observed under light microscope. The observed characters were stomatal density, stomatal index, stomatal length; stomatal width; abaxial epidermis thickness, adaxial epidermis thickness, mesophyll thickness, palisade thickness, palisade ratio, number of calcium oxalate crystal.

Isozyme analysis

Gel and buffer preparation

Acrylamide gel electrophoresis and buffer solutions (extraction buffer, tank buffer, running buffer) were prepared and carried out as described by Suranto (2001) and Setyawan et al. (2014).

Isozyme extraction

Young corms of giant taro were ground in mortar using 0.15-0.35 m l of extracting solution and then transferred to a 1.5 ml microtube. Samples were centrifuged at 3500 g for 15 minutes, and supernatant was transferred to new microtube. The supernatants were then applied into the well of acrylamide gel. The extracting solution consisted of 0.018 g of cysteine, 0.021 g of ascorbic acid, 5 g of sucrose, diluted in 20 ml of borax buffer pH 8.4 (tank buffer).

Electrophoresis

From the centrifuged samples about 200 µl of supernatant was taken and 5µl of bromophenol blue (tracking dye) was added to each sample. About 10-15 ul of prepared samples (for peroxidase) and 15-24 ul (for esterase) was taken and loaded into each well of the gel. Loaded samples were electrophoresed at a constant current of 5 mA for peroxidase and 7 mA for esterase at room temperature for about 60 minutes. Electrophoresis was stopped when the bromophenol blue marker dye had traveled about 56 mm from the well toward the anode (Suranto 2001; Padmanaban et al. 2013; Setyawan et al. 2014).

Staining procedures

After electrophoresis, the gels were stained for the appropriate enzyme as described in Suranto (2001) and Setyawan et al. (2014) with some modifications. Peroxidase staining was prepared by diluting 0.0125 g of O-dianisidine into 25 ml of acetone. Then 50 ml of 0.2 M acetate buffer pH 4.5 was added and 2 drops of H_2O_2 lastly given. Esterase staining was prepared by dissolving 0.0125 g of α-naphthyl acetate in 2.5 ml acetone.After that 50 ml of 0.2 M phosphate buffer pH 6.5 and 0.0125 g of Fast Blue BB Salt were added. Gels were immersed in these staining solutions until bands appeared.

Data analysis

Analysis of variance was performed for quantitative morphological and anatomical observation data in order to test the significance of variation among accessions. The data from zymograms were entered as a matrix of presence/absence of bands for each enzyme. The genetic similarity among giant taro accessions based on morphological, anatomical and isozyme markers was measured by using Group Average Clustering which were integrated in the program Numerical Taxonomy and Multivariate Analysis System (NTSYS) version 2.10. (Rohlf 1998).

RESULTS AND DISCUSSION

Morphological analysis

The analysis of variance revealed significant differences among accessions for all of the tested quantitative morphological traits suggesting that there was a high degree of phenotypic diversity among the accessions. Plant height, leaf length, leaf width, petiole length, sheath length, root length: width ratio showed wide variation while petiole width, sheath width, corm length: width ratio showed a narrower range of phenotypic variation (Table 2).

Plant height exhibited wide range of variation and ranged from 52.5 cm (KRA 2) to 130.5 cm (BYL 3) with an average 89.31 cm. The leaf length varied significantly among accessions and displayed a range from 14.5 cm (KRA 2) to 51.4 cm (BYL 3), with an average 31.26 cm. Leaf width differed significantly among tested accessions and was highest in the accession KRA 1 (46.8 cm) and lowest in the accession SKA (15.3 cm) with an average 29.02 cm. Petiole length also exhibited wide differences among accessions and ranged from 21 cm (KRA 2) to 83 cm (BYL 3) with an average 49.79 cm. Petiole width values showed narrower variation and ranged from 0.8 cm (KRA 2) to 2.87 cm (BYL 3) with an average 1.75 cm. Sheath length displayed wide range of variation and ranged from 10 cm (SKA) to 45.5 cm (BYL 3) with an average 27.22 cm. Sheath width values exhibited narrower differences among accessions and ranged from 1.18 cm (KRA 2) to 4.68 cm (BYL 3) with an average 2.76 cm. Corm length: width ratio displayed narrower differences among accessions and varied from 1 to 4. Root length: width ratio showed wide range of variation and ranged from 23 (WNG 4) to 152 (KTN 3) with an average 88.5. For qualitative morphological characters, most of examined accessions showed flattened abaxial secondary veins, except in WNG 3 accession which has prominet abaxial secondary veins.

Anatomical analysis

Analysis of variance for anatomical characters revealed that there was significant variation for all the tested characters among giant taro accessions, except in case of palisade ratio. However, accessions variation for palisade ratio was non-significant (Table 3).

Comparing the stomatal densities and stomatal index, there was significant variability among the tested accessions. SRG 1 accession displayed the highest value of stomatal density (24.89/mm^2) whereas the lowest one can be found in SKA accession (15.24/mm^2) with an average 20.84/mm^2. The highest stomatal index value was

distributed in WNG 3 accession (40) whereas the lowest one in the KRA 2 accession (15) with an average 24.05.

Leaves are transversally arranged into one layer of upper (adaxial) epidermis cells, mesophyll cells, and one layer of lower (abaxial) epidermis cells. The mesophyll consisted of spongy tissue. Of all examined giant taro accessions was remarkable in having significant variation in leaf tissue layer thickness such as abaxial epidermis thickness, adaxial epidermis thickness, mesophyll thickness and palisade thickness.

Table 2. Morphological character variation among giant taro accessions from Central Java, Indonesia

No.	Accessions	PlH	LfL	LfW	PtL	PtW	ShL	ShW	CoR	RoR	AbV
1	BYL 1	81.2a	31a	29.4a	48a	1.69a	25a	3.66b	1a	127bc	Flat
2	BYL 2	105b	32.8a	30.4a	43.3a	1.94b	23.2a	2.36a	1a	101b	Flat
3	BYL 3	130.5b	51.4b	46.1b	83b	2.87b	45.5b	4.68b	1a	112b	Flat
4	BYL 4	99.9b	36.9b	33.3b	63b	1.72a	34a	3.60b	1a	100b	Flat
5	KTN 1	80.6a	28.3a	27.2a	47a	1.75a	23a	2.55a	1a	46a	Flat
6	KTN 2	102b	37b	38b	61.2b	2.20b	32.5b	3.22b	1a	33a	Flat
7	KTN 3	107b	33.2a	28.2a	56b	1.72a	33b	2.42a	2a	152c	Flat
8	KTN 4	101b	40.5b	36.3b	59b	2.07b	31b	2.99a	1a	82a	Flat
9	WNG 1	102.8b	39.5b	30ab	64.4b	2.36b	39b	3.92b	1a	131c	Flat
10	WNG 2	104.3b	35.4ab	31.3a	47a	1.75a	29a	2.55a	1a	81a	Flat
11	WNG 3	89a	23.2a	22.7a	48.5a	1.62a	25a	2.07a	2a	91ab	Prominent
12	WNG 4	71.6a	29.6a	28a	44a	1.62a	26a	2.87a	2a	23a	Flat
13	KRA 1	105b	46b	46.8b	59b	2.45b	31b	2.87a	2a	52a	Flat
14	KRA 2	52.5a	14.5a	15.6a	21a	0.8a	10.2a	1.18a	1a	91ab	Flat
15	KRA 3	119.7b	40b	36b	73b	2.07b	45b	3.98b	4ab	75a	Flat
16	KRA 4	64a	25a	22a	37a	1.31a	20a	2.48a	4ab	83a	Flat
17	SRG 1	68.5a	23a	19a	46.5a	1.40a	24a	2.48a	1a	141c	Flat
18	SRG 2	71a	19.7a	20.2a	30.5a	1.31a	17a	1.91a	2a	66a	Flat
19	SKH	64a	21.6a	24.5a	40.5a	1.34a	21a	1.91a	1a	128bc	Flat
20	SKA	66.5a	16.5a	15.3a	24a	0.92a	10a	1.43a	1a	48a	Flat
	Average	89.31	31.26	29.02	49.79	1.75	27.22	2.76	1.55	88.15	

Note: * PlH = plant height (cm); LfL = leaf length (cm); LfW = leaf width (cm); PtL = petiole length (cm); PtW = petiole width (cm); ShL = sheath length (cm); ShW = sheath width (cm), CoR = corm length: width ratio; RoR = root length: width ratio; AbV = abaxial secondary veins. ** Values followed by the different lower-case letter in the same column are significantly different (Duncan multiple range test, $p < 0.05$)

Table 3. Anatomical character variation among giant taro accessions from Central Java, Indonesia

No.	Accessions	StD	StI	StL	StW	AbT	AdT	MeT	PaT	PaR	CaO
1	BYL 1	26.44b	32bc	45.08b	30.51b	44.75a	43.79ab	213.22b	61.72ab	0.44a	3a
2	BYL 2	20.14ab	19a	51.86b	32.54b	55.93b	37.41a	206.78ab	52.29a	0.40a	3a
3	BYL 3	16.89a	20a	49.49b	31.19b	49.83ab	40.78a	216.61b	65.00ab	0.44a	3a
4	BYL 4	19.14ab	22a	42.37a	27.46b	47.46a	39.83a	197.29a	52.50a	0.44a	3a
5	KTN 1	21.66ab	24ab	46.10ab	27.80b	52.54b	38.97a	223.05b	73.71b	0.40a	4a
6	KTN 2	19.63ab	22a	46.44ab	32.54b	52.54b	41.72ab	243.39b	75.34b	0.44a	5ab
7	KTN 3	28.28b	27ab	52.88b	30.85b	52.20b	42.59ab	217.63b	65.78ab	0.44a	4a
8	KTN 4	21.19ab	22a	51.53b	32.20b	55.25b	40.95a	220.68b	64.57ab	0.36a	4a
9	WNG 1	25.67b	36b	40.68a	30.85b	47.12a	44.74ab	213.90b	62.41ab	0.44a	4a
10	WNG 2	28.08b	21a	56.95b	32.88b	58.98b	42.33ab	221.02b	61.47ab	0.44a	4a
11	WNG 3	17.93a	40c	34.58a	17.63a	46.44a	37.84a	174.92a	45.00a	0.50a	8b
12	WNG 4	18.73ab	23a	50.51b	36.61b	47.80a	36.72a	199.32a	45.26a	0.44a	3a
13	KRA 1	21.28ab	24ab	44.07a	32.88b	55.93b	41.47ab	219.32	62.41ab	0.40a	2a
14	KRA 2	16.99a	15a	63.39c	27.12b	44.75a	41.38ab	203.73ab	56.64b	0.44a	3a
15	KRA 3	13.62a	23a	46.78ab	29.83b	41.02a	38.36a	212.20b	79.48b	0.40a	3a
16	KRA 4	17.39a	21a	50.51b	30.51b	57.97b	35.69a	164.75a	59.83a	0.44a	4a
17	SRG 1	24.89ab	21a	49.15b	30.85b	49.15ab	40.69a	198.64a	63.62ab	0.44a	2a
18	SRG 2	23.62ab	29b	44.07a	31.19b	45.42a	47.84ab	227.46b	74.48b	0.40a	3a
19	SKH	19.06ab	16a	49.49b	32.54b	51.19b	42.16ab	159.66a	54.48a	0.50a	3a
20	SKA	15.24a	22a	46.44ab	29.15b	44.41a	39.57a	174.24a	60.60ab	0.50a	3a
	Average	20.84	24.05	48.21	30.42	50.33	40.80	207.03	61.89	0.44	3.58

Note: * StD = stomatal density (pore/mm^2); StI = stomatal index; StL = stomatal length (µm); StW = stomatal width (µm); AbT = abaxial epidermis thickness (µm); AdT = adaxial epidermis thickness (µm): MeT = mesophyll thickness (µm); PaT = palisade thickness (µm); PaR = palisade ratio, CaO = number of calcium oxalate crystal (no/mm^2). ** Values followed by the different lower-case letter in the same column are significantly different (Duncan multiple range test, $p < 0.05$).

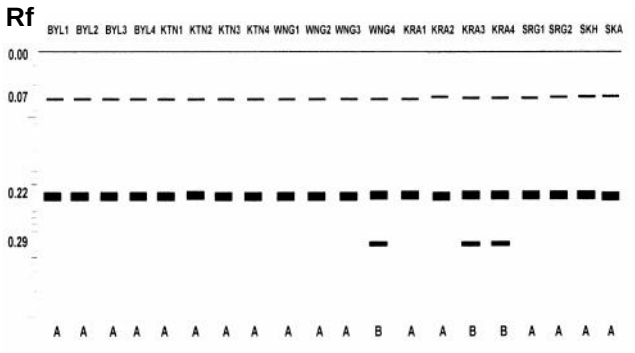

Figure 1. Peroxidase isozymic banding pattern of giant taro accessions from Central Java, Indonesia

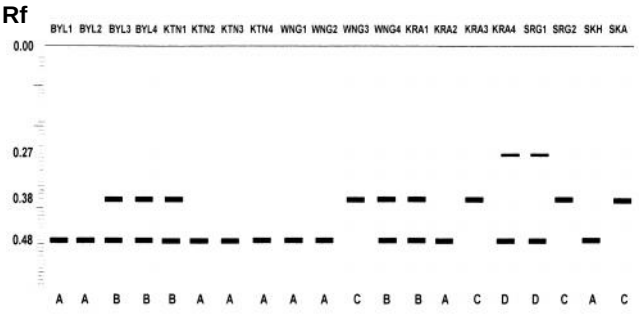

Figure 2. Esterase isozymic banding pattern of giant taro accessions from Central Java, Indonesia

The number of crystal of calcium oxalate per mm^2 also showed significant variation among accessions. The highest number of calcium oxalate was observed in WNG 3 accession ($8/mm^2$), whereas the lowest one was distributed in SRG 1 and KRA 1 accessions ($2/mm^2$).

Isozym analysis

The two enzymatic systems showed a total of six banding patterns, distributed in the whole set of samples as peroxidase with two banding patterns and esterase with four banding patterns. Peroxidase showed three anodic bands, resulting in two patterns zymogram (banding pattern A and B) which distributed in different Rf value varying from 0.07 to 0.29. Banding pattern A consisted of two bands which located at Rf 0.07 and Rf 0.22 whereas banding pattern B consisted of three bands which located at Rf 0.07, Rf 022 and Rf 0.29 from anodal zone (Figure 1).

Two isozymic banding patterns of peroxidase also distributed separately in giant taro accessions (Figure 2). Banding pattern A was seen in majority accessions and occured in 17 tested accessions (BYL 1, BYL 2, BYL 3, BYL 4, KTN 1, KTN 2, KTN 3, KTN 4, WNG 1, WNG 2, WNG 3, KRA 1, KRA 2, SRG 1, SRG 2, SKH, SKA) whereas banding pattern B only distributed in three accessions (WNG 4, KRA 3 and KRA 4). Therefore, peroxidase was considered as a suitable marker for these accessions.

Three bands of esterase at different Rf values varying from 0.27 to 0.48 were observed, which allowed to distinguish four pattern zymograms (banding pattern A, B, C and D) (Figure 2). Banding pattern A only consisted of one band which located at Rf 0.48. Banding pattern B consisted of two bands which located at Rf 0.38 and Rf 0.48. Banding pattern C consisted of one band which located at Rf 0.38. Banding pattern D consisted of two bands which located at Rf 0.27 and Rf 0.38 from anodal zone.

Banding pattern A occured in majority accessions and distributed in nine tested accessions (BYL 1, BYL 2, KTN 2, KTN 3, KTN 4, WNG 1, WNG 2, KRA 2, SKH) whereas banding pattern B in five accessions (BYL 3, BYL 4, KTN 1, WNG 4, KRA 1), banding pattern C in four accessions (WNG 3, KRA 3, SRG 2, SKA) and banding pattern D only lied in two accessions (KRA 4, SRG 1).The observed banding pattern D only distributed in two accessions, so esterase can be considered as a suitable marker for these accessions.

Relationships

In order to study the relationship among accessions, genetic similarity based on morphological, anatomical and isozyme markers was used to predicted a dendrogram for the giant taro accessions from Central Java using NTYSYS software. Based on the dendogram at a level of 50 % similarity, it showed distinct separation of twenty giant taro accessions from Central Java into two major clusters (Figure 3). Cluster I comprised most of tested accessions which originated from Boyolali, Klaten, Sragen, and some parts of Wonogiri and Karanganyar. The closest relationship were showed between KTN 2 and WNG 1 accessions from Klaten and Wonogiri that had 80.95% of similarity coefficient. The five accessions (SKA, SKH, WNG 4, KRA 3, KRA 4) from Surakarta, Sukoharjo, Wonogiri and some parts of Karanganyar were then clustered separately from the another as Cluster II with similarity coefficient of 50% and considered to be genetically unique.

Discussion

Analysis of quantitative morphological characters variation above provide an indication of genetic diversity present among accessions, and such methods have been successfully used to measure phenotypic diversity in germplasm collections. Phenotypic variations provided a good opportunity for genetic improvement (Sabaghnia et al. 2014). In this study, only one qualitative morphological characters was observed i.e. abaxial (lower) secondary veins. The observed abaxial secondary veins showed narrower variation among accessions. These results further indicated the existence of variability among accessions for this trait although their variation was considered low.

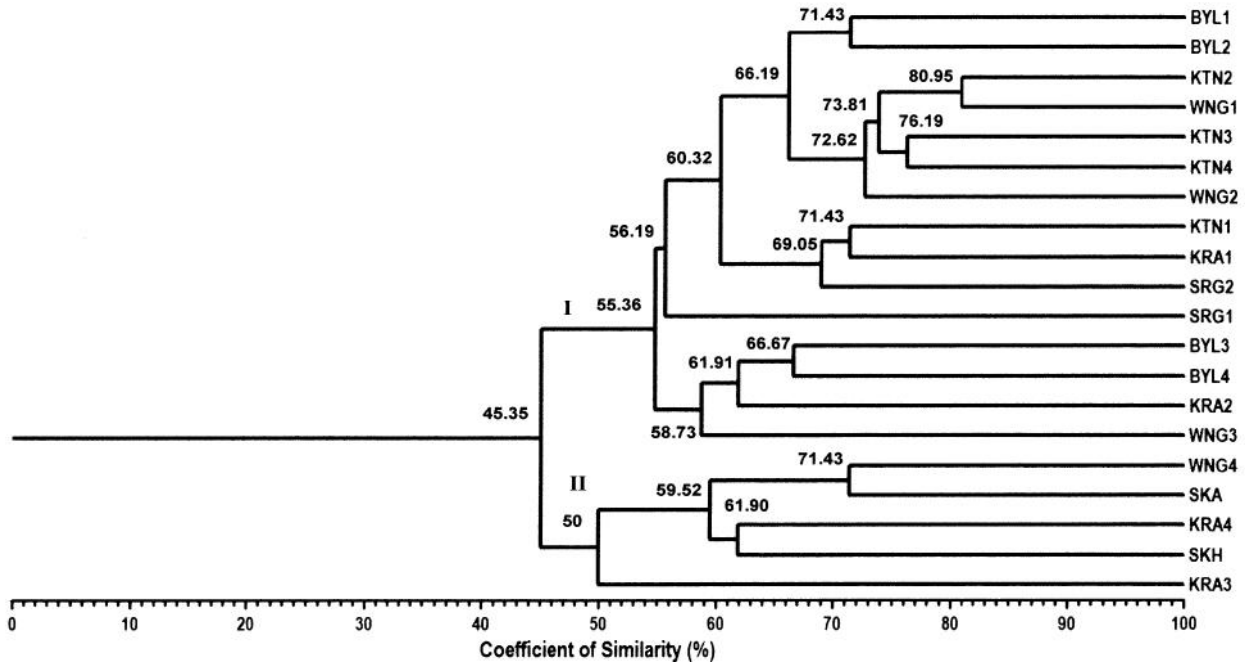

Figure 3. Relationship dendrogram among 20 giant taro accessions from Central Java, Indonesia using morphological, anatomical and isozyme markers

The pattern of variation exhibited for various characters were substantially different. The incidence of highly significant variation among the accessions for the majority of the studied morphological characters is a sign of the presence of high degree of genetic variation implying great potential of the accessions in future breeding programs through selection (Nkansah et al. 2013; Roy et al. 2013). Therefore, this indication showed that there is enough scope for selection of desirable genotypes, where variability exists.

Morphological markers have been commonly used as a first step in germplasm characterisation, but the time required for processing of candidate accessions is significant. Despite this limitation, morphological characters is still useful for preliminary evaluation because it is fast, simple, and can be used as a general approach for assessing genetic diversity among morphologically distinguishable accessions (Beyene et al. 2005).

Stomata of giant taro were found only in lower (abaxial) epidermis layer and distributed among kidney-shaped guard cells. Stomata shape was tetracyclic, with 4 subsidiary cells. In most species, frequency of stomata in the lower epidermis are more than the upper epidermis (Muradoglu and Gundogdu, 2011).

The higher stomatal density or stomatal index can be used as an indicator for higher transpiration rate, highest metabolism and absorption of mineral and water. Stomata characteristics such as frequency and dimensions can be affected by type of species and environmental factors (Munir et al. 2011). Although stomatal features can be affected by multiple ecological factors, as they are directly exposed to the environment, but stomatal differentiation and development are determined by genetic factors (Hetherington and Woodward 2003).

The leaf tissue layer thickness such as abaxial epidermis thickness, adaxial epidermis thickness, mesophyll thickness and palisade thickness exhibited siginificant variation among all examined accessions. The difference in layers thickness of leaves might be attributed to the responses toward environmental factors (Donovan et al. 2007). High levels of genetic variation stimulated the populations or accessions more flexible to fit a variable environment influence.

The number of crystal of calcium oxalat per mm^2 also showed significant variation among accessions. In this study, the observed calcium oxalate presented as fine needle-like crystals or raphides. Calcium oxalate content depends on the cultivars, fertilizers and environmental condition, especially during drought (Bradbury and Holloway, 1988).

Some anatomical characters might be influenced by environment factors but majority of the tested anatomical characters showed highly significant variation among all tested accessions.This information indicated that there is enough scope for selection of accessions on the basis of these characteristics for genetic improvement.

The morphological variation, as a product of genotype and the environment, is an important parameter, but much diversity, which remains unexpressed morphologically, can be revealed by biochemical methods. Study of isozymic variation is one such important and powerful procedure that has often been employed for this purpose (Smila et al. 2007; Johnson et al. 2012).

Isozyme polymorphism was observed in giant taro accessions from Central Java using peroxidase and esterase systems. Peroxidase and esterase have been widely utilized to assess the genetic similarity and to reveal the variation of organisms at the various taxonomic levels. Peroxidase is an easily detected enzyme because of extraordinary activity on plant tissue but in this study peroxidase showed lower variations of isozymic banding pattern among giant taro accessions. Collares et al. (2004) reported that the leaves showed more polymorphism in peroxidase zymograms than that observed in shoot and root samples. In our study, isozymes were extracted from young corms, therefore genetic variation derived from isozymic banding pattern of peroxidase was considered low, due to the small number of polymorphism. Isozyme extraction from leaf tissue of giant taro was difficult to be conducted because of its highly content of mucilage and fenol.

The esterases are a complex and heterogeneous group of enzymes, catalyzing the hydrolysis of the ester link (Smila et al. 2007). Esterase showed most isozymic banding pattern variations compared than peroxidase in this study. According to Desborough and Peloquin (1967), isozymes of esterase in tubers are reliable and valuable as a biochemical markers. Therefore, esterase is considered as a useful diagnostic tool in this study for identification or assesment of genetic variation in view of the extensive polymorphism for this enzyme.

There were some observed bands (both peroxidase and esterase) are fairly thick, but there are also thin bands overlooked in this study. The difference of isozymic banding thickness is probably due to the differences in the copy number of the gene. A thick band may also be caused by two bands coincide, which indicates heterozygote for two alleles of the monomer, and a thin band indicating homozygote (Setyawan et al. 2014).

The availability of isozyme banding pattern has substantially increased our knowledge of the genetics of plant accessions. Differences in isozyme profiles can be used to reveal genetic diversity among accessions. The observed polymorphic zones reflect the validity of the isozyme data to study the genetic diversity at intraspecific levels in giant taro accessions from Central Java. Sher et al. (2010) stated that isozymes are still useful markers for genetic polymorphism identification due to its simplicity and validity for describing genetic structure of groups of plants.

However, the relationship dendrogram showed that the grouping was inappropriate with geographical origins. It is explicit that there is no relationship between geographic distribution and genetic diversity in this study. Thus, the grouping did not always indicate the geographical origins similarity, but possibly showed the genetic similarity (Tikader and Kamble 2008).

One of the main applications of these clusters is the estimation of the genetic similarity among accessions and identification of parents for performing appropriate crosses, and reaching maximum heterosis in hybridization programs (Lombardi et al. 2014; Suratman et al. 2015). Selection of better accessions can be made for species improvement based on its genetic similarity percentage. Two similar genetically accessions or more but possessing distinct characters can be chosen for this purpose.

In this study, morphological, anatomical and isozyme markers showed that this method is informative and can be used to determine genetic variation and the relationships among accessions. The information about genetic similarity will be helpful to avoid any possibility of elite germplasm becoming genetically uniform (Fadoul et al. 2013). Thus, information about genetic diversity through morphological, anatomical and isozyme markers obtained in this study could be valuable for breeding strategies of giant taro in Java. From a conservation perspective, sampling many accessions from all possible agroecologies would be an effective strategy of capturing genetic variation for future collections (Beyene et al. 2005).

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Single Nucleotide Polymorphism within the *LDLR* gene and responsiveness of cynomolgus macaque (*Macaca fascicularis*) to atherogenic diet

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Abstract. *Taher A, Solihin DD, Sulistiyani, Sajuthi D, Astuti DA. 2016. Single Nucleotide Polymorphism within the LDLR gene and responsiveness of cynomolgus macaque (*Macaca fascicularis*) to atherogenic diet. Biodiversitas 17: 430-434.* Genetic variation within low density lipoprotein receptor (*LDLR)* gene has been associated with normal variation of plasma lipid profiles and risks of coronary heart diseases (CHD) in human. Although cynomolgus macaque *(Macaca fascicularis)* is one of non-human primates commonly used as models in atherosclerosis research, little is known about the extent of polymorphism within the *LDLR* gene and its consequences on responsiveness to atherogenic diet. In this study, two regions of *LDLR* gene, namely exon 6 and intron 5, were sequenced in a sample of 22 male cynomolgus macaques which had differences in responsiveness to atherogenic diet. The objective of the study was to identify single nucleotide polymorphism (SNP) within the *LDLR* gene and to evaluate the kinds of haplotypes in relation to the responsiveness of the cynomolgus macaque to atherogenic diet. Sequence analysis revealed that there were two SNPs at exon 6, i.e. IVS5-6C > G and 825C > G, which were distributed in 3 haplotypes, and five SNPs at intron 5, i.e. g.IVS5+99T > C, g.IVS5+173G > T, g.IVS5+327A > G, g.IVS5−96C > T, and g.IVS5−6C > G, which were distributed in 6 haplotypes. It was found that haplotype II (GC) at 6 base pairs prior to the exon 6 and haplotype III (CGGTG) within the intron 5 were associated with hyporesponsiveness to atherogenic diet. The results showed that potential SNP existed within the exon 6 and intron 5 can be used as genetic markers for selecting hypo- from hyperresponders.

Keywords: Hyporesponsiveness, *LDLR* gene, *Macaca fascicularis*, SNP

INTRODUCTION

Cynomolgus macaque (*Macaca fascicularis*), also commonly known as the crab-eating or long-tailed macaque, has a lengthy history of being used as a nonhuman primate model for the study of human atherosclerosis because this species is responsive to dietary cholesterol (Shelton et al. 2012). Even though cynomolgus macaques are responsive, feeding of atherogenic diets to this species results in marked inter individual differences in the response of plasma cholesterol. Certain animals show only small responses (*hyporesponders*) whereas others develop high degrees of hypercholesterolemia (*hyper-responders*) (Beynen et al. 1987). Hyporesponders are constrained in the provision of hypercholesterolemia animals to be used as models to evaluate the effect of diet on plasma lipid profile and its association with the progression or regression of atherosclerosis.

Low density lipoprotein receptor (LDL-R) is a cell membrane glycoprotein that plays a key role in maintaining normal plasma cholesterol levels, mediating the endocytosis of LDL and other cholesterol-carrying particles (Goldstein et al. 1995). Human *LDLR* gene consists of 18 exons and 17 introns with the length of approximately 45 kb, and is mapped onto chromosome 19p13.2. Mature mRNA is 5.3 kb long and encodes a protein of 860 amino acids. Mature receptor (without the signal peptide) is a 839-amino acid protein which can be divided into five functional domains: (i) a 292-amino acid ligand-binding region, (ii) a 400-amino acid region which is homologous to the precursor for epidermal growth factor and is required for dissociation of the receptor from the ligand in lysosomes and for recycling of the receptor to plasma membrane, (iii) a 58-amino acid domain which is extensively glycosylated, (iv) a transmembrane region, and (v) a cytoplasmic domain which is required for targeting the protein to clathrin-coated pits for internalization (Sudhof et al. 1987). Mutations in the *LDLR* gene that disturb the normal functions of the LDL-R protein can cause familial hypercholesterolemia (FH), which is associated with elevated total and LDL-cholesterol and premature coronary heart diseases (CHD) (Hoobs et al. 1990). FH, however, accounts for only about 5% of patients with CHD, and the contribution of genes to CHD in the remaining 95% of cases is still unknown (Ahn et al. 1994). Common single nucleotide polymorphisms (SNPs)

in genes involved in lipid metabolism are potentially important genetic markers in affecting normal variation in plasma or serum lipid profiles and thus determining susceptibility or resistance to CHD in a general population (Kathiresan et al. 2008; Shandu et al. 2008; Talmud et al. 2013).

As with humans, cynomolgus macaques have a diverse genetic background as evidenced by number of genetic polymorphisms that have been reported (Ebeling et al. 2011; Yan et al. 2011; Higashino et al. 2012). Some polymorphisms are functional in gene that involves in metabolic and inflammatory pathway (Uno et al. 2010; Wu and Adkins 2012), and others are related to malaria susceptibility (Flynn et al. 2009). However, very few studies have focused on polymorphism within *LDLR* gene and its consequences on responsiveness to atherogenic diet. In this study, two regions of *LDLR* gene, namely exon 6 and intron 5, were sequenced in a sample of 22 adult male cynomolgus macaques which had differences in responsiveness to atherogenic diet. The primary objective of the study was to identify single nucleotide polymorphism (SNP) within the *LDLR* gene. In addition, a secondary objective of the study was to evaluate kinds of haplotypes in relation to the responsiveness of cynomolgus macaque to atherogenic diet.

MATERIALS AND METHODS

Animals

Blood samples were obtained from 22 adult males of cynomolgus macaques (*M. fascicularis*) from captivity in Primate Research Center of Institut Pertanian Bogor, West Java, Indonesia. The animals were housed in individual cages that were positioned as such so that they can see and hear each other. They were fed with 100-180 g per animal twice a day (08.00 am and 02.00 pm) plus one piece of 70 g banana (12.00 pm). Water was given *ad libitum*. Blood samples were collected by femoral venipuncture about 2 ml using standard techniques while the monkeys were sedated by Ketamin HCl (10 mg/kg body weight, given intramuscular). All treatment procedures applied on the animals had been approved by Institutional Animal Care and Use Committee (IACUC) with protocol number 12-B009-IR. The 22 cynomolgus macaques were devided into 3 groups based on their responsiveness to atherogenic diet (Table 1). They were classified as hyporesponse, hyperresponse, or extreme following a feeding regime of a high cholesterol diet for three months. Animals that had plasma cholesterol concentrations within range of 1.5 SD from mean were classified as hyperresponse (250 to 900 mg/dL), whereas animals with cholesterol levels below or above this range (< 250 mg/dL or > 900 mg/dL) were classified as hyporesponse or extreme.

Genomic DNA extraction

Genomic DNA was extracted from all whole-blood samples using a QIAamp™ DNA Mini Kit (QIAGEN, Hilden, Germany) in accordance with the manufacturer's instructions.

Table 1. Animals used in this study and their groupings based on responsiveness to atherogenic diet

Animals (Tattoo no.)	Respon-siveness	Animals (Tattoo no.)	Respon-siveness
T3707	Hypo-	FE7777	Hyper-
K30	Hypo-	T3536	Hyper-
FC8501	Hyper-	C2480	Hyper-
T3049	Hyper-	T3303	Hyper-
FG7998	Hyper-	FG7909	Hyper-
T3307	Hyper-	T3300	Hyper-
T3700	Hyper-	C0750	Hyper-
T3278	Hyper-	FC9015	Hyper-
FC9113	Hyper-	C4927	Hyper-
9695	Hyper-	C0613	Extreme
C4939	Hyper-	T3535	Extreme

Table 2. Primers used and length in base pairs of expected PCR products

Regions	Primers	PCR product	*Annealing* Temp.
Exon 6	F: 5'-CCTTCCTCCTTCCTCTCTCT-3' R: 5'-ACTCTGCAAGCCGCCTGCAC-3'	184 bp	56°C
Intron 5	F: 5'-AAAATCAACACACTCTGTCC-3' R: 5'-ACTCTGCAAGCCGCCTGCAC-3'	1010 bp	56°C

PCR and sequencing

Two regions within *LDLR* gene of cynomolgus macaque were amplified based on primers from previous study (Hummel et al. 1990). Primers used are shown in Table 2. Reactions were conducted in a 25 µL volume and contained 5µL genomic DNA, 1 µL of each primer 10 pmol, 12.5 µL KAPA HotStart readymix Kit (buffer solution, dNTP and Taq polymerase enzyme) and 5.5 µL nuclease free water. Amplification was performed by using a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA) and the cycling parameters were as follows: denaturation at 94°C for 5 min followed by 40 cycles. Each cycle consisted of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 7 min. The post extension was at 25°C for 4 min. Amplicons was visualized on a transilluminator following agarose gel electrophoresis to check band specificity and sufficiency for subsequent sequence analysis. DNA fragments were then purified using the MinElute Qiagen Kit (QIAGEN, Hilden, Germany) in accordance with the manufacturer's instructions, and sequencing was performed in First BASE Laboratories Sdh. Bhd. (Malaysia).

Sequence and data analysis

Consensus sequences were obtained by combining forward and reverse strands for each amplicon and aligning them to a reference sequences of *Macaca fascicularis* (Genbank accession number XM_005587996.2). We used Geneious 7.0.2 (http://geneious.en.softonic.com, the 30-day trial version) to edit and assess the quality of sequence data, and to generate final consensus sequence for each amplicon. Multiple sequence alignments were obtained by using CLUSTAL W 1.8 in Mega-6, and phylogenetic tree

were conducted in the same program (Tamura et al. 2013). Haplotypes analyses was conducted in DnaSP (Rozas et al. 2003).

RESULTS AND DISCUSSION

Amplification and sequencing of exon 6 region

The amplification of exon 6 region gave results as expected, that was the amplicon with a size of 184 bp. Aligning the consensus sequences to the reference showed that the amplicon contained not only the base nucleotide of exon 6 (125 bp), but also 30 bp of intron 5 and 29 bp of intron 6.

SNP and Haplotype within exon 6 region

The analysis of the nucleotide sequences for the amplicon of 22 cynomolgus macaques had identified two single nucleotide polymorphisms (SNPs), those were IVS5-6C > G (within the intron 5, 6 nucleotides before the beginning of exon 6 nucleotide) and 825C > G (within the exon 6). The identified polymorphic sites as presented in Table 3 show that only 3 out of the 22 animals have nucleotide base sequences possessing polymorphism, i.e. T3535, T3707 and K30. The T3535 animal has two SNPs, i.e. IVS5-6C > G and 825C > G, whereas each of the T3707 and K30 animals have one SNP, i.e. IVS5-6C > G. The 825 polymorphic site in *LDLR* cDNA is located on the third position of codon triplet and it has no effects on the encoded amino acids. Also, the SNP IVS5-6C > G gives no effects on the encoded amino acids because it is located on intron region.

The two sites of polymorphic sites produced three haplotypes i.e. haplotype I (CC), II (GC) and III (GG) with a diversity of 0.255 ± 0.01347. Haplotype I is similar to the one that belongs to the reference cynomolgus macaque in the GenBank and contained in almost all animal's blood samples (Table 3). Animal grouping based on the haplotypes shows an interesting fact when it is related to the responsiveness of cynomolgus macaque to atherogenic diet in Table 1. The fact is that the haplotype II (GC) is a haplotype that belongs to the cynomolgus macaque that possesses hyporesponsiveness i.e. animals T3707 and K30. The similarity of the grouping of these two species based on the haplotype types and the responsiveness to atherogenic diet shows a relationship between the kinds of haplotype and the responsiveness. Electropherogram results from the three kinds of haplotypes are shown in Figure 1.

Amplification and sequencing of Intron 5 region

Amplification using forward and reverse primer for intron 5 results in product sizing of 1010 bp. The sequencing results show that the amplification product consists of 30 bp as part of intron 4, 126 bp is exon 5, 704 bp is intron 5, 126 bp is exon 6 and 24 bp as part of intron 6.

SNP and haplotype within Intron 5 region

An analysis that was performed on the intron 5 sequences of the 22 cynomolgus macaques, had identified 5 polymorphic sites which produce 6 haplotypes. The identified polymorphic sites consists of variables of four parsimony sites (IVS5+99, IVS5+327, IVS5−96 and IVS5−6) and one singleton site (IVS5+173). The resulting haplotype diversity was 0.680 ± 0.095. Six identified haplotypes were haplotypes I (TGACC), II (CGGCC), III (CGGTG), IV (CGACC), V (TGATG) and VI (TTACC). Haplotype I was similar to the reference haplotype belongs to rhesus macaque as in the GenBank and had the highest number of individuals. The rhesus macaque was used as a reference because the intron 5 sequences data of the cynomolgus macaque was not yet available in the Genbank. The Haplotype IV was singleton haplotype and it belonged only to T3535 animals. The total haplotypes and individual groupings based on the haplotypes are shown in Table 4.

Animal grouping based on the haplotypes showed an interesting fact when it is related to the responsiveness of the cynomolgus macaque to atherogenic diet as seen in Table 1. The fact is that the haplotype III (CGGTG) is a haplotype that belongs to the cynomolgus macaque that possesses hyporesponsiveness i.e. animals T3707 and K30. Reconstruction of the phylogenetic tree showed that individuals with haplotype III formed their own groups with high bootstrap value, that is 81% (Figure 2). The high Bootstrop values are benchmarks for the determination of grouping confidence level. This means that T3707 and K30 animals are in separate groups based on the haplotypes owned. The similarity of the groupings of these two species based on the haplotype types and the hyporesponsiveness to atherogenic diet showed a relationship between the types of haplotype and the responsiveness to atherogenic diet.

Table 3. Identified SNPs within exon 6 region aligned to reference GenBank (access number XM_005587996.2).

Haplo-types	Nucleotides base position		No. of ind.	Tattoo no.
	IVS5-6	825		
Ref	C	C	-	-
I	C	C	19	FE7777, T3700, T3536, FC9015, T3303, T3278, FC8501, C0613, T3307, C4927, 9596, T3049, FG7909, FG7998, C0750, T3300, C2480, C4939, FC9113
II	G	C	2	T3707, K30
III	G	G	1	T3535

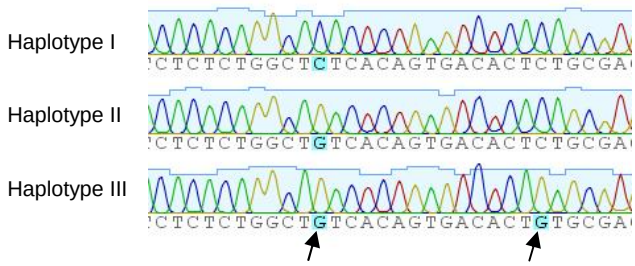

Figure 1. Electropherogram of three patterns of haplotypes

Table 4. Identified polymorphic sites within intron 5 *LDLR* gene and haplotypes, and aligned to the rhesus macaque as a reference (GenBank accession number AY466854).

Haplo-type	Position within the intron 5					No. of ind.	Tattoo no.
	IVS5+99	IVS5+173	IVS5+327	IVS5−96	IVS5−6		
Ref	T	G	A	C	C	-	-
I	T	G	A	C	C	12	9695, C0613, C0750, C2480, FC8501, FC9113, FG7998, T3049, T3278, T3303, T3700, FC9015
II	C	G	G	C	C	4	C4927, C4939, FG7909, T3300
III	C	G	G	T	G	2	T3707, K30
IV	C	G	A	C	C	2	FE7777, T3307
V	T	G	A	T	G	1	T3535
VI	T	T	A	C	C	1	T3536

Discussion

This research showed the existence of common genetic polymorphisms within the regions of exon 6 and intron 5 of the cynomolgus macaque *LDLR* gene. The existence of haplotype II (GC) at 6 base pairs prior to the exon 6 and haplotype III (CGGTG) within intron 5 which are solely possessed by hyporesponders show that common genetic polymorphisms within these regions might be linked to the response variations of the cynomolgus macaque to atherogenic diet. In fact, these evidences have not been reported previously. Generally, reports on identifications of SNPs or haplotypes of the cynomolgus macaque were mainly related to the origin or geographical distribution (de Groot et al. 2011; Fawcett et al. 2011). Investigations on individual genetic variations that affect susceptibility to diseases or other disorders are still less compared to total number of biomedical researches conducted on cynomolgus macaque. Some of them were genetic variations against malaria susceptibility (Flynn et al. 2009), drug safety (Ebeling et al. 2011) and neurobiology reactivity due to stress (Rogers et al. 2013). In humans, the presence of SNP in the *LDLR* gene has been reported to affect normal variation in plasma lipid profile lies in intron 1 and exon 2 (Linsel-Nitschke et al. 2008; Willer et al. 2008).

At the stage of implementation, the similarity of grouping hyporesponder animals based on GC and CGGTG haplotypes allowed us to choose one of the two primer pairs used for genetic variation analysis. In practical aspects, the analysis of genetic variation wihin exon 6 (184 bp) is much more simple than intron 5 (1010 bp). Yet, both polymorphic sites on the exon 6 region differ only in a dozen of nucleotides which makes it easier to identify. Thus, the selection of exon 6 as a region used to identify genetic markers for hyporesponder the cynomolgus macaque becomes the primary choice. Linking the GC and CGGTG haplotypes to hyporesponsiveness in the cynomolgus macaque makes the identified polymorphic sites become potential to be used as genetic markers, although it still needs to be confirmed by using more samples of hyporesponders. The existence of the SNPs within the exon 6 and exon 5 regions that are not functional suggests the possibility of these SNPs to be in *disequilibrium linkage* with other functional SNPs in influencing the hyporesponsiveness to atherogenic diet.

Identification of genetic variation within the exon 6 and intron 5 regions of *LDLR* gene as genetic markers of responsiveness to atherogenic diet is an important breakthrough as it makes a preliminary selection of animals to be simpler and more efficient. Up to this time, the selection of responsiveness to diet in primate centers is done through atherogenic diet intervention in 2 months (Clarkson et al. 1988; Turley et al. 1997). This selection is inefficient as it requires large amount of animals, and strict control on the diet. By the presence of genetic markers as a basis for the selection of hyporesponder monkeys, then no treatment and control of the diet are needed. In terms of time span, the analysis of genetic variation is faster, while on the budget aspect, this technique is cheaper than costs of animal raising and feeding for two months during the selection period in the primate centers.

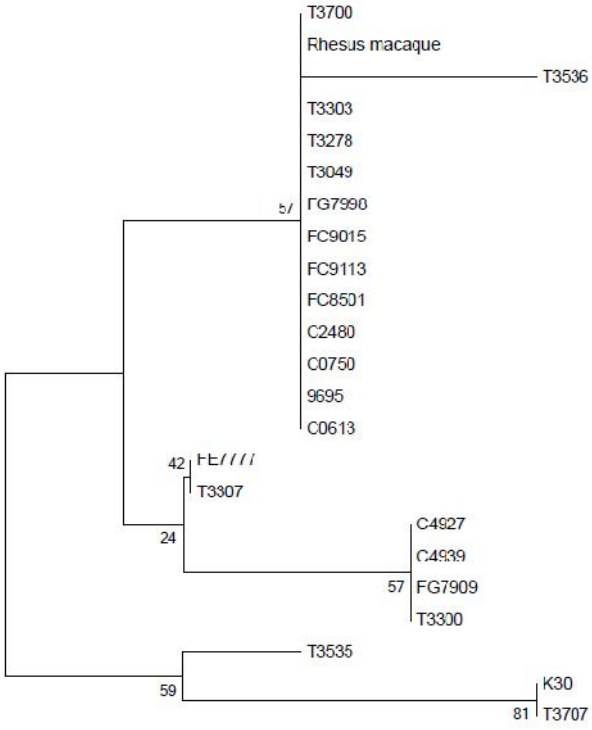

Figure 1. Phylogenetic tree based on intron 5 sequences reconstructed using *Neighbor Joining* method by a 1000-times bootstrap.

Selection of tested animals before conducting research related to atherosclerosis is crucial as it can improve the accuracy and efficiency of the scientific studies that eventually supports the success of the research. Initial selection will also reduces number of tested animals that supports the principles of 3Rs (*reduction*, *refinement* and *replacement*). Furthermore, the selection of animals based on genetic variation may reduce the limitation of using primates as models in genetic studies on complex diseases. The use of animals which are genetically uniform will give more power in statistical analysis of the tested variables, especially on small numbers of samples (Vallender and Miller 2013).

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The local knowledge of the rural people on species, role and hunting of birds: Case study in Karangwangi Village, West Java, Indonesia

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Abstract. *Iskandar J, Iskandar BS, Partasasmita R. 2016.The Local knowledge of the rural people on species, role, and hunting of birds: case study in Karangwangi village, Cidaun, West Java, Indonesia. Biodiversitas 17: 435-446.* Based on the ecological history, in the past many villages of in Indonesia including in West Java had a high diversity of birds. Nowadays, however, the diversity of birds in some villages of West Java has tended to decrease due to many factors, namely habitat loss, the use of pesticides, and intensive illegal bird hunting. The objective of this paper is to elucidate the local knowledge of Karangwangi village, West Java on species, role, and hunting of birds. Method used in this study is the qualitative and ethnoornithological approach with descriptive analysis. Results of study show that the Karangwangi people have a very good knowledge on bird species, particularly on level species/specific. Various bird species are traditionally classified into nine local categories (folk classification), namely based on distinctive voice/vocalization, morphological characteristic, special color, distinctive behavior, time activity, special common habitat, migrant, nest characteristics, and role in the ecosystem. Based on the rural people perception, the role of birds can be divided into two categories, namely notorious and beneficial birds. The diversity of rural birds has tended decrease over time due to various factors, including illegal bird hunting for various purposes of the village people, such as keep a bird in cages and bird trading. The study suggests the perception of rural people on birds have changed caused of socio-economic and cultural changes. Nowadays the bird hunting in the rural area has tended to shift from a purely subsistence form towards a more commercial form and, thus, to conserve bird species the study on ethnoornithology considered as a very important, and socio-economic and cultural rural people aspects might be integrated to national as well as international bird conservation programs.

Keywords: Bird classification, bird hunting, ethnoornithology, Karangwangi, local knowledge

INTRODUCTION

Indonesia recognized as one of the countries that has a high diversity of birds in the world after Brazil. It has been recorded 1.605 species of birds in Indonesia consists of 20 orders and 94 families, representing 16 percent of total bird species in the word (Bird Life International 2003; LIPI 2014). Indeed, Java island one of the major islands in Indonesia has also rich avifauna. According to Delacour (1947) 337 breeding species have been recorded in this island. While Hoogerwerf (1948) recorded 536 species and sub-species of birds in Java and the surrounding island, representing 410 species and sub-species of breeding, 111 species and sub-species of migrants, 13 species and sub-species stragglers, and 2 species and sub-species of unknown status. Various birds have an important role in ecological services or ecological functions, such as seed dispersal, pest predator, pollinator, and indicator of the environmental pollution and environmental changes (cf. Dammerman 1929; Dickinson et al. 1979; Howe and Westley 1988; Iskandar 2007; Iskandar 2015[a]; Sodhi et al.2011). In addition, the birds have socio-economic and cultural functions, such as for food, pet, pet trade, source of fables, tales, stories, folk songs, proverbs, symbolic, myths, and magic (cf. Iskandar 2007; Iskandar 2015a; Kizungu et al. 1998; Jepson and Landle 2005; Alves 2009; van Vloeg and van Weerd 2010; Tidermann et al. 2011; Alves et al., 2013; Bezerra et al. 2013; Roldan Clara et al. 2014; Teixeira et al., 2014; Dandeniya et al. 2015; Partasasmita et. 2016).

Although various birds have an important role both in ecological and socio-economic functions, a lot of birds have been threatened in the village ecosystems of Indonesia, including in West Java in the last several decades. Consequently, some bird species have been recorded became rare or local extinct. Nowadays, it has been recorded some birds considered as globally (near) threatened in Java and Bali, such as Javan hawk eagle (*Spizaetus bartelsi*), grey-headed fish eagle (*Ichthyophaga ichthyaetus*), green peafowl (*Pavo muticus*), yellow-throated hanging-parrot (*Loriculus pusillus*), black-banded barbet (*Megalaema javensis*), white-breasted babbler (*Stachyris grammiceps*), white-bellied fantail (*Rhipidura euryura*) and straw-headed bulbul (*Pycnonotus zeylanicus*) (Van Balen 1999). Many factors have affected bird population in village ecosystems, such as habitat loss, the use of pesticides, and intensive illegal bird hunting (cf. van Balen 1999; Sodhi et al. 2011; Iskandar 2007; Iskandar 2015a; Iskandar and Iskandar 2015; Iskandar 2016). Therefore, the main factor that has caused affected bird

population in the village ecosystem namely human activities (cf. Alves et al. 2013; Iskandar 2015[a]). Originally based on the ecological or environmental history, the village people had utilized birds based on local knowledge (corpus) and cosmos and beliefs (Toledo 2002). For example, most Sundanese villages of West Java had perceived some top predator birds, such as *serak* or barn owl (*Tyto alba*) and *loklok* (Family Strigiformes) as strongly related as mystic or magic (cf. Iskandar 2007; Partasasmita et al. 2016). In addition, traditionally if the village people had very frequently heard voice *uncuing* (cuckoos) and *gagak* (crows) that is considered as early warning there is a person might be pass away in their community member (cf. Iskandar 2007; Muiruri and Maundu 2011; Badriansyah et al. 2015). As a result, culturally those birds had been considered as scary birds and prohibited hunted. Indeed, those birds had rarely kept as pet and indirectly conserved by the village people. In addition, the forests that had culturally considered as sacred places and traditionally managed by the village people recorded owning a high diversity birds compared with that of in non-sacred places (cf. Iskandar 1998; Endri et al., 2015; Badriansyah et al. 2015).

Nowadays, however, some local knowledges as well as cosmoses of belief of village people in birds have eroded. The village birds have been intensively hunted not only for food and live bird keeping in the cages but also for both trading in local villages and trading in urban bird markets (cf. Jepson and Ladle 2005; Jepson 2011; Pangau adam et al. 2011; Iskandar 2015[b], Iskandar and Iskandar 2015). A lot of birds are currently hunted by the village people because various birds can be traded with high prices. Consequently, the populations of the village birds have dramatically decreased. In addition, intensive use of pesticides in the agriculture and habitat loss through land use conversion have seriously affected on the bird populations in the village area.

Because the village bird populations have tremendously decreased mainly caused of the human actions, therefore, to conserve the village bird populations, the socio-economic and cultural aspects must be considered. Indeed, the study on ethnoornithology—the study of bird in culture—useful to support the bird conservation. Due to the study on ornithology that is concerned in the complex of inter-relationship between birds, and all other living and non-living things (Tidemann et al. 2011).

Due to main source of the problems of threatened birds as well as the hope for solution is the human, we cannot talk about bird conservation without incorporating human dimensions (Alves 20012; Alves et al. 2013; Alves and Souto 2015). Ethnoornithology study of birds in cultures or an understanding of the place of birds in cultures, broadly study to the complex of inter-relationships between birds, humans and all other living and non-living things (Tidemann et al. 2010). Therefore, the result of the ethnoornithology studies can provide basic information for designing urgent conservation strategies, as well as promoting public policies (cf. Jepson 2011; Alves 2012; Alves et al. 2013; Bezera et al. 2013). Although the ethnoornithology is a very important, the ethnoornithology has not yet become well integrated within avian conservation (Bonta 2011). Indeed, study on ethno-ornithology studies have rarely undertaken in Indonesia.

The objective of this paper is to elucidate the local knowledge of Karangwangi village, West Java on species, role, and hunting of birds.

MATERIALS AND METHODS

Materials

Some materials were used in this study, including the field guide books to the birds for bird identification, written by Delacour (1947), Hoogerwerf (1949a, 1949b), King et al. (1975); and Mac.Kinnon et al. (1992). In addition, some materials were used, namely binocular, GPS, camera, note book, and ballpoint.

Study area

Study was conducted in the Village (*desa*) of Karangwangi, Sub-district (*kecamatan*) of Cidaun, District (*kabupaten*) of Cianjur, Province (*provinsi*) of West Java, Indonesia. Geographically, the study area, village of Karangwangi lies between 7° 25'- 30'LS 7° and 107° 23'-107° 25 'E (Figure 1). The Karangwangi village is a remote area which has size of approximately 1,527.80 hectares that lies off the south of West Java. It has the distance approximately 120 km from the town of Bandung and approximately 70 km from the town of Cianjur, and to reach this area by vehicle needs a travel time of 5-6 hours from the town of Bandung and approximately 3-4 km from the town of Cianjur. Karangwangi village is directly bordered with Indian Ocean in the south and the nature conservation of Bojonglarang Jayanti in the west. Land used of the Karangawngi comprises the settlement and home garden, mixed garden, rice field, river, and the forest. In 2014, population of the Karangwangi was recorded 5,672 people consists of 2,864 males and 2,808 females, with total 1,691 households.

Procedure

Method was used in this study namely the qualitative with descriptive analysis which the ethno-ornithology or ethnobiology approach was applied (cf. Ellen 1993a; Ellen 1993b; Diamond and Bishop 1999; Newing et al. 2011; Iskandar 2012; Albuquerque et al. 2014). Some collecting field data techniques, such as observation, participant observation, and semi-structure or deep interview were applied. During the field research, the researcher stayed in the village between two and three weeks. For collecting ethnoornithology data, the researcher conducted deep interview with informants who was purposively selected by the snowball technique (cf. Newing et al., 2011; Albuerque et al. 2014). Some informants were selected namely old people, the village formal and informal leaders, farmers, fishermen, bird hunters, and wild bird keepers in the village. Before conducting deep interview with informants, the nature and objective of the research were explained, and permission for the interviewees was requested to record information (cf. Alves et al. 2013). The interview

guideline contained namely the local name/vernacular name of birds, morphological characteristics, distinctive voice, special color, habitat types, and role in ecosystem and socio-cultural, and hunting of birds. To identify and validate bird species for interviewees, the researcher showed bird pictures which are presented in the books of field guide to the birds in Java, Western Indonesia, and Southeast Asia (Hoogerwer 1949a; Hoogerwerf 1949b; King et al. 1975; MacKinnon et al. 1992). In addition, the participant observation was also conducted by researcher during the field research (Newing et al. 2011). For example, the researcher involved in bird hunting activities, namely went to the forests with the informants who were hunting birds.

In addition, to know existing birds and relative population of each bird species in the village study (Karangwangi village), special bird census was conducted by 'IPA' (*Indices Ponctuele d'Abondance*) or "PIA" (*Point Index of Abundance*) method (cf. Blondel et al. 1970; Iskandar 1980, van Helvoort 1981; Bibby et al. 1992). The IPA was undertaken by selecting special points at the different habitats, such as mixed-garden (*kebon tatangkalan*), coastal (*pantai*), and the forest area (*leuweung*) of the Nature Conservation of Bojonglarang, Karangwangi. In each point, researcher recorded all birds seen or heard in 15 minutes. Thus, total birds were collected in 46 point counts or IPAs, representing mixed-garden (6 counts), coastal (5 counts) and the forest of Nature conservation of Bojonglarang Jayanti (35 counts).

The various field data collected by observation and deep interviews were analyzed by cross-checking, summarizing and synthesizing, and to building up a narrative account (cf. Newing et al. 2011). While the data of bird population undertaken by the IPA-censuses were analyzed by calculating the index of dominance of each bird (Jorgensen 1976; Van Helvoort 1981) as follow:

$D_i = N_i/N \times 100\%$ or $Di = 100 \times pi$

Whereby:

D_i = dominance value of bird species i;

N_i = number of individuals belonging to bird species i;

N = total number of bird individual in the community (the sum of all N_i)

P_i = the proportion of the bird individuals of the i-th species of all bird individuals of the community

Moreover bird population can be divided into 3 categories: Di = 0-2% (non-dominant), Di=2-5% (sub-dominant), and Di= over 5% (dominant).

RESULTS AND DISCUSSION

Local knowledge on birds

The Karangwangi people term for birds generally is *manuk*. On the basis of folk classification as mentioned by Berlin (1992), the Karangwangi people recognize three taxonomic levels, namely the level of life form *manuk* (bird), followed by species, equivalent to Western biological classification, such as *cangkurileung* (Sooty-headed bulbul, *Pycnonotus aurigaster*), and divided into two sub-species or variations, culturally named *cangkurileung kapas* (*kapas* literally cotton or whitish meaning the whitish color of *cangkurileung*) and *cangkurileung kotok* (*kotok* literally chicken or dark, meaning dark color of *cangkurileung*) (Table 1).

As it can be seen from an example above that the Karangwangi people well recognize the bird classification particularly at the level two which is analog with species in term of biological scientific classification. The folk classification of Karangwangi people is similar to Karam (Bulmer 1967), Katengan (Diamond and Bishop 2000), and Wola, Papua New Guinea in that it has well recognized the bird classification particularly at the level two, species/specific.

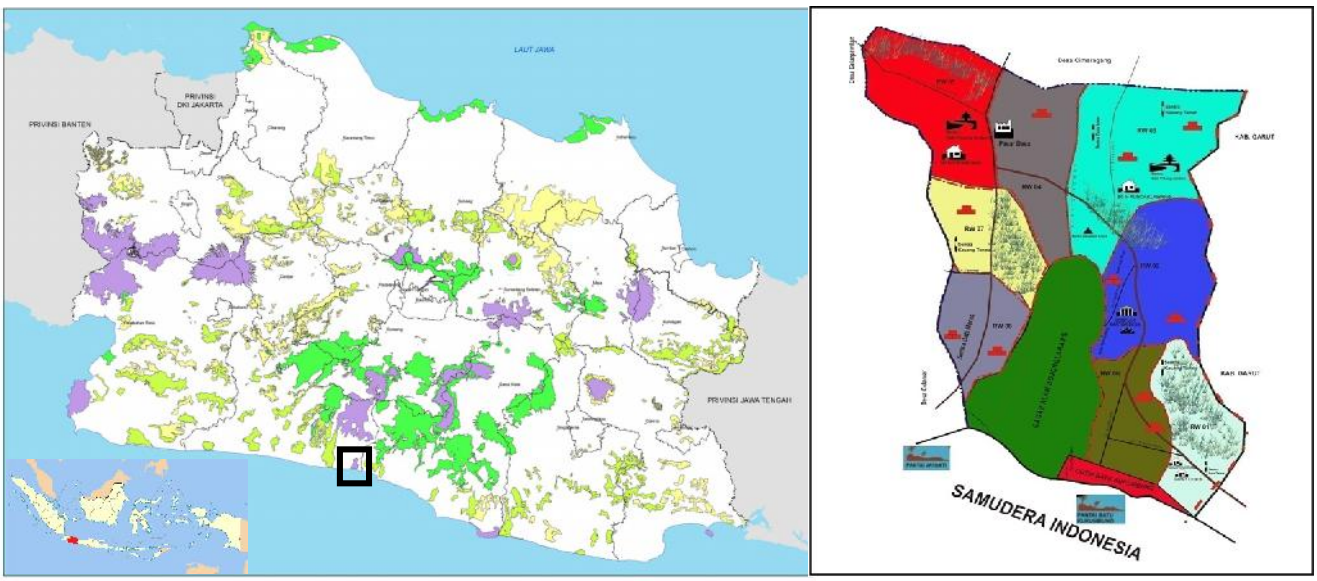

Figure 1. Research location, Karangwangi Village, Cidaun Sub-district, Cianjur District, West Java, Indonesia

On the basis of our interview with informants, it can be revealed that the Karangwangi people recognized at least 41 bird species. While based on the bird population study using IPA-censuses, it was recorded 40 bird species. These can be divided into 3 categorized, Dominant birds (Di= > 5%) recorded 5 species, *Halcyon chloris* (Di=6,87%), *Lonchura leucogastroides* (Di=26.87), *Passer montanus* (Di=7.32%), *Trereron curvirostra* (Di=9.92%), and *Zosterops palpebrosus* (Di=7.48%); sub-dominant birds (Di=2-5%) recorded 8 bird species, Collocalia esculenta (Di=2.13%), *Halcyon cyanoventris* (Di=2,44%), *Megalema australis* (Di=4.42%), *Nectarinia jugularis* (2.44%); *Orthotomus ruficeps* (Di=2.13%), *Orthotomus sepium* (Di=4,58%), *Pycnonotus aurigaster* (Di=4,88%) and *Treron griseicauda* (Di=3.96%), and 27 bird species are categorized as non-dominant birds (Di= < 2%) (Table 2).

Totally bird species recorded both by the IPA-censuses and well recognized by informants of Karangwangi people were 51 bird species representing 14 species (24%) recognized as the protected animals based on Indonesian law (cf. Noerdjito et al. 2001). Of 19 bird species were recorded both by direct observation using IPA-censuses as well recognized by informants. Several bird species, such as *bueuk* (Collared scopsowl, *Otus bakkamoena/lempiji*), *koreak* (Barn owl, *Tyto alba*), and *cuhcur* (Large-tailed Nightjar, *Caprimulgus macrurus*) were well recognized by the informant but were not recorded by IPA-censuses. Due to these birds are recognized as nocturnal birds and were not recoded by the IPA-censuses recorded during the daytime. In addition, other bird species, such as *kerak kebo* (Javan myna, *Acridotheres javanicus*), *kangkareng* (Hornbill, *Anthracoceros* sp.), *heulang hideung* (Black-eagle, *Ictinaetus malayensis*), and *paok* (Banded-pitta, *Pitta guajana*) were not recorded by the IPA-cencuses because these bird populations have been very rare based on the Karangwangi people perception. Conversely, several bird species, such as *Abroscopus superciliaris, Acrocephalus orientalis*, and *Gerygone sulphurea*, were recorded by IPA-censuses but these birds were not well recognized by informants. Because these birds might be have small size and commonly lived in the remote forests, and have not culturally given attention by the Krangwangi people.

Folk classification and naming bird species

On the basis of the deep interview with informants of Karangwangi people, it has been revealed that 41 bird species are well recognized by the local people. These are classified on the basis of distinctive voice/vocalization, morphological characteristic, special color, distinctive behavior, time activity, nest type, habitat, migrant, and role in the ecosystem (Table 3).

Distinctive voice

Bird voice or bird vocalization is the most significant aspects in naming birds in Karangwangi culture. Many bird species, for example, *Cipeuw* (*Aegithina tiphia*), *cuhcur* (*Caprimulgus macrurus*), *dudut* (*Centropos sinensis*), *gagak* (*Corvus enca*), *perkutut* (*Geopelia striata*), *kahkeh/kehkeh* (*Halcyon chloris*), *cekakak* (*Halcyon cyanoventris*), *toed* (*Lanius schach*), *piit* (*Lonchura leucogastroides*), *Ungkut-ungkut* (*Megalema haemacepahala*), and *Prenjak* (*Orthotomus ruficeps*) are culturally given vernacular name based on specific vocalization. Similarly, other bird species, such as *bueuk* (*Ottus bakamoena/lempiji*), *paok* (*Pitta guajana*), *Cininin/pacikrak* (*Prinia familiaris*), *ekek* (*Psittacula alexandri*), *cangkurileung* (*Pycnonotus aurigaster*), *jogjog* (*Pycnonotus goivier*), *tikukur* (*Streptopelia chinensis*), and *koreak* (*Tyto alba*) are attributed by characteristic vocalization (Table 3).

Like Sundanese people of Karangwangi, the Malay people of Malaysia have also recognized some vernacular names of bird species based on characteristic vocalization. For example, *uwak-uwak* (*Amaurornis poenicurus*), *cerewit* (*Lobivanellus indicus*), *tekukor* (*Streptopelia chinensis*), *but-but* or *bubut* (*Centropus sinensis*), *berek-berek* (*Merops viridis*), and *tiong* (*Gracula religiosa*) are culturally given vernacular name based on vocalization characteristic (Madoc 1976). Similarly, local people of Veddah in Sri Lanka have recognized many distinctive bird species based characteristic vocalization (Dandeniya et al. 2015).

Morphological characteristic

Morphological characteristic of bird species has been significantly considered to folk classify and given vernacular name by the Karangwangi people: *Dicrurus macrocercus* and *Zosterops palpebrosa* are case in point. The *Dicrurus macrocercus* (Black drongo) is attributed a vernacular name by the Karangwangi as *saeran gunting* because this bird has a characteristic that its tail has a scissor shap (*gunting*). Similarly, *Zosterops palpebrosus* (Oriental white-eye) is well recognized as *manuk kacamata* because this bird has diagnostic mark, based on the local people it has a very distinct the white eye ring which is similar to glasses sharp (*kacamata*).

Table 1. The three taxonomic levels of bird classification of Karangwangi people

Level	Class	English equivalent	Rank
0	*Sato*	Wild animal	Unique beginner
1	*Manuk*	Bird	Life-form
2	*Cangkurileung*	Sooty-headed bulbul	Species/specific
3	*Cangkurileung kapas*	White-sooty-headed bulbul	Sub-species
	Cangkurileung kotok	Bluish-sooty-headed bulbul	Sub-species

Table 2. Various bird species identified by the local people and recorded by the Point Count Index (PIA) in Karangwangi, Cidaun, West Java, Indonesia

Scientific name	Family	English name	Index of Abundance* (%)	Identified by local people **	Vernacular name
Abroscopus superciliaris	Sylviidae	Yellow-bellied warbler	0.15	-	-
Acridotheres javanicus	Sturnidae	Javan Myna	-	+	*Kerak kebo*
Acrocephalus orientalis	Sylviidae	Eastern Reed Warbler	0.45	-	-
Acrocephalus stentoreus	Sylviidae	Clamarous Reed-Warbler	0.30	-	-
Aegithina tiphia	Aegithinidae	Common-Iora	0.30	+	*Cipeuw*
Alcedo caurelescens (p)	Alcedinidae	Small blue Kingfisher	0.45	-	-
Alcedo meninting (p)	Alcedinidae	Blue-eared Kingfisher	0.91	-	-
Alcippe pyrrhoptera	Timaliidae	Javan Fulvetta	0,91	-	-
Anthracoceros sp.	Bucerotidae	Hornbill	-	+	*Kangkareng*
Antheptes malacensis (p)	Necatariniidae	Plain-throated Sunbird	0.30	-	
Anthreptes singalensis (p)	Nectariniidae	Ruby-cheeked Sunbird	0.30	-	
Cacomantis sp.	Cuculidae	Cuckoo	-	+	*Uncuing*
Caprimulgus macrurus	Caprimulgidae	Large-tailed Nightjar	-	+	*Cuhcur*
Centropus sinensis	Cuculidae	Greater Coucal	0.30	+	*Dudut*
Collocalia esculenta	Apodidae	Glossy swiftlet	2.13	+	*Kapinis*
Collocalia fuciphaga	Apodidae	Edible-nest Swiftlet	-	+	*Kapinis guha*
Copsychus saularis	Turdidae	Magpie Robin	0.45	+	*Kacer*
Corvus enca	Corvidae	Slender-billed Crow	-	+	*Gagak*
Dicaeum trigonostigma	Diceidae	Orange-bellied Flowerpecker	0.30	-	-
Dicaeum trochileum	Diceidae	Scarlet-headed Flowerpecker	0.15	-	-
Dicrurus macrocerceus	Dicruridae	Black Drongo	-	+	*Saeran*
Dicrurus paradisieus	Dicruridae	Greater Racket-tailed Drongo	-	+	*Saeran rame*
Egretta sacra (p)	Ardeidae	Pacific Reef-egret	0.91	+	*Kuntul*
Gallus gallus bankiva	Phasianidae	Red Junglefowl	0.91	+	*Cangehgar*
Geoplelia striata	Columbidae	Zebra-Dove	-	+	*Perkutut*
Gerygone sulphurea	Sylviidae	Golden-bellied Gerygone	0.15	-	-
Halcyon chloris (p)	Alcedinidae	Collared Kingfisher	6.87	+	*Kahkeh/ kehkeh*
Halcyon cyanoventris (p)	Alcedinidae	Javan Kingfisher	2.44	+	*Cekakak*
Haliaeetus leucogaster (p)	Accipitridae	White-bellied Fish-Eagle	0.45	+	*Heulang bodas*
Hirundo striola	Hirundinidae	Striated Swallow	0.15	-	*Kapinis belang*
Hirundo tahitica	Hirundinidae	Pacific Swallow	0.15	-	*Kapinis bodas*
Icitinaetus malayensis (p)	Acciptridae	Black Eagle	-	+	*Heulang hideung*
Lanius scach	Laniidae	Long-tailed Shrike	-	+	*Toed*
Lonchura leucogastroides	Ploceidae	Javan Munia	26.87	+	*Piit*
Macropygia emiliana	Clumbidae	Ruddy Cuckoo-Dove	1.83	-	-
Megalema armilaris (p)	Capitonidae	Orange-fronted Barbet	0.15	-	-
Megalema australis	Capitonidae	Blue-eared Barbet	4.42	-	-
Megalema haemacephala	Capitonidae	Coppersmith Barbet	-	+	*Ungkut-ungkut*
Nectarinia jugularis (p)	Nectariniidae	Olive-backed Sunbird	2.44	-	-
Orthotomus ruficeps	Sylviidae	Ashy Tailorbird	2.13	+	*Prenjak*
Orthotomus sepium	Sylviidae	Olive-backed Tailorbird	4.58	+	*Prenjak*
Orthotomus sutorius	Sylviidae	Common Tailorbird	0.45	+	*Prenjak*
Otus bakkamoena (*lempiji*)	Strigiformes	Collared Scopsowl	-	+	*Bueuk*
Passer montanus	Ploceidae	Eurasian Tree Sparrow	7.32	+	*Galejra*
Pitta guajana (p)	Pittidae	Banded Pitta	-	+	*Paok*
Ploceus sp.	Ploceidae	Munia	-	+	*Manyar*
Prinia familiaris	Sylviidae	Bar-Winged Prinia	0.30	+	*Cininin/pacikrak*
Psittacula alexandri	Psittacidae	Red-Breasted Parakeet	-	+	*Ekek*
Pycnonotus aurigaster	Pycnonotidae	Sooty –headed Bulbul	4.88	+	*Cangkurileung*
Pycnonotus goiavier	Pycnonotidae	Yellow-vented Bulbul	1.37	+	*Jogjog*
Spilornis cheela (p)	Accipitridae	Crested Serpent Eagle	-	+	*Heulang coklat*
Streptopelia bitorquata	Columbidae	Island Collared-Dove	0.61	-	-
Streptopelia chinensis	Columbidae	Spotted-Dove	0.91	+	*Tikukur*
Treron curvirostra	Columbidae	Thick-billed Green-Pigeon	9.92	+	*Walik*
Treron griseicauda	Columbidae	Grey-cheeked Green Pigeon	3.96	+	*Walik*
Tyto alba	Strigiformes	Barn Owl	-	+	*Koreak*
Zoothera citrina	Turdidae	Orange-headed Thrush	-	+	*Anis*
Zosterops chloris	Zosteropidae	Lemon-bellied White-eye	0.61	+	*Manuk kacamata*
Zosterops palpebrosus	Zosteropidae	Oriental White-eye	7.48	+	*Manuk kacamata*

Note: *) – Not recorded by IPA censuses; (p) Protected birds based on Indonesian regulation (Regulation No.5, 1990, on the biodiversity and ecosystem conservation.

Like Karangwangi, the local people Malay of Malaysia have also culturally attributed various vernacular names of bird species based on distinctive morphological diagnostic: *burong botak* (Lesser adjutant, *Leptoptilos javanicus*) and *belatok kecil* (Sunda Woodpecker, *Dendrocopos moluccensis*) (Madoc 1976). In addition, the *Leptoptilos javanicus* is perceived by the local people of Malay has a diagnostic mark as a bald head bird (*burong kepala botak*) due to her head bald (*botak*). Similarly, the *Dendrocopos moluccensis* has been popularly called as *belatok kecil* because this bird has small size compared to other woodpecker bird family (Family Picidae).

Special color

Many birds have been culturally recognized by local people of Karangwangi with own specific vernacular name based on special color. For example, *Haliaeetus leucogaster* (White-bellied fish-eagle) has been culturally named as *heulang bodas* due to general color of feather is white (*bodas*), particularly the head and neck and underparts of the adult bird are white (*bodas*). Similarly, *Ictinaetus malayensis* (Black-eagle) and *Spilornis cheela* (Crested-serpent-eagle) are popularly recognised by Karangwangi as *heulang hideung* and *heulang coklat*, respectively because the *Ictinaetus malayensis* has all part black color (hideung) and *Spilornis cheela* has appearing to be brown (*coklat*), except in the tip of the tail has a broad white band. Like the Karangwang, the local people of Veddah in Sri Lanka (Dandeniya et al. 2015) and the Malay people of Malaysia (Madoc 1976), have also popularly recognized some birds which is given vernacular name based on special color. For example, *Munia maja* (White-headed munia) is famously named as *pipit uban* in the Malay people of Malaysia due to her head has color white or metaphoric as gray hair (*uban*). Another example, *Dinopium javanense* (Common golden back woodpecker) has been culturally called as *belatok mas* in the Malay, because this bird has diagnostic mark at the upper back and the wing-coverts are golden yellow similar golden color (*mas*).

Distinctive behavior

Some birds are culturally classified by local people of Karangwang based on characteristic behavior of those birds. *Manuk kerak kebo* (Javan Myna, *Acridotheres javanicus*), for example, has been given name by the local people because the behavior of this birds usually looking for food types of insects, particularly grasshopper near buffalo being herded in the rice field or grazing grassland. Similarly, this bird has been also commonly called by the Malay people of Malaysia as *gembala kerbau* (grazing buffalo) due to behavior of this bird usually looking for insects in grazing buffalo (cf. Madoc 1976). Another example, *Dicrurus paradiseus* (Greater racket-tailed drongo) has been culturally recognized by local people of Karangwangi as *saeran rame* (noisy drongo) because this bird has specific behavior which is perceived as 'noisy voice'.

Time activity

Local people of Karangwang's taxonomy have two distinct groups as 'diurnal bird species' or active birds at the daytime (*manuk biasa liar siang*) and 'nocturnal bird species (*manuk liar peuting*). On the basis of the local people of Karangwangi most birds are considered as diurnal birds. However, some birds are culturally recognized as nocturnal birds, such as *cuhcur* (Large-tailed nightjar, *Caprimulgus macrurus*), *bueuk* (Collared scopsowl, *Otus bakkamoena/lempiji*), and *koreak* (Barn-owl, *Tyto alba*). Like all nightjar birds, the *cuhcur* starts working for insect at sunset. In the daytime it may be found hiding beneath trees and bushes in the mixed garden or secondary forest. Almost everyone of Karangwangi has been familiar with the vocalization of this bird as *cur-cur-cur*. *Bueuk* (Collared Scopsowl, *Ottus bakkamoena/ lempiji*) is a night bird. At the night time, this bird has frequently heard her voice instead of directly seen. The voice of *bueuk* has culturally herd as*'bueuk-boeuk-bueuk'*. Conversely, this bird usually takes rest in the mixed-garden or in the hole of wood tree. Similarly, *koreak* usually goes out from her resting places in the afternoon, such as house building and other buildings to find foods, namely rate. They usually fly from one place to other places which have distinctive voice as *koreak-koreak-koreak.*

Nesting type

The local people of Karangwangi village have categorized some birds based on nesting type and nesting characteristic. For example, *manuk manyar* (Streaked Weaver, *Ploceus manyar*) has been considered as the builders of wonderful nests. Conversely, *tikukur* (Spotted Dove, *Streptopelia chinensis*) considered as big bird size but it has been well known has bad and simple nesting type. Her nest is usually made of dried tree twig which relative small size. The local people of Karangwangi have also well recognised that *manuk uncuing* (cucoo birds) is a real parasite, and its eggs have usually put in the nest of *manuk Prenjak* (Tailor-birds, *Orthotomus* sp.). While, *manuk kapinis gua* or *walet* (Edible-nest Swiflet, *Collocalia fuciphaga*) recognised has distinctive nest in the cave and edible nest.

Like Karangangi the Veddah of Sri Lanka have culturally categorized birds, such as based on habits of birds and nesting behavior. For example, Streaked Weaver bird has been considered as interesting birds, such as this bird able to make unique nest (Dandeniya et al. 2015).

Habitat type

Some birds have traditionally categorized by the local people of Karangangi based on the habitat characteristic. On the basis of the interview with informants, it has been revealed that some birds have been categorized as village, icefield, forest, and coastal birds. For example, *manuk kangkareng* **(**Hornbill, *Anthracoceros* sp.), *cangehgar* (Red jungle fowl, *Gallus gallus bankiva*), *walik* (Green-pigeon, *Treron* sp.), and *merak* (Green Peafowl, *Pavo muticus*) are categorized as the forest birds. *Heulang bodas* (White-bellied fish-eagle, *Haliaeetus leucogaster*) and *kuntul* (Pacific Reef-egret, *Egreta sacra*) are culturally categorized as the coastal birds. *Manuk gereja* (Eurasian Tree Sparrow, *Passer montanus*), *Prenjak* (Ashy Tailor-bird, *Orthotomus ruficeps*), and *cangkurileung* (Sooty-

headed Bulbul, *Pycnonotus aurigaster*) are recognized as the village birds. While, *piit* (Javan Munia, *Lonchura leucogastroides*), *manyar* (Streaked-Weaver, *Ploceus manyar*), *peking* (Scaly-breasted Munia, *Lonchura punctulata*), and *bondol* (White-headed Munia, *Lonchura maja*) are categorized as the Richfield birds. In addition, *cekakak* (Javan Kingfisher, *Halcyon cyanoventris*) is categorized as the riverbank bird or close to water bodies.

Migrant

Some birds, such as *bondol* (White-headed Munia, *Lonchura maja*), *pipit* (Javan Munia, *Lonchura leucogastroides*), *peking* (Scaly-breasted Munia, *Lonchura punctulata*) and *ekek* (Red-breasted Parakeet, *Psittacula alexandri*), and *kuntul* (Javan Pond Heron, *Ardeola speciosa*) have been categorized by the local people of Karangwangi as local migrant birds. On the basis of the local people perception, *bondol***,** *pipit*, *peking* and *ekek* have been predominantly found in the rice field (*sawah*) and the swidden field (*huma*), but after finishing rice harvesting they have locally migrated to other villages. Similarly, population of *manuk kuntul* has been predominantly found in the many wetlands during the rainy season but they usually move to other places and will return during the rainy season.

Role in the ecosystem

Some birds, such as *ekek* (Red-breasted Parakeet, *Psittacula alexandri*), *pipit* (Javan Munia, *Lonchura leucogastroides*), *peking* (Scaly-breasted Munia, *Loncura punctulata*), *bondol* (White-headed Munia, *Lonchura maja*) and *manyar* (Streaked Weaver, *Ploceus manyar*) are cultural perceived as notorious birds. These birds are categorized as paddy seed eaters. Conversely, bird of *kapinis gua* or *walet* (Edible-nest Swiftlet, *Collocalia fuciphaga*) has been categorized as beneficial bird because the edible nest of this bird can be traded with a very high price. In addition, some raptor birds or top predators, such as *heulang coklat* (Crested Serpent-eagle, *Spilornis cheela*) and *koreak* (Barn owl, *Tyto alba*) have been categorized as beneficial birds due to rat eaters. Similarly, this bird has been considered as a beneficial bird in other Indonesian ethnics, such as village people of Petapahan, Riau, Sumatera (Badriansyah et al. 2015). In other words, based on the local people perception, the rats as paddy pest in the rice fields (*sawah*) and swidden farming fields (*huma*) might be controlled by raptors birds, namely *koreak* and *heulang ruyuk*.

The local people of Karangwangi culturally recognize also some birds, such as *kapinis* (*Hirundo* sp.) and special butterfly (*kukupu*) that are frequently observed in their village can be used as indicator of the beginning of the rainy season. Like, Karangwangi people, the local people the Dayak of Sarawak (Smythies 1960) the bird of wagtails, such as burung *beras-beras* (White wagtail, *Motacilla alba*) have been be used as beginning of the rainy season or paddy season lot of paddy (*beras*) in the swidden fields. While, the *burung ketupong* (Rufous Piculet, *Sasia abnormis*) has been an important role for the Kantu Dayak in selecting the forest are that can be opened for the swidden farming system (*ladang*). For example, by existing a lot of this bird in the certain forest area has been used as indicator such forest is not mature forest and not suitable for the planting paddy which might get a lot of terrestrial weeds (Dove 1988). .

Role in sociocultural

Birds have closely associated with traditions of the local people of Karangawngi or Sundanese people in general. For example, birds have inspired for the local mythology, tale, song, and proverb. On the basis mythology, for example, *manuk lok-lok* (*lok-lok* bird of Family Strigiformes) has been considered as 'dreaded bird 'because it has closely related with the Sundanese mythology, this bird become trans. Some birds also considered as bad omen, namely *uncuing* (Cockoo, *Cacomantis* sp.) and *gagak* (Crow, *Corvus* sp.) (cf.Iskandar 2007; Badriansyah et al. 2015). If people hear continually voice of these birds that is perceived as bad news, may be someone may pass away. Some tales in relation with birds have also been recognized by Sundanese rural people, tale of king of bird (*ratu manuk*) is case in point (cf. Iskandar 2007). Both songs and proverb of Sundanese in relation to birds are also culturally recognized in rural Sundanese of West Java, including the local people of Karangwangi, Cianjur, West Java.

Local knowledge on hunting birds

Traditionally hunting wild animals, including hunting birds have been recognized for a longtime in rural area across cultural in the world, including in West Java (cf Iskandar, 1980; Iskandar 2014; Milton and Marhadi 1989; Alves 2012; Alves et al. 2013; Alves and Souto 2015). On the basis of semi-structure interview with informants of the local people of Karawangi, Cianjur, West Java, the hunting birds had been commonly practiced by the Karangwangi people in the last time but now their hunting activities have tended to decrease due to bird population in their village have not abundant anymore.

Culturally, it has been recognized some techniques are predominantly practiced by local people of Karangwangi, namely to glue birds with sap (*ngaleugeut/ngelem*), to capture birds by nets (*ngajaring*), to catch birds with torch and kerosene lamp (*ngobor*), to hunt with a bamboo blowpipe (*susumpit*), and to hunt with a gun (*bebedil*) (Stachclyda 2015).

Ngaleugeut/ngelem-to glue birds with sap

This technique is aimed to catch live birds which are undertaken during the daytime. The main material commonly used for hunting bird by *ngaleugeut* technique is various sap, such as *karet* (*Hevea braziliensis* (Willd) Muell), *nangka* (*Artocarpus heterophyllus* Lam), *teureup* (*Artocarpus elasticus* Reinw ex Blume) and *sirsak* (*Annona muricata* L). The sap is accommodated by container and simmering. The *ngaleugeut* technique is applied as following. Firstly, the stake bamboo or wood is prepared. Secondly, the bamboo or wood stake is covered by sap. Thirdly, the bamboo or wood stake that is covered by sap is put in the tree that is predominantly visited by birds. Another approach, the selected twigs of tree is covered

Table 3. Diagnostic characteristic used in Karangwangi, West Java, Indonesia bird classification

Folk classification	Vernacular name	Scientific name	English name	Description based on rural people perception (*emic* view)
Distinctive voice/ vocalization	*Cipeuw*	*Aegithina tiphia*	Common Iora	Voice: ciipeuw ciipeuw ciipeuw
	Cuhcur	*Caprimulgus macrurus*	Large-tailed Nightjar	Voice: cuur cuur cuur cuur
	Dudut	*Centropus sinensis*	Greater Coucal	Voice: duut duut duut duut
	Gagak	*Corvus enca*	Slender-billed Crow	Voice: gaaak gaaak gaaak
	Perkutut	*Geopelia striata*	Zebra-Dove	Voice: perkututut perkututut perkututut
	Kahkeh/ kehkeh	*Halcyon chloris*	Collared-Kingfisher	Voice: kahkeh kahkeh kahkeh or kekhkeh kehkeh kehkeh
	Cekakak	*Halcyon cyanoventris*	Javan Kingfisher	Voice: cekakakak cekakakak cekakakak
	Toed	*Lanius schach*	Long-tailed Shrike	Voice: toed toed toed toed
	Piit	*Lonchura leucogastroides*	Javan Munia	Voice: priet priet priet priet
	Ungkut-ungkut	*Megalema haemacephala*	Coppersmith Barbet	Voice: Ungkut-ungkut ungkut or kut kut kut kut
	Prenjak	*Orthotomus ruficeps*	Ashy Tailorbird	Voice: prienjak prienjak prienjak
	Bueuk	*Otus bakkamoena/ lempiji*	Collared Scopsowl	Voice: buueuk buueuk buueuk
	Paok	*Pitta guajana*	Banded Pitta	Voice: paaok paaok paaok
	Cininin/pacikrak	*Prinia familiaris*	Bar-winged Prinia	Voice: cinininin cinininin cinininin or cikrak cikrak cikrak
	Ekek	*Psittacula alexandri*	Red-breasted Parakeet	Voice: keek keek keek keek
	Cangkurileung	*Pycnonotus aurigaster*	Sooty-headed Bulbul	Voice: dret dret kurileung, kurileung kurileung
	Jogjog	*Pycnonotus goiavier*	Yellow vented Bulbul	Voice: jog jog jog jog
	Tikukur	*Streptopelia chinensis*	Spotted-Dove	Voice: tiikukur tikukur tikukukur or tikukukur guk tikukukur guk
	Koreak	*Tyto alba*	Barn Owl	Voice: kooreak kooreak kooreak
	Loklok	Family Strigiformes?		Voice: loklok loklok loklok
Morhological characteristic	*Saeran gunting*	*Dicrurus macroceceus*	Black Drongo	The tail is deeply forked and similar to scissor shape (*gunting*)
	Manuk kacamata	*Zosterops palpebrosa*	Oriental White-eye	The white eye ring is similar to glasses shape (*kacamata*)
Special color	*Heulang bodas*	*Haliaeetus leucogaster*	White-bellied Fish-Eagle	General color of feather is white, particularly the head and neck and underparts of the adult bird are white (*bodas*).
	Heulang hideung	*Ictinaetus ma;ayensis*	Black-Eagle	The magnificent eagle which has appearing to be generally all black color (*hideung*)
	Heulang coklat	*Spilornis cheela*	Crested Serpent-eagle	Generally it has appearing to be brown (*coklat*), except in the tip of the tail has a broad white band.
Distinctive behavor	*Manuk kerak kebo*	*Acridotheres javanicus*	Javan Myna	This bird is frequently seen in rice field and grazing ground very close interaction with buffalo (*kebo*), particularly to find insects in the buffalo body or its surrounding.
	Saeran rame	*Dicrurus paradiseus*	Greater Racket-tailed Drongo	This bird has characteristic behavior, it has been considered as noise voice (*rame*)
	Manuk saleser	*Sitta azurea*	Blue-Nuthatch	This bird can be frequently seen running up the trunt of a tree or it seem to be creeping (*nyaleser*).

Time activity: diurnal and nocturnal	*Cuhcur*	*Caprimulgus macrurus*	Large-tailed Nightjar	Some birds, such as *cuhcur, bueuk*, and *koreak* are active in the night time (nocturnal) and in the day time it may bee found hiding in the rest places. While other birds are considered as diurnal birds.
	Bueuk	*Otus bakkamoena/ lempiji*	Collared Scopsowl	
	Koreak	*Tyto alba*	Barn Owl	
Special habitat	*Kangkareng*	*Anthracoceros albirostris*	Oriental pied hornbill	These birds are perceived by local people as forest birds (*manuk leuweung***)**
	Cangehgar	*Gallus gallus bankiva*	Junglefowl	
	Walik	*Treron* spp.	Green Pigeon	
	Piit	*Lonchura leucogastroides*	Javan Munia	These birds are perceived by local people as rice field birds (*manuk sawah*)
	Manyar	*Ploceus manyar*	Streaked Weaver	
	Kuntul	*Egretta intermedia*	Intermediate Egret	These birds are perceived by local people as the coastal birds (*manuk pantai*)
	Cangkurileung	*Pycnonotus aurigaster*	Sooty-headed Bulbul	These birds are perceived by local people as rural birds (*manuk kamung/desa*)
	Prenjak	*Orthotomus ruficeps*	Ashy Tailorbird	
	Galejra	*Passer montanus*	Eurasian Tree Sparrow	
Nest characteristic	*Manyar*	*Ploceus manyar*	Streaked Weaver	The *manyar* nest is popularly recognized by local people as nice and unique nest. The nest, hung from the tip of branch or palm-frond, is flask-shaped. Conversely, the *tikukur* nest is considered by local people as a simple nest composed by dry branches *Caladi* is recognized by local people as has a nest in the tree hole. *Cekakak* is considered by local people as has nest in the hole of hill soil.
	Tikukur	*Streptopelia chinensis*	Spotted-Dove	
	Perkutut	*Geopelia striata*	Zebra-Dove	
	Caladi	*Dendrocopos macei*	Fulvous-breasted woodpecker	
	Cekakak	*Halcyon chloris*	Collared-Kingfisher	
Migrant	*Ekek*	*Psittacula alexandri*	Red-breasted Parakeet	Many birds, such as *ekek, pipit, peking,* and *kuntul* common locally migrate-out from village to other villages in off paddy farming season and migrate-in to the village during the paddy farming season. Similarly, *kuntul* local migate-out from vill during dry season and migrate-in during the wet aor rainy season.
	Pipit	*Lonchura leucogastroides*	Javan Munia	
	Peking	*Lonchura punctulata*	Scaly-breasted Munia	
	Kuntul	*Egretta intermedia*	Intermediate Egret	
Role in ecosystem: ecosystem sevices and cultural functions	*Ekek*	*Psittacula alexandi*	Red-breasted Parakeet	-Some birds, such as *ekek, piit, peking,* and *manyar* are perceived by local people as 'notorious birds' (*hama pare*-eating paddy seeds)
	Piit	*Lonchura leucogastroides*	Javan Munia	
	Peking	*Lonchura punctulata*	Scaly-breasted Munia	
	Manyar	*Ploceus manyar.*	Streaked Weaver	
	Walet	*Collocalia fuciphaga*	Edible-nest Swiftlet	Conversely, other bird species, such *walet* or *kapinis gua* which has edible nest, perceive as beneficial birds.
	Cangehgar	*Gallus gallus bankiva*	Junglefowl	
	Heulang coklat	*Spilornis cheea*	Crested Serpent-eagle	
	Koreak	*Tyto alba*	Barn Owl	
				Another bird species, such as *cangehgar* which has behavior to eat seeds, perceives as seed dispersal and considered as beneficial bird. Similarly, *heulang coklat* and *koreak* are perceived by local people as rat eaters and considered as beneficial bird. Cultural functions: birds in song, bird in mythos, bird in tale, and birds in bird keeping and trading.

with sap. Forth, the tame bird is put near the bamboo or stake wood or twigs covered by sap. In addition, the bird recording voice of handphone is active. Fifth, the bamboo or stake or twigs are awaited by the bird hunter. Some birds caught by sap are taken and released from the sap using the water or oil. Finally, the hunted birds are collected and put in the cage. In the past, most bird trapped was utilized for bird keeping in the household. Today, however, these bird trapped are traded to middlemen in the village or traded in the urban bird market.

Ngajaring —to capture birds with nets

Ngajaring technique is aimed to catch life birds by using nets. The nets is made by nylon with has along about 20 m. Procedures to catch birds by using nylon nets as follows. Firstly, the nylon nets are placed in areas where many birds, such as forest, river bank, and rice field. The secondly, the nylon nets are placed for a few hours and awaited by the bird hunters. Thirdly, the nylon nets placed are monitored and birds are trapped by nylon nets are released and put into the special cages. The forth, all bird trapped were utilized as source of protein food in the rural people and kept for bird keeping in the cages. Today, however, most bird trapped are sold to the rural middle-man or directly brought to urban and selling in the urban bird markets (Figure 2).

On the basis of information from informants, various bird trapped can be sold in the rural have various price. Some common birds, such as *pipit* (*Lonchura leucogastroides*), peking (*Lonchura punctulata*), kutilang (*Pycnnotus* aurigaster), and *jogjog* (*Pycnonotus goiavier*) are commonly sold in the Krangwangi in a low price (*harga murah*) approximately between Rp. 20,000 and Rp 50,000. Another not-common bird, such as *saeran* (*Dicrurus macrocerceus*) can be sold with moderate price (*harga sedang*) about between Rp 150,000 and Rp 200,000. However, some popular bird songs that are popularly and frequently contested in urban areas, such as *anis* (*Zoothera citrina*) has commonly traded in the Karangwangi in a high price (*harga mahal*) between Rp 400,000 and Rp 500,000. However, this bird if is brought to urban and can be sold in more expensive approximately between Rp 700,000 and Rp 5,000,000. Indeed, the *anis* birds have been docile and good song, have very expensive price, about between Rp 12,000,000-Rp 50,000,000 (Iskandar 2015a).

Ngobor—to catch birds with torch and kerosene lamp

The *ngobor* technique is commonly undertaken by the local people of Karangwangi during the night. Some materials, such as kerosene lamp (*obor*), torch (*lampu senter*), gun (*senapan*), and bag (*kantung*) are commonly used for *ngobor*. The *ngobor* are usually conducted by four persons who each person has special duty, namely as carrying kerosene lamp, torch, gun, and bag, respectively. Culturally, procedure to catch birds with torch and kerosene as follows. Firstly, the appropriate place for hunting birds, particularly bird nesting place is decided. Secondly, the torch beam is directed to resting bird and shouted fire by gun. The dead bird shot by gun is taken by one of the bird hunter members. The dead bird are collected and divided into 4 persons and brought to hamlet (*kampung*) for cooking as chief source protein in the urban area.

Susumpit —to hunt with a bamboo blowpipe

The *susumpit* technique is used several material, particularly a bamboo blowpipe (sumpit) and dart (passer). The bamboo blowpipe (*sumpit***)** is made of a special bamboo called *awi tamiang* (*Schizostachyum iraten* Steud) the long segment of bamboo. The bamboo steak is cut and straightened by heating upper furnaces. To lengthen the bamboo blowpipe is normally spliced by another bamboo segment laced-up by rattan strip and glued to asphalt. The dart (*paser*) is made of bamboo stake that one of tip is sharpened and little bite burned, and in another tip is covered by kapok and tied by banana tree fiber yarn. A number of drat are commonly made because it will be lost in each blown.

The *susumpit* is usually undertaken during the daytime. The procedure of hunting birds with a bamboo blowpipe (*susumpit*) as follows. Firstly, perching bird is observed in different habitats, such as forest, mixed-garden, and river bank. Secondly, the perching bird is carefully approached with appropriate close distance. Thirdly, the targeted perching bird is blown by *sumpit* and dart goes toward the bird target. Forth, the bird in the dart puncture is collected and brought to the home. The birds obtained by *susumpit* are commonly utilized for meet cooking and consumed by the household members.

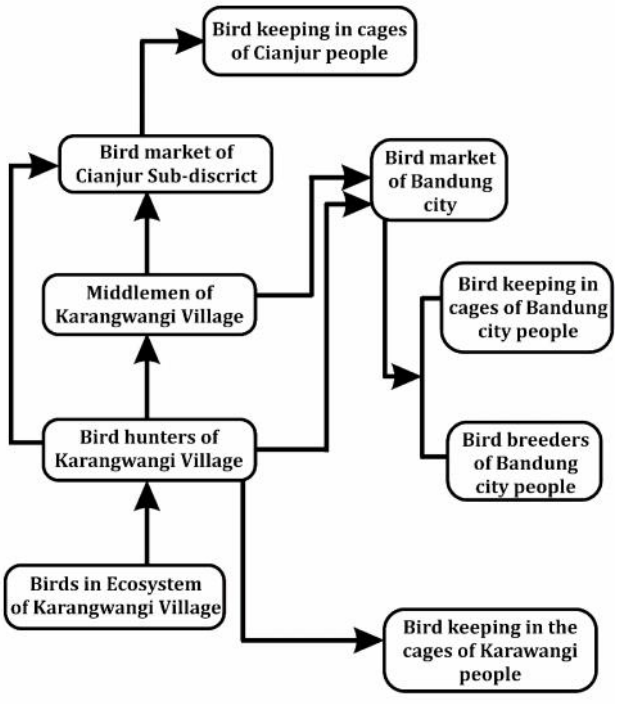

Figure 1. Trading chain of bird in Karangwangi village, West Java, Indonesia

Bebedil—to hunt with a gun

Both to hunt birds with a bamboo blowpipe (*susumpit*) and to hunt a gun (*bebedil*) have similar purpose namely to kill birds. However, unlike the *susumpit***,** the gun (*bedil***)** is mainly used by the bird hunter. Three types of guns are usually used by the local people of Karangwangi, namely *cuplis*, *senapan angin***,** and *senapan modern*. The *cuplis* is traditionally made by the local people of Karngwangi, while the *senapan angin* and *senapan modern* made of urban industry. Procedure of hunting birds a gun (*bebedil*) is similar to that of the *susumpit* as follows. Firstly, perching bird is observed in different habitats, such as forest, mixed-garden, and river bank. Secondly, the perching bird is carefully approached with appropriate close distance. Thirdly, the targeted perching bird is shut by gun. Forth, the killed is collected and brought to the home. Like *susumpit*, the birds obtained by *bebedil* are commonly utilized for meet cooking and consumed by the household members.

The local knowledge of bird conservation

In the past, most Sundanese rural people of West Java utilized birds were based on the local knowledge and cosmos or belief (cf. Toledo 2002). For example, some birds such as *manuk caladi* (Family Picidae), *heulang* (Family Accipitridae) and alap-alap (Family Falconidae) were culturally prohibited (*pamali*) to kill and consume (cf. Iskandar 2014; Ekwochi et al. 2016). In addition, based on the local tradition, looking for some birds such *perkutut* (Zebra dove, *Geopelia striata*) would be provided 'lucky' (*keberuntungan*). Conversely, the looking for *gagak* and *uncuing* was perceived would bad consequences of bad luck or unfortunate. As a result, some birds of raptors, woodpeckers, *gagak* and *uncuing*, have been culturally protected by the Sundanese local people of West Java (Iskandar 2014). Today, however, some culturally prohibitions or taboos (*pamali*) have been eroded due to rapid socio-economic and cultural changes. For example, based of deep interview with informants, almost all bird species including the protected birds by the government are allowed to capture, kill, and to trade by the rural people. As a result, the populations of some birds in the rural area have rapidly decreased due to over exploitation. Indeed, based on the informant perception, some birds, such as *kangkareng* (Hornbill, *Anthracoceros* sp.), *cuhcur* (Large-tailed Nightjar, *Caprimulgus macrurus*), *gagak*, *gelatik* (Great tit, *Parus major*), *merak* (Green-Peafowl, *Pavo muticus*), *paok* (Banded-Pitta, *Pitta guajana*), *ekek* (Red-breasted Parakeet, *Psittacula alexandri*), *kerak kebo* (Javan Myna, *Acridotheres javanicus*), *anis* (Orange-headed-Trush, *Zoothera citrina*), and *ciung* (Whisting-thrush, *Myiophoneus* sp.) have been considered as very rare or already local extinct.

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Mechanisms of antixenosis, antibiosis, and tolerance of fourteen soybean genotypes in response to whiteflies (*Bemisia tabaci*)

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Abstract. *Sulistyo A, Inayati A. 2016. Mechanisms of antixenosis, antibiosis, and tolerance of fourteen soybean genotypes in response to whiteflies (*Bemisia tabaci*). Biodiversitas 17: 447-453.* The attack of whiteflies (*Bemisia tabaci*) in soybean cultivation in Indonesia is one of the limiting factors in increasing the national soybean production. Planting resistant varieties could reduce yield losses due to the damage caused by these pests. This study was conducted to evaluate the resistance of 14 soybean genotypes to the whiteflies. A free-choice and no-choice test was conducted in a green house to study the antixenosis and antibiosis. Meanwhile, field evaluation was conducted to determine the tolerance of soybean genotypes to the whiteflies. Determination of the resistance of soybean genotypes to whiteflies based on the intensity of leaf damage that occurs on fifth weeks after infestation. The results showed that in free-choice test, Gema, IAC-100/Kaba-6, Malabar/IAC-100-85, Kaba/IAC-100//Burangrang-60, and Kaba/IAC-100//Burangrang-63 showed antixenosis mechanism which correlates with length and low density of leaf trichomes as well as leaf thickness. In the no-choice test, antibiosis mechanism can be seen from the small number of adults that develop from nymphs. IAC-100/Kaba-8 and IAC-100/Kaba-14 showed a high degree of antibiosis. In addition, the results of field experiment showed that Gema, IAC-100/Kaba-14, and Tanggamus/Pangrango-78 demonstrated a tolerance to whiteflies. It is shown on a slightly decreasing in yield of these three genotypes (17.33, 19.31, and 19.85%, respectively).

Keywords: decreasing in yield, *Glycine max*, host plant resistance, non-preference, resistance mechanism
Abbreviations: LDI = leaf damage intensity, RC = resistant category, R = resistant, MR = moderately resistant, S = susceptible, HS = highly susceptible, YD = yield decreasing,

INTRODUCTION

Soybean is one of the most important food commodities in Indonesia and ranks third after rice and maize. As a raw material in the food industry in Indonesia, soybean usually processed into tempe, tofu, bean sprouts, soy sauce, and soy milk (Ginting et al. 2009). These five types of processed soybean foods are source of vegetable protein and consumed daily for the majority of Indonesian people. It makes soybean has a strategic role and economic value. According to Sudaryanto and Swastika (2007), soybean consumption in Indonesia reached an average rate of 8.12 kg per year and soybean demand to consumption is expected increase an average of 2.44% per year. Unfortunately, the domestic soybean production is only able to meet 33.33% of the national soybean demand and the rest (66.67%) is met through imports (AMIS 2015). Soybean imports continuously going to make Indonesia relies on imported soybean and can be a serious threat to national food security (Supadi 2009). Increasing the national soybean production is one solution to reduce dependence on imported soybeans.

The efforts to increase soybean production in Indonesia often face of various problems in the field, such as interference from plant intruder organism (pests and diseases) as well as a lack of water as a consequence of the cultivation of soybean that usually falls in dry season following cropping pattern of rice-rice-soybean. The pests on soybean can attack the leaves, pods and stems. One of the pests that attack the leaves of soybean is whiteflies (*Bemisia tabaci* Genn.). Whiteflies can lead to damage either directly or indirectly (Hoodle 2013). Direct damage occurred when the stylet of whiteflies piercing the leaves and suck the liquid that causes chlorosis in plants (Gulluoglu et al. 2010a). While the indirect damage occurs due to the accumulation of honey dew that trigger the growth of sooty mold on the entire surface of the leaf and disrupted the process of photosynthesis (Hilje and Morales 2008). In addition, the whiteflies are also known to play a role as vectors of cowpea mild mottle virus (CMMV) on soybean plants (Rodrigues et al. 2014).

Until now, controlling pests in soybean by spraying insecticides is a method that widely adopted by farmers (Song and Swinton 2009). However, Palumbo et al. (2001) argue that the control of whiteflies by spraying chemical insecticides has not given satisfactory results. According to Norris et al. (2003) this was due to new strains of whiteflies easily formed with increasing levels of resistance to pesticides. Additionally, Bueno et al. (2011) found that the prophylactic use of insecticides in the soybean does not lead to higher productivity in the field when compared with the technique of integrated pest management (IPM) and biological control. Excessive insecticide applications also have a negative impact on the environments, one of them is impairing the efficiency of all existing biological control agents for soybeans (Carmo et al. 2010). One of pest

control techniques in accordance with the principles of IPM is the use of resistant varieties, because this method can be combined with other control techniques that are environmentally friendly, such as the application of biological pesticide or biological agents (Ellsworth and Martinez-Carrillo 2001; Stansly and Natwick 2010; Vieira et al. 2011).

Breeding programs for improvement of soybean varieties that resistant to whiteflies has not been much done in Indonesia (Sulistyo 2014). It can be seen from 84 varieties of soybeans that have been released by the Indonesian government, there is only one variety (Tengger variety) was described as moderately resistant to whiteflies. However, previous studies shown that four out of eight soybean varieties classified as moderately resistant to whiteflies (Sulistyo and Inayati 2014). Resistance information of soybean genotypes to the whiteflies is important to be known by soybean breeders as a guide in selecting source of resistance genes to be used in the improvement of soybean varieties resistant to whiteflies. There are several methods to determine the resistance of soybean genotypes to the whiteflies, among others, by counting the number of population of whiteflies (eggs, larvae, and pupae) per leaf (Gulluoglu et al. 2010b), or the number of nymphs per leaf (Xu et al. 2005; Amro et al. 2007; Xu et al. 2009; Xu 2009), and based on the intensity of leaf damage due to the attack of whiteflies (Inayati and Marwoto 2012; Taggar et al. 2013). The success of the utilization of soybean genotypes that resistant to whiteflies has been reported by researchers in Turkey (Gulluoglu et al. 2010b). The aim of this study was to determine the resistance of soybean genotypes to whiteflies.

MATERIALS AND METHODS

This study consists of two parts, i.e. greenhouse experiments and field experiment. Greenhouse experiments were conducted at Indonesian Legumes and Tuber Crops Research Institute (ILETRI) in Malang district, meanwhile field experiment was done at Muneng Experiment Station, in Probolinggo district. Plant genetic material used was 14 soybean genotypes consisting of 12 lines and two varieties. The two soybean varieties in these research used as a moderately resistant check (Gema), and susceptible check (Anjasmoro) according to previous study (Sulistyo and Inayati 2014). Meanwhile, the 12 lines tested were the offspring of IAC 100 which used as a source of resistance genes to whiteflies (Lima and Lara 2004, Pinheiro et al. 2005).

Greenhouse experiment

The greenhouse experiment was performed through two methods, i.e. free-choice test (tests of attractiveness and preference for oviposition) and no-choice test.

Free-choice test

The entire of plant genetic materials used were grown in plastic pots. Each genotype were planted in 15 plastic pots and arranged in randomized completely block design with three replicates, each replicates consist of five plastic pots. In free-choice test, each replicate caged in a bamboo cage which covered with tile fabric (200 cm of height x 150 cm of width x 350 cm of length) for the purpose of limiting the movement of whiteflies from one replicate to another replicates, but still allows for whiteflies to move from one genotype to another genotypes in accordance with its preferred. Infestation of whiteflies performed at 2-weeks-old plants by laying 10 whiteflies adults on the surface of leaves each individual plant (Mansaray and Sundufu 2009). Weekly observations were carried out on population of whiteflies (egg, nymph, pupae, and adult) following the method of Vieira et al. (2011). Determination of the resistance of soybean genotypes to whiteflies based on the intensity of leaf damage that occurs on fifth weeks after infestation. The intensity of leaf damage was calculated based on a scale of leaf damage following the scores made by Inayati and Marwoto (2012).

No-choice test

This test was done to confirm the resistance of a genotype due to preference or other factors. All of the tested genotypes grown in 15 plastic pots and arranged as randomized completely block design with three replicates. In no-choice test, each plastic pot was individually caged in a bamboo cage which covered with tile fabric (50 cm of diameter x 120 cm of height) with the intention of preventing whiteflies moved to other plant and forced to breed in that plants. Whiteflies infestation and observations of whiteflies population and the intensity of leaf damage was done as in free-choice test which has been described previously.

Field experiment

The field experiment was conducted in the dry season from June to September in 2012. Each genotype were planted on plots measuring width of 2 m and length of 3 m, and laid out as randomized completely block design with three replicates. Planting spacing used was 40 cm between rows and 15 cm within rows, two plants per hole. Fertilization was conducted at planting time to the dose given in accordance with the recommendation, i.e. 50 kg ha^{-1} Urea, 100 kg ha^{-1} SP36, and 100 kg ha^{-1} KCl. Irrigation was done four times, i.e. at planting time, on 3 weeks after planting, during flowering and pods filling. In this study, there were no artificial infestations of whiteflies, but whiteflies allowed to attacking naturally. Therefore, pest control was only performed for other pests besides whiteflies with the purpose of conditioning the whiteflies stress in the field. For the purposes of calculating the percentage of decreasing in soybean yields due to the attack of whiteflies, then a set of the same study conducted in the same time and same location, but separate from the first study with performed pest control optimally including whiteflies. Pest control on this plot carried out by spraying Alika® once a week from 21 to 42 days after planting (dap), and followed by spraying Pegasus® from 49 to 70 dap. Observations were carried out on the intensity of leaf damage due to whiteflies and yield per plot.

Data analysis

The data obtained was statistically analyzed using SAS v.9 software. Duncan Multiple Range Test (DMRT) was done when the F test showed significantly differences among the 14 soybean genotypes tested.

RESULTS AND DISCUSSION

Greenhouse experiment

Observations on free-choice test showed that there are significantly differences in the intensity of leaf damage. The intensity of leaf damage of 14 soybean genotypes varied from 35.18% to 76.59% (Table 1). Anjasmoro as check susceptible showed leaf damage with the most severe intensity. Three lines (G100H/9305//IAC-100-195, IAC 100/Kaba-17, and IAC 100/Kaba-5) showed the intensity of leaf damage with the level of damage as severe as Anjasmoro. Meanwhile, Gema as check moderately resistant indicate the smallest intensity of leaf damage. None of the 12 genotypes with the intensity of leaf damage which is smaller than Gema. Based on the intensity of leaf damage that occurs, then soybean genotypes were tested can be classified into resistant (IAC-100/Kaba-8 and Gema), moderately resistant (IAC-100/Kaba-6, IAC-100/Kaba-14, Malabar/IAC-100-85, Kaba/IAC-100//Burangrang-60, and Kaba/IAC-100//Burangrang-63), susceptible (G100H/9305// IAC-100-271, IAC-100/Burangrang-11, IAC-100/Kaba-5, IAC-100/Kaba-17, and Tanggamus/Pangrango-78), and highly susceptible (G100H/9305//IAC-100-195 and Anjasmoro) (Table 1).

In the free-choice test, differences in resistance of soybean genotypes to whiteflies can be explained by considering the relationship between the number of whiteflies infestations (eggs, nymphs, and adults) with the characteristics of the leaves. Gema and IAC-100/Kaba-8 that classified as resistant to whiteflies (Table 1) have a long leaf trichomes with low density (Table 2). The numbers of eggs were observed on both genotypes relatively small, 2.33 and 1.33 eggs respectively (Table 1). Similarly, the genotypes that is moderately resistant to whiteflies (IAC-100/Kaba-6, Malabar/IAC-100-85, Kaba/IAC-100//Burangrang-60, and Kaba/IAC-100//Burangrang-63) shows the same relationship between the numbers of eggs with the characteristics of trichomes such as those found in Gema and IAC-100/Kaba-8. This indicates that the characteristics of the leaf trichomes determine the preference of whiteflies for oviposition.

In addition, the thickness of the leaves affects the whiteflies in forming colony on the leaf surface of soybean genotypes tested. The leaves were thick, such as those found in all genotypes that classified as resistant and moderately resistant to whiteflies, prevents the colonization of nymphs and adults of whiteflies. This is most noticeable on IAC-100/Kaba-6, IAC-100/Kaba-14, Malabar/IAC-100-85, and Gema which has thick leaves (Table 2). A small number of nymphs or adults that recorded on that four soybean genotypes indicates an antixenosis mechanism (Table 1). It is contrary to Anjasmoro variety, a small amounts of nymphs and adults cause more severe damage up to 76.59% indicating the sensitivity of Anjasmoro to whiteflies.

Observations on no-choice test showed that there are significantly differences in the intensity of leaf damage (Table 3). Among the 14 soybean genotypes tested, the highest intensity of leaf damage was recorded on G100H/9305//IAC-100-195 (61.38%), followed by IAC-100/Burangrang-11 (52.27%), and G100H/9305//IAC-100-271 (51.40%). In this test, the intensity of leaf damage on Anjasmoro variety are 45.15% and not significantly different with the previous three genotypes. Meanwhile, the lowest intensity of leaf damage was found on IAC-100/Kaba-14 (24.85%), followed by Gema variety (26.16%) and Malabar/IAC-100-85 (30.32%). Based on the intensity of leaf damage that occurs, then the soybean genotypes were tested can be categorized as resistant (IAC-100/Kaba-14, Malabar/IAC-100-85, and Gema), moderately resistant (IAC-100/Kaba-5, IAC-100/Kaba-8, IAC-100/Kaba-17, and Tanggamus/Pangrango-78), susceptible (G100H/9305//IAC-100-271, IAC-100/Kaba-6, Kaba/IAC-100//Burangrang-60, Kaba/IAC-100//Burangrang-63, and Anjasmoro), and highly susceptible (G100H/9305//IAC-100-195 and IAC-100/Burangrang-11) (Table 3).

Table 3 shows the number of nymphs, pupae, and adults of whiteflies that found on each surface of the leaves of soybean genotypes in no-choice test. The high intensity of leaf damages which occurs in both susceptible and highly susceptible genotypes is due to the colonization of nymphs. On Anjasmoro variety, although only few number of nymphs and adults were recorded, but it has caused damage as severe as in susceptible and highly susceptible genotypes. This indicates the sensitivity of Anjasmoro to the whiteflies. While in the group of genotypes resistant and moderately resistant to whiteflies, it appears the failure of nymphs to develop into adults. This suggests a mechanism of antibiosis on these genotypes. Antibiosis with high degree was found in IAC-100/Kaba-8 and IAC-100/Kaba-14. The number of nymphs in both genotypes was 66.67 and 53.67, respectively. However, the number of adult were observed on the following observations is only as many as 0.33 for each genotypes (Table 3).

Field experiment

The results of the field test showed that the intensity of leaf damage of 14 soybean genotypes tested varies from 14.10% to 19.12% (Table 4). Based on these results there are three genotypes resistant (IAC-100/Kaba-14, IAC-100/Kaba-17, and Kaba/IAC-100//Burangrang-63), four genotypes moderately resistant (IAC-100/Burangrang-11, IAC-100/Kaba-5, IAC-100/Kaba-6, and Gema), four genotypes susceptible (IAC-100/Kaba-8, Malabar/IAC-100-85, Kaba/IAC-100//Burangrang-60, and Tanggamus/ Pangrango-78), and three genotypes highly susceptible (G100H/9305//IAC-100-195, G100H/9305//IAC-100-271, and Anjasmoro). When compared with the results obtained in free-choice test and no-choice test, it appears that Anjasmoro consistently susceptible and Gema consistently resistant to whiteflies. This means that both varieties can indeed be used as a susceptible check (Anjasmoro) and resistant check (Gema) against whiteflies.

Table 1. The resistance differences of 14 soybean genotypes to whiteflies on free-choice test

Genotype	LDI	RC	Eggs	Nymphs	Adults
G100H/9305//IAC-100-195	68.47^{ab}	HS	1.00^{cde}	44.00^{a}	6.00^{de}
G100H/9305//IAC-100-271	53.54^{bcd}	S	5.00^{b}	16.00^{efg}	4.00^{f}
IAC-100/Burangrang-11	53.98^{bcd}	S	1.33^{cde}	17.00^{ef}	10.33^{c}
IAC-100/Kaba-5	61.14^{abc}	S	0.33^{e}	17.33^{ef}	9.33^{c}
IAC-100/Kaba-6	50.19^{bcd}	MR	2.33^{c}	19.67^{de}	7.33^{d}
IAC-100/Kaba-8	37.34^{d}	R	1.33^{cde}	28.67^{c}	5.67^{e}
IAC-100/Kaba-14	43.41^{cd}	MR	7.00^{a}	16.33^{efg}	4.00^{f}
IAC-100/Kaba-17	61.45^{abc}	S	0.67^{de}	36.00^{b}	3.33^{fg}
Malabar/IAC-100-85	44.17^{cd}	MR	0.67^{de}	16.00^{efg}	1.00^{h}
Kaba/IAC-100//Burangrang-60	53.31^{bcd}	MR	2.00^{cd}	4.33^{h}	4.67^{ef}
Kaba/IAC-100//Burangrang-63	50.34^{bcd}	MR	0.33^{e}	12.33^{g}	1.33^{h}
Tanggamus/Pangrango-78	55.62^{a-d}	S	4.00^{b}	16.00^{efg}	30.67^{a}
Anjasmoro	76.59^{a}	HS	4.33^{b}	23.00^{d}	2.33^{gh}
Gema	35.18^{e}	R	2.33^{c}	15.00^{fg}	16.33^{b}

Note: Means within a column and followed by the same letter (s) are not significantly different based on DMRT at 5%, LDI = leaf damage intensity, RC = resistant category, R = resistant, MR = moderately resistant, S = susceptible, HS = highly susceptible

Table 2. Characteristics of leaf trichomes and leaf thickness of 14 soybean genotypes

Genotype	Length of leaf trichomes (µm)	Number of leaf trichomes	Thickness of leaves (µm)
G100H/9305//IAC-100-195	105^{b}	101^{b}	147de
G100H/9305//IAC-100-271	107^{b}	139^{a}	173^{bcde}
IAC-100/Burangrang-11	89^{cd}	68^{d}	233^{a}
IAC-100/Kaba-5	82^{d}	63de	157^{cde}
IAC-100/Kaba-6	93^{c}	67^{d}	227^{ab}
IAC-100/Kaba-8	94^{c}	56^{def}	143de
IAC-100/Kaba-14	90^{cd}	44^{f}	203^{abc}
IAC-100/Kaba-17	93^{c}	67^{d}	150^{cde}
Malabar/IAC-100-85	156^{a}	65^{d}	220^{ab}
Kaba/IAC-100//Burangrang-60	108^{b}	61de	187^{abcde}
Kaba/IAC-100//Burangrang-63	109^{b}	54^{def}	173^{bcde}
Tanggamus/Pangrango-78	95^{c}	57de	193^{abcd}
Anjasmoro	108^{b}	84^{c}	137^{e}
Gema	107^{b}	51^{ef}	203^{abc}

Note: Means within a column and followed by the same letter (s) are not significantly different based on DMRT at 5%

Table 3. The resistance differences of 14 soybean genotypes to whiteflies on no-choice test

Genotype	LDI	RC	Nymphs	Pupae	Adults
G100H/9305//IAC-100-195	61.38^{a}	HS	32.00^{de}	23.00^{d}	1.67^{b}
G100H/9305//IAC-100-271	51.40^{ab}	S	20.67^{fg}	0.33^{h}	0.00^{c}
IAC-100/Burangrang-11	52.27^{ab}	HS	21.00^{fg}	56.00^{a}	0.00^{c}
IAC-100/Kaba-5	41.04^{b-e}	MR	35.00^{cd}	23.00^{d}	5.00^{a}
IAC-100/Kaba-6	48.55^{abc}	S	27.00^{ef}	0.67^{h}	0.00^{c}
IAC-100/Kaba-8	41.07^{b-e}	MR	66.67^{a}	36.00^{c}	0.33^{c}
IAC-100/Kaba-14	24.85^{e}	R	53.67^{b}	44.33^{b}	0.33^{c}
IAC-100/Kaba-17	35.05^{b-e}	MR	22.00^{fg}	0.33^{h}	0.00^{c}
Malabar/IAC-100-85	30.32^{cde}	R	9.00^{i}	4.67^{g}	0.33^{c}
Kaba/IAC-100//Burangrang-60	43.82^{a-d}	S	16.00^{gh}	15.00^{e}	0.00^{c}
Kaba/IAC-100//Burangrang-63	49.91^{ab}	S	38.67^{c}	15.00^{e}	0.00^{c}
Tanggamus/Pangrango-78	35.08^{cde}	MR	24.00^{f}	0.33^{h}	0.33^{c}
Anjasmoro	45.15^{abc}	S	12.33^{hi}	8.33^{f}	0.33^{c}
Gema	26.16^{de}	R	8.00^{i}	0.00^{h}	0.00^{c}

Note: Means within a column and followed by the same letter (s) are not significantly different based on DMRT at 5%, LDI = leaf damage intensity, RC = resistant category, R = resistant, MR = moderately resistant, S = susceptible, HS = highly susceptible

Table 4. The resistance differences of 14 soybean genotypes to whiteflies on field experiment

Genotypes	LDI	RC	Yield		YD
			Pesticide	Non-pesticide	
G100H/9305//IAC-100-195	17.72^{abc}	HS	935^{a}	597^{cd}	39.34^{abc}
G100H/9305//IAC-100-271	18.26^{ab}	HS	920^{a}	720^{abc}	21.42^{bc}
IAC-100/Burangrang-11	15.05^{bcd}	MR	1,066^{a}	858^{ab}	20.38^{c}
IAC-100/Kaba-5	15.32^{bcd}	MR	1,063^{a}	838^{ab}	21.57^{bc}
IAC-100/Kaba-6	16.05^{abcd}	MR	1,068^{a}	765^{abc}	28.61^{bc}
IAC-100/Kaba-8	16.34^{abcd}	S	1,126^{a}	773^{abc}	31.96^{bc}
IAC-100/Kaba-14	14.10^{d}	R	1,113^{a}	899^{a}	19.31^{c}
IAC-100/Kaba-17	14.76^{cd}	R	953^{a}	746^{abc}	22.85^{bc}
Malabar/IAC-100-85	16.65^{abcd}	S	941^{a}	517^{d}	45.54^{ab}
Kaba/IAC-100//Burangrang-60	16.50^{abcd}	S	972^{a}	696^{bc}	26.46^{bc}
Kaba/IAC-100//Burangrang-63	14.71^{cd}	R	1,065^{a}	824^{ab}	22.76^{bc}
Tanggamus/Pangrango-78	16.71^{abcd}	S	999^{a}	805^{ab}	19.85^{c}
Anjasmoro	19.12^{a}	HS	524^{b}	182^{e}	56.43^{a}
Gema	15.63^{bcd}	MR	985^{a}	809^{ab}	17.33^{c}

Note: Means within a column and followed by the same letter (s) are not significantly different based on DMRT at 5%, LDI = leaf damage intensity, RC = resistant category, YD = yield decreasing, R = resistant, MR = moderately resistant, S = susceptible, HS = highly susceptible

The grain yield of 14 soybean genotypes in the control plot (whiteflies controlled with pesticide) and treatment plot (non pesticide) shown in Table 4. The results showed that Anjasmoro producing the lowest seed in the control plot, while 12 genotypes tested capable of producing seeds that are not significantly different with Gema. Whiteflies attack that occurred on treatment plots causing damage to the leaves of soybean genotypes tested. As a result, the process of forming and filling seed disrupted and resulting in loss of grain yield. The highest grain yield decrease was found in Anjasmoro, amounting to 56.43%, while the lowest grain yield loss encountered in Gema (17.33%), followed by IAC-100/Kaba-14 (19.31%), and Tanggamus/Pangrango-78 (19.85%). The loss of grain yield in small amounts indicates tolerance of the three soybean genotypes to whiteflies.

Discussion

The mechanism of a host plant resistance against insect herbivores divided into three, namely antixenosis, antibiosis, and tolerance (Emden 2002). Antixenosis refers to the absence of the host plant attractiveness for insects to laying eggs and feeding. In other words, antixenosis cause adverse effects on insect behavior. Antibiosis refers to adverse biologically consequences on the life cycle of pests as a result of feeding activity on resistant host plant. Symptoms of antibiosis mechanism among others are the larval mortality, increased mortality of pupae, failure of the adult out of pupae, the low fertility of adult, short life cycle of insects, and other forms of abnormality. While, tolerance is defined as the ability of resistant host plant to produces seeds better than susceptible host plant at the same level of attack of pest.

One of the mechanisms antixenosis affected by the length and density of leaf trichomes. These results are in line with Ihsan-ul-Haq et al. (2003) who found that the leaf trichome is one of the leaf morphology affecting soybean resistance to whiteflies. In this research, genotypes that are resistant to whiteflies have long leaf trichomes with low density. Characteristics leaf trichomes like this are not favored by the whiteflies for oviposition. Lima and Lara (2004) reported the same result that the number of eggs on the soybean genotypes with high density of trichomes is significantly higher when compared to the soybean genotypes with low density of trichomes. Vieira et al. (2011) explained that leaf trichomes with high density preventing the eggs blown off and keep it on the leaf surface. Valle et al. (2012) added that the interaction between the density, size, and angle of inclination of leaf trichomes will determine the resistance of soybean genotypes to the whiteflies. According to War et al. (2012), leaf trichomes function mechanically by disrupting the movement of insect herbivores on the leaf surface, thereby reducing access to the leaf epidermis.

The thickness of the leaves on soybean genotypes that resistant and moderately resistant to whiteflies were also affects antixenosis mechanisms that were found in this study. Thicker leaf would complicate the stylet of whiteflies to penetrate the epidermis of leaves and interrupt the feeding process. It is possible reasons that may explain the small number of colonies of nymphs were found on soybean genotypes that resistant and moderately resistant to whiteflies. These results were contradictory to those reported on other legumes crop. Lakshminarayan et al. (2008) reported that green gram (*Vigna radiata*) genotypes that are resistant to whiteflies have a thin leaf lamina. Similarly in the black gram (*Vigna mungo*), Taggar and Gill (2012) reported that a small amount of whiteflies found in genotypes that moderately resistant with a thin leaf lamina characteristic. Leaves with thinner lamina both in green gram and black gram might be were less succulent and thus were less preferred by whiteflies for feeding and oviposition.

Antibiosis symptom that found in this study is the failure of nymphs to develop into adults. The resistance antibiosis that found on IAC-100/Kaba-8 and IAC-

100/Kaba-14 allegedly originated from IAC-100. Lima and Lara (2004) reported that IAC 100 affect negatively on whiteflies by prolonging the period of nymphs and reducing the appearance of adults. Another antibiosis symptom that has been reported in soybean includes least amount of eggs that hatch into nymphs (Vieira et al. 2011), and the short life cycle of whiteflies (Silva et al. 2012). According to Taggar et al. (2014), a negative correlation between the content of tannins and flavonols with whiteflies population indicate that an increase in the content of these biochemical contribute to the bio-protection of host plants against whiteflies.

The tolerance of host plants against insect herbivores indicated by the ability of the host plants to produce seeds that are relatively stable during the attack of pests. In this study, it was shown by the decrease of grain yield that slightly between the plot with optimal pest control and the plot with no pest control. According to Tiffin (2000), tolerance mechanisms of the host plant may be through increased photosynthesis process, compensatory growth, utilization of stored reserves, and phonological delays. Based on grain yield on two plots with two different treatments (optimal control and non pesticide) in this study, it seems the tolerance of soybean genotypes that tested following the first mechanism (increased photosynthetic activity). The results of field observations showed that the leaves on tolerant genotypes suffered a little damage compared to the susceptible genotypes. This causes tolerant genotypes are still capable of producing seeds. Baldin (2004) reported the same tolerance mechanism in genotype KS-4202. The results of further studies on the genotype KS-4202 demonstrated that the main cause of tolerance on this genotype is not from oxidative enzymes (Cruz 2015).

Based on the results obtained in this research, it was conclude that there are different responses of 14 soybean genotypes tested against whiteflies (*Bemisia tabaci*). The mechanism of resistance of these genotypes can be either antixenosis nor antibiosis, and tolerance. Soybean genotypes resistant to whiteflies can be used as a source of resistance genes in the assembly of the soybean varieties resistant to whiteflies.

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Short Communication: Morphological study of *Fagraea ceilanica* (Gentianaceae) in Mount Nglanggeran, Yogyakarta, Indonesia

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Abstract. *Widodo, Luthfi MJ. 2016. Morphological study of* Fagraea ceilanica *(Gentianaceae) in Mount Nglanggeran, Yogyakarta, Indonesia. Biodiversitas 17: 454-460. Fagraea ceilanica* Thunb. population were found in the Mount Nglanggeran in Gunungkidul, Yogyakarta, Indonesia. Identification was based on the literature and herbarium specimens. The study was conducted through continued exploration and examination on specimen collection. The existence of *F. ceilanica* in Java was only described in Flora of Java by Backer and Bakuizen van den Brink (1965). *Fagraea ceilanica* is a liana climbing on large stone. Characteristic for initial identification were ovate to ellipsoid leaves which were opposite, thick and grayish green; bell-trumpet flower shape with flowering season around March and whitish to yellowish color and also 5-8 cm corolla tube. This paper presents important morphological character, namely leaves, stems, flowers, and fruits of *F. ceilanica*. Study on morphological character of *F. ceilanica* found on Mount Nglanggeran is needed to recognize its potential, benefit and conservation of this species.

Keywords: *Fagraea ceilanica*, Gentianaceae, Mount Nglanggeran, Yogyakarta

INTRODUCTION

In exploration, observation and assessment of wild plants in the Mount Nglanggeran Gunungkidul, Yogyakarta, Indonesia in August 2009; the authors collect liana plants climbing on the rocks with thick leaves and crossed opposite leaf arrangement. The plant was found on main track in S.07.50,319^{o}; E.110.32,186^{o}, 441 m. The authors had difficulty in identifying the species or family of the plant. Through many visits and observations, data were obtained. The process of identification through literature and herbarium study found that these plants are *Fagraea ceilanica* Thunb.

Fagraea is a genus belongs to Gentianaceae family (Takhtajan 2009) while Backer and Bakhuizen van den Brink (1965), Kochummen (1972), Keng (1994), and Steenis (1972) state that this genus belongs to Loganiaceae family. Gentianaceae and Loganiaceae family belong to Gentianales order in Euasterid I clade (APG III 2009). According to The Plant List (2013), *Fagraea* genus belongs to Gentianaceae family. Note: according to the International Plant Names Index (IPNI) (2010), there are 72 species belonging to *Fagraea* genus. Kochummen (1972) states that *Fagraea* genus comprises about 35 species and some 16 species are in Malaysia. Backer and Bakhuizen van den Brink (1965) described 7 species member of *Fagraea* genus found in Java, namely: *Fagraea auriculata* Jack, *Fagraea fastigiata* Bl., *Fagraea fragrans* Roxb., *Fagraea racemosa* Jack ex Wall., *Fagraea elliptica* Roxb., *F. ceilanica*, and *Fagraea blumei* G. Don. Hassler (2016) informed that the distribution of *Fagraea ceilanica* including China (Guangdong, Guangxi, Hainan, Yunnan), Taiwan, Cambodia, India, Darjeeling, Laos, Burma, Thailand, Vietnam, Java, New Guinea (alpine), Sri Lanka, peninsular Malaysia (Kelantan, Perak, Pahang, Selangor, Johor), India (Assam), Deccan, Sumatra, Borneo, Sulawesi, Lesser Sunda Isl., Moluccas, New Guinea, Philippines (throughout).

Leaf characteristics, life forms, and corolla tube size of *Fagraea* in Nglanggeran are in accordance with the description of Backer and Bakhuizen van den Brink (1965) concerning *F. ceilanica*. According to Backer and Bakhuizen van den Brink (1965), *F. ceilanica* is the same type as *Fagraea litoralis* Bl. and *Fagraea obovata* Wall. Based on illustrations (Blume 1836), *Fagraea* of Mount Nglanggeran have the same characteristics with *Fagraea coromandelina* Wight, while the characteristics of the leaves and stems are in accordance with *F. ceilanica*, traits of fruit are in accordance with *F. litoralis, F. ceilanica, F. obovata.* Based on herbarium specimens from Nglanggeran, the plants have similarity with *F. coromandelina* that lately identified as *F. ceilanica*. According to The Plant List (2013), *F. ceilanica* is synonymous with *F. coromandelina, Fagraea gardneri* Thwaites, *Fagraea khasiana* Benth., *Fagraea malabarica* Blume, *F. obovata*, *Fagraea prainii* Gand., *Fagraea rostrata* Blume, and *Fagraea sasakii* Hayata. It is needed to check the identification of Nglanggeran *Fagraea* as well as the accuracy of the statement about *Fagraea* synonymous.

This paper presented a detailed description of the characteristics of the plant and herbarium specimens of

Fagraea from Mount Nglanggeran completed with illustrations/pictures, herbarium types and descriptions in the existing literature to clarify the identification. *Fagraea* species discoveries in the Mt. Nglanggeran, Baturagung Mountains, Yogyakarta needs to be disseminated to present the status of flora in Java. The status of flora in Java, especially non-cultivated plants, is now no longer recognized either by its name or its specimen despite documented in the books of flora and herbarium hundreds of years ago by European explorers. Publication of species of plants in nature is required to complete the data of world's flora, re-check and rediscovery of the flora, and to improve and recheck the description of the characteristics for a further research on biodiversity of plants, plant structure, and plant systematic. The study of biology is the basic material to support the activities of conservation and exploration of earth sustainable plant use.

MATERIALS AND METHODS

Study area

This research is primarily conducted in the climbing track area of mount Nglanggeran that is the ecotourism region in Gunungkidul, Yogyakarta Province, Indonesia (Figure 1). This study uses continuous exploration visits (exploration and collection trip) (Singh 2010). The image is taken for the first step of identification. The sample specimens for the herbarium are also brought home with regard to the sustainability of the population. Along with the identification process, observation on inflorescence and fruit formation are carried out.

Equipments and materials

Equipment for observation and collection comprises: digital cameras of Sony NEX F3, Sony Cyber-Shot DSC-W180 and Canon DSLR, rulers, micrometers, calipers, roll meter, plastic collection, scissors, cutter, label paper, GPS (Global Positioning System), dried herbarium collecting equipment, bottles, stereo microscope Nikon SMZ 1500 equipped with a camera, Nikon light microscope equipped with Nikon Eclipse 50 DSF1. Specimen collection procedure implemented with dried herbarium method refers to Simpson (2006) and Singh (2010). Herbarium specimens deposited by the authors in the Universitas Islam Negeri Sunan Kalijaga, Yogyakarta, Indonesia (Herbarium Baturagung, BAW501II16A1).

Methods

Plant image at the site, dried herbarium, pictures of flowers/fruit, making of herbarium dried, shooting dried herbarium specimens, observation of the structure plant, are identified, checked and matched with herbarium catalogue of Royal Botanic Gardens, Kew (K), (http://www.kew.org/herbcat) (RBGK 2015), Muséum National d'Histoire Naturelle, Paris (France) Collection of Vascular Plants (P) (http://coldb.mnhn.fr/catalognumber/mnhn/p) (NMHN 2016), Backer and Bakhuizen van den Brink (1965), Steenis (1972), and Kochummen (1972).

RESULTS AND DISCUSSION

The observation and assessment of the initial specimen by the authors on exploration of wild plants in Mount Nglanggeran Yogyakarta since August 2009 found a kind of liana plants that climbing a rock. This plant is interesting because it has thick leaves like bark, obovate to elliptic leaf shape, unclear bone on secondary leaves, and face crossed leaf arrangement. These plants often grow together with *Hoya* sp. and leaf morphology of both plants has similarities. In exploration on February 2012, authors found flowering plants in the location S.07.50,319°; E.110.32,186°, 441 m. Later, on the April 2012, the plant had young fruit and on August 2012, the fruit was in a state of its maximum size.

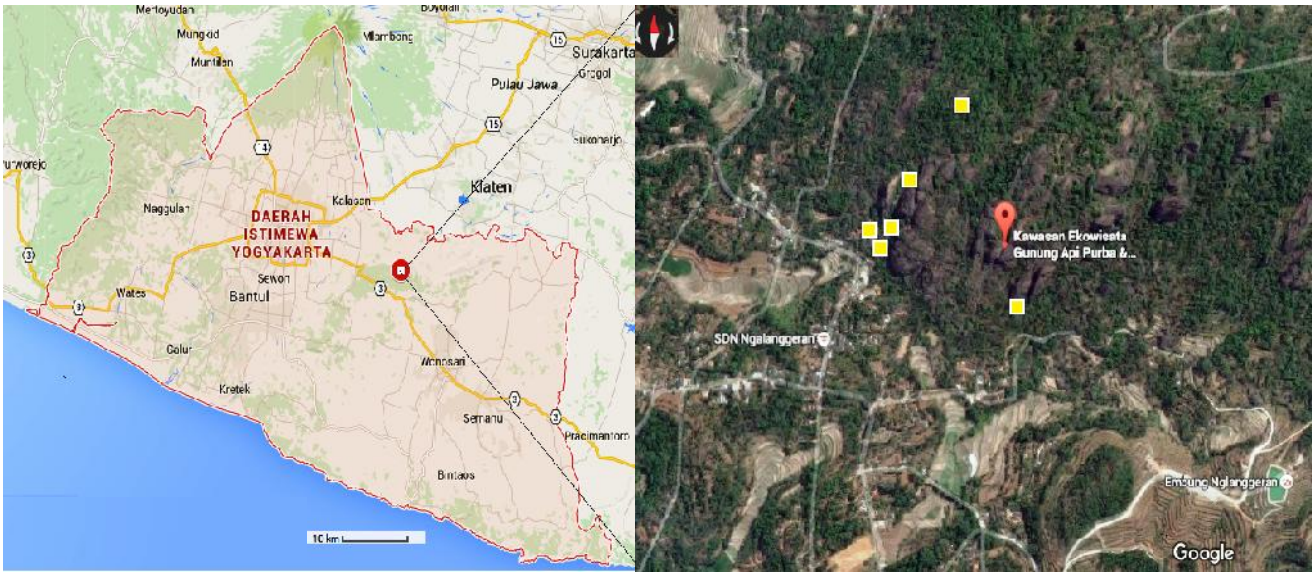

Figure 1. A. Yogyakarta province, B. The western part of Mount Nglanggeran in Gunungkidul (□ = *Fagraea ceilanica* location)

Identification using Backer and Bakhuizen van den Brink (1965) concluded that this plant is *Fagraea*. This *Fagraea* shows characteristics of *F. litoralis* or *F. obovata* or *F. ceilanica*. Figure 2 shows a specimen of *F. ceilanica* of Mount Nglanggeran. Description of *F. ceilanica* by Backer and Bakhuizen van den Brink (1965) are shown in Table 1.

Compared to descriptions by Backer and Bakhuizen van den Brink (1965), *F. litoralis* or *F. obovata* or *F. ceilanica* that is found in Mount Nglanggeran has a longer corolla tube sizes which is about 4-8 cm, while the smaller fruit size is about 3 cm long. According to Slik (2016), *F. ceilanica* can have variations in the size of 2-5 cm with the shape of crown tube, such as variation found in Sri Lanka, and of 8.5-10 cm, such as variation found in southwest Decan (India), the variation in length of 3-5 cm and in Assam (India) only 1.8 cm.

Herbarium specimens of Mount Nglanggeran *Fagraea* are shown in Figure 3A. Based on the information of Backer and Bakhuizen van den Brink (1965), herbarium of *Fagraea* from Mount Nglanggeran is identical to type of herbarium of *F. litoralis* or *F. obovata* or *F. ceilanica* (Figure 3.B, 3.C). Information from The Plant List (2013) and the Catalogue of Life (2016) was that *F. ceilanica* also

Table 1. Description of *Fagraea litoralis* Bl. or *Fagraea obovata* Wall or *Fagraea ceilanica* Thunb. (Backer and Bakhuizen 1965)

Part	Description
Character	Flower is much larger Nerver of leaves are obsolete
Leaves, petiolus	Leaves are elliptic to oblong, thick coriaceus, acute base, acuminate apex, 5-15 cm, 2-9 cm; ½-3 cm petiolus; axyllary which is small scale, rounded, appressed and against the twig,
Habitat, habitus	Ephyfitic, 3-15 m height, flowering on January-June, 0-1700 m asl of forest, forest edges, secondary forest, bushes, beaches, littoral cliff.
Flower, fruit	small Inflorescences, subsessile, few flower, campanulate calyx, 1-1 ¾ cm long, over ¼-1/2 cm, conate; corolla slender, thrumpet shape, 2 ½-5 cm tube, stamen and style which are not much exerted from the throat; ½-¾ cm anther, variate stigma, fruit ellipsoid to globose, 3-5 cm, sordidly white, lobes patens calyx.
Distribution in Java	West, Central and East Java

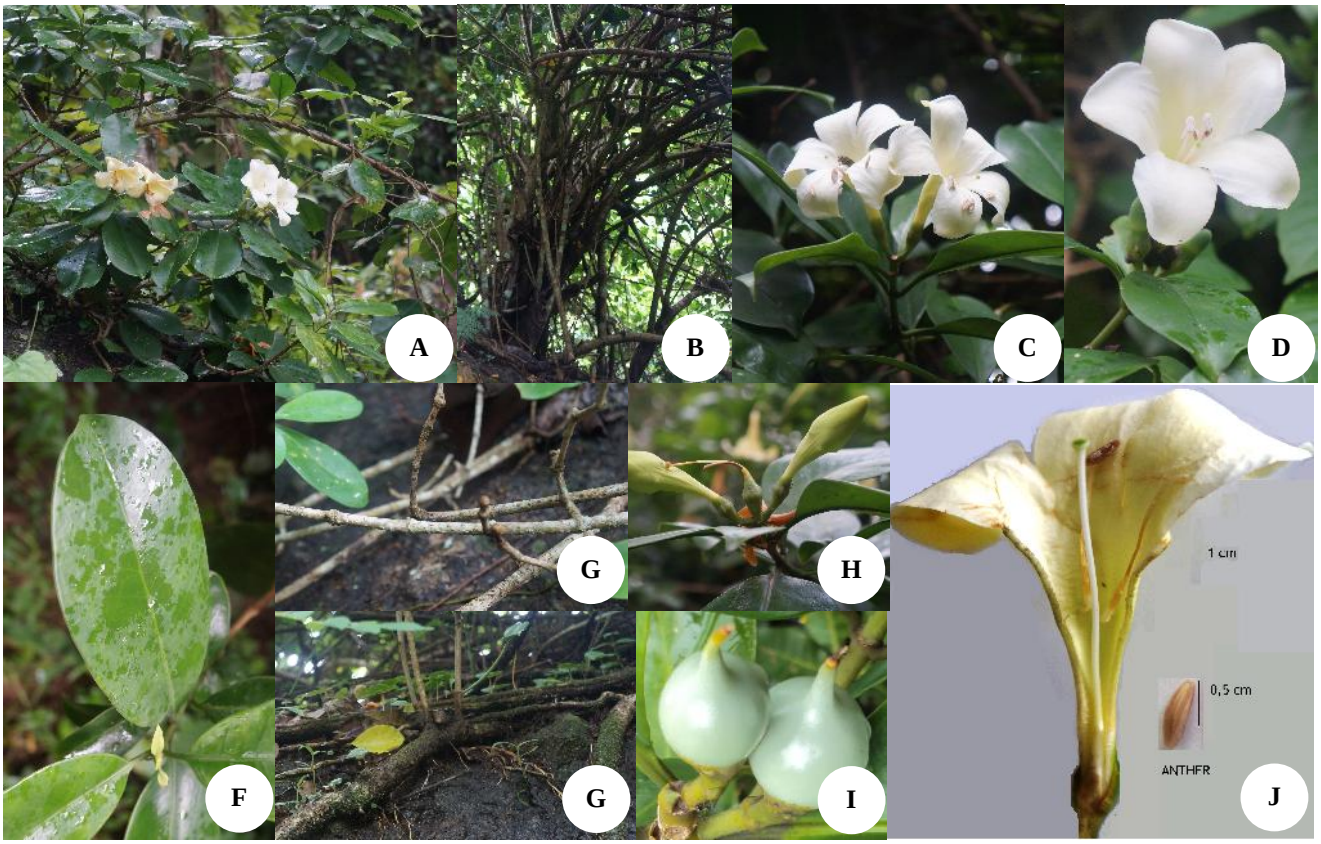

Figure 2. *Fagraea ceilanica* form Mount Nglanggeran, Yogyakarta, Indonesia. A. Habitus, B. Stem, C. Inflorescense, D. Flower, E. Leaf, F. Branch, G. Root, H. Cymose Inflorescense, I. Fruit, J. Section of flower

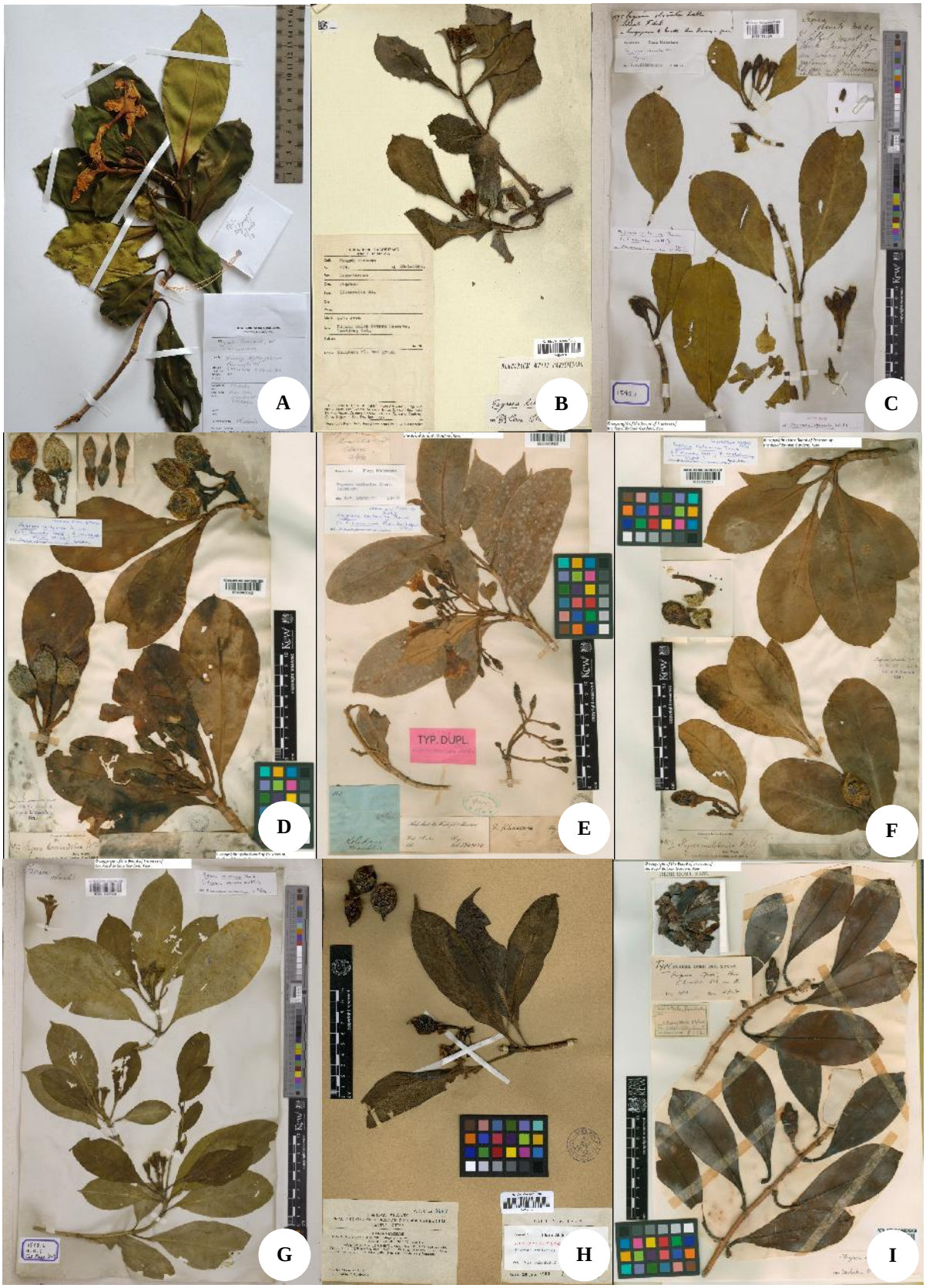

Figure 3. Comparison of herbarium of *Fagraea* of Mount Nglanggeran (A) with herbarium type *Fagraea ceilanica* (B, C, D, E, F. G, H, I). A. Herbarium *Fagraea* from Mount Nglanggeran (BAW501II16A1), author collection. B. *Fagraea litoralis* Bl., MNHN-BO (P0456462), C. *Fagraea obovata* revised to *Fagraea* ceilanica, KEW (K001113547), D. *Fagraea coromandelina* revised to *Fagraea ceilanica*, KEW (K000883562), E. *Fagraea khasiana* revised to *Fagraea* ceilanica, KEW (K000883560), F. *Fagraea malabarica* revised to *Fagraea ceilanica*, KEW (K000883561), G. *Fagraea obovata* revised to *Fagraea* ceilanica, KEW (K001113549), H. *Fagraea chinensis* revised to *Fagraea ceilanica*, MNHN (P00349420), I. *Fagraea lanceolata* revised to *Fagraea* ceilanica, KEW (K00438439).

Figure 4. Comparison photograph of the *Fagraea ceilanica* from Mount Nglanggeran (A, B, C, D) with ancient botanical illustration (D, E, F, G, H), A. Terminal inflorescence, B. Characteristic of flower buds, C. Corolla, D. Young fruits, E. *Fagraea ceilanica* (Curtis's Botanical Magazine, vol. 100 [ser. 3, vol. 30]: t. 6080 (1874) [W.H. Fitch], F. *Fagraea obovata* (Curtis's Botanical Magazine, vol. 72 [ser. 3, vol. 2]: t. 4205 (1846) [W.H. Fitch]), G. *Fagraea coromandelina* (Beddome, R.H., The flora sylvatica of southern India, vol. 2: t. 244 (1869-1874) [Govindoo]), H. *Fagraea malabarica* (Wight, R., Icones Plantarum Indiae Orientalis, vol. 4(2): t. 1317 (1846) [Govindoo]), I. *Fagrae litoralis* (Blume, C.L., Rumphia, vol. 2: t. 74 (1836)), J. *Fagraea lanceolata* (Blume, C.L., Rumphia, vol. 2: t. 77 (1836)).

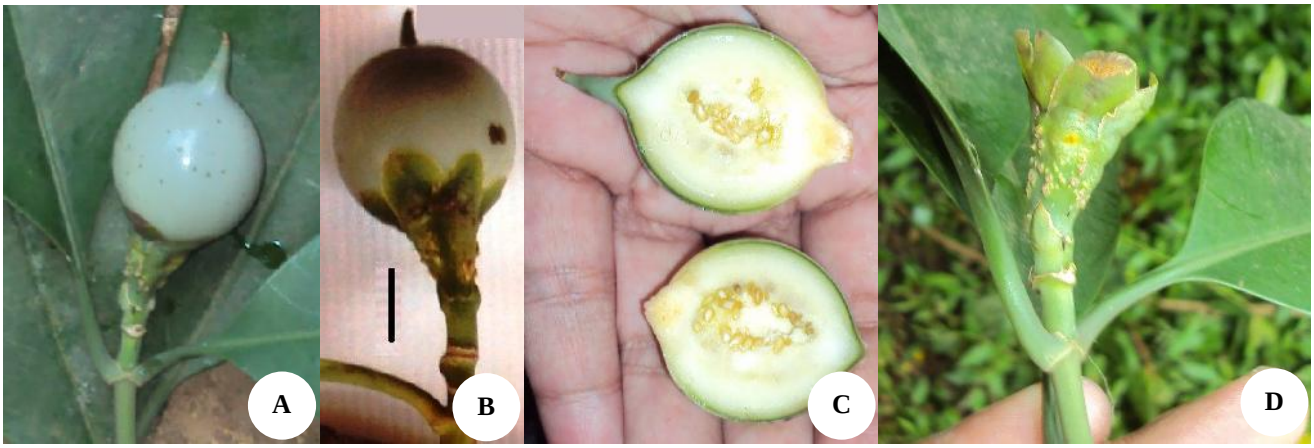

Figure 5. Fruit of *Fagraea* from Mount Nglanggeran. A. Fruit shape, B. Fruit section, C. Characteristic of fruit stalk

had similarity with *F. coromandelina*, *F. khasiana*, *F. malabarica*, *F. obovata*, *Fagraea chinensis* Merr. Herbaria which is shown in Figure 3. D, E, F, G, H, I. According to The Plant List (2013), *F. litoralis* is identical to *Fagraea lanceolata* Bl. Herbarium *F. lanceolata* from Singapore can be observed in Figure 2.I. However, synonymy *F. litoralis*, *F. obovata*, *F. coromandelina*, *F. khasiana*, *F. chinensis*, *F. malabarica* to *F. ceilanica* have a remarkable consequence. If all the synonyms belong to *F. ceilanica*, it is ignoring the wide variety of all synonyms that occur consistently all over the world.

From comparison with herbarium, it is concluded that type of Mount Nglanggeran *Fagraea* have similarity with herbarium *F. coromandelina*, especially in terms of flower size. *Fagraea*, herbarium of Mount Nglanggeran, have flowers with a relatively large size compared to other herbaria. It has cymose inflorescence with fewer flower units (3 or 5). Figure 4 shows a comparison photograph of the Mount Nglanggeran *Fagraea* specimen with ancient botanical illustration.

From the comparison, it appears that *Fagraea* of Mount Nglanggeran are more resemblance to the *Fagraea coromandelina* in term of flower buds and the inflorescences structure. Flower size of a Mount Nglanggeran *Fagraea* is relatively accordance with *F. ceilanica*, *F. obovata*, *F. coromandelina* and *F. malabarica*. Morphological structure of fruit and fruit color of Nglanggeran *Fagraea* (Figure 5) are equal to *F. litoralis* and *F. lanceolata*, *F. coromandelina* (Figure 4 I. J; Figure 3.G). Herbarium types of *F. ceilanica* in the herbarium center KEW and MNHN obtained from this research did not exist. Herbarium *F. ceilanica* is a reidentification of *F. obovata*, *F. khasiana*, *F. coromandelina*, *F. malabarica*, *F. litoralis*, *F. chinensis*, and *F. lanceolata*. Based on the characteristics of herbarium and *ancient illustrations* (Antheunisse 2016) on *Fagraea*, the author identifies *Fagraea* of Mount Nglanggeran as *F. ceilanica* ssp. *coromandelina*.

From the discussion above, the findings of *Fagraea* of the Mount Nglanggeran, need further examination to become the basis of re-identification of herbarium specimen types. There is a high diversity of traits among herbarium types of *F. ceilanica*. The name or identity of the species in the old manuscript illustration or early herbarium should be considered carefully to correct the identification of herbarium specimens. In the case of this *Fagraea*, early authors' illustrations and identification has higher accuracy rate than the present existing identification. Herbarium is an important base in identifying and determining the identity of the plant.

Taxonomic information

Fagraea ceilanica Thunb., Kongl. Vetensk. Acad. His Handl. 132. 1782; *Fagraea coromandelina*. Type: Indian Peninsular, 1812. Wight. KEW K0005883562.

Description

Climber or small tree, epiphytic shrub. Simple, opposite, decussate leaves which are clustered at end of branchlets, elliptic to oblong, thick-coriaceous, acute base, acute and acuminate apex, 5-15 cm by 2-9 cm; ½-3 cm petiole, obsolete nerves, raised midrib; secondary nerves obscurely visible, tertiary and higher order nerves not visible; small scale, rounded, appressed against twig, subconnate axillary. Inflorescences, terminal cymes, subsessile, few flower, 3-5; large flowers, campanulate calyx, 1-1 ½ cm long, connate, slender corolla, trumpet shape; 3-5 cm tube; yellow outside and white inside petals; stamen and style are not much exerted from the throat; ½-¾ cm anther; green stigma; ellipsoid to globose fruit, 3-4 cm, sordidly white green, lobes patent calyx.

Note

Fagraea ceilanica in Mount Nglanggeran are flowering on February-March.

Conservation

The existence of this plant at the site is threatened by human activity due to the increased interest in the site as a tourist destination. It is necessary to the provision of adequate information to the community about the position this plant in local ecosystems.

In conclusion, *F. ceilanica* are found on Mount Nglanggeran Yogyakarta. Morphological characteristics stature (habitus), leaves, twigs, flowers of *F. ceilanica* from Mount Nglanggeran show similarity to *Fagraea coromandelina* collection of Wight (1812) from India (KEW K0005883562). The existence *F. ceilanica* on Mount Nglanggeran complements and recovers the description of Backer and Bakhuizen van den Brink (1965) about *F. ceilanica*.

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Short Communication: Evaluation of quantitative and qualitative morphological characters of sunflower (*Helianthus annuus*) germplasm

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Abstract. *Purwati RD, Herwati A. 2016. Evaluation of quantitative and qualitative morphological characters of sunflower (*Helianthus annuus*) germplasm. Biodiversitas 17: 461-465.* Sunflower (*Helianthus annuus* L.) germplasm collection in ISFCRI was characterized aiming to distinguish the morphological characters of each genotype. Based on that information it would be possible to observe the diversity and to choose appropriate parent genotypes for successful hybridization. The investigation was carried out in the Pasirian Experimental Station, Lumajang District, East Java, Indonesia located at 110 m above the sea level (113° E, 8° S) in the 2015 growing season. Thirty-three germplasm accessions were characterized. Each accession was planted in 32 m² plot size with four lines. Fertilizer dose was 75 kg Nitrogen + 30 kg P_2O_5 + 30 kg K_2O per ha. The results showed that the low variation value in some quantitative characters such as seed size, weight of 100 seeds, seeds thickness, plant height, leaf size, ray floret length, bract length, head diameter, the flowering time, and seed oil content. The qualitative characters exhibited high coefficient of variation values with only one exception-pollen formation in sunflower inflorescences. These results indicated that on the base of their qualitative morphological characters, sunflower accessions possessed high diversity. The seed size and seed thickness showed significant positive correlation with 100 seeds weight. These two characters might be used as selection criteria in sunflower breeding programs for appropriate screening of parental genotypes included in hybridizing process aiming the increase of plant productivity.

Key words: Characters, diversity, quantitative, qualitative, sunflower

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an important oil seed crop that belongs to family Asteracae (Compositae) originated from North America (Bukhsh et al. 2011). Domesticated (cultivated) sunflowers have a single stalk topped by a large flower (inflorescences). The wild sunflowers from the genus Helianthus are branched annuals and perennials species with different ploidy level (Aboki et al. 2012). Sunflower plants have great potential and have been utilized for different purposes. In Malaysia, a research of biodiesel production from waste sunflower cooking oil and pure sunflower cooking oil had been done (Hossain and Boyce 2009). Meanwhile smallholder farmers in Nigeria have been using sunflower for animal feed, seed oil extraction, snack production, manure or fertilizer, ornamental, and traditional medicine (Torimiro et al. 2014). Recently sunflower oil was evaluated for anti-microbial properties on different pathogenic organisms (Aboki et al. 2012). Sunflower also appeared as an economically important crop in Pakistan due to its significant portion in vegetable oil production (Nasim et al. 2012).

In Indonesia, sunflower has been studied since 1970 but in the beginning it was known only as ornamental plant. Recently, many farmers and stakeholders are interested in developing this crop due to their usefulness. Most people cultured sunflower for both human consumption and as a raw material for the processing industry. But, the development of sunflower was faced in several constraints mainly limited varieties appropriated for Indonesia. Indonesian Sweetener and Fibre Crops Research Institute (ISFCRI), Malang, East Java, Indonesia is a research institue which have mandate to carry out the experiments of this crop and have started in sunflower breeding aiming the development of some high yield varieties.

The success of a breeding program depends on the variability of the initial materials. Selection of parents is the most important stage in any breeding program to develop new varieties having desirable trait. For instance, the most important goal of sunflower breeding in Croatia is increasing of oil yield (Mijic et al. 2009). Study on genetic variability of germplasm collection was very important activity in identification different genotypes (Siddiqi et al. 2012). Characterization of the existing collection by phenotype is essential for the breeders to identify different genotype. The evaluation of sunflower referred to Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability. The International Union for The Protection of New Varieties of Plants (UPOV 2000) and Germplasm database of The International Plant Genetic Resources Institute (IPGRI 2005). This study was conducted to evaluate the sunflower germplasm collection on the basis on quantitative and qualitative morphological characters.

MATERIALS AND METHODS

Study area

The field study was performed in Pasirian Experimental Station, Lumajang District, East Java, Indonesia located at 110 m above the sea level (113°E, 8°S). The annual rainfall was 1.700 mm with 120 rainy days per year (C type climate according to the classification of Schmidt Ferguson). The soil type was sandy loam clay (0-15 cm, top soil) as classified of entisol/regosol.

Procedures

The research was conducted from March to December in the 2015 growing season. Thirty-three accessions, each one of 32 m² plot size were included in the study (Table 1). In this study, some accessions from abroad were included due to the limited local accessions. Fertilizer was applied three times as follow: 75 kg Nitrogen + 30 kg P_2O_5 + 30 kg K_2O h^{-1} (full dose) on sowing date, 1/3 dose of N on 14 days after planting (DAP), and 2/3 dose of N -on 30 DAP.

The quantitative characters observed were: leaf size, plant height at full flowering, time of flowering (50% of the plants are in flower), ray floret length, bract length of tip, head diameter, seed size, seed thickness, weight of 100 seeds, and seed oil content. Seed oil content was measured by soxhlet method (Akpan et al. 2006) and calculated on dry matter (D.M.) basis. Leaf size was measured using modified gravimetric method (Chaudhary et al. 2012)

The qualitative characters measured were: absent or present hypocotyls anthocyanin coloration, hypocotyls intensity of anthocyanin coloration, leaf green color, leaf blistering, leaf serration, leaf shape of cross section, leaf shape of distal part, leaf auricles, leaf wings, leaf angle of lowest lateral veins, leaf height of the tip of the blade compared to insertion of petiole (at 2/3 height of plants), stem hairiness at the top (last 5 cm). Also the characteristic of inflorescence were monitored such as ray florets density, shape, floret disposition, and color, disk flower color, absent or present of anthocyanin coloration of stigma, intensity of anthocyanin coloration of stigma, production of pollen, bract shape, bract green color of outer side, bract attitude in relation to head, plant branching (excluding environmental branching), type of branching, plant natural position of highest lateral head to the central head, head attitude, head shape of grain side, seed shape, seed main color, seed stripes on margin, seed stripes between margins, seed color of stripes, and seed spots on pericarp. All characters were measured according to "Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability" of sunflower (*H. annuus*) (UPOV 2000) and Germplasm data base (IPGRI 2005). In each accession, ten plants in the center of population were observed as samples.

Data analysis

Data analysis of characters observed was conducted using average $\pm$ standard deviation. The correlation between each character was analyzed using Pearson correlation.

Table 1. The sunflower accessions included in the study

Accessions	Origin
Ha.1	Pati, Central Java, Indonesia
Ha.2	Australia
Ha.3	Pati, Central Java, Indonesia
Ha.4	Netherland
Ha.5	Indonesia
Ha.6	Australia
Ha.7	Australia
Ha.10	Waingapu, NTT, Indonesia
Ha.12	NTB, Indonesia
Ha.14	Malang, East Java, Indonesia
Ha.15	Pati, Central Java, Indonesia
Ha.16	Malang, East Java, Indonesia
Ha.17	Malang, East Java, Indonesia
Ha.18	Malang, East Java, Indonesia
Ha.19	Indonesia
Ha.21	Indonesia
Ha.23	Indonesia
Ha.25	Australia
Ha.29	Malang, East Java, Indonesia
Ha.34	Pekanbaru, Riau, Indonesia
Ha.39	Pekanbaru, Riau, Indonesia
Ha.41	Pekanbaru, Riau, Indonesia
Ha.42	Pekanbaru, Riau, Indonesia
Ha.43	Pekanbaru, Riau, Indonesia
Ha.44	Pekanbaru, Riau, Indonesia
Ha.52	Turkey
Ha.54	Turkey
Ha.56	Turkey
Ha.58	Turkey
Ha.60	Turkey
Ha.62	Turkey
Ha.65	Turkey
Ha.70	Turkey

RESULTS AND DISCUSSION

All investigated quantitative characters showed low differences between accessions, thus indicating the low diversity of germplasm investigated (Table 2). This result was different from Terzić et al. (2006) who obtained highly variability in plant height and type of branching of F1 generation. Encheva et al. (2008) also observed significant differences in plant height of various sunflower lines and hybrids. Onemli and Gucer (2010) found significant differences in plant height, head diameter, and period of flowering of sunflower wild genotypes. Highly significant differences was also reported in leaf number, plant height, days to flowering and days to maturity in sunflower by Siddiqi et al. (2012). It might be concluded that the results obtained in the current study reflect the origin of germlasm accessions which evidently have close genetic background. The above mentioned researchers used wild species, parental lines and their hybrids. It is well known that wild species and hybrid genotypes normally have highly genetic diversity.

Time of flowering character was important for selection of early maturity accessions. Accessions with time of flowering less than 60 days after planting indicated as early maturity accessions. In this study, 19 accessions were identified as early maturity (Table 2.). These accessions

were potential as parental in sunflower hybridization to produce new high yield early maturity varieties.

From Table 2. could be identified three accessions as short type plants with plant height less than 150 cm. Accessions with short type had some superiority e.g. the plants were not easy damage by wind flow and easier to harvest. There were also found that four accessions produced 100 seed weight more than 16 g, and nine accessions had high oil content (> 55%). Accessions which have high seed weight and oil content are categorized as potential accessions because seed weight is one of yield components (Dehkhoda et al. 2013; Rafiei et al. 2013; Ion et al. 2015).

Coefficient of variation (CV) was used to measure genetic variability of sunflower genotypes. The low genetic variability (2.60-12.06%) was observed for quantitative characters of all accessions (Table 3). According to Hadi et al. (2014), the genetic variability is low when CV varied from 0 to 25%. Similar results were reported by Sudrik et al. (2014) sunflower germplasm characterization showed that no accession was found to be promising for all quantitative characters.

The results indicated that sunflower accessions have higher variability based on the qualitative morphological characters. All investigated qualitative traits exhibited differences between accessions except formation of pollen (Table 4). The results were in conformity to previous report of Makane et al. (2011), characterization of sunflower germplasm indicated wide variation for all the qualitative characters among accessions. Tan and Tan (2011) also reported that the morphological variation on the observed characters was found highly variable for some characters. There was no variation of pollen fertility. All accessions released the fertile pollen. Diederichsen (2010) found the variability of qualitative characters i.e. leaf dimensions and leaf margin serration in wild species (*Helianthus tuberosus* L). These two characters could be used to distinguish extreme genotypes.

The diversity of qualitative characters between the different lines was also investigated by Khoufi et al. (2013) on 73 adapted sunflowers and 7 hybrids. Shamshad et al. (2014) analyzed 31 lines of sunflower germplasm and obtained a lot of diversity between these lines which can be exploited in hybrids breeding program. Meanwhile Presotto et al. (2009) reported that the populations of *H. annuus* naturalized in Argentina presented a high degree of phenotypic variability.

Table 3. Variability of quantitative morphological characters of sunflowers germplasm

Quantitative characters	Initial	Average ± SD	CV (%)
Leaf size (cm²)	LS	332.25 ± 91.01	8.85
Plant height (cm)	PH	191.60 ± 39.47	9.62
Time of flowering (day)	TF	64.43 ± 12.63	4.37
Ray floret length (cm)	RF	6.72 ± 0.81	7.32
Bract length of tip (cm)	BL	3.37 ± 0.72	12.06
Head diameter (cm)	HS	18.15 ± 4.76	6.87
Seed size (mm²)	SS	105.08 ± 33.25	3.51
Weight of 100 seeds (g)	WS	11.08 ± 3.82	2.60
Seed thickness (mm)	ST	4.07 ± 0.58	4.82

Table 4. Variability of some qualitative morphological characters of sunflower germplasms

Characteristics	Expression	Percentage (%)
Hypocotyl: anthocyanin coloration	Absent	81.82
	Present	18.18
Leaf: size	Small	12.12
	Medium	84.85
	Large	3.03
Leaf: green color	Light	10.00
	Medium	60.61
	Dark	30.30
Stem: hairiness at the top (last 5 cm)	Weak	24.24
	Medium	51.52
	Strong	15.15
	Very strong	9.09
Time of flowering	Early	60.60
	Medium	18.18
	Late	6.06
	Very late	15.15
Ray floret: shape	Fusiform	51.52
	Narrow ovate	48.49
	Broad ovate	0
	Rounded	0
Ray floret: color	Light yellow	12.12
	Orange yellow	39.39
	Orange	48.49
Disk flower: color	Yellow	45.46
	Orange	24.24
	Purple	30.30
Disk flower: production of pollen	Absent	0.00
	Present	100
Bract: length of tip	Short	6.06
	Medium	21.21
	Long	54.55
	Very long	18.18
Plant: natural height	Very short	12.12
	Short	51.51
	Medium	27.27
	Tall	9.09
Plant: type of branching	Predominantly basal	9.09
	Overall	18.18
	Predominantly apical	9.09
	Only one	54.54
Head: size	Small	30.30
	Medium	60.61
	Large	6.06
Head: shape of grain side	Weakly concave	18.18
	Flat	69.70
	Weakly convex	6.06
	Strongly convex	3.03
	Deformed	3.03
	Elongated	15.15
Seed: shape	Narrow ovoid	45.46
	Broad ovoid	36.36
	Rounded	3.03
Seed: main color	White	9.09
	Whitish grey	6.06
	Grey	18.18
	Light brown	6.06
	Medium brown	12.12
	Dark brown	1818
	Black	30.30

Table 2. Quantitative characters of sunflower accessions

Accession	Leaf size (cm^2)	Time of flowering (day)	Ray floret length (cm)	Bract length of tip (cm)	Plant height (cm)	Head diameter (cm)	Seed size (mm^2)	Seed thickness (mm)	100 seed weight (g)	Oil content (%)
Ha.1	536.3±97.0	96.8±5.7	7.2±0.2	2.4±0.2	242±39.3	23.3±3.3	75.2±6.1	3.4±0.5	7.2±0.2	48.17
Ha.2	349.5±83.8	61.5± 4.5	7.5±0.9	3.5±0.5	176±29.1	20.2±4.3	126.7± 7.9	4.2±0.3	13.8±0.6	58.81
Ha.3	312.0±35.0	90.2±4.2	6.6±0.3	1.7±0.4	252±11.1	17.5±4.5	96.2±8.3	3.6±0.4	9.7±0.2	51.98
Ha.4	333.3±40.8	59.4± 6.9	6.5±0.6	3.2±0.8	168±29.1	21.7±1.7	116.9± 9.9	4.0±0.5	13.9±0.4	49.99
Ha.5	418.0±46.7	56.4±2.7	6.7±0.8	3.1±0.6	185±18.1	9.2±2.2	149.9±12.7	5.5±0.7	14.6±1.1	48.02
Ha.6	277.5±39.9	65.9±4.1	7.4±0.9	3.7±0.6	219±33.7	23.0±4.2	73.3±7.7	3.2±0.3	7.2±0.3	57.33
Ha.7	271.0±12.9	55.4±5.3	5.8±0.8	3.5±0.6	125±26.7	11.4±1.9	53.6±9.4	2.9±0.4	3.9±0.1	62.68
Ha.10	402.3±30.1	62.9±5.8	6.7±0.7	3.7±0.9	195±32.4	21.2±2.4	118.8±21.2	4.2±0.7	17.5±0.6	50.20
Ha.12	336.0±50.4	56.0±5.8	7.1±0.9	3.5±0.5	137±25.8	23.4±3.3	99.4±13.6	4.2±0.5	11.7±0.4	44.11
Ha.14	255.3±48.7	51.9±4.9	6.9±1.0	3.8±0.9	117±16.6	16.0±5.8	157.3±13.8	5.5±0.8	16.4±1.3	44.50
Ha.15	434.5±63.6	85.5±3.8	6.5±0.7	4.1±0.3	172±11.0	13.7±3.8	50.4±3.9	3.2±0.4	4.1±0.1	60.13
Ha.16	516.6±47.2	61.9±6.4	7.2±0.8	3.6±0.7	219±33.5	11.0±1.8	63.1± 6.7	3.5±0.4	7.8±0.4	56.91
Ha.17	316.3±34.1	59.7±4.5	7.2±1.1	3.3±0.4	214±38.4	20.7±2.7	124.2±12	3.5±0.4	12.7±0.6	47.87
Ha.18	347.5±5.0	74.4±8.3	7.3±1.1	4.2±0.8	242±39.3	11.8±2.4	79.4±7.1	4.2±0.6	9.2±0.2	57.97
Ha.19	332.5±44.0	57.2±4.4	6.7±0.9	4.4±0.9	184±35.0	17.8±3.4	152.7±16.2	4.6±0.4	16.7±0.6	51.38
Ha.21	229.8±39.4	54.0±3.8	5.7±1.5	2.4±0.8	127±41.2	13.4±1.5	105.4±7.5	4.3±0.6	13.2±0.3	40.00
Ha.23	319.5±8.0	57.2±2.9	5.3±1.1	2.5±0.8	154±15.9	11.1±0.9	84.9±4	3.6±0.4	13.7±0.4	56.75
Ha.25	347.5±1.0	85.0±3.8	7.2±0.8	3.7±0.4	254±37.2	13.2±2.0	71.1±5.1	3.6±0.5	7.5±0.7	53.01
Ha.29	383.3±44.2	61.3±7.3	7.0±1.1	3.9±0.6	232±22.0	20.9±1.4	97.8± 8.6	3.7±0.5	9.8±0.2	56.00
Ha.34	327.0±39.0	58.4±4.1	6.3±0.9	3.4±0.7	172±23.3	17.4±2.8	95.0± 7.2	3.9±0.7	5.9±0.8	55.81
Ha.39	305.5±26.5	54.8±4.6	7.0±1.0	3.5±0.7	175±23.3	17.6±2.5	139.7±16.8	4.4±0.5	15.0±0.2	48.09
Ha.41	327.0±25.2	55.6±3.6	6.1±1.0	3.0±0.7	173±14.4	9.1±1.3	118.6±15.6	3.9±0.6	11.7±0.6	41.71
Ha.42	316.0±25.2	55.0±7.6	8.4±1.5	3.3±0.6	190±44.0	23.8±2.0	140.5±7.9	4.2±0.6	14.2±0.4	49.62
Ha.43	319.8±34.0	58.3±4.4	7.1±1.1	3.9±0.9	204±22.4	22.2±1.2	184.7±20.2	5.0±0.7	16.7±0.3	44.95
Ha.44	311.8±49.4	84.1±5.3	8.4±0.5	5.0±0.4	224±46.9	17.7±1.8	129.2±8.2	4.9±0.6	15.1±0.8	38.20
Ha.52	299.3±37.3	54.8±2.9	6.5±1.2	3.1±0.6	175±22.0	22.1±2.7	81.1±31.4	4.3±0.6	7.1±0.4	45.31
Ha.54	237.8±60.5	56.4±2.1	6.8±1.2	3.1±0.9	180±31.2	24.0±1.9	79.1±14.9	4.2±0.7	7.6±0.5	50.00
Ha.56	319.3±45.1	74.1±7.6	7.3±0.9	5.0±1.1	260±21.2	17.4±4.2	90.1±6.1	4.4±0.5	10.6±0.2	45.87
Ha.58	304.3±13.9	60.2±4.4	6.8±0.9	3.3±0.8	198±26.5	21.4±4.2	132.1±11.6	4.4±0.5	13.9±0.6	46.16
Ha.60	254.3±23.3	58.1±3.3	5.4±1.2	3.3±0.5	152±16.7	22.5±3.2	72.5±7.3	4.3±0.3	7.3±0.3	46.22
Ha.62	320.3±30.9	56.0±2.4	7.3±1.0	2.7±0.5	180±27.5	24.3±4.8	139.8±18.6	4.1±0.7	12.6±0.5	41.91
Ha.65	291.5±35.4	59.2±3.2	6.7±0.9	3.0±0.9	169±17.8	20.7±2.6	109.7±15.3	4.0±0.3	11.9±0.4	49.55
Ha.70	164.3±9.0	89.2±3.1	5.8±0.3	2.0±0.4	239±13.6	18.2±1.2	70.0±6.3	3.7±0.4	7.7±0.3	41.03

Table 5. Correlation coefficients of quantitative morphological characters of sunflower genotypes

Characters		HS	BL	RL	LS	PH	ST	WS	SS
Time of flowering (day)	TF	-0.058	-0.052	0.041	0.233	0.724**	-0.362*	-0.365*	-0.428**
Head diameter (cm)	HS		-0.037	0.352*	-0.101	0.074	0.088	0.142	0.189
Bract length of tip (cm)	BL			0.321	0.013	0.111	0.308	0.145	0.120
Ray floret length (cm)	RL				0.260	0.443**	0.335	-0.399*	0.415*
Leaf size (cm^2)	LS					0.341*	-0.174	-0.051	-0.079
Plant height (cm)	PH						-0.199	-0.156	-0.181
Seed thickness (mm)	ST							0.769**	0.808**
Weight of 100 seeds (g)	WS								0.904**
Seed size (mm^2)	SS								

Note: * significant at $p \leq 0.05$, ** significant at $p \leq 0.01$

Tan and Tan (2010) reported that sunflower landraces have significant diversity in Turkey as one of the micro-gene centers for sunflower. Since some accessions evaluated in this experiment were also originated from Turkey, it was found the significant diversity of qualitative characters. According to Vear et al. (2011), the phenotypic and genotypic differentiation between accessions must be managed in the best way since it was very useful as resources in breeding.

Correlation coefficients are useful since it allow to determine the component character on which selection can be based, thus improving seed yield (Jockovic et al. 2012). In the current study, plant height showed negative correlation with weight of 100 seed and seed size (Table 5). These results are similar to Arshad et al. (2007) who found that association between plant height and seed yield was negative at both genotypic and phenotypic levels. Also, plant height was highly significant and positively

Table 6. Promising accessions of sunflower for different characters

Characters	Germplasm accessions
Time to flowering (< 60 days)	Ha.4, Ha.5, Ha.7, Ha.12, Ha.14, Ha.17, Ha.19, Ha.21, Ha.23, Ha.34, Ha.39, Ha.41, Ha.42, Ha.43, Ha.52, Ha.54, Ha.60, Ha.62, Ha.65
Plant height (<150 cm)	Ha.7, Ha.12, Ha.21
100-seed weight (>16 g)	Ha.10, Ha.14, Ha.19, Ha.43
Seed size (≥ 141 mm2)	Ha.5, Ha.14, Ha.19, Ha.43
Seed thickness (≥ 4.6 mm)	Ha.5, Ha.14, Ha.19, Ha.43, Ha.44
Seed oil content (>55%)	Ha.2, Ha.6, Ha.7, Ha.15, Ha.16, Ha.18, Ha.23, Ha.29, Ha.34.

correlated with days to flowering. This result was in agreement with finding of Jockovic et al. (2012) who reported a significant and positive correlation between plant height and days to flowering. The longer flowering of plant will have more time to grow. On the other hand, plant height has negative correlation with 100 seed weight which corresponded to the yield.

Seed size and seed thickness have highly significant and positive correlation with weight of 100 seeds. Based on this result, seed size and seed thickness showed the highest positive effect on seed yield. These two characters could be used as selection criteria in sunflower breeding programs for screening the parental genotypes for development of sunflower cultivars with higher productivity.

Based on quantitative characters, it can be concluded that some accessions were identified as promising for different characters (Table 6.). These accessions can be used to generate a gene pool by constituting the germplasm lines of interest or by creating a broad based cross. Such accessions were very useful as a base population to develop promising populations and lines.

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Dendrochronology of young *Swietenia macrophylla* and the variation of its growth response to the past wet climate in Bengkulu, Indonesia

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Abstract. *Susatya A, Yansen. 2016. Dendrochronology of young* Swietenia macrophylla *and the variation of its growth response to the past wet climate in Bengkulu, Indonesia. Biodiversitas 17: 466-472.* Dendrochronology had long been studied in temperate regions to know tree growth responses to the past climate, and to predict the future effects of climate change. In the wet tropics, dendrochronology studies were rarely carried out because of the lack of distinct annual growth rings or wide variation of growth ring forms. Our research was aimed to know the variations of width of growth rings, and growth response of young Big-Leaf Mahogany (*Swietenia macrophylla*) to the past wet climate in Bengkulu, Indonesia. Wood disc specimens of cross sections were collected from seven different mahogany trees from campus forest, University of Bengkulu, and then were dried, sanded, and digitally photographed. Growth rings were measured to the nearest 0.001 cm with ImageJ software. The annual ring width data were cross-dated visually by synchronizing and aligning the width patterns of all wood specimens. The results showed that the average of annual growth rings varied from 0.679 cm/year to 1.047 cm/year, and was not significantly different among trees. The width of growth ring of Big-Leaf Mahogany trees demonstrated periodicity through ages, and increased until 9 years old and then started to decline. Individual tree responded differently to climate through out the ages. In the stand level, the average annual growth ring was very sensitive to climate, and positively correlated to rainfall in the first six years, but was independent to rainfall in the past five years. It was speculated that local environments and ecological processes were attributed to obscure the influence of rainfall to the annual growth ring of the older stand.

Keywords: Annual growth ring, cross date, dendrochronology, Indonesia, tropics

INTRODUCTION

Mitigation and adaptation for climate change rely on the health of ecosystems. In many aspects, species compositions of forests will determine the end result of mitigation, because the species will control the total carbon sequestered and balance to the ecosystems. Carbon sequestration capability can be reflected by the variation of annual growth rings of trees (Fritts and Swetnam 1989; Sesler 2009). These growth rings are results from the distinct differences between cell division of vascular tissues at growing and dormant seasons. The sequence of annual growth rings within trees is then studied by dendrochronology (Norton and Ogden 1987). The core of dendrochronology is cross dating, which is referred as a method to conduct comparisons and synchronizations the similarity of the width patterns of the annual growth ring of the different trees, and then to relate the patterns to ages (Fritts and Swetnam 1989; Laroque 1995). This method is based on the assumption that climate will similarly affect the growth of all trees in a given area, and therefore, the trees will produce similar patterns of the growth rings (Norton and Ogden 1987; Boninsegna et al. 2009). Dendrochronology is widely used to understand the relationship between radial growth and past environment, past climate and hydrological regimes (Fritts and Swetnam 1989; Fritts and Dean 1992), forest community changes through time (Guindon and Kit 2012), and species' responses to climate change (Ettl 1994; Guindon and Kit 2012). Dendrochronology studies also allow reconstruction of past climates (Martinelli 2004; Sano et al. 2008; Boninsegna et al. 2009; D'Arrigo et al. 2011; Pumijumnong 2012b), and estimations for carbon sequestration (Bascrietto et al. 2004; Martinelli 2004). For example, Fritts and Dean (1992) analyzed wood cores of Pines to estimate past climates between the years of 900 to 1200, and summarized that the very dry spring and summer of mid-1100 caused the distinctive variation of growth rings in Southwest USA. A similar pattern was also reported by Lopez et al. (2012), where the presence of annual rings in seven tree species of tropical moist forests was the result of dry months. A strong correlation between past climates with growth rings was also shown by *Arbutus menziesii* (Ettl 1994), teak, *Tectona grandis* (Stahle 1999; Worbes 2002), and *Nothofagus pumilio* and *Polylepis tarapacana* (Boninsegna et al. 2009). However, climate was not the only factor influencing the variation of growth rings. The ring width variation within a single tree of *Pinus merkusii* and *P. kesiya* was more influenced by local soil moisture than by rainfall as well as temperature (Pumijumnong and Wanyaphet 2006). The ring widths of *Abies lasiocarpa* were also known to have a weak correlation with regional climate, but to perform a strong sensitive to microclimate (Guindon and Kit 2012).

In the contrary to temperate regions, where dendrochronology has been extensively studied (Norton and Ogdnen 1987; Fritts and Dean 1992; Martinelli 2004; Guindon and Kit 2012), dendrochronology has been practiced less in the tropics, even though some early works in the tropics can be traced back to the early 1900s, when Dutch scientists carried out a study in Java, Indonesia (Worbes 2002). Because of increasing role of forest in mitigating effects of climate change and of the need for information of the relationship between tree growth and climate, dendrochronology has been growingly conducted in various tropical regions such as South America (Worbes 2002; Boninsegna et al. 2009), Tibet (Liang and Eckstien 2009), Ethiopia (Wils et al. 2010), and Eastern Guatemala (Sigal 2011). In South East Asia region, dendrochronology and its various techniques were also gaining attention (Pumijumnong 2012a). For example, D'Arrigo et al. (2006) used wood rings data from 9 living teak to understand drought monsoon variation in the past two centuries in Java Indonesia. Sono et al. (2008) utilized the growth rings of *Fokienia hodginsii* to reconstruct the eighteenth century climate in Northern Vietnam. D'Arrigo et al. (2011) used growth rings from 20 living teaks to study the past three centuries monsoon variability in Myanmar. Furthermore, Pumijumnong and Wanyaphet (2006) and Pumijumnong (2012b) also explored Teak and two species of Pinus to study past climate in Northern Thailand. Unfortunately, dendrochronology in South East Asia was limitedly conducted based on data from very few tree species. Pumijumnong (2012a) listed only 4 tree species consisting of *Pinus merkusii, P. kesiya, Tectona grandis,* and *Fokienia hodginsii* that showed responsive to past climate. In such a high tree species diversity of Sumatra rain forest (Susatya 2010), information on the response of growth of the other species to climate is necessary to gain a better understand the role past and future climates to the ecosystem.

In the wet tropics, people reluctantly explored dendrochronology to study past climatic and ecological events related to annual growth rings. This was partly caused by reasons that the growth rings of trees were not distinctively formed, the annual variation of climate was not strong enough to form distinct rings in the wet tropics (Lopez et al. 2012), and that false, incomplete, poorly defined or multiple rings were abundant (Wils et al. 2010; Azim and Okada 2014). However, Baguinon et al. (2008) found interesting results concerning to the distinctiveness of growth rings of Southeast Asia trees. Baguinon et al. (2008) conducted a comprehensive study to determine growth rings of trees from various forest formations including wet tropical forests from Sri Lanka, India, Thailand, Malaysia, and The Philippines. They reported that of 424 tree species, ninety-eight (23%) showed distinct rings, while the rest had either indistinct or missing rings. A similar study was also conducted in Peninsular Malaysia by Azim and Okada (2014). They reported that among 29 trees observed, only two tree species showed distinct growth rings, the other trees had either absence or poorly defined growth rings.

Big-leaf Mahogany, *Swietenia macrophylla* King, occurs through out the tropics of Asia, Central and South America, and is able to thrive in many soil types from very poor to well-drained and fertile in dry as well as wet tropical regions (Krisnawati et al. 2011). Distinct growth rings have been reported for this species (Baguinon et al. 2008), and therefore can be explored as a model to determine the various influence of the past climate to growth of species in the wet tropics. The objective of this research was to know the variation of the width of tree rings, and the growth response of young Big-Leaf Mahogany to climate and rainfall in the wet climate of Bengkulu, Sumatra, Indonesia.

MATERIALS AND METHODS

The study area was located in the campus forest of the University of Bengkulu in Bengkulu, Indonesia (3°45'30"S -102°16'22"E). The forest was a remnant of young secondary lowland rain forest, with its main tree species consisted of *Cinnamomum iners, Alstonia scholaris, Rhodamnia cinerea, Cassia siamea, Peronema canescens, Oroxylum indicum,* and *Vitex pinnata.* Other species associated with disturbance such as *Croton argyratus,* and *Endospermum diadenum* were also present. *Swietenia macrophylla* along with *Paraserianthes falcataria,* and *Shorea leprosula* was part of enrichment program planted during the early 2000 to 2008. Topography of the site was varied from gently to moderately sloping.

The site had wet climate, where over the past 10 years, the average annual rainfall in this area was 2620 mm with the maximum and minimum annual rainfalls reached 3750 mm (2005) and 2286 mm (2011), respectively. The average of monthly rainfall over the past 10-year period was 232 mm, with the highest in December (403 mm) and the lowest in September (144 mm). The months of May to September received relatively low average of monthly rainfalls ranging from 144 mm to 262 mm. The absence of the average of monthly rainfall with less than 100 mm indicated a seasonal climate of the site. During the last decade, unusual dry month or month with less than 100 mm rainfall was very rare, being only recorded in June to October of 1994 and 1997; June, July, and September of 2003; and June and July of 2008. Monthly rainfall varied through time, and months with relatively low rainfall showed high variations (Figure 1). Even though, dry month, which defined seasonality of the climate, generally did not occur, people considered May to September as drier periods, and October to April as wetter periods.

Seven trees of *Swietenia macrophylla* with diameters from 10 to 30 cm were selected and harvested in March to April 2013 in the study area to collect wood disc specimens. Each wood specimen of each tree was collected from a stump at 10 cm the above soil surface. All wood specimens were dried, sanded, and digitally photographed. Wood specimens were coded m1 (diameter at breast height, DBH, =10.04 cm), m2 (12.70 cm), m3 (12.98 cm), m4 (13.47 cm), m5 (13.50 cm), m6 (27.90 cm), and m7 (31.76 cm). A cross section of each disc wood specimen that had the longest distance from its pith to the outer wood was selected for measurements of the radial growth rings. The

radial growth rings were measured to the nearest 0.001 cm by using ImageJ software. The measurement data of the growth rings were then used to carry out cross dating visually (Fritts and Swetnam 1989). Cross dating was initiated by plotting all widths of growth rings of all seven wood specimens (Y) against time (X). The position of each growth ring of all seven wood specimens was then adjusted and aligned according to the similarity of width patterns of the rings. The aligned width patterns of each wood specimen were then used to determine the age of growth ring by placing the most recent year (2012) at the outermost ring and subsequently increasing ages towards to the inner most ring. The results of the alignment and adjustment of growth rings and determination of ages were used to calculate the mean of annual growth rings, and to conduct a one-way analysis of variance (ANOVA) to determine whether widths of growth rings differed among the trees.

We calculated autocorrelation and mean sensitivity to know the relationship the growth rings and climate. For more detailed analysis to know what time period showing more sensitive growth ring to climate, we calculated the index of ring width. Autocorrelation was calculated to know whether a radial growth ring was influenced by its previous radial growth ring. Low autocorrelation value indicated low influence of the previous growth, and therefore indicated the influence of climate to the growth ring. Meanwhile, mean sensitivity refers to the proportion of change in width of annual ring from two successive growth rings. It reflected how sensitive growth rings to climate fluctuations. Therefore, we used the combination of low autocorrelation and high mean sensitivity of the ring width of wood specimens to indicate whether ring width data were good for dendroclimatology (Laroque 1995). We followed Cook and Pederson (2011) to calculate autocorrelation and mean sensitivity.

$$\text{Autocorrelation } (r) = [\text{sum } (X_i - X_m)(X_{i-1} - X_m)] \, [\, (n-1)\, S_x^{-2}]^{-1}$$

$$\text{Mean sensitivity } (ms) = [\, \text{sum } 2\,(X_i - X_{i+1})\,(X_i + X_{i+1})^{-1}] \, [\, (n-1)\,]^{-1}$$

Where X_{i-1}, X_i, X_{i+1}, X_m, S_x^2 respectively stands for the width of the growth ring at age i-1, i, i+1, the average of annual width, and variance of width.

The index or standardization was developed to remove the variation of the width of growth ring associated with the increasing age of the trees (Fritts and Dean 1992). The Index of ring width (Y axis) was calculated, plotted against age (X) for each wood specimen to know width index pattern. The pattern was used to determine sensitive and complacent series, which reflected growth behavior for all individual trees to the climate. Sensitive and complacent series were age periods respectively showing strong and steady variation of the index values. The sensitive series indicated that the growth of trees was influenced by the climate, while the complacent series showed that climate had a very weak influence to the growth (Schweingruber 1989). The index was calculated by dividing the actual width of a growth ring with its corresponding width value estimated from the model of the growth ring of a wood specimen. The model of the growth ring was developed based on the cubic polynomial equation of the width of the growth rings and their ages. For the purpose to determine the general pattern of relationship between rainfall and stand growth of Big-Leaf Mahogany, we averaged the index of all wood specimens according to its ages (AI). We used this averaged index to know sensitive series, which was then used to run regression analysis between the value of the averaged index (Y axis) and rainfall (X axis).

RESULTS AND DISCUSSION

Results

The average of annual growth rings of young Big-Leaf Mahogany trees ranged from 0.679 cm yr $^{-1}$ to 1.047 cm yr $^{-1}$. The highest average annual growth rings were 1.048 cm yr $^{-1}$ and 1.003 cm yr $^{-1}$, and respectively occurred in trees with DBH of 12.98 cm (m3) and 12.70 (m2). The lowest values, 0.597 cm yr $^{-1}$ and 0.679 cm yr $^{-1}$, were respectively found in trees with DBH of 31.76 cm (m4) and 27.90 cm (m7) (Figure 2). There was no statistically significant result indicating correlation between diameter and the annual growth ring (Table 1).

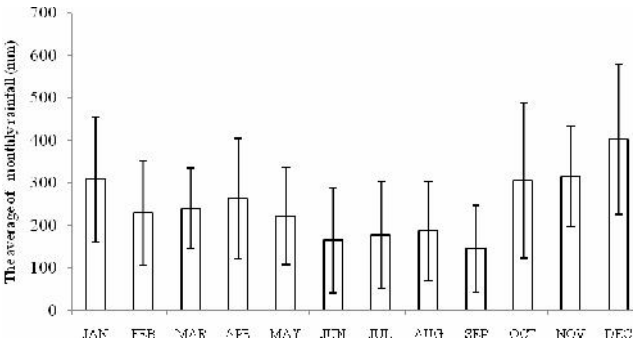

Figure 1. The Average of monthly rainfall recorded at Climatology Station of The University of Bengkulu. Indonesia from 1993-2013

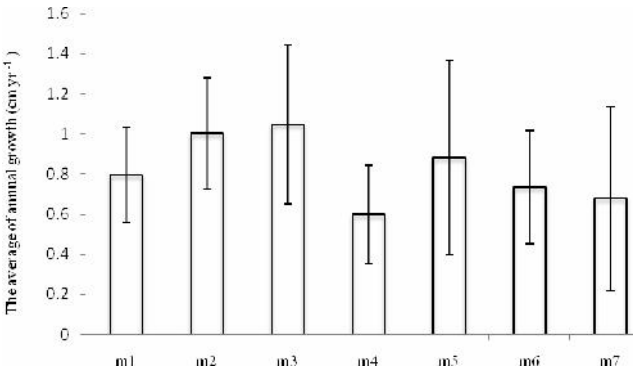

Figure 2. The average of annual growth rings (cm yr $^{-1}$) of Big-Leaf Mahogany trees in Bengkulu, Sumatra, Indonesia. The seven trees were coded as m1 (DBH=10.04 cm), m2 (12.70 cm), m3 (12.98 cm), m (13.47 cm), m5 (13.50 cm), m6 (27.94 cm), and m7 (31.76 cm)

Table 1. The result of ANOVA of regression analysis between the average of growth ring and diameter of Big-Leaf Mahogany trees

	df	SS	MS	F	Significance F
Regression	1	0.036	0.035	1.359	0.296^{ns}
Residual	5	0.131	0.026		
Total	6	0.166			

Table 2. The result of ANOVA to compare the average of annual growth ring width of Big-Leaf Mahogany trees

Source	df	Sum of squares	Mean of squares	F-test
Tree	6	1.292	0.215	1.533^{ns}
Error	54	7.588	0.141	
Total	60	8.880		

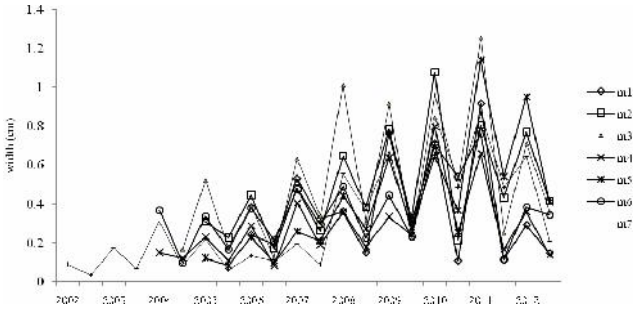

Figure 3. The variation of the widths of growth rings of Big-Leaf Mahogany trees according to ages in Bengkulu, Sumatra, Indonesia. The seven trees were coded as m1, m2, m3, m4, m5, m6, and m7.

Furthermore, the average of the annual growth ring was not significantly different among seven Big-Leaf Mahogany trees (Table 2). Coefficient variation of the width of annual growth rings varied among trees and was considered high. Trees with DBH of 13.50 cm (m5) and 27.90 cm (m6) respectively showed the lowest and highest coefficient variations, while both the smallest (10.04 cm, m1), and the largest DBH (31.76 cm, m7) appeared to have high variations (61.22% and 63.11%). Our result on the variation of growth ring through time showed three interesting patterns (Figure 3): (i) The width of growth rings showed periodicity, where broad width was followed by narrow one; (ii) The width appeared narrow in the young ages and became wider as trees grew older; and (iii) The width steadily increased over time until 2011, after which time the widths somewhat narrowed.

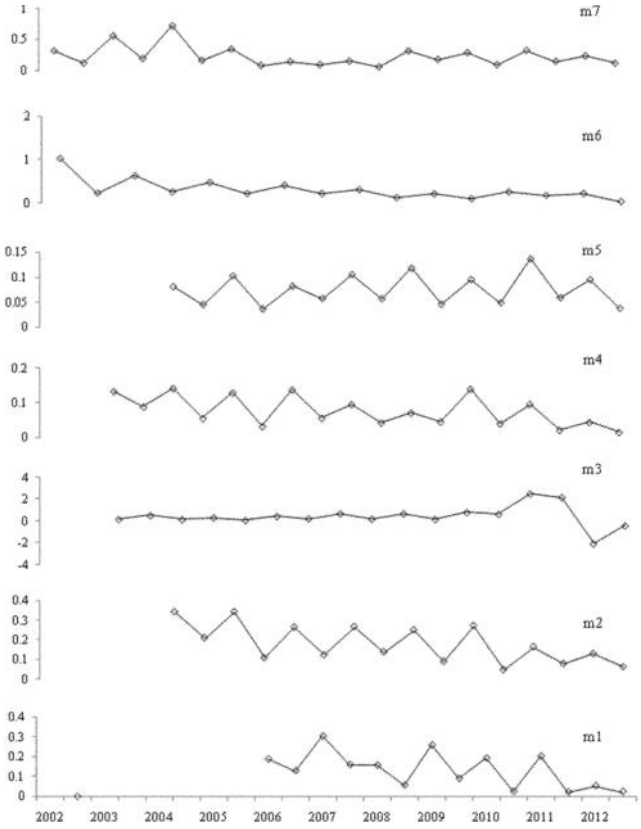

Figure 4. The index of annual ring width of Big-Leaf Mahogany trees (Y-axis) in Bengkulu, Indonesia. The seven trees were coded as m1, m2, m3, m4, m5, m6, and m7.

All wood specimens had high mean sensitivity (0.693-0.988) and low autocorrelation (0.076-0.42), except to trees of m2 and m7, which respectively had high autocorrelations (0.943. and 0.896) (Table 3). These figures reflected that the width of annual growth rings of all trees showed good responses to the variation of climate, except to m2 and m7 trees.

In the individual tree level, the index of growth ring width of all wood specimens showed interesting patterns based on sensitive and complacent series. The index of m1, m4, and m5 showed that they varied through ages. It also indicated that these trees were relatively responsive to climate through the time. Meanwhile, trees of m3, and m6 responded differently. The index of m3 was steady in the first eight years (complacent series), and then was varied in the last three years (sensitive series). In the other hand, the index of m6 had sensitive series in the early stages, and showed complacent series at the later ages (Figure 4).

Table 3. The mean sensitivity and autocorrelation of growth ring width for Big-Leaf Mahogany. The seven trees were coded as m1, m2, m3, m4, m5, m6, and m7. AI refered to the averaged values of the growth ring widths of all wood specimens (m1 to m7)

	m1	m2	m3	m4	m5	m6	m7	AI
Mean sensitivity	0.8963	0.7718	0.9883	0.8042	0.6933	0.7730	0.8275	0.8478
Autocorrelation	-0.4203	-0.9429	0.3938	-0.2137	0.2247	-0.1316	0.8967	0.0761

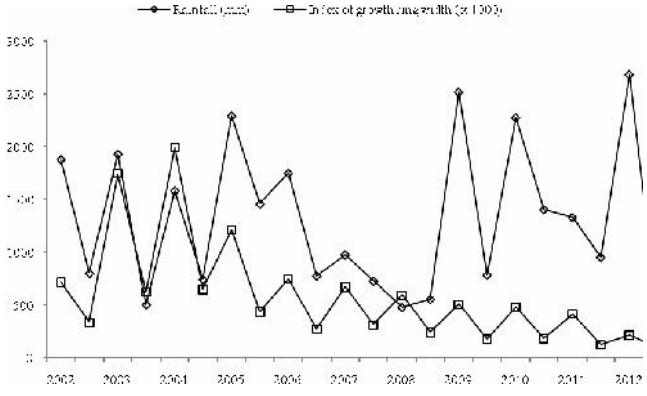

Figure 5. The Index of annual growth ring width for Big-Leaf Mahogany stand and annual rainfall (mm).

Table 4: The results of analysis of correlation between the index of growth ring width of Big-Leaf Mahogany stand and rainfall

	df	SS	MS	F	Significance F
Regression	1	1.301	1.301	6.153	0.033*
Residual	10	2.114	0.211		
Total	11	3.415			

In the stand level, sensitive series were apparent between 2002 and 2007, and followed by complacent series occurring from 2008 to 2012 (Figure 5). We run only regression analysis between index and rainfall during 2002 to 2007. The result of our regression analysis between the index of growth and rainfall demonstrated that during this first six years, the index of growth was positively correlated with rainfall (R= 0.617) (Table 4). On the other hand, we did not run regression analysis data between 2007 and 2012, because the growth ring width appeared independent to rainfall variation (Figure 5).

Discussion

Based on the results of our study, the average of annual growth rings (0.679 to 1.047 cm yr $^{-1}$) of trees was within the range of the observed diameter growth rates of the same species in Belize, Central America. The diameter growth rate in the Belize's study ranged from 0.69 cm yr $^{-1}$ to 1.21 cm yr $^{-1}$, where the fastest growth of individual trees reached 2 cm yr $^{-1}$ (Shono and Snook 2004). The average of annual growth was highly varied among trees, and tendency that larger diameter had smaller width increments was not presence. This was partly caused by the high variation of the width of growth rings within a tree. Such high variation was reflected by the fact that both trees with the smallest and largest DBH had considerably similar high coefficient variations of the width of growth rings. The variation of width as expressed the growth of Big-Leaf Mahogany was also reported by Sebastian et al. (2015). Ruger et al. (2011), who studied impacts of light availability and diameter to diameter growth of 274 woody plants of Barro Colorado Island, Panama, reported that diameter was less responsible to the variation growth rate, than that of soil characteristics. They further predicted that half of the trees performed faster growth rate either in smaller and bigger diameters, respectively. It appeared that the diameter did not produce a strong influence on the variation of width series, but climatic fluctuation and tree maturity.

Our result indicated that the rhythmic pattern of the width of growth rings through time was presence, despite of wet climate. This was contrary with the common beliefs, where in wet tropical climate, the annual growth was continuous, and therefore the distinct tree rings were very rare. The very rare distinct tree rings were caused by the lack of seasonal climate under high rainfall condition (Groenendijk et al. 2014). However biological periodicities were not uncommon in the Southeast Asia tropical forests as reflected by the incidence of flowering and fruiting phenologies (Bawa and Krugman 1991). Furthermore, the result of our research also supported the findings of Fichtler and Clark (2003), who carried out research in La Selva wet tropical forests, and Lopez et al. (2012), who studied tree rings in Bolivian tropical moist forests. Fichtler and Clark (2003) found that six observed species showed distinctive tree rings, and the other two species displayed indistinctive rings. Meanwhile, Lopez et al. (2012) found that seven species showed annual rings. Fichtler and Clark (2003) further explained that even in the wet tropics, plants could experience drought stress, which further caused distinctive tree rings. The drought stress was triggered by the presence of relatively drier and wetter periods and the incidence of unusual low rainfall in drier periods. In La Selva, drier and wetter periods were months with rainfall of 156-271 mm month $^{-1}$ and 353-527 mm month $^{-1}$, respectively. Both similar conditions appeared to occur in the research site. These reasons could explain the distinctive tree ring in our research.

Our result showed increasing width of growth rings with increasing ages, except to the last two years. This pattern was comparable to the result of similar research on Big-Leaf Mahogany at Belize (Shono and Snook 2004). This pattern was common growth development in trees, where they tended to grow higher than to grow larger in diameter at younger ages, but they then gradually switched to grow larger in diameter as they grew older (Halle et al. 1978). However, this result was deviated with the general pattern showing that older and larger diameter trees tended to have smaller width increments than younger and smaller ones (Norton and Ogden 1987; Helama et al. 2004). The deviation of the general pattern could be explained by several reasons. We speculated that all the trees was classified as juvenile phase, which generally performed accelerating growth, therefore the growth rate was higher at older ages. On the other hand, trees of maturity phase demonstrated de-accelerating rate, therefore the growth rate was slower at older ages. In our study, all wood samples also came from young Big-Leaf Mahogany trees (6-12 years), and their canopy developments may attribute to the growth pattern. Their leaf crown structures and volumes

were still developing and responsible to the increasing width in the young ages. As the leaf crown approached to its full development, the annual growth would be decreased. Worbes (2002) also reported that young trees have different physiological responses compared to the old trees. Furthermore, the index of growth ring width also indicated that the growth ring of Big-Leaf Mahogany stand tended to be more positively responsive to rainfall in the first six years than that of the later ages; therefore it was expected to have increasing widths in younger ages. These ecological and physiological aspects may cause the annual growth ring pattern of the young Big-Leaf Mahogany. This pattern of the annual growth ring was apparently common growth behavior among Big-Leaf Mahogany stands elsewhere. Data from South Kalimantan, Java, and Nusa Tenggara, Indonesia showed that growth rate of Big-Leaf Mahogany increased until 10 years old, but declined after that age, and finally leveled after 30 years old (Krisnawati et al. 2011). Furthermore, Sebastian et al. (2015) reported that the quadratic growth model of Big-Leaf Mahogany planted in an agroforestry system in Gunungkidul, Yogyakarta, Indonesia, showed that the diameter growth increased with increasing ages, but its rate started to decrease after 20 years old.

A more detail examination of the index of growth ring width revealed interesting growth variations for each tree. The index basically eliminated only age-influenced growth, and therefore reflected effects of climate, local disturbance, environmental conditions, as well as unknown factors, to the annual growth ring (Fritts and Swetnam 1989). The strong association between climate and annual growth ring had been reported by Stahle (1999), Worbes (2002), Pumijumnong and Wanyaphet (2006), Baguinon et al. (2008), Liang and Eckstien (2009), Wils et al. (2010), and Sigal (2011). However, all of these studies came from areas with distinct dry and wet seasons, therefore the climate especially rainfall was expected to cause significant effects to generate distinctive growth rings. For a seasonal region such in Bengkulu, the effects of climate did not necessarily occur throughout ages in the stand level. The positive influence of rainfall to the annual growth ring of Big-Leaf Mahogany appeared in the first six years. In the first six years, the annual growth was increased accordingly with rainfall. Furthermore, a strong positive correlation between rainfall and growth of Big-Leaf Mahogany in natural forests in Belize, Central America was also reported by Shono and Snook (2004). They found that in a wetter year, Big-Leaf Mahogany showed higher annual growth than that of in drier years. In our study, after six years old, the annual growth ring was not influenced by rainfall, and was apparently independent to rainfall. Furthermore, based on the pattern of index at individual level, trees of m1, m4, and m5 showed similar sensitive responses to climate through ages, while the two other trees, m3 and m6, had strong responses either in the early or later ages. On the other hand, the effects of climate to the growth of trees of m2 and m7 were compounded by the effects of diameter as shown by their high autocorrelations. It can be inferred that individual trees did not perform growth responses similarly to climate through ages, even though they grew in the same site and climatic regime. Climate can affect the growth of Big-Leaf Mahogany trees either throughout ages or a certain period of ages. These different responses suggested that the growth pattern cannot be sufficiently explained by climate only. Other factors could play more important roles than the climate to influence the growth of trees. The variation of local environments including microclimate, nutrient distribution, soil moisture (Guindon and Kits 2012), genetic variability, competition among trees for limiting factors (Bascrietto et al. 2004), and canopy closure (Fritts and Swetnam 1989) can cover up the influence of climate on the tree growth. The role of local environments hindered the influence of climate has been reported by Pumijumnong and Wanyaphet (2006). They found that intra-annual variation of tree growth was influenced by local soil moisture, and not by rainfall and temperature. Meanwhile, Sebastian et al. (2015) reported that low soil pH was responsible to generate retarded growth of Big-Leaf Mahogany in agroforestry system in Gunungkidul, Yogyakarta, Indonesia. High variation of annual growth rings as a result to different responses of each tree to climate and local environments may be attributed to insignificantly different mean annual growth rings among trees.

To conclude, the average of annual growth rings varied from 0,679 cm/year to 1,047 cm/year, and was not significantly different across sampled trees. Presence of growth periodicity, high mean sensitivity and low autocorrelation of majority of trees and growth periodicity indicated that Big-Leaf Mahogany was a good species for dendrochronology study in the wet tropics. The width of growth ring increased with ages, but somewhat narrowed in the last two years. In the individual tree level, each tree responded differently to climate. Two of them were responsive to the climate variations through out ages; the others were responsive either in the young or the older ages. The stand of Big-Leaf Mahogany appeared to have strongly sensitive to climate in the early ages or the first six years. Its growth ring had a positive correlation to rainfall in the first six years. The last five years, the annual growth ring of Big-Leaf Mahogany stand was independent, or not affected by rainfall. We speculated that local environments and ecological processes could mask the influence of rainfall to the annual growth ring in the older Big-Leaf Mahogany.

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Soil invertebrate diversity in coffee-pine agroforestry system at Sumedang, West Java, Indonesia

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Abstract. *Kinasih I, Cahyanto T, Widiana A, Kurnia DNI, Julita C, Putra RE. 2016. Soil invertebrate diversity in coffee-pine agroforestry system at Sumedang, West Java, Indonesia. Biodiversitas 17*: *473-478.* In order to maintain natural habitat while provide economic benefit for community near forest, some agroforestry systems were developed. This system depends on service provided by ecosystem such as nutrient cycling by soil invertebrates. One of important factors of healthy ecosystem services at particular agroecosystem is local biodiversity of the area. In this study we carried out biodiversity survey of soil invertebrates at local coffee - pine agroforestry system at Rancakalong Sub-district, Sumedang District, West Java, Indonesia. Soil invertebrates were collected from coffee plantation, coffee and pine (*Pinus merkusii*) plantation and pine plantation by 40 pitfall trap per location. Results showed the highest abundance was recorded at coffee plantation (2477 individuals) and the lowest was at pine plantation (1372 individuals). All collected soil invertebrates grouped into 3 classes (Arachnida, Chilopoda and Insecta), 16 orders, 47 families, and 124 morphospecies. Soil invertebrates were dominated by Formicidae, Scarabaeidae, Blattidae, Forficulidae, and Phalangiidae. The average diversity index of soil invertebrates was 2.25 (coffee plantation), 2.64 (coffee and pine plantation) and 1.85 (pine plantation). The evenness value was 0.30 (coffee plantation), 0.49 (coffee and pine plantation) and 0.39 (pine plantation). This study showed agroforestry may improve soil invertebrate abundance and diversity of monoculture pine forest through creation of additional and alternative nutrition and microhabitats.

Keywords: Agroforestry, biodiversity, coffee, soil invertebrate

INTRODUCTION

Agroforestry is land management where trees, shrubs, field crops and/or animal production are intentionally integrated on the same area at the same time. This integrated farming system takes the advantage of the productive, protective, and other services provides by its local biodiversity. This practice is a classic system in which farmer have long applied intercropping of economic crops with surrounding forest as a way to satisfy their need for food, wood products, fodder, and economic stability (Gliessman 2007). Among various crops, coffee is, along with cocoa, the most commonly cultivated crops in this system.

This perennial and woody plant (Bagyaraj 2015) is originated from Ethiopia, where they found grows as a natural understorey shrub of rainforest (De Beenhouwer 2014). In Indonesia, at coffee production region, they are grown under shade of canopies of trees in agroforestry system (Verbist et al. 2005; Hanisch et al. 2011; Evizal et al. 2016). Plants cultivated in agroforestry will have advantage from ecosystem services provided by plants and animals of surrounding forest, namely protection from excess sunlight (Felipe dos Santos et al. 2015), nutrient cycles (Lopez-Rodriguez et al. 2015), conservation of soil fertility (Lin and Richards 2007), waste regulation (Evizal et al. 2009), and pollination (Philpott et al. 2006).

Some studies showed agroforestry systems could improve biodiversity as they served as a refugia and buffer zone for mobile species (Cullen et al. 2001, Cruz and Sutherland 2004). Furthermore, this system also believed to improve soil fertility and microclimate of crop plantation area while provide more habitats for wild organisms than conventional monocultures (Tscharntke et al. 2011). Improvement of soil quality, as results of organic material input from both tree and crop species will stimulate establishment of soil invertebrate community. These invertebrates, with different proportion and function, maintain soil fertility through their interaction with each other and microbial community (Bardgett 2005; Bardgett et al. 2005; Lavelle et al. 2006). However, soil invertebrates population are sensitive to changes in plant cover (Barros et al. 2003), management regime (Aquino et al. 2008; Farska et al. 2014; Zaitsev et al. 2014), and microclimate (Vasconcelos et al. 2009).

West Java has long history of conversion of natural forest into plantation forest, i.e. pine forest (*Pinus merkusii*). This management practice may affect soil invertebrate diversity and function through direct (litter quality) and indirect effects (microhabitats and environmental factors like pH, soil humidity, soil fertility). In last 10 years, as part of community development and protection of plantation forest, pine forests have been utilized as part of agroforestry. Previous study on pine

forest showed high level of soil nitrification of Indonesia pine forests (Krave et al. 2002). Soil nitrification results in instability of nitrogen supply to plants as nitrate is easily removed from soil by denitrification and leaching (Watts and Seitzinger 2000). Furthermore, high nitrification rates in N-saturated soils, which in common in Indonesia, can produce soil acidification which affect soil invertebrate population (Abeliovich 1992; Zaitsev et al. 2014). However, despite their importance in ecosystem processes, very few studies focused on the diversity of soil invertebrates on this agroforestry especially on under pine forest. Thus, the objectives of this study were to explore the diversity of soil invertebrates of coffee-pine plantation and the effect of this diversity to soil quality.

MATERIALS AND METHODS

Study area

The research was conducted from September to December 2015 at an urban coffee plantation located 500 meters from Rancakalong Sub-district, Sumedang District, West Java, Indonesia. The plantation was located at 6°49'27.2"S and 107°48'34,7"E, and about 1100-1200 m above sea level.

Soil invertebrates were sampled at three regions, (i) coffee plantation without shade/under direct sun (C), (ii) coffee plantation under the shade of *Pinus merkusii* (CP), and (iii) pine forest dominated with *P. merkusii* (P) (Figure 1).

Procedures

Soil invertebrates sampling

Pitfall traps were used to obtain surface soil invertebrates (Maftu'ah et al. 2005). Samples were collected from 4 pitfall traps placed at 10 subplots, selected randomly. Each trap's distance was 2 meter. Thus, total number of sample per land use type was 40 samples (Table 1). Samples were collected 10 times during study period (which further referred as sampling effort in this study). During study period, total number of 1200 samples from all study areas was collected.

All sampled specimen was grouped by hand sorting and then was identified its morphospecies level based on Borror et al. (1989) and Dindal (1990).

Environmental factors measurement

At each habitat, three soil samples were collected and several soil characteristics were measured, both the characters of physics and chemistry, namely: (i) physical characteristics (soil texture, soil humidity, soil temperature); (ii) chemical characteristics (soil pH, C, N, C/N, and P) (Table 2).

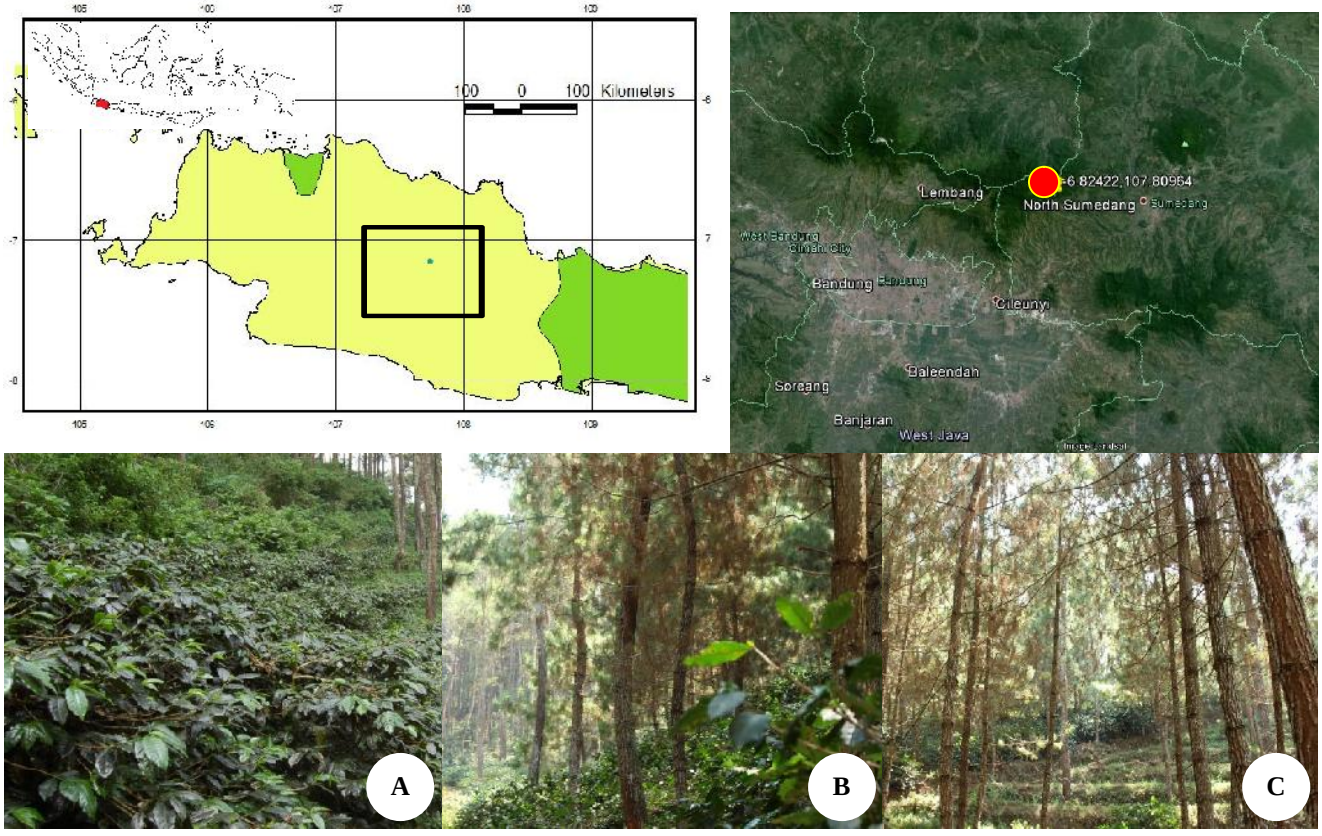

Figure 1. Study site in Rancakalong Sub-district (●), Sumedang District, West Java, Indonesia. A. Coffee plantation without tree shade, B. Coffee plantation under shade of *Pinus merkusii* trees, C. *Pinus merkusii* forest

Table 1. Sampling area characteristic and sampling effort

Habitat	Habitat code	Area size	Total no. of samples	Litter thickness (cm)
Coffee plantation	C	3,375 ha	40	1.13
Coffee-pine agroforestry	CP	4,572 ha	40	1.46
Pine forest	P	3,112 ha	40	0.98

Table 2. Soil characteristics of sampling area

Site	Soil pH	Soil temperature (°C)	Soil humidity (%)	Soil texture			C	N	C/N	P_2O_5
				Sand	Dust	Clay				
C	5	27	27	42	54	5	7.99	0.79	10	73.4
CP	4.5	26	28	48	45	8	9.59	0.66	14	8.9
P	5.4	28	26	35	24	41	5.61	0.62	9	52.1

Data analysis

Species diversity index was calculated to compare diversity among sampling areas. Species diversity was represented by Shannon diversity index (Ludwig and Reynolds 1988) by:

$$H' = -\sum_{i=1}^{R} p_i \ln p_i$$

Where, *p*i: the proportion of individuals belonging to the *i*th species to total sample

We calculated species evenness to evaluate variation in communities among sampling areas which represented by Pielou's evenness index (J') (Mulder et al. 2004).

$$J' = \frac{H'}{H'_{\max}}$$

Where, H'_{max} is the number derived from previously calculated by Shannon diversity index.

$$H'_{\max} = -\sum_{i=1}^{S} \frac{1}{S} \ln \frac{1}{S} = \ln S.$$

Where, S is total number of species in the community.

Species evenness index, then, was supplemented with Simpson's dominance index (Morris et al. 2014) in order to find possibility of dominance of some species at sampling areas.

$$D = \sum_{i=1}^{S} p_i^2.$$

Where, *p*i: the proportion of individuals belonging to the *i*th species to total sample.

All species abundance data was subjected to statistical analysis of variance. First test was normality test by Shapiro-Wilk test with alpha level $P < 0.05$. Our test obtained P value of Shapiro-Wilk test with 0.01483 indicating non normal distribution of sample. Thus, Kruskal Wallis test was applied to evaluate differences between separate means. This test followed by Mann-Whitney pair wise test to establish the significance of differences among study areas. Differences obtained at levels of $P < 0.05$ were considered significant. All statistical analysis was carried out with Past 3.12

Multivariate statistic method of CCA, calculated with CANOCO software (Microcomputer, Ithaca, N.Y.) , was used to explore the variability of taxa related to environmental variables (soil humidity, soil temperature, soil pH, nitrogen, carbon, phosphat).

RESULTS AND DISCUSSION

We collected 7,379 individuals during sampling period. Among all study areas, coffee plantation (C) had the highest abundance of individuals (3,486 individuals, 47%), followed by coffee-pine agroforestry (2,519 individuals, 34%), and pine forest (1,374 individuals, 19%) (Figure 2.A). The similar pattern also showed on the number of morphospecies and number of soil invertebrate family (Figure 2.B). Further, statistical test showed that mean abundance of soil invertebrates at coffee plantation was significantly higher than other sampling areas (Table 3). Normality test indicated non normal data distribution of sampling area as soil invertebrate population at each sampling area dominated by some taxa, like Formicidae and Scarabaeidae. This finding was also supported by low evenness value and high dominance value of all sampling area (Table 4).

This study showed benefit of agroforestry management for soil invertebrates, even though its abundance and richness was higher at coffee plantation. Diversity index of both coffee plantation and coffee-pine agroforestry indicated more diverse and equally distributed soil

invertebrates compare to pine forest. Studies showed that plantation management practices that promote the maintenance of plant residues on soil provide more favorable environment for soil invertebrates (Moco et al. 2005). This study showed increasing litter quantity in mixed stands which was agree with most studies (Scheu et al. 2003; Albers et al. 2004; Gartner and Cardon 2004). Thicker litter was found on coffee plantation and coffee-pine agroforestry providing more habitats for soil invertebrates while maintaining soil temperature and humidity which was important for many soil invertebrates.

Differences on soil invertebrate abundance and diversity could be explained by litter quality. Litter quality was considered as important resources for soil invertebrates and could shape soil communities (Kaneko and Salamanca 1999; Salamon et al. 2004). Coffee litter which was much easier to decompose seemed to provide better environmental condition to encourage and maintain higher number and more diverse soil invertebrates compared to other area. Furthermore, coffee litters having lower tannin and polyphenol content could offset the unfavorable condition for decomposition and improve environmental condition for soil invertebrates (Hattenschwiler et al. 2005; Korboulewsky et al. 2016). Higher tannin and polyphenol content of pine needle might lead to slower decomposition process which lowered the abundance and activities of soil invertebrates and it explained low population and diversity on pine forest (Hattenschwiler et al. 2005; Vivanco and Austin 2008; Cesco et al. 2012).

Both litter quantity and quality may influence the soil invertebrate community as they create specific microclimate on soil surface. Highly specific litter and microclimate in pine forest made it favorable only for some specific species with ability to decompose, feed on microorganism life on pine litter, or live on the humus of pine forest which creates unique spatial distribution of soil invertebrates. This specialization of soil invertebrates created by differences in humus characteristic was also reported to be occurred on other agroforestry in East Java (Peritika et al. 2012). With possibility of higher rate of nitrogen lost from nitrification (soil of pine forest had lowest N) (Krave et al. 2002) made this area considered as barren area compared to other area and it lowered soil invertebrate population and diversity. Our result showed that only scavenger like Blattidae and Formicidae thrived well in this area (Table 4). Coffee-pine agroforestry system provided various litter types which allow different decomposer species to coexist and share the resources (Wardle et al. 2006), and it is showed by an increasing population and diversity of soil invertebrates in our study. Our study also showed that humus condition of coffee plantation provides best condition for many soil invertebrate. Furthermore, coffee plantation area where coffee trees were planted with specific distance had more patches (where litters were physically separated) than coffee-pine area (where different litters were thoroughly mixed) and it provided more microhabitat for soil invertebrates (Sulkava and Huhta 1998).

In some studies, soil invertebrate is negatively correlated with soil pH (Wu et al. 2011; Peritika et al. 2012) which may explain higher abundance on coffee plantation. CCA analysis showed long term application of agroforestry system already created specific habitat for some taxa at both coffee-pine and coffee plantation, i.e. Anisolabididae and Apidae which were only found at coffee-pine area while Berytidae, Calliphoridae, Dolichopodidae, Dytiscidae, and Histeridae which were only found at coffee plantation (Table 4, Figure 3).

Based on CCA, it could be concluded that distribution of most soil taxa was influenced by amount of carbon at humus, while high nitrogen content of coffee plantation creating specific niche for specific taxa were found at that area (Figure 3). Furthermore, this result also showed high mobility of taxa to move on all area as pit fall trap designed for trapping active surface soil invertebrates.

This study showed benefit of agroforestry to improve soil invertebrate population and diversity especially for forest which produced low quality litter. Abundance and diversity of soil invertebrates could be increased through creation of additional nutrition and microhabitats. The possibility of similar pattern found in agroforestry and located in rich natural forest should be observed in order to find best management practice for agroforestry in tropical regions like Indonesia.

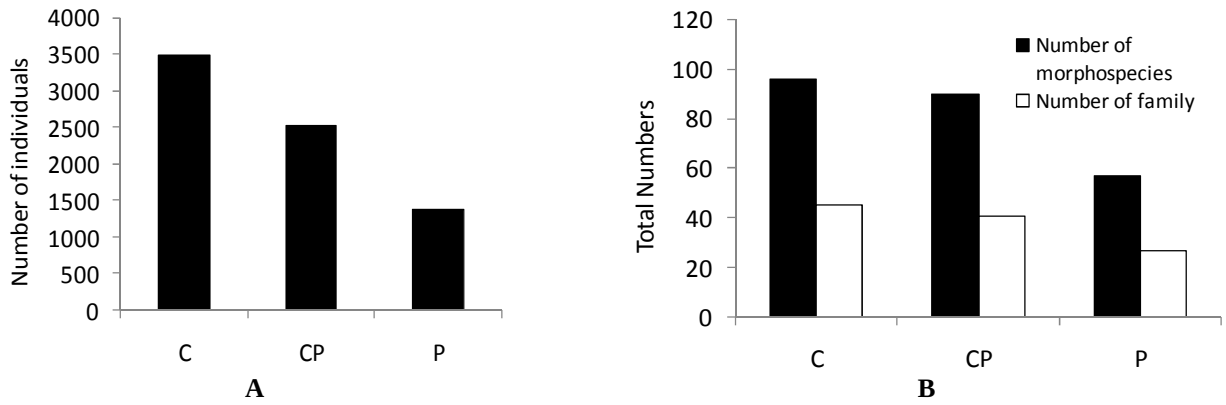

Figure 2. Abundance and diversity of soil invertebrates per sampling area

Table 3. Mean of Soil Invertebrate Abundance among sampling area

Site	Mean abundance (mean ± SD)
Coffee plantation	348.6 ± 118.57a
Coffee-pine agroforestry	251.9 ± 109.3b
Pine forest	137.4 ± 61.17c

Note: Different letter indicates significant difference with P < 0.05

Table 4 Number of individual, families and diversity index at each site

Families	C	CP	P
Acrididae	8	13	1
Anisolabididae		1	
Apidae		3	
Berytidae	8		
Blattellidae	9	43	8
Blattidae	674	453	161
Calliphoridae	1		
Carabidae	5	18	1
Cecidomyiidae	8	3	5
Cerambycidae	1	1	
Cheliferidae	5	7	
Chrysomelidae	1		1
Coccinellidae	3	2	
Cossidae (larvae)	7	2	3
Curculionidae	21	13	
Deinopidae	2	2	1
Dermestidae	1	1	
Dipluridae	93	85	44
Dolichopodidae	3		
Drassidae	5	1	3
Drosophilidae	149	93	28
Dysderidae	2	1	
Dytiscidae	4		
Forficulidae	227	101	33
Formicidae	753	980	981
Gryllidae	28	41	6
Hepialidae	47	39	12
Histeridae	1		
Hypodermatidae	11	8	
Ichneumonidae	3	5	
Lepidoptera	2	5	2
Lithobiidae	2	5	1
Lymantriidae	16	13	1
Mantidae	1	2	
Noctuidae (larvae)	2	2	
Nymphalidae	29	34	1
Phalangiidae	111	245	33
Phasmatidae	2	1	
Pompilidae	7	11	4
Scarabaeidae	1162	230	7
Scytodidae	23	20	11
Sparassidae	13	10	10
Sphecidae	4	2	4
Staphylinidae	24	17	10
Tenthredinidae	1	1	2
Thelyphonidae	4	4	
Therevidae	3	1	
Number of individual	3486	2519	1374
Diversity index (H')	2.24	2.64	1.85
Pielou's evenness index	0.30	0.49	0.39
Simpson's dominance	0.79	0.86	0.74

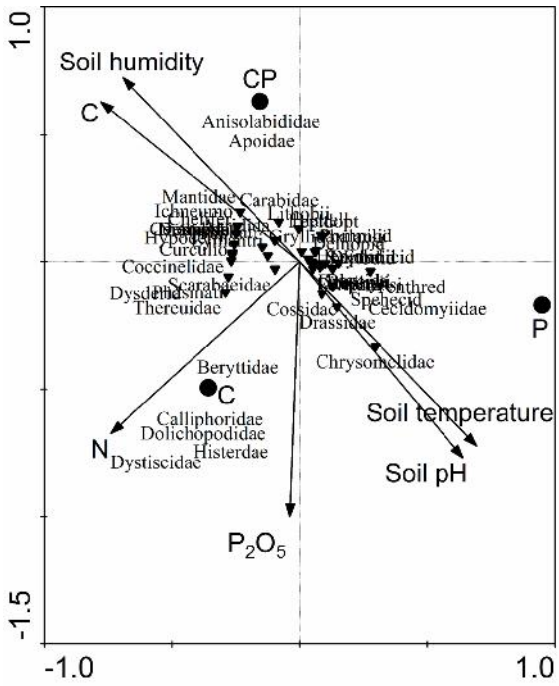

Figure 3. Ordination plots of CCA results for taxa distribution related with sampling area and soil characteristics. The direction of an arrow indicates the steepest increase in the variable and the length indicates the strength relative to other variables.

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Diversity of faunal communities in the Biodiversity Park of Ciherang, Bogor, West Java, Indonesia

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Abstract. *Gunawan H, Sugiarti, Rianti A, Sihombing VS. 2016. Diversity of faunal communities in the Biodiversity Park of Ciherang, Bogor, West Java, Indonesia. Biodiversitas 17*: *479-486.* A Biodiversity Park is a new concept for ex situ conservation in Indonesia which was first launched in 2012. The purposes of a Biodiversity Park are to conserve indigenous and threatened species of flora, provide habitat for a diversity of animals, and to provide opportunities for economic benefit, recreation, education and research. The main goal of Biodiversity Park is to increase flora and fauna diversity in the midst of human settlement and industrial precincts. This research was directed at studying the diversity of faunal communities in the Biodiversity Park of Aqua Danone Ciherang**,** Bogor, West Java, Indonesia. Line transects, walk transects, terrestrial transects and point count methods were combined to census the mammals, reptiles, amphibians and birds in the Biodiversity Park. Twenty five families of fauna were identified, consisting of 28 genera and 32 species. The Shannon diversity index for the total faunal community was 2.82. Composition of the faunal community consisted of birds (66%), reptiles (16%), mammals (12%) and amphibians (6%). This finding supports the goal that Biodiversity Park can increase flora and fauna diversity. The diversity index of 2.37 for the bird community indicates a beneficial contribution to habitat quality within an urban environment.

Keywords: Biodiversity Park, fauna, habitat, green space

INTRODUCTION

Java is the most populated island in Indonesia, with 136.45 million people or 57.44% of its total population (237.56 million) (Center Bureau of Statistic 2010). Due to the high density of human population, Java is facing two serious challenges namely, consumptive behavior leading to over-exploitation of natural resources; and limited understanding of the importance of conservation, which leads to low implementation of conservation principles in the planning and implementation of development (Whitten et al. 1996). The threat of environmental degradation and biodiversity loss comes from habitat alteration, invasive alien species, pollution, over-exploitation and climate change (Widjaja 2014). Fragmentation of the environment is also leading to local extinction of species (Morrison et al. 1992; Turner 1996; Gu et al. 2002; Henle et al. 2004; van Houtan et al. 2006; Parker et al. 2008).

The rate of biodiversity loss must be curtailed, and priority given to rehabilitation of degraded ecosystems both inside conservation areas and in surrounding human settlements (BAPPENAS 2003). Some effort has been initiated by the Government of Indonesia to protect biodiversity through in situ and ex situ conservation methods. Besides protecting the biodiversity in its natural habitat through designation of in situ conservation areas, the Government of Indonesia has also established ex situ conservation areas such as Botanical Gardens and Biodiversity Parks (Widjaja 2014).

A Biodiversity Park is a reserved area for preserving local biodiversity, especially the flora and associated fauna that act as pollinators and seed dispersal agents. The goals of Biodiversity Parks are to conserve local and threatened species of flora, develop habitat to increase faunal diversity, and provide opportunities for tourism, research, education and local wealth generation (Ministry of Environment 2012).

AQUA Danone Plant Ciherang is a producer of bottled drinking water under the management of the Aqua Danone Group. Aqua Danone Plant Ciherang has established a Biodiversity Park as part of its effort to achieve Green Company status within the Green PROPER (Performance Rating in Environmental Management) award scheme promoted by the Ministry of Environment and Forestry. To achieve a Green PROPER award as a Biodiversity Park, various activities and outputs must match with the criteria set out in PROPER assessment (Ministry of Environment 2014). One of the award criteria for a Biodiversity Park is that it must impact positively to increase the status of flora and fauna diversity in the local environment. Objective measures of diversity based on the variety and relative abundance of species are frequently used as indicators of the well-being of ecological systems (Magurran 1988; Spellerberg and Fedor 2003).

A program of research is needed to determine whether faunal diversity is increasing as a result of the development of Biodiversity Parks. A quantitative census of wildlife species is a key tool used to establish a base-line description of the site, to estimate population sizes, and to monitor population changes. It also provides basic information for determining the habitat requirements of identified species, for determining if and why species have declined, and for monitoring the effects of habitat management (Sutherland 2004). The objective of this research was to study the diversity of faunal communities in the Biodiversity Park of Aqua Danone Ciherang, Bogor, West Java, Indonesia. This was to be used to provide evidence as to whether or not the implementation of the Biodiversity Park concept at this site has been effective in increasing species diversity.

MATERIALS AND METHODS

The research was conducted from July to December 2015 in the Biodiversity Park of Ciherang which is located in the industrial and sub-urban settlement environment of Ciherang Pondok Village, Sub District of Caringin, Bogor District, West Java Province, Indonesia. The Biodiversity Park of Ciherang is managed by Aqua Danone which covers 3.76 hectares allocated as open green space. Identification of fauna was based on various field guides, namely 'Guide to the Tracks of Mammals of Western Indonesia' (van Strien 1983); field guides for identification of Indonesian birds (Mackinnon 1991; MacKinnon et al. 1992); field guides for the identification of amphibians (Iskandar 2002; Kusrini 2013), and for identification of snakes (Suhono 1986; Supriatna1995). Binoculars, SLR camera with tele-lens and camera trap were used in spotting fauna.

Line transects were used to sample individual mammals detected on and to each side of the transect line, followed by other specific methods for identifying particular species. Faecal pellet investigation was used to identify mammals, particularly carnivores which use their droppings for territory marking. Observation of feeding signs was used to identify those species that leave conspicuous markings on food sources or remains. The track count method was also used to identify a variety of species. Systematic searching for signs along transects provides an indication of presence and activity (Hill et al. 2005). Camera trap were also installed in strategic sites to monitor those species of fauna that are difficult to find because of secretive or nocturnal behavior (McDonald 2004; Meek 2012).

The walk transect method was used to survey reptiles. All reptiles seen along transects were identified and recorded (Hill et al. 2005). Terrestrial transect searches were used to estimate amphibian numbers on land. Transects were searched carefully (i.e. on hands and knees) for signs of amphibians within a specified distance of the transect line (Hill et al. 2005). The point counts method was applied for the bird survey (Hill et al. 2005) with observation radius of 50 m. The result was a list of bird species and a number of records for each species (van Lavieren 1982).

Data was processed and analyzed using Microsoft Excel. Data was analyzed and interpret to provide information of Relative Abundant (RA); Relative Frequency of occurrence (RF); Important Value (IV), diversity index (H') and evenness index (E) for each community. The diversity and evenness indices were based on the Shannon formula (Ludwig and Reynolds 1988; Magurran 1988; Spellerberg and Fedor 2003). The equation for Shannon function, which uses natural logarithms (ln), is

$$H' = -\sum pi \ln pi$$

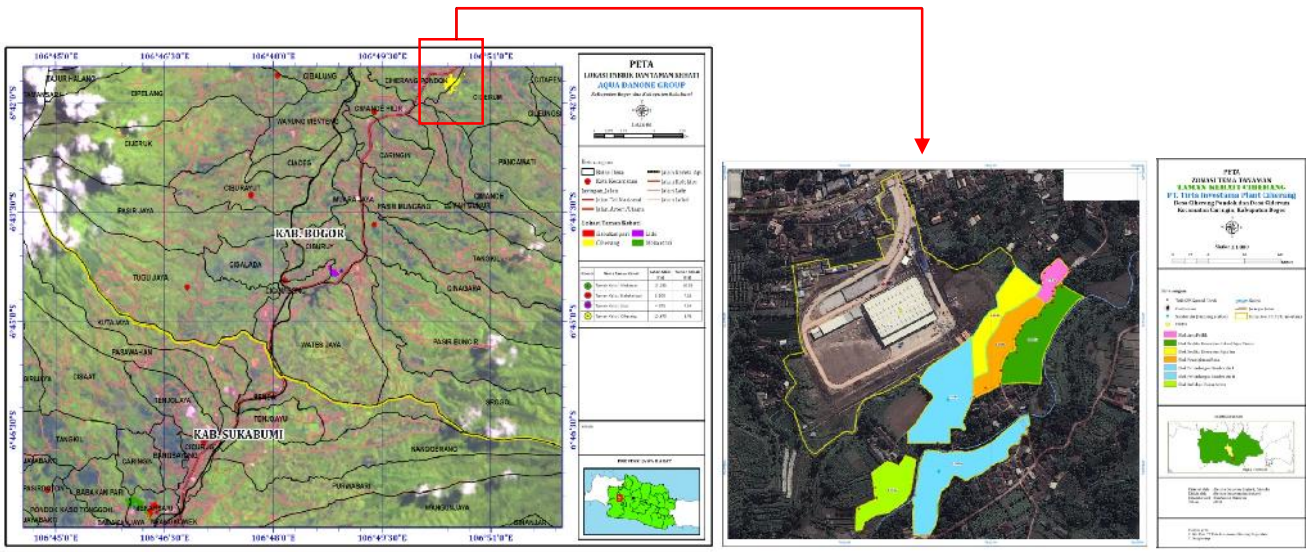

Figure 1. Research location at Ciherang Pondok Village, Sub District of Caringin, Bogor District, West Java Province, Indonesia

Where, *pi* is the proportion of individuals found in the i^{th} species which is estimated as ni/N. The formula of evenness index (E) used in this research is

$$E = \frac{H'}{lnS}$$

Where, S is number of species.

Relative abundance (RA) was determined by using formula (Zakaria et al. 2009):

$$RA\ (\%) = \frac{n}{N} x100\%$$

Where, *n* is number of particular recorded animal and *N* is total recorded animal. In the same way, we get formula for Relative Frequency of occurrence (RF) as follow:

$$RF = \frac{f}{F} x100\%$$

Where, *f* is frequency of detection of particular recorded animal and *F* is total recorded animal. Important value is a sum of relative abundant and relative frequency of occurrence.

Fauna was listed and categorized according to the IUCN Red List status (i.e. LC = Least Concern; NT = Near Threatened; NE = Not Evaluated) (IUCN-WCU 2001) and Government Protection status (i.e. L=Protected; TL=Unprotected) (Ministry of Forestry 1999). Birds were also categorized according to feeding guilds (i.e. carnivorous, nectarivorous, granivorous, frugivorous, and insectivorous) (Wiafe and Nutsuakor 2012; Edison et al. 2016).

RESULTS AND DISCUSSION

The diversity of faunal communities

A serial survey of fauna at the Biodiversity Park of Aqua Danone Ciherang found 25 families consisting of 28 genera and 32 species. For comparison, at the similar type of vegetation in Biodiversity Park of Aqua Danone Babakan Pari, West Java with 4.15 hectares area only found 22 species of fauna consisted of four mammalian, four herpetofauna and 14 avifauna (Gunawan and Sugiarti 2015a). Both of biodiversity parks are located in the midst of industrial and human settlement environment. The diversity index for the total fauna community at Biodiversity Park of Aqua Danone Ciherang was 2.82, higher than Biodiversity Park of Aqua Danone Babakan Pari (2.62). Other research at Biodiversity Park of Aqua Danone Lido with 4.34 hectares area found 21 species with diversity index of 2.58 (Gunawan and Sugiarti 2015b). In general, the Biodiversity Park of Aqua Danone Ciherang is more succeed in providing habitat for animal life.

Bird community was dominant which comprised 66% (21 species) of the total number of faunal species (32) in the Biodiversity Park. Avifauna had the highest diversity index (2.37), followed by reptiles (1.52) and mammals (1.28) and the lowest was for amphibians (0.56). In general, the Biodiversity Park has provided habitats for many species of fauna, including mammals, reptiles, amphibians and bird. Diversity in the species of plants occurring in the Biodiversity Park of Aqua Danone Ciherang creates habitats which provide a variety of feed types, nesting trees, foraging trees and shelter for fauna.

Most of the fauna in Biodiversity Park of Aqua Danone Ciherang are listed as Least Concern in IUCN Red list; three species are listed as Not Evaluated and one species is listed as Near Threatened. Sixteen percent of the species recorded are protected by law due to their rarity and threatened status. This means that the Biodiversity Park represents an oasis of biodiversity in a marginal environment of dense human settlement and industry.

The Biodiversity Park has also demonstrated its significant role for carnivores such as Javan mongoose (*Herpestes javanicus javanicus* É. Geoffroy Saint-Hilaire) and common palm civet (*Paradoxurus hermaphroditus* Pallas) which are now becoming difficult to be found. Javan mongoose is a carnivore which controls populations of pest like rat (*Rattus rattus* Linnaeus). Common palm civet is a species of carnivore that mostly feeds on fruits and seeds of many trees, palms and rattans, such as *Vitex glabrata* R.Br, *Coffea arabica* L, *Pinanga kuhlii* Blume and *Pinanga javana* Blume; so this species is an important agent of seed dispersal (Thohari and Santoso 1996; Gregory and van Strien 2010; Chakravarthy and Ratnam 2015).

The monitor lizard (*Varanus salvator* Laurent) and snake species (*Rhabdophis subminiatus* Schlegel) are predatory animals which live in the Biodiversity Park. These species have a role as controllers of their prey populations in its ecosystem. Monitor lizard is carnivorous and scavenger (Kulabtong and Mahaprom 2015), it has an extremely broad diet and will scavenge food left over from residents (Uyeda 2009). As scavenger, monitor lizard has important role in cleaning the environment from potential pests and diseases arising from dead carcasses. On the other side, monitor lizard is also facing threat from hunting for food, medicine and leather.

Other reptiles (i.e. *Bronchocela jubata* Duméril and Bibron, *Draco volans* Linnaeus, and *Eutropis multifasciata* Kuhl), as well as amphibians (i.e. *Hylarana chalconota* Schlegel and *Duttaphrynus melanostictus* Schneider) have important roles in ecosystems. Manned forest lizard (*Bronchocela jubata)* preys on butterflies, moths, dragonflies, flies and other small insects. Common gliding lizard (*Draco volans*) feeds mainly on ants, and possibly termites (McGuire and Kiew 2001). Common sun skink (*Eutropis multifasciata)* specializes on spiders, insect larvae, snails, grasshoppers and crickets (Ngo et al. 2015).

The role of amphibians in ecosystems is their contribution to supporting services. Amphibians can affect ecosystem structure through soil burrowing and aquatic bioturbation and ecosystem functions such as decomposition and nutrient cycling through waste excretion and indirectly through predatory changes in the food web. They also can control primary production in aquatic ecosystems through direct consumption and nutrient cycling (Hocking and Babbitt 2014).

The existence of carnivorous and insectivorous species in an ecosystem suggests the possibility of maintaining a harmonious food chain. Scavengers play an important role in the ecosystem by consuming the dead animal and plant material. This imply that the presence of scavengers has also tends to keep the ecosystem healthy. Based on these facts, the Biodiversity Park has successfully provided a habitat for some carnivorous species and insectivorous reptiles and amphibians.

Structure and composition of bird community

Avifauna plays an important link of food chain in ecological unit of nature. Hence, it is very important to know their diversity, migratory status, population size, distribution pattern and conservation status (Jeevan et al, 2013). There were 15 family of birds found in Aqua Danone Ciherang Biodiversity Park, consisting of 17 genera and 21 species. Twenty species are listed as Least Concern (LC) status in the Red list of IUCN; one species is listed as Near Threatened (NT). Referring to the Government Regulation No. 7/1999, there are five species which are protected by law and the rest are unprotected. The Shannon diversity index of the bird community in Aqua Danone Ciherang Biodiversity Park is 2.37 with an evenness index of 0.78.

Table 1. Diversity of fauna in the Biodiversity Park of Aqua Danone Ciherang at Ciherang Pondok Village, Sub District of Caringin, Bogor District, West Java Province, Indonesia

Classes	Family	Genus	Species	Diversity Index	Evenness Index
Mammal	4	4	4	1.28	0.92
Reptile	4	5	5	1.52	0.94
Amphibian	2	2	2	0.56	0.81
Aves	15	17	21	2.37	0.78
Total community	25	28	32	2.82	0.81

Table 2. List of fauna in the Biodiversity Park of Aqua Danone Ciherang at Ciherang Pondok Village, Sub District of Caringin, Bogor District, West Java Province, Indonesia

Local Name	Latin Name	Family	Status	RF	RA	IV
Mammal						
Garangan jawa	*Herpestes javanicus javanicus* (É. Geoffroy Saint-Hilaire, 1818)	Herpestidae	LC/TL	2.56	1.35	3.92
Bajing kelapa	*Callosciurus notatus* (Boddaert, 1785)	Sciuridae	LC/TL	1.28	2.70	3.98
Musang luwak	*Paradoxurus hermaphroditus* (Pallas, 1777)	Viverridae	LC/TL	3.85	2.03	5.87
Tikus	*Rattus rattus* (Linnaeus, 1758)	Muridae	NE/TL	1.28	0.68	1.96
Reptile						
Biawak	*Varanus salvator* (Laurenti, 1768)	Varanidae	LC/TL	1.28	0.68	1.96
Bunglon	*Bronchocela jubata* (Duméril and Bibron, 1837)	Agamidae	LC/TL	2.56	2.03	4.59
Cecak terbang (haphap)	*Draco volans* (Linnaeus, 1758)	Agamidae	NE/TL	2.56	1.35	3.92
Kadal kebun	*Eutropis multifasciata* (Kuhl, 1820)	Scincidae	NE/TL	5.13	2.70	7.83
Ular picung	*Rhabdophis subminiatus* (Schlegel, 1837)	Colubridae	LC/TL	2.56	1.35	3.92
Amphibians						
Kongkang kolam	*Hylarana chalconota* (Schlegel, 1837)	Ranidae	LC/TL	1.28	0.68	1.96
Katak buduk	*Duttaphrynus melanostictus* (Schneider, 1799)	Bufonidae	LC/TL	3.85	2.03	5.87
Aves						
Walet sapi	*Collocalia esculenta* (Linnaeus, 1758)	Apodidae	LC/TL	7.69	12.84	20.53
Walet linchi	*Collocalia linchi* (Horsfield and Moore, 1854)	Apodidae	LC/TL	10.26	17.57	27.82
Tekukur	*Spilopelia chinensis* (Scopoli, 1768)	Columbidae	LC/TL	2.56	2.03	4.59
Madu kuning	*Cinnyris jugularis* (Linnaeus, 1766)	Nectariniidae	LC/L	2.56	1.35	3.92
Burung gereja	*Passer montanus* (Linnaeus, 1758)	Passeridae	LC/TL	2.56	4.73	7.29
Rajaudang jawa	*Halcyon cyanoventris* (Vieillot, 1818)	Halcyonidae	LC/L	3.85	2.03	5.87
Meninting	*Alcedo meninting* (Horsfield, 1821)	Alcedinidae	LC/L	1.28	0.68	1.96
Bondol jawa	*Lonchura leucogastroides* (Horsfield and Moore, 1856)	Estrildidae	LC/TL	8.97	20.95	29.92
Wiwik lurik	*Cacomantis sonneratii* (Latham, 1790)	Cuculidae	LC/TL	2.56	1.35	3.92
Wiwik uncuing	*Cacomantis sepulcralis* (S. Muller, 1843)	Cuculidae	LC/TL	2.56	1.35	3.92
Cinenen pisang	*Orthotomus sutorius* (Pennant, 1769)	Cisticolidae	LC/TL	3.85	2.70	6.55
Cipoh	*Aegithina tiphia* (Linnaeus, 1758)	Aegithinidae	LC/TL	3.85	2.03	5.87
Cucak kuning	*Pycnonotus melanicterus* (Gmelin, 1789)	Pycnonotidae	LC/TL	1.28	0.68	1.96
Cerucuk	*Pycnonotus goiavier (*Scopoli, 1786)	Pycnonotidae	LC/TL	1.28	1.35	2.63
Kutilang	*Pycnonotus aurigaster* (Vieillot, 1818)	Pycnonotidae	LC/TL	3.85	3.38	7.22
Kacamata jawa	*Zosterops flavus* (Horsfield, 1821)	Zosteropidae	NT/TL	2.56	1.35	3.92
Kacamatan biasa	*Zosterops palpebrosus* (Temminck, *1824)*	Zosteropidae	LC/TL	1.28	1.35	2.63
Alap-alap kecil	*Microhierax fringillarius* (Drapiez, *1824)*	Falconidae	LC/L	1.28	0.68	1.96
Burung cabe	*Dicaeum agile* (Tickell, 1833)	Dicaeidae	LC/TL	3.85	2.03	5.87
Puyuh tegalan	*Turnix sylvaticus* (Desfontaines, 1789)	Turnicidae	LC/TL	1.28	0.68	1.96
Cekakak sungai	*Todiramphus chloris* (Boddaert, 1783)	Halcyonidae	LC/L	2.56	1.35	3.92
			Total	100.00	100.00	200.00

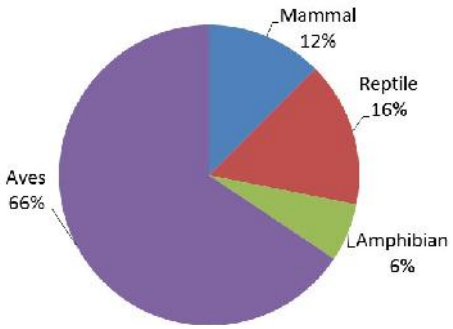

Figure 2. Composition of faunal diversity in Biodiversity Park of Ciherang

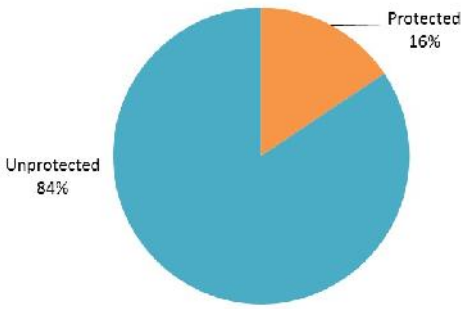

Figure 4. Protection status of fauna in Biodiversity Park of Ciherang

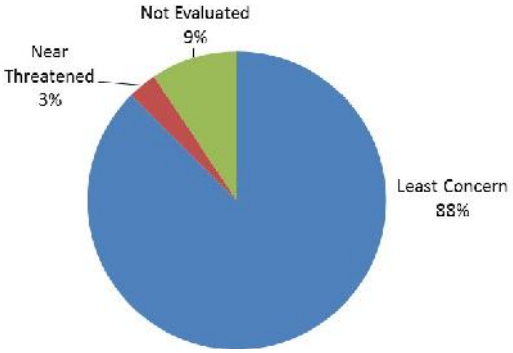

Figure 3. Red List status of fauna in Biodiversity Park of Ciherang

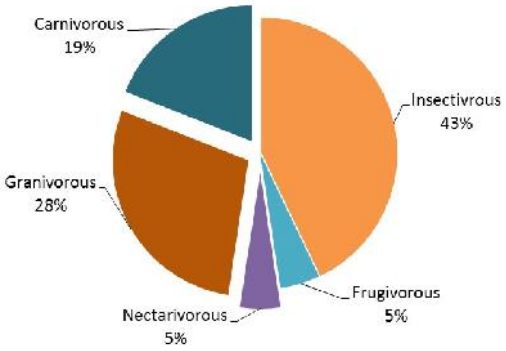

Figure 5. Composition of feeding guilds of the bird community in Biodiversity Park of Ciherang

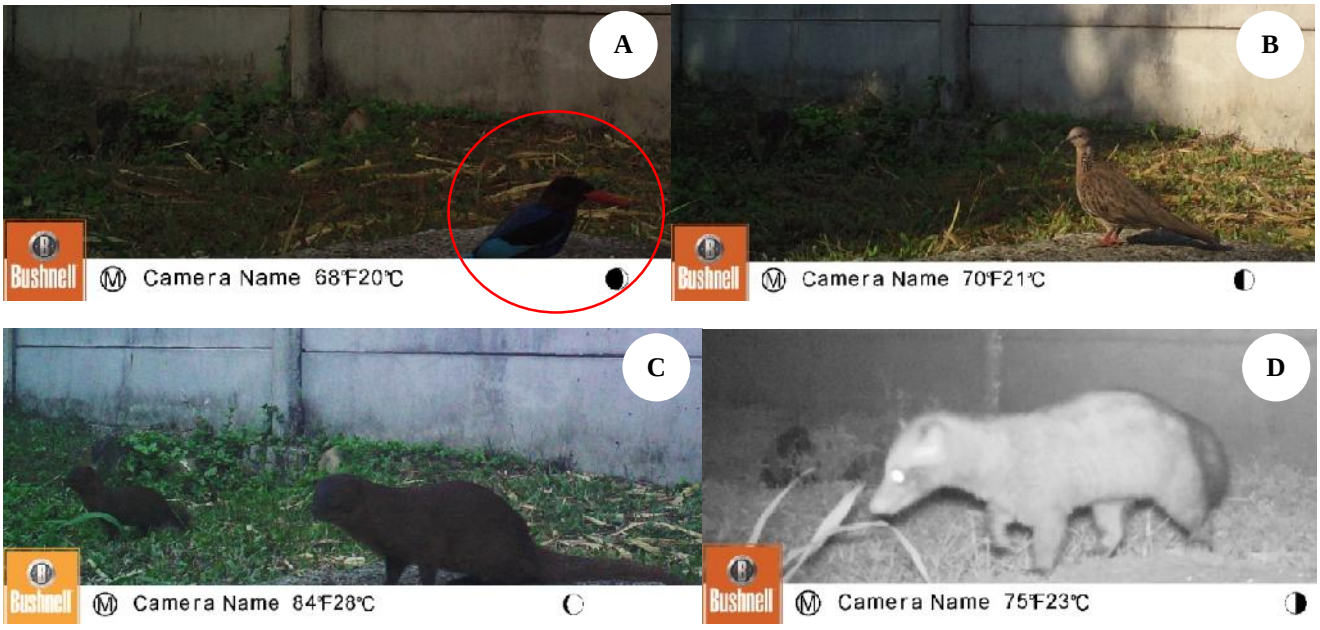

Figure 6. Some fauna species which have been caught on camera traps at the Biodiversity Park of Ciherang. A. *Halcyon cyanoventris* Vieillot, B. *Spilopelia chinensis* Scopoli, C. *Herpestes javanicus javanicus* É. Geoffroy Saint-Hilaire, D. *Paradoxurus hermaphroditus* Pallas

These indices are relatively high for a habitat situated in the middle of human populations and an industrial environment. For comparison, the diversity index of bird community in Biodiversity Park of Aqua Danon Lido is 1.92 (Gunawan and Sugiarti 2015b) and in Aqua Danone Mekarsari Biodiversity park is 2.22 (Gunawan and Sugiarti 2015c).

In accordance with feeding guilds, the insectivorous birds are dominant (42%) in the Biodiversity Park of Aqua Danone Ciherang (Figure 5). This fact means that the area is most suitable for insectivorous birds. At the initial stage of the vegetation succession, the dominant habitat is usually open shrub and grass land, dominated by young trees with not much flowering or fruit production. Open land with shrubs and grasses is a suitable habitat for many species of insects such as grasshoppers, butterflies, moths, and dragonflies, as well as spiders. Insectivorous birds that feed on harmful insects and other pests in agro-ecosystems are beneficial to agriculturists (Rajashekara and Venkaesha 2014). Insectivorous birds act as important biological control agents of insect pests in agriculture, floriculture, horticulture and forests (Thakur et al. 2010).

Granivorous birds are also abundant (28%), but most of the granivorous birds feed on small seeds of wild shrubs and on ferns, which are still available in the Biodiversity Park. The impact of granivorous birds on any ecosystem can be viewed from different points but the best known impact of granivorous birds is an economical one. Granivorous birds in fact cause some damage to cultivated plants and, therefore, to primary production (Turcek 2010). On the other side, granivorous birds, especially the small sized species, will probably be the only free-living, surviving birds on cultivated land and in an environment dominated by man in the next centuries, then the importance of these birds is even more evident (Turcek 2010).

Carnivorous birds represented 19% of the bird community in the Biodiversity Park of Aqua Danone Ciherang. Carnivorous birds feed on other fauna including small mammals, birds, reptiles, fishes, etc. All carnivorous birds are protected by law due to their role as predators in the food chain of ecosystems (Ministry of Forestry 1999).

Nectarivorous birds are also protected due to their role as pollination agents of flowering plants (Ministry of Forestry 1999). Interactions between honeyeaters and flora may have important ecological consequences for native forest communities. Drastic reduction in the abundance and diversity of honeyeaters may be limiting the regenerative capacity of a wide range of native flowering species (Anderson 2003).

Spilopelia chinensis Scopoli is the only frugivorous bird (i.e. feeding purely on fruits) in the Biodiversity Park of Aqua Danone Ciherang. *Ficus benjamina* L. is the main source of food for *Spilopelia chinensis.* Most of the plants in the Biodiversity Park are not producing fruits yet, so the carrying capacity of the habitat for supporting frugivorous birds is still limited. Frugivorous birds play important role as seed dispersal agent in an ecosystem. Frugivore seed dispersal is a common phenomenon in many ecosystems (Wutherich et al. 2001)

Cinnyris jugularis Linnaeus is the only nectarivorous bird in Aqua Danone Ciherang Biodiversity Park. This species mainly feeds on *Heliconia psittacorum* L.f. which is available in the Biodiversity Park. In the Biodiversity Park of Aqua Danone Ciherang, there are some flowering plants such as *Spathodea campanulata* P.Beauv, *Delonix regia* (Hook.) Raf, *Calliandra tetragona* (Willd.) Benth, *Erythrina crista-galli* L, *Bauhinia purpurea* L, and *Heliconia psittacorum* L.f.

Monitoring the bird community is important because birds can be used as indicators of environmental changes (Furness et al. 1994). Birds have been proposed, assessed or used as indicator species for a range of environmental parameters, including biodiversity and species richness; environmental contamination by pollutants; the condition of ecosystems; ecosystem responses to disturbances and processes including urban expansion; replacement of endemic ecosystems with plantations and habitat restoration programs (Chambers 2008).

Bird numbers and diversity indices can be used as indicators in monitoring pollutants and radionuclide contamination. Birds also can be used as indicators of change in water quality and change in marine prey stocks (Furness et al. 1994) and of course good indicators of wetland status and change (Mistry et al. 2008). Birds are also good Indicators of the Effects of Climate Change (Lemoine et al. 2007).Birds have advantages as indicators due to their easiness to identification, their classification and systematics are well established, so there is a little risk of monitoring being confounded by uncertainties regarding the identities of, or relationship between the species being studied (Furness et al. 1994).

Birds in most cases are good indicators of general change in the quality and quantity of habitat (Furness et al. 1994) including forests (Canterbury et al. 2000) and urban areas or mosaics (Jedicke 2000). Hence, birds are good indicator for ecosystem restoration program (Fredrick et al. 2009). The fact that bird populations and communities change as habitats are altered can be a basic indicator for the monitoring and evaluation of Biodiversity Parks. When diversity of birds increases, we can conclude that the Biodiversity Park has improved in its quality of bird habitat, which means that the quality of the ecosystem is getting better. This means that Biodiversity Park which located in the midst of agriculture land can provide ecosystem services such as pest control, pollination and soil fertility (Power 2010).

In conclusion, faunal diversity in the Biodiversity Park of Aqua Danone Ciherang is 2.82. There were found 25 families of fauna consisting of 28 genera and 32 species. Birds were dominant representing 66% of the total number of faunal species identified; reptiles were 16% of the total, mammals 12% and amphibians 6% of the total. The Biodiversity Park has successfully provided habitat for all tropic levels of the food chain i.e. herbivores, carnivores, omnivores and scavengers. The existence of birds in the Biodiversity Park indicates that there is improvement of habitat.

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Short Communication: Genetic diversity and conservation strategy considerations for highly valuable medicinal tree of *Taxus sumatrana* in Indonesia

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Abstract. *Rachmat HH, Subiakto A, Kamiya K. 2016. Genetic diversity and conservation strategy considerations for highly valuable medicinal tree of* Taxus sumatrana *in Indonesia. Biodiversitas 17*: *487-491.* Genetic variation is considered to be the key factor for long-term survival of the species. The recognition of the existing genetic diversity is the preliminary phase in development of an effective strategy for conservation of forest tree species. *Taxus sumatrana* or is confined to grow naturally only in Asia, it is a rare and endangered species that in several Asian countries needs both ex situ and in situ protection program. In its natural distribution, *T. sumatrana* is the only *Taxus* species that reached its southernmost distribution to Sumatran forest-Indonesia and locally named as Sumatran Yew. The objective of this research was to determine the genetic variation of *T. sumatrana* as baseline information for designing conservation strategy of the species. Leaves samples were collected from two natural population of *T. sumatrana* in Mt. Kerinci (Sungai Penuh, Jambi) and Mt. Dempo (Pagaralam, South Sumatra), both sites are located along Bukit Barisan Mountain Ranges of Sumatra. We sequenced two non-coding chloroplast DNA (cpDNA) regions of *trnL-trnF* and *psbC-trnS* that each yielded 808 bp and 1092 bp, and *rbcL gene* of 523 bp, in which the total length covered 2423 bp. Surprisingly, we found no variation for all individuals and population, which means that the species is similar and both populations are not genetically structured. This study also revealed on how a proper conservation strategy should be practiced for the species as we know that without a sufficient amount of genetic variation, a population cannot evolve in response to changing environmental conditions. In situ conservation program is a must that can maintain the existence of the species while at the same time keeping the sustainability of the entire systems; in other side ex situ conservation strategy can take place as an additional effort to secure the genetic resources in case of the catastrophic events that might diminish their limited natural habitat.

Key words: Genetic variation, *cpDNA, rbcL* gene, Sumatran Yew

INTRODUCTION

Plants of the genus *Taxus* are sources of a number of physiologically and pharmacologically active compounds of different classes, especially the anti-cancer paclitaxel and many other taxane derivatives. The species of *Taxus* are more geographically than morphologically separable. The genus *Taxus* has included eight geographically defined species; including *T. sumatrana* (Miq.) de Laub (Spjut 2007). *T. sumatrana* (Miq.) de Laub, locally names as Sumatran Yew, is naturally distributed in Taiwan, Sulawesi, and reached its southernmost distribution to Sumatra mainland (de Laubenfels 1988). In Sumatra, *T. sumatrana* is an endangered conifer with a scattered distribution. The highly valuable timber is usually distributed in shady valleys and slopes at high altitudes, e.g at 1700- 2200 m asl. in Mt. Kerinci-Jambi.

Plants within the Genus of *Taxus* are highly known for their taxol production. Taxol is a blockbuster anticancer drug which is widely used for clinical application against different types of cancer (Zhou et al. 2010). The drug is known to bind to microtubules and essentially freeze them in place, prevent them from separating the chromosomes when cell divides. This mechanism will kill dividing cells, particularly cancer cells (Weaver 2014). In other part of the world, species in the Genus have been facing serious threats because of human overexploitation and habitat destruction that lead to the decline and fragmentation of populations. Yet, there have been any reports of the exploitation for its valuable bark or other tree parts. However, increasing pressure on forest and land and also their narrow and scattered distribution at only several spots have made the species to be the priority for conservation (Hidayat et al. 2014). Field exploration on the potency and distribution of *T. sumatrana* (Rachmat 2008; Hidayat et al. 2013) in Mt. Kerinci and Mt. Dempo of Sumatra, Indonesia found that this species occurred in a narrow habitat range with low numbers of mature individuals consisting of 13-19.

Genetic variation refers to all the different gene versions that are present in a population. Over long time scales, decreased genetic variation can be a problem for a population because genetic variation is the raw material of

evolution (Fisher 1930). Furthermore, loss of genetic diversity in small populations of threatened species is predicted to reduce their ability to evolve, and increase their extinction risk in response to environmental change. While experimental evidence validates this prediction, there are only a few examples where extinctions of natural populations can be directly attributed to lack of genetic variation (Farkham et al. 2004).

Understanding genetic variation within and between populations is essential for the establishment of effective and efficient conservation practices for rare and or endangered species. Several aspects of conservation biology, such as loss of genetic diversity in conservation programs and restoration of threatened population, can only be addressed by detailed population genetic studies (Hamrick and Godt 1996). The objectives of this study are to examine the levels of cpDNA (chloroplast DNA) variation and genetic differentiation among *T. sumatrana* population growing in Sumatra. This molecular information will provide effective and efficient measures for protecting the species.

MATERIALS AND METHODS

Plant material

Leaf samples of adult trees were collected from two populations of *T. sumatrana* in Mt. Kerinci (Sungai Penuh, Jambi) and Mt. Dempo (Pagaralam, South Sumatra); both sites are located along The Bukit Barisan Mountain Ranges of Sumatra, Indonesia. We took leaf samples from adult trees with diameters of over 25 cm at breast height, and the minimum distance between individuals sampled was 50 m. In total, 27 individuals of *T. sumatrana* were analyzed in this study: 14 individuals were sampled from Mt. Kerinci and 13 individuals from Mt. Dempo.

Loci studied

At the beginning we evaluated the performance of six candidate plastid DNA regions those: *trnT-trnL*, *trnL-trnF* (Taberlet et al. 1991), *psbC-trnS*, *tnH-trnK* (Demesure et al. 1995), *trnH-psbA* (Kress and Erickson 2007) and *rbcL gene* (Hasebe et al. 1994). However, only three loci gave a good amplified product, those were *trnL-trnF*, *psbC-trnS* and *rbcL* and used for further analysis.

DNA isolation, amplification, and sequencing

Genomic DNA was isolated from adult leaves following the company procedure using DNeasy® Plant Mini Kit (Qiagen, Germany). PCR amplifications were performed in a volume of 20 µl containing 10 ng of genomic DNA, 5 pmol of each forward and backward primer, and 10 µl of Go Taq® Hot Start Colourless Master Mix (Promega, Madison, WI, USA) according to the manufacturer's instructions. Initial denaturation was performed at 95°C for 2 min, followed by 30-35 cycles of denaturation at 95 °C for 1 min, annealing 52°C for *trnL-trnF* and 56°C for *psbC-trnS* and *rbcL* and polymerization at 72°C for 2 min, and final extension at 72°C for 7 min. Prior to sequencing, the PCR products were purified using rAPid Alkaline Phosphatase™ (Roche, Germany) and Exonuclease I (New England Biolabs, Ipswich, MA, USA). Purified products were directly sequenced on both strands using an ABI Prism 3100 automatic sequencer (Applied Biosystems, Foster City, CA, USA).

Data analysis

DNA sequences were checked visually, forward and reverse traces were assembled using the ATGC program (Genetyx Corporation, Japan). Single nucleotide polymorphism would clearly be distinguishable from the electropherograms showed for each sequences in ATGC. However it was more apparent when all sequences were exported into fasta file and read in BioEdit (Hall 1999). Further analysis could not be executed from all loci studied because of the absence of nucleotide variation from all individuals in both populations.

RESULTS AND DISCUSSION

Sequences of 808, 1092 and 523 bp (2423 bp in total) were determined, for two non-coding regions of cpDNA, *trn*L-*trn*F, *psb*C-*trn*S, and *rbcL* gene respectively. The sequence for each of the region are described below.

(i) sequence of *trn*L-t*rn*F region of all studied *T. sumatrana* individuals:

```
CCTTGGTATGGAAACTTACTAAGTGATAGCTTCCAAATA
CAGGGGAACCCTGGAATATTTTGAATGGGCAATCCTGAT
CCAAATCCGTATTATAGGAACAATAATTTTATTTTCTAG
AAAAGGGATAGGTGCAGAGACTCAACGGAAGATATTCTA
ACGACTTAATATCATTTGAATTTGAACCAATATTCTATC
TACAGAGTGTAGTATGTTATTGAAAACTTTGAAGTGTTC
TGTATCATCGTTAAAACTTGTTTCAACGATTAGAACTTG
AGTTGTTCTAGGCTTGCCTAGCTTAATGAATACTTAATT
AAAGTAATTCAATTAAGAAATAAATAGAATTTATTCCAT
TTTTGAATTATTGGACGAGGATAAAGATAGAGTCCAATT
CTACATGTAAATGCCAACAACAACAATGCAAATTGCAGT
AGTCGGAAAATCCGTTGGTTTTATAAACCGTGAGGGTTC
AAGTCCCTCTATCCCCAGGTGTATTTCCGAATTAAAGAA
AGATCAAATATTATTACTCTTGACAATTTTATAAGCAAT
CCAGAATATAGAGCTATATTCCCATAAATTTAGAAAGGT
TGATCGTAAGATCAACTCATACATTTTGTCAGATATAAC
ATTTGTGTATGTATAATTGTATTATACATACAATTTAAA
TTTATAATAGAAATTGATAATGGTAACTTACCAATCCAA
AAGTATAATTTAAAAAGGGAAATAAAAAAAGGATTTTCT
TTTGTCTTTTTAGTTGACCTGAGCTCAGGTTCTGCGCTA
GGATGATAAACAGGGAAGAGTCGGGATAGCTC;
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(ii) Sequence of *psbC-trnS* region of all studied *T. sumatrana* individuals:

```
TAAATTACTTGGGGCTCACGTGGCTCATGCCGGATTAAT
TGTATTCTGGGCTGGAGCAATGAATCTATTTGAAGTGGC
TCATTTTGTATCGGAAAAGCCTATGTATGAACAAGGATT
GATTTTACTTCCCCATCTAGCTACTTTAGGATGGGGAGT
CGGTCCTGGTGGGGAAATTGTGGACACTTTTCCCTATTT
TGTATCTGGGGTACTTCACTTAATTTCTTCTGCAGTTTT
AGGTTTTGGTGGTATTTATCACGCACTAATCGGACCCGA
AACTTTAGAAGAATCTTTTCCATTTTTTGGTTATGTCTG
GAAAGATAGAAATAAAATGACTACAATTTTAGGTATTCA
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CTTAATTTTGCTAGGTGTTGGTGCTTTTCTTCTAGTCTT
CAAGGCTTTGTATTTTGGTGGCATATATGATACCTGGGC
TCCTGGTGGTGGAGATGTAAGAAAAATTATGAACCTTAC
GCTTAACCCAGTTGCTATATTTGGTTATTTGCTCAAGTC
TCCTTTTGGAGGAGAGGGATGGATTGTTAGCGTGGACAA
TCTAGAAGATATAATCGGAGGACATGTATGGTTAGGTTC
CACCGAGCAATAAGGACTCAGTTGGAAAAAAATATCGAA
GGATCCTGCTATCCCGTCTCCCAACATGGTAAATGAAAA
GAAATTAGATGAATTATGATTTTATCTAGTTTATTTTAT
CGTTTAATTAAGAGGGGTCATGGAAAGAACAGGTTCAAA
ATCACGATCAATTCCTTTCTCAAATCCTGCTGCAGCTGC
GCGAGCTCTTCCTGCATGCCACAAATGACCCACGAAAAA
GAAAAATCCTAGAACAAAATGAGAGGTGGCTAACCAACT
TCGGGGTGATACATAATTCACCGCATTAATCTCGGTAGC
TACACCACCCACAGAATTTAAAGAACCTAAAGGAGCATG
AGTCATATATTCCGCTGAACGTCGTTCTTGCCAGGGTTG
TATGTCCTTTTTCAACTTACTCAGGTCCAAACCATTAGG
ACCCCTTAGAGGTTCCAACCAGGGAGCACGAAGATCCCA
AAAACGCATTGTTTCTCCTCCGAAGATAATTTCTCCAGT
;

(iii) Sequence result of *rbcL* gene of all studied *T. sumatrana* individuals:

CGGCTCTACCATAATTTTTGGCAGATAGGCCCAATTTTG
GTTTTATAGTACATCCCAGCAAAGGACGACCATATTTGT
TTAATTTATCTCTTTCCACTTGGATACCATGTGGTGGGC
CTTGGAAAGTTTTTGAATAAGCAGGAGGAATTCGTAGAT
CTTCCAGACGTAGAGCTCGTAGGGCTTTGAATCCAAAGA
CATTACCTACAATGGAAGTGAACAGGTTAGTCACAGAAC
CTTCTTCGAAAAGATCTAAGGGGTAAGCTACATAGGCAA
TAAATTGATTTTCCTCTCCAGGAACGGGTTCGATATCAT
AGCATCGTCCCTTGTAACGATCAAGACTGGTAAGTCCAT
CGGTCCAAACAGTGGTCCATGTACCAGTGGAAGATTCGG
CAGCTACTGCTGCTCCCGCTTCCTCGGGGGGCACTCCCG
GTTGAGGAGTGACTCGGAATGCTGCCAAGATATCAGTAT
CTTTGGTCTGATATTGTGGAGTATAATAAGTTAGTCTGT
AATCTTTAACACCAGC.

There were no variants for all individuals and population studied. *T. sumatrana* growing in Sumatra-Indonesia occupies specific sites and very restricted with clumped or scattered distribution. Species with this kind of characteristic would show low levels of genetic variation as compared to other species in the genus with wider distributions. This is indeed the case for *T. sumatrana*. There were no variations observed both the population and species level. Our result conformed to Hamrick and Godt (1996) who stated small population size tends to have low level of genetic diversity. Our study also supports the general expectation of reduced genetic diversity in the species with a narrow geographic distribution (Hamrick et al. 1992).

Genetic structure within and between populations is important for developing a conservation strategy for endangered species, especially if not all populations can be protected. Species with low levels of population structure could be simplified, as the loss of single population may have little impact on the species-wide genetic diversity. Molecular and morphological studies of *Taxus* have distinguished genotypes that differentiate (i) individuals within populations (Collins et al. 2003; Lewandowski et al. 1995; Spjut 2007), (ii) distinct populations within geographic regions (El-Kassaby and Yanchuk 1994; Li et al. 2006, Zu et al. 2006; Spjut 2007; Zarek 2009), and (iii) alleged geographically distinct species (Collins et al. 2003; Spjut 2007). However, little attempt has been made to determine genetic variation of the *Taxus* growing in their southernmost distribution, *T. sumatrana* in Indonesia.

Genetic diversity is attributed to the capacity of long term survival of the species. Low genetic diversity in the species will increase inbreeding rate, while the reduction of genetic diversity will affect the adaptability level to the environment change (Furlan et al. 2012). Our study revealed that no variation and no population differentiation could be detected in *T. sumatrana* based on cpDNA variation, suggesting that both population from Mt. Kerinci and Mt. Dempo harbored similar genetic characteristics. It could be that the low level of genetic variation is specific in the *cpDNA* regions. However, among plant DNA regions, non-coding regions, such as the chloroplast markers *trnH-psbA* and *trnL-trnF* usually exhibit high levels of variation, including indel polymorphism (Graham et al. 2000), and for several cases can provide good capacity even for species identification (Hollingsworth et al. 2011; Taberlet et al. 2007). Moreover, in a previous study of DNA barcoding for Eurasian *Taxus* species based on five DNA regions (*rbcL, matK, trnL-trnF, trnH-psbA* and *ITS*), eleven species were clearly identified (Liu et al. 2011). Population genetic study of several *Taxus* species were also recorded using *trnL-trnF* and *petA-psbE* showed significant genetic variation (Gao et al. 2007; Liu et al. 2013; Poudel et al. 2012; Cheng et al. 2015). This observation suggests that the extremely no genetic variation in the *cpDNA* regions examined here is specific for *T. sumatrana* since those loci yielded some extent of genetic variation when assessed to other *Taxus* and non *Taxus* species.

In Sumatra, Indonesia, the species grows naturally inside the protected area or nature reserved. We can simply determine that this condition was strong enough to conserve the species. However, this fact does not reflect factual condition because protected areas and or nature reserves in actual condition are not fully free from disturbances. There are high pressures on habitat destruction of both protected and nature reserves. In this case, effort to conserve outside its natural habitat is worth to be considered and those implemented within the concepts of ex situ conservation strategy. In Indonesia we can see ex situ conservation effort for *T. sumatrana* has been conducted in Cibodas Botanical Garden, West Java.

When habitat is highly narrow and limited, in situ conservation effort is a compulsory to carry out with emphasizing on genetic considerations. Many plants, especially rare taxa, exhibit microhabitat preferences (Maliakal-Witt et al. 2005). When these microhabitats occur in the landscape in discrete and small-scale patches, along the time they can create opportunities for genetic divergence at a small spatial scale. To avoid this phenomenon for the narrowly scattered-clumped distribution of the *T. sumatrana*, maintaining the connectivity among clumps is a must. This will allow gene flow and might be impacted to maintain viability or even to

increase population size. Intact and free-perturbation habitat need to be secured. In a simple word, in situ conservation is the core strategy for species conservation. How this should me managed properly to assure the species conservation would require several actions in the field as described below.

Soft flesh fruit of *Taxus* species (aril) are highly preferred by certain birds and rodents. As seeds are the main key for natural regeneration, predatory mechanism should be checked scientifically. This will give insight to how and what actions need to overcome the predatory problems. Rarity to find natural seedling during field sampling indicated the need of special concern on this aspect. In this case, it is clearly seen the need of additional treatments to support natural regeneration. Related to their natural regeneration capability, soil condition is one of the important factors that need to be taken into account as soil moisture can be extremely limiting factor for seedling survival. Genetic variation is one of the most important factors for the survival of the population. In case of less or even no variation, artificial regeneration is essential. In addition that the population size of *T. sumatrana* known to be small, limited, and showed narrow habitat range, artificial regeneration could be an important way to increase the population size and yet increase the genetic variation. The success of regeneration both natural regeneration and artificial regeneration should be evaluated by regeneration survey in at least 5-10 year cycles.

Dharr et al. (2006) stated that appropriate light and microclimatic condition are needed to maintain yew population. To maintain the light availability, a continuous selective thinning reducing competition with other tree species is advocated to improve the population status. This also can be applicable for *T. sumatrana* growing in Indonesia when they exhibit similar ecological niche, the trees usually fill the spots on shady ridges of the hills. Artificial management on promoting light availability might support species growth and lessen the competition with others trees.

If in certain condition the trees need to be cut, it should be done at least 25 cm above from the ground as in general *Taxus* species can produce more sprouting buds from that origin. During field surveys it is commonly found that many sprouting comes from the fallen branches and stems. Actually this condition is beneficial, especially when alternative propagation by cutting is considered to carry out for producing new plants.

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Identification of growth hormone gene variation in exon region at Indonesian Local Cattle based on PCR-SSCP method

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Abstract. *Rahmatullah SN, Jakaria, Noor RR. 2016. Identification of growth hormone gene variation in exon region at Indonesian Local Cattle based on PCR-SSCP method. Biodiversitas 17*: *492-497.* The aim of this study was to identify the polymorphisms of the growth hormone gene (GH) of Indonesian local cattle as well as two exotic cattle, as the outside group, using polymerase chain reaction and single strand conformation polymorphism (PCR-SSCP) and using five primers DNA to identify polymorphism of GH gene. Twenty DNA samples of each Indonesian local cattle, consists of Bali, Pesisir, Madura and Katingan, and ten DNA samples of Simmental and Limousine cattle were used. The results showed that the polymorphism of the GH gene was found in three exons which are exon 1, 2 and 5 for Indonesian local cattle except for the Bali and Madura cattle that showed polymorphism was only at exon 2. Bali and Madura cattle also showed monomorphism in exon 3 and 4. On the other hand, the exotic breed showed the polymorphism in all exons, except for exon 2 in Simmental cattle which was found to be monomorphic.

Keywords: Growth hormone gene, Indonesian local cattle, PCR-SSCP, polymorphism

INTRODUCTION

Indonesia has a diverse local cattle population including Bali, Aceh, Pesisir, Madura and Katingan cattle and their genetic information is still limited. Cattle breeds mentioned above have enormous potential as local genetic resources. There are two main factors that affect performance of local cattle, namely; genetic and environmental factors. From the genetic aspects, there are several genes that have a large influence (major gene) on the properties of economic value. One of the most influential genes is the growth hormone gene for they produce growth hormone (Carnicella et al. 2003). Growth hormone gene (GH) plays an important role as a regulator of feed and nutrient metabolism and absorption in the growth processes (Pawar et al. 2007). In addition GH gene also plays a role in the development of mammary gland cells, lactogenesis and mammary cell proliferation (Lagziel et al. 2000). The other function of GH gene is as a candidate gene to associate with sperm quality traits and polymorphisms of GH gene that could be potential markers for testicular growth after puberty and the on set puberty in bulls (Gorbani et al. 2009; Unanian et al. 2002)

The structure of the growth hormone gene consists of 5 exons and 4 introns and is located on chromosome 19 in the bovine (Hediger et al. 1990). Exon is segment of the eukaryotic gene that encodes a portion of the final product of the gene that especially produces amino acid (protein), whereas intron is part of the genes that is not translated at the time of formation of amino acid/protein and, until recent day, part of intron is unknown for its function (Nicholas 2009).

Exploration and information on the diversity of the gene growth hormone (GH) in exons, either in exon 1, 2, 3, 4 or exon 5, on the Indonesian local cattle are still limited. It is important to know this genetic data that is expected to be used in breeding and developmental programs of Indonesia local cattle. This short report determine the diversity of the growth hormone (GH) gene in exons is by using PCR-SSCP (Polymerase Chain Reaction-Single Strand conformation Polymorphism) in Indonesian local cattle.

MATERIALS AND METHODS

Blood samples used were from the Indonesia local cattle namely Bali, Madura, Pesisir and Katingan cattle. Twenty samples were taken from each Indonesian local cattle, while the ten samples were from each of Simmental and Limousin cattle (imported cattle) which are used as comparing cattle. These samples were collected and stored at the Laboratory of Molecular Genetics and Animal Breeding, Faculty of Animal Science, Institut Pertanian Bogor, West Java, Indonesia.

Primer amplification of GH gene in exons 1, 2, 3, 4, and 5 uses base primer Lagziel et al. (1996) and modified primer of Kioka et al. (1989). It can be seeing in Table 1.

Table 1. Position, fragment length and primer sequences used for amplification of GH gene.

Exon	Fragment length (bp)	Primer sequence [a]
1	315	F: 5′-TGG TGG CAG TGG AGA CGG GA-3′
		R: 5′-GGA CAC GCG AAT GGA GGG GA-3′
2	283	F: 5′-GCC CTG CTC TGC CTG CCC TG-3′
		R: 5′-CCC CAC ACA CCC CCG TTT CT-3′
3	158	F: 5′-GTG TGT TCT CCC CCC AGG AG-3′
		R: 5′-CTC GGT CCT AGG TGG CCA CT-3′
4	198	F: 5′-GGA AGG GAC CCA ACA ATG CCA-3′
		R: 5′-CTG CCA GCA GGA CTT GGA GC-3′
5	392	F: 5′-GCT GCT CCT GAG GGC CCT TC-3′
		R: 5′-CCA CCC CAC CCC CCA GAA TA-3′

The extraction of DNA from blood samples followed the standard method of phenol chloroform (Sambrook et al. 1989) that have been modified. There were five sets of primers used for amplification of GH gene fragment in the exons (Table 1). Mix reagent used 1 ml samples of DNA, 25 pmol of primers (forward-reverse), 200 µM dNTP mixture, 1 mM $MgCl_2$, and 0.5 units of *Taq* polymerase and buffer with a total volume of 12 ml. PCR conditions for amplification were as follows: denaturation at a temperature of 95°C for 5 minutes, annealing at a temperature of 60-66°C for 45 seconds and extension at 72°C for 1 minute, and extension end at 72°C for 5 minutes. DNA amplification was cycled 35 times. PCR products were then electrophorezed on 6% of polyacrylamide gel.

SSCP analysis (Single Strand Conformation Polymorphism)

Application of the Single Strand Conformation Polymorphism (SSCP) is a simple and reliable yet sensitive technique because in this technique, the relation of the electrophoretic mobility of a single strand DNA is important for detection of mutation in genomic DNA. SSCP is a powerful method in screening single strand and much more sensitive to the replication of DNA (Zhu et al. 2006). Identification (genotyping) of PCR products with SSCP technique (single strand conformation polymorphism) was performed with 12 mL of PCR product mixed with 10 mL loading dye (98% formamide, 10 mM EDTA, 0.025% bromophenol blue, 0.025% xylene-cyanol), then denatured at 95^0C for 5 minutes. Samples were cooled immediately in iced water for 3 minutes, then electrophorezed on the SSCP gel polyacrylamide 10-12%. Electrophoresis was carried out using Protean II xi cells (Bio-Rad) at 200-300 V with temperature of 5^0C for 8-14 h at 0.5 x TBE buffer solutions. After gel has undergone electrophoresis and genotyping, the results based on the SSCP banding pattern were appeared on polyacrylamide gel that has undergone silver staining method (Byun et al. 2009) with some modification.

Data analysis

Frequencies of allele and genotype

Frequencies of allele and genotype are calculated using the method of Nei and Kumar (2000) i.e.:

Heterozygosity value

Genetic diversity (genetic variability) is done by estimating the frequency of observed heterozygosity (Ho) Weir (1996).

Frequencies of expected heterozygosity

$$H_e = 1 - \sum_{1=1}^{n} p_{1i}^2$$

Note:

H_e = frequencies of expected heterozygosity

P_{1i} = frequencies of allele number individuals to-iin locus 1

n = number of sample in locus-1

Expected heterozygosity variance

$$V_{s1}(H_e) = \frac{2}{2_n(2n-1)}\left\{2(2_n-2)\left(\sum x_i^3 - \left(\sum x_i^2\right)^2\right) + \sum x_i^2 - \left(\sum x_i^2\right)^2\right\}$$

Note:

V_{sl} (H_e) = expected heterozygosity variant

x_i = frequency of gene to-i

Standard error = $\sqrt{V_{s1}(H_e)}$ (Weir 1996).

RESULTS AND DISCUSSION

Amplification of GH gene

The results of study on genetic markers could be applied in breeding since the use of GH gene is as molecular assisted selection to increase daily gain production and improve milk production and composition (Bastos et al. 2001). GH gene amplification results in fragment length shown in each exon are as follows: exon 1: 315 bp, exon 2: 283 bp, exon 3: 158 bp, exon 4: 198 bp, and exon 5: 392 bp. Results of GH gene PCR amplification product in each exon is showed in Figure 1. Based on the results of amplification performed on each fragment in the GH gene, annealing temperature found in exon 1 is at temperature of 62°C, exon 2 is at temperature of 66°C, exon 3 is at temperature of 61°C, exon 4 and exon 5 are at temperature of 60^0C, whereas the time needed by five exon was 45 seconds for each. The results are different from those performed by Yao et al. (1996), Lagziel et al. (1996), Malveiro et al. (2001), Muhaghegh et al. (2006) that the temperature and time of annealing in exon 1, 2, 3, 4and 5 are, respectively, 63°C for 50 seconds, 68°C for 50 seconds, 60°C for 30 seconds, 70^0 C for 30 seconds, and 63°C for 50 seconds. Amplification of gene fragments had succeed and was determined by: the condition of annealing to DNA target, PCR machine conditions and reagents used, in addition, the type/breeds of cattle delivered different amplification gene fragment process (Viljoen et al. 2005). Amplification of GH gene fragment product was subjected to identify variations in number of bands of DNA sample (Muhaghegh et al. 2006).

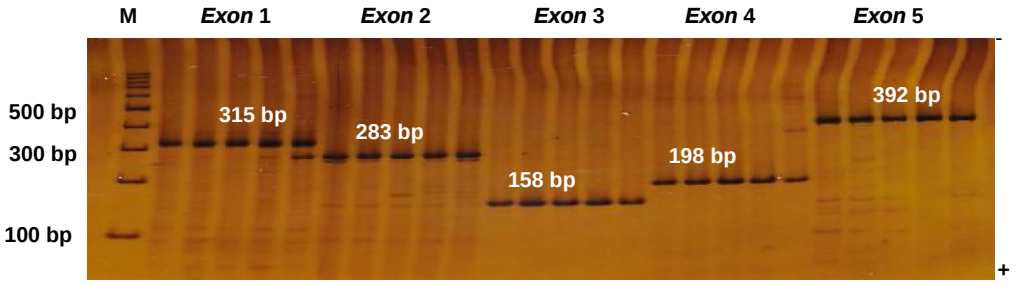

Figure 1. Results of PCR amplification products fragments of GH gene on 6% polyacrylamide gel. M=Marker 100 bp

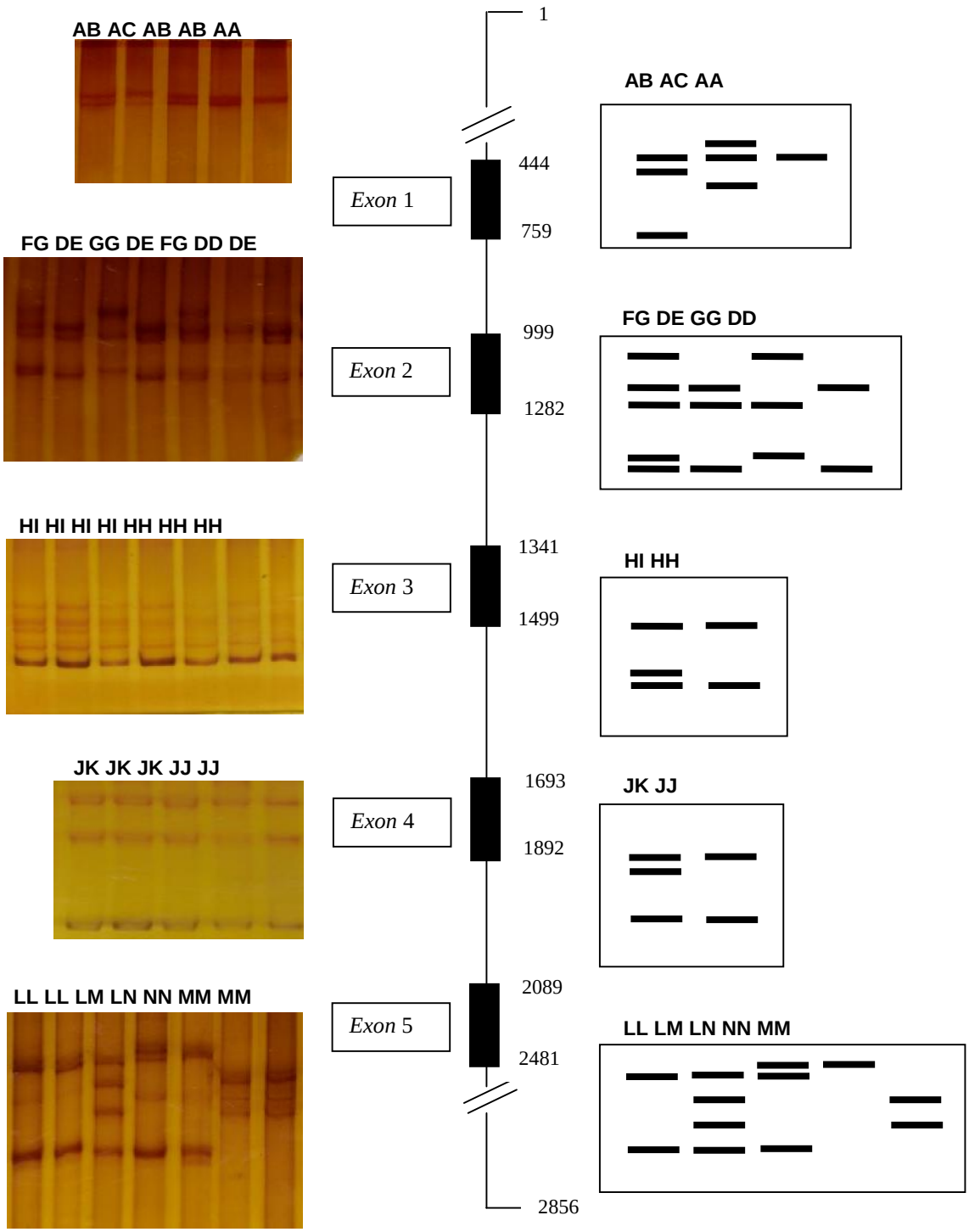

Figure 2. Pattern genotype of GH gene which was found in Indonesian local cattle

Frequencies of genotype and allele GH gene

Genotype and frequencies of genotype in GH gene obtained from the results of this study indicate that there is variation in range of genotypes and frequencies of genotype on the Indonesian local cattle (Bali, Pesisir, Madura and Katingan cattle) and imports cattle (Simmental and Limousin cattle) (Figure 2 and Table 2). The number of genotypes found in the GH gene exon 1, 2, 3, 4 and 5 are, respectively, three genotypes (AA, AB, AC), four genotypes (FG, DE, GG, DD), two genotypes (HH, HI), two genotype (JJ, JK), and five genotype (LL, LM, LN, NN, MM).

Several alleles are found in each exon and it is shown in (Table 3). The number of alleles found in Indonesian local cattle for each exon varies from 1 - 4 alleles, whereas the Simmental and Limousin cattle have 1-3 alleles. Several studies have shown that the number of alleles found in FH cattle is 2-3 alleles (Lagziel et al. 1996; Yao et al. 1996), while Malveiro et al. (2001) reported 2-6 alleles at the goat Algarvia in Portugal. The results of this study had shown that alleles variation in the Indonesian local cattle are more than that of imported one, and this is like to be caused by several factors from every domestication center, including the use of Indonesian local cattle and the selection system (Talib 2002).

The Indonesian local cattle had high frequencies with value of 1.000 i.e. in Bali cattle, in exon 1, 3, 4 and 5 of alleles A, D, H, J and L. In imported cattle breeds, there are variations in the value frequencies of allele, especially on allele B, C and D. The highest frequencies for allele B is 0.800 in Limousin cattle in exon 3. In Simmental cattle, allele C in exon 5 has the highest frequencies of allele with 0.300, and the highest frequencies value of allele D is 1.000 in Simmental cattle in exon 2. There is something special about Indonesian local cattle, namely the frequencies of allele A at a Indonesian local cattle (Bali, Pesisir, Madura and Katingan cattle) was the highest compared to the imported cattle from the *Bos taurus* (Simmental and Limousin cattle). Like the Bali cattle, other local cattle like Pesisir, Madura and Katingan cattle are polymorphic in each exon in GH gene. Nei and Kumar (2000) states that an allele in a population said to be polymorphic if it has two or more alleles with frequencies of more than 1%. One cause of the GH gene polymorphisms in several breeds such as the breeds cattle of *Bos taurus* and *Bos indicus* is the occurrence of mutations of amino acid leucine-valine (Leu/Val) (Yardibi et al. 2009; Kovacs et al. 2006) and histidine/arginine (Beauchemin et al. 2006). In bovine breeds, in GH polymorphism, some associations between different GH gene genotypes with production traits, growth traits, milk production have been made and the effects of different genotypes were estimated (Bastos et al. 2001).

Allele and genotype variations of GH gene in cattle that was shown on monomorphic Bali cattle is caused by domestic cattle in tropical regions of Indonesia and Bali cattle is an indicator that the cattle is different from the breed cattle of *Bos taurus* and *Bos indicus*. Bali cattle's adaptability is obtained from natural selection and the effect of the natural proliferating (Talib 2002).

About Pesisir cattle, the results of this study found that there was polymorphic nature of the bovine GH gene of entire exon of Pesisir cattle. This was evident that the frequencies of allele and genotype of GH gene in the four exons of Pesisir cattle having value less than 0.99 except in exon 2 which was monomorphic. GH gene polymorphism was found in samples of Madura cattle was consistent with the results of research of Purwoko et al. (2003), which stated that there were polymorphisms in the GH gene locus 2 which was positioned at 329 bp.

About Katingan cattle, the results of the study found that there were variations in frequencies alleles namely polymorphic in exon 1, 2, 4 and 5, except exon 3 which was monomorphic. Katingan cattle were one of special genetic diversity cattle due to their genetic specific location and they had associated with the other Indonesian local cattle, for example, Bali cattle, Madura cattle. Katingan cattle are predicted as the crossbreeding results of Bali cattle and native cattle in Katingan region in Central Kalimantan Province, Indonesia.

Within the Indonesian local cattle (Bali, Pesisir, Madura and Katingan), the banding and electrophoretic mobility of the population is analyzed and identified in exon (1, 2, 3, 4 and 5), SSCP patterns were observed and it showed that difference in genotype frequency may be influenced by some factors such as genetic drift and location (Muhaghegh et al. 2006).

Degree of heterozygosity

The degree of heterozygosity is the average percentage of heterozygous lociper individual or the average percentage of heterozygous individuals in the population. Estimation of heterozygosity values is important to know so to get an overview of genetic variability and to determine the level of an allele polymorphism (Nei and Kumar 2000). High heterozygosity showed high genetic diversity within a population.

The average value of the highest heterozygosity was found in Pesisir cattle for 0.390, and the lowest was found in cattle of Madura at 0.0800. The highest heterozygosity values found on the import cattle, Limousin cattle, was at 0.720 and the lowest was in Simmental cattle with 0.620. Based on the results obtained (Table 5), it is shown that Bali cattle had the lowest average of heterozygosity when compared to Pesisir, Madura, Katingan, Limousin and Simmental cattle in each exons 1, 3, 4 and 5. Heterozygosity values obtained from the Indonesian local cattle (Bali, Pesisir, Madura and Katingan cattle) and from the imported cattle (Simmental and Limousin cattle) are polymorphic based on estimations of heterozygosity values. Estimating the value of heterozygosity has important significance namely to know a description of genetic variability (Marson et al. 2005), to know the level of allele polymorphism and the future prospect of population (Falconer and Mackay 1996). The value of heterozygosity could be an indication of the existence of an intensive selection process (Machado et al. 2003). Tambasco et al. (2003) states that if the value of the observed heterozygosity (Ho) is much lower than the expected heterozygosity (He), then it indicates breeding within the group is as a result of an intensive selection process.

Table 2. Number of alleles and genotypes of GH gene in five exons of all cattle breeds

Fragment	Breed cattle											
	Bali cattle		Pesisir cattle		Madura cattle		Katingan cattle		Simmental cattle		Limousin cattle	
	Allele	Genotype	Allele	Genotype	Allele	Genotype	Allele	Genotype	Allele	Genotype	Allele	Genotype
Exon 1	1	1	3	3	2	2	3	3	3	2	2	1
Exon 2	2	2	1	1	1	1	2	3	1	1	2	2
Exon 3	1	1	2	2	1	1	1	1	2	2	2	2
Exon 4	1	1	2	2	1	1	2	2	2	2	2	1
Exon 5	1	1	2	2	2	2	2	3	3	4	3	4

Table 3. Frequency of GH gene genotype in five exon of all breeds

Population	Fragmen																					
	Exon 1 Genotype						*Exon* 2 Genotype					*Exon*3 Genotype			*Exon* 4 Genotype				*Exon* 5 Genotype			
	AA	AB	BB	AC	CC	DD	DE	EE	FF	FG	GG	HH	HI	II	JJ	JK	KK	LL	LM	MM	LN	NN
Bali	1.000	0.000	0.000	0.000	0.000	0.450	0.550	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000
Pesisir	0.300	0.600	0.000	0.100	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.950	0.050	0.000	0.400	0.600	0.000	0.400	0.600	0.000	0.000	0.000
Madura	0.850	0.000	0.000	0.150	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000	1.000	0.000	0.000	0.750	0.250	0.000	0.000	0.000
Katingan	0.200	0.650	0.000	0.150	0.000	0.000	0.000	0.000	0.200	0.250	0.550	1.000	0.000	0,000	0.700	0.300	0.000	0.450	0.350	0.200	0.000	0.000
Simmental	0.000	0.800	0.000	0.200	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.300	0.700	0,000	0.100	0.900	0.000	0.300	0.300	0.000	0.200	0.200
Limousin	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.300	0.700	0.200	0.800	0,000	0.000	1.000	0.000	0.400	0.200	0.000	0.200	0.200

Table 4. Allele frequencies in five exon GH genes in all breeds

Population	Fragment													
	Exon 1 Allel			*Exon* 2 Allele				*Exon* 3 Allele		*Exon* 4 Allele		*Exon* 5 Allele		
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Bali cattle	1.000	0.000	0.000	0.725	0.275	0.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000
Pesisir cattle	0.650	0.300	0.050	1.000	0.000	0.000	0.000	0.950	0.050	0.700	0.300	0.700	0.300	0.000
Madura cattle	0.930	0.000	0.070	0.000	0.000	0.000	1.000	1.000	0.000	1.000	0.000	0.875	0.125	0.000
Katingan cattle	0.600	0.325	0.075	0.000	0.000	0.325	0.675	1.000	0.000	0.850	0.150	0.625	0.375	0.000
Simmental cattle	0.500	0.400	0.100	0.000	0.000	0.000	1.000	0.300	0.700	0.550	0.450	0.550	0.150	0.300
Limousin cattle	0.500	0.500	0.000	0.000	0.000	0.150	0.850	0.200	0.800	0.500	0.500	0.650	0.100	0.250

Table 5. Observed heterozygosity (Ho) values and expected heterozygosity (He) values of gene fragment in five exon GH gene in all breeds

Population	n	GH *exon* 1		GH *exon* 2		GH *exon* 3		GH *exon* 4		GH *exon* 5	
		H_{obs}	H_{exp} ± SE	H_{obs}	H_{exp}± SE	H_{obs}	H_{exp}±SE	H_{obs}	H_{exp}± SE	H_{obs}	H_{exp}±SE
Bali cattle	20	0.000	0.000 ±0.000	0.550	0.399±0.028	0.000	0.000±0.000	0.000	0.000±0.000	0.000	0.420±0.000
Pesisir cattle	20	0.700	0.485±0.029	0.000	0.000±0.000	0.050	0.095±0.012	0.600	0.420±0.028	0.600	0.420±0.028
Madura cattle	20	0.150	0.139±0.018	0.000	0.000±0.000	0.000	0.000±0.000	0.000	0.000±0.000	0.250	0.219±0.023
Katingan cattle	20	0.800	0.529±0.028	0.450	0.349±0.028	0.000	0.000±0.000	0.300	0.255±0.025	0.350	0.469±0.026
Simmental cattle	10	1.000	0.580±0.053	0.000	0.000±0.000	0.700	0.420±0.055	0.900	0.495±0.050	0.500	0.585±0.053
Limousin cattle	10	1.000	0.500±0.050	0.300	0.255±0.050	0.800	0.320±0.055	1.000	0.500±0.050	0.500	0.505±0.059

GH gene is polymorphic in five exons (exon 1, 2, 3, 4 and 5) of Indonesian local cattle. The study found allele A, D, H, J and L in Indonesian local cattle which have higher allele frequency value than that in imported cattle. Simmental and Limousin cattle are polymorphic for all the exon (exon 1, 2, 3, 4, 5) except in Simmental cattle that are purely monomorphic in exon 2. The degree of heterozygosity of Indonesian local cattle is the lowest compared to the imported cattle (Simmental and Limousin cattle), so it can be concluded that imported cattle have high polymorphism.

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Short Communication: Fecundity of freshwater prawn (*Macrobrachium rosenbergii*) in selected rivers of Sarawak, Malaysia

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Abstract. *Khairul Adha AR, Nicholas FF, Long SM, Naqiuddin AS, Esa Y. 2016. Fecundity of freshwater prawn (*Macrobrachium rosenbergii*) in selected rivers of Sarawak, Malaysia. Biodiversitas 17: 498-502*. Giant freshwater prawn (Macrobrachium rosenbergii) is one of the important species of freshwater aquaculture in Malaysia. However, the sustainability of freshwater prawn farming is currently threatened by low production efficiency. In addition, the degradation of natural habitats and the use of illegal catching methods have caused great threats to freshwater giant prawn populations. Thus, the main objective of this study was to examine the wild population, ecology, and fecundity of giant freshwater prawn in natural water bodies in Sarawak's rivers namely Samarahan, Sadong and Kayan rivers. The mean values of the physicochemical water parameters, such as dissolved oxygen, pH values, conductivity, turbidity and temperature from three rivers surveyed were differenced significantly ($P< 0.05$). However, the characteristics of water quality measured were found to be within the ideal range for freshwater prawn to survive and grow. There were significant differences ($P < 0.05$) of total length, total body weight and eggs weight of prawn population among three rivers. There was no significant difference ($P > 0.05$) of prawn fecundity among the three rivers. The present study showed that berried female particularly from Kayan and Kerang river are suitable as potential brood stock from the wild population for breeding program.

Keywords: Giant freshwater prawn, *Macrobrachium rosenbergii*, fecundity, length and weight

INTRODUCTION

Giant freshwater prawn (*Macrobrachium rosenbergii*), which is indigenous to South and Southeast Asia, parts of Oceania and some Pacific islands has been farmed commercially both within and outside its natural range (Short 2004). In Malaysia, the giant freshwater prawn can be found in most inland freshwater areas including lakes, rivers, swamps, irrigation ditches, canals and ponds, as well as in estuarine areas (New 2002). This prawn requires brackish water in the initial stages of their life cycle, although some complete their cycle in inland saline and freshwater lakes (Ling and Merican 1961; New et al. 2000).

Due to the importance of *M. rosenbergii* for commercial fisheries and aquaculture, much is known about their ecology, biology physiology and behavior (Rao 1991; Cavalli 2001; Sithee et al. 2006). In addition, there are many publications and manual on the culture and growth development of freshwater prawn farming (Rao 1965; Ling 1969b; Costa 1980; Ang and Law 1991; Kurup et al. 1996; New and Valenti 2000; Krasindh et al. 2008; Pillai et al. 2011). With the widespread use of hatchery-reared seeds, the production and demand of farmed prawn has gradually increased (Department of Fisheries 2010). Despite the potential for increase production, the sustainability of freshwater prawn farming is currently threatened by low production efficiency and low quality of brood stock from grow-out ponds which resulting high levels of inbreeding (Mather and Bruyn 2003).

However, there is still a lack of studies about the fecundity, brood stock quality and ecology of freshwater giant prawn from natural habitat such as in the rivers of Sarawak. The fecundity study is not only important in estimating the reproductive potential of prawn brood stock development in the hatcheries but also as an assessment on the stock size of their natural population (Patra 1976; Lobão et al. 1985; Valenti et al. 1989; Ang and Law 1991).

Furthermore, the degradation of natural habitats, reclamation of mangroves, water pollution and the use of illegal methods for catching prawn have caused great threats to freshwater prawn and fish populations in Malaysia (Zakaria-Ismail 1994; Khairul Adha 2012). Thus, examining the environmental parameters may contribute to understanding the current status and population structure of this giant prawn in natural habitat throughout Sarawak.

MATERIALS AND METHODS

Description of study area

The studies were carried out in estuaries and the main river basin in Sarawak namely; Samarahan River (N 01° 27.286'E 110° 03.206'), Kayan River (N 1° 39.50 E 109°

Figure 1. Map showing four sampling locations in Sarawak. A. Kayan River, B. Samarahan River, C. Kerang River

51.13) and Kerang River (N 1° 11.02 E 110° 41.19) (Figure 1). All sampling locations were determined and relocated with a differentially corrected Global Positioning System (GPS) receiver (Model Garmin, GPS 76, SN 80308437, Olathe USA). Selections of these locations were made based on the representation of prawn habitat, accessibility, history, and the popularity of the location as giant prawn fishing ground.

Physicochemical water parameters

The characteristics of habitats chosen were recorded for all stations. A set of basic physicochemical water parameter variable including pH, temperature (°C), dissolved oxygen (D.O) (mg/L), conductivity (µS) and Turbidity (NTU)) were determined *in situ* using WP81 Waterproof pH-Conductivity-TDS-Temp meter (Model 121132/1), HANNA Dissolved Oxygen Meter (Model HI 9146), Eu-Tech Portable Turbidity Meter (Model ECTN 100 1R) and Eu-Tech ORP Testr10.

Prawn sampling and identification

Surveys were carried out from September 2014 to March 2015 when relative prawn density was highest. Surveys were focused on collecting only on the adult prawns. The prawn collections were made using cast nets, gill nets and traps as well as purchasing samples from local fishermen. This is reduced the sampling bias through the use of one collecting method. A cast net (3m long with 5 to10 mm mesh sizes) was used at shallow pool of the river systems. Approximately 10 throws of the cast nets were made at each station. Monofilament gill nets with different mesh sizes (0.5, 1.0, 1.5 and 2.0 cm) were applied in pool and deeper parts of study sites for 3 to 6 hours. The traditional fishing method such as trap with 150 m length and 3 meter height were mostly set parallel to the river banks during low water and submerged during high water. The traps were placed in the water for 12 hour period overnight.

Adult specimens were preserved in 70% ethanol before being identified using specific taxonomic keys according to classification developed by (Holthuis 1980; Ling 1969a; Wowor and Ng 2007). Total weight (g), total length (cm), sex (based on presence or absence of the male appendix on the second pair of pleopods) (Ismael and New 2000) and male morphotype stage (following the coloration of the claws: SM-transparent, OC-orange, BC-blue) (Kuris et al. 1987) was recorded for each individual. Gravid females (egg bearing females) were kept in separate plastic bags to prevent egg loss during transportation. The eggs adhered to the pleopods were removed and preserved in 70% ethanol (Glaúcia et al. 2011). The eggs were then added to 1 liter of

water, homogenized and sampled using a 10 ml pipette. The eggs were captured under stereomicroscope using Moticam 2.0.and counted by ImageJ software. Three subsamples were counted from each female. Fecundity was calculated using the formula: N=M/VP × VT (Glaúcia et al. 2011) where N = number of eggs, M = mean number of eggs across all sub-samples, VP = volume of the sub-samples and VT = total volume of the sample.

Data analyses

Descriptive statistics including minimum and maximum values, means and standard error of pH, Dissolved Oxygen (D.O), water temperature (°C), conductivity (µS), and Turbidity (NTU) from each survey station were calculated. Analyses of variance (ANOVA) were used to compare the differences in the physicochemical water parameters of all stations surveyed. For the morphometric analysis (body length, weight and egg weight) descriptive statistics were applied. Data were expressed in terms of mean and standard deviation. Chi-square test was used to test the significance of the sex ratio of the prawn collected. Pearson's linear regression was used to assess the correlation between prawn weight and fecundity and total length and fecundity. All statistical analyses were done using SYSTAT Version 7.0 (Wilkinson 1996). All differences are significant at $P < 0.05$, unless otherwise stated.

RESULTS AND DISCUSSION

Physicochemical water parameters

The mean values of the physicochemical water parameters from three habitats surveyed are summarized in Table 1. The highest pH was recorded in Kerang River and the lowest pH was in Samarahan River, with mean pH value 6.32 ± 0.21 and 5.33 ± 0.10, respectively. The dissolved oxygen (mg/L) concentration ranged from 4.59 ± 0.12 mg/L in Samarahan River to 6.11 ± 0.10 mg/L in Kerang River. The highest mean temperature recorded was in Kayan River and the lowest mean temperature was in Kerang River with mean value 33.45 ± 0.26°C and 22.87 ± 0.78°C, respectively. The conductivity (µS) ranged from 38.40 ± 1.76 µS in Kerang River to 80.30 ± 9.15 µS in Samarahan River. The highest Turbidity (NTU) value was recorded in Kayan River and the lowest turbidity value was in Kerang River with mean turbidity 240.49 ± 6.27 NTU and 125.60 ± 12.21 NTU, respectively.

Generally, there were significant differences ($P < 0.05$) of pH value, dissolved oxygen, turbidity, conductivity and water temperature among the river surveyed. Although the physicochemical water parameter measured is slightly significant differenced, the characteristics of water quality measured were found to be within the ideal range for freshwater prawn to survive and growth (Sampaio and Valenti 1996; New 2002; Alam and Alam 2014). Oben et al. (2015) found that the water quality parameters did not seem to have an influence on the variation in the natural population and composition of *M. vollenhovenii* collected from the Yoke River. However, the degradation of natural habitats, deforestation, reclamation of mangroves and peat swamps, water pollution, overexploitation of biological resources and the use of illegal methods for catching fish have caused great threats to freshwater fish populations in Malaysia (Khairul Adha 2012). For instance, Ho (1994) estimated that the catch of freshwater giant prawns had been reduced to about 25% in the Tanjung Tualang, Perak over the past 20 years, due to deterioration in water quality.

Fecundity of freshwater giant prawn population

A total of 680 individual freshwater giant prawn including 264 male, 350 female and 66 berried female were collected from the three rivers, namely Samarahan, Kayan and Kerang rivers. The overall sex ratio during sampling showed that female prawn significantly greater than male prawn population ($P < 0.05$). Table 2 shows the individual collection, mean total length, mean total weight, mean total eggs weight and fecundity for berried female from the three rivers. However, the mean length and weight of male and unberried female prawn were not included for detailed analyses.

The mean total length and weight of berried prawn were ranged from 13.27 ± 1.84 cm to 15.58 ± 2.25 cm and 25.98 ± 9.21 g to 43.72 ± 16.91g, respectively. The mean weight of eggs was ranged from 2.11 ± 1.36 to 4.06 ± 1.49 g. The prawn from Kerang River shows the greatest weight, length and eggs weight and the samples from Samarahan River is the least weight, length and eggs weight. There were significant differences ($P < 0.05$) of total length, total body weight and eggs weight of berried prawn population among the three rivers. The greatest fecundity of the berried prawn was recorded from Kerang river and the least fecundity was from Samarahan river with the fecundity value of 30633 and 23523, respectively. However, there were no significant differences ($P > 0.05$) of prawn fecundity among the three rivers. According to Graziani et al. (1993) the fecundity of *Macrobrachium* species associated with the female age and maturity. Bal and Rao (1990) also stated that individual of the same species produces varying number of eggs depending on their age, length, weight and environmental condition.

The differences of berried prawn fecundity in this study are almost similar reported in literature. According to Ling (1969b), Patra (1976) and Ang and Law (1991) the fecundity of *M. rosenbergii* of wild population is ranged from 60000 to 130000. The variations found in prawn fecundity may be attributed to the different conditions of female maintenance in the laboratory, female physiological conditions and season (Lobão et al. 1986). In addition, Babu (2014) stated that there was no significant variation of the fecundity of female prawn which was recorded from 2010-2012 in Bhairavapalem, India.

The correlation between fecundity with length and weight of *M. rosenbergii* from Samarahan, Kayan, and Kerang Rivers is shown in Table 3. The fecundity of prawn population from Samarahan, Kayan and Kerang showed positive correlation with the total weight and length. According to Mahapatra et al. (1996) fecundity was more closely related to weight than to length of the prawn. The present study indicated that the fecundity of the prawn

Table 1. Mean of physicochemical water characteristics of Samarahan, Kayan and Kerang rivers in Sarawak (Mean ± SD)

Parameters	Samarahan River	Kayan River	Kerang River
Dissolved oxygen (mg/L)	4.59 ± 0.12	4.61 ± 0.10	6.11 ± 0.10
Temperature (°C)	26.64 ± 0.10	33.45 ± 0.26	22.87 ± 0.78
pH	5.33 ± 0.10	5.67 ± 0.08	6.32 ± 0.21
Conductivity (µS)	80.30 ± 9.15	58.27 ± 0.06	38.40 ± 1.76
Turbidity (NTU)	142.00 ± 9.15	240.49 ± 6.27	125.60 ± 12.21

Table 2. Number of individual, mean total length (cm), mean total weight (g), mean total eggs weight (g) and fecundity of berried prawn collected from Samarahan, Kayan and Kerang rivers

	Location		
	Samarahan River	Kayan River	Kerang River
N	20	30	16
Total length (cm)	13.27 ± 1.84	14.22 ± 1.52	15.58 ± 2.25
Total weight (g)	25.98 ± 9.21	26.93 ± 8.63	43.72 ± 16.91
Total eggs weight (g)	2.11 ± 1.36	2.20 ± 1.21	4.06 ± 1.49
Fecundity	23523 ± 12175	28251 ± 11000	30633 ± 12068

Table 3. The correlation between fecundity with length and weight of *Macrobrachium rosenbergii* from Kayan, Kerang and Samarahan rivers, Sarawak

	Length vs fecundity ($ln\ F_T = b\ ln\ TL + a$)				Body weight vs fecundity ($ln\ F_T = b\ ln\ BW + a$)			
	a	b	r^2	*p*	a	b	r^2	*p*
Kayan River	1.220	3.369	0.516	< 0.05	6.350	1.172	0.607	< 0.05
Kerang River	4.892	1.958	0.471	< 0.05	7.320	0.792	0.626	< 0.05
Samarahan River	3.224	2.607	0.453	< 0.05	6.256	1.151	0.573	< 0.05

from the three rivers was correlated closely with weight than length. Sureshkumar and Kurup (1998) also found that fecundity of *M. rosenbergii* showed a positive correlation with total weight, total length and carapace length. In addition, Babu (2014) also stated that the fecundity and relation with length and weight of *Penaeus monodon* significantly varied from different geographical location. Bhuiyan et al. (2007) found that the number of eggs and the length of the female body in *M. dayanum* were positively correlated. The increase of fecundity with body size seems to be a rule that is applicable to many crustaceans (Oben et al. 2015).

The fecundity data can be used to access the reproductive potential of the prawn spawning stock (Ang and Law 1991). The present study showed that berried female particularly from Kayan and Kerang rivers are suitable as the potential brood stock from the wild population for prawn breeding. Khairul Adha et al. (2014) also found that the berried female of giant prawn from Kerang Rivers is one of the potential brood stocks for breeding programs. The wild stocks of giant prawn brood stock could be an important resource for genetic improvement of culture stocks in the future. Sourcing brood stock from grow-out ponds which resulting in high levels of inbreeding over time was believed to be the reason for growth decline in Thailand (Mather and Bruyn 2007). Thus, efforts should be done to exploit the reproductive output of wild broodstock of this species for breeding program. Although the physicochemical water properties measured were differed significantly among the three stations, the various size and growth stage of prawn found from the rivers have indicated that the freshwater giant prawn is still available and can be sustain for future resources.

The present study has showed relationships between fecundity and body weight, fecundity and total length, as well as between fecundity and eggs weight in *Macrobrachium rosenbergii* from the rivers in Sarawak regions. Differences of physicochemical water properties from three river studied probably not influence the variation of prawn fecundity, body weight, length and eggs weight of giant freshwater prawn in that habitat. However, the berried female of giant prawn from Kerang and Kayan rivers has a potential as brood stock resources from the wild for breeding program.

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Vegetative and generative growth of groundnut genotypes under biotic environmental stress

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Abstract. *Rahmianna AA, Yusnawan E. 2016. Vegetative and generative growth of groundnut genotypes under biotic environmental stress. Biodiversitas 17: 503-509.* The decrease in groundnut pod yield is mainly influenced by disease infestation, especially bacterial wilt and foliar diseases. The objectives of this experiment were to determine the response and tolerance of groundnut genotypes to bacterial wilt, leaf spot and rust diseases, and seed infection by *Aspergillus flavus*. The planting materials were 25 genotypes (11 Indonesian cultivars, 12 lines introduced from ICRISAT, 1 Indonesian promising line, and 1 local cultivar) with various superiorities on diseases resistance. This study was arranged in a randomized completely block design with triplicate. The results indicated that both genotypes from ICRISAT and Indonesia had similar response to leaf spot i.e. ranging from susceptible (score 6-7) to highly susceptible (8-9). The score for rust ranged from moderately resistant to susceptible. The average pod yield was 23.1 g/plant (from 11.9 g to 29.5 g), and 13 and 12 genotypes produced pods higher and lower than the average value, respectively. ICGV 86158 and ICGV 95322 had the highest and lowest seeds as well as pod productivity, respectively. The ICRISAT genotypes were susceptible to *Ralstonia solanacearum* infection, except for ICGV 86590. Among the Indonesian cultivars, those with Valencia type of growth, relatively had better resistance to bacterial infection. These cultivars were also resistant to rust and *A. flavus* infection.

Keywords: Biotic stress, generative, groundnut, vegetative performance

INTRODUCTION

The national productivity of groundnut in Indonesia was lower compared to the world production, and even lower compared to China, Argentina, USA, and Australia, which are the main groundnut production countries in the world. The productivity was considered to be low because a number of constraints both abiotic and biotic factors. The major biotic factors limited pod yields are pests, diseases and weeds. Among these factors, the reduction of pod yield of groundnut in Indonesia is mainly influenced by disease infection, especially bacterial wilt and foliar diseases.

The most prevalent foliar diseases in Indonesia are late leaf spot and rust caused by *Cercospora arachidicola* (*Phaeoisariopsis personata*) (Berk & M.A. Curtis) van Arx, and *Puccinia arachidis* Speg., respectively (Mehan and Hong 1994). The infection of *P. arachidis* is able to reduce to 60% of groundnut pod yield. The same figure also occurs when the crop is infected by *C. arachidicola* or *Cercosporium personatum* (Thakur et al. 2013). When rust and leaf spot infect together, there will be 50-70% pod yield reduction. Instead of pod yield, these diseases also reduce kernel quality and produce severe damage of the foliage.

Bacterial wilt caused by *Ralstonia solanacearum* has been becoming serious yield limiting factor for groundnut production since ten of years ago in Indonesia. This incidence was firstly reported in year 1905 at Cirebon region, West Java. The yield loss due to Ralstonia bacterial wilt was 15-35% for resistant variety, and 60 to 100% for susceptible variety grown in endemic area with high infection (Machmud and Rais 1994; Nugrahaeni and Purnomo 2013).

Aspergillus flavus is a saprophytic fungi that produces secondary metabolite called aflatoxin under unfavorable environment. This toxin is very harmfull to human health as of its carcinogenic, teratogenic and immune suppressive which causes liver cancer to human and early death to cattle, duck and chicken.

The important role of foliar diseases and bacterial wilt on the success of groundnut production was noted since early phase of breeding works in Indonesia in 1950's. Instead of high pod yield, the resistance status of these diseases had been included as the main parameters in developing new varieties. The journey of breeding works since then until recently, has been consistently put those three important diseases into the main parameter in developing new varieties despite the additional parameters especially from the abiotic factors such as tolerance to suboptimal soil conditions (acid soil with and without high Aluminum saturation, tidal swamp areas, dry areas and dry season cropping, wet/waterlogged condition left after wetland rice, drought during generative growth phase, suitability to multiple cropping, and shading tolerance, as well as alkaline soils with high iron (Fe) content and high pH. Since the last 15 years, another biotic factor i.e. the tolerance status to *A. flavus* infection has been considering as an important aspect (Wahyar et al. 2015).

Due to the important status of those diseases to groundnut crops and its pod yield, the experiment was

aimed to determine the response as well as the tolerance of groundnut genotypes to bacterial wilt, leaf spot and rust diseases, and seed infection by *A. flavus* under wet condition in developing-central production areas.

MATERIALS AND METHODS

The experiment was conducted at the farmer's field in dryland area at Central Java Province, Indonesia during wet season from January to May. In this region, groundnut is grown once a year intercropped with cassava where cassava is grown 3-4 weeks after groundnut sown. A total of 25 genotypes with various superiorities especially on diseases resistance, were grown under rainfed condition. The planting materials consisted of 11 Indonesian improved cultivars, 12 lines (ICGV's, IC and J) obtained from International Center for Research Institute for Semi Arid Tropic (ICRISAT), 1 Indonesian promising line, and 1 local cultivar (Table 1).

This study was arranged in a randomized completely block design with triplicate. Each genotype was planted in a 12 m^2 plot with plant spacing of 40 x 15 cm, and 1 plant was maintained in every hole. The basal fertilizers of 22.5 kg N, 36 kg P_2O_5 and 50 kg K_2O ha^{-1} were applied just after sowing by broadcasting the fertilizers in the furrow along the row. The dosage of 1000 kg farmyard manure and 500 kg lime ha^{-1} were incorporated into the soil during land cultivation. Weeding was conducted twice, i.e. at 25 and 59 days after sowing (DAS). Insecticides were applied at 15, 22, 40, and 50 DAS. *A. flavus* inoculation was conducted to guarantee the presence of abundant population in the *geocarphosphere*. The inoculation was conducted at 55 DAS along the plant's rows. Meanwhile, leaf spot and rust incidence merely depend on the natural infection. Water irrigation was intermitted at 60 DAS then the soil was left drying out until harvesting time at 90 DAS. Harvesting was undertaken when 75% of the filled pods had already matured. The pods then immediately separated from the plants, and sun dried. The observations were undertaken on agronomic parameters (plant height, haulm weight, 100 seed weight, filled pod number, empty pod number, and pod yield/plant), and number of plants at harvesting time (the plants that survived/interference from bacterial wilt infection), the incidence of leaf spot and rust, and *A. flavus* infection on the seeds. The bacterial wilt incidence was observed by counting the number of wilted plants every 2 weeks and expressed as percentage of wilted plants to its full population. The resistance criteria was rated according to Machmud and Rais (1994) that resistant (R) genotypes with ≤15% wilt incidence; moderately resistant (MR) with 16-25% wilt incidence; moderately susceptible (MR) with 26-35% wilt incidence, and susceptible (S) with >35% wilt incidence. Assessment on the severity of leaf spot and rust diseases were scored at 85 DAS using 1-9 scale developed by Subrahmanyam et al. (1995), where 1 = no disease and 9 = plants severely affected. The percentage of kernels infected by *A. flavus* was determined by plating 100 kernels per sample onto the *A. flavus* and *parasiticus* agar (AFPA) media in 10 petri

Table 1. List of genotypes and their characteristics used as planting materials. Banjarnegara, wet-early dry season (February-May)

Genotype	Remarks
ICRISAT lines	
ICGV 86158	Fresh seed dormancy
ICGV 93291	Short duration
ICGV 95322	Short duration
ICGV 86590	Resistant to rust and tolerant to leaf spot, a released cultivar in India
J 11	Resistant to in vitro seed colonization by *A. flavus*
ICGV 93280	Resistant to in vitro seed colonization by *A. flavus*
ICGV 95494	Tolerant to in vitro seed colonization by *A. flavus*
ICGV 99029	Large seed, suitable for confectionery uses
IC 48	Tolerant to drought
ICGV 91278	Tolerant to drought
ICGV 91284	Short duration
ICGV 89104	Tolerant to pre-harvest seed infection by *A. flavus*
Indonesian cultivars	
Anoa	Resistant to wilting caused by *R. solanacearum*; resistant to rust and leaf spot
Bima	Moderately resistant to wilting caused by *R. solanacearum*; susceptible to rust and moderately susceptible to leaf spot
Bison	Moderately resistant to rust, leaf spot and seed infection by *A. flavus*; Tolerant to 25% shading; tolerant to Fe shortage and adapted in alkaline Alfisols
Jerapah	Resistant to wilting caused by *R. solanacearum*; resistant to rust and leaf spot; tolerant to drought, tolerant to acidic soil condition
Kelinci	Moderately resistant to wilting caused by *R. solanacearum*; resistant to rust and leaf spot
Komodo	Resistant to wilting caused by *R. solanacearum*; susceptible to PSTV; suitable to drylands; dry season planting
Kancil	Resistant to wilting caused by *R. solanacearum*; tolerant to rust, leaf spot, and seed infection by *A. Flavus*
Panter	Resistant to wilting caused by *R. solanacearum*; tolerant to rust, leaf spot, drought, broad adaptation
Singa	Tolerant to wilting caused by *R. solanacearum*; resistant to rust and moderately resistant to leaf spot, tolerant to drought, broad adaptation
Turangga	Resistant to wilting caused by *R. solanacearum*; moderately resistant to rust, leaf spot, seed infection by *A. flavus*, drought and shading
Zebra	Tolerant to rust and leaf spot, suitable to wet and dry lands
Indonesian promising line	
GH 51	Tolerant to drought; Moderately susceptible to rust, leaf spot and wilting caused by *R. solanacearum*; low aflatoxin contamination; resistant to pre-harvest seed infection by *A. flavus*
Local variety	
Lamongan	Tolerant to drought

dishes (as replications) or 10 seeds per each petri dish. The seeds were incubated for 3 days when the fungal infection was easily identified i.e. by the presence of dark yellow or orange color fungal colonies on the seeds. The number of seeds with yellow fungal colony was recorded. All the data obtained were subjected to analyses of variance (Anova), the significance of treatment differences was found out using Duncan's Multiple Range Test at 5% level of probability.

RESULTS AND DISCUSSION

Vegetative growth

Plant height and fresh haulm (above ground biomass) weight were two parameters that were mostly used to express the performance of vegetative growth of the crop. These two parameters were significantly different among genotypes tested. Plant height of those genotypes varied from 25.1 cm to 56.1 cm with 35.3 cm in average (Table 2). As many as 17 genotypes were shorter and eight genotypes were taller than the average height. In addition, fresh haulm weight ranged from 25 g to 91.7 g (Table 2), with average weight was 56.2 g, where 13 and 12 genotypes had lower and higher weight than the average value, respectively.

Both genotypes from ICRISAT and Indonesia had similar response to leaf spot i.e. ranging from susceptible (score 6-7) to highly susceptible (8-9). In connection to rust disease, the score ranged from moderately resistant to susceptible (Table 3). The score of leaf spot disease was higher compared to that of rust disease in all genotypes tested, as leaf spot incidence was more prominent during wet season that last from October to April.

Generative growth

The average pod productivity (pod yield per plant) was 23.1 g with the lowest and highest values were 11.9 g and 29.5 g, respectively. A total of 13 and 12 genotypes produced pods higher and lower than the average value, respectively. In terms of seed yield per plant, the highest and lowest yields were 19.0 g and 6.9 g, respectively with 14 genotypes gave higher seed yield and 11 genotypes gave lower seed yield than the average, i.e. 13.3 g. ICGV 86158 and ICGV 95322 had the highest and lowest seed as well as pod productivity, respectively (Table 4). Contrary to vegetative growth, pod and seed yields per plant of all genotypes did not significantly different.

Singa cultivar which was superior in its vegetative growth (plant height and fresh haulm weight) was not followed by the highest pod yield, number of filled pods, and seed weight of individual plant. These components were less superior compared to those of ICGV 86158 which produced highest pod yield of individual plant (29.1 g). This success was supported by high values of the yield components i.e. seed yield per plant, number of mature pods per plant, and shelling outturn (Table 4). These results were in agreement with the finding of Padmaja et al. (2013) for seed yield per plant, number of mature pods per plant.

The study revealed correlations between leaf spot and the number of filled pods, shelling outturn, and seed yield. Pod yield, separately, correlated to plant height, fresh haulm weight, number of filled pods, and seed yield. The direct correlation of leaf spot and seed yield was reported by Thakur et al. (2013). Meanwhile, rust disease correlated to the number of filled pods only (Table 5). Despite the presence of correlation to vegetative and yield components, these two diseases did not correlate to pod yield. This is in agreement of the findings of Padmaja et al. (2013) who noticed the negative indirect effect of leaf spot to pod yield via number of mature pods per plant and hundred seed weight.

Table 2. Vegetative components of 25 peanut genotypes grown at dryland. Banjarnegara, wet-early dry season (February-May)

Genotype	Plant height (cm)	Fresh weight of haulm (g/plant)
Local Lamongan	34.0 de	38.3 de
ICGV 86158	35.1 cde	83.3 a
ICGV 93291	30.1 de	47.9 cde
ICGV 95322	25.4 e	38.3 de
ICGV 86590	46.8 abc	65.0 a-d
J 11	32.8 de	71.7 a-d
ICGV 93280	31.6 de	50.0 b-e
GH 51	34.3 de	50.0 b-e
ICGV 95494	34.6 cde	61.7 a-d
ICGV 99029	28.7 de	42.5 cde
IC 48	36.1 cde	72.7 abc
ICGV 91278	29.8 de	43.8 cde
ICGV 91284	33.3 de	66.7 a-d
ICGV 89104	30.2 de	61.7 a-d
Anoa cultivar	34.3 de	43.3 cde
Bima cultivar	32.1 de	45.8 cde
Bison cultivar	25.1 e	25.0 e
Jerapah cultivar	32.3 de	48.3 b-e
Kelinci cultivar	40.3 bcd	63.3 a-d
Komodo cultivar	32.9 de	40.0 cde
Kancil cultivar	40.9 bcd	43.3 cde
Panter cultivar	39.3 cd	68.3 a-d
Singa cultivar	56.1 a	91.7 a
Turangga cultivar	51.1 ab	81.7 ab
Zebra cultivar	36.3 cde	60.0 a-d
DMRT 5%	10.6	27.9

Note: Numbers in the same column followed by the same letter indicated not significantly different based on Duncan test at 5%

Table 3. The scores of leaf spot and rust diseases in 25 genotypes. Banjarnegara, wet-early dry season (February-May)

Genotype	Leaf spot score*)	Rust score*)	Genotype	Leaf Spot score[1)]	Rust score[1)]
ICGV 86158	7.3	6.3	Lamongan	8.3	6.7
ICGV 93291	7.0	5.7	Anoa	8.0	7.7
ICGV 95322	6.7	5.3	Bima	7.0	6.0
ICGV 86590	6.7	5.7	Bison	7.7	7.0
J 11	7.7	6.3	Jerapah	8.0	7.3
ICGV 93280	7.7	6.3	Kelinci	6.7	5.3
GH 51	8.0	7.0	Komodo	8.0	7.3
ICGV 95494	8.0	6.7	Kancil	8.0	6.3
ICGV 99029	7.0	6.7	Panter	7.0	5.3
IC 48	7.3	6.3	Singa	7.0	5.0
ICGV 91278	7.7	6.3	Turangga	7.0	5.0
ICGV 91284	7.0	6.3	Zebra	6.7	5.0
ICGV 89104	7.7	6.7			

Note: *) Scored on a modified 1-9 scale where 1 = no disease and 9 = plants severely affected (scoring system followed Subrahmanyam et al. 1995). 1 = highly resistant, 2-3 = resistant, 4-5 = moderately resistant, 6-7 = susceptible, and 8-9 = highly susceptible (Subrahmanyam et al. 1995).

Plant population

The number of established plants of all genotypes at 14 DAS was high, i.e. >90% except those of Kelinci, Komodo, Bima, Bison varieties, ICGV 93291, ICGV 95322, ICGV 91278, J 11, ICGV 93291 genotypes (Tabel 6). Lower crop establishment of those nine genotypes was closely related to germination failure rather than plant wilting caused by *R. solanacearum* as the wilt symptom by this bacterial infection can be observed at least 3 weeks after sowing and the peak of occurrence will appear in 40-50 DAS (Wang et al. 2009). The level of infection was high as shown by high number of wilted plants at around harvesting time.

When looking at the number of wilted plants, ICRISAT genotypes had higher number of wilted plants compared to those of Indonesia cultivars (Table 6). All ICRICAT genotypes were heavily infected as figured out by >35% of wilt incidence and therefore classified as susceptible to bacterial wilt according to Machmud and Rais (1994) classification. These genotypes were maintained at ICRISAT Pathancheru, Andhra Pradesh, India where this country does not a geographical site for *R. solanacearum* infestation. Conversely, that bacteria is the important yield constraint in China, Indonesia and Vietnam, certain part of Malaysia and Uganda. Recently, bacterial wilt is observed in the Philippines, Thailand, Sri Lanka, Papua New Guinea, and India (Mehan et al. 1994).

Only two Indonesia cultivars (i.e. Singa and Turangga cultivars) showed moderately susceptible while the rest of Indonesia varieties, including Kelinci variety, were in susceptible category (Table 6). Previous study conducted by Saleh and Nugrahaeni (1996) in different site during dry season cropping, Kelinci cultivar had 28.3% of wilt

Table 4. Generative growth of 25 genotypes of groundnut grown at dryland. Banjarnegara, wet-early dry season (February-May)

Genotype	No. of filled pods/plant	No. of empty pods/plant	No. of filled/ total pods (%)	Seed weight (g/plant)	Shelling out turn (%)	Dry pod yield (g/plant)
Lamongan	24.3 a-d	7.5 a-f	76.4	13.2 a	38.6	21.5 a
ICGV 86158	30.9 a	9.9 a-e	75.7	19.0 a	35.6	29.5 a
ICGV 93291	22.8 a-d	6.8 b-f	77.5	9.4 a	40.1	15.7 a
ICGV 95322	14.7 d	5.3 def	73.5	6.9 a	32.1	11.9 a
ICGV 86590	19.8 bcd	10.5 a-d	65.3	10.5 a	55.3	23.5 a
J 11	25.9 a-d	10.7 abc	70.7	16.8 a	40.6	28.3 a
ICGV 93280	24.6 a-d	6.5 b-f	79.1	11.6 a	49.6	23.0 a
GH 51	26.7 abc	9.7 a-f	75.4	15.7 a	40.8	26.5 a
ICGV 95494	22.8 a-d	9.3 a-f	71.0	14.6 a	43.4	25.8 a
ICGV 99029	17.7 bcd	6.5 b-f	73.1	9.6 a	66.9	29.0 a
IC 48	16.1 cd	6.5 b-f	71.2	11.8 a	42.2	20.4 a
ICGV 91278	16.2 cd	5.5 c-f	74.6	10.8 a	42.6	18.8 a
ICGV 91284	27.7 ab	7.8 a-f	78.0	16.6 a	40.1	27.7 a
ICGV 89104	27.6 ab	11.1 ab	71.3	16.7 a	26.8	22.8 a
Anoa	24.4 a-d	5.1 ef	82.4	13.4 a	32.0	19.7 a
Bima	19.6 bcd	7.7 a-f	71.8	11.7 a	53.2	25.0 a
Bison	16.3 cd	6.3 b-f	72.1	10.3 a	48.0	19.8 a
Jerapah	25.7 a-d	6.1 b-f	80.8	15.4 a	35.1	23.7 a
Kelinci	18.3 bcd	12.2 a	60.0	13.8 a	38.2	22.3 a
Komodo	22.1 a-d	4.6 f	82.7	12.4 a	37.7	19.9 a
Kancil	23.1 a-d	7.2 a-f	76.2	14.5 a	39.1	23.8 a
Panter	19.1 bcd	8.6 a-f	68.9	15.8 a	43.8	28.1 a
Singa	19.7 bcd	6.6 b-f	74.9	13.9 a	42.3	24.1 a
Turangga	18.8 bcd	7.5 a-f	71.5	13.4 a	42.0	23.1 a
Zebra	17.7 bcd	10.0 a-e	63.8	14.6 a	39.5	24.1 a
DMRT 5%	9.2	4.3	--	ns	--	ns

Note: Numbers in the same column followed by the same letter indicated not significantly different based on Duncan test at 5%

Table 5. The correlation coefficients among vegetative and generative parameters obtained from 25 genotypes. Banjarnegara, wet-early dry season (February-May)

	Plant height	Fresh haulm weight	No. of filled pods	No. of empty pods	Shelling outturn	Seed yield	Leaf Spot	Rust	Pod yield/ plant
Plant height	--	0.542 ***	0.101	0.048	0	0.207	-0.201	-0.384 ***	0.235 **
Fresh haulm weight		--	0.305 ***	0.449 ***	0.145	0.442 ***	-0.296 **	-0.342 **	0.378 ***
No of filled pods			--	0.273 **	0.298 ***	0.601 ***	0.431 ***	0.371 ***	0.488 ***
No of empty pods				--	0.121	0.295 ***	-0.181	-0.175	0.233 **
Shelling outturn					--	0.642 ***	0.312 ***	0.137	-0.083
Seed yield						--	0.292 **	0.121	0.688 ***
Leaf spot							--	0.682 ***	0.122
Rust								--	0.067
Pod yield/plant									--

Table 6. Number of emerged plants, number of wilted plants and the resistance status to bacterial wilt of 25 peanut genotypes. Banjarnegara, wet-early dry season (February-May)

Genotype	Number of emerged plants at 14 DAS (%)	Bacterial wilt incidence[1)] (%)	Disease reaction [2)]
Lamongan	91.5 a-e	80.2 cd	S
ICGV 86158	92.0 a-d	90.5 abc	S
ICGV 93291	82.8 c-g	98.0 ab	S
ICGV 95322	84.6 b-g	97.6 ab	S
ICGV 86590	90.8 a-f	51.7 e	S
J 11	82.3 d-g	95.9 a	S
ICGV 93280	88.3 a-g	88.9 a-d	S
GH 51	92.1 a-d	78.5 d	S
ICGV 95494	93.1 abc	94.6 ab	S
ICGV 99029	90.0 a-f	95.1 ab	S
IC 48	94.4 ab	97.8 ab	S
ICGV 91278	80.5 fg	96.3 ab	S
ICGV 91284	95.1 ab	95.5 ab	S
ICGV 89104	95.1 ab	96.5 ab	S
Anoa	93.1 abc	41.1 efg	S
Bima	84.6 b-g	94.0 ab	S
Bison	80.8 ef	87.8 a-d	S
Jerapah	93.6 ab	44.8 ef	S
Kelinci	78.0 g	46.3 ef	S
Komodo	87.4 a-g	86.6 bcd	S
Kancil	95.9 a	39.6 fg	S
Panter	91.0 a-f	52.1 e	S
Singa	90.5 a-f	32.6 g	MS
Turangga	89.3 a-f	33.9 g	MS
Zebra	91.8 a-d	45.1 ef	S

Note: Values in the same column followed by the same letters did not significantly different based on Duncan test at P≤0.05. [1)]. Bacterial wilt incidence (%) was calculated from the number of wilted plants over the number of plants at 12 DAS. [2)]. Resistant (R): 0-15% wilt incidence; Moderately Resistant (MR): 16-25% wilt incidence; Moderately Susceptible (MS): 26-35% wilt incidence; Susceptible (S):>35% wilt incidence (Machmud and Rais, 1994).

incidence and therefore categorized as moderately susceptible. This different reaction probably due to different pathotypes between the sites as reported by Wang et al. (2009).

The number of harvested plants varied from 2.0% to 67.4%. This figure explained that plant population at harvesting time was very low. Due to this situation, the pod yield did not available for all 25 genotypes, but only for genotypes with higher number of survival plants (Table 7). In other words, pod yield at harvesting time was available only to some genotypes that had higher plant populations. These survival plants were the plants that showed some resistance to bacterial wilt incidence. The data showed that Kancil cultivar resulted in highest pod production, while ICGV 86590, Anoa, and Jerapah cultivars came afterward with lower yields. These higher pod productions seem to correlate to higher number of harvested plants and higher plant productivity. On the other hands, the yield reduction positively correlated to high reduction in plant population (Table 7).

Tabel 7. Number of harvested plants and pod yield per plant. Banjarnegara, wet-early dry season (February-May)

Genotype	Dry pod yield (g/plant)	No. of harvested plants (%)	Production (t ha^{-1} of dry pods)	The predicted production (t ha^{-1} of dry pods) *)]	Yield reduction (%)
Lamongan	21.5	19.8	0.287	1.449	80.2
ICGV 86158	29.5	9.5			
ICGV 93291	15.7	2.0			
ICGV 95322	11.9	2.4			
ICGV 86590	23.5	48.3	1.125	2.329	51.7
J 11	28.3	4.1			
ICGV 93280	23.0	11.1			
GH 51	26.5	21.5	0.559	2.599	78.5
ICGV 95494	25.8	5.4			
ICGV 99029	29.0	4.9			
IC 48	20.4	2.2			
ICGV 91278	18.8	3.7			
ICGV 91284	27.7	4.5			
ICGV 89104	22.8	3.5			
Anoa	19.7	58.9	1.180	2.003	41.1
Bima	25.0	6.0			
Bison	19.8	12.2			
Jerapah	23.7	55.2	1.055	1.911	44.8
Kelinci	22.3	53.7	0.753	1.402	46.3
Komodo	19.9	13.4			
Kancil	23.8	60.4	1.304	2.158	39.6
Panter	28.1	47.9	0.970	2.025	52.1
Singa	24.1	67.4	0.978	1.451	32.6
Turangga	23.1	66.1	1.025	1.550	33.9
Zebra	24.1	54.9	0.567	1.032	45.1
Average	22.7	46.8			

Note: *) predicted under full population (100% harvested plants)

Aspergillus flavus infection

The observation of *A. flavus* infection on seeds was undertaken only to the genotypes with high number of plant population at harvesting time, as those genotypes provided enough amounts of seeds. The experiment shows that Local Lamongan, ICGV 86590, GH 51, Jerapah, Kelinci, Turangga, Anoa, and Zebra were highly resistant with seeds infected by *A. flavus* lower than 15%. Mean while Kancil, Panter, and Singa cultivars were grouped as moderately resistant to *A. flavu*s infection (Table 8).

ICISAT lines which were resistant to in-vitro seed colonization (ICGV's 93280, 95494, J11) and tolerant to pre-harvest seed infection by *A. flavus* (ICGV 89104) were devastated by *R. solanacearum* bacteria. Only one line, ICGV 86590, was survived.

Singa cultivar grew vigorously as shown by the tallest plant (56.1 cm) with the heaviest fresh fodder (91.7 g). Conversely, Bison had the lowest values both its height and weight, with 25.1 cm height and 26.7 g weight only. One of the ultimate reasons for this difference was the type of growth where Singa variety is Valencia type and Bison variety is Spanish type. Generally, Valencia type grows taller with more leaves and stronger stems compare to that of Spanish type. Highest green fodder yield per plant and

Table 8. Number of *A. flavus* incidence on some peanut genotypes. Banjarnegara, wet-early dry season (February-May)

Genotype	Seed infected by *A. flavus* (%)	Rust score*)	Genotype	Seed infected by *A. flavus* (%)	Rust score*)
ICGV 86158	-	-	Lamongan	0	HR
ICGV 93291	-	-	Anoa	11.0	HR
ICGV 95322	-	-	Bima	-	-
ICGV 86590	3.0	HR	Bison	-	-
J 11	-	-	Jerapah	4.0	HR
ICGV 93280	-	-	Kelinci	9.7	HR
GH 51	9.3	HR	Komodo	-	-
ICGV 95494	-	-	Kancil	19.7	MR
ICGV 99029	-	-	Panter	19.3	MR
IC 48	-	-	Singa	27.3	MR
ICGV 91278	-	-	Turangga	7.0	HR
ICGV 91284	-	-	Zebra	4.5	HR
ICGV 89104	-	-			

Note: *) Highly resistant (HR): <15% seed infection; moderately resistant (MR): 15-30% infection; moderately susceptible (MS): 30-50%; highly susceptible (HS): >50% (Siulin et al. 1996).

its tallest plant of Singa cultivar was supported by Özyiğit and Bilgen (2013) who reported that cultivar with highest green fodder yield had tallest plant.

It is summarized that more genotypes had shorter and lighter than its average values. Based on the data of the experiment, it should be mentioned that plant height tent to positively correlate to fresh haulm weight (r: 0.542).

The dominance of leaf spot in wet season crops was supported by earlier study in Indonesia by Saleh and Nugrahaeni (1996) as well as the screening for leaf spot resistant lines conducted at ICRISAT (Subrahmanyam et al. 1995), and recent study in Nepal (Thakur et al. 2013). Meanwhile, Saleh and Trustinah (1996) reported that rust incidence was more dominant during dry season planting. The development of leaf spot disease is contributed by rainfall or irrigation, and high humidity during the growing season (Kumar et al. 2013). Even in drier areas (with average minimum and maximum air temperatures of 23 and 31^{o}C) like in Ghana, groundnut crops also suffered from foliar diseases as the presence of rainfall 42-305 mm/month in 3-17 rain days/month during 6 months of cropping season.

The scores of leaf spot and rust diseases in groundnut with Valencia type (such as Kelinci, Panter, Singa, Turangga, Zebra, and ICGV 86590) was lower compared to those of cultivars with Spanish type. One of the reasons was Valencia type has thicker leaflet. Early study at ICRISAT reported that almost all resistant lines to both diseases were the fastigiata or Valencia type (Subrahmanyam et al. 1995).

Leaf spot disease will end up with defoliation, and rust disease causes necrotic. These two conditions reduce fresh weight of leaves. Therefore, the incidence of leaf spot or rust individually affects haulm weight; the similar result was obtained by Sunkad and Kulkarni (2006). In this experiment, there was significantly negative correlation between fresh haulm weight and leaf spot. Thakur et al. (2013) reported that genotypes that produced highest haulm weight had low leaf spot incidence. In regard to rust disease, the significant negative correlation to plant height and fresh haulm weight existed (Table 5). Instead of reducing weight, leaf spot infection reduces the quality (crude fiber, crude protein, fat and dry matter content of haulm) of groundnut haulm (Bdliya 2007).

Leaf spot disease reduced both quantity and quality of haulm, while rust disease resulted in short plant with less fresh haulm weight of groundnut plants. Therefore, applying fungicide to control the disease or growing a resistant cultivar is suggested to ensure good quality and quantity of haulm. Apart from haulm weight reduction, rust incidence also negatively correlated to plant height. It means that severe rust incidence was followed by short plants.

In conclusion, there were no introduced genotypes from ICRISAT which had the resistant response to the wilt infection better than that of Indonesian cultivars. All the genotypes from ICRISAT were susceptible to *R. solanacearum* infection when cultivated in Indonesia. Valencia type of growth of the Indonesian cultivars relatively had better resistance to bacterial infection. These cultivars also resistant to rust and *A. flavus* infection into their seeds. Three genotypes, i.e. Kancil, Anoa cultivars and ICGV 86590 produced higher pod yield among those genotypes which relatively resistant to bacterial wilt incidence, therefore, could be considered as one of the parents in groundnut breeding program.

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Short Communication: Georeferencing orchids specimen history cards in Bogor Botanic Gardens to increase their use for conservation efforts

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Abstract. *Rahayu EMD, Yusri S. 2016. Georeferencing orchids specimen history cards in Bogor Botanic Gardens to increase their use for conservation efforts. Biodiversitas 17: 510-514.* Orchids are considered valuable plant resource but overharvesting and habitat conversion have threatened their population. Bogor Botanic Gardens (Kebun Raya Bogor; BBG) stores millions of plant specimens, including orchids, taken from the wild or captivity. Origin of specimens is recorded in specimen tags and cards, where each of these can be converted to species occurrence datum for investigations of biodiversity, its relationship with the environment, evaluating conservation efforts and anthropogenic disturbances along spatial or temporal scales. However, data from tags and cards available are often insufficient because localities are typically being recorded as textual descriptions, without geographic coordinates, thus making analysis using Geographical Information System (GIS) tools difficult. In this paper, we reviewed the use of online resources (i.e. GoogleMaps™, ProtectedPlanet.net) for georeferencing specimen cards and Quantum GIS as a GIS tool to store and display the data. Specimen cards from the chosen genera of orchid in BBG were reviewed. The georeferencing process encountered several obstacles, includes: geographically biased locations, changes in spatial-administrative borders, unregistered location name, unavailability of location name in online resources, and typographic errors during specimen recording process. We also encounter quality difference along georeferenced records, some are good quality (i.e. record coordinates or nearest village) and some are poor (only record the provinces). Georeferencing is an underappreciated task, but once it is done, it can be used for future expeditionary research, national conservation planning, species status review, and other large scale analysis for both spatial and temporal scales.

Keywords: Bogor Botanic Gardens, georeferencing, orchids, plants, specimen collections

INTRODUCTION

The orchid's family is the most diverse family within the plant kingdom. O'Byrne (1994) estimated that there are 17000-35000 species of orchids in the world that consist of 750-850 genera. The islands in Indonesia with known number of species of orchids are Java, it has 731 species (Comber 1990), Sumatra has 1118 species (Comber 2001), Borneo has 2000 species (Chan et al. 1994), Sulawesi and Maluku have 820 species (Thomas and Schuiteman 2002), and Papua which has 3000-3500 species of orchids (O'Byrne 1994).

Orchids have high commercial value. They are known for its beauty and specific appearance (Irawati 2012). Some of the orchids also have medicinal properties, such as *Dendrobium nobile* Lindl., *Bletilla striata* (Thunb). Rchb.f., and *Gastrodia elata* Blume (Bulpitt et al 2007; Pant 2013). Therefore, searching for new orchid species is constantly done and leads to over harvesting of the orchids in the wild. Moreover, orchids species are also facing a great pressure to extinction caused by various disturbance and habitat encroachment. Therefore, there is an urgent need for orchids conservation (Irawati 2012).

Herbaria, museums, and botanic gardens regularly collect specimen from the wild. These collections are the source of primary research archives for biodiversity, conservation, and sustainable use (Beaman and Conn 2003). These species occurrence data are important in order to understand the spatial relationships between plants and various environmental variables within plant communities (Buonopane 2005). Therefore, Global Biodiversity Information Facility (GBIF 2016) has proven that species occurrence data can be used in a wide range of research, addressing the key scientific questions related to biodiversity, such as the spread of invasive alien species, the relationship between climate and biodiversity, conservation, food and farming, and human health. Species occurrence data are recorded in specimen cards and tags. Sadly, most biological collection locality data are written in the form of descriptive localities and very difficult for spatial analysis and often geographically, temporally, and taxonomically biased (Beaman and Conn, 2003; Wieczorek et. al. 2004; Garcia-Milagros and Funk, 2010; van Erp et. al. 2014). Georeferencing is the process of translating a locality description into a mappable representation of a feature (Chapman and Wieczorek 2006). Through georeferencing process, legacy data without coordinates, can be used for quantitative analysis of specimen data with spatial data, using geographical information systems (GIS) (Wieczorek et. al. 2004). Internet is an effective source of

dissemination of various geospatial informations (Qiu and Thakkar 2004). Online geographical gazetteers such as Google Maps (maps.google.com and Open Street Map (openstreetmap.org) have been used for various georeferencing purposes (van Erp et. al. 2014, Fleet et. al. 2012; Tsioukas 2009). Therefore, georeferencing orchid collection can be done using online gazetteers.

Center for Plant Conservation - Bogor Botanic Gardens plays an important role in conserving Indonesia's plants species (Irawati 2011). One of the conservation efforts that were done is collecting plants, including orchids, from the wild to be conserved ex situ at Bogor Botanic Gardens (BBG). During the collection activities, all the information about the specimens collected was recorded.

BBG has conserved Indonesian orchids in an ex vitro and in vitro condition. Wati and Mursidawati (2015) stated that BBG has 94 genera, 499 species and 6004 specimens. Meanwhile, around 100 species of orchids have been tried to be propagated in vitro in BBG's tissue culture laboratory (Mursidawati and Handini 2008). The purpose of our research is to understand the orchids distribution in Indonesia, based on BBG's orchids collection. By georeferencing orchids specimens in BBG, it can be used for future expeditionary research, national conservation planning, species status review, and other large scale analysis for both spatial and temporal scales.

MATERIALS AND METHODS

A number of 300 orchids collection cards are selected. Genera used for analysis is chosen randomly from spreadsheets of orchids collection records. The first thing we did was adding latitude, longitude, and altitude fields along with URL (Universal Resource Locator) of each georeference source.

Google Maps has a ranking mechanism that will show the most relevant information first. The GeoNames geographical database covers all countries and contains over eight million place names. There is also a need for a special type of gazetteer, especially when dealing with protected areas. The World Database on Protected Areas (WDPA, available in protectedplanet.net) provides the names and polygon shape files of terrestrial and marine protected areas on Earth. Each card approximation of coordinates was done using the gazetteers available based on available information, such as altitude, placemark/ geographic features, habitat, habitus, planting date, cultivated date, nursery, reported, flowering time, fruitting time, previous names, vernacular names, literature, herbarium, and notes. Coordinates were stored in decimal degree. Once spatial coordinates had been assigned, these records were mapped with Geographic Information Systems (GIS) program, which was Quantum GIS Lyon, designed to manage and analyze spatial information. The map projection used is WGS-84. Species occurrence data was overlaid over Indonesian Coastline and Administrative borders.

RESULTS AND DISCUSSION

A number of 300 collections are georeferenced. The records include collection from eight genera, which are: *Acanthephippium*, *Acriopsis*, *Adenoncos*, *Appendicula*, *Dendrobium*, *Dipodium*, *Grammatophyllum*, and *Phalaenopsis*. Georeferenced data of eight orchids genera of BBG's collection are shown in Figure 2. It shows that the orchids expedition conducted by the BBG already covered the area of Sumatra, Java, Nusa Tenggara, Kalimantan, Sulawesi, Maluku, and Papua. Orchids expedition in Sumatera, Java, Kalimantan, and Sulawesi seemed already covered a representative area of Sumatra. Unfortunately, the area of Nusa Tenggara and Papua were less explored by the BBG. Based on Figure 1, BBG should conduct more orchids expedition to the eastern part of Indonesia, especially Papua, whether by itself or in collaboration with other botanic gardens in Indonesia. By doing so, hopefully the BBG's orchids collection can represent all part of Indonesia.

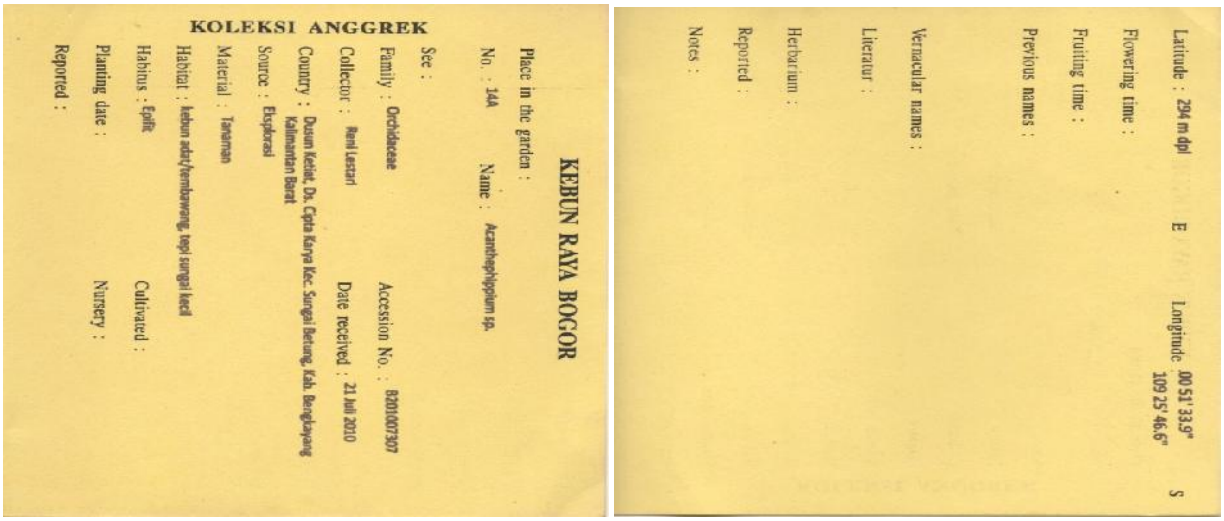

Figure 1. Orchids collection card of Bogor Botanic Gardens

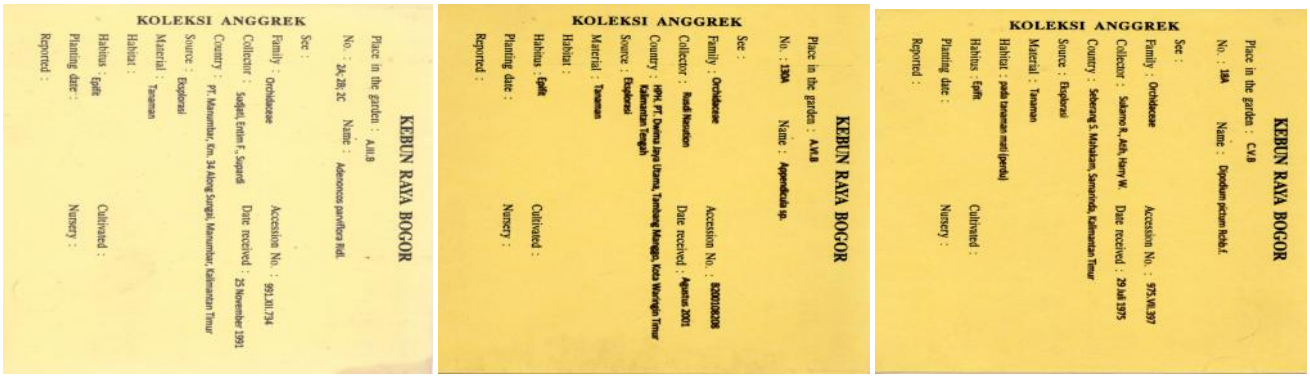

Figure 3. Orchid collection cards with geographically biased location

The orchids distribution is shown in Figure 2. *Acanthephippium* comprises of about 15 species, found throughout tropical Asia and the Pacific Islands (O'Byrne 1994). BBG already collected 3 species from Sumatera, Java, Kalimantan, Sulawesi and Papua which are *A. javanicum* Blume, *A. lilacinum* J.J. Wood & C.L. Chan, and *A. splendidum* J.J. Sm. *Acanthephippium lilacinum* is endemic species of Borneo (Chan et al. 1994). O'Byrne (1994) stated that Papua has one species, *A. splendidum*.. Unfortunately, BBG doesn't have the collection yet. Java only consists of three species, none is endemic. They are *A. parviflorum* Hassk., *A. javanicum*, and *A. striatum* Lindl. (Comber 1990). *Acanthephippium* collection from Java only represents *A. javanicum*. This species distribution includes Sumatera, Malay Peninsula, Java, and Borneo.

Another genus studied is *Adenoncos*, which are small epiphytic monopodial orchids with thick pencil like leaves (Comber 1990). The members of this genus have small greenish to yellowish flower which have long lasting bloom. This orchid genus is distributed from Thai Peninsula and West Malaysia through the Islands of Malaysia and Indonesia to Eastern New Guinea, where just a single species is known. Sumatra records five species, Peninsular Malaysia (and Thailand) have four, there are four more from Borneo but apparently none from the Philippines. One only is known from Java, *Adenoncos virens* Blume. Unfortunately, BBG doesn't have the *A. virens* collection from Java. BBG has collected *A. parviflora* Ridl., *A. sumatrana* J.J. Sm, *A. vesiculosa* Carr., and *A. virens*. All of the collections are from Sumatra, Kalimantan, and Sulawesi.

Appendicula is sympodial orchid without pseudobulbs, usually epiphytic with leaves all along the stems placed in two rows and the flowers are small (Comber 1990). More than 60 species of *Appendicula* have so far been named and this number is likely to increase as more species are found in the future (Comber 1990). Sumatra has to be the centre of development of the genus with 33 species so far found there. Java has 19 species, three of them endemic and the rest are all recorded also from Sumatra, and many from elsewhere as well. The genus ranges from the Himalayas to Micronesia, with a considerable number in New Guinea. Most species grow at lower to middle altitudes in the mountains, particularly on the more humid slopes. BBG has collected 14 species of *Appendicula* from Sumatera, Java, Kalimantan, Sulawesi, Nusa Tenggara, and Papua (Figure 2).

Phalaenopsis consists of 64 species (Cribb and Schuiteman 2012a). According to Cribb and Schuiteman (2012b), the highest diversity of *Phalaenopsis* is in the Philippines (21 species) and then followed by Borneo (16 species). Indonesia has 25 species of *Phalaenopsis*, 10 of them are endemic to Indonesia (Christenson 2001). *Phalaenopsis* could have various shapes, colors, and sizes of flowers. Therefore, *Phalaenopsis* is becoming one of the most popular ornamental commodities. *Phalaenopsis* also has potency as a parent in orchids breeding to produce a new variety of orchids hybrid (Tang and Chen 2007). Some species of *Phalaenopsis* are included in the priority for the conservation of plant species Indonesia (Risna et al. 2010) and all *Phalaenopsis* are listed on Appendix II of CITES (CITES 2015). Rahayu (2015) stated that BBG has conserved endemic *Phalaenopsis*, both ex vitro and in vitro. Those endemic *Phalaenopsis* are *Phalaenopsis javanica* J.J. Smith, *Phalaenopsis floresensis* Fowlie, and *Phalaenopsis viridis* J.J. Sm. *Phalaenopsis javanica* is endemic to West Java (Comber 1990). It is also suspected to be extinct in the wild (Cribb et al. 2003). Whereas *P. floresensis* is endemic to Flores Island and *P. viridis* is endemic to Sumatra (Christenson 2001).

The georeferencing process encountered several obstacles which made data quality varies. Some of the cards record geographically biased location (Figure 3), such as PT. Manumbar (a company name, not a geographic place name or feature), HPH PT. Dwima Jaya Utama (a forest concession which changes overtime), and Seberang Sungai Mahakam (the opposite riverbank of Mahakam, which is a large area without certainty of its specific position). There were also changes in spatial-administrative borders, such as divisions of provinces, regencies, and villages. Changes also happened due to an update of administrative borders. Some of the records also contains typographic errors (such as Mandiangin, should be Mandi Angin; or Karanginten, should be Karang Intan). Some of the cards recorded place names that only familiar with local villagers (such as, Sungai Sambat Kiri - The Left Sambat River), therefore they are either unregistered or unavailable in online resources.

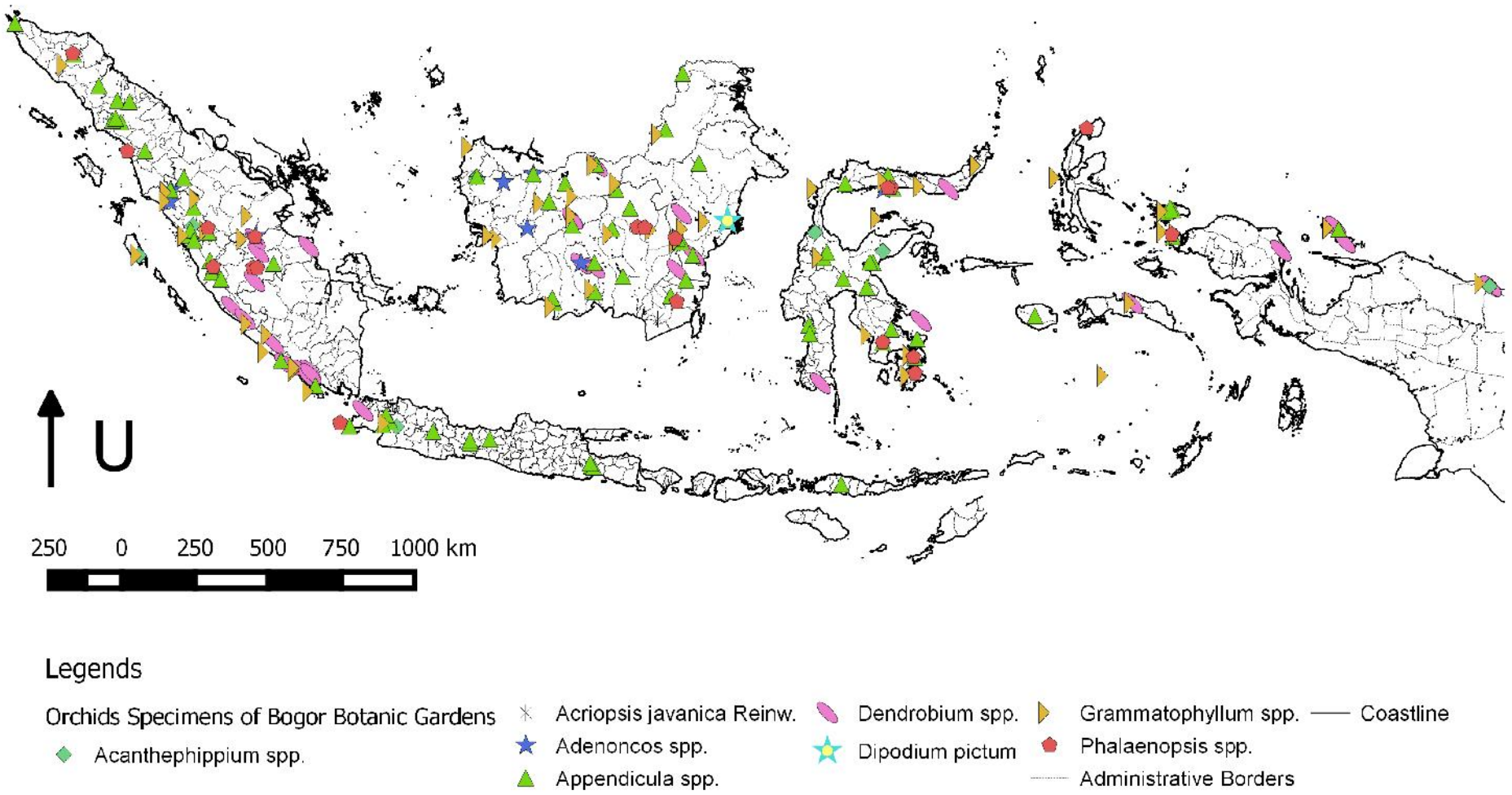

Figure 2. BBG's orchids specimen distribution according georefenced data

These types of records require further research to find the closest geographic name possible. Geographically biased location records are the most difficult to handle. Records with company names will only produce company address, and finding the real collection area requires time consuming research since company names should be traced to their work area (i.e. concessions) at a particular time. Records from more than 10 years ago are difficult to trace because the probability for the company to move their work area is high. Other obstacles are easier to research. Most of the name hints did come out on from search engine's query. Hints on place names usually acquired from travel bloggers, press releases from Ministry of Forestry, and forestry or tourism news. Hints and species specific habitat information were then used for searching in gazetteers to find the closest possible match for coordinate approximation.

After the georeferencing process was completed, we found that the data quality varied due to different scale of closest place names used. Some records provided good quality data due to the detail of the location names in the specimen collection cards. These records provided good hints on finding their approximate location. Researcher doing the collection realized the importance of the detailed information of location record, firstly they are a good source of explanation when dealing with ecological phenomena. Some collection cards provide poor quality data, these cards include information with geographically biased location. Some cards are even unusable since they only record the province. Georeferencing these cards requires exhaustive literature study of each field expedition. Thus making them unsuitable for analysis required for this study.

In conclusion, utilization of online gazetteers has proven to be effective to speed up the georeferencing process of orchid collections. Even though in recent expeditions, the use of GPS becomes the standard procedure, legacy data from past expeditions still requires the georeferencing process. Good locality description will lead to more accurate georeferences and provide higher quality data. On the other hand, bad locality description will lead to increased effort for coordinate approximation. The best practice would be to keep providing descriptive localities even if geographic coordinates are available. The locality should be as specific, succinct, unambiguous, complete and as accurate as possible. Never use a temporary location as a reference. Although the georeferencing orchid collection is underappreciated, it showed areas where BBG's orchids collection is lacking. BBG need to increase the efforts of expeditions to the eastern parts of Indonesia through joint expeditions or collaborations. Aside from orchids collection in this study, other collections in BBG will also benefit from georeferencing process.

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Relationship of physicochemical factors with fish biomass and production in Shadegan Wetland, Iran

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Abstract. *Hashemi S, Ghorbani R, Kymaram F, Hossini SA, Eskandari G, Hedayati A. 2016. Relationship of physicochemical factors with fish biomass and production in Shadegan Wetland, Iran. Biodiversitas 17: 515-522.* The biomass of fishes was estimate in the Shadegan Wetland with Leslie model. Also the relationships between fish biomass and physic-chemical parameters of were studies. Sampling was carried out seasonally at five stations; include Atish, Khorosy, Mahshar, Rogbe, and Salmane from April 2013 to March 2014. During this study, 2795 specimens were measured and weighed. The highest fish biomass and lowest fish biomass were in spring and winter seasons and Khorosy and Rogbe stations have highest and lowest fish biomass. The mean biomass of fish in four seasons Shadegan, 243±35 (kg/ha) and the amount of biomass in different seasons were not significantly different ($P>0.05$). Average values of water physicochemical parameters in different seasons were no significant ($P>0.05$), however average values of salinity stations were significant differences ($P<0.05$). Fish biomass regressions was estimated as Fish Biomass = 0.41 (temperature) $^{2.56}$. CCA ordination explained temperature, salinity, PH and DO, as the most important variables influencing the variation of fish composition in the Shadegan Wetland. Multi-layer artificial neural network showed four parameters (temperature, salinity, depth and DO) have the greatest impact on fish biomass.

Key words: Artificial neural network, fish biomass, water physic-chemical parameters

INTRODUCTION

The renewal of fish biomass is provided by production which is the "amount of tissue elaborated per unit time per unit area, regardless of its fate". It is thus of interest to fisheries ecologists to know how fish production varies among ecosystems and populations. A first step toward this goal is to determine which characteristics of ecosystems have the greatest impact on this rate of renewal (Downing and Plante 1993). Waters (1997) reviewed and proposed the application of annual production, annual P/B ratio, and eco-trophic coefficient (annual angler harvest/annual production) to management of trout fisheries. Incorporating fish production and it relations physicochemical factors may provide a broader perspective on the dynamics of harvested fishes.

Freshwater resources comprise a mere 2.5% of the earth's water, together with wetlands, around 8% of the land area and contain about 40% of all fish species, but the relative productivity of lakes, reservoirs, rivers, and wetlands is enormous, contributing about 15% of the world fisheries production (Kolding and Zwieten 2006). Shadegan Wetland is located in the very flat territories at the furthermost downstream reach of the Jarrahi River in Khuzestan Province. It is the largest wetland in Iran, and following the recent demise of the Mesopotamian marshes, has become the largest wetland in the Middle East (Kholfenilsaz 2009). Wetlands exist in the terrestrial-aquatic interface and are associated with high nutrient levels, high primary productivity and diversity of structural habitats which are utilized by a variety of organisms (Prince et al. 1992).

Maramazi (1997), Ansari and Mohamadi (2001), Ansari et al. (2009), and Hashemi et al. (2011, 2012) were searched fish stock assessment and capture conditions of Shadegan Wetland. Lotfe et al. (2003) were considered human activity and effect and also diversity and capture situation of Shadegan Wetland. The aim of the present study was twofold: (i) to estimate its stock assessment status and fish production, (ii) to determine, relations of fish production with physicochemical factors. Results will greatly contribute to elaborate management programs for this economically important fish species and preserve other fish species.

MATERIAL AND METHODS

Study area

Estimation of biomass and production of fish species was carried out from April 2013 to March 2014 in the Shadegan Wetland. Samples were collected from at five stations, Mahshar (Doragh)(48°,45′ E, 30°,33′ N), Rogbe (48°,33′ E, 30°,41′ N), Khorosy (48°,40′ E, 30°,39′ N), Salmane (48°,28′ E, 30°,40′ N) and Atish (48°,40′ E, 30°,54′ N) in the Shadegan Wetland in Khuzestan Provinces (Figure 1).

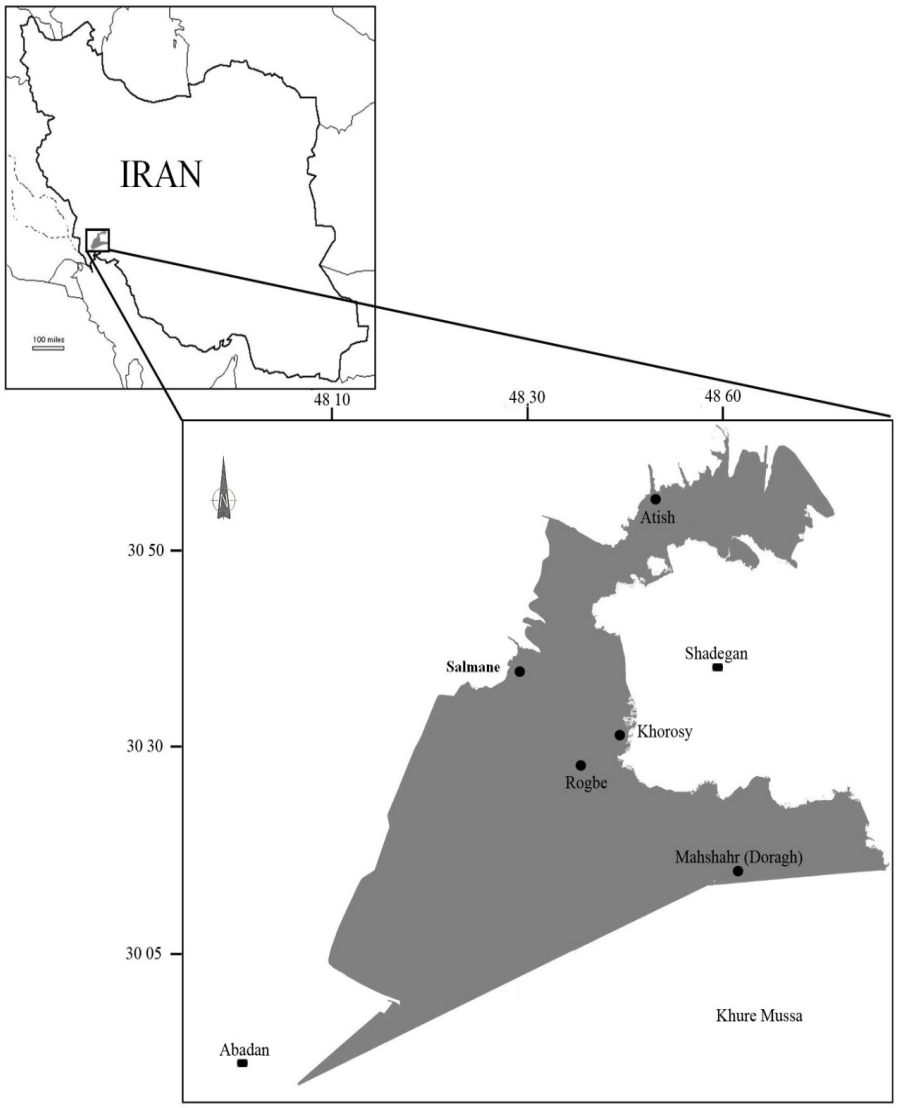

Figure 1. The map of Iran, location of five capture sites was sampled in Shadegan Wetland, Khuzestan Pprovince, South West of Iran

Data collection

Seasonally water samples for analysis of environmental parameters were collected from each station using a Nansen bottle sampler and analyses as per standard analytical procedures (Clesceri et al. 1989). Eighteen environmental parameters (Table 1) were considered in this research and included water temperature (WT), water depth (WD), water salinity (WS) phosphorus (TP), nitrate (TN), pH, biological oxygen demand (BOD) and dissolved oxygen (DO). In each season, 5 stations were selected for sampling. Sampling was carried out by using fixed gill net with 45 mm mesh (100-120 m length and 50-70m width) and then transported to lab with dry ice. Total length with ±l mm and total weight with ±0.01 g were measured for each fish. Amount of 800-1500 m^2 (enclosed area) was changed in different seasons and at each station according to environmental conditions.

Fish biomass

In the Leslie method, the relationship between catch per unit effort and population size is defined by: Ct/ft = qN, where t = time period under consideration, q = catchability, N = initial population number. The population at any time t, is equal to the initial population less what has been caught up to time t (cumulative catch), $N_t = N_0 - \sum C$; By substituting N_t from the catch per unit relationship into the above expression, a linear relationship is obtained: $Ct/ft = q(N_0 - \sum C)$. The initial population size can therefore be derived from: $N_0 = a/q$ (Leslie and Davis 1939). The adjusted cumulative catch (x)—the cumulative catch to interval i plus one half of the catch during interval i proposed by Chapman (1961) compensates for the decline in catchability during each time interval (King 2007). Biomass is estimated as: $B = N.\bar{W}$. Where B = estimated biomass (g); N = estimated abundance; and w = mean weight of fish in the population (g) (Anderson and Neumann 1996).

Fish production

Production is estimated by the size-frequency method for fishes as (Garman and Waters 1983): P = 0.5×C[W1(N1-N2)+∑W k(Nk-1-N k)+Wc(Nc-1-Nc)] (1/CPI), where P = production for a given population or multispecies group within a specified interval, N = estimated mean density (arithmetic mean of estimates) for a specific length-group, w = estimated mean weight (arithmetic mean of estimates) of individuals in a specific length-group, k = index for length-groups, c = number of length-groups, and CPI = the cohort production interval (average maximum age of fish in the population or multispecies group in years).

Modeling procedure

CANOCO 4.5 (ter Braak and Smilauer 1998) was used to run canonical correspondence analysis (CCA) as in Seilheimer and Chow-Fraser (2006). Canonical correspondence analysis (CCA) was used to explore the distribution of the fish biomass in relation to the environmental variables. Environmental variables selected for the CCA analysis included continuous variables, such as water quality data (e.g., pH and BOD). All continuous environmental variables were log (1 + x) transformed and standardized to have a zero mean and unit variance.

Artificial neural network (ANN) is a form of artificial intelligence that is composed of a network of connected nodes (Rumelhart et al. 1986). In this model, non-linear elements (neurons) are arranged in successive layers, with a one-way flow of information (i.e., weights) from input layer to output layer, through a hidden layer (Lek and Guegan 2000). The ANN models were performed using the same data matrix as the input (environmental variables). The ANN model predicts biomass of fish populations. Correlation coefficient (R) between observed and estimated values in artificial neural network (ANN) training and testing for the fish biomass (Brosse et al. 2001). Comparison of *fish biomass* during different spatial and temporal carried out by analysis of variance (ANOVA). Statistical analyses were performed with SPSS 21 software package and a significance level of 0.05 was adopted.

RESULTS AND DISCUSSION

A total of 2795 fish individuals comprising 26 species from 6 families were sampled from Shadegan Wetland throughout the entire study period (Table 1).

Table 1 showing the list of fish species caught and the percentage composition for fish population in Shadegan Wetland. The gradient of comprises the wetland species are native (N), exotic (E), and introduce (I) and it show in table 1. Maximum and minimum capture was *Carasobarbus luteus* and *Cyprinion macrostomus*, *Cyprinion kais, Barbus luciobarbus*, and *Tenualosa ilisha*, respectively.

Fish biomass

The biomass in each station of the Shadegan Wetland with Leslie model were calculated (Figure 2). The results of fish biomass in Shadegan Wetland stations indicated that maximum and minimum fish biomass was found in Khorosy (mean 409±44 kg/ha) and Rogbe (mean 102±53 kg/ha) respectively. The mean biomass of fish in four seasons Shadegan 243 (kg/ha) and the amount of biomass in different seasons were not significantly different ($F = 22$, $P<0.05$).

Average values of fish biomass in each season presented in Table 2. Maximum and minimum fish catch was *Silurs triostegus* and *Acanthopagrus arabicus,* respectively. This could not be attributed to changes in fishing method, or current strength, all of which remained constant. Value Catchability Coefficients (q) differs in each season and each station in Shadegan Wetland. Mean and standard deviation in q values for Leslie model in different stations were 0.007±0.005.

The *S. triostegus* has highest value of biomass to seem than can adapt with Shadegan Wetland condition in different season. The biomass was calculated in each season of the Shadegan Wetland. Maximum and minimum biomass values were spring (479±9) and winter (181±5). A comparison of value biomass between different season is significant ($F = 10.22$, $P<0.05$).

Fish production

The maximum and minimum fish production was showed in Table 2. Generally, the maximum and minimum fish production in Shadegan Wetland was *S. triostegus* and *A. arabicus*, respectively. Overall, *S. triostegus*, *Carasobarbus luteus*, *Carassius auratus*, *Cyprinus carpio*, *Mesopotamichthys sharpeyii* are near 90% production of Shadegan Wetland species. P/B ratio of fish species presented in table 2. Average values production and P/B of fish species were 328±31 kg.ha/yr and 0.99 respectively (Table 2).

Environmental parameters and fish biomass

Fish biomass was strongly correlated with temperature (Figure 3) and Fish biomass regressions with one parameter mentioned below (temperature) were obtained ($P<0.05$). Fish Biomass = 0.41 (temperature) $^{2.56}$. Fish biomass regressions and temperature with different fish species are shown in Table 3 and the highest value of R^2 this regression were observed in *S. triostegus* species. Other species is no significant value of R^2 ($P>0.05$).

Physic-chemical parameters and different station are shown in Table 4. A comparison of value salinity is significant between different station ($F = 18.21$, $P<0.05$) and other parameters is no significant different station ($P>0.05$).

Fish biomass and CCA

An ordination of the main fish species and *five stations from* with eight environmental variables produced significant correlations between species, station and variables associated with environmental degradation for AB data (Table 1). The position of a species and station on the CCA tree plot is a reflection of the environmental conditions where it was found. The first axis of the CCA was strongly correlated with environmental conditions, where the positive end of CCA axis 1 was associated with

species normally found in degraded conditions, while the negative end was associated with species that are intolerant of water-quality impairment. This location can be interpreted as representing the species' affinity for degraded vs. unimpacted habitat. It seems, distribution of *Carassius auratus* and *Chelon abu* species are associated with temperature; *C. carpio, C. luteus, Hypophthalmichthys molitrix*, *S. triostegus* species are associated with PH and salinity and also, *M. sharpeyii* species with Po4 respectively (Figure 4).

The ANN models yielded correlation coefficients ($P<0.05$) and in the training procedure and testing procedure value for r for the fish biomass were 90% and %88, respectively. Multi-layer artificial neural network showed four parameters (temperature, salinity, DO and deep) have the greatest impact on fish biomass (Figure 5).

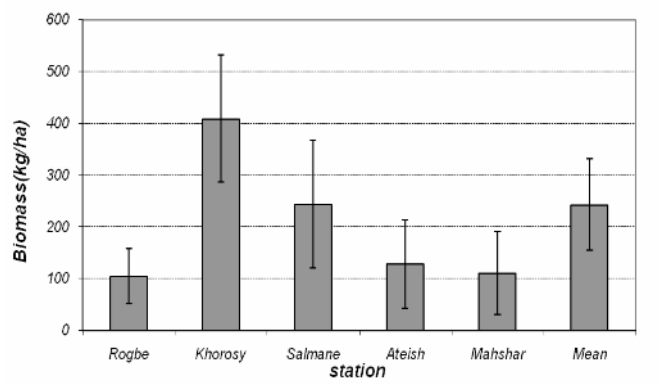

Figure 2. Value fish biomass estimates in different station from Shadegan Wetland (2013-2014)

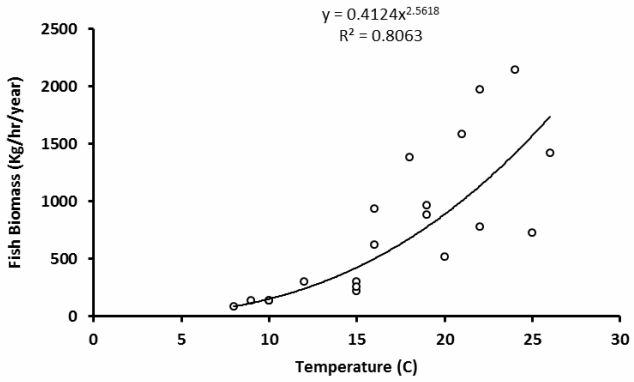

Figure 3. Fish biomass and Temperature in different station from Shadegan Wetland (2013-2014)

Table 1. List of fish species caught and the percentage composition for fish population in Shadegan Wetland (2013-2014)

Family	Species	Status	N	%N
Cyprinidae	*Carasobarbus luteus*	-	634	22.68
	Cyprinus carpio	-	513	18.35
	Carassius auratus	-	417	14.92
	Mesopotamichthys sharpeyii	-	335	11.99
	Leuciscus vorax	-	161	5.76
	Hypophthalmichthys molitrix	-	71	2.54
	Tor grypus	-	70	2.50
	Hypophthalmichthys nobilis	Introduce	51	1.82
	Ctenopharyngodon idella	-	24	0.86
	Luciobarbus pectoralis	Native	14	0.50
	Luciobarbus xanthopterus	-	3	0.11
	Acanthobra mamarmid	-	2	0.07
	Alburnoides bipunctatus	-	2	0.07
	Cyprinion macrostomus	-	1	0.04
	Cyprinion kais	-	1	0.04
	Barbus luciobarbus	-	1	0.04
Clupeidae	*Tenualosa ilisha*	-	1	0.04
	Sardinellas indensis	Exotic	2	0.07
Engraulidae	*Chelon abu*	Native	339	12.13
	Thryssa hamiltonii	Exotic	5	0.18
Mugilidae	*Ellochelon vaigiensis*	-	7	0.25
	Chelon subviridis	Exotic	7	0.25
Mastacembelidae	*Mastacembelus mastacembelus*	Native	21	0.75
Sparidae	*Silurs triostegus*	-	60	2.15
	Acanthopagrus arabicus	Exotic	46	1.65
Siluridae	*Heteropneustes fossilis*	Native	3	0.11

Table 2. Average values catch, total biomass and production of fish species from the Shadegan Wetland (2013-2014)

Species	Spring (kg/ha)	Summer (kg/ha)	Autumn (kg/ha)	Winter (kg/ha)	Mean biomass (kg/ha/yr)	Production (kg/ha/yr)	Production per biomass (P/B)
Acanthopagrus arabicus	0.2	6.8	1.1	-	2±3	2±1	1.39
Aspius vorax	-	17.8	55.1	14.3	21±2	16±5	0.75
Carasobarbus luteus	167.4	43.9	27.7	11.5	62±7	65±1	1.05
Carassius auratus	22.8	62.4	24.1	12.7	30±2	41±7	1.35
Chelon abu	22.8	7.1	8.1	7.9	11±7	9±1	0.81
Cyprinus carpio	57.4	74.8	40.1	14.6	46±2	66±2	1.43
Mesopotamichthys sharpeyii	63.3	53.7	32.7	29.4	44±2	44±6	1.00
Silurs triostegus	93.3	141.2	13.1	90.8	84±5	71±3	0.85
Tor grypus	21.5	-	-	0.5	5±1	4±2	0.76
Others	31.1	7	-	-	9.5±1.5	4±0.5	0.52

Table 3. Fish biomass and temperature in different species from Shadegan Wetland (2013-2014)

Fish species	Regression formula	R^2
Cyprinus carpio	$y = -0.6215x^2 + 38.854x-319.88$	0.5059
Hypophthalmichthys molitrix	$y = 0.8108x^2-21.582x + 132.01$	0.4256
Mesopotamichthys sharpeyii	$y = 0.0296x^2 + 6.6605x-52.136$	0.3641
Silurs triostegus	$y = 1.3568x^2-27.411x + 153.68$	0.5434
Tor grypus	$y = 0.6235x^2-17.028x + 111.21$	0.5155

Table 4. Physic-chemical parameters in different station from Shadegan Wetland (2013-2014)

Variable	Value	Atish	Khorosy	Mahshar	Rogbe	Salmane	P-values
	Max	8.1	10	8.05	6.2	7.1	>0.05
DO	Min	4.5	3	2	2	4.5	
	Mean	6.8±1.6	6.9±3.3	5.2±3.2	3.3±1.8	6.4±1.3	
BOD	Max	4.6	6.6	3.1	2.6	3.4	>0.05
	Min	2.5	4	2	1	2.5	
	Mean	3.7±0.8	4.7±0.9	2.4±0.4	1.7±0.6	2.9±0.4	
pH	Max	8.2	8.8	7.6	8.4	8.1	>0.05
	Min	8	7.2	7.2	8	7.5	
	Mean	8.1±0.1	7.9±0.6	7.3±0.1	8.1±0.1	8.1±0.1	
Tem.	Max	22	21	24	25	22	>0.05
	Min	8	15	9	10	10	
	Mean	16±6.2	18.2±2.6	16.7±6.3	17.5±6.2	16.5±5.2	
Sal.	Max	6	8	50	17	40	< 0.05
	Min	5	2	10	6	6	
	Mean	5.25±0.5	3.68±2.9	18.75±10.2	10.5±4.8	23±2	
NO_3	Max	8.5	8	5.5	5	4.5	>0.05
	Min	4	4.5	4	4.5	3	
	Mean	5.7±1.4	6.7±1.3	4.8±1.1	4.6±0.4	4±0.7	
PO_4	Max	0.7	1.2	0.8	0.6	0.8	>0.05
	Min	0.3	0.2	0.2	0.1	0.2	
	Mean	0.4±0.1	0.6±0.2	0.5±0.2	0.5±0.3	0.5±0.2	
Depth	Max	2.7	2.1	2.6	2.5	2.3	>0.05
	Min	2.1	1.4	1.4	1.4	2	
	Mean	2.4±0.2	1.7±0.3	1.9±0.5	1.9±0.4	2.1±0.1	

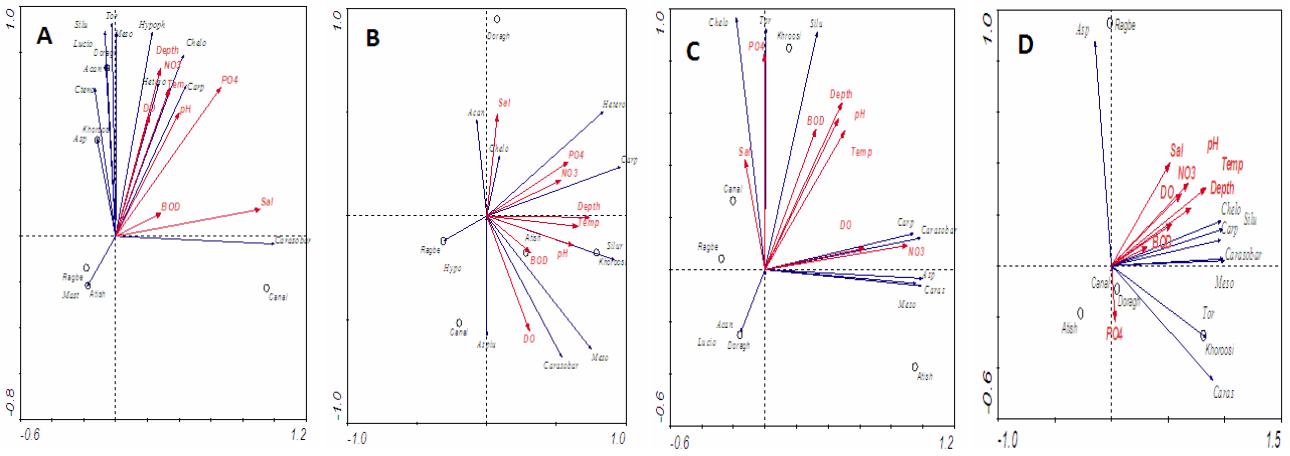

Figure 4. Ordination three plot of main fish species (see Table 1 for species numbers) and five stations from canonical correspondence analysis (CCA) with 8 environmental variables in Shadegan Wetland (A = Spring, B = Summer, C = Autumn, D = Winter).

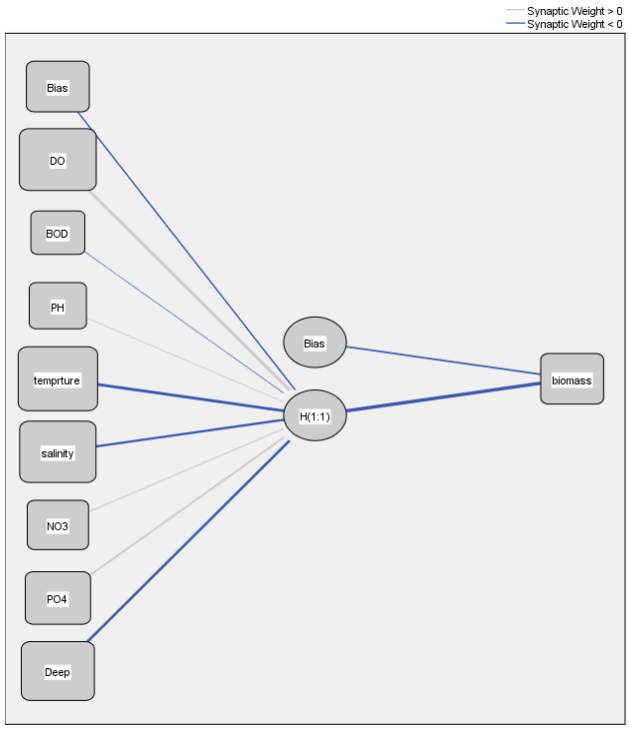

Figure 5. Artificial neural network (ANN) with one input layer corresponding to the input (independent), one hidden layer and one output layer to estimate the output (dependent). Solid lines show connections between neurons. Bias neurons are also shown their input value is biomass

Discussion

The native marshland fish populations were originally dominated by Cyprinid fish of the genus *Barbus*. Overall, *B. luteus, M. sharpeyii, C. carpio, C. carasus, A. vorax* and *Chelon abu* are included over 70% biomass and fish main species of Shadegan Wetland species (Hashemi et al. 2015). The dominance of cyprinids in tropical reservoirs has been observed in Sri Lankan reservoirs, where the family formed over 50% of the species present (Amarasinghe 1992). Abundance of fish populations in river, lake with river source and reservoirs widely changed from year to year and the relative frequency of different species is different in population. The increasing area and flood flow time is improved spawning, growth and survived rate (Welcomme 2001). Bias associated with fishing gear types can greatly influence comparisons of aquatic habitats, especially when meaningful community information is desired for habitat restoration research (Jackson and Harvey 1997).

Average fish biomass in spring and summer of 1997, 70.2 kg/ha, 109.2 kg/ha, and in 2001, 186.5 kg/ha and 269.4 kg/ha and in 2009, 249 kg/ha, 216 kg/ha was calculate, respectively (Maramazi 1997; Ansari et al. 2001; Hashemi et al. 2012). In spring and summer were increased of biomass comparing 1997, 2001 and 2009. It seems, climate change and wetland nutrient elements are very effective factor that influenced on biomass. The Khorosy stations in different seasons have high amount of fish biomass. It seems, that entering the Jarahi river for east side of the wetland and location of Khorosy station in near the river month and entering of nutrition element was caused to increase phytoplankton and phytobentozic production that caused to increase fish biomass in these areas (Kholfenilsaz 2009). The dynamics of wetland fish communities are determined by periodically changing abiotic factors, especially water temperature and water level, and biotic factors, especially food availability. Water level fluctuations have several important functions and result in pulses of nutrient input and fish abundance. Wetland fish stocks can usually be sustained as long as the pristine flood regime is retained, but disruption of the flooding pattern interferes with fish breeding and nutrient flow (Bruton and Jackson 1983).

Mean ± S.D fish production values were 325±33 kg ha/yr. Productive reservoir fisheries have developed in small reservoirs in Africa with yields of up to 329 kg ha/yr, in Latin America and the Caribbean with yields up to 125 kg ha and in Asia with yields up to 650 kg ha/yr (SOFIA 2002). Fish production estimates are valuable statistics for understanding population dynamics and elucidating

ecological relationships and have great potential for improving fisheries management.

Based on P/B value were as 0.52-1.43. Typical values of P/B for freshwater invertebrates range from 2.5 to 5, with a mean of 3.5. Values for fishes generally are lower. (Waters et al. 1990) The P/B (per year) ratio indicates how quickly biomass is potentially changing. For fish populations in lakes, most P/B ratios varied between 0.2 and 5.0, and were inversely related to maximum size of the fish in the populations and positively related to lake productivity (Downing and Plante 1993).

In the CCA ordination, axes 1 and 2 together explained a high percentage of variance of the species-station-environment tree plot, with temperature, salinity, PH and DO, as the most important variables influencing the variation of fish composition in the Shadegan Wetland (Figure 5). It seems in multivariate indices; depth and Po4 have low affect associated with species distribution (Hashemi et al. 2015). Thus, the fish assemblage of the freshwater-influenced habitat was characterized by the presence of numerous species that are tolerant to low-salinity conditions, and enter the system mainly for food and protection. The fish assemblage of the marine-influenced habitat was characterized by the presence of occasional and seasonal species (Simon 1999).

Fish biomass regressions and ANN model was showed four parameters (temperature, salinity, DO and depth) have the greatest impact on fish biomass. Among the physic-chemical factors, water salinity, temperature, dissolved oxygen, and their regular or irregular fluctuations at different time scales, have been identified as determinants in fish ecology (Blaber 2000). The associations between fish biomass and water quality variables owing to a complex array of stochastic and/or deterministic effects, long-term studies are ideal because they incorporate both annual and seasonal variation (Leash and Pigg 1990).

The successful application of ANN at various spatial scales, and for a range of aquatic ecosystems (lakes and rivers), organisms (invertebrates and fish), and ecological descriptors (abundance, Shannon diversity index, and community composition) demonstrated in this study opens new fields for the application of ANN in aquatic ecology (Lek and Guegan 2000). Due to their ability to mimic non-linear systems, ANNs proved far more effective in modeling the distribution of these species in the marine ecosystem (Brosse et al. 2001). The ANN models could be developed using information measured in undisturbed reference sites (environmental parameters and fish biomass), and deviations between reference and test sites may be interpreted with respect to potential anthropogenic impacts.

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Identification and expression of two types of chicken GnRH-II genes in mature hard-lipped barb, *Osteochilus hasselti*

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Abstract. *Prayogo NA, Wijayanti GE, Sulistyo I. Sukardi P. 2016. Identification and expression of two types of chicken GnRH-II genes in mature hard-lipped barb,* Osteochilus hasselti. *Biodiversitas 17: xxx.* Gonadotropin-releasing hormone (GnRH) is synthesized in the brain and acts in the anterior pituitary to stimulate the release of gonadotropins in fishes as well as in other vertebrates. Genomic DNAs and cDNAs of two chicken-type GnRH-II genes of hard-lipped barb, namely cGnRH-II type 1 and type 2, were cloned. The length of cloned genomic DNA of cGnRH-II type 1 was 580 bp and cDNA was 206 bp. The length of cloned genomic DNA of cGnRH-II type 2 was 570 bp and cDNA was 196 bp. The cGnRH-II type 1 and type 2 cDNAs encode precursors of 68 and 63 amino acids, respectively. Those precursors consist of a signal peptide, cGnRH-II decapeptide and a GnRH-associated peptide (GAP) linked by a Gly-Lys-Arg proteolytic site. Using quantitative Real Time-PCR, expression levels of these two cGnRH-II genes were detected in the brain, liver and gonad of hard-lipped barb. Expression of the GnRH-II type 1 gene was found only in the brain and liver, on the other hand, expression of the cGnRH-II type 2 gene was found in the gonad, in addition to the brain and liver. The expression of the cGnRH-II genes outside the brain suggested that cGnRH-II might act as an autocrine or paracrine regulator.

Keywords: cGnRH-II, type 1 and 2, Real Time-PCR, amino acid

INTRODUCTION

Gonadotropin-releasing hormone (GnRH) is a conserved neuro-decapeptide family, which plays a crucial role in regulating gonadal development and controlling the final sexual maturation in vertebrates (Gibson et al. 1997; Sayed et al. 2010; Gharaei et al. 2011). The GnRH decapeptide is synthesized by neuro-secretory cells in hypothalamus and secreted into portal vessels, transported to the pituitary gland where it stimulates secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from pituitary gonadotrophs (Yaron et al. 1995).

The presence of either two or three forms of GnRH in teleost fishes has been well documented (Kah et al. 2007). The so-called GnRH-I system is regarded as a species specific form and includes mammalian GnRH (mGnRH), seabream GnRH (sbGnRH), chicken GnRH-I (cGnRH-I), and pejerrey GnRH (pjGnRH) (White and Fernald 1998; Morgan and Millar 2004; Kah et al. 2007; Sayed et al. 2010). The GnRH-I system is generally localized in the forebrain and is considered to exert the neuroendocrine control over LH secretion. Another form of GnRH designated as GnRH-II (Sherwood et al. 1993; Sealfon et al. 1997; Volkoff and Peter 1999) has been reported in all major vertebrate groups, including mammals and is mainly expressed in the midbrain (Sherwood et al. 1993). GnRH-II appears to have direct effects on sexual behavior in mammals, birds, and fish (Rissman et al. 1997; Muske 1998; Russell and Richard 1999; Troskie et al. 1998; Wang and Lin 1998), and this effect is believed to be its primary function. Finally, GnRH-III is represented by salmon GnRH (sGnRH) (Sherwood et al. 1983; Adam et al. 2002) and is found in the forebrain either alone or together with GnRH-I depending on the species (Adam et al. 2002; Morgan and Millar 2004). GnRH peptides are also reported in the ovary and testis of fish and in the ovary, testis, mammary gland and placenta of mammals (Sherwood et al. 1993). cGnRH-II exists in the brain tissues of all the fishes, in which cDNA sequences of GnRH have been characterized, and are distributed mainly in the midbrain. Both the function of cGnRH-II and the cycle variations of expression levels during gonad development are still controversial.

Hard-lipped barb (*Osteochilus hasselti* C.V.) species is an indigenous tropical fish and is synchronous batch spawner fish (Prayogo et al. 2008), which is capable of spawning several times during the peak of the spawning period. This fish, a familiar economical freshwater fish in Indonesia, is used as the model of endocrine regulation of freshwater fish (Prayogo et al. 2012). In our laboratory, cGnRH-II cDNAs have been cloned from hard-lipped barb brains for the first time, and all of them are encoded by two different gene loci. This study reports the isolation and identification of two differing cGnRH-II cDNAs and genes in the hard lipped barb. Expression levels of the cGnRH-II genes are assayed in the brain, liver and gonad by real time-PCR. The research results offer novel evidence for two types of cGnRH-II genes for understanding further the

function and regulation mechanism of cGnRH-II genes in the HPG axis in hard-lipped barb.

MATERIALS AND METHODS

Brain, liver and gonad collection

Total RNA and genomic DNA were isolated from brain, liver and gonad. Total of 30 sexually mature female Hard-lipped Barb weighing of 100 g in average were purchased from local market in Banyumas District, Central Java, Indonesia. Fish brains were removed, snapped frozen, and stored at-150°C with liquid nitrogen until the time for RNA and genomic extraction. Isolation, cloning, and sequencing of two cGnRH-II genes were conducted at the Laboratory of Molecular Biologi, Universitas Jenderal Soedirman, Purwokerto, Banyumas, Indonesia.

Genomic DNA isolation

Total genomic DNA was extracted from whole brain, liver and gonad. The tissue was mixed with 400 µL TNES (Tris, NaCl, EDTA, and SDS), and 0.5 µL RNAse and 3 µL Proteinase K were added to the sample. The sample was incubated in 37^0C for 2 hour and then centrifuged for 15 minutes. Then the sample was extracted with phenol chloroform followed by centrifugation for 5 minutes. DNA in the water phase was precipitated with ethanol. The integrity of the DNAs was verified by agarose gel electrophoresis and staining with ethidium bromide.

RNA isolation and RT-PCR

Total mRNA was extracted from whole brain, liver and gonad using Blue Sepasol R-RNA super 1 reagent (nacalaitesque) based on ethanol-phenol-chloroform extraction method. The prepared RNA was treated with RNase-free DNase (Takara). The quality and concentration of RNA were assayed by denaturing agarose gel electrophoresis and optical density reading at 260 and 280 nm. The RNA was aliquoted in batches and frozen at-70^0 C. Total RNA samples (1.5 ng each) were reversely transcribed using cDNA synthesis kit (PrimeScript™ Reverse Transcriptase) from Takara.

Amplification of GnRH-II genomic DNA and cDNA

The primer pairs, Cyprinidae cGnRH-II Type 1F containing an *EcoR*1 site and Cyprinidae cGnRH-II T1 R containing a *Xho1* site were designed based on cGnRH-II cDNA sequences of Cyprinidae (*Cyprinus carpio* AY189961.1) and *Carassius auratus*, U30386.1. The primer pairs, Cyprinidae cGnRH-II Type 2 F containing an *EcoR1* site and Cyprinidae cGnRH-II T2 R containing a *Xho1*site were designed from cGnRH-II cDNA sequences of *Carassius auratus* (AB017271.1), and *Cyprinus carpio*, (AF521130.2). The sequences were aligned with MultAlin to identify the conserved sequences in the ORF region. The primers to amplify the cGnRH-II type 1 and type 2 cDNAs were designed using Primer 3 software (Table 1). The same primer pairs were used to amplify cGnRH-II type 1 and type 2 genes of hard lipped-barb (Table 1).

PCR for both genomic DNA and cDNA was carried out using a thermal cycler (Robocycler, Stratagene) according to the following cycle; 95°C for 2 min, 35 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 60 s, followed by a 5 min extension at 72°C. After amplification, the PCR products was electrophoretically separated on a 1.5% agarose gel and stained with ethidium bromide.

Cloning and Sequencing of PCR Products

PCR amplified fragments of genomic DNA and cDNA were separated by agarose gel electrophoresis. DNA was extracted from the incised gels using the DNA gel extraction procedure (Green and Sambrook 2012.). The desired DNA fragments were subcloned into BSKS Eco R1/Xho 1 vector (10 ng) (Takara) using ligation with T4 ligase. The plasmid was transfected into *E. coli* and the bacteria were spread on LB medium plates (Mohamed et al. 2008). The recombinant positive colonies were screened using ampicillin. Plasmid DNAs were purified from positive colonies with mini scale plasmid preparation. DNA sequences were determined using the Big Dye version 3.1 sequencing method with specific primers. Primers used for sequencing of cGnRH-II type 1 were *Cyprinidae F cGnRH-II T1* and *Cyprinidae R cGnRH-II T1*, and those for cGnRH-II type 2 were *Cyprinidae F cGnRH-II* F2 and *Cyprinidae F cGnRH-II R2* (Table 1). The sequence data were automatically collected on the ABI PRISM 3100 Genetic Analyzer (PE Applied Bio-systems).

Sequence analysis

The genomic and cDNA sequences for two cGnRH-II genes were analyzed using BLASTN (http: //www.ncbi.nlm.nih.gov/BLAST/) with default settings on the complete, non-redundant GenBank database nucleotide sequences. The genomic and cDNA sequences were aligned using CLUSTALW software to identify introns and exons.

Table 1. The primers used to amplify the two cGnRH-II genes and cDNAs and to sequence their PCR products.

Primer	Code	Sequences	Tm	PCR Product
F cGnRH-II T1	F2	TGGGGATGTTGCTGTGTCTA	64.18	580 bp
R cGnRH-II T1	R2	TCTTTTGGAAATCCCGTATG	57.55	
F cGnRH-II T2	F3	GGTGATGGGGATGTTGATGT	59.28	580bp
R cGnRH-II T2	R3	TCTTTTGGAAATCCCGTATG	58.43	

Phylogenetic analysis

For phylogenetic analyses, hard-lipped barb cDNAs of cGnRH-II type 1 and type 2 were compared to cDNA sequences of cGnRH-II from nineteen fish species. All sequences were retrieved from NCBI GenBank (Appendix 1). The relationship between hard-lipped barb GnRH and other teleost GnRH was generated with CLUSTAL W with scoring method percent, and the unrooted tree was generated using Treeview version 1.5.2. (Magdy et al. 2007).

Quantitative Real Time analysis

The primers were designed using the Primer 3.0 software. The used primers were as follows: type 1 cGnRH-II forward, 5-TGGGGATGTTGCTGTGTCTA-3; type 1 cGnRH-II reverse, 5-TCTTTTGGAAATCCCGTATG-3; type 2 cGnRH-II forward 5-GGTGATGGGGATGTTGATGT-3; type 2 cGnRH-II reverse, 5-TCTTTTGGAAATCCCGTATG-3. Goldfish actin (GenBank accession number AB039726.2), used as endogenous control, was amplified using the following primers: actin forward, 5-GAGCTATGAGCTCCCTGACGG-3; actin reverse, 5-AAACGCTCATTGCCAATGGT-3, and were used to normalize variations in RNA. After optimization, PCR was performed in a 10 µL solution containing 2 µL cDNA, 5 µL SYBR mix (Applied Biosystem), 0.3 µL forward primer, 0.3 µL reverse primer and 2.4 µL DDW using the following conditions: 95°C for 45 s, 45 cycles of 95°C for 15 s and 60°C for 1min, then 95°C for 15s, 60°C for 15s and 95°C for 15s. The results were analyzed using the standard curve mode, according to the manufacturer's recommendations (Applied Biosystems).

Data analysis

The mRNA levels for each sample were expressed as the ratio of cGnRH-II mRNA to actin mRNA. The data were subjected to ANOVA followed by Turkey's multiple-comparisons tests. Differences were considered significant when $P<0.05$.

RESULTS AND DISCUSSION

Cloning of Genomic DNA and cDNA of two cGnRH-II genes in Hard Lipped Barb

The two types of cGnRH-II genes of the hard-lipped barb were successfully amplified from genomic DNA and cDNA. The agarose gel electrophoresis of PCR products from the two types of cGnRH-II genomic DNAs showed specific bands, approximately 580bp in size, which were designated as cGnRH-II type 1 (JN867722) and cGnRH-II type 2 (GenBank accession 1697609) (Figure 1). The genomic sequences of the two cGnRH-II genes were analyzed with BLAST and we found that they were different from each other and also different from GnRH genes of other species. The nucleotide sequence identity of cGnRH-II type 1 cDNAs was 92% with cGnRH-II of carp (*Cyprinus* carpio, AY189961.1), 90% with goldfish (*Carassius auratus*, U30386.1), 92% with roach (*Rutilus rutilus*, U60668.1), and 90% with grass carp (*Ctenopharyng odonidella*, EU981284.1).

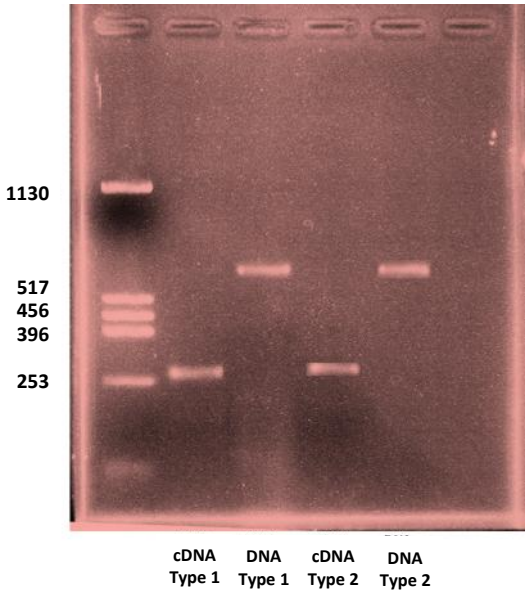

Figure 1. PCR products amplified from genomic DNA using the primer sets designed for each of the two cGnRH-II genes of hard-lipped barb (*Osteochilus hasselti* C.V).

The nucleotide sequence identity of cGnRH-II type 2 cDNAs was 94% with cGnRH-II of carp (*Cyprinus carpio*AY189961.1), 92% with goldfish (*Carassius auratus*, U30386.1), 94% with roach (*Rutilus rutilus*, U60668.1), and 92% with grass carp (*Ctenopharyng odonidella*, EU981284.1). These results indicate the presence of two different genes and cDNAs encoding cGnRH-II in the brain of hard-lipped barb for the first time.

The nucleotide sequence identity of cGnRH-II type 2 cDNAs was 94% with cGnRH-II of carp (*Cyprinus carpio*AY189961.1), 92% with goldfish (*Carassius auratus*, U30386.1), 94% with roach (*Rutilus rutilus*, U60668.1), and 92% with grass carp (*Ctenopharyng odonidella*, EU981284.1). These results indicate the presence of two different genes and cDNAs encoding cGnRH-II in the brain of hard-lipped barb for the first time.

Gene Structure of cGnRH-II

The two cGnRH-II genes share the same basic structure. The genomic DNA fragments both contained 3 exons (coding region) and 2 introns (non coding region). The first exon encoded a signal peptide (17 amino acids for type 1 and 13 amino acids for type 2), GnRH-II decapeptide, the proteolytic cleavage recognition site (3 amino acids for both types) and N-terminus of GnRH-associated peptide (GAP) (first 9 amino acids for both types). Exon 2 encoded the central portion of GAP and exon 3 encoded the C terminus of GAP (Figure 2). All intron-exon boundary sequences conformed to the GT-AG rule.

Structures for two types of cGnRH-II had a high similarity in length for exon 1 and 2, but the intron sizes of cGnRH-II type 1 were different from cGnRH-II type 2 (Figure 2). The level of similarity in the coding sequences can be seen as the distance at the phylogenetic tree (Figure 6). The greatest differences within the preprohormone are within the GAP coding sequences. The striking contrast

between the conservation of the GnRH coding sequences and the lack thereof in the GAP coding sequences is the evidence of differential selective pressure within the gene (Figure 5). This is evident in cases where the identity and similarity of the GnRH and GAP coding sequences have been compared for mRNAs of GnRH-II genes from different species (Figure 4) (White and Fernald 1998; Russell and Richard 1999).

Phylogenetic analyses

Phylogenetic analyses were performed to establish an evolutionary context for the two cGnRH-II genes. Genetic distances (measured as substitutions per site) showed moderate low values, and the topology was well supported by strong bootstrap values. As expected, two types of cGnRH-II in hard-lipped barb were included within a sub-cluster of the *carp* (*Cyprinus carpio*, *Carassius auratus*) with high bootstrap values (Figure 6).

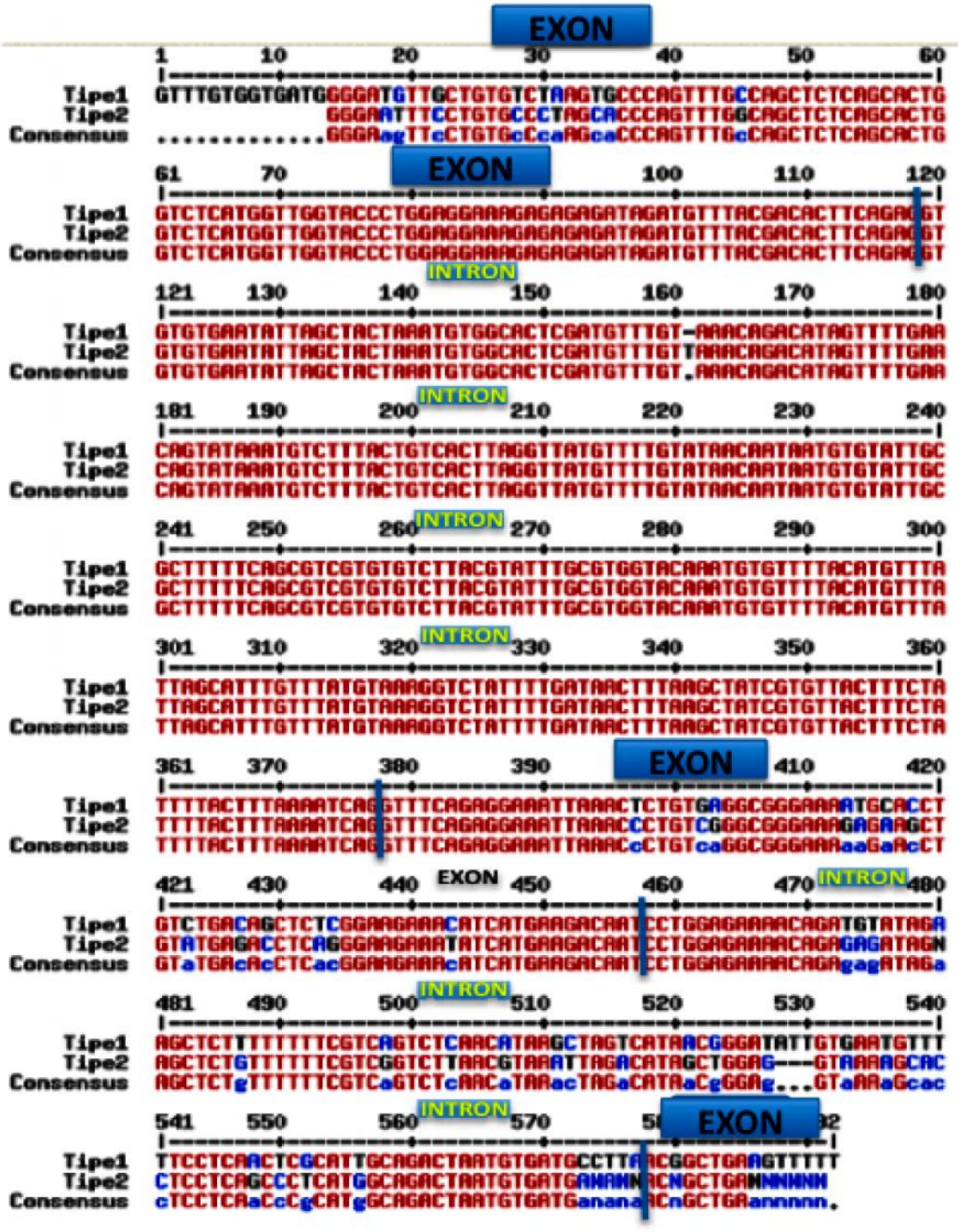

Figure 2. Nucleotide sequences and exon/intron structure of two cGnRH-II genes in hard-lipped barb

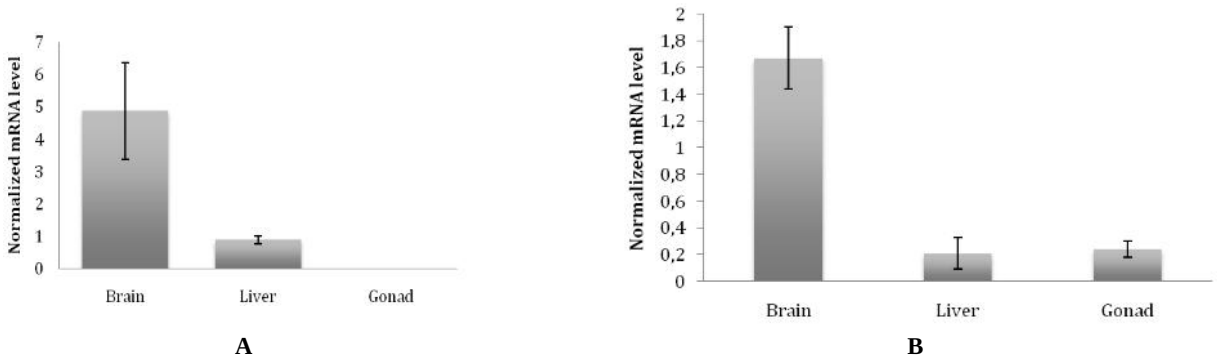

Figure 3. Expression of two types of cGnRH-II mRNA in the brain, liver and gonad of mature female hard-lipped barb. (A) Expression of cGnRH-II type 1 mRNA. (B) Expression of cGnRH-II type 2 mRNA

Expression of two cGnRH-II genes in the brain, liver and gonad of mature fish

Total RNA was isolated from the brain, liver, and gonads of mature female hard lipped barb, and the reverse transcription products of total RNA were amplified by primers F2 and R2 for cGnRH-II type 1, and primers F3 and R3 for cGnRH-II type 2 (Figure 3). The results of qRT-PCR analysis showed that two types of cGnRH-II genes were coexpressed in the brain of mature hard-lipped barb. cGnRH-II type 2 mRNA was expressed in the liver and gonad, and type 1 mRNA was expressed in the liver, but not in the gonad. The expression levels of the two types of cGnRH-II mRNA in the brain were much higher than those in the liver and gonad. It is also reported that two types of cGnRH-II mRNAs were expressed in the liver of female common carp, but only cGnRH-II type 2 mRNA is expressed in the gonad (Lin et al. 2003).

Discussion

This paper reports for the first time that hard-lipped barb had two forms of cGnRH-II namely cGnRH-II type 1 and cGnRH-II type 2, similar to *Cyprinus carpio* (Lin and Lin 1994; Wang and Lin 1998), and goldfish (Kim et al. 1995; Lin and Peter 1996; Chik et al. 1997; Yu et al. 1998). The two cGnRH-II genes and cDNAs cloned in this study are missing 5' and 3' sequences, due to the design of the PCR primers. Our analyses hence have limitation, but still give deep insights into the evolution and physiological functions of these genes. The newly identified hard-lipped barb type 1and type 2 genes show a high conservation with other GnRH-II genes previously reported (Figure 4). The nucleotide sequence of cGnRH-II type 1 cDNA shows, based on BLAST search, 96, 95, 94, 94% similarity to cGnRH-II cDNAs of carp (*Cyprinus carpio*AY189961.1), goldfish (*Carassius auratus*, U30386.1), roach (*Rutilus rutilus*, U60668.1), and grass carp (*Ctenopharyng odonidella*, EU981284.1), respectively. The nucleotide sequence of cGnRH-II type 2 cDNA is also very similar to other cGnRH-II cDNAs, 95, 94, 92, 92, 91% similarity to cGnRH-II of goldfish (*Carassius auratus*, AB017271.1), carp (*Cyprinus carpio*, AF521130.2), roach (*Rutilus rutilus*, U60667.1), grass carp (*Ctenopharyng odonidella*, EU981295.1) and zebrafish (*Danio rerio*, AY557019.1), respectively (Figure 5). The structural characteristics of GnRH-II type1 and type 2 genomic loci in hard lipped barb are similar with GnRH genes in other species. The results show that GnRH-II genes might evolve from a common ancestral molecule.

The hard-lipped barb cGnRH-II type 1 and type 2 precursor peptides are composed of, as predicted from the partial cDNA sequence, at least 68 and 64 amino acid residues, respectively, which consist of a signal peptide, cGnRH-II decapeptide, and a GAP linked by the highly conserved processing site (Gly-Lys-Arg) (Figure 5). The signal peptides and GAP are only partially cloned. The amino acid sequences of the precursors were compared with previously identified fish GnRH-II precursors including roach (*Rutilus rutilus*), goldfish (*Carassius auratus*), carp (*Cyprinus carpio*), grass carp (*Ctenopharyng odonidella*), zebrafish (*Danio rerio*) (Table 2). The results show that the amino acid homology of GnRH-II type 1 precursors within Cyprinoids is 85-92%, but only 50-71% among other teleosts. The amino acid homology of the cGnRH-II type 2 precursor within Cyprinoids was 83-96%, but only 50-64% among other teleosts (Table 2).

The decapeptide is the minimal structural requirement for gonadothropin releasing activity (Raymond et al. 1986). The processing site (Gly-Lys-Arg) is essential for releasing GAP. The decapeptides and processing sites of the two hard-lipped barb GnRH-II precursors were entirely conserved in vertebrate evolution. However, signal peptides that direct the transport of proteins and GAP are diverged between the two cGnRH-II precursors as well as among other teleosts. The amino acid divergence in the signal peptides and GAP was much higher between the two types of cGnRH-II than between those of neighboring species. It is presumed therefore that the cGnRH-II type 1 and type 2 precursors could have different functions obtained through adapting to natural selections during evolution.

```
cfathead        ATGGTGCACATCTGCAGGCTGCTTGTGCTGATGGGGATGTTGCTGTGTTTAAGTGCCCAG 60
cPimephales     ATGGTGCACATCTGCAGGCTGCTTGTACTGATGGGGATGTTGCTGTGTTTAAGTGCCCAG 60
croach          ATGGTGCACATCTGCAGGCTGCTTGTGCTGATGGGGATGTTGCTGTGTCTAAGTGCCCAG 60
cgrass          ATGGTGCACATCTGCAGGCTGCTTGTGCTGATGGGGATGTTGCTGTGTCTAGGTGCCCAG 60
cred            ATGGTGCACATCTGCAGGCTGTTTGTGGTGATGGGGATGTTGCTGTGTCTAAGTGCCCAG 60
cgoldfish       ATGGTGCACATCTGCAGGCTGTTTGTGGTGATGGGGATGTTGCTGTGTCTAAGTGCCCAG 60
cCyprinus       ATGGTGCACATCTGCAGGCTGTTTGTGGTGATGGGGATGTTGCTGTGTCTAAGTGCCCAG 60
cZebrafish      ATGGTGCTGGTCTGCAGGCTGCTGTTGGTCATGGGGCTGATGCTGTGTCTGAGTGCTCAG 60
type1           --------------------GTTTGTGGTGATGGGGATGTTGCTGTGTCTAAGTGCCCAG 40
type2           ---------------------------------GGGAATTTCCTGTGCCCTAGCACCCAG 27
                                                  ***.: :* *****    .* .* ***

cfathead        TTCGCCAGCTCTCAGCACTGGTCTCATGGCTGGTACCCTGGAGGAAAGCGAGAGATAGAC 120
cPimephales     TTCGCCAGCTCTCAGCACTGGTCTCATGGCTGGTACCCTGGAGGAAAGCGAGAGATAGAC 120
croach          TTTGCCAGCTCTCAGCACTGGTCTCATGGCTGGTACCCTGGAGGAAAGCGAGAGATAGAC 120
cgrass          TTTGCCAGCTCTCAGCACTGGTCTCATGGCTGGTACCCTGGAGGAAAGCGAGAGATAGAC 120
cred            TTTGCCAGCTCTCAGCACTGGTCTCATGGCTGGTATCCTGGAGGAAAGAGAGAGATAGAC 120
cgoldfish       TTTGCCAGCTCTCAGCACTGGTCTCATGGCTGGTATCCTGGAGGAAAGAGAGAGATAGAC 120
cCyprinus       TTTGCCAGCTCTCAGCACTGGTCTCATGGCTGGTACCCTGGAGGAAAAAGAGAGATAGAC 120
cZebrafish      TTGAGCAGCGCTCAGCACTGGTCTCACGGCTGGTATCCTGGAGGAAAGAGAGAGATAGAC 120
type1           TTTGCCAGCTCTCAGCACTGGTCTCATGGTTGGTACCCTGGAGGAAAGAGAGAGATAGAT 100
type2           TTTGGCAGCTCTCAGCACTGGTCTCATGGTTGGTACCCTGGAGGAAAGAGAGAGATAGAT 87
                ** . **** ***************** ** ***** ***********..**********

cfathead        ATTTACGATACATCAGAGGTTTCAGAGGAAATTAAACTCTGTGAGGAAGGAAAATGCAGC 180
cPimephales     ATTTACGATACATCAGAGGTTTCAGAGGAAATTAAACTCTGTGAGGAAGGAAAATGCAGC 180
croach          ATTTACGATACATCAGAGGTTTCAGGGGAAATTAAACTCTGTGAGGCAGGAAAATGCAGC 180
cgrass          ATTTACGATACCTCAGAGGTTTCAGAGGAAATTAAACTCTGTGAGGCAGGAAAATGCAGC 180
cred            GTTTACGATTCCTCAGAGGTTTCAGGGGAAATTAAACTCTGTGAGGCAGGAAAATGCAGC 180
cgoldfish       GTTTACGATTCCTCAGAGGTTTCAGGGGAAATTAAACTCTGTGAGGCAGGAAAATGCAGC 180
cCyprinus       GTTTACGATACCTCAGAGGTTTCAGAGGAAATTAAACTCTGTGAGGCAGGAAAATGCAGC 180
cZebrafish      CTCTACGACACCTCAGAGGTTTCAGAGGAAGTGAAGCTCTGCGAGGCAGGAAAATGCAGT 180
type1           GTTTACGACACTTCAGAGGTTTCAGAGGAAATTAAACTCTGTGAGGCGGGAAAATGCACC 160
type2           GTTTACGACACTTCAGAGGTTTCAGAGGAAATTAAACCCTGTCGGGCGGGAAAGAGAAGC 147
                 * ***** :* *************.****.* **.* ***  .**..*****.:*.*

cfathead        TATCTGAGACCCCAGGGAAGAAACATTCTGAAGACAATACTGCTGGATGCCCTCATACGG 240
cPimephales     TATCTGAGACCCCAGGGAAGAAACATTCTGAAGACAATACTGCTGGATGCCCTCATACGG 240
croach          TACCTGAGACCCCAGGGACGAAACATTCTGAAGACAATACTGCTGGATGCCCTCATACGG 240
cgrass          TACCTGAGACCCCAGGGAAGAAACATTCTGAAGACAATACTGCTGGATGCCCTCATACGG 240
cred            TACCTGAGACCCCAGGGAAGAAACATTCTGAAGACAATACTGCTGGATGCCCTCATACGG 240
cgoldfish       TACCTGAGACCCCAGGGAAGAAACATTCTGAAGACAATACTGCTGGATGCCATCATACGG 240
cCyprinus       TACCTGAGACCCCAGGGAAGAAACATTCTGAAGACAATACTGCTGGATGCCCTCATACGG 240
cZebrafish      TACCTGAGACCGCAGGGAAGAAACATCCTCAAGACAATACTGCTGGATGCCCTCATTCGT 240
type1           TGTCTGACAGCTCTCGGAAGAAACATCATGAAGACAATACGGCTGA-------------- 206
type2           TGTATGAGACCTCAGGGAAGAAATATCATGAAGACAATACGGCTGA-------------- 193
                *. .*** * * *: ***.**** ** .* ********** ****.
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Figure 4. Nucleotides alignment of two types of cGnRH-II cDNA of hard-lipped barb with those of other teleosts

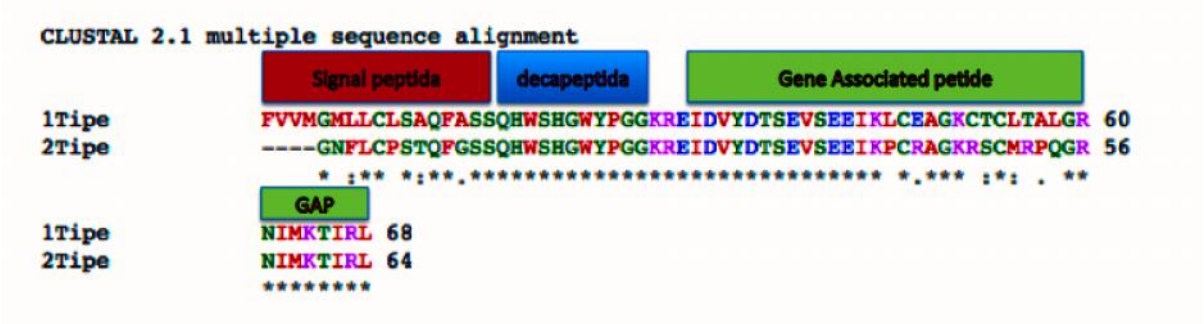

Figure 5. Amino acids alignment of cGnRH-II type 1 and type 2 of hard-lipped barb.

Table 2. Amino acid homology of hard-lipped barb cGnRH-II type 1 and type 2 with those of other species

Species	Type 1		Type 2	
	Accession no.	Homology (%)	Accession no.	Homology (%)
Cyprinus carpio	AY189961.1	92	AAO39975.2	94
Carassius auratus	U30386.1	91	BAB18904.1	92
Ctenopharyng odonidella	EU981284.1	90	ACH78254.1	91
Pimephales promelas	EF672264.1	90	ABV45418.1	88
Rutilus rutilus	U60668.1	90	AAR18405.1	83
Danio rerio	AY094357.1	85	NP_878307.2	90
Oncorhynchus mykiss	AF125973.1	71	AAK82957.1	58
Coregonus clupeaformis	AY245102.1	71	ABP04042.1	96
Anguilla japonica	AB026990.1	61	AAR20401.1	64
Anguilla marmorata	GQ422803.1	61	ACN88548.1	56
Thunnus thynnus	EU239502.1	68	ABX10868.1	50
Oreochromis niloticus	AB101666.1	69	AAD02425.1	51
Mugil cephalus	AY373451.2	50	AAQ83268.1	54
Gadus morhua	GU332294.1	59	ADD92007.1	62

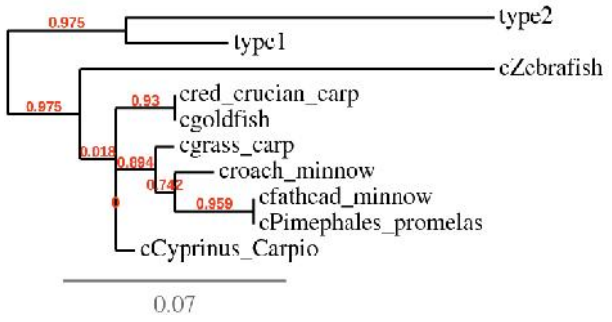

Figure 6. Phylogenetic relationship of hard-lipped barb cGnRH-II type 1 and type 2 precursors with known GnRH. The relationship was generated with CLUSTAL W and the unrooted tree was generated using Treeview version 1.5.2. The scale bar represents the estimated evolutionary distance as 0.1 amino acid substitutions per site

The present study is the first to describe the two types of cGnRH-II genes in hard-lipped barb, and provides new evolutionary information on this gene family. The cGnRH-II type 1 and type 2 genes in hard-lipped barb can be grouped together with other teleost cGnRH-II genes in the phylogenetic tree, suggesting a common ancestor for both groups of genes. Phylogenetic analysis showed that the cGnRH-II type 1 gene is highly homologous to cGnRH-II genes of goldfish (*Carassius auratus*), carp (*Cyprinus carpio*), red carp, and the cGnRH-II type 2 is highly homologous to that of zebrafish (*Danio rerio*) (Figure 6).

The distribution of cGnRH-II peptides and the expression pattern of cGnRH-II genes in brain regions of teleost fishes has indicated that cGnRH-II mainly acts as a neurotransmitter and/or neuromodulator. mRNAs of two goldfish cGnRH-II genes are detected not only in brain regions, but also in the ovary and testis (Lin and Peter 1996; Yu et al. 1998). In hard lipped barb, the two cGnRH-II genes are expressed in the brain and liver. The cGnRH-II Type 2 gene, but not the type 1 gene, is expressed in the ovary, although at much lower levels than in the brain. cGnRH-II should mainly work as the neurotransmitter and neuromodulator and, therewith, operate in the regulation of the GnRH release. The expression of the cGnRH-II genes in the liver and gonad suggests that cGnRH-II stimulate the release of other hormones, such as estradiols and testosterone, in an autocrine or paracrine manner

In summary, the present study has revealed the genomic and cDNA sequences of two cGnRH-II variants namely cGnRH-II type 1 and cGnRH-II type 2 in hard-lipped barb. The phylogenetic analyses support the idea that the two cGnRH genes share the same basic structure with other teleost cGnRH-II genes. It means that the two cGnRH-II genes of hard-lipped barb are conserved, assuming a similar function with other teleost cGnRH-II genes.

ACKNOWLEDGEMENTS

This work was supported by the RISIN Grant from Universitas Jenderal Soedirman, Purwokerto, Banyumas, Central Java Indonesia. Also thanks to Mega Dissa and Diah from their assistance and knowledge.

APPENDIX 1

Accession numbers of the GnRH sequences from teleost fishes, downloaded from GenBank.

GnRH II clade: Anguilla japonica: AB026990; Carassius auratus: U30386; Clariasgariepinus: X78047; Coregonusclupeaformis: AY245102; *Cyprinus carpio*: AY147400; Danio rerio: AF511531; Dicentrarchuslabrax: AF224281; Macacamulatta: AF097356; Micropogoniasundulatus: AY324669; Monopterusalbus: AY786183; Moronesaxatilis: AF056313; Mugilcephalus: AY373451; Odontesthesbonariensis: AY744687; Oncorhynchusmykiss: AF125973; Oreochromisniloticus: AB101666; Oryziaslatipes: AB041330; Rutilus rutilus: U60668; Sci-aenopsocellatus: AY677171; Sparus aurata: U30325; Suncusmurinus: AF107315; Trichosurusvulpecula: AF193516; Tupaiabelangeri: U63327; Typhlonectesnatans: AF167558; Veraspermoseri: AB066359.

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Plant diversity after sixteen years post coal mining in East Kalimantan, Indonesia

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Abstract. *Komara LL, Choesin DN, Syamsudin TS. 2016. Plant diversity after sixteen years post coal mining in East Kalimantan, Indonesia. Biodiversitas 17: 531-538.* Post coal mining areas need to be rehabilitated through reclamation and revegetation. The objective of this study was to evaluate plant diversity after 16 years of revegetation activities in a coal mining reclamation site in East Kalimantan. In an effort to restore plant diversity, the coal mining company began by planting fast growing species as pioneers, then planting local species after three years. This study compared a 20 hectare reclamation site with conditions in the pre-mining area, which covered 14,988 hectares. Vegetation sampling was conducted in 20 plots measuring 20x20 m^2 along line transects, with 100 m distance between plots. A total of 104 plant species were found in the reclamation site, consisting of 76 tree species and 28 herbaceous species. Tree species consisted of 35 planted local species (e.g., *Dryobalanops aromatica, Eusideroxylon zwageri, Macaranga gigantea*), 25 planted non-local species, and 16 local species that grew spontaneously (e.g., *Leucaena glauca, Lansium domesticum, Shorea laevis*). In comparison, 133 species were found in the pre-mining site, consisting of 132 local tree species, one non-local tree species (*Acacia mangium*) and 52 herbaceous species. Tree species diversity index in the reclamation site after 16 years post mining (i.e., 3.54) was still lower than in the pre-mining area (4.29); while the diversity indices for herbaceous plants were relatively similar (2.97 and 2.67 in the reclamation and pre-mining sites respectively). The slightly higher diversity of herbaceous plants in the reclamation site may be attributed to higher coverage per species in this site, despite lower species richness.

Keywords: Kalimantan, local species, plant diversity, reclamation, revegetation

INTRODUCTION

Plant species composition in an area depends on environmental factors, such as temperature, humidity, nutrition, sunlight, topography, bedrock geology, soil characteristics, canopy structure and land use history (Hutchinson et al. 1999). Tropical rain forests are the most important plant species diversity centers in the world (Turner 2004). Situated in the tropics, the island of Borneo, including within it the Indonesian provinces of Kalimantan, contains a highly diverse flora, due partly to its unique geological and climatic history. The island is known to have 15,000 flowering plant species, and more than 3,000 species of trees, including 267 Dipterocarpaceae species (WWF 2005). However, this high diversity is currently being threatened by human activity and changes in land use.

Coal mining is a major activity that is changing landscapes. Coal mining causes changes in biodiversity (Cooke and Johnson 2002); soil profile (Makineci et al. 2011) and geological structure permanently (Shrestha and Lal 2011) by leaving large overburden areas (Graham and Haynes 2004; Sheoran and Sheoran 2009; Alday et al. 2011). Considering the impact of mining on the environment, post-mining areas need to be rehabilitated by conducting reclamation and revegetation (Hazarika et al. 2006). Reclamation in post coal mining areas involves moving the overburden to its original contour and spreading top soil over it (Shrestha and Lal 2011; Malakar et al. 2015). After the reclamation process is completed, the reclamation site is then ready to be revegetated (Shrestha and Lal 2011; Wardana 2008). At reclamation sites, soil nutrients are generally limited, soil pH is low, and there are often metal contaminants; therefore, revegetation activity must be carried out with plants selected on the basis of their ability to survive and regenerate or reproduce under severe conditions. Normally the revegetation process is started by selecting plants that are resistant to drought, or fast growing crops or fodder which can grow with limited nutrients (Sheoran et al. 2010).

In East Kalimantan province, Indonesia, coal mining covers an area of almost 3.27 million hectares (Nugroho and Adman 2011). Previous reclamation efforts in Kalimantan have shown that directly planting local tree species in reclamation sites is not successful, compared to planting pioneer plants such as *Acacia mangium*, *Paraserianthes falcataria* (Mansur 2010). According to Indonesian regulation, the success of post mining reclamation is indicated by 90% growth of vegetation and vegetation conditions that are close to pre-mining conditions (Regulation of State Minister for The Environment no. 4/2012). According to Claassen et al. (2008) one of the indicators of reclamation success is the presence of vegetation. Specifically, Perrow and Davy (2002) mention plant species composition and richness as criteria for evaluating the success of restoration. These parameters are easy to measure and are quite sensitive (Dale and Beyeler 2001; Ludwig et al. 2003). The objective

of this study was to evaluate plant diversity after 16 years of revegetation activities in a coal mining reclamation site in East Kalimantan.

MATERIALS AND METHODS

Study area

The study was conducted at a post coal mining area in the lowlands (approximately 58-200 m above sea level) of East Kutai District. East Kalimantan Province (Figure 1). The mining area was previously a production forest, i.e., a forest concession area, loaned from the Ministry of Forestry under condition that at the end of mining activities, the area should be returned to its pre-mining condition. This study was conducted in two sites: 1) a pre-mining area, i.e., a production forest dominated by *Acacia mangium* (mainly for pulp and paper production) which has been abandoned for a period of 16 years, and 2) a reclamation site, i.e., a post mining area which has been prepared for reclamation by forming it following its original contour. The latter site was covered with 30 cm of top soil in order to plant vegetation. The distance between the two locations was approximately 3 km. At the reclamation site, about 48 tree species were planted, including fast growing species (*Acacia mangium*, *Cassia siamea*, *Paraserianthes falcataria* etc.). In the reclamation program, plant survival was monitored every three months for a period of one year. Trees showing unsuccessful growth were replaced using the same species. In order to restore plant diversity to its pre-mining condition, in the third year of revegetation, a total of 56 local species, e.g., *Dryobalanops aromatica* and *Shorea leprosula*, were planted.

Procedures

Plant diversity in both the pre-mining and reclamation sites was studied for a period of six months (from March to September 2013). In each location, vegetation sampling was conducted in 20 plots measuring 20x20 m^2 along line transects (Soerianegara and Indrawan 1998). The distance between plots was 100 m (Fig.2). The 20x20 m^2 plots were used to sample tree species within these plots, 5x5 m^2 subplots were used to sample non-tree woody plants and 1x1 m^2 subplots to sample herbaceous plants. Non-tree woody plants were later grouped together with herbaceous plants. Plant samples (both tree and herbaceous species) were collected if needed in order to identify unknown species for identification. Plant species were identified using references in Herbarium Bandungense, School of Life Sciences and Technology, Institut Teknologi Bandung, West Java, Indonesia.

Degree of vegetation cover was measured as percentage of area occupied by a plant's crown, stem (basal area) or patch. Tree basal area was determined after measuring diameter at breast height (Phillips 1959). The data collected from plots were analyzed for frequency, density and abundance (Kent and Coker 1992), their relative values were calculated as follows:

$$Relative\ density = \frac{Number\ of\ individuals\ of\ species}{Total\ number\ of\ inviduals} x100$$

$$Relative\ dominance = \frac{Dominance\ of\ a\ species}{Dominance\ of\ all\ species} x100$$

$$Relative\ frequency = \frac{Frequency\ of\ species}{Frequency\ of\ all\ invidiuals} x100$$

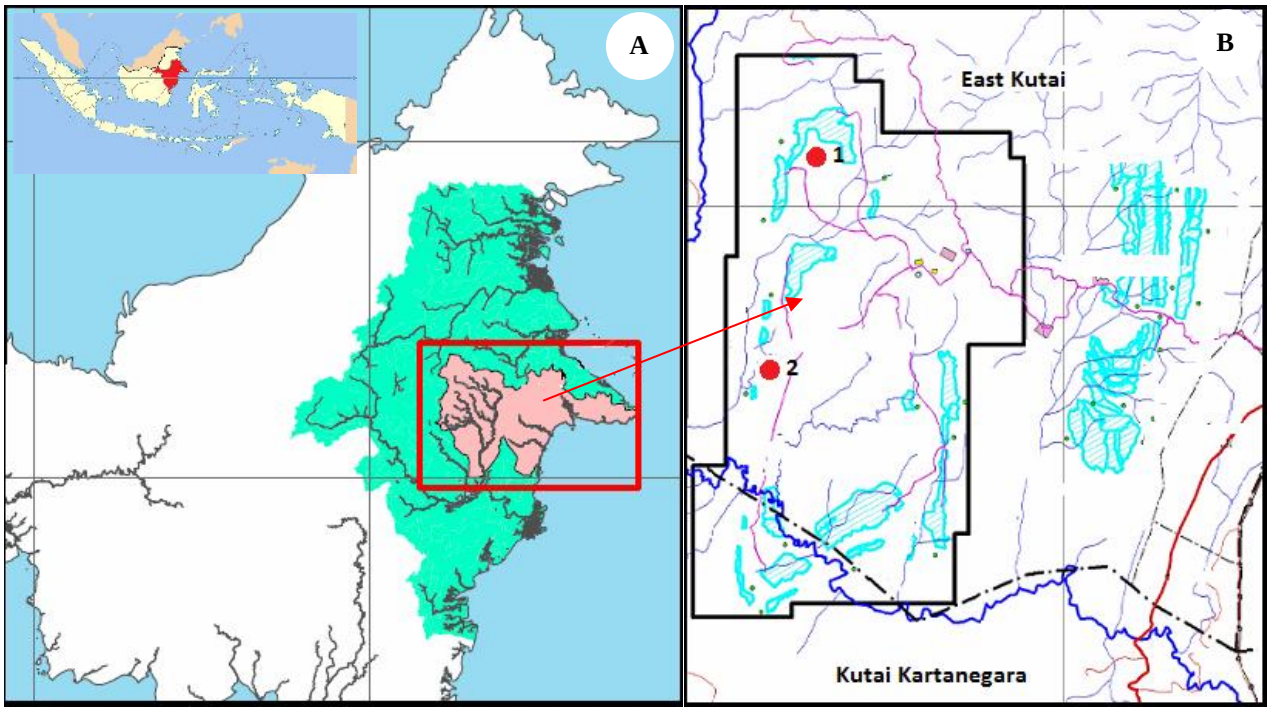
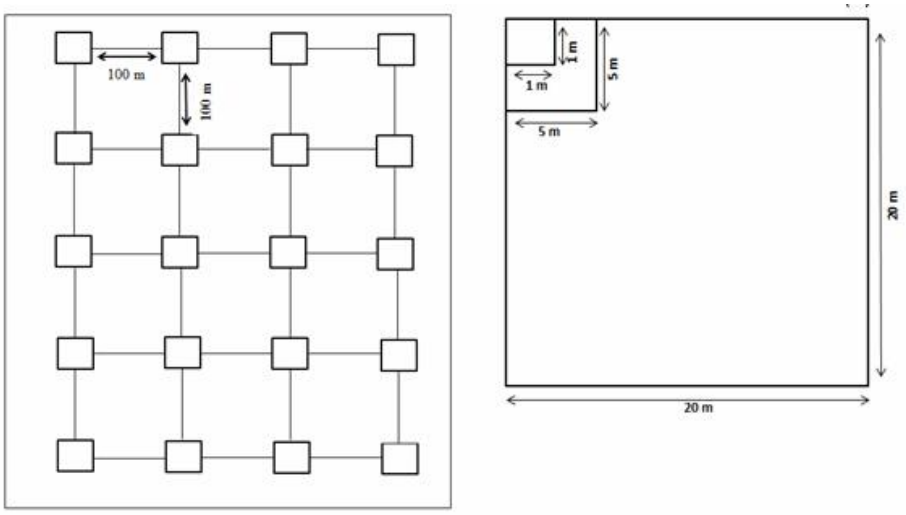


Figure 1. Location of the study area in East Kalimantan at 117º12'50"-117º23'30" EL and 00º02'20"-00º13'00" NL. A. Borneo island, B. Red circle 1 is the pre-mining site and red circle 2 is reclamation site location.



A **B**

Figure 2.A. Plots in line transects in pre-mining and reclamation sites, each plot measuring 20x20 m^2 with 100 m distance between plots; B. Nested plot measuring 20x20 m^2 for trees, 5x5 m^2 for non-tree woody plants and 1x1 m^2 for herbaceous plants.

Importance value for each species = relative density + relative dominance + relative frequency

Where, dominance is defined as the mean basal area per tree times the number of trees of the species.

The species diversity index (H') was determined following Shannon-Wiener (Kent and Coker 1992; Hazarika et al. 2006; Ekka and Behera 2011), i.e., as follows:

$$H' = -\sum_{i=1}^{S}(pi \ln pi)$$

Where, H' = observed species diversity, S = the number of species; pi = the proportion of individuals or the abundance of the i^{th} species expressed as a proportion of total cover; ln = log $base_n$.

Data analysis

All data were calculated using Excel for Windows 7 for the relative density, relative frequency, relative dominance, Importance value index and diversity index (H').

RESULTS AND DISCUSSION

Plant diversity in pre-mining area

The pre-mining area was formerly a production forest dominated by *Acacia mangium* and *Cassia siamea*. After the change of management from production forest to mining company, no activities were conducted in the area. The land was abandoned and vegetation grew naturally for 16 years without any human interference. In this area, we found 185 plant species, consisting of 133 tree species and 52 herbaceous plant species (Table 1 and 2). Among the tree species, there were 132 local species and one non-local species (*Acacia mangium*) which was present since the area was still a production forest. The importance value indices of plant species in this area varied from 0.64% to 46.08%. Fifteen species of tree species had importance value indices higher than 20%, while 15 species of herbaceous plants had indices higher than 5% (Figure 3). Specifically, tree species were dominated by *Macaranga gigantea* (46.08 %), *Eusideroxylon zwageri* (40.96%), *Cananga odorata* (39.04 %), *Euodia speciosa* (36.48 %) and *Dillenia excelsa* (33.92 %); while the herbaceous understorey was dominated by *Blumea balsamifera* (29.04 %), *Pandanus* sp. (23.05%), *Donax cannaeformis* (19.27%), *Phrynium placentarium* (19.10 %) and *Selaginella plana* (17.76 %).

Plant diversity in reclamation site

In the first year of reclamation, 48 non-local species were planted, consisting of pioneer plants and fast growing trees. Three years later, 52 local tree species were planted. After 16 years of reclamation, we found 104 species which consist of 76 tree species and 28 herbaceous species (Table 1). The importance value indices of plant species in this area varied from 0.75% to 66.75%. The top 15 species with highest importance value indices (higher than 4.35% for tree species and more than 7.51% for the herbaceous plants) are presented in Figure 4. tree species were dominated by *Acacia mangium* as shown by the highest importance value index of 37.75%, followed by *Cassia siamea* (35.08%), *Paraserianthes falcataria* (28.28%), *Dryobalanops aromatic* (18.85%) and *Samanea saman* (15.23%). Herbaceous species were dominated by *Diplazium esculentum* with importance value index of 66.75%, followed by *Blumea balsamifera* (30.01%), *Ageratum conyzoides* (18.75%), *Acmella oleracea* (14.25%) and *Merremia peltata* (12.75%).

Table 1. Diversity of tree species at pre-mining and reclamation site (√ for present or × for absent)

Tree species	Pre-mining	Reclamation
Acacia mangium Willd.	√	√
Actinodaphne diversifolia Merr.	√	×
Actinodaphne glomerata (Bl.) Nees.	√	√
Aglaia grandis Korth.ex Miq.	√	×
Aglaia tomentosa Teijsm.& Binn.	√	×
Aleurites moluccana (L.) Willd.	√	√
Alstonia angustiloba Miq.	√	√
Anthocephalus chinensis (Roxb) Bosser.	√	√
Aquilaria malaccensis Lam.	√	√
Archidendron havilandii (Ridl.) I.C.Nielsen.	√	√
Artocarpus altilis(Parkinson) Fosberg.	√	√
Artocarpus champeden (Lour.) Stokes.	√	√
Artocarpus rigidus Blume, Bijdr.	√	√
Averrhoa carambola L.	√	√
Baccaurea macrocarpa (Miq.) Müll.Arg.	√	×
Baccaurea sp.	√	√
Baccaurea stipulata J.J.Sm.	√	×
Barringtonia sarcostachys (Blume) Miq.	√	×
Bauhinia sp.	√	√
Beilschmiedia rivularis Kosterm.	√	×
Bischofia javanica Blume.	√	√
Callicarpa pentandra Roxb.	√	√
Cananga odorata (Lam.) Hook.f. & Thomson	√	√
Canarium odontophyllum Miq.	√	×
Canthium confertum (Burm.f.) Alston	√	×
Casuarina equisetifolia L.	√	√
Cleistanthus myrianthus (Hassk.) Kurz.	√	√
Clerodendrum confusum Hallier f.	√	×
Cratoxylum arborescens (Vahl) Blume.	√	√
Croton argyratus Blume, Bijdr.	√	×
Cryptocarya sp	√	×
Cyathea contaminans (Wall. ex Hook.) Copel	√	×
Dacryodes rostrata (Blume) H.J.Lam	√	×
Dehaasia incrassata (Jack) Nees.	√	×
Dillenia excelsa (Jack) Martelli ex Gilg.	√	×
Dillenia reticulata King.	√	×
Dillenia sumatrana Miq.	√	×
Dimocarpus longan Lour.	√	×
Diospyros borneesis Hiern.	√	×
Diospyros macrophylla Blume.	√	×
Diospyros sp.	√	×
Dipterocarpus confertus Slooten.	√	×
Dipterocarpus cornutus Dyer.	√	×
Dipterocarpus humeratus Slooten	√	×
Disepalum anomalum Hook.f.	√	×
Dracontomelon dao (Blanco) Merr. & Rolfe.	√	√
Drimycarpus luridus (Hook. f.) Ding Hou.	√	×
Dryobalanops aromatica Gaertn.*f., nom cons.*	√	√
Drypetes longifolia (Blume) Pax & K. Hoffm.	√	×
Drypetes subcubica (J.J.Sm.) Pax & K.Hoffm.	√	×
Duabanga moluccana Blume.	√	√
Durio zibethinus Rumph. ex Murray.	√	√
Dyera costulosa (Miq.) Hook. film.	√	×
Euodia alba Hook. f.	√	×
Euodia speciosa Rchb.f. & Zoll. ex Teijsm. & Binn.	√	×
Elmerrillia tsiampacca (L.) Dandy.	√	×
Eusideroxylon zwageri Teijsm. & Binn.	√	√
Evodia latifolia DC.	√	√
Ficus fistulosa Reinw. ex Blume	√	×
Ficus geocarpa Teijsm. ex Miq.	√	×
Ficus sp.	√	√
Ficus uncinata (King) Becc.,	√	×
Ficus variegata Blume	√	×
Flacourtia rukam Zoll. & Moritzi	√	×
Glochidion sp.	√	√
*Gluta renghas*L.	√	√
Hibiscus similis Bl.	√	√
Homalanthus populneus (Giesel.) Pax.	√	×
Horsfieldia grandis (Hk. f.) Warb.	√	√
Ilex cymosa Blume	√	√
Knema conferta (King) Warb.	√	×
Knema latericia Elm.	√	×
*Koompassia malaccensis*Maingay ex Benth.	√	√
Koordersiodendron pinnatum (Blanco) Merr. Bull.	√	√
Lansium Domisticum Corr.	√	√
Lansium parasiticum (Osbeck) Sahni et. Bennet	√	√
Leea aculeata Blume	√	√
Lepisanthes alata (Blume) Leenh.	√	√
Leucaena glauca (Linn.) Benth.	√	√
Lithocarpus sp.	√	×
Litsea accendens (Blume) Boerl.	√	√
Litsea sp.	√	√
Macaranga gigantea (Reichb.f. & Zoll.) Muell.	√	√
Macaranga hypoleuca (Rchb.f. & Zoll.) Müll.Arg.	√	×
Macaranga pruinosa (Miq.) Mull Arg	√	×
Macaranga sp.	√	×
Macaranga tanarius (L.) Müll.Arg.	√	×
Macaranga triloba Thunb.) Müll.Arg.	√	√
Maranthes corymbosa Blume.	√	×
Merremia peltata (L.) Merr.	√	×
Memecylon garcinioides Blume.	√	×
Mitrephora fragrans Merr.	√	×
Myristica elliptica Wall.ex. Hook. f. Thoms.	√	×
Myristica guatteriifolia A.DC.	√	×
Myristica maxima Warb.	√	√
Nauclea calycina Bartl. ex DC	√	×
Nauclea purpurascens Korth.	√	×
Neouvaria acuminatissima (Miq.) Airy Shaw	√	×
Nephelium cuspidatum Blume.	√	×
Nephelium lappaceum L.	√	×
Nypa fruticans Wurmb	√	×
Ochanostachys amentacea Mast.	√	×
Octomeles sumatrana Miq.	√	×
Homalanthus sp.	√	√
Orophea corymbosa (Blume) Miq	√	×
Palaquium quercifolium (de Vriese) Burck	√	√
Planchonia valida (Blume) Blume	√	×
Pholidocarpus sp.	√	×
Polyalthia obliqua Hook.	√	×

Polyalthia sumatrana (Miq.) Kurz.	√	×
Pternandra rostrata (Cogn.) M.P.Nayar.	√	×
Pterospermum diversifolium Blume.	√	×
Pterospermum javanicum Jungh.	√	×
Sageraea lanceolata Miq.	√	×
Sageraea glabra Merr.	√	×
Saraca hullettii Prain.	√	×
Saurauia nudiflora DC.	√	×
Schima wallichii (DC.) Korth.	√	×
Semecarpus glaucus Engl.	√	×
Shorea dispar Ashton.	√	√
Shorea laevis Ridl.	√	√
Shorea leprosula Miq.	√	×
Shorea pinanga Scheff.	√	√
Shorea seminis (de Viese) v.Slooten	√	×
Syzygium acuminatissima (Blume) Merr. & Perry.	√	√
Syzygium sp. 1	√	×
Tetramerista glabra Miq.	√	√
Trema orientalis (L.) Blume	√	×
Vatica sp.	√	×
Vernonia arborea Buch.-Ham.	√	×
Vitex pubescens Vahl.	√	√
Walsura pinnata Hassk.	√	×
Xanthophyllum vitellinum (Blume) D.Dietr.	√	×

Comparison between pre-mining and reclamation sites

After 16 years of reclamation, plant diversity in the reclamation site was still lower than in the pre-mining site which was used as reference. Comparison of species diversity indices (H') between the two sites indicate a significant different in tree species, however, diversity indices for herbaceous plants were similar between the two sites. The diversity index for tree species in pre-mining area was 4.29 and in reclamation site was 3.54. Table 3 presents species richness and diversity indices in the two sites.

It is interesting to note that 16 species of tree species plants in the reclamation site grew spontaneously without being planted by the reclamation program. These were *Leucaena glauca*, *Lansium domesticum*, *Shorea laevis*, *Homalanthus populneus*, *Durio zibethinus*, *Casuarina equisetifolia*, *Averrhoa carambola*, *Artocarpus champeden*, *Bauhinia* sp, *Palaquium quercifolium*, *Horsfieldia grandis*, *Evodia latifolia*, *Cleistanthus myrianthus*, *Bischofia javanica*, *Koompassia malaccensis* and *Actinodaphne glomerata* (Figure 5). Considering the seed form and dispersal process, it is assumed that the presence of these species in the reclamation site is related to the activity of animals which are found near the reclamation site and act as dispersal agents. Both the pre-mining and reclamation sites are in fact not too far from a forest system outside the area. This forest system could be considered as a source of plant diversity, so plant colonization at the reclamation site was presumably assisted by animals from its surroundings. This phenomenon may have benefited the reclamation program, although actual plant dispersal processes need to be confirmed.

Table 3. Diversity of herbaceous species at pre-mining and reclamation site (√ for present or × for absent)

Herbaceous	**Pre-mining**	**Reclamation**
Acmella oleracea (L.) R.K.Jansen,	√	√
Ageratum conyzoides L.	√	√
Amomum maximum Roxb	√	√
Asplenium sp.	√	×
Begonia sp.	√	×
Blumea balsamifera L.	√	√
Boehmeria nivea (L.) Gaudich.	√	√
Borreria sp.	√	×
Calathea bachemiana E.Morren.	√	×
Callicarpa longifolia Lamarck.	√	√
Colocasia esculenta (L.) Schott;	√	√
Colocasia sp.	√	×
Commelina sp.	√	×
Curculigo latifolia Dryand.	√	√
Cyperus sp.	√	√
Diplazium esculentum (Retz.) Sw.	√	√
Donax cannaeformis (G.Forst.) K.Sch.	√	√
Dryopteris sp.	√	×
Fimbristylis sp.	√	×
Gleichenia linearis (Burm. f.) S.W. Clarke.	√	×
Globba leucantha Miq.	√	×
Helminthostachys zeylanica (L.) Hook.	√	√
Hornstedtia affinis Riedl.	√	×
Hornstedtia irya (Gaertn.) Warb.	√	×
Labisia pumila Benth. & Hook	√	√
Lantana camara L	√	√
Mapania sp	√	×
Melastoma malabathricum L.	√	√
Merremia peltata (L.) Merr.	√	√
Mimosa pudica L.	√	√
Pandanus sp.	√	×
Pandanus tectorius Parkinson.	√	√
Peperomia pellucida (L.) Kunth.	√	√
Phacelophrynium maximum (Blume) K. Schum	√	√
Phyllanthus urinaria L.	√	×
Phrynium hirtum Riedl.	√	√
Phrynium placentarium (Lour.) Merr.	√	√
Phrynium sp.	√	×
Piper betle L.	√	√
Piper nigrum L.	√	√
Plagiostachys albiflora Riedl.	√	×
Pleomele angustifolia (Roxb.) N.E.Br.	√	×
Pteris sp.	√	×
Rhaphidophora minor Hook.f. Climber.	√	×
Saccharum spontaneum L.	√	√
Scoparia dulcis L.	√	√
Selaginella plana Hieron.	√	√
Stenochlaena palustris (Burm.f.) Bedd.	√	√
Tectaria sp.	√	×
Tetrastigma sp.	√	×
Zingiber longipedunculatum Riedl.	√	√
Zingiber sp.	√	×

Table 3. Plant species diversity indices in pre-mining and reclamation site

Site	**Pre-mining**		**Reclamation**	
	Number	**H'**	**Number**	**H'**
Tree species	133	4.29	76	3.54
Herbaceous plants	52	2.67	28	2.97
Total species	185		104	

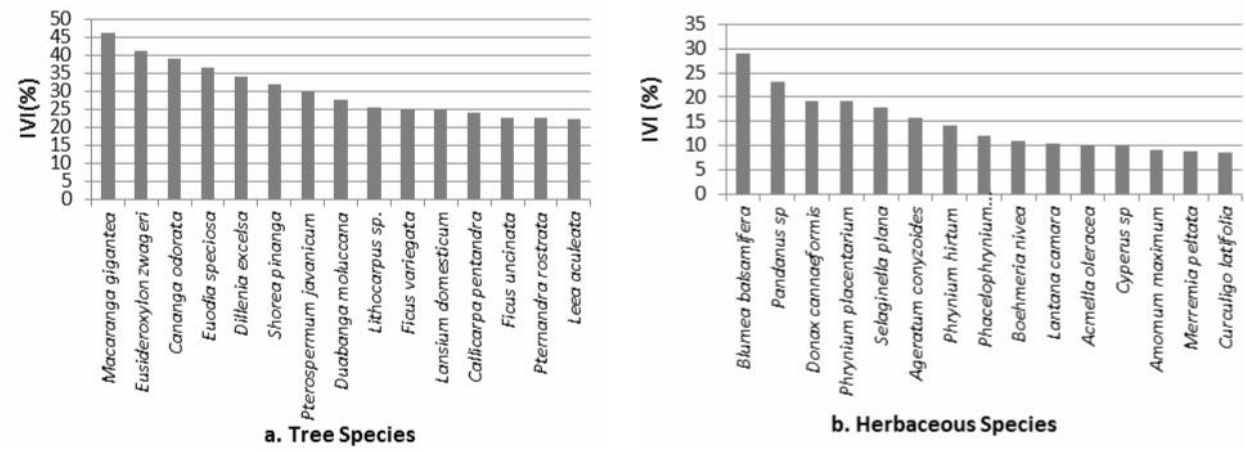


Figure 3. The importance value indices (IVI) of 15 species of tree species (a) and 15 species of herbaceous plants (b) in pre-mining area

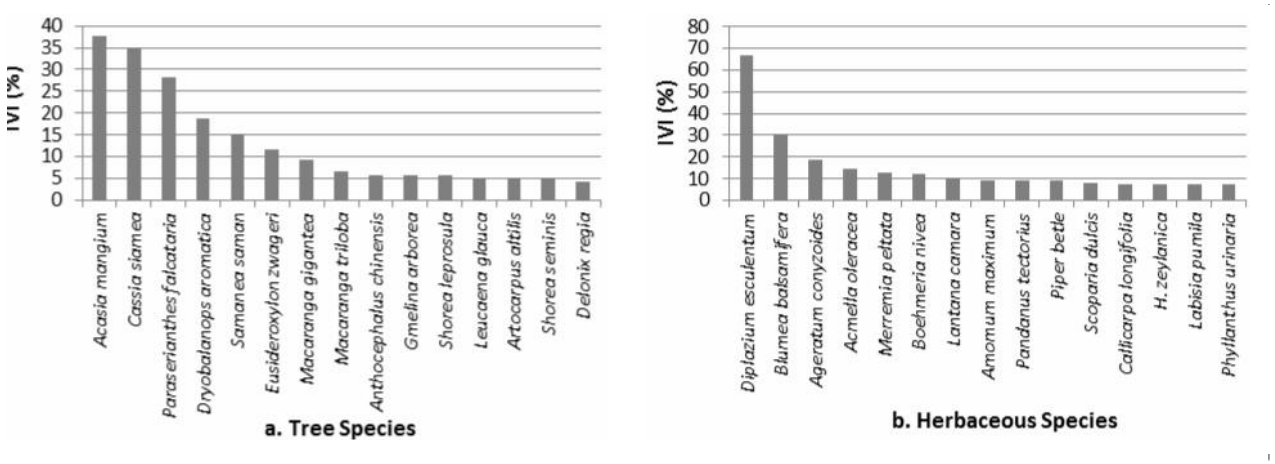


Figure 4. The importance value indices (IVI) of 15 species of tree species plants (a) and 15 species of herbaceous plants (b) in reclamation area

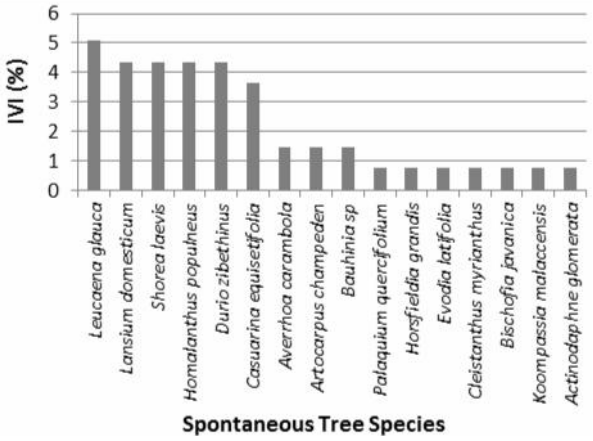


Figure 5. Importance value indices (IVI) of 16 tree species that grew spontaneously at reclamation site

Discussion

The mahang tree (*Macaranga gigantea*) is a local species which dominated in the pre-mining area, as shown by its highest importance value index. *Macaranga* is known as a pioneer and fast growing plant, with soft wood, which sprouts all year round and is able to reach a height of up to 20 meters (Zakaria et al. 2008). In addition, many species of *Macaranga* sp. favor high light intensity, indicating its relative tolerance to open areas. Several species of *Macaranga*, such as *Macaranga tanarius* and *Macaranga javanica* have been used as indicator species for disturbed forest areas (Zakaria et al. 2008). In the reclamation site, the dominant species was *Acacia mangium*. However, *Macaranga* sp. was also quite abundant although not many seedlings were planted (Komara et al., unpublished data). The high number of seedlings of local species planted in the reclamation site was one of the factors contributing to the importance value indices of these plant species. In the case of *Macaranga* sp., however, abundance may also have been supported by the natural availability of seedlings in the site. This phenomenon is in accordance with the generalization of major factors affecting succession, as stated by Noble and Slatyer (1980), i.e. that the composition of species immediately after a disturbance depends on propagules

which have either dispersed from elsewhere or have persisted through the disturbance or on vegetative resprouting of organs surviving the disturbance. Three groups of vital attributes are important to vegetation replacement, i.e., the method of arrival or persistence of a species during and after disturbance; the ability to establish and grow to maturity; and the time taken fo them to reach critical stages in their life history (Noble and Slatyer 1980).

The same phenomenon was found in *Eusideroxylon zwageri*, known as the ulin tree. This local species was the second dominant species in the pre-mining site. This plant is well known for its high quality wood but slow growth rate. The high importance value index of ulin in this area indicates that this area could conserve the ulin tree in its natural habitat. In the reclamation site, *Eusideroxylon zwageri* was the sixth rank. The slow growth rate of this species seems to have contributed to its relatively low importance value in the reclamation site.

In the pre-mining site, the dominant herbaceous species was *Blumea balsamifera* with the highest importance value index (29.04%), while in the reclamation site this plant was the second dominant species with an importance value index of 30.01%. The dominant species in the reclamation site was the fern *Diplazium esculentum* with an importance value index of 66.75%. *Blumea balsamifera* has abundant leaves, grows relatively fast, and is extremely tolerant to minimum light intensity (shade plant). In contrast, *Diplazium esculentum* is tolerant to high light intensity. The presence of *Diplazium esculentum* could be related to its dispersal ability, i.e., dispersed by wind from the forest system. However, this needs to be reconfirmed.

The presence of herbaceous plants benefits the reclamation program through their role as ground cover. Plants such as *Diplazium esculentum, Blumea balsamifera, Ageratum conyzoides, Acmella oleracea* and *Merremia peltata* easily grow in relatively poor soil conditions under sufficient sunlight. Besides its rapid growth, *Diplazium esculentum* seems to be an adaptable species for post-mining areas. Several herbaceous species from the pre-mining area were found in the reclamation site, e.g., *Piper betel, Selaginella plana, Selaginella deoderleinii* and *Piper nigrum.*

The ability of plants to grow in the reclamation site can be attributed to seeds already present in the top soil during reclamation process, or by seed dispersal. For example, *Leucaena glauca* (5.08 %) is propagated by seeds, and is easy to grow after being cut, felled or burned. *Lansium domesticum* (4.35%), *Durio zibethinus* (4.35%), *Averrhoa carambola* (1.45%) and others are several fruits species with seeds that could be dispersed by animals. *Shorea laevis* (4.35%) seeds contain a lot of fat that is commonly eaten by animals. Dispersion of plant species depends on animal species and the distance between the reclamation site and the nearby vegetation source, e.g., the nearest forest that will affect the distribution of the species (Novianti 2013; Traveset et al. 2014).

Based on the number of plant species that successfully grew at the reclamation site, the following findings could be used to suggest further management of this study site: in terms of species richness, there were 132 local species in the pre-mining area as reference site, while at the reclamation site there were 35 planted local species and 16 local species which grew spontaneously. In other words, a total of 51 local species successfully grew at the reclamation site after a period of 16 years. To restore plant diversity in the reclamation site to its assumed pre-mining conditions, it should be planted with 81 local species of tree species. The next step is to select the local tree species from the pre-mining area by considering the species importance value indices. In the pre-mining area, only eight local species had importance value indices higher than 20%, e.g., *Euodia speciosa* (36.48%), *Shorea pinanga* (31.80%), *Lithocarpus* sp. (25.44%), *Ficus variegata* (25.01%), *Ficus uncinata* (22.72%), *Pternandra rostrata* (22.40%), *Shorea dispar* (21.76%) and *Canarium odontophyllum* (21.76%); however the reclamation program must consider the availability of seedlings and the ability of the plant to grow successfully in reclamation conditions.

To conclude, after 16 years of reclamation, 104 plant species were found in the reclamation site, consisting of 76 tree species and 28 herbaceous species. tree species that successfully grew in this site consisted of 35 planted local species (e.g., *Dryobalanops aromatica, Eusideroxylon zwageri, Macaranga gigantea*), 25 planted non local species, and 16 local species that grew spontaneously (e.g., *Leucaena glauca, Lansium domesticum, Shorea laevis*). In comparison, data from the pre-mining area indicate the presence of 133 plant species, consisting of 132 local tree species, one non local tree species (*Acacia mangium*) and 52 herbaceous species. Tree species diversity index in the reclamation site after 16 years post mining (i.e., 3.54) was still lower than in the pre-mining area (4.29); while the diversity indices for herbaceous plants were relatively similar (2.97 and 2.67 in the reclamation and pre-mining sites respectively). The slightly higher diversity index in the reclamation site can be attributed to higher coverage per species in this site, despite lower species richness.

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Sequence-Related Amplified Polymorphism (SRAP) analysis for studying genetic characterization of *Bouea macrophylla*

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Abstract. *Kaewpongumpai S, Poeaim S, Vanijajiva O. 2016. Sequence-Related Amplified Polymorphism (SRAP) analysis for studying genetic characterization of* Bouea macrophylla. *Biodiversitas 17: 539-543. Bouea macrophylla* Griff. is well-known as one of native typical fruits in Southeast Asia which needs to be preserved and continuously cultivated because of economical and ecological significances. More recently, sequence-related amplified polymorphism (SRAP) markers have been developed, which are used to amplify coding regions of DNA with primers targeting open reading frames. This technique has proven to be robust and highly variable and is attained through a significantly less technically demanding process. In this research, SRAP method was preliminary applied to assess genetic characterization of *B. macrophylla*. Genomic DNA was extracted from fresh leaf samples. The result clearly showed that at 100 ng template DNA and $MgCl_2$ 5 mM concentration are suitable for further PCR analysis. Thirty SRAP primer combinations were initially screened for analysis and 26 primer combinations were chosen for further analysis. A total of 222 DNA fragments, varying from 90-2500 bp, were amplified. The produced band number for each optimal primer set ranged from 3 to 12 with a percentage of polymorphic bands spanning from 33.33 to 80.00%. Therefore, SRAP analysis is suitable for further analysis method on genetic study of *Bouea* species and related genera.

Keywords: *Bouea macrophylla*, SRAP, genetic characterization

INTRODUCTION

Bouea macrophylla Griff. is a tropical fruit tree indigenous to Southeast Asia. The species belongs to the cashew family (Anacardiaceae) (Chayamarit 2010). In appearance it closely resembles the mango, to which it is related, but its size, foliage and fruit are all smaller. It is commonly known as Marian plum or plum mango, also called ramania or gandaria in Indonesia and kundang, rembunia or setar in Malaysia, mayun in Myanmar, and maprang, mayong or mayongchid in Thailand, respectively (Lim 2012; Rajan et al. 2014). The species is one of the oldest fruit crops, which has been cultivated in Southeast Asia region for more than hundred years. The immature fruit is pale green when the fruit is small and becomes dark green as the fruit develops. The ripe fruit is yellow-orange, mango-like in character, roundish, and juicy with a sour to sweet taste according to the variety, and has a faint turpentine smell. There is one seed in a fruit; the seed is similar to that of the mango but smaller in size (Rifai 1992). The endosperm is white and pinkish purple, and has a bitter and astringent taste. Ripe fruits are consumed fresh, but sometimes they are made of whole or pieces of fruit in sugar syrup. On the other hand, unripe fruits are also consumed by local people as an ingredient of chillies paste as well as traditional salad dish. Many researchers found that an extract of unripe and ripe fruit exhibited various bioactive compound and antioxidant activity (Khoo et al. 2008; Rajan et al. 2014, 2016).

With the introduction of sweet-flesh ripe fruits, *B. macrophylla* has received more attention in recent years. This edible fruit species may have good potential for commercial development if subjected to more research on marketing and postharvest storage. Marian plum is gaining popularity among local consumers in recent years particular in ASEAN countries. The Thai government, for example, is trying to help in exporting this fruit as many exporter firms have started to advertise Marian plum fruit for export. This suggests that *B. macrophylla* has good prospects for wider commercialization. Over the centuries, various *B. macrophylla* cultivars have arisen in Thailand. The plant is normally cultivated in small-scale mixed orchards and is usually grown together with other economic crops and usually sold at local markets when in season (Subhadrabandhu 2001). *B. macrophylla* used to be grown with mixed results from seeds of trees bearing superior quality fruit, but are now propagated by layering, or more commonly, by grafting, including bud, veneer, wedge, whip or grafting onto seedlings of randomly selected rootstocks. One of the reasons for this is the selection of cultivars with high quality fruits. Growers in the central and lower northern regions of Thailand cultivate these high quality Marian plum cultivars in their orchards. More than 50 *B. macrophylla* cultivars have been named in Thailand. However, the difference between its cultivars is practically not studied. There is not much information available on the genetic characterization between cultivated Marian plum cultivars in Thailand.

Knowledge of genetic characterization within crop species is a fundamental resource, which has been employed in breeding programs for the improvement of the crops. Detection of polymorphism among germplasm collections for selected species will provide insight into the genome evolution, origin of cultivated species, and current level of diversity in modern agricultural crops. One of the most promising is the molecular marker technique as it offers great possible to the analysis of plant genetic structure, diversity, and functionality that are required for marker assisted breeding schemes. Nowadays, molecular markers have been incorporated in conventional breeding programs or utilized as a substitute for conventional phenotypic selection on the assumption that efficiency and precision of the genetic improvement could be greatly increased. The available molecular marker techniques include random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), inter primer binding site (iPBS), inter simple sequence repeat (ISSR), and simple sequence repeat (SSR) and sequence-related amplified polymorphism (SRAP) (Agarwal et al. 2008; Kalendar et al. 2010; Zheng et al. 2015). Of these, the SRAP technique is recognized as a simple, efficient, and cost-effective marker system that could be used in multiple molecular biology studies, including genetic-diversity analysis, genomic and cDNA fingerprinting, map construction, gene tagging, and map-based cloning. Compared to other marker systems, this technique is specifically targeted to genome open reading frame (ORF) sequences, which provide more genetic information associated with phenotypes. For successful application of SRAP, the most critical step is the selection of optimal forward and reverse primer pairs that permit an effective polymorphism characterization of various fruit species, such as apple (Si et al. 2010), pear (Zhang et al. 2013), citrus (Hazarika et al. 2014) and guava (Padmakar et al. 2015).

The objectives of this study was to survey the variability of *B. macrophylla* collected from major cultivated area in Thailand and to evaluate the availability of SRAP technique in terms of genetic characterization and significant marker-trait associations, aiming to profile these cultivars properly for further utilize. Currently, no specific SRAP marker is available for Anacardiaceae including this species, and the development of a new marker for this species would be time-consuming and costly. Therefore a more practical approach is to use SRAP marker on *Bouea.* To our knowledge, this is the first report on application of SRAP marker to detect variations among *Bouea* species and related genera.

MATERIALS AND METHODS

Plant materials

Bouea macrophylla was mainly collected from central and lower northern regions of Thailand (Table 1). As for the 30 accessions collected in our survey, 29 samples are under cultivation and one sample (MP18) is uncultivated. The obtained fresh leaves were stored at -20 °C until further processing.

Table 1. The names *Bouea macrophylla* cultivar and origins of samples for SRAP analysis

Cultivar name	Origin	Code
Maprang-Mae Anong	Nakhon Nayok, Thailand	MP01
Maprang-Thong Nopparat	Nakhon Nayok, Thailand	MP02
Maprang-Thong Yai	Nakhon Nayok, Thailand	MP03
Maprang-Maha Chanok	Nakhon Nayok, Thailand	MP04
Maprang-Suwan Nabat	Nakhon Nayok, Thailand	MP05
Maprang-Chao Sua	Nakhon Nayok, Thailand	MP06
Maprang-Lung Chit	Nakhon Nayok, Thailand	MP07
Maprang-Waan Yai	Phetchabun, Thailand	MP08
Maprang-Waan Thong	Phetchabun, Thailand	MP09
Maprang-Patum Thong	Sukothai, Thailand	MP10
Maprang-Rung Arun	Prachinburi, Thailand	MP11
Maprang-Phet Wan Yao	Kamphaengphet, Thailand	MP12
Maprang-Waan Kom	Kamphaengphet, Thailand	MP13
Maprang-Puang	Phetchabun, Thailand	MP14
Maprang-Phet Noppakao	Kamphaengphet, Thailand	MP15
Maprang-Cheong Lan	Kamphaengphet, Thailand	MP16
Maprang-Yai	Phetchabun, Thailand	MP17
Maprang	Ayutaya, Thailand	MP18
Mayongchid-Suan Waan	Nakhon Nayok, Thailand	MY01
Mayongchid-Chit Sanga	Nakhon Nayok, Thailand	MY02
Mayongchid-Tan Kao	Nakhon Nayok, Thailand	MY03
Mayongchid-Bang Khun Non	Nakhon Nayok, Thailand	MY04
Mayongchid-Tan Tawai	Nakhon Nayok, Thailand	MY05
Mayongchid-Tadaan	Nakhon Nayok, Thailand	MY06
Mayongchid-Tan Kao	Lopburi, Thailand	MY07
Mayongchid-Neang Siam	Sukothai, Thailand	MY08
Mayongchid-Mae Ya	Sukothai, Thailand	MY09
Mayongchid-Bang Khun Non	Kamphaengphet, Thailand	MY10
Mayongchid-Phet Kang Dong	Kamphaengphet, Thailand	MY11
Mayongchid-Phet Cheong Lan	Kamphaengphet, Thailand	MY12

Genomic DNA isolation

Total genomic DNA was extracted individually from young leaves of 30 accessions using the CTAB method (Doyle and Doyle, 1987) with minor modification. The leaves (500 mg) were ground in a mortar with a pestle. Extraction buffer [(1% (w/v) CTAB, 50 mM Tris-HCl (pH 8), 0.7 M NaCl, 0.1% β-mercaptoethanol)] 500 µL was added and the solution was incubated at 60 ºC for 30 min. The homogenate was mixed with 25: 24: 1 phenol: chloroform: isoamyl alcohol (v/v/v) by gentle inversion. After centrifugation at 13,000 rpm for 15 min, the upper aqueous layer was transferred to a fresh tube. RNA was removed by treating with 2.5 µL of the RNase (10 µg/µl) for 30 min at 37 ºC. The extraction of DNA with phenol/chloroform/isoamyl alcohol was repeated one more time. DNA in the solution was precipitated with 0.6 volume of ice-cold isopropanol and washed with 70% ethanol. Following this, the DNA was extracted using CTAB DNA extraction protocol without RNase. The process was repeated until the DNA pellet was free of color (two to three times) and the final pellet was dissolved in sterile deionized water. DNA quality and quantity were determined on 0.8% agarose gel. The DNA was stored at -20 ºC, for further use as templates for PCR amplification. The quality of DNA was also evaluated by reading the absorbance at 260 and 280 nm.

Table 1. SRAP primers used in this study

Forward primer	**Sequence (5'-3')**
me1	TGAGTCCAAACCGGATA
me2	TGAGTCCAAACCGGAGC
me3	TGAGTCCAAACCGGAAT
me4	TGAGTCCAAACCGGACC
me5	TGAGTCCAAACCGGAAG
Reverse primer	**Sequence (5'-3')**
em1	GACTGCGTACGAATTAAT
em2	GACTGCGTACGAATTTGC
em3	GACTGCGTACGAATTGAC
em4	GACTGCGTACGAATTTGA
em5	GACTGCGTACGAATTAAC
em6	GACTGCGTACGAATTGCA

SRAP analysis

Primers pairs used in this study were synthesized by Ward Medic Ltd., Part. Thailand (Table 1). The PCR was performed using a Thermohybaid Px2 (Roche Molecular Systems, Inc., USA). The PCR reaction mixtures (25 µL total volumes) consisted of 10x Reaction Buffer, 100 ng template DNA, 0.6 mM dNTP mixture, 5 mM $MgCl_2$, 1 unit of Taq polymerase and 0.6 µM of each primers. The SRAP amplification conditions were 5 min initial denaturation at 94ºC and 5 cycles consisting of 1 min denaturation at 94ºC, 1 min primer annealing at 35ºC, and 2 min extension at 72 ºC. In the following 30 cycles, the annealing temperature was increased to 50ºC and a final 8 min extension at 72 ºC.

The SRAP products were all analysed by agarose (1.8% w/v) gel electrophoresis at 150 volts for 30 minutes in 0.04 M TAE (Tris-acetate 0.001 M-EDTA) buffer pH 8. The gels were stained with ethidium bromide (10 mg/ml). The gels were viewed and photographed by Bio-Imaging System (Syngene, Genegenuis). To determine SRAP profiles, the size of each DNA band was inferred by comparison with a 100 bp DNA ladder (Promega), used as a molecular weight marker (M). Polymorphisms at all loci were confirmed by three repeating tests for each primer at different times.

RESULTS AND DISCUSSION

DNA isolation and optimization of SRAP-PCR parameters

The extraction of high quality DNA from *Bouea macrophylla* is challenging because presence of high polyphenolics in the tissues. A high throughput DNA extraction protocol is prerequisite. The presence of polyphenols, which are influential oxidizing agents show in many tropical plant species, can decrease the yield and purity by binding covalently with the extracted DNA making it useless of most research applications (Vanijajiva 2011). The extraction of high quality DNA was optimized by re-extracting the DNA using CTAB DNA isolation protocol and phenol: chloroform: isoamyl alcohol extraction instead of chloroform: isoamyl alcohol extraction. The polyphenolics with the DNA were simply removed and good SRAP electrophoretograms were obtained with all samples. DNA extracted from *B. macrophylla* leaf using an above modified gave a good and sufficient quality DNA for SRAP-PCR reaction. DNA isolated by minor modification method yielded strong and reliable amplification products and the amount of DNA extracted from the accessions ranged from 125 to 245 µg/g fresh weight leaf material. The ratios of A260/A280 varied from 1.84 to 1.98. The quality of DNA was also tested by PCR, which confirmed that the DNAs were suitable for PCR reaction. The parameters for the sequence-related amplified polymorphism protocol from *B. macrophylla* cultivars were also studied. Several parameters had an effect on banding patterns and reproducibility such as concentration of dNTPs, magnesium chloride concentration, concentration of enzyme, concentration of primer and concentration of template DNA (Sun et al. 2011), but the concentration of template DNA and magnesium chloride were most important. The result clearly showed that at 100 ng template DNA and $MgCl_2$ 5 mM concentration are suitable for further PCR analysis.

SRAP analysis

Genetic characterization is one of the key successes to crop breeding programs. Knowledge of the genetic variation between the different accessions supplying this diversity can greatly assist the development of efficient germplasm-management and -utilization strategies. Currently, genetic marker technology designed to detect naturally occurring polymorphisms at the DNA level had become an invaluable and revolutionizing tool for both applied and basic studies of plant. In this study, *Bouea macrophylla* was used for analyzing molecular characterization using a novel molecular marker sequence-related amplified polymorphism (SRAP). The selected primers were based on earlier reports of Li and Quiros (2001) and Vanijajiva and Kundee (2014). There were 30 sets of primer combinations that combined 5 forward primers and 6 reverse primers. Based on preliminary test, 26 sets of combination primers which steadily produced well-defined and scorable amplification products showed polymorphisms in all 30 *B. macrophylla* cultivars. Figure 1 was the illustration amplification electrophoretograms of MP18 accession of *B. macrophylla*.

Total and polymorphic band number and polymorphism ratio of *Bouea macrophylla* accessions were processed in Table 2.The main criteria by which the primer selection was made are: clarity, reproducibility of amplified bands and high rates of polymorphism. The 26 primer combinations generated 222 electrophoretic profiles, of which 150 bands were polymorphic (57.27%). A high degree of polymorphism was revealed by these combinations that ranged from 33.33 to 80.00% across all the genotypes studied. The size of amplified bands ranged from 90 to 2,500 bp. The number of fragments amplified by each primer ranged from 3 to 12 with the average of 8.53 per primer combination. By scoring the bands from forward or reverse primer directed primer combinations, the results showed that the amplification ability of either

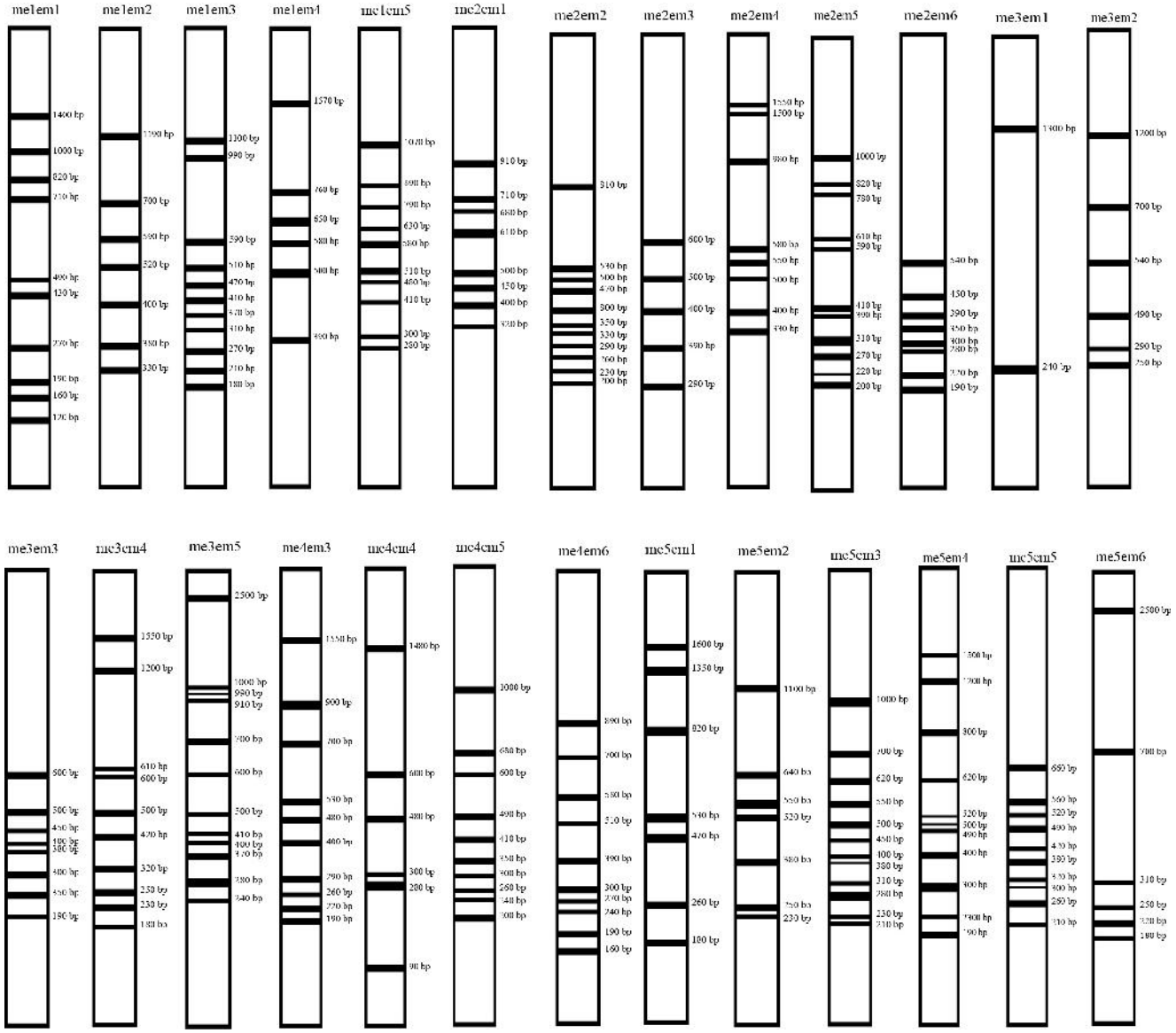

Figure 1. The representative SRAP profiles in this study. The electrophoretograms are employed as representative of clear, distinguished, stable profiles from 26 combination primers of MP18 wild accession

the forward or reverse primers varied significantly with each other, revealing a genomic bias of *B. macrophylla* cultivars on different forward- and reverse-primer nucleotides. In fact, primer preference for successful SRAP-PCR assays has been widely recognized in many plant species (Agarwal et al. 2008).

This preliminary result indicated that the SRAP technique could be used as an alternative molecular tool on *B. macrophylla*. Recently, ISSR and SSR has been applied in the molecular biology studies of *Bouea* species (Damodaran et al. 2013; Ghazali et al. 2015). However, SSR and ISSR techniques provided limited functional gene information associated with the traits of interest. In other plant species, such as buffalograss cultivars, SRAP technology prove useful for varietal identification than SSR and ISSR markers (Budak et al. 2004) as well as *Dianthus* accessions, the information given by SRAP markers was more concordant to the morphological variability and to the evolutionary history of the morphotypes than that of ISSR markers (Fu et al. 2008). These contrasting levels of SRAP, SSR and ISSR correlation with morphological traits may be related to the fact that, unlike SSR and ISSR which are targeted to microsatellite regions, SRAP markers preferentially amplify ORFs (open reading frames). Exons are usually GC rich and, thus, the 'CCGG' sequence in the core of the forward SRAP primers is designed to target such coding regions (Li and Quiros 2001; Shao et al. 2010). Thus, SRAP technique may be helpful in deciphering the genomic basis of complex traits that are related to the economic value of *B. macrophylla* and are likely to better reflect genetically determined morphological variation.

Table 2. Total and polymorphic band number and polymorphism ratio of *Bouea macrophylla* accessions

Primer combinations	Total bands	Poly-morphic bands	% Poly-morphism	Size (bp)
me1em1	10	7	70.00	120-1400
me1em2	7	6	85.71	330-1190
me1em3	11	8	72.72	180-1100
me1em4	6	3	50.00	390-1570
me1em5	10	7	70.00	280-1070
me2em1	8	6	75.00	320-910
me2em2	11	6	54.55	200-810
me2em3	5	4	80.00	290-600
me2em4	8	3	37.50	330-1550
me2em5	11	8	72.73	200-1000
me2em6	8	6	75.00	190-540
me3em1	3	1	33.33	240-1300
me3em2	6	1	16.67	250-1200
me3em3	8	3	37.50	190-600
me3em4	10	7	70.00	180-1550
me3em5	11	8	72.72	240-2500
me4em3	10	4	40.00	190-1550
me4em4	6	4	66.67	90-1480
me4em5	10	8	80.00	200-1000
me4em6	10	2	20.00	160-890
me5em1	7	5	71.43	180-1600
me5em2	7	3	42.86	230-1100
me5em3	12	8	66.67	210-1000
me5em4	11	6	54.55	190-1500
me5em5	10	4	40.00	210-660
me5em6	6	2	33.33	180-2500
Total	222	130		90-2500
Mean	8.53	5	57.27	

In conclusion, the present study is, to the best of our knowledge, the first report of genetic investigation of *Bouea macrophylla*, using SRAP markers. It is concluded that SRAP is a useful DNA fingerprinting tool for evaluation of genetic diversity of species, cultivars and breeding lines, especially for species with underdeveloped marker systems. It is a fast, low-cost and efficient molecular method applicable to plant breeding.

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Suitability and availability analysis of tropical forest wood species for ethanol production: a case study in East Kalimantan

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Manuscript received: 1 January 2016. Revision accepted: 30 June 2016.

Abstract. *Amirta R, Mukhdlor A, Mujiasih D, Septia E, Supriadi, Susanto D. 2016. Suitability and availability analysis of tropical forest wood species for ethanol production: a case study in East Kalimantan. Biodiversitas 17: 544-552*. Fifteen species of woody biomass from tropical forest of East Kalimantan, Indonesia and identified as *Acacia mangium, Aleurites moluccana, Alstonia scholaris, Anthocephalus cadamba, Artocarpus altilis, Artocarpus elasticus, Cananga odorata, Gmelina arborea, Lagerstroemia speciosa, Leucaena leucocephala, Macaranga gigantea, Macaranga tanarius, Paraserianthes falcataria, Shorea leprosula* and *Swietenia macrophylla* were characterized and studied to find out and discover their potential utilization as suitable feedstocks for biofuel (ethanol) production. Characterization was done by evaluation of lignin, holocellulose and cellulose contents of woody biomass including the yield of reducing sugar (saccharification) after pretreated with alkaline (NaOH) at moderate temperature. Among 15 species of tropical forest wood biomass evaluated, our findings showed that *M. gigantea* was gave the highest yield of saccharified sugar (42.22%, weight of original wood dry basis) and also yield of theoretical ethanol (± 273 L/ton). We also found growth of *M. gigantea* was very fast to produce approximately 26,119 kg ha^{-1} dry biomass within 3 years. In general, the tropical wood biomass such as *M. gigantea, A. moluccana, G. arborea, A. cadamba*, and *P. falcataria* are suitable and potentially to be used as feedstocks for ethanol production due to their fast growing ability, availability and attractive chemical composition to produce high saccharified sugar and yield of ethanol.

Keywords: Ethanol, suitability, tropical forest, wood biomass, East Kalimantan

INTRODUCTION

Nowadays, energy crisis is one of the most serious threats towards the sustainability of human kinds and civilization. Although industrial revolution has changed the world to its sophisticated edge, excessive dependent on fossil fuels as the main source of energy has leads to the diminishing of this non-renewable supply. Furthermore, demand for petroleum-derived fuels is not slowing down but instead increases substantially over the past few decades (Goh et al. 2011). Regarding to this issue, as one of the countries which have abundant reserves of forest biomass and agricultural residues, Indonesia government has declared to start production of fuels and energy from renewable sources. The government realizes that the biofuels and bioenergy industries will increase the amount of domestic supply of fuels and electricity with decrease in subsidy for promotion of the biofuels (DGEEU, Indonesia Ministry of Energy and Mineral Resources 2005; Watanabe et al. 2008). Thus far, natural and industrial forest plantation in Indonesia has been managed and designed to produce construction wood materials, boards and papers, and potency of wood biomass as a source for biofuel production has received much less attention. However, tropical rain forest includes a wide variety of wood species which has no values for the current industry but may have a great deal of potential for production of biofuels and chemicals. From the view point of biodiversity and potential of the bioresources for sustainable society, WWF Indonesia pointed out importance of the tropical forests in Indonesia, particularly in Kayan Mentarang Forest, Malinau East Kalimantan, which includes around 15,000 species of plants (Pio and D'Cruz 2005). The biodiversity value of forest is the highest compared to other places on the earth. Forest in Kalimantan is characterized also by richness in endemic species. There are at least 6,000 endemic species of plants, including 155 dipterocarp trees species. The lack of information on the basic properties, function and suitability as the feedstock for the fuels and energy production including conversion process, are believed as the main reason and barrier factor for utilization of those wood species (Amirta et al. 2016a,b). Therefore, herein this preliminary study the fifteen species of wood biomass that commonly growth in primary forest, secondary, and also plantation forest in East Kalimantan, Indonesia were characterized with the special emphasize to point out and discover their potential utilization as suitable feedstocks for biofuel (ethanol) production. The growth ability of wood species was also discussed to get more information about their availability as potential feedstock for ethanol production in the near future.

MATERIALS AND METHODS

Study area

The field observation and plant material including wood biomass was collected from Universitas Mulawarman Education Forest located at Samarinda Botanical Garden (Kebun Raya UNMUL Samarinda-KRUS), Samarinda, East Kalimantan, Indonesia (0°25'10"LS – 0°25'10"LS and, 117°14'00"BT-117°14'14"BT-300 ha) (Figure1).

Wood material

Wood samples (biomass) from 15 species of tropical wood biomass with diameter about 10-20 cm were collected from Universitas Mulawarman Education Forest located at Samarinda Botanical Garden, Samarinda, Indonesia. Their leaves and wood samples were identified as *Acacia mangium* Willd., *Aleurites moluccana* (L.) Willd., *Alstonia scholaris* (L.) R.Br., *Anthocephalus cadamba* (Roxb.) Miq., *Artocarpus altilis* (Parkinson) Fosberg, *Artocarpus elasticus* Reinw. ex Blume, *Cananga odorata* (Lam.) Hook. f. & T. Thomson, *Gmelina arborea* Roxb., *Lagerstroemia speciosa* (L.) Pers., *Leucaena leucocephala* (Lam.) de Wit, *Macaranga gigantea* (Rchb.f. & Zoll.) Müll.Arg., *Macaranga tanarius* (L.) Mull.Arg., *Paraserianthes falcataria* (L.) Nielsen, *Shorea leprosula* Miq., and *Swietenia macrophylla* King (Figure 2) in the Laboratory of Forest Dendrology, Faculty of Forestry, Universitas Mulawarman, Samarinda, Indonesia. The wood samples were debarked, cutted, chipped and air dried up to approximately 12% moisture content (MC), and used throughout this study.

Alkaline pretreatment of woody biomass

Alkaline pretreatment of wood biomass was carried out for 60 min at 160ºC using a liquid-to-solid ratio 8:1 (w/w) and NaOH concentration between 3.5% and 5.0% based on dry weight of the woody biomass. The reactions were carried out using a rotary digester equipped with a controller for pressure, rotary speed and temperature. After the reaction, the pulp fraction was separated by filtration and washed extensively with tap water until neutral pH.

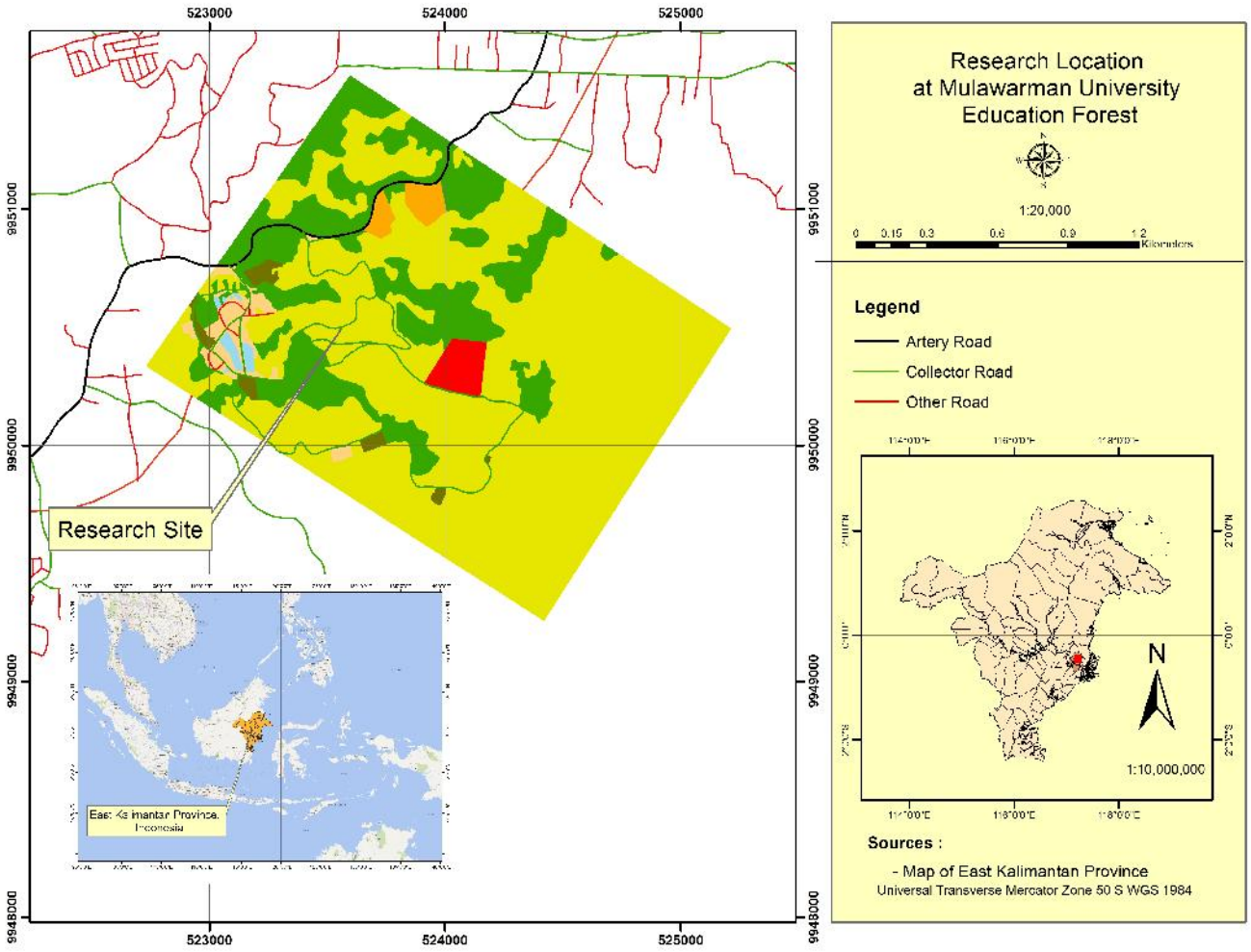

Figure 1. Sampling location at Samarinda Botanical Garden-Education Forest of Universitas Mulawarman, East Kalimantan, Indonesia

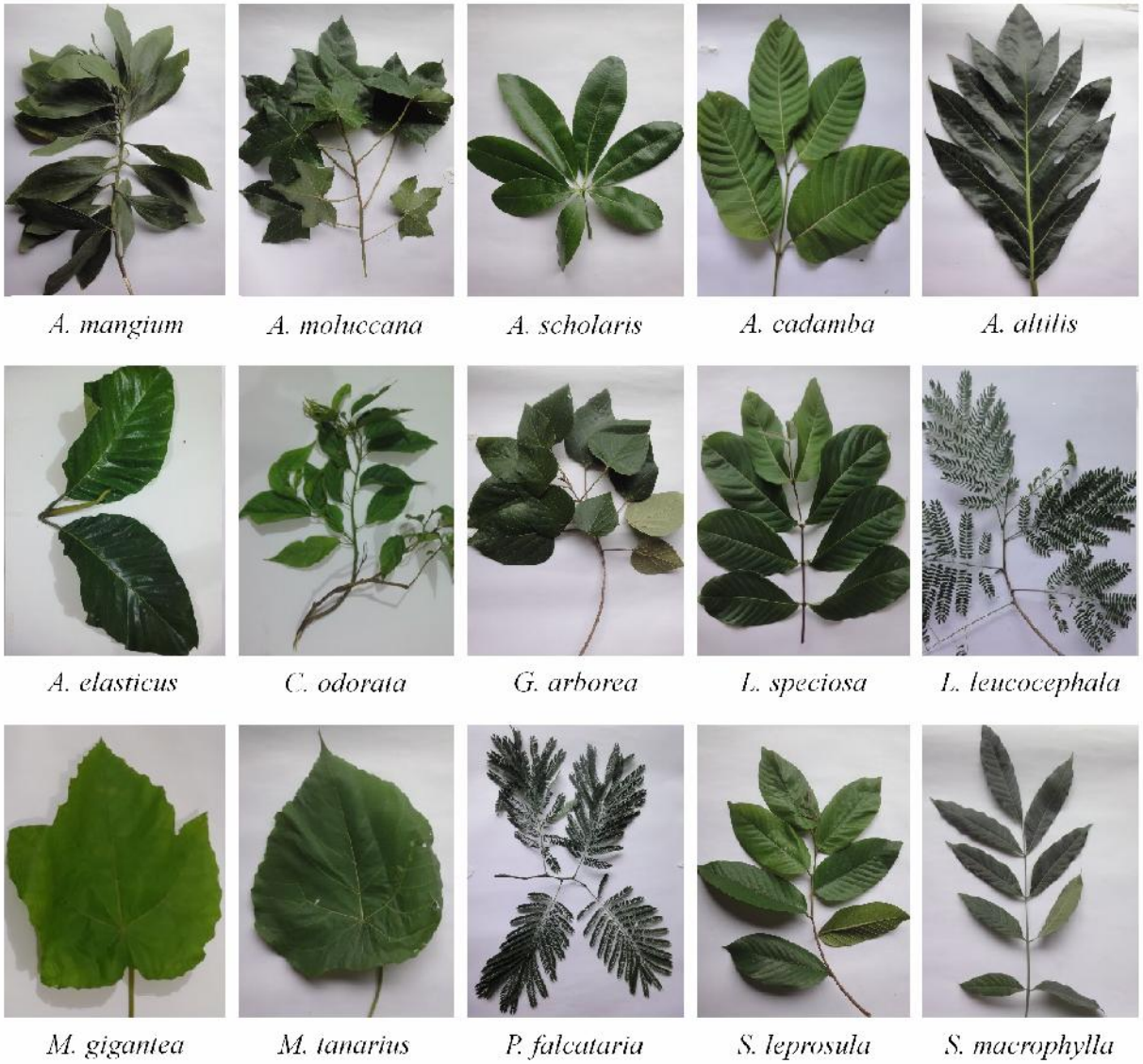

Figure 2. Leaves shape of fifteen species of tropical wood biomass studied

Wood component analysis

The Klason lignin content was determined by the TAPPI standard method (TAPPI 1998a). The holocellulose and α-cellulose contents were determined according to Wise's chlorite method (Wise et al. 1946) and the TAPPI standard method (TAPPI 1988b), respectively. The reducing sugar content was determined by the Somogyi–Nelson method (Somogyi 1952).

Enzyme activity

Filter paper unit activities were assayed in reaction mixture containing 50 mg (w/v) Whatman filter paper number 1, 50 mM tartrate buffer, pH 4.5 and the enzyme. After incubation at 50ºC for 30 min., the reducing sugars produced were determined by Somogyi-Nelson method (Somogyi 1952). One unit (U) of each enzyme activity is defined as the amount of enzyme, which produce 1 µmol reducing sugar as glucose in the reaction mixture per minute under above specific condition.

Saccharification of wood biomass

The wet pulp fraction was hydrolyzed with a commercial cellulose preparation, meisellase from *Trichoderma viride* (Meiji Seika Co., Ltd., 224 filter paper units (FPU)/g, β-glucosidase activity 264 IU/g). The cellulase enzyme loading was an 8-FPU/g substrate. Enzymatic hydrolysis was performed at a substrate concentration of 2% in 0.05 M sodium citrate buffer (pH 4.5) containing 0.02% sodium azide at 45ºC on a rotary shaker (NTS-4000C, Rikakikai, Japan) at 140 rpm for 48 h (Itoh et al. 2003). The saccharification ratio per pulp was calculated according to the NREL LAP-009 procedure (Brown and Torget 1996). The sugar yield per wood is based on the weight percentage of the reducing sugars to the original wood. The overall yield of sugars per wood is calculated by multiplying the saccharification ratio per pulp and the pulp yield. All enzymatic hydrolysis experiments were performed in triplicate.

Estimation of ethanol production from woody biomass

Potential ethanol production from Macaranga wood was estimated based on the amount of hexose sugar (HXTEL) in the lignocellulosic material obtained by enzymatic saccharification of the insoluble pulp fraction. Due to the high content of glucose in the pulp fraction, HXTEL was approximated by the amount of reducing sugars obtained from the pulp fraction (equation 1). The ethanol yields (ETOHBIO) based on the weight of original biomass was calculated from equation (2)

$$HEXTEL = HEX \times a \quad (mg/kg) \quad eq.1$$

$$ETOHBIO = HXTEL \times Ye.h/b \quad (mL/kg) \quad eq.2$$

Where, HEX is the hexose (D-glucose) yield upon saccharification from hexosan (w/w, of original wood basis), a is the weight of substrate (1000 mg, 1kg), Ye.h is the theoretical ethanol yield from hexose (D-glucose) (0.511), and b is the ethanol density (0.789 kg/L) (modified from Premjet et al. 2013).

Estimation of dry wood biomass production

The potency of above ground tree dry biomass was estimated using allometric equations that previously reported by Hiratsuka et al. (2006). The equation was specifically developed to estimate the above ground tree dry biomass of Macaranga, particularly M. gigantea and M. hypoleuca. The equation is (equation 3):

$$M = 5.64 \times 10^{-2} (D)^{2.47}; \quad r^2 = 0.96 \quad eq.3$$

Where, M is the total aboveground dry mass of an individual tree (kg) and D is the trunk diameter at 1.3 m aboveground of the tree (cm).

RESULTS AND DISCUSSION

Lignocellulosic components of wood biomass

In this work we evaluated lignin, holocellulose, and also cellulose contents of wood biomass to point out their correlations to saccharification and estimated yield of ethanol. Among 15 species of wood biomass tested, we found that the highest lignin content was 31.97% from *A. elasticus* and followed by 31.07% (*S. leprosula*), 30.40% (*M. tanarius*), 29.32% (*L. leucocephala*), 29.22% (*L. speciosa*), and 28.15% (*S. mahagoni*) (Table 1). On the other hand, the lowest lignin content was obtained from *A. moluccana* (22.23%). Our finding also demonstrated that the highest cellulose content was 51.41% from *P. falcataria*, and followed by 49.88% from *A. scholaris*, 49.05% (*A. elasticus*), 47.54% (*L. leucocephala*), 46.95% (*S. leprosula*), 46.81% (*S. mahagoni*), 46.73% (*A. altilis*), and 46.67% (*M. gigantea*). These values were higher than several weed and wood biomass that previously reported (Premjet et al. 2013; Tye et al. 2016). We also found that *A. cadamba* was gave the highest hemicellulose content (31.86%) and followed by *A. moluccana* (30.60%), *S. mahagoni* (28.87%) and *G. arborea* (28.13%). The present results were also showed that the amounts of cellulose, and lignin were variable for the biomass species examined. In line with our findings, the previous papers reported that the diversity of biomass composition was dependent on the plant species, soil nutrients, climate and competition (McKendry 2001; Premjet et al. 2013).

Effects of alkaline pretreatment on lignocellulosic components of wood biomass

Effects of alkaline pretreatment on the change of wood components were also analyzed. We found the alkaline pretreatment was effectively defibrillated and delignified the woody biomass that marked by the residual pulp fractions and decreased of Klason lignin content (Table 2,3). In general discussion, we found two different types of the residual pulp fraction, hard fiber and soft fiber. The formation of residual pulp fraction was really dependent on the concentration of alkaline and also biomass species used (Table 2, Figure 3). The highest decrease of lignin content was obtained when 5.0% NaOH applied at 160ºC to gave the lowest residual lignin content at 1.81~6.19%, respectively (Table 3). Decrease of lignin during alkaline pretreatment correspond to the cleavage of hydrolysable linkages such as α-and β-aryl ethers in lignin and glycosidic bonds in carbohydrates constitute the primary reactions that lead to the dissolution of lignin and carbohydrate with lower alkali stability (Lai 1991; Wang et al. 2008). The removal of lignin is beneficial for enzymatic saccharification due to increased accessibility of hydrolases to cellulose and hemicelluloses and decrease in non-productive binding between lignin and the enzymes (Zhao et al. 2007; Taherzadeh and Karimi 2008; Kumar et al. 2009; Gupta and Lee 2010; Alvarez et al. 2013; Rahikainen et al. 2013; Bali et al. 2014; Amirta et al. 2016a; Jönsson and Martin 2016).

Figure 3. Pulp properties: A. Hard fiber (left); B. Soft fiber

Table 1. Lignocellulosic compositions of 15 species of tropical wood biomass (sound wood or original wood components)

Wood biomass (species and local name)		Lignin (%)	Holocellulose (%)	Cellulose (%)
Acacia mangium Willd.	Akasia	27.80 ± 0.38	64.37 ± 0.18	38.94 ± 0.41
Aleurites moluccana (L.) Willd.	Kemiri	22.23 ± 0.14	76.08 ± 0.06	45.48 ± 0.19
Alstonia scholaris (L.) R.Br.	Pulai	25.05 ± 0.17	70.05 ± 0.04	49.88 ± 0.05
Anthocephalus cadamba (Roxb.) Miq.	Jabon	24.92 ± 0.12	69.22 ± 0.06	37.36 ± 0.61
Artocarpus altilis (Parkinson) Fosberg	Sukun	26.87 ± 0.47	69.39 ± 0.33	46.73 ± 0.98
Artocarpus elasticus Reinw. ex Blume	Terap	31.97 ± 0.05	68.80 ± 0.06	49.05 ± 0.88
Cananga odorata (Lam.) Hook. f. & T. Thomson	Kenanga	24.40 ± 0.14	64.14 ± 0.25	43.05 ± 0.61
Gmelina arborea Roxb.	Gmelina	25.73 ± 0.14	71.30 ± 0.20	43.17 ± 0.50
Lagerstroemia speciosa (L.) Pers.	Bungur	29.22 ± 0.17	70.36 ± 0.03	43.22 ± 1.11
Leucaena leucocephala (Lam.) de Wit	Lamtoro	29.32 ± 0.78	68.90 ± 0.11	47.54 ± 0.68
Macaranga gigantea (Rchb.f. & Zoll.) Müll.Arg.	Mahang	24.14 ± 0.27	71.14 ± 0.42	46.67± 0.55
Macaranga tanarius (L.) Mull.Arg.	Mara	30.40 ± 0.28	69.32 ± 0.28	45.49 ± 0.12
Paraserianthes falcataria (L.) Nielsen	Sengon	23.82 ± 0.45	69.64 ± 0.03	51.41 ± 0.86
Shorea leprosula Miq.	Meranti	31.07 ± 0.19	68.89 ± 0.04	46.95 ± 0.73
Swietenia macrophylla King	Mahoni	28.15 ± 0.21	75.68 ± 0.20	46.81 ± 1.34

Table 2. Pulp yield and pulp properties of wood biomass pretreated with 3.5% and 5.0% NaOH at 160°C for 60 min

Wood biomass		3.5% NaOH		5.0% NaOH	
Species	**Local name**	**Pulp (%)**	**Pulp Properties**	**Pulp (%)**	**Pulp Properties**
A. mangium	Akasia	58.86	hard fiber	48.64	soft fiber
A. moluccana	Kemiri	57.60	soft fiber	52.76	soft fiber
A. scholaris	Pulai	56.81	soft fiber	54.34	soft fiber
A. cadamba	Jabon	50.38	soft fiber	46.58	soft fiber
A. altilis	Sukun	66.12	soft fiber	53.24	soft fiber
A. elasticus	Terap	66.23	hard fiber	53.94	soft fiber
C. odorata	Kenanga	56.15	soft fiber	34.29	soft fiber
G. arborea	Gmelina	65.71	soft fiber	53.53	soft fiber
L. speciosa	Bungur	57.49	hard fiber	44.78	soft fiber
L. leucocephala	Lamtoro	70.01	hard fiber	59.11	soft fiber
M. gigantea	Mahang	75.90	hard fiber	58.86	soft fiber
M. tanarius	Mara	69.61	soft fiber	63.00	soft fiber
P. falcataria	Sengon	61.61	soft fiber	49.02	soft fiber
S. leprosula	Meranti	57.99	hard fiber	44.87	soft fiber
S. macrophylla	Mahoni	59.00	hard fiber	49.82	soft fiber

Table 3. Residual lignin, holocellulose and cellulose of wood biomass pretreated with 3.5% and 5.0% NaOH at 160°C for 60 min

Wood biomass		Lignin (%)		Holocellulose (%)		Cellulose (%)	
Species	**Local name**	**3.5% NaOH**	**5.0% NaOH**	**3.5% NaOH**	**5.0% NaOH**	**3.5% NaOH**	**5.0% NaOH**
A. mangium	Akasia	6.39 ± 0.07	4.96 ± 0.02	47.03 ± 0.03	42.44 ± 0.06	35.73 ± 0.45	35.30 ± 0.02
A. moluccana	Kemiri	5.85 ± 0.07	2.51 ± 0.04	51.09 ± 0.44	50.20 ± 0.28	42. 95 ± 0.60	38.79 ± 0.98
A. scholaris	Pulai	6.51 ± 0.02	6.15 ± 0.01	43.77 ± 0.07	43.70 ± 0.23	38.95 ± 0.29	40.98 ± 0.66
A. cadamba	Jabon	4.34 ± 0.01	2.88 ± 0.06	45.62 ± 0.26	42.42 ± 0.30	35.29 ± 0.16	30.37 ± 0.05
A. altilis	Sukun	7.16 ± 0.08	4.97 ± 0.07	53.27 ± 0.22	47.61 ± 0.30	45.55 ± 0.23	41.86 ± 0.64
A. elasticus	Terap	10.82 ± 0.03	5.79 ± 0.01	50.86 ± 0.18	43.90 ± 0.36	43.71 ± 0.34	40.20 ± 0.11
C. odorata	Kenanga	5.29 ± 0.01	1.81 ± 0.01	47.40 ± 0.28	32.37 ± 0.06	39.61 ± 0.12	28.44 ± 0.85
G. arborea	Gmelina	6.97 ± 0.07	3.60 ± 0.02	52.40 ± 0.07	49.57 ± 0.26	41.20 ± 0.24	38.66 ± 0.25
L. speciosa	Bungur	5.78 ± 0.04	4.58 ± 0.01	46.03 ± 0.36	39.30 ± 0.06	38.65 ± 0.63	32.34 ± 0.06
L. leucocephala	Lamtoro	6.35 ± 0.08	5.97 ± 0.05	62.51 ± 0.54	52.59 ± 0.41	43.39 ± 0.13	41.94 ± 1.12
M. gigantea	Mahang	6.12 ± 0.09	6.17 ± 0.27	55.75 ± 0.52	53.52 ± 0.33	45.56 ± 0.22	44.43 ± 0.48
M. tanarius	Mara	7.74 ± 0.06	6.19 ± 0.03	56.52 ± 0.28	55.03 ± 0.01	49.65 ± 0.20	36.48 ± 0.39
P. falcataria	Sengon	5.98 ± 0.03	2.14 ± 0.09	53.39 ± 0.22	44.37 ± 0.32	47.71 ± 0.21	34.61 ± 0.28
S. leprosula	Meranti	6.68 ± 0.02	4.63 ± 0.03	46.55 ± 0.52	39.70 ± 0.23	43.55 ± 0.03	28.64 ± 0.13
S. macrophylla	Mahoni	8.98 ± 0.01	4.27 ± 0.04	48.11 ± 0.03	45.26 ± 0.60	41.48 ± 0.15	33.48 ± 0.34

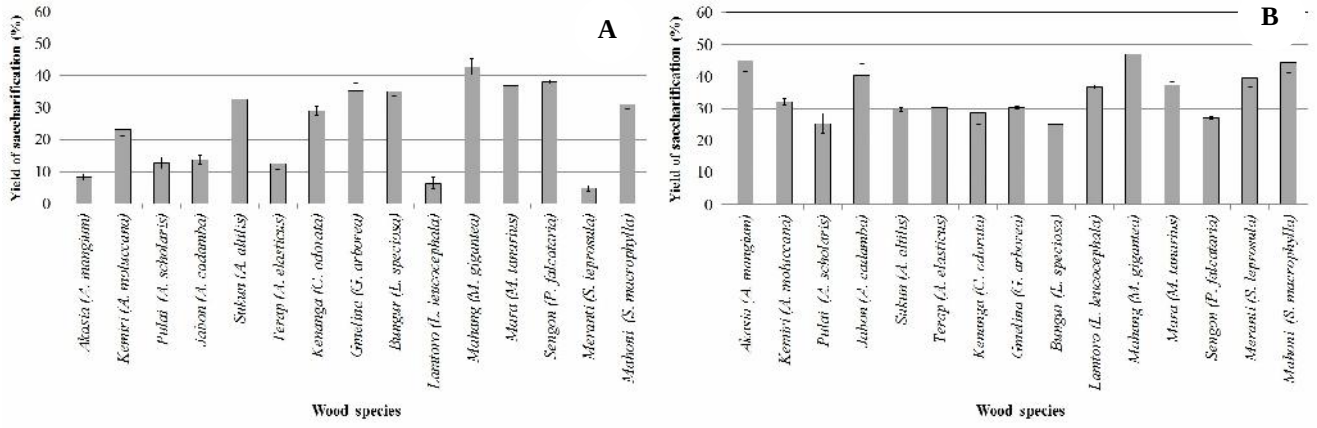

Figure 4. Yield of saccharification of tropical wood biomass pretreated with alkaline (original wood basis): A. 3.5% NaOH and B. 5.0% NaOH

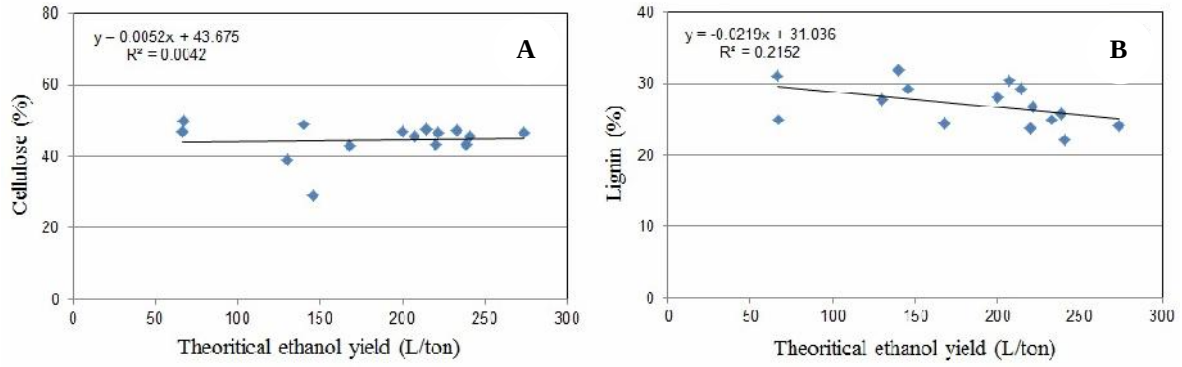

Figure 5. Correlation between: A. Cellulose and theoretical yield of ethanol, B. Lignin and theoretical yield of ethanol pretreated with 5.0% NaOH

Table 4. Saccharification yield of wood biomass pretreated with 3.5% and 5.0% NaOH at 160°C for 60 min

Wood biomass		Pulp saccharification (w/w, pulp basis)	
Species	**Local name**	**3.5% NaOH**	**5.0% NaOH**
A. mangium	Akasia	13.58 ± 1.84	45.87 ± 6.01
A. moluccana	Kemiri	42.95 ± 1.98	78.29 ± 1.07
A. scholaris	Pulai	10.39 ± 3.07	21.13 ± 3.10
A. cadamba	Jabon	68.30 ± 7.05	85.67 ± 3.59
A. altilis	Sukun	27.36 ± 4.07	71.42 ± 0.51
A. elasticus	Terap	10.49 ± 2.20	44.46 ± 0.09
C. odorata	Kenanga	59.07 ± 1.46	84.05 ± 3.68
G. arborea	Gmelina	40.78 ± 2.50	76.58 ± 0.33
L. speciosa	Bungur	35.00 ± 1.36	55.92 ± 0.23
L. leucocephala	Lamtoro	26.62 ± 3.04	62.15 ± 0.17
M. gigantea	Mahang	26.84 ± 2.58	79.70 ± 5.48
M. tanarius	Mara	23.48 ± 4.63	56.48 ± 0.91
P. falcataria	Sengon	47.33 ± 0.59	77.11 ± 0.37
S. leprosula	Meranti	10.16 ± 1.67	25.21 ± 2.62
S. macrophylla	Mahoni	42.66 ± 1.34	68.90 ± 3.05

Enzymatic saccharification of wood biomass

In order to evaluate the effective alkaline pretreatment on sugar production, enzymatic saccharification of wood biomass was studied. The effects of alkaline pretreatment on enzymatic saccharification was analyzed at two different concentrations of NaOH, 3.5% and 5.0% at 160°C for 60 min using a commercial cellulase from *T. viride*, Meicelase (Fig. 4). General trend for promotion of sugar yield by higher concentration of NaOH with the decreased amount of remaining lignin was found (Fig. 4 and Table 4), in accordance with the previous report (Taherzadeh and Karimi 2008; Wang 2008; Zhao et al. 2007; Mirahmadi et al. 2010; Chiaramonti et al. 2012; Sing and Trivedi 2013; Amirta et al. 2016a), who described that the hydrolisability of treated hardwood increased with decrease in lignin content. The alkaline pretreatment was effectively facilitated enzyme to digested tropical rain forest wood biomass to produce reducing sugar. The results also demonstrated that increase in the NaOH concentration from 3.5% to 5.0% increased the sugar yield by 8.24% (*A. mangium*), 10.63% (*A. moluccana*), 5.02% (*A. scholaris*), 4.96% (*A. cadamba*), 17.94% (*A. altilis*), 15.93% (*A. elasticus*), 12.78% (*G. arborea*), 4.43% (*L. speciosa*), 16.29% (*L. leucocephala*), 23.89% (*M. gigantea*), 17.31% (*M. tanarius*), 7.78% (*P. falcataria*), and 4.88% (*S. leprosula*). On the contrary, sugar yield of *C. odorata* with 3.5% NaOH was higher (29.85%) than the pretreatment

with 5.0% NaOH to gave the sugar yield only 25.94%. Among the fifteen species of tropical wood biomass tested, our finding showed that five species of wood biomass such as *M. gigantea*, *A. moluccana*, *G. arborea*, *A. cadamba*, and *A. altilis* were gave more than 35% of sugar yield based on the original weight of wood used. The highest sugar yield, 42.22% (weight of original wood basis) was obtained for *M. gigantea* at the alkaline concentration 5.0% NaOH and followed by *A. moluccana* (37.18%), *G. arborea* (36.89%), *A. cadamba* (35.91%) and *A. altilis* (34.22%).

Theoretical ethanol production from wood biomass

In this study, we found that the lowest theoretical yield of ethanol was from *S. leprosula* (66 L/ton) and *A. scholaris* (67 L/ton). On the other hand, the highest theoretical ethanol yield was from *M. gigantea* (273 L/ton) and followed by *A. moluccana* (241 L/ton), *G. arborea* (239 L/ton), *A. cadamba* (233 L/ton), *A. altilis* (222 L/ton) and *P. falcataria* (220 L/ton). The higher theoretical yield of ethanol was obtained from the biomass with the lower lignin content (22.23%~25.73%) and the high cellulose content (43.17%~47.36%), respectively. There have been correlations between lignin, cellulose and yield of ethanol (Fig. 5). Similar with results that we mentioned earlier, general trend for higher theoretical yield of ethanol by higher cellulose content and lower Klason lignin were also found. This finding was in line with the previous results reported (McKendry 2001; Premjet et al. 2013; Amirta et al. 2016a). *M. gigantea, A. moluccana, G. arborea, A. cadamba,* and *P. falcataria* are attractive for their high conversion efficiency and susceptibility to the pretreatment.

Availability of wood biomass

Sustainable feedstock availability is one of the important factors that should be considered on the idea to utilize wood biomass for ethanol production in the near future. In term of that in this study, availability of *A. moluccana, G. arborea, A. cadamba, P. falcataria,* and *M. gigantea,* that are attractive for their high conversion efficiency and susceptibility to the pretreatment and produced high saccharified sugar and ethanol yield were also discussed. In general practices we knew *G. arborea, A. cadamba,* and *P. falcataria,* were planted for the mass production of wood for the construction purposes and also pulp and fiber production in the scheme of industrial forest plantation and also community forest development programs in Indonesia, including in East Kalimantan. In addition, *A. moluccana*, was traditionally planted for the production of candle nut since thousands years ago. The waste of wood biomass that annually obtained from the thinning and harvesting activities of the industrial forest industry and unproductive and mature cutting stem waste of *A. moluccana* for replanting purposes are potential feedstock that can be used for ethanol production. The wood biomass feedstock could also obtain by putting the additional sharing portion (quota) of forest plantation and wood production for ethanol as well as regulated for CPO (crude palm oil) production. Nowadays, CPO production was not only utilized for the domestic cooking oil production and exported to foreign countries. The new regulation released by the Indonesia Government and also the East Kalimantan Province was clearly stated that 20% of total CPO production should be provided and shared for domestic feedstocks for biodiesel production purposes. In term of this, more than 500,000 cubic meters of wood biomass will be available annually (Table 5) and number of feedstock potentially increased due to development target of the East Kalimantan Government to have more than two millions hectares of plantation forest in 2016 (East Kalimantan Planning Agency-BAPPEDA 2015). The source of plantation forest developed was not only belongs of the private companies but also owned by communities (community forest).

Fast growing ability and productivity of *M. gigantea*

Different then *G. arborea, A. cadamba, P. falcataria* and *A. moluccana,* the pioneer wood species *M. gigantea* was not commercially planted yet. The tropical fast growing species *M. gigantea* was reported growth dominantly on the secondary forest of Kalimantan (Borneo Island). *M. gigantea* was commonly used for natural indicator on succession process of secondary forest in this area (Slik et al. 2003, 2005). Our result also demonstrated growth of pioneer species was very fast to rise of plant diameter approximately 3-6 cm annually (Fig. 6 and Table 6). Based on the data of plant growth collected from the trial planting plot of *M. gigantea* located at Universitas Mulawarman Education Forest, we estimated the production of dry weight wood biomass were 1,297 kg ha^{-1} (1^{st} year), 17,154 kg ha^{-1} (2^{nd} years) and 26,119 kg ha^{-1} (3^{rd} years), respectively (Table 6). The high yield production of dry wood biomass was very promising for sustainable ethanol feedstock in the near future.

Table 5. Biomass production from Industrial Forest Plantation in East Kalimantan FY 2014

Wood species	Wood production (m^3)	Harvesting waste		20% Sharing production quota (m^3)
		%*	m^3	
A. mangium	1,971,292.22	4.39	86,539.73	394,258.44
E. pelita	200,193.69	2.64	5,285.11	40,038.74
G. arborea	95,281.77	3.50	3,334.86	19,056.35
P. falcataria	26,167.77	3.50	915.87	5,233.55
	2,292,935.45		**96,075.58**	**458,587.09**

Note: *Waste biomass from harvesting activity of industrial forest plantation in East Kalimantan (Syahrinudin 2014)

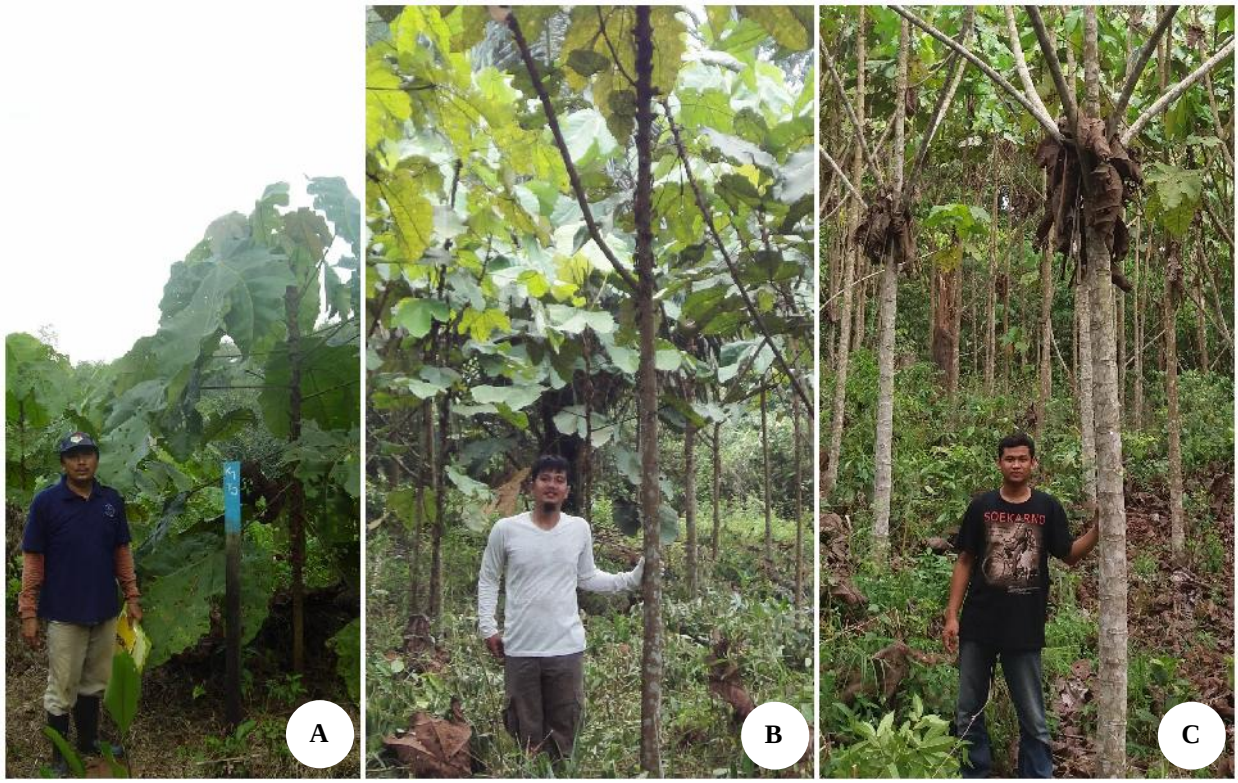

Figure 6. Description of fast growing ability of *M. gigantea*. A. 1st year, B. 2nd year, C. 3rd year

Table 6. Growth of diameter, high and biomass production of *M. gigantea* for 3 years of plantation

Growth indicators	1st year	2nd years	3rd years
Plant density (g/cm^3)	-	0.30 ± 0.05	0.33 ± 0.07
Plant diameter (cm)	3.41 ± 0.53	9.70 ± 0.25	11.50 ± 2.10
Plant high (m)	1.76 ± 0.34	7.51 ± 1.60	9.00 ± 1.70
Plant Biomass (kg ha^{-1} dry wood)	1,297	17,154	26,119

The present study gives a new role in the *M. gigantea*, and other secondary forest wood species as a resource for ethanol production (bio refinery). Design of the sustainable cycle including forest plantation of the *Macaranga* and other wood species, and their conversion into fuels and chemicals will activate the local economy in the tropics with concomitant contribution to the global environment. This is an essential point because of those woody materials were not be considered as potential biomass feedstock for the biofuel production as far, except for *P. falcataria*, *G. arborea* and *A. cadamba* which are planted in forest plantation for years in Indonesia especially in East Kalimantan. Nowadays, *A. moluccana* and *A. altilis* and *A. elasticus* are daily used by rural people in East Kalimantan for tree fruit (candle nut, bread fruit and keledang fruit). *M. gigantea* and *M. tanarius* have been used as firewood species by local people in East and North Kalimantan Provinces, instead of the higher density wood species such as *Vitex pinnata*, *Nephelium lappaceum*, *Blumeodendron kurzii* and *Dipterocarpus* sp. (Yuliansyah et al. 2012). Furthermore, the dried root and fresh leaves of *Macaranga* was also used to cover wounds to prevent inflammation, as an emetic agent, antipyretic, antioxidant and antitussive in Thailand and Malaysia (Chulaborn et al. 2002; Lim et al. 2009). The bioactive compound of *M. tanarius* was also reported effective to be used as an antidiabetic (Puteri and Kawabata 2010). Instead of firewood and medicine, since many years ago *Macaranga* was traditionally used by Dayak people in East Kalimantan as the natural plant indicator to determine the end of the recovery period of forest land after ground fire or shifting cultivation activities.

The tropical wood biomass particularly *M. gigantea*, *A. moluccana*, *G. arborea*, *A. cadamba* and *P. falcataria*, are attractive for their fast growth ability, availability, chemical composition and also conversion process. These wood species are promising and potentially to be used and developed widely as suitable feedstocks for ethanol production, chemical and also other bio refinery purposes in the near future. Further investigation required to explore and find more attractive conversion system for the tropical wood biomass plant species that abundance in the rain forest area of East Kalimantan, Indonesia.

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Short Communication: Conservation of mangrove gobies in Lesser Sunda Islands, Indonesia

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Abstract. *Zamroni Y, Soewardi K, Suryobroto B, Jaafar Z. 2016. Short Communication: Conservation of mangrove gobies in Lesser Sunda Islands, Indonesia. Biodiversitas 17: 553-557.* Ecosystems goods and services from mangrove forests are especially vital to coastal communities. Yet mangrove areas continue to be deforested at unprecedented rates. Using gobioid fishes associated with mangrove forests as focal organisms, we assessed their diversity in 14 selected sites within the Lesser Sunda group of islands. We applied Correspondence analysis to determine the relationships between ecosystems based on the occurrence of these fishes and complementarity analysis to identify the minimum number of sites to conserve maximum diversity based on a rarity algorithm. We recovered 55 gobioid fish species at these mangrove areas, and proposed six mangrove areas within the Lesser Sunda group of islands as areas of conservation priority: Loh Sebita, Oebelo, Bipolo, Lembar Bay, Selindungan, and Kawangu. The three former areas are already within protected zones while the remaining latter three areas are at present unprotected. The argument for the conservation of these three remaining areas is a compelling one, based on our data (diversity of gobioid fishes), and corroborating data (diversity of corals, reef fishes, stomatopods, seagrasses, and marine birds) from other studies.

Keywords: Brackish, mangroves, deforestation, Nusa Tenggara, Threatened Species, Gobiidae

INTRODUCTION

Ecosystem goods and services from mangrove forests, estimated to be at least USD 1.6 billion a year, are vital to the livelihood of coastal communities and general society at large (Costanza et al. 1997; Wilkie and Fortuna 2003). Yet mangrove forests remain one of the most imperiled ecosystems globally and face extinction risks in areas where they occur (Polidoro et al. 2010). Drivers of deforestation and degradation of mangrove forests in Southeast Asia include the conversion of such habitats to aquaculture ponds, oil palm plantations, and urban areas (Richards and Friess 2015). Within the Lesser Sunda Islands, more than 25 000 hectares of mangrove forests were deforested over a span of just five years, from 2007 to 2009 (Bakosurtanal 2009). Threats to the mangrove ecosystem are expected to escalate in the future, as global demands for food, biofuels, and raw materials continue to increase (Richards and Friess 2015; Polidoro et al. 2010).

We propose the conservation of the mangrove ecosystem in the Lesser Sunda Islands. Using gobioid fishes (Teleostei: Gobioidei) as focal organisms, we demonstrate that mangrove areas within the Lesser Sunda Islands are rich in biodiversity, and are biogeographically significant. Gobioid fishes are one of the dominant fish groups within mangrove habitats, with many undiscovered cryptic species (Lim and Larson 1994). Many gobioid fish species are reliant on mangrove habitats; mangrove deforestation was reported to be the cause for the decline of *Boleophthalmus pectinirostris* populations in Japan (Nanami and Takegaki 2005) and *Periophthalmodon septemradiatus* populations in Peninsular Malaysia (Khaironizam and Norma-Rashid 2003), and possibly the cause for extinction of *Periophthalmus malaccensis* in Singapore (Polgar 2012). Gobioid fishes are also ecologically and economically significant. Throughout Asia for example, many gobioid fish species are consumed (Nanami and Takegaki 2005; Kizhakudan and Shoba 2005; Polgar and Lim 2011). Gobioid fishes, such as species of *Periophthalmus*, are bio-indicators for the health of mangrove habitats (Kruitwagen et al. 2006). Some fishes have been used in bio-prospecting, for example, compounds within the mucus of *Boleophthalmus* spp. possess anti-bacterial properties which can be activated against human pathogens (Ravi et al. 2010).

Given the immense value of, and imminent threats to, the mangrove ecosystem, these habitats must be slated for conservation. With the data obtained in this study, and from literature review of other mangrove biota, we assess mangrove areas within the Lesser Sunda Islands and recommend areas that should be considered conservation priorities.

MATERIALS AND METHODS

Sampling of gobioid fishes

Gobioid fishes were sampled from fourteen mangrove areas in six islands throughout the Lesser Sunda group of

islands (specific localities within parentheses): Lombok Island (Lembar Bay, Selindungan, Sepi Bay, and Jor Bay), Sumbawa Island (Labuan Alas, and Cempi Bay), Komodo Island (Loh Sebita), Flores Island (Terang Bay), Sumba Island (Bugis Village, and Kawangu), and Timor Island (Paradiso Beach, Bipolo, Oebelo and Atapupu) (see Fig. 1). Fishes were collected using hand nets and fish traps within mangrove forests, and on adjacent mudflats and in littoral creeks. Sampling duration followed local tidal cycles, starting on the mudflat during the low tide and finishing in the back mangrove forest during the incoming tide. Specimens were preserved in 10% buffered formalin solution for two weeks before being transferred to 70% ethanol for long term storage. Specimens were deposited in the teaching collection of Mataram University. Fishes were identified using Jaafar and Larson (2008), Larson (2001; 2010), Murdy (1989; 2006; 2008a, b), Murdy and Shibukawa (2001; 2003) and Pezold and Larson (2015).

Data analyses

A taxon occurring in two or more areas indicates that those areas share closer relationships than areas without the taxon (Parenti and Ebach 2009). Based on this premise, the biogeographical history of the Lesser Sunda Islands was inferred from a data matrix of presence/absence data of sampled gobioid species at each location. The ordination of each mangrove area or location was evaluated using two-way correspondence analysis (CA). This analysis provides a graphic method of exploring the relationship between rows (location) and columns (species occurrence) in a contingency table. The distance between locations is positively correlated to the degree of similarity in species composition. Package 'ca' in R 3.0.0 was used to conduct the two-way correspondence analysis (Nenadic and Greenacre 2007).

Complementarity analysis was administered to identify the minimum number of sites required to conserve a set of species (Pressey et al. 1993), using the 'rarity algorithm', which reserves sites with the highest total rarity score in each iteration (see Turpie et al. 2000).

RESULTS AND DISCUSSION

We recovered 55 species of gobioid fishes in 34 genera from mangrove forests and mudflat habitats throughout the Lesser Sunda Islands (see Tab. 1). Of the sites assessed, Lembar Bay and Labuan Alas has the highest and lowest species richness respectively, with 31 species recovered in Lembar Bay, and four species recovered in Labuan Alas. Fifteen of these species were assessed and listed in the IUCN Red List of Threatened Species. One specie, *Pandaka pygmaea*, is listed as 'Critically Endangered'; one specie, *Favonigobius reichei* is 'Near Threatened'; eleven species, *Boleophthalmus boddarti*, *Bostrychus sinensis*, *Butis butis*, *Caragobius urolepis*, *Eleotris melanosoma*, *Eugnathogobius illotus*, *Eugnathogobius mindora*, *Mugilogobius chulae*, *Mugilogobius mertoni*, *Ophiocara porocephala* and *Redigobius bikolanus*, are listed as 'Least Concern'; and two species, *Mangarinus waterousi* and *Oxyeleotris urophthalmoides*, are listed as 'Data Deficient' (IUCN 2015). The conservation status of the remaining

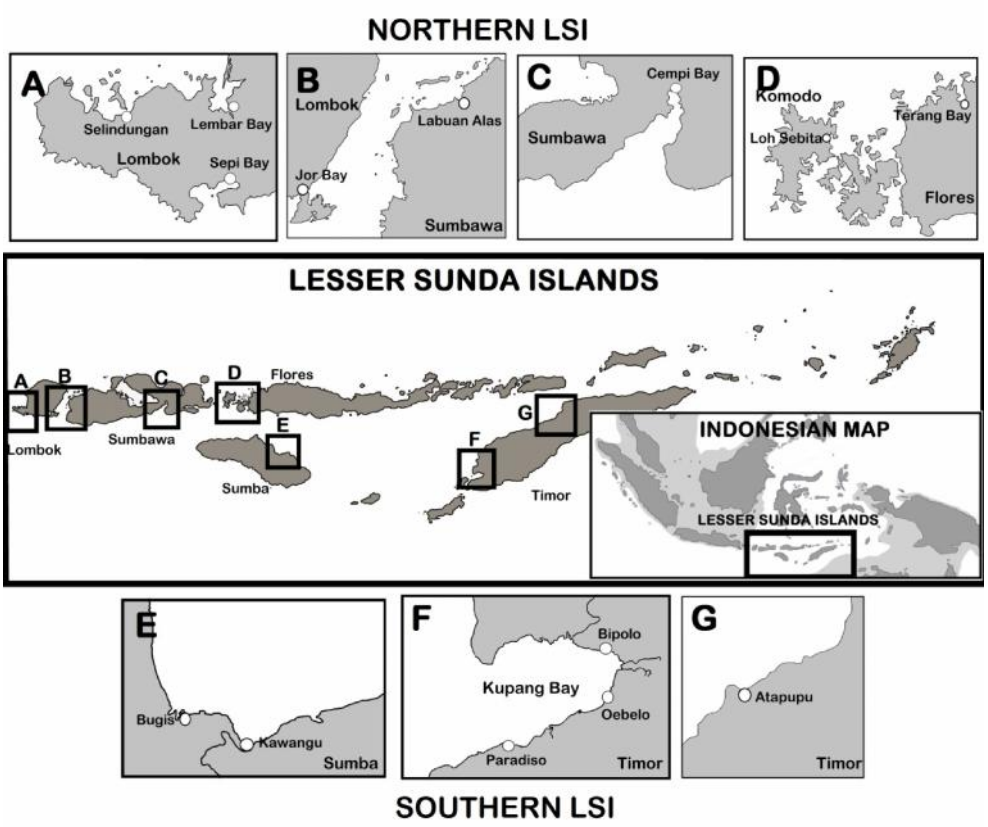

Figure 1. Sampling areas throughout the Lesser Sunda Islands, Indonesia, represented by open circles

40 species has not been assessed, but the close association between these fishes and the mangrove ecosystem make them likely candidates for future conservation assessments.

Correspondence analysis of the 55 species of gobioid fishes reveals that the Lesser Sunda Islands can be considered two distinct biogeographical zones. The first zone comprises Lombok, Sumbawa, Komodo, and Flores islands. The second zone comprises Sumba and Timor islands (Figure 2). These two zones reflect the geological history of islands in the region. The northern islands of the Lesser Sunda group of islands, corresponding to the first zone described above, are formed by volcanic activities during the Miocene to Pliocene 15-5 MYA. The southern islands of the Lesser Sunda group of islands, corresponding to the second zone, are made up of continental fragments from the Australian and Asian tectonic plates (Hall 2002).

Thirty-nine gobioid fish species were collected from mangrove habitats in the first zone, or the northern Lesser Sunda group of islands; 14 of these species were not recovered from the second zone, or the southern Lesser Sunda group of islands. Of the 40 species of gobioid fishes found in the southern Lesser Sunda group of islands, 16 species were not recovered from the northern Lesser Sunda group of islands. All gobioid fishes recovered in this study have wide ranges; no taxon is endemic to the Lesser Sunda group of islands. *Boleophthalmus boddarti*, for example, is widely distributed throughout coastal areas of south and Southeast Asia; the northern Lesser Sunda group of islands represents their eastern-most distribution limit. The southern Lesser Sunda group of islands represents the western-most distribution limits of *Periophthalmodon freycineti* and *Trypauchenichthys larsonae*. All the gobioid fishes recovered in this study can be conserved in a minimum of six areas spanning both biogeographical zones: Loh Sebita, Oebelo, Bipolo, Lembar Bay, Selindungan, and Kawangu (Table 2). Three of these six suggested sites, Loh Sebita, Oebelo, and Bipolo, are already established conservation areas. Loh Sebita is within the Komodo National Park. This national park was set-up to protect the habitat of *Varanus komodoensis* and encompasses three main islands in the area, Komodo, Rinca, and Padar, as well as numerous smaller islands in the vicinity. Mangrove areas within the park experience the least amount of anthropogenic disturbance when compared to other sites sampled in this study (Monk et al. 2000). Oebelo and Bipolo, are part of the Kupang Bay Wildlife Reserve. This reserve was established as a marine coastal management area for the conservation of coral reef habitats (Monk et al. 2000). Mangrove areas within the Kupang Bay Wildlife Reserve face moderate to large-scale anthropogenic threats (Bakosurtanal 2009).

We propose the three remaining sites, Lembar Bay, Selindungan, and Kawangu as areas of conservation priorities (Table 2). The inclusion of these three additional areas ensures the protection of maximum diversity of gobioid fish species within the Lesser Sunda group of islands. Parts of the mangrove areas in Lembar Bay, Selindungan, and Kawangu have been converted to small marinas, aquaculture ponds, human settlement, and tourism facilities.

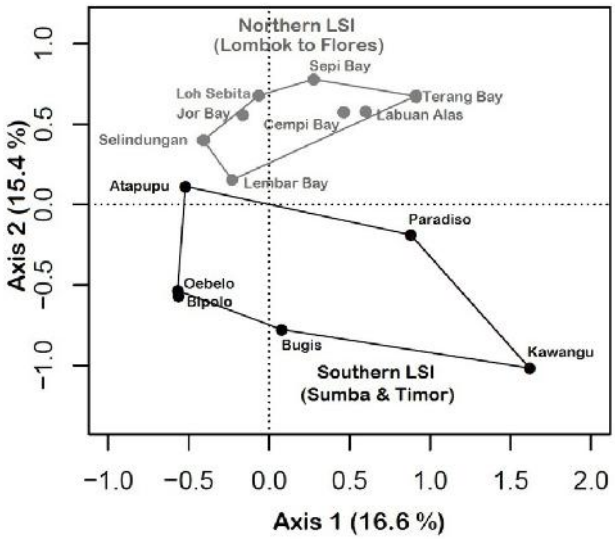

Figure 2. Divergence of mangrove habitats within the Lesser Sunda Islands based on gobioid fishes

Additionally, ten species of mangrove flora with conservation interest were reported from Lembar Bay and Selindungan reproduced here with the IUCN Red List status included: *Avicennia lanata* listed as 'Vulnerable'; *Ceriops decandra* listed as 'Near Threatened'; *Avicennia alba, Avicennia marina, Avicennia officinalis, Rhizophora apiculata, Rhizophora mucronata, Rhizophora stylosa* and *Sonneratia alba* listed as 'Least Concern' (Zamroni and Rohyani 2007; Syarifuddin and Zulharman 2012; IUCN 2015). Further, *Avicennia lanata* and *Ceriops decandra* are two of 14 floral mangrove species protected in Indonesia due to their rarity and slow growth (Noor et al. 1999). *Avicennia lanata* is currently known only from Peninsular Malaysia, and the islands of Borneo, Bali, Lombok, and Singapore (Noor et al. 1999). *Ceriops decandra* has a wide distribution from India eastward to Australia, but are low in densities in areas where they occur (Noor et al. 1999).

Lembar Bay and Selindungan have been previously proposed as part of a larger marine protected area, the Labuan Tereng Reefscape, based on high diversity of corals, reef fishes, stomatopods, and seagrasses (de Vantier et al. 2008). Moreover, marine avian fauna such as *Numenius madagascariensis* and *Calidris tenuirostris* listed as 'Endangered' and *Fregata andrewsi* listed as 'Critically Endangered' have also been reported to forage in the Lesser Sunda group of islands (Monk et al. 2000; Myers and Bishop 2005; IUCN 2015).

The conservation of the mangrove ecosystem is important especially for an archipelagic area such as the Lesser Sunda group of islands. These islands are susceptible to strong winds and storms and to hydrological forces and coastal erosion. Further, being in a zone with high tectonic activity, these communities are susceptible to tsunamis and volcanic activities. The mangrove forest has been shown to ameliorate the devastating impacts of most of these natural disasters (Gunawan et al. 2005). Mangrove areas within the Lesser Sunda groups of islands are already at risk from anthropogenic impacts. We propose that some

of these areas are conserved for species and genetic diversity. The conservation of the mangrove ecosystem will of great human benefit, especially because of the ecosystem goods and services rendered.

Table 1. List of gobioid fishes recovered in this study with locality data. Species already assessed by the IUCN Red List of Threatened Species are in bold

Localities/Species	Lembar Bay (Lombok)	Selindungan (Lombok)	Sepi Bay (Lombok)	Jor Bay (Lombok)	Labuan Alas (Sumbawa)	Cempi Bay (Sumbawa)	Loh Sebita (Komodo)	Terang Bay (Flores)	Bugis Village (Sumba)	Kawangu (Sumba)	Bipolo (Timor)	Paradiso (Timor)	Oebelo (Timor)	Atapupu (Timor)
Acentrogobius audax	+	+					+							
Acentrogobius caninus	+												+	
Acentrogobius janthinopterus	+	+	+	+	+	+	+	+	+			+		
Acentrogobius nebulosus							+							
Acentrogobius viridipunctatus	+	+		+	+	+		+	+		+		+	+
Amblygobius sp.							+							
Amoya gracilis	+		+	+			+	+						+
Amoya madraspatensis	+				+							+		
Amoya moloanus	+	+												
Apocryptodon madurensis	+										+		+	+
Boleophthalmus boddarti	+					+								
Bostrychus sinensis		+												
Butis butis	+			+			+					+	+	
Butis humeralis											+			
Callogobius sp.	+		+				+							
Caragobius urolepis	+										+		+	
Cristatogobius aurimaculatus	+										+			
Cristatogobius lophius							+							
Cristatogobius nonatoae	+													
Cristatogobius rubripectoralis	+	+									+			
Drombus triangularis									+		+		+	
Eleotris melanosoma	+	+		+			+				+		+	
Eugnathogobius illotus	+													
Eugnathogobius mindora									+	+				
Eugnathogobius polylepis	+													
Favonigobius reichei	+						+		+			+		
Glossogobius sp.				+							+			
Gobiopterus sp.											+			
Hemigobius hoevenii										+	+			
Mangarinus waterousi		+												
Mugilogobius chulae	+													
Mugilogobius mertoni	+								+					
Mugilogobius sp.											+			
Odontamblyopus rubicundus	+										+		+	
Ophiocara porocephala	+	+					+		+		+		+	
Oxyeleotris urophthalmoides	+													
Oxyurichthys cornutus	+	+	+				+				+			
Oxyurichthys ophthalmonema									+			+	+	
Oxyurichthys tentacularis	+	+							+		+	+	+	
Oxyurichthys sp.											+			
Pandaka pygmaea										+				
Paratrypauchen microcephalus													+	
Periophthalmodon freycineti													+	+
Periophthalmus argentilineatus	+	+	+	+	+	+	+	+	+	+	+	+	+	
Periophthalmus gracilis			+			+								
Periophthalmus kalolo	+							+				+		
Periophthalmus malaccensis			+			+	+	+		+				
Periophthalmus minutus	+	+	+									+	+	
Periophthalmus pusing										+	+	+	+	+
Pseudogobius javanicus	+	+	+	+		+	+		+		+		+	+
Redigobius bikolanus	+								+	+	+		+	
Scartelaos histophorus									+				+	
Taenioides sp.													+	
Trypauchen vagina	+													
Trypauchenichthys larsonae													+	
Trypauchenopsis intermedia													+	
Total Species	**31**	**14**	**9**	**8**	**4**	**7**	**16**	**6**	**13**	**7**	**21**	**10**	**23**	**6**

Table 2. The minimum number of mangrove areas to conserve all gobioid fish species recovered in our study, determined by complementarity analysis

Number	Mangrove ecosystems	Number of species	Cumulative species reserved
1	Lembar Bay	31	31
2	Oebelo	9	40
3	Bipolo	6	46
4	Loh Sebita	4	50
5	Selindungan	3	53
6	Kawangu	2	55

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Fish community structure in high water temperature around Bontang Industrial Estate, East Kalimantan, Indonesia

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Abstract. *Suyatna I, Sidik AS, Almadi FA, Rizal S, Sukarti K. 2016. Fish community structure in high water temperature around Bontang Industrial Estate, East Kalimantan, Indonesia. Biodiversitas 17: 558-564.* We have conducted studies on fish community and their physical and chemical properties of coastal waters in the work area of the industrial estate from 2012 to 2014. At least four industries are known to use the coastal waters in cooling their processing equipments, the hot wastewater is then discharged back into the coastal environment. The current study reports the result of a survey undertaken around the work area of PT Blackbear Resources Indonesia. The result showed that high water temperature and tidal fluctuation are affecting the fish community in the area. By sampling results, during HWL of the spring tide at the maximum of water temperature of 35,9^{o}C, fish were the most populated and found 5999 ind. or 72,4% of the total number of 8291 fish. During LWL of the same tide type fish were observed 1931 ind (23.3%) and during the neap tide 361 ind (4.3%) at the maximum of water temperature of approximately 40^{o}C, this ambient showed that the fish population drastically change in number and the environment became undesirable.

Keywords: Bontang, industry, fish community, high water temperature, leiognathids

INTRODUCTION

Geographically, Bontang City stretches between 117^{o}23' and 117^{o}38' E and 0^{o}01' and 0^{o}12' N, and is located between the ecosystems of Mahakam Delta and Sangkulirang Bay of East Kalimantan, Indonesia. Defined as a centre for petrochemical industries, raw materials for such industries are transported through pipes from Oil and Gas exploitation companies in the Mahakam Delta and Balikpapan. The coastal zone of Bontang City is continuously transformed with new constructions such as industrial plants, land reclamation, ports, and channel dredging (Suyatna and Sidik 2013). In the process of petrochemical production, the industry uses sea water to cool their processing machines and utilities in a cooling water system, and discharges the hot waste water to the sea environment. Cooling water system applied in the industrial processes in Bontang is in two forms i.e. once through system or blowdown cooling system. Once through system usually discharge huge volume of 'still hot' wastewater to the environment around or not far from the outlet, sea water temperature was recorded high. Blowdown cooling system, different from once through system, discharges only small volume of waste water, since the sea water used in the system is reused by recirculation. A higher water temperature accelerates both biological and chemical processes in the sea, and reduces the solubility of dissolved oxygen in the water (Boyd 1995). This can affect the physiological life of fish such as growth, reproduction and distribution. However, the environmental factors change according to photoperiod, tidal cycle and climatic change (Lam et al. 2005). Hot water flowing from the outlet of industries has already been recorded to be responsible for the rising of sea water temperature around an industrial estate and may harm fish in Bontang.

A study to explain the actual fish composition structure of a community is required, since the mentioned industries are not only responsible for the increase in sea water temperature but also had potential of causing chemical pollution, thereby, altering the population and species of the fish community.

The study was carried out to understand (i) the community of fish species, (ii) the most fish population and (iii) the physical and chemical properties of sea water around the work area of PT Blackbear Resources Indonesia (PT BBRI).

MATERIALS AND METHODS

The study was undertaken in August 2013 and May to June 2014 in Guntung village, Bontang Utara Subdistrict, Bontang City, East Kalimantan, Indonesia around the work area of PT BBRI (Figure 1). Even the cooling water system of PT BBRI is blowdown cooling system; the sea water area surrounding this industry is also influenced by wastewater from other outlets from once through system of the neighboring industries.

Hydrographic parameters such as tide, water depth and current velocity were surveyed using tidal pole, echo-sounder GPSmap 2108 Garmin and Braystoke BFM001 Current Flow Meter, Valeport Marine Scientific Ltd made in UK 1985. Tide was observed manually in every 30 minutes. Physical and chemical factors such as temperature,

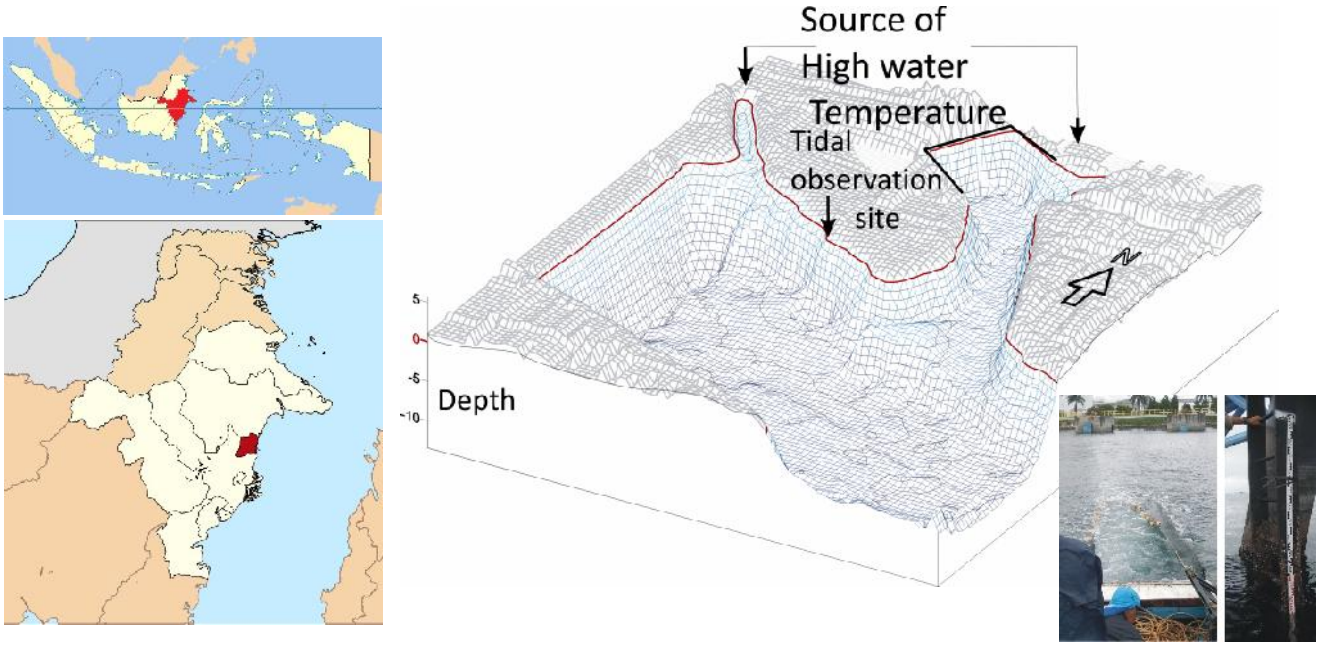

Figure 1. Study site in Guntung village, North Bontang Subdistrict, Bontang City, East Kalimantan, Indonesia and partly bottom profile, fish sampling gear and tide pole.

salinity, pH and dissolved oxygen (DO) was taken approximately every 25 m interval using Water Checker Horiba U-50 series starting from the outlet extending 250 m (three directions) with angle of 37.5°. Fish were sampled during high water level (HWL) and low water level (LWL) in spring tide as well as during neap tide three times each, using a trawl net with size of 10 m length and performed by a motorized boat to tow the net. Trawl net was towed approximately 30 minutes to avoid torn net due to hard substrate (area of reefs dredged). Small fish were weighed with a digital precision balance ACS AD-300i (capacity: 300 g x 0.01 g). Fish identification was referred to to the field manual according to Anam and Mostarda (2012), Matsunuma et al. (2011), Allen (2000), Peristiwady (2006), and Masuda et al. (1975), Chakrabarty et al. (2008), Chakrabarty et al. (2010a), Chakrabarty and Sparks (2008), Seah et al. (2009) and Chakrabarty et al. (2010b). The swept area was applied to find fish density (Can et al. 2005): a = *D.h.X*, where h is the length of the head-rope, D is the cover of distance. X is the fraction of the head-rope length. The value of X varies from 0.4 to 0.66 (commonly used 0.5). Garmin GPSMap 60CSx was used to record the geographic position of sampling sites and to determine the distance. Diversity index (Shannon Winner_H', Dominancy and Margalef richness) of fish was analyzed using software of the PAlaentological Statistics 'PAST' version 3.05 (Hammer 2015).

RESULTS AND DISCUSSION

Physical and chemical properties

The physical, chemical and hydrographic conditions of waters influencing fish in their habitats were studied by Kane et al. (2010), Madeira et al. (2012) and Hsieh (2012) and therefore some properties of water in the study area were measured. After measuring water depth and corrected by water level position that resulted from tide observation during 164.5 hours or seven days (Table 1), of the 3476 data recorded, sounding depth showed the maximum water depth of -16.39m (actual depth) and -13.78m (chart depth) and bottom profile. The bottom profile lying in long, narrow and deep waters is shown in Figure 1.

Regular changes of water level were known as two low tides and two high tides in 24 hours and tidal cycle duration at the location was recognized to occur between 5.5 and 8.0 hours and this type of tide according to Hicks (2006) is classified as mixed semidiurnal. As tide, local current velocities were monitored to describe water dynamics. Currents in the coastal area can have various origins such as tides and temperatures (Santema 1964). As seawater is heated molecular activity increases and thermal expansion occurs, reducing the density, different density of water causes water current. The velocities are commonly in km, m or cm per second (Harris 1978). In general, the result of the current velocity measurement is presented in Table 2. Suyatna et al. (2012) monitored current velocities 24 hours continuously in estuarine waters (at surface, middle and the bottom) during spring tide using the same current meter, and the current velocities ranged from 0.117 to 0.765 m/sec in day time and from 0.704 to 1.467 m/sec at night. Compared to this result, the current velocities around the study area were very weak.

The result of the physical and chemical properties measured during the study is shown in Table 3. The table shows that the highest temperature (39.6°C) and salinity (35 ‰) were recorded around the outlet, and they indicate a gradual decrease according to the distance from the source

of high water temperature. Temperature, along with salinity affects almost every physical property of seawater (Canadian Council of Ministers of the Environment, 2007) including pH, DO and solubility of gases (FGDC 2012). The CTMax from all estuarine and coastal fish species is reported between 36.4°C and 37.9°C (Murchie et al. 2011), 27.4°C and 38°C and the elevation of 2°C may cause stress of fish (Madeira et al. 2012). Contamination resulting to fish mortality has not been recorded in this area in past two decades. However, the non-toxic algal bloom was observed many times along the coast of Bontang, this can be either a natural phenomenon (Anderson 2005) or link to excessive pollution inputs such as nutrients (Anderson et al. 2002) and change of sea temperature (Silk 2015).

The salinity measured in the study area ranged from 28 to 35 ‰, and this range belongs to offshore waters when referring to Nordlie and Haney (1999) who classify coastal waters salinities to be between 16 and 32 ‰ and offshore waters 25 to 35 ‰. The pH values from all observations in the area were most similarly, ranging from 7.71 to 8.19. The growth of fish is greatly affected by pH (Majeed et al. 2015), and the ideal water pH for biological productivity is from 7.0 to 8.5 (Kane et al. 2010). Furthermore, the measured DO ranges from 4.89 to 5.00 mg/L and shows that the farther the distance from the outlet, the higher the concentration. Most species of fish are distressed when DO falls to 2 to 4 mg/L (Francis-Floyd 2014) and the minimum DO requirement for tropical marine fish is 5 mg/L, or 75% saturation (Mallya 2007).

Table 1. The result of tide observation in the study area

Date	Duration	Water level observed					Tide type
		First	Last	Lowest	Highest	Tide range	
	(hour)	(m)	(m)	(m)	(m)	(m)	
2 to 4 August 2013	52.0	2.32	2.52	1.96	3.31	1.35	Spring tide
22 to 24 May 2014	47.5	1.30	1.75	1.07	1.87	0.80	Neap tide
1 to 4 June 2014	65.0	1.28	1.05	0.70	2.53	1.83	Spring tide
7 days	164.5					2.61	

Table 2. The result of current velocity measurement in the study area

Date	Duration (hour)	'n' data	Current velocity (m/sec) Surface water (0.0m)	(cm/sec)	Tide type and Measurement time
2 to 4 August 2013					Neap tide
11.00 am to 23.00 pm	12.5	26	0.033-0.036	3.3-3.6	Every 30'
00.00 am to 23.30 pm	24.0	48	0.033-0.038	3.3-3.8	Every 30'
00.00 am to 15.30 pm	15.5	31	0.033-0.039	3.3-3.9	Every 30'
1 to 4 June 2014					Spring tide
15.00 pm to 03.00 am					
Station A	12	13	0.036-0.115 at 0.0m	3.6-11.5	Hourly
		13	0.038- 0.163 at -4.0m	3.8-16.3	Hourly
		13	0.038-0.117 at -9.0m	3.8-11.7	Hourly
Station B	12	13	0.157-0.333 at 0.0m	15.7-33.3	Hourly
		13	0.053-0.291 at -0.5m	5.3-29.1	Hourly
		13	0.053-0.269 at -1.0m	5.3-26.9	Hourly

Table 3. The mean concentration of some surface water quality parameters measured at the spring tide (high and low) and the neap tide during the study period.

Distance from outlet	Temperature (°C)		Salinity (‰)		pH		DO (mg/L)	
(m)	Spring	Neap	Spring	Neap	Spring	Neap	Spring	Neap
25	35.9	39.6	30	35	8.01	8.07	4.90	5.49
50	34.2	35.0	31	35	7.98	8.06	5.04	5.47
75	33.9	34.4	31	35	7.98	8.13	4.91	5.59
100	33.8	35.6	30	35	8.01	8.09	4.89	5.55
125	33.8	34.4	30	35	8.02	8.17	5.06	5.47
150	34.0	35.6	30	35	7.97	8.09	5.26	5.61
175	34.1	34.6	30	35	7,82	8.19	5.28	5.56
200	34.5	36.3	31	35	7.71	8.10	5.37	5.63
225	35.3	36.4	28	33	7.91	8.09	4.98	5.65
250	35.2	37.0	29	33	7.95	8.15	5.22	5.61

Fish composition, diversity and density

Fifty-three (53) species belonging to 7 orders, 22 families, 32 genera from 8291 fish caught were identified, and fishes were dominated by the members of order Perciformes (Figure 2 and Table 4). The overall difference in the size distribution of fish was not significant, and based on fish species most of them were categorized as young fish as shown in Table 5.

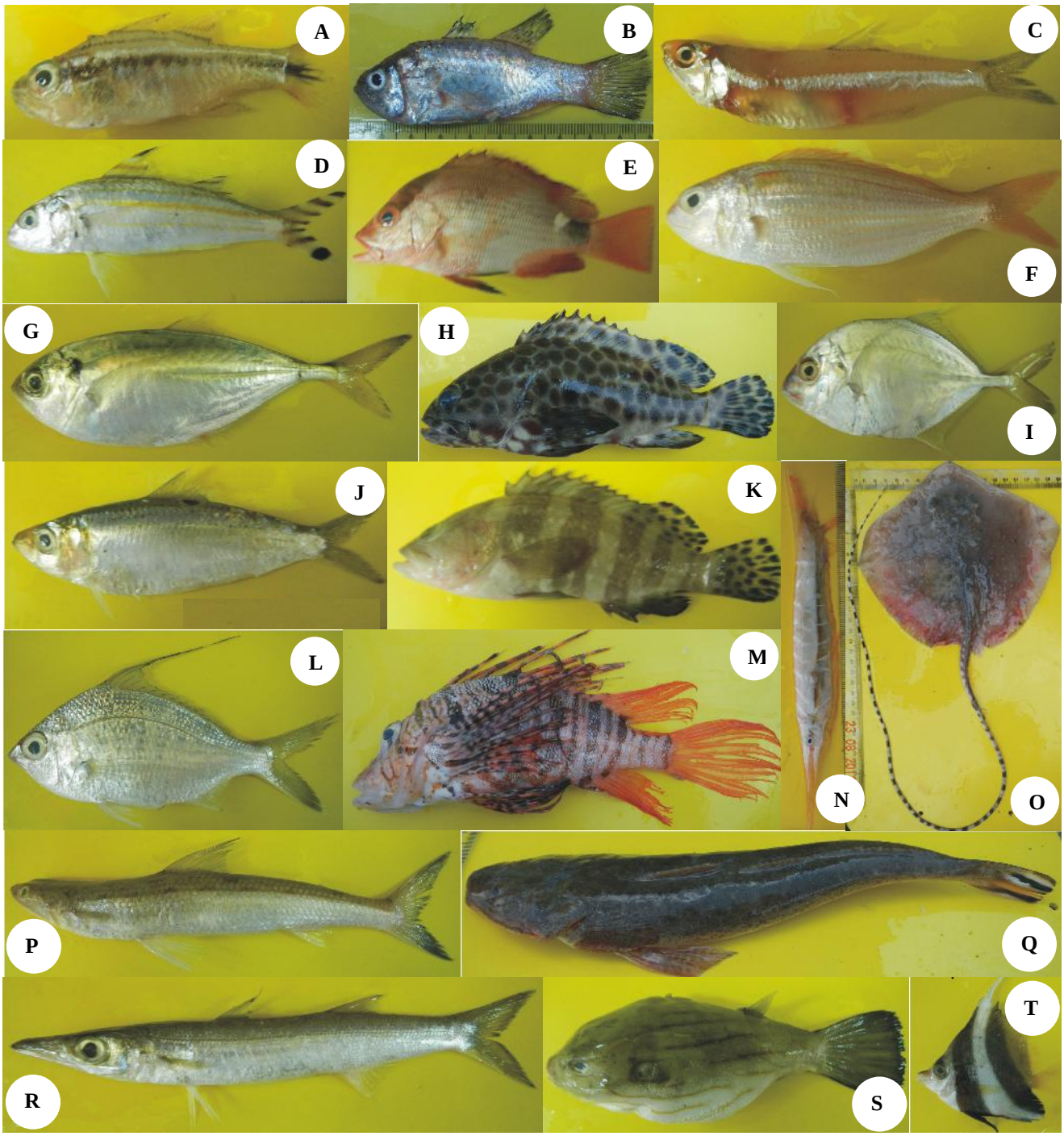

Figure 2. Some fish species other than leiognathids caught during the study. A. *Apogon kiensis*, B. *A. poecilopterus*, C. *Stolephorus indicus*, D. *Upeneus vittatus*, E. *Lutjanus erythropterus*, F. *Nemipterus hexodon*, G. *Megalaspis cordyla*, H. *Epinephelus megachir*, I. *Ulua mentalis*, J. *Sardinella fimbriata*, K. *E. sexfasciatus*, L. *Gerres filamentosus*, M. *Pterois russelli*, N. *Centriscus scutatus*, O. *Himantura gerrardi*, P. *Saurida tumbil*, Q. *Platycephalus endrachtensis*, R. *Sphyraena obtusa*, S. *Arothron manillensis*, T. *Heniochus diphreutes*.

Table 4. Length-weight size distribution and mean individual number of fishes caught

No	Common name	Family	Genus	Size distribution		Total number
				Length (cm)	Weight (g)	
HWL in spring tide, four samplings						
1	Ponyfish	Leiognathidae	a. *Leiognathus* (3)	3.0-14.3	0.2-35.7	1997
			b. *Secutor* (1)	3.0-14.3	0.2-35.7	3433
			c. *Gazza* (2)	3.7-8.0	0.8-8.9	262
2	Silver biddy	Gerreidae	*Gerres* (2)	5.0-9.1	1.7-11.9	75
3	Goatfish	Mullidae	*Upeneus* (2)	5.4-8.8	2.2-22.5	112
4	Lizardfish	Harpodontidae	*Saurida* (1)	7.5-24.0	3.0-104.0	15
5	Threadfin beam	Nemipteridae	*Nemipterus* (3)	6.9-20.5	3.6-139.5	34
6	Cardinalfish	Apogonidae	*Apogon* (3)	5.7-9.0	1.6-4.6	13
7	Flathead	Platycephalidae	*Platycephalus* (2)	9.5-16..5	2.1-28.7	10
8	Flounder	Bothidae	*Pseudorhombus* (2)	5.2-6.5	1.0-2.1	9
9	Stingray	Dasyatidae	*Dasyatis* (1)	33	77.7	4
10	Grouper	Serranidae	*Cephalopholis* (1)	10.5-22.5	15.8-137.0	3
11	Grouper	Serranidae	*Epinephelus* (3)	13.5-26.5	16.9-147.0	3
12	Puffer	Tetraodontidae	*Arothron* (2)	4.5-16.0	1.6-122.2	6
13	Hairtail	Trichiuridae	*Trichiurus* (1)	15.7	1	3
14	Sardinella	Clupeidae	*Amblygaster* (2)	8.1-10.0	7.1-9.1	5
15	Queenfish	Carangidae	*Scomberoides* (1)	10	6.7	3
16	Snapper	Carangidae	*Lutjanus* (4)	6.0-8	2.0-4.9	6
17	Trevally	Carangidae	*Alectis* (1)	18.5	72	6
				Total individuals:		5999
LWL in spring tide, three samplings						
1	Ponyfish	Leiognathidae	a. *Leiognathus* (3)	2.7-13.0	0.3-23.2	181
			b. *Secutor* (1)	4.5-13.0	0.4-23.2	1517
			c. *Gazza* (2)	2.7-6.4	0.3-4.5	143
2	Silver biddy	Gerreidae	*Gerres* (1)	5.5-8.5	1.0-8.6	26
3	Goatfish	Mullidae	*Upeneus* (2)	5.4-10.0	1.6-13.9	24
4	Lizardfish	Harpodontidae	*Saurida* (1)	7.0-25.6	1.0-134.9	7
5	Threadfin beam	Nemipteridae	*Nemipterus* (2)	9.3-19.0	1.3-89.7	10
6	Cardinalfish	Apogonidae	*Apogon* (2)	7.7-8.2	4.2-5.4	4
7	Flathead	Bothidae	*Pseudorhombus* (2)	5.9	5.9	4
8	Stingray	Dasyatidae	*Dasyatis* (1)	51	516.4	2
9	Grouper	Serranidae	*Cephalopholis* (1)	6.2-16.8	2.0-69.9	4
10	Snapper	Lutjanidae	*Lutjanus* (3)	11.2	17.2	2
11	Hairtail	Trichiuridae	*Trichiurus* (1)	25.1-81	8.4-516.8	3
12	Trevally	Carangidae	*Scomberoides* (1)	4.9	0.4	2
13	Lion fish	Scorpaenidae	*Pterois* (1)	11.5	14.9	2
				Total individuals:		1931
Neap tide, three samplings						
1	Ponyfish	Leiognathidae	a. *Leiognathus* (3)	3.5-10	0.3-16.2	135
			b. *Secutor* (1)	3.5-10	0.5-16.2	15
			c. *Gazza* (2)	4.0-5,5	0.3-2.5	130
2	Silver biddy	Gerreidae	*Gerres* (2)	4.5-10	0.9-11.1	15
3	Lizardfish	Harpodontidae	*Saurida* (1)	3.4-18.0	2.3-49.2	11
4	Threadfin beam	Nemipteridae	1) *Nemipterus* (1)	9.0-29.0	4.2-138.9	5
			2) *Pentapodus* (1)	7.0-20.0	3.2-116.9	4
5	Cardinalfish	Apogonidae	*Apogon* (1)	5.5-7.0	2.4-5.5	4
6	Glass perchlet	Channidae	*Ambassis* (1)	5.0-6.0	0.9-1.8	3
7	Flathead	Platycephalidae	*Platycephalus* (2)	8.0-31.0	10.8-200.3	7
8	Flathead	Bothidae	*Pseudorhombus* (1)	6.5	1.2	2
9	Grouper	Serranidae	*Cephalopholis* (1)	4.0-29.0	0.5-306.6	6
10	Snapper	Lutjanidae	*Lutjanus* (2)	4.0-7.0	1.1-6.6	3
11	Puffer	Tetraodontidae	*Arothron* (2)	18.5-19.0	48.3-150.0	7
12	Hairtail	Trichiuridae	*Trichiurus* (1)	53	116	2
13	Trevally	Carangidae	*Alectis* (1)	2.5-6.0	0.6-5.4	4
14	Anchovy	Engraulidae	*Stolephorus* (1)	8	0.3	2
15	Sardinella	Clupeidae	*Amblygaster* (1)	10	10.8	3
16	Razorfish	Centriscidae	*Aeoliscus* (1)	10	10.8	3
				Total individuals:		361

Note: Number in paranthesis: species number.

Table 5. Composition of community member of fish species caught from the study area

Orders	Families	Genera	Species	Environment origin (commonly found)
Perciformes	14	19	38	Demersal, pelagic, muddy, sandy, coral substrate
Scorpaeniformes	2	3	3	Demersal, muddy and sandy substrate
Clupeiformes	2	3	4	Pelagic, coastal and estuarine water
Tetraodontiformes	1	3	4	Demersal, coastal and estuarine water
Syngnathiformes	1	1	1	Demersal, seagrass meadow
Myliobatiformes	1	2	2	Demersal, muddy and estuarine water
Aulopiformes	1	1	1	Demersal, muddy and estuarine water
	22	32	53	

Table 6. The diversity indices of fishes caught around the waters of industrial estate

Diversity	Taxon	No of ind.	Dominance_D	Shannon_H'	Margalef_R
HWL (spring tide)	23	5999	0.441	1.075	1.954
LWL (spring tide)	19	1931	0.632	0.841	1.850
Neap tide	22	361	0.276	1.765	2.887

Table 7. Diversity of fishes caught around the waters of industrial estate

Tide	*Leiognathus* sp	*Secutor* sp	*Gazza* sp	Total Number	Density (ind/km^2)
HWL spring tide	1997	3433	262	5682	476,741
LWL spring tide	181	1517	143	1841	154,195
Neap tide	135	15	130	280	23,452

Leiognathids from one family were caught 7813 fish (95.1%) and the remaining from 21 families only 406 fish (4.9%). Leiognathids of three genera (*Leiognathus*, *Gazza* and *Secutor*) were composed of seven species: *Leiognathus nuchalis*, *L. splendens*, *L. fasciatus*, *Gazza achlamys*, *G. minuta*, *Secutor ruconius* and *S. indicius*. In the previous study all these leiognathids were also observed at the same place (Suyatna and Sidik 2013).

Wantiez and Kulbicki (1995) found leiognathids of soft bottom communities were the major species in density and biomass like *L. rivulatus* and *S. ruconius.* In India, pugnose ponyfish (*Secutor* sp) is one of the major by-catch composition (Muddula 2015) from the country leiognathids were known 16 species (Abraham et al. 2011), in Sri Lanka 13 species (Chakrabarty et al. 2008), in Malaysia 22 species (Seah et al. 2011) that were identified from eight or nine genera (Seah et al. 2012), at certain location of Malaysia such as the coastal waters of Pulau Sibu-Tinggi nine species (Mazlan et al. 2006), and in Thailand found in intertidal mudflats seven species (Sichum and Tantichodok 2013). In Indonesia, these fishes from various places were reported 11 species (Suyatna et al. 2010), 10 species (Wedjatmiko 2007), 20 species (Pauly 1977). The Table 6 showing a result analysis of the diversity indices of the total diversity of fishes and estimated the density of leiognathids. The value of dominance, Shannon (H') and richness is almost similar at the high and low tide of the spring tide, but higher at the neap tide, except the dominance. This indicated no extreme difference of fish population size at the neap tide (Table 4).

The highest density of leiognathids species observed at HWL in the spring tide (Table 7) could be as a result of passive migration aiming at finding new area for feeding, at the same time water temperature that decreases at site due to dilution by sea water coming from outside (the sea) made the environment more comfortable for living. Juliani and Suyatna (2014) reported stock potency of leiognathids in waters of East Kutai district but did not mention the density.

To conclude, in high water temperature, 53 species of various fish were found. Leiognathids were the most abundance and different based on the tidal range level (spring and neap tide), from seven species belonging to three genera identified, *Secutor* sp was the largest. Around the outlet, physical and chemical water properties were in tolerable limit except water temperature.

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Identification of soybean genotypes adaptive and productive to acid soil agro-ecosystem

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Abstract. *Adie MM, Krisnawati A. 2016. Identification of soybean genotypes adaptive and productive to acid soil agro-ecosystem. Biodiversitas 17: 565-570.* Optimalization of acidic land for soybean development can be performed through the provision of soybean variety adapted to low pH. A total of 13 soybean genotypes was identified for its performance on three acid soil sites in Lampung Province, Indonesia, from February to June 2015. Soybean variety adapted to acid soil (Tanggamus and Demas 1) were used as check varieties. The experiment was using Randomized Block Design, 15 traits and four replicates. The concentration of pH (H_2O) in locations L1, L2 and L3 were 5.87, 5.04, and 4.73, respectively. The average yield in L1, L2 and L3 were 1.96 t/ha, 2.17 t/ha, and 1.92 t/ha, respectively. This showed that yield decrease as soil pH value decline. Genotype G4AB was consistently produced highest yield at pH 5.04 as well as at pH 4.73, hence the genotype G4AB was not only adaptive at low pH but also relatively productive. Based on yield in three locations, G4AB categorized as less stable. On the contrary, genotype G115H/Kaba//Kaba///Kaba-8-6 produced average yield of 2.23 t/ha, and categorized as stable in three sites of acid soil. Soybean genotype adaptive to acid soil was characterized by its ability to maintain the plant height, and followed by a high number of node per plant and pod per plant.

Keywords: *Glycine max*, acid soil, pH, yield

INTRODUCTION

Soybean development in acid soils is very potential due to the world-wide availability of acidic land, including in Indonesia. The potential acidic upland in Indonesia reached 148 million hectares, which about 102.8 million ha of the land can be classified into acid soil and the rest of 45.2 million ha as non-acid upland area (Mulyani 2006). The soybean development in acid soil is limited by low pH (< 5.5), low cation exchange capacity (CEC), susceptibility to erosion, poor biotic elements, and high in aluminum (Al) content (Mulyani 2006; Utama 2008). High Al content can cause detrimental effects for soybean plants, such as toxicity and root damage which lead to drought susceptibility and nutritional unbalance (Spehar and Souza 2006). Optimizing the development of soybean in acid soils can be performed through two approaches, by providing soil conditioner to increase soil pH for optimal plant growth, or by using soybean varieties adaptive to low pH. The first approach affects increasing cost of soybean production, and in addition, the application of soil conditioner should be done continuously. Bromfield and Ayamaba (1980) showed that soybean in acid soil which treated without Rhizobium inoculation and liming resulted in fewer number of nodules, nitrogen deficiency and very low seed yield (0.3-0.4 t/ha). Provision of soybean varieties adaptable to dry acid soil conditions are considered more profitable than the use of soil conditioner (Akinrinde et al. 2004; Ezeh et al. 2007). This is possible because of the availability of these varieties do not require additional cost of farming, the effects of adaptive varieties are for long periods, and compatible with other components of soybean cultivation technology.

The strategy on the provision soybean adapted to dry acid soil is initiated by identification of gene source, characterization to obtain morphological characters as tolerance determinant to acid soil, and appropriate selection method. The major constrain of soybean plant in acid soil is Al toxicity which inhibit the cell elongation and division, shorten the root growth, and affected to absorption of water and nutrient (Zheng 2010). Various studies have identified that adaptability in acid soil was determined by plant ability to make morphological changes and root architecture, root symbiosis, activation of high-affinity phosphate (Pi) transporters, enhancement of internal phosphatase activity, and secretion of organic acids and phosphatases into the rhizosphere (Raghothama 1999; Vance et al. 2003; Gahoonia and Nielsen 2004). The soybean root system in acid soil is also important related to the use of phosphor as efficiently. Evaluation on the soybean tolerance to acid soil by Uguru et al. (2012) concluded that root length, root weight and the number of root nodules were as the adaptation characters of soybean in the acid soil; and by using all of these three characters have successfully mapped the level of soybean genotypes tolerance to low pH. Other research revealed that difficult to obtain equality in assessing the soybean tolerance in acid soil based on solution culture than the field screening (Horst and Klotz 1990). This shows that an improvement is still needed in the screening method using solution culture. In the segregated population derived from crossing, Spehar and Souza (2006) performed selection using hydroponic

solution in F2 population using characters of root growth, and able to obtain F3 population tolerant to low pH.

Foy et al. (1993) screened soybean germplasm and obtained the range of tolerance to pH 4.0 based on absolute dry shoot weights, relative shoot dry weights, and absolute root dry weights. The evaluation of soybean tolerance to acid soil in Indonesia (with pH H2O 4.3, exchangeable-Al 3.92 me/100 g, and Al saturation 56.48%) which performed by Kuswantoro and Zen (2013) was successfully obtained two soybean lines which produced higher yield than the resistant check variety (1.53 t/ha). A similar result also reported by Uguru et al. (2012) in acid soil of South eastern Nigeria (pH < 5.5), that acid-tolerant soybean was indicated by normal root growth and relatively high yield. However, the tolerance to acid soil is a complex multigenic trait, hence the identification method should be able to produce genotype with high degree of tolerance, productive, and have broad adaptation. The objective of the research was to identify and classify soybean genotypes with high yield and adaptive in acid soil.

MATERIALS AND METHODS

Field experiment

The research material consists of 13 soybean genotypes (11 AB, 13 ED, 14 DD, 19 BE, 25 EC, G4AB, G2BB, G3CB, G5EB, G1DB, G115H/Kaba//Kaba///Kaba-8-6, G511H/Anj//Anj-2-10, G511H/Anj-1-3) and two check varieties adapted to acid soil, i.e. Tanggamus and Demas 1. The field experiment was conducted in three locations of Lampung Province (Indonesia) in 2015, i.e. South Lampung, Pesawaran (dry season 1), and Pesawaran (dry season 2). The experimental design in each location was randomized completely block design with four replicates. The plot size was 2.4 × 4.5 m, 40 cm × 15 cm plant distances, two plants/hill. Fertilizer of 250 kg/ha Phonska and 100 kg/ha SP 36 were applied before sowing time. Seed treatment by the ametoxam. The land used was upland; therefore soil management was optimally performed. Before sowing, a drainage channels was made. Insect and disease were controlled intensively. Weed control was done at two and four weeks after planting. The parameter measured on days to maturity, days to flowering, 100 seed weight, plant height, number of branches, number of nodes, and number of pods per plant.

Characteristics of location

The level of soil acidity in three locations varied from medium acid (pH 5.87) in South Lampung, pH 5.04 (very strong acid) in Pesawaran at dry season 1, and in Pesawaran at dry season 2 categorized as extremely acid (pH 4.73) (Table 1). The availability of P2O5 varied from low to very high in Pesawaran and South Lampung, respectively. The Al-dd concentration only detected in Pesawaran at dry season 2, whereas the H concentration in the soil (H-dd) was from 0.54 up to 1.40. Based on those nutrient characteristics, therefore all three locations were feasible to detect and identify the soybean genotypes adapted to various soil pH.

Data analysis

Data were subjected to analysis of variance using a general linear model. The stability assessment in three environments following Francis and Kannerberg (1978), that is mapping between the coefficient of variation and seed yield from each genotype.

RESULTS AND DISCUSSION

Analysis of Variance

Analysis of variance for yield and yield component as shown in Table 2, location was significantly affect all observed characters, i.e. days to maturity, days to flowering, plant height, number of branches, number of nodes, number of filled pods, 100 seed weight, and seed yield. The effect of genotype was not significant on characters of plant height, number of branches, and number of nodes. The effect of genotype by location interaction was significant on characters of days to maturity, plant height, number of nodes, 100 seed weight, and seed yield (Table 2). The significant effect of genotype by location interaction for seed yield reflecting the availability of adaptation data for each genotype in certain location or specific pH.

Seed yield

The average seed yield of 15 soybean genotypes in South Lampung (pH 5.87) was 1.96 t/ha, in Pesawaran dry season 1 (pH 5.04) was 2.17 t/ha, and pH 4.73 in Pesawaran MK2 reached 1.92 t/ha (Table 3). The seed yield in South Lampung ranged from 1.71 to 2.28 t/ha. Seed yield of two check varieties adapted to acid soil of Tanggamus and Demas 1 were 2.01 and 1.90 t/ha, respectively. Tanggamus variety was released in 2001, whereas Demas 1 was released in 2014. The highest yield at pH 5.87 was genotype 13 ED, which reached 2.28 t/ha, followed by 25 EC, i.e. 2.18 t/ha.

Table 1. Soil analysis of three acid soil locations, in 2015

Code	Location	Actual pH H_2O	Potential pH KCl	P_2O_5 Bray I (ppm)	Concentration	
					Al-dd	H-dd
L1	Hajimena, Natar, South Lampung	5.87 (medium acid)	5.15	19.2 (very high)	0.00	0.54
L2	Masgar, Tegineneng, Pesawaran (DS1)	5.04 (very strong acid)	4.70	14.4 (high)	0.00	0.54
L3	Masgar, Tegineneng, Pesawaran (DS2)	4.73 (extremely acid)	4.20	6.47 (low)	0.43	1.40

Note: DS1 = dry season 1, DS2 = dry season 2.

Table 2. Combined analysis of variance for yield and yield component of 15 soybean genotypes in acid soil, in 2015

Trait	Mean Square				CV (%)
	Location (L)	Replication/R	Genotype (G)	L × G	
DTF	66.6000 **	1.6000 *	10.5095 **	0.4095 ns	2.74
DTM	551.6222 **	0.6685 ns	14.9698 **	8.3901 **	1.49
PHT	2700.8509 **	24.8138 ns	372.0337 **	136.9473 **	15.15
NOB	40.1349 **	2.2280 ns	2.4326 ns	1.2542 ns	39.51
NON	1776.1075 **	11.0981 ns	11.1537 ns	24.2740 *	23.58
NOP	17236.5261 **	228.2982 ns	213.0542 ns	244.0468 ns	36.38
W100	26.8170 **	2.7031 ns	28.5135 **	7.7216 **	13.15
T/H	1.0553 **	1.0167 **	0.4014 **	0.6633 **	17.08

DTF = days to flowering (days), DTM = days to maturity (days), PHT = plant height (cm), NOB = number of branches/plant, NON = number of nodes/plant, NOP = number of filled pod/plant, W100 = 100 seed weight (g). T/H = seed yield (t/ha), CV = coefficient of variation, * = significant at p = 0.05; ** = significant at p = 0.01, ns = not significant.

Table 3. Seed yield of 15 soybean genotypes in acid soil. Lampung, in 2015

Genotype	Yield (t/h)			
	L1	L2	L3	Mean
11 AB	2.05	1.84	2.15	2.01
13 ED	2.28	1.52	2.04	1.95
14 DD	1.83	2.41	2.06	2.10
19 BE	2.05	1.60	2.04	1.90
25 EC	2.18	1.00	1.81	1.66
G4AB	1.97	2.97	2.14	2.36
G2BB	1.93	2.58	1.97	2.16
G3CB	1.76	1.35	2.09	1.74
G5EB	1.71	2.75	2.04	2.16
G1DB	1.94	1.99	1.80	1.91
G115H/Kaba//Kaba///Kaba-8-6	1.91	2.81	1.97	2.23
G511H/Anj//Anj-2-10	2.04	2.53	1.66	2.08
G511H/Anj-1-3	1.85	2.09	1.80	1.91
Tanggamus	2.01	2.80	1.35	2.05
Demas 1	1.90	2.29	1.93	2.04
Mean	**1.96**	**2.17**	**1.92**	**2.02**

Note: L1 = South Lampung, L2 = Pesawaran DS1, L3 = Pesawaran DS2

The seed yield in Pesawaran during dry season 1 at pH 5.04 ranged from 1.35 to 2.97 t/ha. The highest yield at pH 5.04 was G4AB (2.97 t/ha), followed by G115H/Kaba//Kaba///Kaba-8-6 (2.81 t/h). Within two locations, Tanggamus variety produced high yield than Demas 1. The seed yield in Pesawaran at dry season 2 ranged from 1.35 to 2.15 t/ha. The highest yield genotype was 11AB (2.15 t/ha), followed by G4AB (2.14 t/ha). Changing of seed yield superiority within three locations at different pH is a consequence of the interaction between genotype with the environment as stated in Table 2. This means that each genotype has a different adaptation to different environments.

The average seed yield in three locations was 2.02 t/ha, with a range of 1.66-2.36 t/ha. Seed yield of Tanggamus and Demas 1 were 2.05 and 2.04 t/ha, respectively. A total of six genotypes produced yield higher than the best check variety (Tanggamus), and seed yield range of those six genotypes was 2.08-2.36 t/ha. If the selection was based on seed yield increase of 10% higher than the check variety Tanggamus (2.05 t/ha), then it will be obtained only one genotype, i.e. G4AB (2.36 t/ha). A relationship between soil pH, average yield per location, and highest yield of genotype from each location was presented in Figure 1. All the tested soybean genotypes showed adaptability at pH greater than 5, but began to show decreasing yield at pH less than 5. Under a low pH conditions, the aluminium, P fixation, iron, and manganese concentration increases to the toxic level (Keyser and Munns 1979). Furthermore, increase in soil acidity can reduce root growth, reduce nutrient availability and thus, would result in poor crop performance (Ezeh et al. 2007; Duressa et al. 2011). The result agrees with the report of Uguru et al. (2012) that soil pH had strong impact on the soybean root growth, agronomic performance, and yield traits.

The best genotype at pH 5.87 have seed yield differences of 0.32 t/ha with the average yield. Yield differences was showed at pH 5.04, i.e. 0.80 t/ha, and at pH 4.73 was 0.23 t/ha, respectively. Foy et al (1992) conducted a screening of soybean tolerance in the field without liming, and successfully obtained PI248511 (Japan), Perry (USA), PI381674 (Uganda), Amcor (Ohio USA) and Hernon 147 (Zimbabwe, Africa). In this research, genotype G4AB was considered as adaptive to acid soil and as well as productive to be developed in acid soil, followed by genotype G115H/Kaba//Kaba///Kaba-8-6.

Yield stability

Yield stability intended to assess the performance of a genotype which has smallest yield difference between one locations to another. Francis and Kannerberg (1978) combines between the coefficients of variation with yield to map a genotype into four quadrants. Quadrant I is characterized by genotypes which has a relatively stable and high yield at three locations. Quadrant II showed genotypes with high yield but unstable. Quadrants III and IV characterized by seed yield below average, but the genotypes in quadrant IV were considered more stable than the genotypes that were in quadrant III (Figure 2).

Based on those combination, five genotypes were stable and produced high yield, three genotypes produced high yield but less stable. The highest yielding genotype (G4AB) was less stable. On the contrary, G115H/Kaba//Kaba///Kaba-8-6 as a stable genotype in three locations of acid soil.

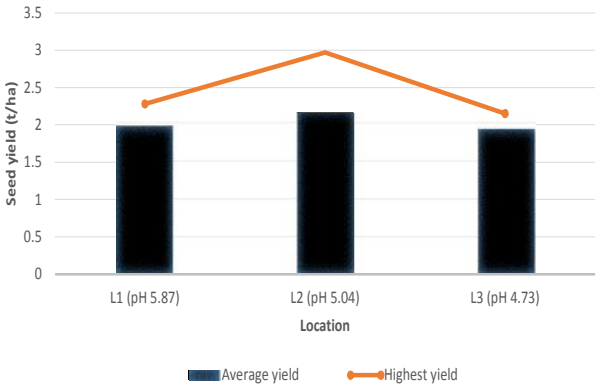

Figure 1. Average yield and the best genotype in three acid soil locations

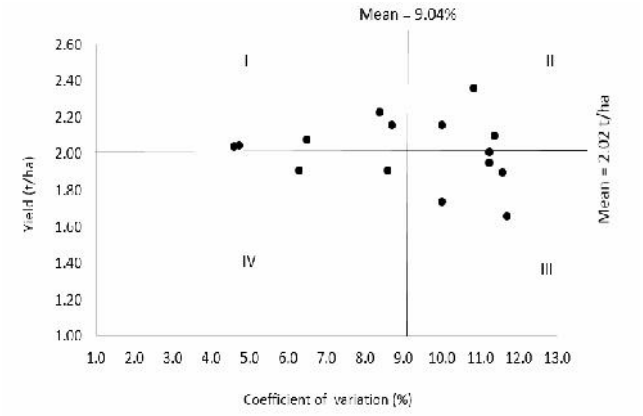

Figure 2. Yield stability of 15 soybean genotypes in three acid soil locations

Yield component

The yield components which consists of days to flowering, days to maturity, plant height, number of branches, number of nodes, number of filled pods, number of empty pods, and 100 seed weight of 15 soybean genotypes in three acid soil were presented in Table 4, Table 5, Table 6, and Table 7; respectively.

Character of days to flowering was more influenced by the genetic factor of each genotype, but for the days to maturity have tendency more influenced by pH. It means that the lower soil pH will tend to extend the plant age. Soybean varieties adapted to acid soil which have been released in Indonesia, have days to maturity over 85 days (Iletri 2012). The current farmers' preferences are soybean with early maturing day (<80 days) and large seed size (>14 g/100 seed). In this study, soybean with early maturing day was not obtained. High yielding soybean in acid soil have 81 days to maturity.

Plant height is often to be used as tolerance indicator of soybean genotype to low pH. Decreasing in soil pH tends to increase plant height. Soybean genotype which has identified producing high yield (G4AB) shows a relatively higher plant height than other genotypes, and followed by high number of branches and number of pods per plant. According to Samac and Tesfaye (2003), aluminium tolerance is a complex multigenic trait, therefore the selection method and selection indicator become something important. In this study, the observed morphological characters were those parts of the plant above ground. Wang et al. (2010) stated that the root system plays an important role in the efficiency of phosphorus in soybean, so it requires root breeding program.

Another interesting point, the best two soybean genotypes (G4AB and G115H/Kaba//Kaba///Kaba-8-6) have high number of empty pods and relatively small seed size, respectively. It seems that those characters have less effect on yield.

Table 4. Days to flowering and days to maturity of 15 soybean genotypes in acid soil, in 2015

Genotype	Days to flowering (days)				Days to maturity (days)			
	L1	L2	L3	Mean	L1	L2	L3	Mean
11 AB	32	31	31	31	78	82	78	79
13 ED	33	32	31	32	78	83	81	81
14 DD	33	31	30	31	79	82	82	81
19 BE	33	32	31	32	79	82	82	81
25 EC	33	32	31	32	79	83	82	81
G4AB	33	32	31	32	79	83	82	81
G2BB	34	33	32	33	78	83	84	82
G3CB	35	34	34	34	80	84	85	83
G5EB	35	34	33	34	79	84	87	83
G1DB	34	33	32	33	79	83	85	82
G115H/Kaba//Kaba///Kaba-8-6	35	33	31	33	79	84	85	83
G511H/Anj//Anj-2-10	34	33	32	33	79	84	85	83
G511H/Anj-1-3	35	33	32	33	78	84	83	81
Tanggamus	34	33	32	33	78	83	88	83
Demas 1	35	34	33	34	78	83	88	83
Mean	**34**	**33**	**32**	**33**	**78**	**81**	**84**	**81**

Note: L1 = South Lampung, L2 = Pesawaran dry season 1, L3 = Pesawaran dry season 2

Table 5. Plant height and branches number of 15 soybean genotypes in acid soil, in 2015

Genotype	Plant height (cm)				Number of branches/plant			
	L1	L2	L3	Mean	L1	L2	L3	Mean
11 AB	49.00	75.25	57.85	60.70	2.25	3.25	2.30	2.60
13 ED	50.50	51.90	52.20	51.53	2.75	3.45	1.45	2.55
14 DD	37.00	55.95	47.00	46.65	2.75	3.90	2.90	3.18
19 BE	43.25	59.55	56.25	53.02	2.25	2.50	2.20	2.32
25 EC	50.00	38.05	41.60	43.22	1.75	3.65	3.75	3.05
G4AB	45.50	64.45	62.95	57.63	2.75	4.35	3.00	3.37
G2BB	45.75	55.75	44.65	48.72	2.50	3.25	3.40	3.05
G3CB	41.75	46.10	48.55	45.47	2.50	4.10	3.10	3.23
G5EB	41.75	57.60	55.05	51.47	2.00	5.75	3.60	3.78
G1DB	42.50	55.35	50.40	49.42	2.50	4.90	2.75	3.38
G115H/Kaba//Kaba///Kaba-8-6	40.50	69.40	66.50	58.80	2.00	3.95	2.45	2.80
G511H/Anj//Anj-2-10	41.75	49.65	49.15	46.85	2.75	4.00	3.10	3.28
G511H/Anj-1-3	37.50	47.05	44.00	42.85	1.50	3.30	1.85	2.22
Tanggamus	36.75	54.80	42.50	44.68	2.50	3.55	2.70	2.92
Demas 1	40.50	62.15	51.00	51.22	2.50	5.05	3.05	3.53
Mean	**42.93**	**49.35**	**51.31**	**47.87**	**2.35**	**3.11**	**2.77**	**2.75**

Note: L1 = South Lampung, L2 = Pesawaran dry season 1, L3 = Pesawaran dry season 2

Table 6. Number of node and 100 seed weight of 15 soybean genotypes in acid soil, in 2015

Genotype	Number of node/plant				100 seed weight (g)			
	L1	L2	L3	Mean	L1	L2	L3	Mean
11 AB	11.25	23.80	12.00	15.68	15.24	15.61	17.63	16.16
13 ED	14.50	21.50	10.45	15.48	15.85	15.86	17.43	16.38
14 DD	11.00	20.15	17.65	16.27	15.85	13.68	15.09	14.88
19 BE	10.75	23.25	14.20	16.07	14.77	15.63	13.12	14.50
25 EC	9.50	17.45	17.30	14.75	15.98	12.54	17.90	15.47
G4AB	10.50	21.20	19.55	17.08	13.05	11.40	16.29	13.58
G2BB	11.50	21.25	13.05	15.27	13.18	15.41	13.96	14.18
G3CB	10.00	22.70	17.95	16.88	12.93	9.84	11.34	11.37
G5EB	9.50	27.75	15.50	17.58	13.38	10.21	12.95	12.18
G1DB	13.50	19.30	14.05	15.62	12.82	12.68	13.44	12.98
G115H/Kaba//Kaba///Kaba-8-6	8.50	22.55	16.55	15.87	13.63	12.51	13.93	13.35
G511H/Anj//Anj-2-10	11.75	19.65	16.25	15.88	12.83	12.64	12.92	12.80
G511H/Anj-1-3	9.00	19.40	12.60	13.67	13.85	15.94	17.48	15.75
Tanggamus	10.50	22.95	13.80	15.75	12.74	12.96	11.53	12.41
Demas 1	11.25	22.20	16.60	16.68	15.05	11.28	12.91	13.08
Mean	**10.87**	**16.10**	**15.17**	**14.04**	**14.08**	**13.66**	**14.53**	**14.09**

Note: L1 = South Lampung, L2 = Pesawaran dry season 1, L3 = Pesawaran dry season 2

Table 7. Number of filled and empty pods of 15 soybean genotypes in acid soil, in 2015

Genotype	Number of filled pod/plant				Number of empty pod/plant			
	L1	L2	L3	Mean	L1	L2	L3	Mean
11 AB	34.50	69.60	24.50	42.87	5.50	9.65	2.25	5.80
13 ED	35.00	45.50	15.30	31.93	6.75	20.85	2.00	9.87
14 DD	28.25	59.90	26.55	38.23	5.00	10.20	5.30	6.83
19 BE	26.50	58.50	28.75	37.92	5.25	6.30	2.95	4.83
25 EC	27.75	45.30	21.40	31.48	5.00	10.15	8.70	7.95
G4AB	28.50	48.45	38.85	38.60	6.50	14.55	2.85	7.97
G2BB	29.00	40.30	20.05	29.78	5.50	10.80	2.10	6.13
G3CB	18.25	60.40	23.55	34.07	6.00	9.60	4.35	6.65
G5EB	24.25	73.15	29.20	42.20	5.50	22.30	2.90	10.23
G1DB	22.75	59.95	23.10	35.27	5.25	9.40	1.25	5.30
G115H/Kaba//Kaba///Kaba-8-6	20.75	46.15	34.40	33.77	4.75	17.25	1.90	7.97
G511H/Anj//Anj-2-10	32.75	45.10	18.60	32.15	4.00	11.20	0.75	5.32
G511H/Anj-1-3	22.25	46.65	17.95	28.95	5.75	8.75	0.45	4.98
Tanggamus	26.75	65.40	13.65	35.27	5.75	12.35	1.45	6.52
Demas 1	20.25	53.30	23.70	32.42	7.50	10.30	3.15	6.98
Mean	**26.50**	**40.05**	**23.97**	**30.17**	**5.60**	**8.81**	**2.82**	**5.75**

Note: L1 = South Lampung, L2 = Pesawaran dry season 1, L3 = Pesawaran dry season 2

From this study, it can be concluded that soybeans are still able to produce optimally on soil acidity to a pH of 5.0. At pH below 5.0, the soybean productivity has declined and suggested to use soybean genotypes adapted to these conditions. Plant height is one of the morphological indicators to identify soybean genotype adaptive to acid soil. Furthermore, genotype G4AB was adaptive and productive to acid soil to a pH of 4.7, and therefore recommended to be developed as high-yielding variety for acid soil.

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Molecular identification of commercially important species of *Nemipterus* (Perciformes: Nemipteridae) in surrounding seas of Malaysia

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Abstract. *Imtiaz A, Duong TY, Nor SAM, Naim DM. 2016. Molecular identification of commercially important species of Nemipterus (Perciformes: Nemipteridae) in surrounding seas of Malaysia. Biodiversitas 17: 571-577.* The genus *Nemipterus* is a group of coral fishes which are morphologically diversified in coloration and distribution pattern. In this study, the mitochondrial Cytochrome c oxidase-I (COI) gene was analyzed for genetic identification of 127 samples of genus *Nemipterus* from Malaysia waters. Sequence analysis of COI based data clearly distributed ten putative species into four distinct clusters clades. Intra-specific genetic distance values of 2.7%, 3.4% were observed in *N. japonicus* and *N. nemurus* which require more detailed analysis of the taxonomic status of some of the individuals attributing to slightly atypical values. Neighbor joining (NJ) tree shows a low genetic structuring in *N. japonicus*. Populations from Indian Ocean and South China Sea are isolated from each other but both share genetic structure with the population from the Straits of Malacca, suggesting the possibility of the latter acting as a barrier to the movement of this species between the two neighboring seas.

Keywords: Coral fishes, genetic structure, intra-specific, Cytochrome c oxidase-I, Malaysia, *Nemipterus*, South China Sea

INTRODUCTION

Integrated land of Malaysia is a combination of the southeastern tip of the Asian mainland (Peninsular Malaysia) and the states of Sabah and Sarawak in the western part of Borneo Island. Peninsular Malaysia faces the Strait of Malacca (extension of the Indian Ocean) towards the west and the South China Sea towards the east. Sarawak faces the South China Sea while Sabah faces the South China Sea, Sulu Sea and Celebes Sea. Malaysia has the potential to be one of the leading fishing nations in the Southeast Asian region, and is poised to step into a new era of development in the marine fishing industry. However, the ill-effects from past management programs have created many problems and conflicts for Malaysia in managing her own resources to achieve optimum utilization. One of the main issues is conservation planning. In the early stages of development, the country failed to take conservation measures into serious consideration, which resulted in inshore waters being over-exploited.

One of the impacted fish genus is *Nemipterus* from the family Nemipteridae. They - are fished throughout the year and are very popular with Malaysian consumers. According to Abu Talib (2003) and Mohd Taupek (1996), decline of *Nemipterus* has been occurring for some time as observed through trend landing analysis. Family Nemipteridae worldwide consists of five genera comprising 67 species. Within genus *Nemipterus* 25 species are present worldwide with 19 species documented in Malaysia (Edward 1992; Russell 1990). Members under this genus are well known for their delicious taste and commercial importance. They are commonly called threadfin breams and can be identified by their pinkish body coloration with variable yellow streaks on the whole abdominal length and fins. Threadfin breams are demersal fish and are residents of the Indo West Pacific biogeographical region (Russell 1990). They feed mainly on crustaceans. They are small to medium sized fish and can be easily caught through trawling. Although the taxonomy of the genus has been largely defined and resolved on the basis of morphological characters (Russell 1986, 1990, 1993), a number of taxonomic issues still remain. By relying solely on external morphology, subtle differences may not be detected and could lead to misidentification, for example *N. randalli* was misidentified as *N. japonicus* (Lelli 2008).

Misidentification is a common occurrence at fish trading places such as the market and landing sites. Precise identification of fish is important for the satisfaction, value for money and well-being, in the case of allergies, to the consumers. Often a species may be known by alternative vernacular names and synonyms which could lead to much confusion. Kannuchamy (2015) detected mislabeling of 22% of frozen seafood prevailing in Indian markets. Changizi (2013) revealed incorrect labelling of the Narrow-barred Spanish mackerel samples in Iranian fish products. Data on the genetic identity of populations/species are

essential when designing programs for the conservation and management of fish. DNA barcoding is a molecular technique that is now widely accepted as a tool for taxonomic identification of various organisms including fish species. It involves the amplification of a specific segment of the mitochondrial DNA which is sufficiently variable to distinguish between species but conserved within species. In fish, the DNA barcoding region is a 650 base pair fragment of the mitochondrial cytochrome oxidase I (COI) gene and has been widely utilized to authenticate for species identification, particularly in the case of cryptic species and ambiguous morphological characteristics (Hubert et al. 2008, Aquilino et al. 2011, Sanciangco et al. 2011). The COI gene group is considered to be efficient for species level taxonomic identification because it consists of only protein encoding genes (Brown 1979) and have slow evolutionary rate (Saccone et al. 1999) which can help in elucidating evolutionary divergences (Lynch and Jarrell 1993). DNA barcoding has proven to be very successful for systematic investigation of a wide variety of marine fish taxa (Ward et al. 2005, Lakra et al. 2010, Wang et al. 2012). In South East Asia DNA barcoding has not been applied widely although there are few reports on marine organisms i.e. Suzanna et al. (2011) used DNA barcoding to discriminate oysters species. Jaafar et al. (2012) conducted a comprehensive DNA barcoding study on family Carangidae.

Thus, the current research aims to genetically identify the species of genus *Nemipterus* in the surrounding seas of Malaysia. To the fisheries managers, our study contributes invaluable complementary data for biodiversity assessment and recognition of cryptic species for sustainable fisheries of this genus.

MATERIALS AND METHODS

Sample collection

A total of 127 samples of genus *Nemipterus* were obtained from Malaysian waters consisting of Straits of Malacca, South China Sea, Sulu Sea and Celebes Sea (Figure 1). All samples were morphologically identified into nine species (*N. japonicus, N. hexodon, N. tambuloides, N. peronii, N. thosaporni, N. nematophorus, N. bipunctatus, N. marginatus,* and *N. furcosus*). In addition, we included fifteen specimens belonged to four species (*N. hexodon, N. japonicus, N. furcosus, N. tambuloides*) from Vietnam and seven specimens of one species (*N. japonicus*) from Pakistan as regional conspecific comparison with our samples (Table 1) and a specimen identified as *Scolopsis vosmeri* as an out-group. All samples were photographed and have been kept as voucher specimens at Zoological Museum in Biodiversity Centre, Universiti Sains Malaysia after preservation in 95% alcohol. The morphological identification was based on FAO catalog of genus *Nemipterus* (Russell 1990).

DNA Isolation

Total genomic DNA was extracted by salt extraction (Animal Genomics Laboratory, Liverpool University, United Kingdom, (2001) in the presence of proteinase K (Nacalai Tesque, Japan) with some modification on the amount of TNES-Urea used to improve the yield and quality of the extracted DNA. The DNA pellet obtained was then eluted with deionized water. Quality and quantity of isolated DNA were measured using spectrophotometer (Quawell, Korea) and stored at-20°C until further use.

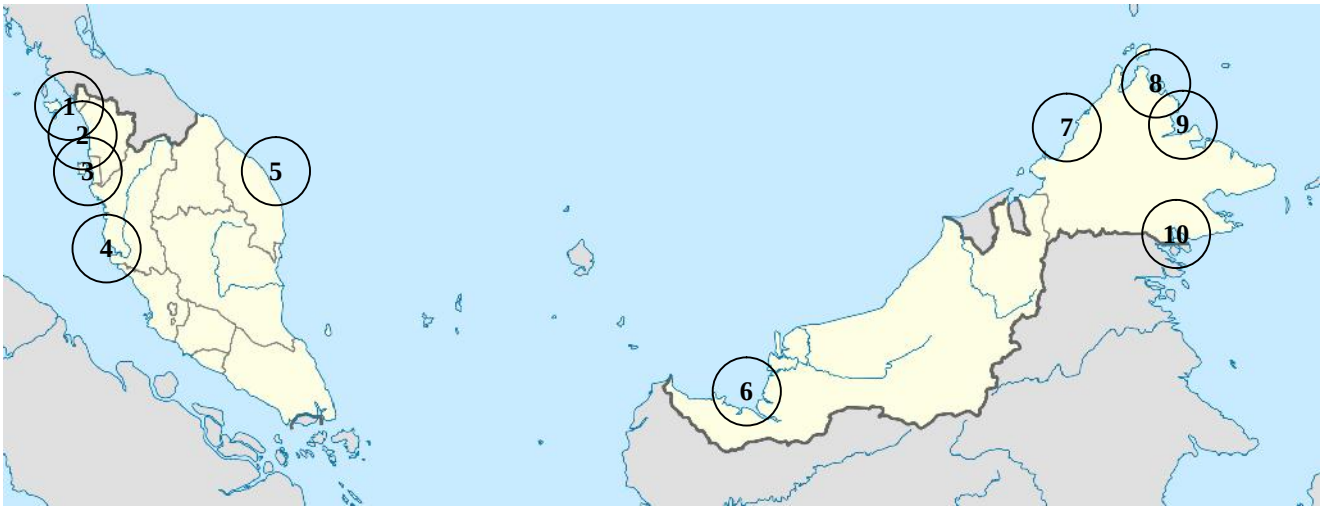

Figure 1. Sampling locations of species collected from Malaysian waters (Strait of Malacca, South China Sea and Sulu Sea, Celebes Sea). Note: 1. Kuala Kedah, Kedah (KK), 2. Kuala Perlis, Perlis (KP), 3. Batu Lanchang, Pemang (BL), 4. Lumut, Perak (ML), 5. Kuala Terengganu,Terengganu (TBK), 6. Kuching, Sabah (KCH), 7. Kota Kinabalu, Sabah (KTK), 8. Kudat, Sabah (KD), 9. Tawau, Sabah (TW), 10. Sandakan, Sabah (SDK), 11. Vietnam (NRC,VCM,VBL), 12.Pakistan (Pak)

Table 1. Total number of *Nemipterus* species and their respective locations

Species name	Locations of sampling												Total no. of Samples
	1	2	3	4	5	6	7	8	9	10	11	12	
N. hexodon	2	2	0	0	0	0	1	0	1	1	2	0	9
N. japonicas	6	6	6	8	0	0	1	1	4	5	5	7	47
N. tambuloides	0	0	0	0	0	0	0	2	0	0	2	0	4
N. bipunctatus	0	3	0	9	0	0	0	0	0	0	0	0	12
N. furcosus	0	0	0	9	0	0	0	0	6	4	6	0	25
N. nematophorus	0	1	0	0	0	0	1	0	0	0	0	0	2
N. marginatus	0	2	0	0	3	0	2	1	1	1	0	0	10
N. thosaporni	0	0	0	0	0	0	0	1	5	5	0	0	11
N. peronii	0	0	0	0	0	0	1	0	2	1	0	0	4
N. nemurus	0	0	0	0	0	1	1	1	0	0	0	0	3

Note: 1. Kuala Kedah, Kedah (KK), 2. Kuala Perlis, Perlis (KP), 3. Batu Lanchang, Pemang (BL), 4. Lumut, Perak (ML), 5. Kuala Terengganu, Terengganu (TBK), 6. Kuching, Sabah (KCH), 7. Kota Kinabalu, Sabah (KTK), 8. Kudat, Sabah (KD), 9. Tawau, Sabah (TW), 10. Sandakan, Sabah (SDK), 11. Vietnam (NRC,VCM,VBL), 12.Pakistan (Pak)

Table 2. Sequences Accessed from Bold and Genbank with species names and areas of Collection sites

BOLD/Genbank Accession numbers	Species name	Collection site
ANGEN128-15	*N. japonicus*	Indian Ocean
ANGEN16115	*N. japonicus*	Indian Ocean
LGEN09314	*N. japonicus*	Indian Ocean
EF609556	*N. japonicus*	Indian Ocean
EF609553	*N. japonicus*	Indian Ocean
FJ347947	*N. japonicus*	Indian Ocean
JQ691509	*N. japonicus*	South China Sea
EU871686	*N. japonicus*	South China Sea
JF493971	*N. japonicus*	South China Sea
EU871687	*N. japonicus*	South China Sea
HQ676778	*N. marginatus*	Indian Ocean
JQ681506	*N. marginatus*	South China Sea
HQ423413	*N. bipunctatus*	Indian Ocean
JQ350137	*N. bipunctatus*	Indian Ocean
EF609414	*N. hexodon*	Pacific Ocean*
FJ237848	*N. virgatus*	Unknown
JN992286	*N. nematophorus*	Indian Ocean
JQ681467	*N. bathybius*	South China Sea
JN992287	*N. zysron*	unknown
EF609557	*N. mesoprion*	Indian Ocean
JX866609	*N. mesoprion*	Indian Ocean
EF609561	*N. mesoprion*	Indian Ocean
EF609415	*N. peronii*	Pacific Ocean*
EF609413	*N. furcosus*	Pacific Ocean*
JQ681525	*N. furcosus*	South China Sea
JN992288	*N. japonicus*	Indian Ocean

Note: *Pacific Ocean = Near Australia

Amplification and sequencing

The COI gene was amplified in a 50 µL volume solution with 5 µL of 10X PCR buffer, 3.5 µL of 50mM MgCl2, 2 µL of 0.05mM dNTP, 1 µL of 0.01mM each primer, 0.5 units of *iTaq* plus DNA polymerase and 50-100ng of genomic DNA template. The primers used for the amplification of the COI gene were FishF1-5'TCAACCAACCACAAAGACATTGGCAC3' and FishR1 5'TAGACTTCTGGGTGGCCAAAGAATCA3' (Ward et al. 2005). The thermal regime consisted of an initial step of 2 min at 95°C followed by 35 cycles of 45 s at 94°C, 45 s at 50°C and 1 min at 72°C and a final extension of 10 min at 72°C. The PCR products were visualized on 2 % agarose gels, and only intense and sharp bands were selected for purification as recommended in Intron Purification Kit (Intron, South Korea). The cleaned PCR products were sequenced by a service provider, 1st BASE Sequencing Service Sdn. Bhd.

Data analysis

The obtained sequences (Tamura et al. 2007) were edited by combining the forward and reverse sequences and ambiguous sites were deleted. Multiple alignments were then performed and Kimura 2-Parameter was selected to infer genetic variability as estimated by nucleotide diversity, haplotype diversity and pairwise genetic distance among haplotypes. Neighbors-joining (NJ) tree was then constructed using 10, 000 bootstrap replications. Twenty-six sequences (ANGEN128-15, ANGEN16115, LGEN09314, EF609556, EF609553, FJ347947, JQ691509, EU871686, JF493971, EU871687, HQ676778, JQ681506, HQ423413, JQ350137, EF609414, FJ237848, JN992286, JQ681467, JN992287, EF609557, JX866609, EF609561, EF609415, EF609413, JQ681525, and JN992288) were retrieved from GenBank and BOLD systems for comparison (Table 2). All analyses were conducted in MEGA 6.06 program (Tamura et al. 2013).

RESULTS AND DISCUSSION

Sample collection

A total of 127 samples of genus *Nemipterus* were obtained from Malaysian waters consisting of Straits of Malacca, South China Sea, Sulu Sea and Celebes Sea. All samples were morphologically identified into nine species (*N. japonicus*, *N. hexodon*, *N. tambuloides*, *N. peronii*, *N. thosaporni*, *N. nematophorus*, *N. bipunctatus*, *N. marginatus*, and *N. furcosus*). In addition, we included fifteen specimens belonged to four species (*N. hexodon*, *N. japonicus*, *N. furcosus*, *N. tambuloides*) from Vietnam and seven specimens of one species (*N. japonicus*) from Pakistan as regional conspecific comparison with our

samples and a specimen identified as *Scolopsis vosmeri* as an out-group. All samples were photographed and have been kept as voucher specimens at Zoological Museum in Biodiversity Centre, Universiti Sains Malaysia after preservation in 95% alcohol.

COI divergence analysis

A total of 153 sequences (127 sequences from current study and 26 sequences retrieved from NCBI and BOLD) of various species from genus *Nemipterus* were analyzed in this study. All specimens were successfully amplified and cross referenced to GenBank and BOLD systems. Most sequences showed > 98% identity to the species sequences from both databases as had been morphologically determined. However, three samples which had been morphologically been classified as *N. japonicus* were genetically identified as *N. nemurus* making up a total of 10 species compared to only 9 species from the initial morphological identification. Overall, a consensus length of 639 base pairs was used for analysis. The investigated sequences clustered into several haplotypes for each putative species generating a combined 52 unique haplotypes (Table 3). We found 294 variable positions with 255 parsimonious sites (39.9%) for further use in constructing phylogenetic tree. No insertions/deletions, stop codon or heterozygous sites were detected. Hence, all of the amplified sequences represent functional mitochondrial COI sequences.

Table 3. Number of samples, haplotypes and haplotype ID of *Nemipterus* spp. analysed in the study

	N. hexodon	*N. japonicus*	*N. tambuloides*	*N. bipunctatus*	*N. furcosus*	*N. nematophorus*	*N. marginatus*	*N. thosaporni*	*N. peronii*	*N. nemurus*
No. of samples	9	47	4	12	25	2	10	11	4	3
No. of haplotypes	4	22	2	4	5	2	3	5	2	3
Haplotype ID	H21, H22, H23 H24,	H28,H29, H30,H31 H32,H33, H34,H35 H36,H37, H38,H39 H40,H41, H42,H43 H44,H45, H46,H47 H48,H52	H1 H2	H3, H4 H5, H6	H16, H17 H18, H19, H20	H7,H8	H25,H26, H27	H11, H12 H13, H14 H15	H9, H10,	H49,H50, H51

Table 4. Inter-specific and intra-specific genetic distances of *Nemipterus* spp.

Species name	*N. japonicas*	*N. nemurus*	*N. furcosus*	*N. tambuloides*	*N. marginatus*	*N. thosaporni*	*N. hexodon*	*N. bipunctatus*	*N. nematophorus*	*N. peronii*	*N. zysron*	*N. mesoprion*	*N. virgatus*	*N. bathybius*	*S. vosmeri* (outgroup)
N. japonicus	**0.027***														
N. nemurus	0.175	**0.034***													
N. furcosus	0.167	0.183	**0.005**												
N. tambuloides	0.169	0.218	0.171	**0.001**											
N. marginatus	0.136	0.212	0.184	0.209	**0.015**										
N. thosaporni	0.188	0.192	0.187	0.155	0.193	**0.018**									
N. hexodon	0.195	0.208	0.169	0.143	0.213	0.137	**0.018**								
N. bipunctatus	0.200	0.216	0.162	0.138	0.205	0.149	0.131	**0.002**							
N. nematophorus	0.179	0.218	0.170	0.141	0.206	0.120	0.144	0.142	**0.023***						
N. peronii	0.164	0.161	0.100	0.184	0.187	0.206	0.216	0.178	0.199	**0.024***					
N. zysron	0.186	0.211	0.171	0.148	0.198	0.116	0.120	0.150	0.110	0.187	**0.000**				
N. mesoprion	0.183	0.199	0.173	0.145	0.195	0.105	0.108	0.144	0.110	0.183	0.013**	**0.003**			
N. virgatus	0.178	0.200	0.150	0.126	0.197	0.138	0.131	0.131	0.111	0.196	0.122	0.121	**0.000**		
N. bathybius	0.172	0.225	0.183	0.133	0.192	0.112	0.117	0.144	0.105	0.198	0.082	0.076	0.105	**0.000**	
S. vosmeri (outgroup)	0.244	0.230	0.237	0.241	0.245	0.245	0.236	0.236	0.265	0.232	0.245	0.233	0.262	0.248	**0.000**

Note: Value in bold represents intra-specific genetic distance values, * indicates high intra-specific distance value, ** minimum inter-specific genetic distance obtained

Genetic distances

Table 4 summarizes the genetic distances within and between genera and species. Intra-specific distances ranged from 0.0-3.4% while inter-specific distances ranged between 1.3-21.8%. Intra-specific distances in *N. japonicus*, *N. nemurus*, *N. nematophorus* and *N. peronii* were > 2% (2.7%, 3.4%, 2.3% and 2.4% respectively). Low interspecific genetic distance of 1.3% was obtained between *N. mesoprion* and *N. zysron* obtained from GenBank (EF609557, EF609581, JX866609, and JN992287).

Phylogenetic analysis

We used the 52 newly generated unique haplotypes and 26 additional sequences from GenBank and BOLD which comprised of 14 species of threadfin breams to construct a Neighbor-Joining tree. All samples generated from this study were clustered into their presumed species (Fig 2) according to BOLD data system and generally GenBank, including *N. nemurus*, *N. nematophorus* and *N. peronii* which have very small sizes. All haplotypes clustered with their respective species. However, a few sequences from GenBank require further taxonomic clarification. For instance, the presumed *N. japonicus* specimen, JN992288 had a low genetic distance with *N. nemurus* was more closely related to the basal taxon, *N. nemurus*. Furthermore, GenBank sequences, *N. zysron* and *N. mesoprion* with a genetic distance of only 1.3% clustered together.

We found two major clusters clades dividing *N. japonicus*. Cluster 1 Clade 1 was comprised of samples from the Arabian Sea (Indian Ocean) and Straits of Malacca. Cluster 2 Clade 2 clustered all South China Sea, Sulu Sea and Celebes Sea individuals. The intra-specific distance between the two clusters clades was 2.7%. Despite the smaller sample sizes, a similar trend was observed in *N. nematophorus* and *N. bipunctatus*, *N. hexodon*, *N. furcosus*, *N. marginatus* where samples from the Indian Ocean and the three seas (South China Sea, Sulu Sea and Celebes Sea) grouped into two distinct clusters, both of which are interspersed with specimens from the Straits of Malacca. For other species, samples were clustered in mixed localities. However, sample sizes were low compared to *N. japonicus*.

Discussion

Initially we identified 127 samples of *Nemipterus* into nine species through morphological key (Russell 1990). However, using DNA barcoding approach, three putative *N. japonicus* were re-classified and identified as *N. nemurus* (KTK6, KCH5 and KD05). Such misidentification is not an uncommon occurrence for morphologically similar species. Often diagnostic characters of specimens are lost due to preservation method or handling during capture. We did not observe the golden yellow stripes which extend from posterior nostril through eye and from upper lip to lower eye, characteristic of *N. nemurus*. Instead, a pale golden-yellow stripe along the body from behind head to base of caudal fin which is typical of *N. japonicus* was observed during the morphological inspection. We suspect found that

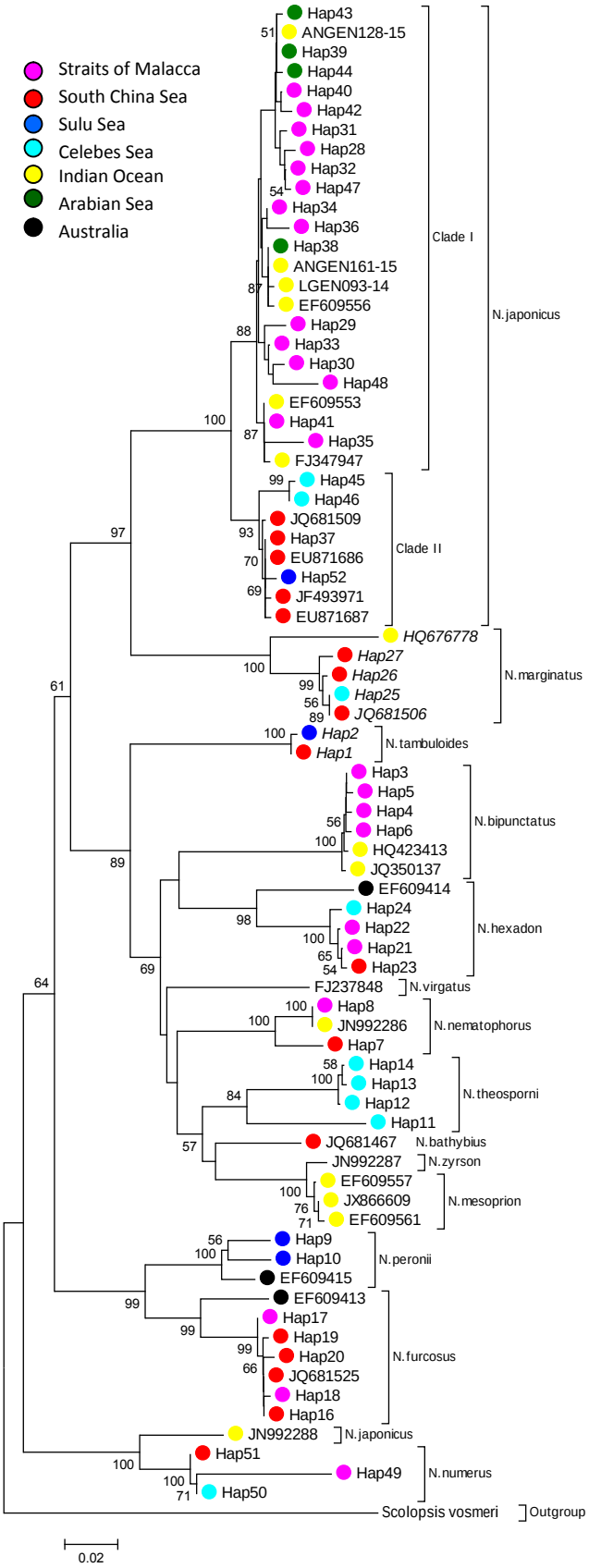

Figure 2. Neighbor-joining tree of species of *Nemipterus* using the cytochrome oxidase c subunit I

this coloration is unstable as specimens may have been out of the waters for a longer period. Alternatively, hybridization between the two species could have occurred, reflecting the maternal origin of the three specimens. This has proven the utility of the DNA barcoding method in identifying *Nemipterus* species when diagnostic morphological characters are not perfect or complete. Similarly, Becker et al. (2011) also reported that this approach can be used for fish identification for various stages of the life cycle and forms of seafood product whole fish, fillets, fins, fragments, juveniles, larvae, eggs, or any properly preserved tissue available. Such advantages can aid in fisheries management and conservation (Moura et al. 2008) as well as to prevent seafood fraud.

Several sequences from GenBank require further taxonomic validation. This include a voucher specimen of *N. japonicus* (ID JP992288) that clustered with *N. nemurus*, similar to our observation for the three samples and we believe is attributable to the same factors discussed. Becker et al. (2011) identified errors in FISH-BOL barcode data. He predicted that contradiction in identifications of same taxa can be seen when many laboratories are working on similar taxa and this misidentification of voucher specimens can also have serious implications for end users of reference libraries. Likewise, the genetic distance value calculated between *N. mesoprion* and *N. zysron* which atypically low (1.3%) between marine species and might also be due to misidentification.

Higher than typical marine fish intraspecific divergence of 2.7% and 3.4% were observed in *N. japonicus* and *N. nemurus*. Environmental heterogeneity and life-history traits could be factors elevating the variability. A similar divergence value of 2.7% had been previously reported (Ning et al. 2015) in *N. japonicus* populations from the Indian Ocean and West Pacific Ocean which was attributed to presence of cryptic species. Lim et al. (2014) reported that while the populations of *N. japonicus* in the Straits of Malacca were panmictic from the Perlis waters in the Northwest to Kuala Sedili in the Southeast, a distinct cluster clade was observed in the South China Sea population of Tok Bali. In the current study clustering of *N. japonicus* in the NJ tree into two separate clusters clades separating the South China Sea, Sulu Sea and Celebes Sea from Indian Ocean (populations from Pakistan) suggests limited sharing gene pool between the two regions. Haplotypes retrieved from GenBank belonging to South China Sea also clustered in same Cluster II Clade II. Interestingly, haplotype sharing was observed in the Straits of Malacca with only Indian Ocean populations. Whereas the South China Sea populations are isolated.

The parallel findings with Ning et al. (2015) and Lim et al. (2014) signifies the presence of a genetic barrier between the Indian Ocean and South China Sea for this demersal fish with the Straits of Malacca being the focal point of the two groups. Although on a smaller sizes, a detailed inspection showed that the same trend was also observed in *N. japonicus, N. bipunctatus, N. hexodon, N. furcosus, and N. marginatus.* This is in contrast to the lack of structuring of the pelagic Indian mackerel, *Rastrelliger kanagurta* (Akib et al. 2015). Ravitchandirane et al. (2012) reported genetic divergence values sufficient to differentiate various species of threadfin breams. Future studies on increased number of species and populations within this region are required to verify this.

To conclude, this study has contributed important data for the management of the threadfin breams in the Malaysian waters in the aspects of precise identification and genetic variability. Furthermore, it has provided additional data to the major databases of GenBank and BOLD. We confirm that DNA barcoding can efficiently diagnose genetic differences and genetic distances among and within species as well as resolving the issue of ambiguousness in catch identification. We found BOLD as an authentic genetic sequence library of voucher specimens. We recommend further validation of GenBank sequences with respect to their voucher specimen to prevent future misidentification of fish species.

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Identification of denitrifying bacteria from sediments of Rawa Jombor waters, Central Java and its trophic status

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Abstract. *Sunarto, Setyaningsih R, Yanti A. 2016. Identification of denitrifying bacteria from sediments of Rawa Jombor waters, Central Java and its trophic status. Biodiversitas 17: 578-584.* Pooled freshwater (lentic) is susceptible to contamination. One of pollution contributors on freshwater is the agricultural sector. One of them is the inclusion of waste in the form of organic and inorganic materials. The waste will increase the nutrient in waters causing sedimentation, eutrophication and pollution. The pollution is from nitrates derived from agricultural wastes using inorganic fertilizers. An alternative solution for the prevention and tackling of it is to utilize aquatic microorganisms namely denitrifying bacteria. These bacteria can convert nitrate into nitrogen gas (N_2) in the anaerobic state so that it can handle the pollution of nitrate in water. This study aimed to identify the denitrifying bacteria isolated from aquatic sediments of Rawa Jombor and to determine trophic status of Rawa Jombor waters. Identification of denitrifying bacteria was done through the morphology and physiology testing phase and also molecular analysis of 16S rRNA gene sequence. Based on parameters of total nitrogen, phosphor and brightness referring to the criteria of the lake trophic status attached to Ministry of Environment (PerMNLH) No. 28 2009, Rawa Jombor aquatic trophic status was analyzed. The results showed that as many as 6 denitrifying bacteria were isolated from sediments in Rawa Jombor waters, Klaten. Characteristics of bacteria colonies were translucent, round in shape, Gram-negative, rod and motile shaped cells. Based on the analysis of 16S rRNA gene sequence, all of denitrifying bacteria isolates were identified as having the highest similarity to the *Shewanella* genus. TmD isolate was identified as *Shewanella putrefaciens*, while TmE, TmG and TmI isolates were identified as *Shewanella* genus and TmA isolates was new species. Trophic status of Rawa Jombor aquatic was hypereutrophication.

Keywords: denitrifying bacteria, sediment, Rawa Jombor, nitrate, 16S rRNA gene.

INTRODUCTION

Rawa Jombor is a semi-artificial lake in the Dutch period of almost 12.7 km^2 in width and is located in the village of Krakitan, Bayat Sub-district, Klaten District, Central Java, Indonesia. Rawa Jombor has extensive pool of 180 hectares surrounded by a ring road, drainage channels, hills, trees, residential and some paddy fields. Rawa Jombor is used as a source of irrigation for the east area since 1967, as the *keramba* (floating net) fishery activity since 1986 and as the floating food stall business since 1998 (Ganjarsari 2008).

Rawa Jombor is a pooled freshwater (lentic) which is a form of aquatic ecosystems where flow/stream of water does not play an important role. Problem often occurred in the waters of Rawa Jombor is water pollution due to the inclusion of pollutant sources coming from sewage of floating food stalls, from the tourists and from *keramba* fishery as well as from agricultural waste and domestic waste around the waters. The high contamination of organic and inorganic wastes increases the content of nitrogen compounds that are harmful to aquatic organisms such as nitrate, nitrite, and ammonia. Nitrate is a form of nitrogen compounds and one of essential elements for protein plants synthesis. In high concentrations, nitrates can stimulate an unlimited growth of phytoplankton if some conditions, such as phosphorus concentration, can be met. Nitrate levels of more than 0.2 mg/L may result in eutrophication of waters which can stimulate the growth of algae and aquatic plants rapidly or blooming (Effendi 2003). Eutrophication is the enrichment of water due to the presence of nitrogen and phosphorus that are badly needed by plants and can causes an increase in the waters primary productivity (Mason 1993). In addition, high nitrate in waters can also cause decrease of water quality such as lowering of dissolved oxygen, fertilizing waters.

One attempt to control high nitrate compounds in the water is by utilizing the activity of denitrifying bacteria. Denitrifying bacteria can reduce nitrate to nitrogen gas so as to reduce nitrate levels in water. Denitrifying bacteria is a group of nitrate reducing bacteria. These bacteria are heterotrophic; require an organic carbon source such as acetic acid, propionic acid, succinic acid, glycerol and glucose for growth (Teixeira and Oliveira 2002). The ideal denitrifying bacteria in the process of nitrate control are the bacteria that produce N_2 gas as their end product.

Denitrifying bacteria lives well on the environment having relatively low oxygen content. According to Teixeira and Olivera (2002), denitrifying bacteria is anaerobic facultative or anaerobic obligate. In a system of freshwater environment, groups of denitrifying bacteria can live well on a waters base region or sediment. The oxygen content in the sediment is relatively low and at night, it can reach 0 ppm.

This study aimed to identify the denitrifying bacteria using the gene coding of 16S rRNA and to test the trophic status of Rawa Jombor waters based on environmental parameters such as total nitrogen, total phosphor and brightness. Trophic status needs to be tested in order to know the quality of the water and its allocation level.

MATERIALS AND METHODS

Sampling and analysis of water quality

The study was conducted on July till November 2013, in the waters of Rawa Jombor, Bayat, Klaten, Central Java, Indonesia. Samples of sediment and water were taken in five sampling points, namely: inlet, middle, outlet, western part and eastern part of floating food stalls (Figure 1). The analysis of water samples were conducted at the Central Laboratory of Sciences, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia. As the parameters of environment, the sediment and water of Rawa Jombor waters were measured. In the sediment, parameters measured were pH, nitrate level, nitrite level, and ammonia level. In water, the parameters measured were pH, brightness level, nitrate level, nitrite level, total nitrogen (N) and total phosphor (P). Parameters of pH and brightness were measured on site. The pH was measured using pH meter; the electrode, which was previously calibrated with distilled water, was dipped into the sample. Brightness was measured with Secchi disk; it was a disc with a rope in the middle. Secchi disk was inserted into the water, if the disc became invisible, the rope was marked and if the disc became visible again when it was pulled upward, the rope was marked too. Brightness values can be determined by measuring the distance of the disc visibility from invisible into visible again. While the parameters of nitrate level, nitrite level, total N and total P were tested at the Center for Environmental Engineering and Contagious Disease Control (BBTKL PP) Yogyakarta, Indonesia.

Isolation and characterization of denitrifying bacteria

The medium used for the isolation of denitrifying bacteria was denitrification liquid and solid media consisting of $CH_3COOHNa.3H_2O$, K_2HPO_4, KH_2PO_4, NH_4Cl, $MgSO_4.7H_2O$, yeast extract, $NaNO_3$, distilled water, and agar were added for solid media (Setyaningsih et al. 2013) and also nitrient agar (NA) media. Isolation of denitrifying bacteria was carried out using a liquid denitrification medium and was added by nitrogen gas. To isolate bacteria which were fermentative negative then the molecular test was carried out for identification process using the gene coding for 16S rRNA.

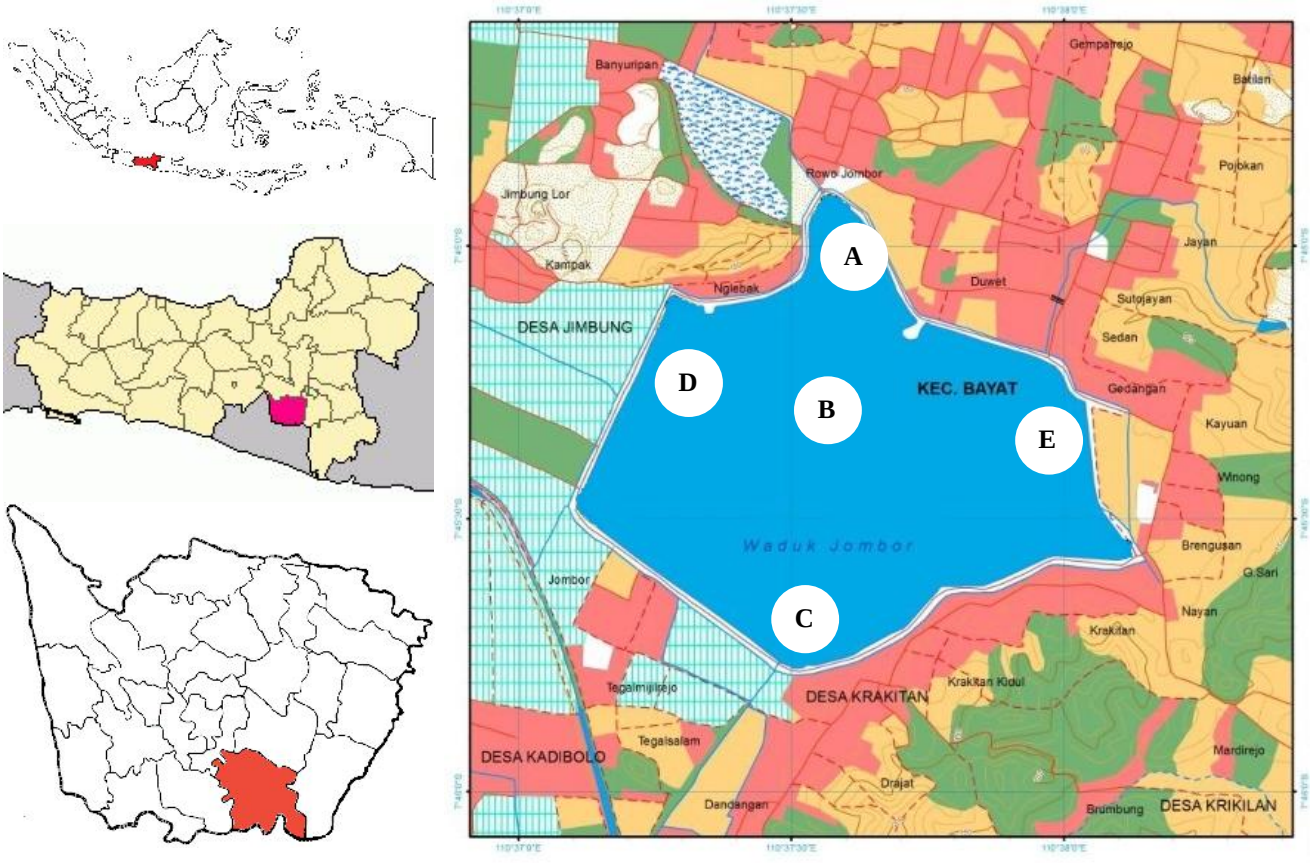

Figure 1. The study site in Rawa Jombor, Bayat, Klaten, Central Java, Indonesia. A. inlet, B. middle, C. outlet, D. western part and D. eastern part of floating food stalls

Sediment samples were put into sterile glass bottles of 100 mL for as much as 20 grams and mixed with 80 mL of liquid denitrification medium. Anaerobic condition was created by removing the oxygen in the bottle, by inserting nitrogen gas at a pressure of ± 250 kPa, via syringe aseptically into the bottle for 15 minutes. Then the mixture was homogenized in an orbital shaker (130 rpm, 28-30^{o}C) for 5 days (Widiyanto et al. 2008). With inoculating needle, 1 loopful supernatant was streaked onto Petri dishes containing solid denitrification media with quadrant method and was incubated at a temperature of 28^{o}C for ± 2 to 3 days in an incubator (Waluyo 2008). The colonies which were grown separately were streaked and incubated again on NA medium with the same incubation conditions for purification. The obtained pure isolates were stored on oblique NA media as cultured stocks.

Nitrate reduction test

The next selection phase was to test the nitrate reduction which aimed to select bacteria that could reduce nitrate to nitrite. Nitrate reduction test was performed in the Laboratory of Microbiology Health Laboratory (BLK) Yogyakarta. Bacterial isolates were inoculated into the medium of nitrate broth (DifcoTM) and were incubated for 24 h at 37 ° C in an incubator. The bacterial cultures were dripped with 2-3 drops of solution A, and then were dripped again with 2-3 drops of solution B. If nitrite were found in the tested cultures, it would be marked by the formation of red which meant that nitrate was reduced into nitrite (positive test). If there were no visible change in color within 3-5 minutes, a bit of zinc powder would be added into the culture. If red color were formed, it would mean that the test was negative, whereas if there were no color change, it would mean that the test was positive (Hanum 2005).

Oxidative/fermentative (O/F) test

The last selection stage was the oxidative-fermentative test (OF) which aimed to select bacteria isolates that were fermentative. Oxidative/fermentative test was performed to select oxidative bacteria since most denitrifying bacteria were generally oxidative and also to distinguish the fermentative bacteria which reduce $NO_3^-$to NH_4^+. The medium used was O/F media (Hugh and Leifson 1953). A total of 5 mL of medium was poured into a 12 mL test tube with a screw lid, then bacteria isolates were inoculated by a puncture. On culture, liquid paraffin was poured into to create anaerobic conditions. Incubation was performed at room temperature for 1-2 days. Group of fermentative bacteria produced acids so that green medium changed to yellow.

Characterization of morphology and nature of bacteria Gram

The characterization of colonies and cells were the observation of cell morphology and colony morphology of denitrifying bacteria. Observation of cell morphology included Gram staining and observation of the bacteria cell shape. Observations on colony morphology included colony color observations, observation on the form and on the edge of colony. Gram staining was performed by passing an object glass over a Bunsen flame. Then 1 drop of sterile physiological saline was dripped on the object glass and continued by a drop of pure isolates. Isolates and the physiological saline were spread out evenly and then dried up and a fixation was carried out on it. 1-2 drops of Gram **A** was dripped on its surface and left for 30-60 seconds. Preparation was washed with flowing water, and then was dried up. 1-2 drops of Gram B solution was dripped onto the surface of preparations, left for 30-60 seconds, washed with flowing water and dried up. Furthermore, Gram C (96% alcohol) was dropped on the preparation for as much as 1-2 drops and left for 30 seconds, then it was dripped with Gram D of 1-2 drops and left for 30-60 seconds. Finally, preparation was washed with flowing water and was observed under a microscope to find out the microscopic character of the bacteria in the form of Gram reaction and cell shape of bacteria (Waluyo 2008). If the results of the bacteria cell staining were red, it would mean that the cells are Gram-negative, while if the color were purple, it would indicate that the nature was Gram-positive.

Identification of denitrifying bacteria using sequences gene coding for16S rRNA

Bacteria DNA extraction

Prior to the extraction of DNA, bacteria isolates were cultured in Luria Bertani media (LB) for 24 hours on an orbital shaker (130 rpm, 37 ° C). DNA extraction of 6 denitrifying bacteria isolates from aquatic sediments of Rawa Jombor, Klaten used geneJET genomic DNA purification kit (Fermentas). One mL of liquid culture was poured into 1.5 mL eppendorf tube and was centrifuged at 5000 rpm for 10 minutes. Supernatant was discarded and the pellet was added with 180 µL of digestion solution and 20 µL of proteinase K, and then was mixed using a vortex. The mixture was incubated in an incubator shaker 56^{o}C for 30 minutes with a speed of 150 rpm. After incubation 20 µL of RNase solution was added to the previous solution and was mixed using a vortex before it was incubated at 37 ° C for 10 minutes. After the second phase incubation 200 µL of lysis solution was added to it and was mixed using a vortex for 5 seconds until homogeneous. After it was homogeneous, the solution was added with 400 µL of 50% ethanol and was mixed using a vortex. Supernatant was poured in geneJet tube which was equipped with a flow-through tube, and then it was centrifuged for 1 minute at a speed of 6000 rpm. Furthermore, the liquid inside the flow-through tube was removed and the flow-through tube was reassembled. A total of 500 µL of washing buffer I was added and the solution was centrifuged for 1 minute at a speed of 8000 rpm. The residue (liquid) inside the flow-through tube was removed and reassembled. A total of 500 µL of washing buffer II was added and was re-centrifuged for 3 minutes at a speed of 12,000 rpm. If the residue (liquid) were still present, the flow-through tube could be emptied and was centrifuged again for 1 minute at a speed of 12,000 rpm. Furthermore, the flow-through tube was

removed and replaced with a new 1.5 mL eppendorf. The next phase 200 µL of elution buffer was added, and then was incubated for 2 minutes at room temperature, and then was centrifuged again for 1 minute at a speed of 8000 rpm. At the last phase, the genJet tube was removed and the supernatant which was left inside eppendorf tubes (microtube) was genomic DNA. Results of the extraction or isolation of genomic DNA was visualized on a 0.8% agarose gel and was stored at-21 ° C.

Amplification of 16S rRNA Gene

Amplification was performed with universal primers for group of bacteria namely primer 63F (5'CAG GCC CAC TAA GTC ATG CAA) and 1387R (WTG 5'GGG GTA CAA CGG GGC) (Marchesi et al. 1998). The reaction mixture consists of: 18.8 µL ddH2O, 10x 2.5 µL dream taq buffer, 0.5 µL dNTP, 0.5 µL of each primer, 0.2 µL DNA polymerase (Fermentas) dream taq and 1 µL DNA samples (DNA Template), so that the total volume was 25 µL. PCR conditions took place in the following stages: pre-PCR for 5 minutes at a temperature of 95° C, denaturation for 30 seconds at 94° C, annealing for 30 second at 55^{o} C, elongation for 30 seconds at 72^{o} C, post PCR for 7 minutes at 72^{o} C and storage at 4° C. The PCR process lasted for 30 cycles. PCR products were visualized on 0.8% agarose gel (Salupi 2011). DNA amplification product was then sequenced to obtain sequences of DNA isolates of denitrifying bacteria isolated from aquatic sediments of Rawa Jombor, Klaten. The purification and sequencing of rRNA 16S genes were conducted by PT Genetika Science Indonesia-1st Base, Singapore. The base sequence of the sequencing results were then compared to sequences in the data bank of the National Center for Biotechnology Information (NCBI) using the program of Basic Local Alignment Search Tool for Nucleotides (BlastN) (http:// www.ncbi.nlm.nih.gov/BLAST/).

The test of trophic status

In this study, water samples were taken from waters of Rawa Jombor at 5 locations of sampling namely; inlet, middle, outlet, western and eastern of floating food stalls. The parameters tested to determine the trophic status in these waters were the total nitrogen, total phosphorus and brightness. The results obtained were in accordance to the Regulation of the Minister of Environment No. 28 of 2009 on Water Pollution Load Capacity Lake and/or reservoir.

RESULTS AND DISCUSSION

The condition of water quality of sampling sites

The observation of water quality in aquatic sediments in Rawa Jombor of five different sampling locations was presented in Table 1. In general, the sediment in the waters of Rawa Jombor has a neutral pH of 6.91 to 7.05, but the pH at the inlet was higher with 8.27. The highest content of ammonia was at the west of floating foodstall and the lowest was at the outlet. The content of nitrate and nitrite at the east of floating foodstall was the highest and the lowest was at the inlet. The highest result of DO measurement was at the outlet, and the lowest was at the west of the floating foodstall.

Table 1. Condition of water quality in Rawa Jombor, Klaten

Sample location	pH	Ammo-nia (mg/L)	Nitrate (mg/L)	Nitrite mg/L)	DO (ppm)
Inlet	8.27	0.28	0.08	0.06	7.0
Middle	6.91	0.20	0.23	0.17	6.5
Outlet	6.95	0.02	0.10	0.07	8.0
West of floating foodstall	7.05	0.43	0.31	0.23	5.5
East of floating foodstall	6.99	0.33	0.54	0.40	6

The observation on the trophic parameters, namely ammonia, nitrite and nitrate resulted that the highest number was at the vicinity of the floating foodstalls. It was closely related to the waste produced by the activity of the floating foodstalls and agriculture. In an anaerobic circumstance and the availability of high nutrient especially nitrate, denitrifying bacteria could grow and perform optimal denitrification process. The presence of ammonia, nitrates and nitrites in the water was not the only the result of the nitrogen cycle, but mostly was from pollutants originating from outside of waters that goes into the waters such as the remnants of the pesticides use flowing in with rain water, fertilizer use and domestic waste from surrounding waters.

A total of 45 bacteria isolates were obtained from 5 sediment samples from different locations, namely 5 isolates were found in the inlet (In), 8 isolates were found in the middle (Te), 6 isolates were found in outlet (Ot), 11 isolates were found at west of floating foodstall (Br), and 15 isolates were found at east of floating foodstall (Tm). Isolation was also as an initial selection stage to obtain denitrifying bacteria isolates. Based on results of nitrate reduction test, it was shown that as much as 35 bacteria isolates were positive results as nitrate-reducing bacteria and one isolate was weak nitrate reducing.

Oxidative-fermentative test results showed that out of 34 isolates tested, 28 isolates were fermentative and 6 of them were negative fermentative. These six isolates which were negative fermentative were denitrifying bacteria and all obtained from sediment samples at east of floating foodstalls (Tm). At this point of sampling, the number of nitrate and nitrite were the highest, so the denitrifying bacteria could be found at this location. Denitrifying bacteria live and grow in an environment that has a high content of nitrogen compounds, especially nitrates and nitrites in an anaerobic circumstance (Effendi 2003). Denitrification was carried out by microorganisms using nitrate and nitrite compounds as an electron acceptor and gaseous nitrogen compounds. Group of positive fermentative bacteria will reduce nitrate to ammonium (Rusmana and Nedwell 2004). Group of positive fermentative bacteria could not use nitrate as an electron acceptor compounds, on the contrary, they used it as a source of electrons (Widiyanto et al. 2008).

Characteristic of colonies and isolates cell of denitrifying bacteria

A total of six isolates of denitrifying bacteria found in aquatic sediments of Rawa Jombor had similar characteristics, namely translucent colored colonies, round with flat edges, rod-shaped bacterial cell and Gram-negative bacteria (Figure 2 and Table 2). Characteristics of denitrifying bacteria obtained in this study was relatively similar to that reported by Widiyanto et al. (2008) which states that the denitrifying bacteria found in the shrimp ponds have rod-shaped cell, motile and Gram negative.

Denitrifying bacteria identification base on 16S rRNA gene sequence

A total of six isolates of bacteria which were capable of reducing nitrate and were thought to be group of denitrifying bacteria that isolate the TmA, TmD, TmE, TmG, TmI and TmK were identified base on the sequences of 16S rRNA gene. Fragment of PCR product had a size of about 1300 bp which was the expected size by using a combination of 63F primer for forward direction and 1387R primer for reverse direction (Figure 3).

PCR products which could be analyzed further by sequencing were DNA fragment of the five bacterial isolates. One PCR product was damage, so it was not sequencing analyzed, namely DNA fragment of TmK. The data results which were analyzed by BlastN showed that all five isolates have the highest similarity to the genus *Shewanella*. One isolate which was identified as *Shewanella putrefaciens* was TmD isolates with the percentage of similarity of 99%, two other isolates, namely the isolates TmE and isolates TmG has similarities with *Sh. putrefaciens* with the percentage of similarity respectively 98% and 97%, while the isolates TmI

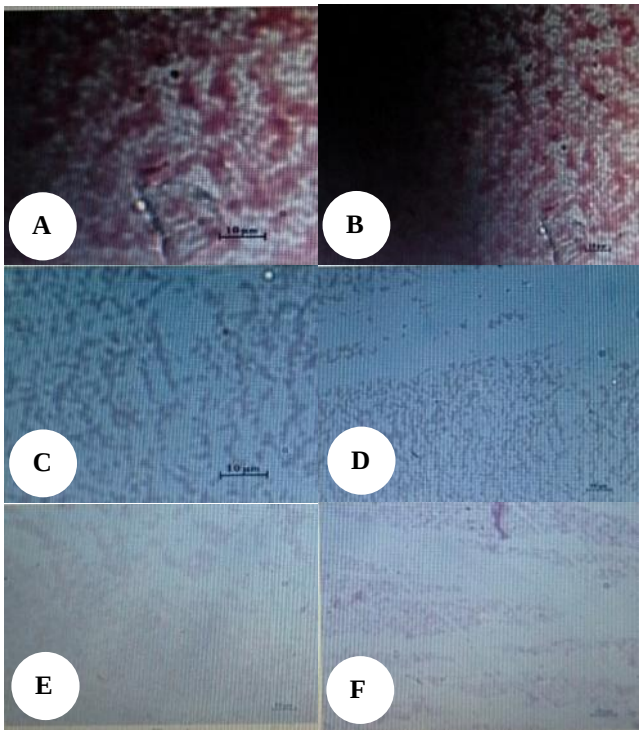

Figure 2. The shape of the cell of 6 denitrifying bacteria. A. Tm A, B. Tm D, C. Tm E, D. Tm G, E. Tm I, F. Tm K.

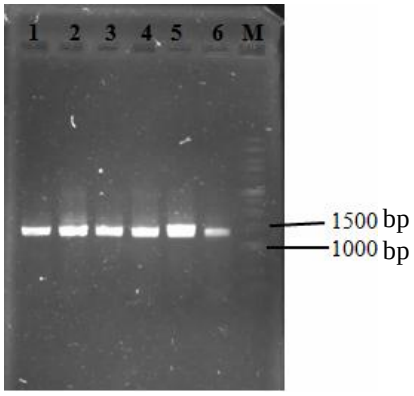

Figure 3. The DNA fragment as a result of amplification of 16S rRNA gene on six denitrifying bacteria isolates. M = 1 kb DNA Marker; 1 = isolate the TmA; 2 = isolates TmD; 3 = isolates TmE; 4 = isolates TmG; 5 = isolates TmI; 6 = isolates TmK

Table 2. Characteristics of denitrifying bacteria isolated from aquatic sediment of Rawa Jombor, Klaten, Central Java

Bacteria isolates	Colony color	Colony shape	Colony shape	Cell shape	Gram
Tm A	Yellowish Trans.	Round	Flat	Rod	Negative
Tm D	Brownish Trans.	Round	Flat	Rod	Negative
Tm E	Translucent	Round	Flat	Rod	Negative
Tm G	Yellowish Trans.	Round	Flat	Rod	Negative
Tm I	Translucent	Round	Flat	Rod	Negative
Tm K	Translucent	Round	Flat	Rod	Negative

Note: Trans. = Translucent

Table 3. Identification of denitrifying bacteria on sediment water of Rawa Jombor using BlastN

Isolate code	Closest relatives	Access number	% Simila-rity
Tm A	*S. putrefaciens* strain KOl2	KC607511.1	96
Tm D	*S. putrefaciens* strain KOl2	KC607511.1	99
Tm E	*S. putrefaciens* strain K717	KC607526.1	98
Tm G	*S. putrefaciens* strain KOl2	KC607511.1	97
Tm I	*S. xiamenensis* strain H3	HQ418493.1	98

Note: *S.* = *Shewanella*

resemblance to the *Shewanella xiamenensis* with 98% similarity percentage. One other isolates namely isolates TmA had a percentage of similarity of 96%. This isolates was possibly a new species (Table 3). According to Dancourt et al. (2000), if the similarity with the database were ≥ 99%, then the isolates could be identified at the species level. If it were ≥ 97%, it could be identified at the genus level, whereas if the similarity were <97%, it might be a new species due to the lack of data on the database or the size of the sequencing results was too short to be compared to database.

Shewanella genus was one of metal-reducing bacterium genus. These bacteria were found many in the marine environment, freshwater, lakes, ground or terrestrial, rivers, Arctic and Antarctic oceans, the rusty or corroded oil pipeline and contaminated uranium aquifer environment. These bacteria were widely used for bioremediation or for cleaning the environment from pollutants such as compounds that underwent chlorination, radionuclides and other environmental pollutants (Venkateswaran et al. 1999). *Shewanella* belong to the Gram-negative bacteria and some species were pathogens that cause disease in humans. Other characteristics of these bacteria were rod-shaped, motile (moving) with polar flagella and had a metabolism as a facultative anaerobic organisms (Huang et al. 2010). To survive, these bacteria were capable of using a variety of electron acceptors such as oxygen, iron, manganese, uranium, nitrate, nitrite, fumaric and others. *Shewanella putrefaciens* could be found in the marine environment, motile, and was facultative anaerobic bacteria that had the ability to reduce iron and manganese as a terminal electron acceptor in the electron transport chain. This bacterium was also an organism associated with the stench of decaying fish, such as marine organisms that produce trimethylamine (Johansen et al. 1996). S. *putrefaciens* could grow on solid media as well as on liquid media. On solid media, bacteria colonies were round, pink and grew fast. These bacteria also grew fast on liquid medium and the liquid medium was made entirely into pink (Khashe and Janda 1998). *Shewanella xiamenensis* was motile with single nonpolar flagella and was facultative anaerobic. Colonies were circular-shaped, brown, grew at temperatures between 4 ° C-37 ° C but if it were below 37 ° C, they would not grow. pH growth ranged from 6.0 to 9.0 with a pH optimum of 7.0, positive to hydrolyze gelatin, DNA and Tween 80 and could reduce nitrate, nitrite, fumaric (Huang et al. 2010).

Trophic Status of Rawa Jombor Waters

Rawa Jombor had high level of total-N and total-P high and low brightness making it to have hypereutrophication status according to the Regulation of the Minister of Environment No. 28 of 2009 (Table 4). Status hypereutrophication (very fertile) is a status of waters containing nutrients in very high levels.

Table 4. Total nitrogen, total phosphor and brightness on water of Rawa Jombor, Klaten

Sample	Level of Total-N (µg/l)	Level of Total-P (µg/l)	Brightness (m)
Inlet	5643	667.1	0.12
Middle	19903	260.4	0.12
Outlet	4807	416.1	0.18
West of floating foodstall	6273	542.2	0.14
East of floating foodstall	3827	601.6	0.17
Average	8090.6	497.48	0.146
Criteria of Regulation of the Minister of Environment (Hypereutrophication)	>1900	$\geq$ 100	< 2.5

According to Suryono et al. (2008), the amount of nutrients contained in the waters of lakes or reservoirs can be used for the assessment of the trophic status. This status indicates the water was heavily contaminated by elevated levels of nitrogen and phosphate. Machbub et al. (2003) suggested that the occurrence of eutrophication in the waters of the lake and reservoirs can be detected by a variety of indicators, namely: (i) decrease in the concentration of dissolved oxygen in the zone of hypolimnion, (ii) increase of nutrients i.e. nitrogen and phosphorus in bodies of waters, (iii) the decrease of water transparency, and (iv) increase in suspended solids, especially those containing organic material. The indicators are a common sign, but the monitoring of water quality parameter remains to be done, especially parameters associated with the process of eutrophication.

In conclusion, a total of six isolates of denitrifying bacteria were found in aquatic sediments of Rawa Jombor waters with characteristic colonies are round, translucent color, are Gram negative and rod-shaped cells. Five isolates were identified as having the highest similarity to the genus *Shewanella*. Isolates TmD is identified as *Shewanella putrefaciens* with the percentage of similarity of 99%, isolates TmE and TmG has similarities with *Shewanella putrefaciens* with percentages respectively 98% and 97%, isolates TmI has similarities with *Shewanella xiamenensis* with a percentage of 98% and isolates TmA having percentage of similarity of 96 % by *Shewanella putrefaciens* has possibility as a new species. The trophic status of Rawa Jombor aquatic based on parameters of total nitrogen, total phosphate and brightness is hypereutrophication (very fertile), that these waters contain nutrients with very high levels.

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Seagrass biodiversity at three marine ecoregions of Indonesia: Sunda Shelf, Sulawesi Sea, and Banda Sea

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Abstract. *Kawaroe M, Nugraha AD, Juraij, Tasabaramo IA. 2016. Identification of soybean genotypes adaptive and productive to acid soil agro-ecosystem. Biodiversitas 17: 585-591.* Seagrass is one of the coastal ecosystems in marine ecoregions of Indonesia that has very important ecological and economical functions. This study aimed to illustrate the diversity of seagrass ecosystems through its distribution, coverage, and density, in three marine ecoregions of Indonesia, namely Sunda Shelf/SHS (Bintan Island/SHS-B and the Seribu Islands/SHS-S), Sulawesi Sea/SS (Talaud Island), and Banda Sea/BS (Tanimbar Islands). The study was conducted at 16 stations in SHS, 20 stations in SS, and 30 stations in BS. A line transect method was used. Three line transects (length 50m) were deployed in each station perpendicular to the shoreline towards the sea with a distance of 20 meters between transect lines. In each line transect, quadrate transects were placed (0.5mx0.5m) along the line, alternating left and right line up to the edge. Species identification and seagrass density were calculated in each quadrate transect. Similarity indexes were calculated and analyzed between ecoregion on seagrass coverage and abundance through dendrogram graphic. Results showed that 10 species of seagrass were found in three marine ecoregions. In SHS-B, 10 species with a coverage cover of 61% were found and *Thalassia hemprichii* was of the highest abundance. In ShS-S, 6 species with a coverage cover of 37% were found and *Enhalus acoroides* species was of the highest abundance. In SS, 5 species with a coverage cover of 43% were found and *Cymodocea rotundata* was of the highest abundance. Finally, in BS, 7 species with coverage of 60% were found and *Thalassia hemprichii* was of the highest abundance. These results indicated that seagrass biodiversity found in 3 Indonesia marine ecoregions were still in a healthy condition. One of the implications of this healthy condition of seagrass was that the very important functions of seagrass as a habitat for economically important organisms and a food source for herbivores, particularly *Dugong dugong*, living in seagrass was still secured.

Key words: biodiversity, Indonesia, marine ecoregion, seagrass

INTRODUCTION

Twelve of 57 worldwide marine ecoregions are found in Indonesia (Huffard et al. 2012). All ecoregions are determined based on their biological diversity, including seagrass. Seagrass is part of an ecosystem found in coastal regions and the only flowering plant capable to live a submerged life in sea water (Kawaroe et al. 2016). Seagrass ecosystems have important roles as a source of primary productivity and a foraging and nursery ground for some marine biota (Erftemeijer et al. 1993; Christianen et al. 2014). In addition, seagrass plays a role in carbon cycle in the atmosphere (Duarte 2005; Kennedy et al. 2010).

Seagrass is so widespread in the world, but the highest biodiversity is found in the Indo-Pacific region, including Indonesia (Waycott et al. 2004). As a country with high biodiversity, Indonesia has 12 of 69 species of seagrass found in the world. Seagrass in Indonesia is found in coastal areas and small islands where it is able to live up to a depth of 40 meters. Seagrass lives in sand, sandy mud, mud, and rubble substrate. Based on the data validated by Indonesian Science Institute (2016), seagrass distribution in Indonesia reaches 25.742 ha in 29 locations around the country. Distribution and diversity of seagrass is found fairly throughout the coastal areas of Indonesia. Several studies have noted the presence of seagrass distribution but it remained unconnected with existing ecoregions.

Seagrass ecosystem conditions in some parts of Indonesia are found to be under threats from human activities such as tourism, ports, aquaculture, and sand mining. It was estimated that 58% seagrass ecosystems in the world has decreased the extents number (Waycott et al. 2009). According to Vo et al. (2013), seagrass bed areas in Indonesia are declined by about 30-40%, and the biggest damage of seagrass beds is found in Java Island. Pari Island is another place in Indonesia where seagrass is found to be reducing. This reduction occurs continuously every year as the effect of anthropogenic pressures (Kawaroe et al. 2008; Rustam 2014). The protection of seagrass ecosystems needs to be done immediately and this can be initiated by in-depth studies on the distribution and diversity. This study aimed to analyze the diversity of seagrass in three marine ecoregions of Indonesia including Sunda Shelf (Riau and the Seribu Islands), Sulawesi Sea (Talaud Island), and Banda Sea (Tanimbar Island). The diversity of seagrass was illustrated by its distribution, coverage percentage, density, and functions as well as threats to the existence of the seagrass ecosystems.

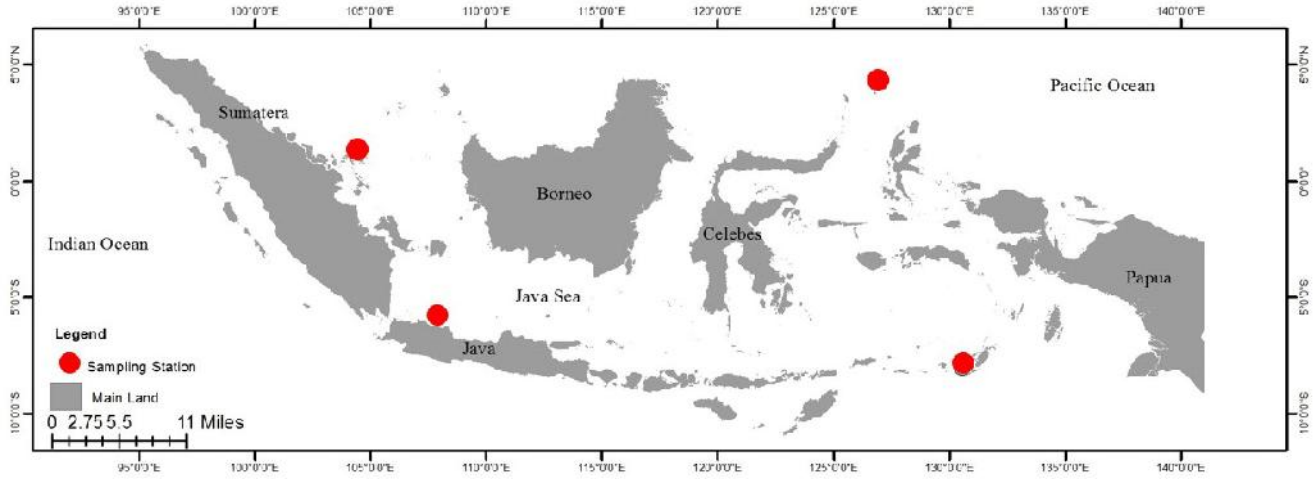

Figure 1. The research location of seagrass distribution in Indonesian marine ecoregion at the Lesser Sunda Shelf ecoregion, Sulawesi Marine Ecoregion and the Banda Sea Ecoregion

MATERIALS AND METHODS

Research locations

This research was conducted in 2015 in 3 marine ecoregions in Indonesia, namely Sunda Shelf represented by Riau Islands and Seribu Islands, Celebes Sea represented by Talaud Islands, and Banda Sea represented by Tanimbar Islands (Figure 1).

Seagrass observation methods

Observations were made using the line transect method when the lowest ebb occurred (English et al. 1997). Each study site was represented by five observation stations and each station consisted of three transect lines. The distance between each transect line was 50 meters. In each transect, a perpendicular line was drawn along 100 meters starting from the beginning of the shoreline where the seagrass was near perpendicular to the sea.

Seagrass density

Observations on seagrass density on each transect line were done by using squares sized 0.5mx0.5m (English et al. 1997; McKenzie and Yoshida 2009). Each square in transect lines was placed at a distance of 5 m. Seagrass density was calculating by the number of stands or shoots of any seagrass species that are found in the squares. Seagrass found on any squares were directly identified at the study sites from the shape of the leaves, rhizomes, flowers and fruits (McKenzie and Yoshida 2009; Waycott et al. 2004). The density of seagrass species was the total number of individual seagrass species in a unit area measured. Seagrass species density was determined by the following formula (English et al. 1997):

$$\mathbf{D} = \sum \frac{Ni}{A} \quad \text{..... (1)}$$

Where:
D = Species density (shoot/m2)
N_i = Number of shoot of species-i
A = Sampling area (m^2)

Seagrass coverage

Seagrass coverage (%) was observed using Seagrass-watch method on the same square of seagrass density observations. Seagrass coverage of each seagrass species was calculated with the comparation to the picture available in each square on observation area (McKenzie and Yoshida 2009).

Seagrass diversity, evenness and dominance

Seagrass diversity was calculated using the following Shannon Wiener index (Odum 1983):

$$H' = -\sum \left(\frac{ni}{N}\right) \ln \left(\frac{ni}{N}\right) \quad \text{..... (2)}$$

Where:
n_i = number of shoot in each species
N = total number of shoots

Table 1. Biodiversity Index Category (Odum 1983)

Biodiversity	Category
H' < 2.0	Low
2.0 < H' < 3.0	Moderate
H' ≥ 3.0	High

Seagrass similarity was calculated with the formula as follows (Odum 1983):

$$E = \frac{H'}{\ln S} \quad \text{..... (3)}$$

Where:
E = Similarity index
H' = Diversity index
S = number of species

Table 2. Similarity index category (Odum 1983)

Similarity	Category
0,00 < E < 0,50	Depressed community
0,50 < E < 0,75	Labile community
0,75 < E < 1,00	Stabile community

Seagrass dominant was calculated with the formula as follows (Odum 1983):

$$C = \sum\left(\frac{ni}{N}\right) \quad \text{..... (4)}$$

Where:
C = dominance index
n_i= number of shoots species
N = total number of individuals

Table 3. Dominant index category (Odum 1983)

Dominant	Category
0.00 < C < 0.50	Low
0.50 < C < 0.75	Moderate
0.75 < C < 1.00	High

Data analysis

Density and seagrass cover were analyzed using a cluster analysis. This analysis is a part of multivariate statistics, which was done to classify the seagrass in each region observed by its characteristics, namely density and seagrass cover.

RESULTS AND DISCUSSION

Species distribution and seagrass conditions

Results showed that in three marine ecoregions of Indonesia, 10 species of seagrass of two families were found (Table 4). These seagrass species were *Enhalus acoroides, Thalassia hemprichii, Halophila minor, H. ovalis, H. spinulosa, Cymodocea rotundata, C. serrulata, Halodule uninervis, H. pinifolia*, and *Syringodium isoetifolium.*

Seagrass species found throughout the ecoregions studied were *T. hemprichii, C. rotundata, H. uninervis and S. isoetifolium.* Four of these species were seagrasses that predominate in the Indo-Pacific region (Short et al. 2007). In addition, there was a seagrass species, *H. pinifolia* that was only found in Riau Islands waters. According to UNEP (2008) *H. pinifolia* seagrass was considered as a pioneer seagrass species and one of its distribution areas was Riau Islands. This seagrass species was also found in some coastal areas of Malaysia (Bujang et al. 2006) and Singapore (Yaakub et al. 2013). Geographically, the coastal

Table 4. Seagrass species distribution in three marine ecoregions (represented by 4 islands) of Indonesia

Family/Species	Sunda Shelf		Sulawesi Sea	Banda Sea
	Riau Islands	Seribu Islands	Talaud Islands	Tanimbar Islands
Hydrocharitaceae				
Enhalus acoroides	+	+		+
Thalassia hemprichii	+	+	+	+
Halophila minor	+			+
H. ovalis	+		+	+
H. spinulosa	+			
Potamogetonaceae				
Cymodocea rotundata	+	+	+	+
C. serrulata	+	+		
Halodule uninervis	+	+	+	+
H. pinifolia	+			
Syringodium isoetifolium	+	+	+	+

Note: + : found

Regions of Malaysia and Singapore have a location adjacent to Riau Islands and it is a Sunda Shelf ecoregion. Therefore, the similarity of seagrass species found in these three locations was the evidence of similar environmental conditions the regions shared. Most seagrass species was found in Riau Islands and Tanimbar Islands. This was presumably because of the fact that these regions were adjacent to the open sea. Riau Islands are close to the South China Sea and Tanimbar Islands are located adjacent to Arafuru Sea. The existence of strong current in locations adjacent to high seas allows the connectivity process in biodiversity (Chiu et al. 2013).

Seagrass cover found in 4 islands of 3 marine ecoregions in Indonesia was different (Figure 2). Seagrass cover in Riau Islands waters was 61%, Seribu Islands 37%, Talaud Islands 43%, and Tanimbar Islands 60%. The differences in seagrass covers were caused by several factors including seagrass topography, physical and chemical water condition, coastal community activities around the seagrass, and distribution of seagrass species (UNEP 2008). Another factor that could affect the distribution of seagrass was predation of seagrass by biota associations (Heck and Valentine 2006). Based on the obtained seagrass cover, Riau Islands and Tanimbar Islands had seagrass cover higher than did the other two islands. The lowest seagrass cover was found in Seribu Islands. The close location of Seribu Islands to Jakarta Bay was presumably the cause of this. There are 13 rivers flowing into the Gulf of Jakarta with high enough pollutant loads to affect the biodiversity of coastal ecosystems in the surrounding waters of Jakarta Bay. According to Ambo-Rappe (2014), if the location of seagrass ecosystems was very close to the mainland it had the potential to receive the negative effects of anthropogenic activities from that mainland. In addition to the base of substrate and water conditions, number of residents in a region, which was correlated with anthropogenic stress, was another factor affecting seagrass cover and distribution. (Campbell and McKenzie 2004; Waycott et al. 2005).

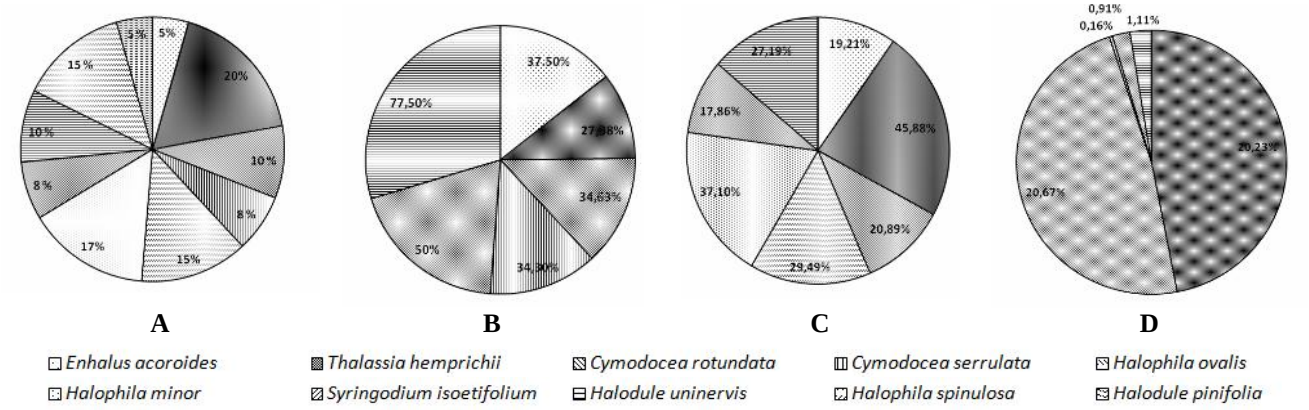

Figure 2. Seagrass cover (%) at 4 islands (3 marine ecoregions in Indonesia): A. Riau Islands, B. Seribu Islands, C. Talaud Islands, and D. Tanimbar Islands.

It was shown from the seagrass cover in each species (Figure 2) that in Riau Islands, *T. hemprichii* was the highest (20%) followed by *H minor* (17%) while in Seribu Islands, *H. uninervis* was the highest (77.5%) followed by *E. acoroides* (37.5 %). Talaud Islands had *C. rotundata* and *T. hemprichii* with the highest cover percentage of 20.67 and 20.23%, respectively. In *Tanimbar* Islands, *T. hemprichii* and *H minor* were found by 45.88and 37.10%, respectively. *T. hemprichii* was the species found to have a high coverage in all locations. Seagrass species had a very wide distribution in Indonesian waters (UNEP 2008) and this was suspected to be caused by its cosmopolitan nature in the Indo-Pacific region and its ability to adapt to the environment (Klumpp et al. 1993). In addition to *T. hemprichii, C. rotundata* was found to have high coverage as it could tolerate a wide range of water conditions (Tomascik 1997).

The highest density of seagrass species was found in Riau Islands and the lowest one in Talaud Islands (Table 5). It was suspected that the finding caused the high density of seagrass in Riau Islands that the substrate in the region was sandy clay that was a suitable substrate for seagrasses to grow. This condition was different from that in the other three locations where sand was the dominant substrate. Substrate is one of the important factors affecting the density of seagrass (Kaewsrikhaw and Prathep 2014).

The number of individual seagrass species found in a region was affected by diversity, uniformity and dominance of seagrass (Figure 3). Results showed that the diversity of seagrass in three Indonesian marine ecoregions belonged to medium and low categories. Riau Islands and Tanimbar Islands had a diversity index of seagrass of medium category while those of Seribu Islands and Talaud Islands belonged to low category. This might be caused by the finding that the uniformity of seagrass in three marine ecoregions belonged to the instability category indicating that the condition of the waters was under threats, both from natural factors and human activities. There was highly dominating species found in Talaud Islands. This indicated that certain types of seagrass had a tendency to dominate and have a wide distribution in the area. *C. rotundata* and *T. hemprichii* segrasses had the most extensive distribution and were often found in Talaud Islands. According to Hemminga and Duarte (2000), seagrass, which had extensive deployment, had a high adaptability so that it could grow well in different habitat types with various environmental conditions.

Table 5. Seagrass species density in three marine ecoregions (four islands) in Indonesia

Family/Species	Seagrass density (ind/m^2)			
	Riau Islands	**Seribu Islands**	**Talaud Islands**	**Tanimbar Islands**
Hydrocharitaceae				
Enhalus acoroides	62±16.83	372±63.04	0	45±06.65
Thalassia hemprichii	417±17.37	608±50.91	123±13.02	358±26.80
Halophila minor	280±12.00	0	0	161±9.97
H. ovalis	350±10.87	0	2	191±2.55
H. spinulosa	244±43.00	0	0	0
Potamogetonaceae				
Cymodocea rotundata	166±7.07	844±178.19	131±22.23	150±2.06
C. serrulata	120±5.37	784±339.41	0	0
Halodule uninervis	222±17.45	0	16±1.7	323±25.85
H. pinifolia	112±10.09	0	0	0
Syringodium isoetifolium	318±17.82	612±32.75	11±2.8	430±2.64

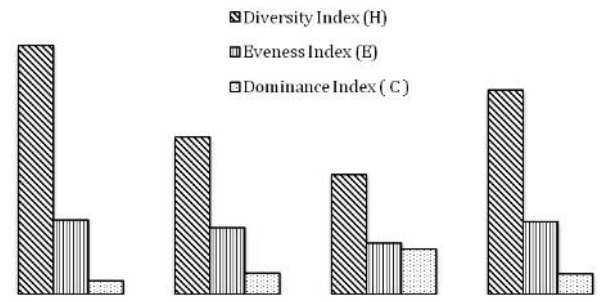

Figure 3. Diversity (H'), evenness (E), and dominance (C) of seagrass at three marine ecoregions in Indonesia

Results of cluster analysis based on the density showed that there were large seagrass ecosystems. The first group consisted of Talaud Islands (Sulawesi Marine Ecoregion) and Seribu Islands (Sunda Shelf Ecoregion) (Figure 4). The second group with the highest similarity scores consisted of Riau Islands (Sunda Shelf) and Tanimbar Islands (Banda Sea Ecoregion). Results of a cluster analysis showed that the groups with the highest percentage of seagrass cover were Tanimbar Islands and Talaud Islands as the first group, Riau Islands as the second group and Seribu Islands as the third group. It was also shown that the conditions of seagrass ecosystems, represented by both density and seagrass cover percentage, were influenced by environmental factors such as habitat, topography, type of substrate, distribution of seagrass species, and human activities around the seagrass (UNEP 2008; Gonzalez-Correa et al. 2007; Waycott et al. 2005).

Utilization of and threats to seagrass ecosystems

Seagrass ecosystem have very important role both ecologically and economically. Considering that role then it needs to be protected from threats that could undermine its existence and reduce its functions. Threats that can damage seagrass can be caused by human activities and events that occur naturally. Based on observations in the field, the important role and the threat contained in seagrass ecosystems in some areas located in three ecoregions Indonesia are presented in Table 6.

Seagrass ecosystems in three ecoregions in Indonesia have a role as catchment area of biota, which can be used, as a food source for coastal communities. It shows that seagrass plays a role as a feeding and nursery area ground making many-associated biota live in seagrass. The number of biota living in seagrass ecosystems indicates that these ecosystems bring benefits as a source of food security for coastal communities (Cullen-Unsworth et al. 2013). Most people use gears such as nets (gill net), spear gun or a hand to catch organisms in seagrass and consume them as food. If the catch result is in excess, it is sold to the public. The majority of captured organisms are squid, gastropods, fish and shellfish.

Another important point is the role of seagrass as a feeding area for *Dugong dugong*. Dugong is one of the marine herbivorous mammals, which have a very high dependence on seagrass (Preen 1995). Dugongs like high-density seagrass areas (Bujang and Mutaharah 2002), and they are founding Riau Islands (Sunda Shelf) and Tanimbar Islands (Banda Sea). *Halophila* sp., *Halodule* sp., and *Syringodium* sp. are seagrass species favored by Dugong (Heinsohn and Birch 1972). These seagrass species are very common to be found in Riau Islands and Tanimbar Islands.

The threats to seagrass in Seribu Islands (Sunda Shelf) were mostly derived from human activities. Seribu Islands region is a tourist destination and located near the Bay of Jakarta making it prone to conditions that threaten the sustainability of seagrass ecosystems. Some of seagrass ecosystems found in Riau Islands are now considered as protected areas and this has made seagrass remain in good condition and alive. Meanwhile, seagrass at Talaud Islands (Celebes Sea) and Tanimbar Islands (Banda Sea) tended to be free from threats from human activities as human population in the two islands was still low. This condition

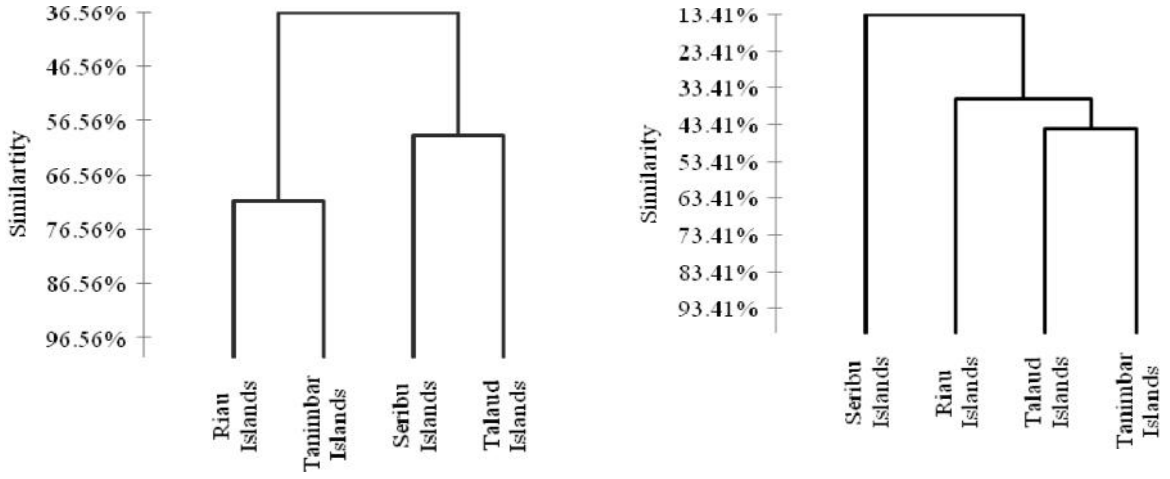

Figure 4. Dendogram of seagrass cluster analysis in three ecoregions of Indonesia (4 islands) based on the density (left), the cover percentage of seagrass (right)

Table 6. The important role of and the threats to seagrass in three ecoregions (4 Islands) in Indonesia

Ecoregion/Island/ Location	Habitat Association	The important role of seagrass	Threat	
			Human activity	Natural
Sunda Shelf (Riau Islands): Penggudang, Busung	Inter-tidal	• Habitat for gastropods, bivalves, fish, sea cucumber, echinoderms • Feeding ground for dugongs • Small-scale fisheries and traditional fishing, recreation area • Some areas are used for seagrass conservation	• Oil spill • Ocean pollution	• Sedimentation
Sunda Shelf (Seribu Islands): Pramuka Island, Pari Island	Inter-tidal	• Habitat for gastropods, bivalves, fish, sea cucumber, echinoderms • Capture fisheries and small-scale traditional learning area • Recreation area	• Ocean pollution • Reclamation • Transportation • Tourism	• Macroalgae invation • Lowest ebb • Transportation
Sulawesi Sea (Talaud Islands): Karokotan Islands	Inter-tidal	• Habitat for gastropods, bivalves, fish, sea cucumber, echinoderms • Capture and small-scale traditional fisheries	• Transportation	• High Wave
Banda Sea (Tanimbar Islands): Yamdena Island, Selaru Island, Larat Island	Reef flat	• Habitat for gastropods, bivalves, fish, sea cucumber, echinoderms • Feeding Ground for Dugong and turtle • Capture fisheries and small-scale traditional • Local wisdom "SASI" to protect seagrass.	• Marine culture • Transportation	• Sedimentation • Lowest ebb

was also supported by the local knowledge on local community applied in managing marine resources. One of these local wisdoms was called SASI. Closing fishing access for a certain period of time did management with SASI system. During this closure public were forbidden to take biota that live in the seagrass. This aimed to provide opportunity for organisms to reproduce and maintain the survival of organisms that live in the seagrass. People who violated SASI would be socially punished. Communities were allowed to take biota found in seagrass when SASI was revoked.

To summary, there were 10 species of seagrass found in three marine ecoregions of Indonesia (Sunda Shelf, Sulawesi Sea, and Banda Sea). Distribution and seagrass density was highest (61%) in Riau Islands waters (Sunda Shelf Ecoregion) and was dominated by *T. hemprichii*. Meanwhile, the value of seagrass cover in Tanimbar Islands was 60%, in Talaud Islands43%, and in Seribu Islands 37%. Based on their seagrass density, these marine ecoregions could be put in two groups namely Talaud Islands (Sulawesi Marine Ecoregion) and Seribu Islands (Sunda Shelf Ecoregion) as group one and Riau Islands (Sunda Shelf Ecoregion) and Tanimbar Islands (Banda Ecoregion) as group two. Meanwhile, based on the lack of coverage three groups were obtained, namely Tanimbar Islands and Talaud Islands as the first group, Riau Islands as the second group, and Seribu Islands as the third group. Marine ecoregions in Indonesia did not really affect seagrass, but the magnitude of the threat from human activities was the major cause of damage to the aquatic environment and substrate conditions. Seagrass had a very important role in maintaining the survival of some aquatic biota. Some predators could be used as a food source for coastal communities. Therefore, seagrass brought a very important benefit as a direct positive impact to the coastal communities in the territory of Indonesia.

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Morphology, anatomy, and mycorrhizal fungi colonization in roots of epiphytic orchids of Sempu Island, East Java, Indonesia

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Manuscript received: 18 February 2016. Revision accepted: 25 July 2016.

Abstract. *Nurfadilah S, Yulia ND, Ariyanti EE. 2016. Morphology, anatomy, and mycorrhizal fungi colonisation in roots of epiphytic orchids of Sempu Island, East Java, Indonesia. Biodiversitas 17: 592-603.* Roots of orchids have important role for survival, adaptation, water and nutrient absorption, and as a place of symbiosis with mycorrhizal fungi. The present study aimed to investigate the morphology, anatomy, and mycorrhizal status in roots of orchids of Sempu Island, Indonesia (*Ascochilus emarginatus, Taeniophyllum biocellatum*, and *Thrixspermum subulatum*), in relation to their adaptation to their habitat of coastal forests of Sempu Island. These orchids have different morphological characters; *Ascochilus emarginatus* and *Thrixspermum subulatum* are leafy orchids, while *Taeniophyllum biocellatum* is a leafless orchid. The results showed that all orchids have small number of velamen layers (1-2 layers) as an adaptation to the relatively humid condition. Cell wall thickenings of velamen, exodermis, and endodermis are structural adaptation of all orchids to the relatively high intensity of illumination, to reduce water loss because of transpiration. Mycorrhizal fungi colonization which is important for nutrient acquisition occurs in cortical cells. All orchids have differences in their cell shape, size, and specific characters, such as chloroplasts. The leafless *Taeniophyllum biocellatum* has many chloroplasts in the cortical root cells that support the photosynthesis process, while *A. emarginatus* and *T. subulatum* are lack of chloroplasts in their cortical root cells.

Keywords: Anatomy, morphology, orchids, Sempu Island, symbiotic association

INTRODUCTION

Orchidaceae is one of the most diverse and the greatest plant families containing 25,000-30,000 species worldwide. They have various morphologies with specialized features that allow the family to thrive in different environments and to occupy diverse habitat types. The morphological structures of vegetative organs are specifically variable among species (Dressler 1993). Some orchids are leafy, while some others are leafless.

Root of orchids is a vital vegetative part that has important role for survival, adaptation, water absorption, nutrient acquisition, and as a place of symbiosis with mycorrhizal fungi. There is specialization in the anatomical structure of orchid roots consisting of components that support the function of the roots and to adapt to specific environments (Figueroa et al. 2008; Moreira et al. 2013). For example, large number of velamen layers is related to the orchids growing in arid and dry areas, while small number of velamen layers is associated with orchids from relatively humid areas (Dycus and Knudson 1957; Sanford and Adanlawo 1973). Another specialization in orchid roots is the colonization of mycorrhizal fungi in the cortical cells of orchid roots. Orchidaceae is characterized by its symbiotic association with mycorrhizal fungi partly or in its entire life cycle (from early development to the adult stage of orchids). Orchids are highly dependent on the mycorrhizal fungi as it is a nutrient supplier for the orchids. Mycorrhizal fungi are known to have capacity to absorb nutrients from soil or other substrates and transfer a proportion of the nutrients to the orchids (Smith et al. 1994; Rasmussen 2002; Nurfadilah et al. 2013). In the early development of orchids, mycorrhizal fungi colonize orchid seeds that are tiny and lack of nutrient reserves. Colonization of mycorrhizal fungi in the orchid seeds is important for seed germination and seedling development (Arditti 1991; Dearnaley and McGee 1996; Swarts and Dixon 2009; Steinfort et al. 2010). In the adult stage of orchids, mycorrhizal fungi colonize the orchid roots on the organ that contacts with soil or other substrates, in which mycorrhizal fungi live and grow (Brundrett 1991; Batty et al. 2002; Kristiansen et al. 2004; Stark et al. 2009; Steinfort et al. 2010; Sakamoto et al. 2015). The mycorrhizal fungi facilitate to absorb nutrients from soil or substrates for more effective nutrient uptake.

The aim of the present study was to investigate the morphology, anatomy, and mycorrhizal status in roots of epiphytic orchids of Sempu Island (*Ascochilus emarginatus*, *Taeniophyllum biocellatum*, and *Thrixspermum subulatum*). These orchids have different morphological characters; *Ascochilus emarginatus* and *Thrixspermum subulatum* are leafy orchids, while *Taeniophyllum biocellatum* is a leafless orchid. Sempu Island is a small island off the south coast of East Java province, Indonesia and has an area of 877 ha. Little is known about the biology and ecology in this small island, especially the biology and ecology of orchids. It is administratively located in Malang Regency, East Java. The coastline is mainly composed of limestone cliffs, off the southern part of East Java in Indian Ocean.

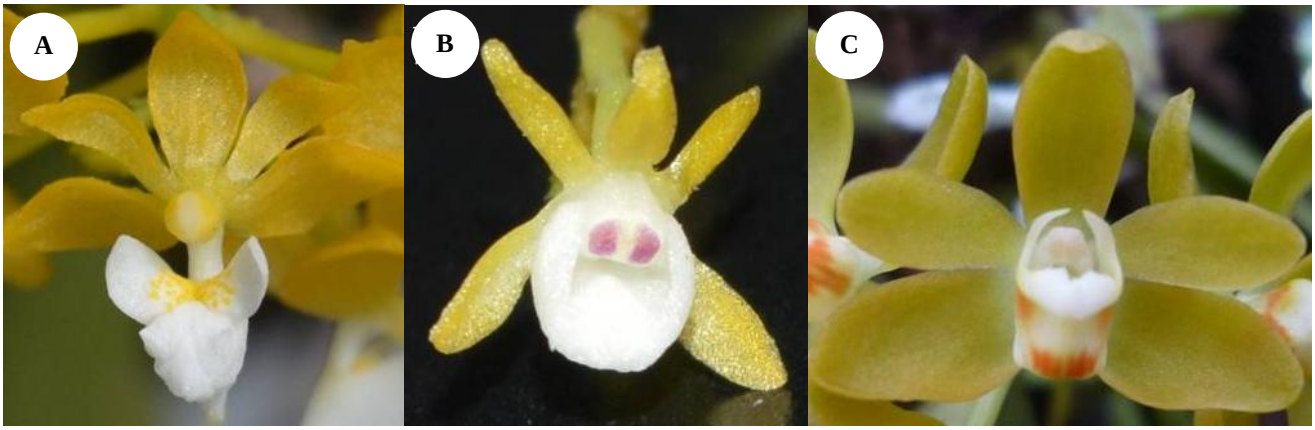

Figure 1. Orchids of Sempu Island, East Java. A. *Ascochilus emarginatus*, B. *Taeniophyllum biocellatum*, C. *Thrixspermum subulatum*

Figure 2. The vegetative organs of orchids of Sempu Island. A. The leafy orchid *Ascochilus emarginatus*, B. The leafless orchid *Taeniophyllum biocellatum*, C. The leafy orchid *Thrixspermum subulatum*

The ecosystems are characterized by coastal forests. The island is a nature reserve under the Ministry of Forestry. Present study also aimed to reveal biology and ecology of orchids in this small island to support orchid conservation programs.

MATERIALS AND METHODS

Materials

Roots of orchids of Sempu Island (*Ascochilus emarginatus* (Blume) Schuit, *Taeniophyllum biocellatum* J. J. Sm., and *Thrixspermum subulatum*), FAA (Formaldehyde Acetic Acid), 70% ethanol, 0.01% Fuchsin acid, glycerol, microtome, object glass, cover glass.

Methods

Roots of epiphytic orchids from areas of Air Tawar and Teluk Semut of Sempu Island (*Ascochilus emarginatus*, *Taeniophyllum biocellatum*, and *Thrixspermum subulatum*) were collected. These areas are relatively humid. The orchids grow on host trees in illuminated areas.

The orchid roots were fixed in FAA (Formaldehyde Acetic Acid) for several days, and transferred to 70% ethanol for several days. The roots were sectioned transversally with a microtome. The slices were stained with 0.01% Fuchsin acid or Methylene blue for one night, mounted in glycerol, and observed under microscope.

The characterization is based on the morphological features, anatomical characters and mycorrhizal fungi colonization. The morphological features of all orchids were characterized. Anatomical characters of velamen, exodermis, passage cells, tilosomes, cortex, endodermis, vascular bundles, and other characters (such as the presence of tilosomes, chloroplasts, and supraendodermal cells) were observed under light microscope. The cell size of orchid roots was measured using micrometer. Mycorrhizal fungi colonization was also screened from the outer part to the inner part of orchids.

RESULTS AND DISCUSSION

The results showed that all orchids of Sempu Island (*Ascochilus emarginatus*, *Taeniophyllum biocellatum*, and *Thrixspermum subulatum*) in the study had similarity and difference in the morphological and anatomical characters (Table 1, 2, and 3). The study also demonstrated that mycorrhizal fungi were present in orchid roots, colonized orchid roots and formed symbiotic association with the orchid roots. The anatomical features of roots of these orchids are related to their habitat in the coastal forests of Sempu Island.

Morphological characters

All orchids of Sempu Island in the study has morphological similarity in the presence of roots, while differences of morphological characters among orchids are clearly seen in the presence and absence of leaves, the shape and the color of the roots (Table 1). Leaves are present in *Ascochilus emarginatus* and *Thrixspermum subulatum*, while they are absent in *Taeniophyllum biocellatum.* Although leaves are absent in *T. biocellatum*, it has green roots, indicating the presence of the chloroplasts in its roots. Chloroplast is known as an essential and indispensable component for photosynthesis. The presence of chloroplasts in *T. biocellatum* is confirmed in the anatomical characters of roots of *T. biocellatum* (Table 2; Figure 4.D). *Ascochilus emarginatus* and *T. subulatum* have white greyish colored roots. Their anatomical characters show that chloroplast cells are not clear or absent in their roots.

Anatomical characters of roots of epiphytic orchids

The anatomical organization of roots of orchids of Sempu Island showed that components forming roots consist of velamen, exodermis with the passage cells, cortex, endodermis, vascular bundles, and pith (Table 2). There are specific characters, such as chloroplast, spiral thickening, and supraendodermal cells for particular orchid species (Table 2).

Ascochilus emarginatus

The anatomical characters of *A. emarginatus* root showed that velamen of *A. emarginatus* is uniseriate with cell wall thickening, polygonal shaped cells. There was no epivelamen (outward extension of velamen cells) (Table 2). The exodermis is a single layer with passage cells (smaller cells than exodermal cells, located between exodermal cells). The shape of the exodermis cells is elliptical. The exodermis had ∩ wall cell thickening. The cortex had six layers that can be divided into two types of cortex (outer cortex that had smaller cell size (three layers) and inner cortex that had bigger cell size (three layers)). It has rounded to polygonal shaped cortical cells. There were specific characters in *A. emarginatus* root; the presence of spiral thickening in the cortical cells and supraendodermal cells above endodermis (Figure 3.B and 3.E). Mycorrhizal fungi (pelotons; the hyphae of fungi penetrating orchid roots forming a coiled configuration) were present in cortical cells (Figure 3.D). Endodermis is 1 layer with O

Table 1. Comparative morphological characters of three orchids of Sempu Island

Anatomical characters	*Ascochilus emarginatus*	*Taeniophyllum Biocellatum*	*Thrixspermum subulatum*
Leaf	Yes	No	Yes
Root	Yes	Yes	Yes
Shape	Cylindrical	Flattened	Cylindrical
Color	White grayish	Green	White grayish

Table 2. Anatomical characters (transverse section) of the roots of orchids of Sempu Island

Anatomical characters	*Ascochilus emarginatus*	*Taeniophyllum biocellatum*	*Thrixspermum subulatum*
Epivelamen			
Epivelamen layer	-	1 layer	1 layer
Epivelamen cell shape	-	Rectangular	Round
Epivelamen cell size			
Velamen			
Velamen layer	1 layer	1 layer	1 layer
Velamen cell shape	polygonal	Rectangular	polygonal elongate;
Velamen thickening	Yes	Yes	Yes
Exodermis			
Exodermis layer	1 layer	1 layer	1 layer
Exodermis cell shape	Ellips	Polygonal	Ellips to polygonal
Exodermis cell size			
Exodermis thickening	∩	∩	O
Passage cell			
Passage cell	Yes	Yes	Yes
Tilosomes			
Tilosomes	Not clear	Not clear	Yes
Type of tilosomes	-	-	-
Cortex			
Outer cortex cell layer	3 layers	2 layers	2 layers
Outer cortex cell shape	Round to polygonal	Polygonal	Polygonal
Outer cortex cell size			
Inner cortex layer	3 layers	5 layers	6 layers
Inner cortex cell shape	Round to polygonal	Round to polygonal	Polygonal
Inner cell cortex size			
Total cortex layer	6 layers	7 layers	8 layers
Width of cortex			
Specific characters			
Chloroplast	No	Yes	No
Spiral thickening	Yes	No	No
Supra endodermal cell	Yes	No	No
Mycorrhizal fungi colonization	Yes	Yes	Yes
Endodermis			
Endodermis	1 layer	1 layer	1 layer
Endodermis thickening	O	O	O
Pericycle	1 layer	1 layer	1 layer
Vascular bundles			
Vascular bundles (archs)	8	6	20
Pith	Parenchy-matous	Parenchy-matous	Parenchy-matous

cell wall thickening. It has 1 layer of pericycle. There were 8 archs of vascular bundles composed of phloems and xylems that were embedded in the schlerenchymatous tissues. Pith is parenchymatous (Figure 3).

Taeniophyllum biocellatum

Transverse section of *T. biocellatum* root revealed that velamen is uniseriated with wall thickenings. There was a single layered of epivelamen (the extension of velamen to out side). Exodermis is one-layered with ∩ cell wall thickening, and polygonal-shaped cells. The passage cells are smaller than exodermal cells and have tilosomes (thickening above the passage cells). There were two types of cortical cells, outer cortical cells (2 layers), and inner cortical cells (5 layers). The shape of cortex is polygonal, with cell wall thickenings. Chloroplasts were present in cortical cells (Figure 4.D). Endodermis is uniseriate with cell wall thickenings. 1 layered pericycle. Vascular bundles with 6 archs consisted of phloem and xylem that were embedded in the schlerenchymatous tissues. Pith is parenchymatous (Figure 4).

Thrixspermum subulatum

Thrixspermum subulatum had uniseriate velamen periclinally with 1 layer epivelamen. The shape of velamen was polygonal to elongate, with cell wall thickenings. Exodermis was 1 layer with passage cells. The shape of exodermal cells was elliptical to polygonal. The exoermis had O cell wall thickening. Passage cells had tilosomes. Two types of cortex; outer cortex (2 layers) and inner cortex (6 layers). Endodermis was 1 layer with O thickening. Pericycle 1 layer. Vascular bundles 20 archs were composed of phloems and xylems that were embedded in the schlerenchymatous tissues. Pith was parenchymatous.

Comparison between anatomical characters of three species

Orchids of Sempu Island (*Ascochilus emarginatus*, *Taeniophyllum biocellatum*, and *Thrixspermum subulatum*) had similarity and difference in their anatomical features (Table 2 and Table 3).

Velamen

Velamen is the outermost of orchid roots. Components of velamen cell consist of cellulose with various proportions of lignin and suberin. The main functions of velamen are mechanical protection, water and nutrient absorption, reduction of transpiration and water loss, and infra red reflection (Dycus and Knudson 1957; Benzing et al. 1982, 1983; Pridgeon 1986; Moreira et al. 2013).

The orchids in the present study have 1-2 velamen layers (2 velamen layers = 1 velamen layer and 1 epivelamen: the outerward extension and the development of velamen). The velamen of these orchids had cell wall thickenings. Other orchids; both epiphytic and terrestrial orchids are reported to have velamen, with various number of velamen layers. The number of velamen layers of epiphytic orchids was various: *Dichaea cogniauxiana* (2 layers), *Epidendrum secundum* (4-5 layers) (Moreira et al. 2013), *Lueddemannia pescatorei* (11-13 layers), *Acineta densa* (12-15 layers), *Coeliopsis hyacinthosma* (4-5 layers), *Coryanthes macrantha* (7-8 layers), *Gongora galeata* (6-7 layers), *Kegeliella atropilosa* (4-5 layers) (Stern and Whitten 1999), *Catasetum fimbriatum* and *Stanhopea lietzei* (15 layers) (Oliveira and Sajo 1999). Terrestrial orchids also have various number of velamen layers: No velamen in *Zeuxine gracilis* (Muthukumar et al. 2011), single layer of velamen, but was partly replaced with an exodermis in *Neottia nidus-avis*, *Limodorum abortivum*, *Serapias orientalis* (Aybeke 2012). *Ophrys iricolor* and *O. morio* have epivelamen without any velamen (Aybeke et al. 2010). Other terrestrial orchids have a uniseriate velamen, such as *Habenaria rhodocheila* (Stern 1997) *Cranichis cochleata*, *Ponthieva ephippium*, *Goodyera brachyceras*, and *Ludisia discolor* (Figueroa et al. 2008). Other terrestrial orchids are reported to have more than one layer of velamen; such as *Bonatea steudneri* (3-4 layers) (Stern 1997), *Calypso bulbosa* (2 layers), *Tipularia discolor* (4 layers) (Stern and Carlsward 2008), *Sauroglossum nitidum* (9- 10 layers)(Moreira and Isaiaas 2008).

The difference in the number of layers of velamen indicates the adaptation of orchids to specific environments. Orchid species from arid and dry habitats were associated with multilayers of velamen, while orchid species from humid habitats were related to lack velamen or only one layer of velamen (Dycus and Knudson 1957; Sanford and Adanlawo 1973). The epiphytic orchids of Sempu Island in this study (*Ascochilus emarginatus*, *Taeniophyllum biocellatum*, and *Thrixspermum subulatum*) had similar number of velamen layers (1-2 layers). The low number of velamen layers of these orchids is related to the habitat condition of these orchids which are relatively humid in the coastal forests of Sempu Island.

All orchids of Sempu Island in the present study had velamen cell wall thickening. Velamen cell wall thickening is the result of suberin impregnation with lignified thickenings (Benzing et al. 1983; Noel 1974). Other orchids (both epiphytic and terrestrial orchids) also showed velamen thickening, while some others did not exhibit velamen cell wall thickening. The epiphytic orchids: *Catasetum fimbriatum*, *C. matogrossense*, *C. schmidtianum*, *C. apolloi*, *C. juruense*, *C. longifolium*, *C. osculatum*, and *C. saccatum* had velamen cell wall thickening (da Silva et al. 2015). Thickened velamen was also reported in other epiphytic orchids; such as *Encyclia patens*, *Sophronitis pumila*, *Polystachia estrellensis* (Moreira and Isaiaas 2008), *Epidendrum secundum* (Moreira et al. 2013). In addition, terrestrial orchids were reported to have conspicuous velamen thickening, such as orchids from tribes Spiranthinae and Prescottiinae (except Pseudocranichis) (Figueroa et al. 2008). However, other orchids showed thin-walled velamen, such as *Dichaea cogniauxiana* (Moreira et al. 2013).

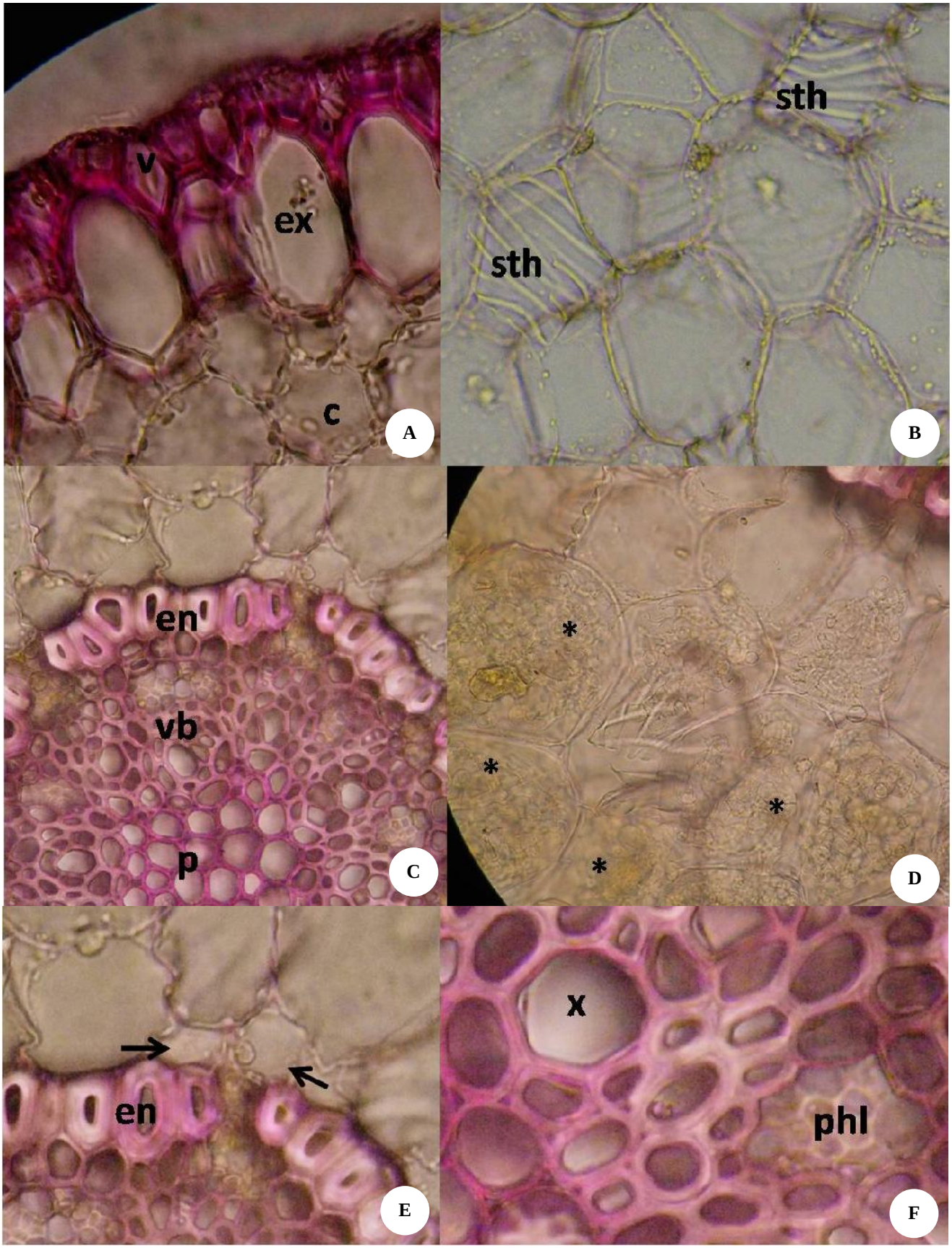

Figure 3. Root anatomy (transverse section) of *Ascochilus emarginatus*. A: epivelamen (ep); exodermis (ex); cortex (c), B: spiral thickening (sth) in cortical cells, C: endodermis (en); vascular bundles (vb); pith (p) D: cortical cells colonized by mycorrhizal fungi are marked with *, E: supraendodermal cells above endodermis (arrow), F: xylem (x); phloem (phl)

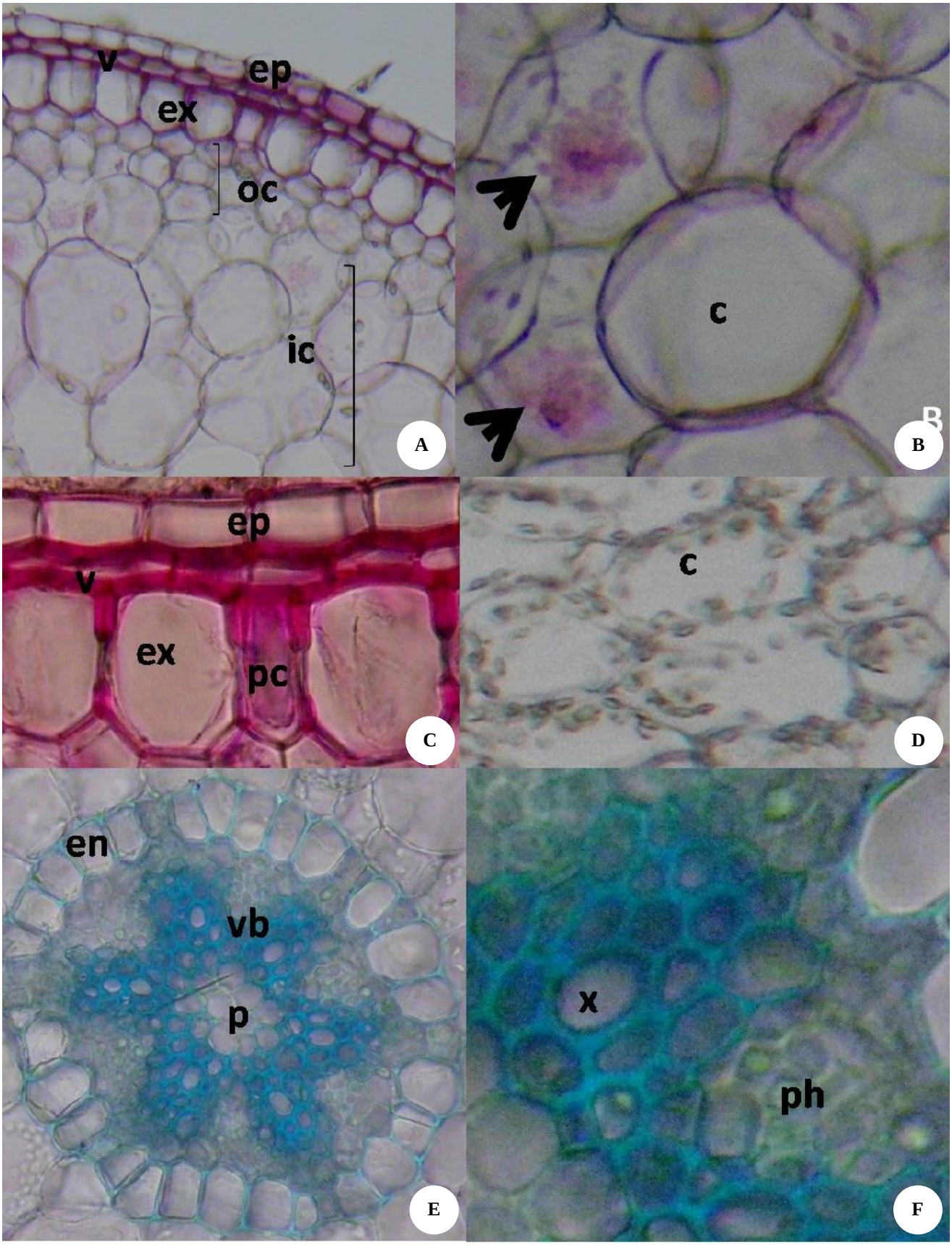

Figure 4. Root anatomy (transverse section) of *Taeniophyllum biocellatum*. A: epivelamen (ep); velamen (v); exodermis (ex); outer cortex (oc); inner cortex (ic); B: cortical cells colonized by mycorrhizal fungi (arrow head) C: passage cell (pc); D: chloroplasts in cortical cells E: endodermis (en), vascular bundles (vb), pith (p); F: xylem (x); phloem (ph)

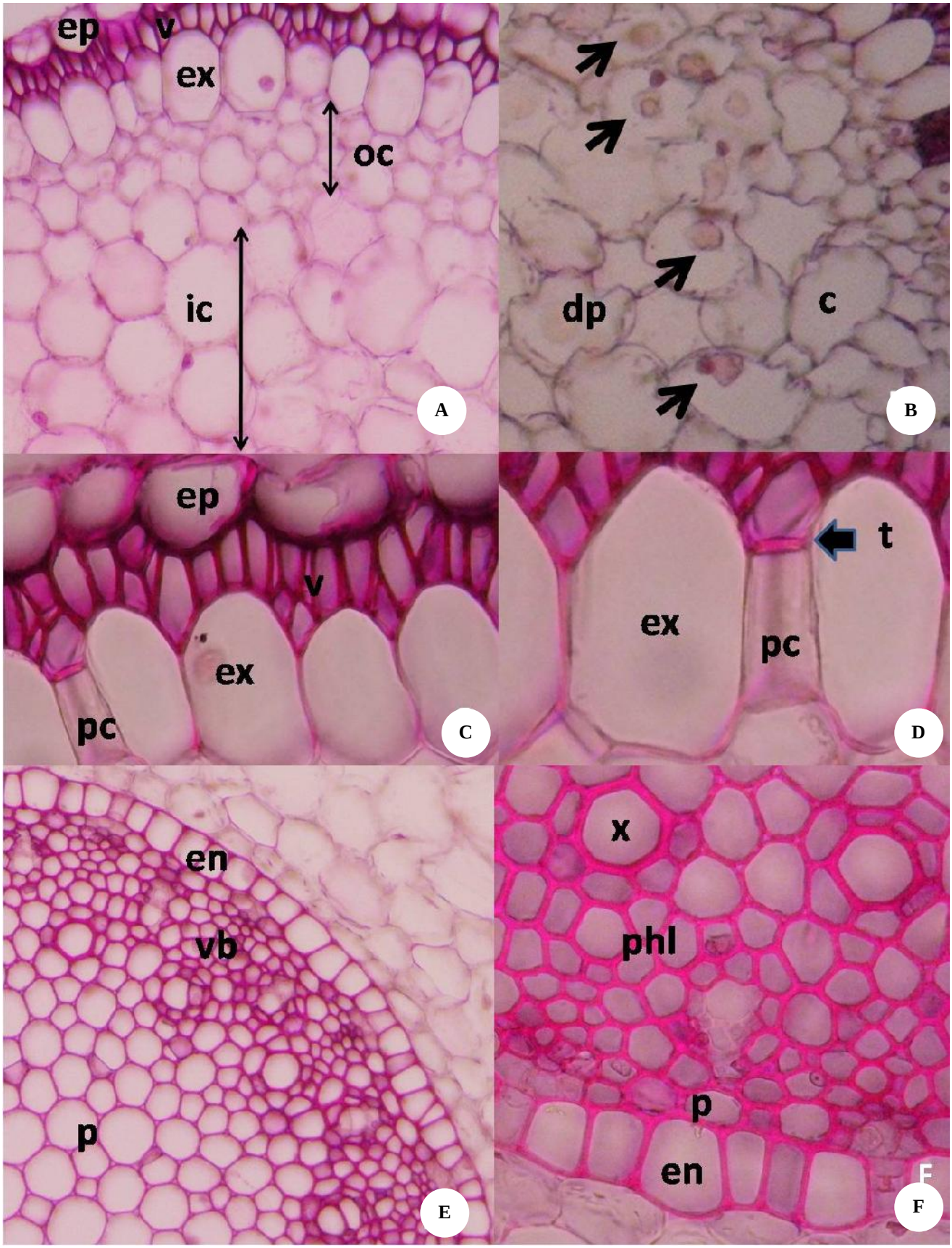

Figure 5. Root anatomy (transverse section) of *Thrixspermum subulatum.* A: epivelamen (ep); velamen (v); exodermis (ex); outer cortex (oc); inner cortex (ic), B: cortical cells colonized by mycorrhizal fungi, degenerative pelotons (dp) (arrow), C: passage cell (pc); exodermis (ex); D: passage cell (pc), tilosome (t) (arrow) E: endodermis (en), vascular bundles (vb), pith (p), F: xylem (x); phloem (phl); pericycle (p), endodermis (en)

The role of velamen cell wall thickening is for mechanical support to avoid water loss (Noel 1974; Benzing et al. 1983). The velamen cell wall thickening in orchids of Sempu Island (*Ascochilus emarginatus, Taeniophyllum biocellatum,* and *Thrixspermum subulatum*) is related to their habitat and environment. As they grow in illuminated areas and are exposed to high intensity of light, the velamen cell wall thickening is vital to reduce root transpiration and water loss. It is a part of structural adaptation to their habitat in illuminated areas in the coastal forest of Sempu Island.

Exodermis

Below the velamen layers, there is exodermis layer, which is the outer layer of cortex (Engard 1944). The exodermis cell had secondary cell wall thickenings that are empty and dead at maturity (Pridgeon 1986). The exodermis cell wall thickening is caused by lignin and suberin impregnation (Fahn 1990). The function of exodermis cell wall thickening is for mechanical protection against water evaporation, to retain moisture in the cortex, and to control the entrance of mycorrhizae in cortical cells (Benzing et al. 1983; Sanford and Adanlawo 1973; Moreira and Isaiass 2008).

Exodermis of orchids of Sempu Island (*Ascochilus emarginatus, Taeniophyllum biocellatum*, and *Thrixspermum subulatum*) in the present study is uniseriate. Most orchid species have 1 layer of exodermis. The number of exodermal layers can be more than one layer, such as in some *Ophrys* ranged from 1 to 4 (Aybeke et al. 2010). Orchids of Sempu Island in the present study exhibited exodermis cell wall thickening with various patterns of exodermis cell wall thickening. *Ascochilus emarginatus* and *Taeniophyllum biocellatum* had ∩ cell wall thickening, *Thrixspermum subulatum* had O cell wall thickening. Other orchids, both epiphytic and terrestrial orchids also had exodermis thickening with various patterns (Moreira and Isaiaas 2008). Other epiphytic orchids were reported to have thickened exodermis; such as *Epidendrum campestre* with ∩ thickening, and *Pleurothallis smithiana, Vanda discolor,* and *Encyclia calamara* with O thickening (Oliveira and Sajo 1999), *Aerangis confusa, A. coriacea, A. kirkii, Angraecum calceolus, A. conchiferum, A. teres* with ∩ cell wall thickening (Carlsward et al. 2006); *Epidendrum campestre* (Oliveira and Sajo 1999). Furthermore, terrestrial orchids also showed exodermis thickening, such as *Sobralia macrantha* (Benzing et al. 1982); *Cranichis cochleata, Ponthieva ephippium, Goodyera brachyceras,* and *Ludisia discolor* (Figueroa et al.); *Zeuxine gracilis* (Muthukumar et al. 2011).

Similar to velamen thickening, the cell wall thickening of exodermis of orchids of Sempu Island in the present study is important as the mechanical protection to reduce transpiration and water loss from cortex, as these orchids grow in illuminated areas and exposed to high intensity of light. Such as velamen thickening, exodermis thickening is also a structural adaptation in the coastal forest with high intensity of light (Moreira and Isaiass 2008).

The exodermis size and shape of orchids of Sempu Island in the present study were different (Table 2 and 3). Ellips, polygonal, ellips-polygonal are the shape of exodermis of *Ascochilus emarginatus, Taeniophyllum biocellatum,* and *Thrixspermum subulatum*, respectively. Other orchids also had various shape of exodermal cells, such as elongate to isodiametric in *Angraecum calceolus, Angraecum conchiferum, Bolusiella iridifolia, Aerangis confusa, A. coriacea, A. kirkii* (Carlsward et al. 2006).

Passage cells

Between exodermis cells, there are shorter cells that are living and have thin cell wall. Like other orchids, orchids of Sempu Island in the present study also had pasage cells that were alternately disposed between exodermis cells. Pasage cells in the exodermis layer are important for the passing of water and nutrient, and attracting mycorrhizal fungi (Peterson and Enstone 2006; Senthilkumar et al. 2000).

Tilosomes

Tilosome is the extension from the innermost cell wall of velamen cells attached to the pasage cells of exodermis. The function of tilosome is to protect from water loss via root transpiration (Pridgeon et al. 1983). The presence and absence of tilosome is one of key characters in the classification, systematics and phylogenetics of orchids (Figueroa et al. 2008). Of the three species of orchids of Sempu Island in the present study, tilosome was clearly seen in *Thrixspermum subulatum*, while it was not clear or absent in *A. emarginatus* and *Taeniophyllum biocellatum*. The presence or absence of tilosomes was also reported in other orchid species. Tilosomes were present in *Prescottia tubulosa* and *Prescottia stachyodes* (Prescottiinae) and in many species of Spiranthinae (Figueroa et al. 2008; some *Ophrys* (Aybeke et al. 2010), while tilosomes were absent in some species of Goodyerinae, Cranichidinae and Manniellinae (Figueroa et al. 2008).

Cortex

Cortex is a tissue beneath exodermis which is formed by thin walled parenchymatous cells with various sizes. Outer cortex layers are composed of small size cells, while inner cortex layers are formed by large size cells (Muthukumar et al. 2011). Number of cortex layers varied among orchid species of Sempu Island in the present study. *Ascochilus emarginatus* had 6 cortex layers (3 outer and 3 inner cortex layers), *Taeniophyllum biocellatum* exhibited 7 cortex layers (2 outer and 5 inner cortex layers), and *Thrixspermum subulatum* possessed 8 cortex layers (2 outer and 6 inner layers). Other orchid species (both epiphytic and terrestrial orchids) were reported to exhibit different number of cortex layers; such as the epiphytic orchids *Dichaea cogniauxiana* (14-16 layers); *Epidendrum secundum* (6-12 layers) (Moreira et al. 2013); while the terrestrial counterparts: *Zeuxine gracilis* (16 layers) (Muthukumar et al. 2011), *Neottia nidus-avis* (9 layers), *Cephalanthera epipactoides* (10-18 layers), *Limodorum abortivum* (18-27 layers), *Platanthera chlorantha* (layers) (Aybeke 2012).

Table 3. Comparison of the anatomical characters of roots of 3 orchids of Sempu Island based on quantitive measurements of cell and layer size of roots

Characters	*Ascochilus emarginatus*	*Taeniophyllum biocellatum*	*Thrixspermum subulatum*
Transverse section width	935,13 ± 37,51 (a)	1787,83 ± 2,94 (c)	1285,5 ± 2,81 (b)
Transverse section length	1021,8 ± 20,13 (b)	605,63 ± 3,67 (a)	1267,53 ± 10,11 (c)
Epivelamen			
Epivelamen length	0 ± 0 (a)	11,58 ± 1,11 (b)	20,85 ± 1,79 (c)
Epivelamen breadth	0 ± 0 (a)	22 ± 1,06 (b)	22 ± 1,32 (b)
Passage cell			
Passage cell length	31,68 ± 1,81 (a)	22,68 ± 1,66 (a)	32,68 ± 1,58 (a)
Passage cell breadth	15,87 ± 1,02 (a)	14,38 ± 1,18 (a)	16,12 ± 1,38 (a)
Velamen			
Velamen length	22,55 ± 1,33 (b)	6,13 ± 0,81 (a)	14,6 ± 1,24 (c)
Velamen breadth	10,93 ± 0,69 (b)	18,57 ± 1,8 (c)	6,4 ± 0,36 (a)
Velamen width	22,77 ± 4,69 (a)	18,57 ± 1,80 (a)	21,03 ± 1,90 (a)
Exodermis length	55,05 ± 2,14 (c)	27,2 ± 1,13 (a)	42,32 ± 1,11 (b)
Exodermis breadth	31,53 ± 1,46 (b)	24,4 ± 3,13 (ab)	22,83 ± 1,05 (a)
Cortex			
Outer cortex length	23,1 ±1,91 (b)	17,83 ± 0,7 (a)	17,47 ± 0,74 (a)
Outer cortex breadth	21,02 ± 2,17 (a)	20,13 ± 1,47 (a)	18,05 ± 1,41 (a)
Inner cortex length	67,95 ± 3,94 (b)	62,62 ± 5,35 (ab)	45,88 ± 2,59 (a)
Inner cortex breadth	67,93 ± 6,27 (a)	50,6 ± 4,59 (a)	50,92 ± 2,33 (a)
Outer cortex layer	59,68 ± 5,97 (ab)	39,4 ± 4,08 (a)	70,78 ± 6,13 (b)
Inner cortex layer	242,95 ± 8,08 (b)	161,38 ± 8,41 (a)	221,02 ± 12,58 (b)
Stele			
Stele width	201,33 ± 1,61 (b)	108,72 ± 3,44 (a)	511,73 ± 4,97 (c)
Endodermis length	19,22 ± 0,65 (a)	19,58 ± 3,57 (a)	15,68 ± 1,00 (a)
Endodermis breadth	10,8 ± 0,68 (a)	17,23 ± 5,45 (a)	13,7 ± 0,97 (a)
Pith	73,25 ± 4,09 (b)	18,58 ± 1,81 (a)	339,97 ± 3,53 (c)
Vascular bundle	8 ± 0 (b)	6 ± 0 (a)	20 ± 0 (c)

Spiral thickening in root cortical cells can be a key character of orchid species. In the present study, *Ascochilus emarginatus* had spiral thickening in the cortex, while *Taeniophyllum biocellatum*, and *Thrixspermum subulatum* did not exhibit the presence of spiral thickening in their cortex. Other orchid species were reported to have spiral thickening in their root cortical cells, such as *Eulophia epidendraea* and *Malaxis acuminata* (Uma et al. 2015), *Catasetum schmidtianum*, and *C. juruense* (da Silva et al. 2015). Leroux et al. (2010) suggested that spiral thickening in cortical cells functions as mechanical protection, prevention from desiccation because of root transpiration, and for more efficient water and nutrient uptake.

In the present study, chloroplasts were present in cortical cells of *Taeniophyllum biocellatum*, but they were absent in cortex of *Ascochilus emarginatus* and *Thrixspermum subulatum.* Chloroplasts contain chlorophyll that is important for photosynthesis. They usually occur in leaf cortical cells,. The presence of chloroplasts in root cortical cells of *T. biocellatum* may be related to the life form of *T. biocellatum* that do not have leaves (leafless), and they evolve green roots containing chloroplast for photosynthesis to survive. Chloroplasts were not clear or absent in root cortex *A. emarginatus* and *Thrixspermum subulatum* may be related to their life form in possessing leaves that contain chloroplasts. The color of roots of *A. emarginatus* and *T. subulatum* were white grayish (Table 1). This indicated there were no chloroplasts, as chloroplasts are associated with green colored parts.

Endodermis

Endodermis is a layer beneath cortical cells that protect the inner parts (vascular bundles and pith). Some orchids have secondary endodermal cell wall thickening, while some others exhibit thin walled endodermis. Epiphytic orchids *Angraecopsis parviflora, Microcoelia bulbocalcarata, M. corallina* and *M. stolzii* had thin walled endodermis (Carlsward et al. 2006). Terrestrial orchids that had thin walled endodermis included *Zeuxine gracilis* (Muthukumar et al. 2011); *Habenaria arenaria, H. cornuta, H. odontopetala, H. snowdenii, Stenoglottis fimbriata, S. longifolia, S. woodii* (Stern 1997) Other orchids exhibited secondary cell wall thickening of endodermis, such as *Bolusiella batesii, B. iridifolia, Microcoelia aphylla* (Carlsward et al. 2006). Endodermal thickening also occurred in *Acineta densa, Lueddemannia*

pescatorei, *Polycynis gratiosa*, *Stanhopea candida* with ∩ endodermal thickening, *Cirrhaea dependens*, *Stanhopea pulla*, *Stanhopea panamensis* (Stern and Whitten 1999). Similar to exodermis, the pattern of cell wall thickening varied, such as ∩ and O thickening.

In the present study, the endodermis of orchids of Sempu Island (*A. emarginatus*, *Taeniophyllum biocellatum*, and *Thrixspermum subulatum*) had O cell wall thickening. The function of cell wall thickening in endodermis is similar to that of exodermis and velamen, as mechanical protection and as prevention against water loss because of root transpiration. This feature of endodermal cell wall thickening in orchids of Sempu Island can be an adaptation to their habitat that are exposed to high intensity of light in the coastal forest of Sempu Island.

Supraendodermal spaces were observed above endodermis of *A. emarginatus*, while they were absent in *Taeniophyllum biocellatum*, and *Thrixspermum subulatum.* Supraendodermal spaces are small intercellular spaces that occur outside the endodermis. This feature is also a key character in the classification and phylogenetic information (Figueroa et al. 2008). Some orchid species have supraendodermal spaces, while some others do not. It was reported that some orchids having supraendodermal spaces were *Pseudocranichis thysanochila*, *Aulosepalum pyramidale*, *Mesadenus lucayanus*, *Microthelys constricta*, *Sacoila lanceolata*, while those having no supraendodermal were *Cranichis cochleata*, *Goodyera brachyceras*, *Ludisia discolor*, *Manniella gustavi*, *Prescottia tubulosa* (Figueroa et al. 2008). Most of species having supraendodermal spaces occur in high transpiration areas in seasonally dry habitats (Figueroa et al. 2008). The presence of supraendodermal spaces in roots of *A. emarginatus* indicated for more effective protection against root transpiration as this orchid grow in areas of high intensity of light in the coastal forests of Sempu Island.

Vascular bundles

Vascular bundle is a transport system containing xylem and phloem that are important in the transport of water and nutrients. The number of archs in vascular bundles of orchids of Sempu Island in the present study was different. *Ascochilus emarginatus*, *Taeniophyllum biocellatum*, and *Thrixspermum subulatum* had 8, 6 and 20 archs, respectively. Number of archs in vascular bundles is notably various between orchid species (Oliveira and Sajo 1999). Other orchid species showed different number of archs in the vascular bundles, such as *Neottia nidus-avis* (3 archs), *Cephalanthera epipactoides* (7-11 archs), *Limodorum abortivum*, (9-25) *Platanthera chlorantha* (5-10 archs) (Aybeke 2012).

Pith

Pith of orchid is the central part in roots and is composed of parenchym or schlerenchym. The pith of all orchids of Sempu Island in the present study is parenchymatous. Pith of *Microcoelia corallina* was parenchymatous, while the pith of *Angraecopsis breviloba*, *Microcoelia globulosa*, *Microterangis hildebrandtii* was schlerenchymatous (Carlsward et al. 2006).

Mycorrhizal fungi colonization

The results of the present study showed the presence of mycorrhizal fungi (pelotoons) in orchid roots of Sempu Island (*A. emarginatus*, *Taeniophyllum biocellatum*, and *Thrixspermum subulatum*) (Figure 3, 4, and 5). There is a process in the colonization of mycorrhizal fungi in orchid roots. The mycorrhizal fungi entered the roots through the velamen layers and moved into the inner layers of the roots (exodermis). The exodermis was not colonized by mycorrhizal fungi. This is related to the thick structure of exodermis because of cell wall thickening (Schreiber and Franke 2011). The mycorrhizal fungi were able to penetrate the exodermis through *passage cells* in the exodermis layer (Senthilkumar et al.2000). Passage cells have thin wall that make them possible to be penetrated by mycorrhizal fungi. Passage cells in the exodermis also have specific function to attract mycorrhizal fungi (Peterson and Enstone 2006). The thickened structure of exodermis is important as mechanical protection and to avoid unwanted compounds such as toxin and pathogen microorganisms. Wanted compounds (ions, nutrients) and symbiotic mycorrhizal fungi were able to penetrate the exodermis through the passage cells in the exodermis layer. After entering the passage cells, mycorrhizal fungi colonized the inner layer (cortex cells) (Oliveira and Sajo 1999; Schreiber and Franke 2001; Moreira and Isaias 2008; Matsuda et al.2009; Muthukumar et al.2011)

The distribution and the pattern of mycorrhizal fungi colonization in roots of *A. emarginatus*, *T. biocellatum*, and *T. subulatum* in the present study were similar. The mycorrhizal fungi only colonized the cortex of the roots. The colonization of mycorrhizal fungi forms a coiled configuration of mycorrhizal fungi hyphae (pelotons) in cortex cells. Pelotons were not found in the endodermis or stele (endodermis, phloem, xylem, and pith). The pelotons were not found in epidermis and exodermis either. Other studies reported that orchids from Japan, India, Brazil, South America, and Europe also showed similar results that colonization of mycorrhizal fungi occurred in cortex cells and was not found in other root layers (Oliveira and Sajo 1999; Senthilkumar et al. 2000; Yagame et al. 2008; Fracchia et al.2009; Látalová and Balaz 2010; Muthukumar et al. 2011; Hadley and Williamson 1972).

In the symbiotic association between orchids and the mycorrhizal fungi, orchids absolutely rely on the mycorrhizal fungi in their entire life cycle, while the mycorrhizal fungi can survive without the orchids as the mycorrhizal fungi are saprophytic that have a capacity to absorb nutrients from soil or other substrates (Smith et al. 1994; Rasmussen 2002; Nurfadilah et al.2013). In the relationship with the orchids, mycorrhizal fungi transfer nutrients to the orchids and obtain photosynthat (carbon) transfer from the orchids (Rasmussen 2002; Cameron et al. 2006).

In the perspective of orchids in the symbiotic association with the mycorrhizal fungi, orchids highly depend on the mycorrhizal fungi from the early growth and development of the orchids to the adult stage. Orchid seeds are tiny and lack of nutrient reserves, thus they need external nutrients for seed germination and seedling

development. The external nutrients are fulfilled by nutrients transferred by the mycorrhizal fungi. While the orchids have a photosynthetic capacity, the symbiotic association still has an important role to maximize the nutrient uptakes (Arditti 1991; Dearnaley 2007; Swarts and Dixon 2009).

Implication for conservation

Understanding the biology and ecology of orchids is important in the conservation of orchids. The present study is one part to increase understanding on the morphology, anatomy of orchids of Sempu Island in relation to the ecology of orchids in the coastal forests of Sempu Island.

The finding of the present study on the symbiotic association between orchids and the mycorrhizal fungi increases understanding of the biology and ecology of orchids. The implication of this finding is important in the management of orchid conservation that the conservation of orchids needs to include the conservation of the mycorrhizal fungi as their associates. Orchids are known highly rely on the mycorrhizal fungi in orchid's entire life cycle. Thus, for the existence and the survival of orchids, the conservation of both orchids and mycorrhizal fungi associates is required.

The present study has demonstrated that the colonization of mycorrhizal fungi of all epiphytic orchids of Sempu Island (*Ascochilus emarginatus*, *Taeniophyllum biocellatum*, and *Thrixspermum subulatum*) was similar. These results indicate the symbiotic association of orchid roots and mycorrhizal fungi. The present study increases understanding of the biology of orchids in Indonesia, in terms of its symbiotic association with mycorrhizal fungi. The implication of this finding is important in the management of orchid conservation that the conservation of orchids needs to include the conservation of the mycorrhizal fungi associates.

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Short Communication: Resistance of eleven new hybrid maize genotypes to Turcicum leaf blight (*Exserohilum turcicum*)

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Abstract. *Setyawan B, Suliansyah I, Anwar A, Swasti E. 2016. Short Communication: Antidiabetic screening of some Indonesian marine cyanobacteria collection. Biodiversitas 17: 604-608.* Turcicum leaf blight (TLB) is a leaf disease caused by the fungus *Exserohilum turcicum* (Pass.) Leonard and Suggs. In Indonesia, TLB was first discovered in North Sumatra in 1917 (Van Hall 1929), and now is found throughout Indonesia (Semangun 2008). Losses due to yield decrease will be greater when the plant is infected at the time of flowering and grain filling phase. Resistant varieties are the most effective way of controlling TLB. The purpose of this research was to test 11 new hybrid maize genotypes to determine the level of TLB resistance. The research was conducted in 2 season, using randomize complete block design, 3 replication and 2 control genotypes. Based on statistical examinations and CIMMYT (1999) scoring system, it could be concluded that 10 prospective genotypes (90.9%) which were SSU3X28871, SSU3X29131, SSU3X30735, SSU3X45172, SSU3X68276, SSUSX02791, SSUSX06145, SSUSX48274, SSUSX68849 and SSUSX76844 were significantly better than both control genotypes at LSD 5% (α=0.05).

Keywords: Disease, genotype, leaf blight, maize, turcicum
Abbreviations: CIMMYT: Centro Internacional de Mejoramiento de Maiz y Trigo (International Maize and Wheat Improvement Center), DAP: days after planting, DS: dry season, OPV: open pollinated variety, LR: less resistant, LSD: least significant difference, R: resistant, RS: rainy season, S: susceptible, TLB: turcicum leaf blight, VR: very resistant, VS: very susceptible.

INTRODUCTION

Turcicum leaf blight (TLB) is a leaf disease caused by the fungus *Exserohilum turcicum* (Pass.) Leonard and Suggs. (Semangun 2008). TLB can result in decreased yield when infects during the flowering phase. The development of this disease is strongly influenced by the resistance of varieties, cultivation systems and the weather/climate (Carson 1995). The disease also infects the corn plant in India with a loss rate from 28% to 91% (Pant et al. 2001; Singh et al. 2012; Ishfaq et al. 2014; Nwanosike et al. 2015) and until 2005 had been found at least three races, i.e. Race 2, Race 3 and Race 4 (Dutta et al. 2005).

Right now the disease has spread throughout the world with several different races such as Race 0,1,2,3, N, 12,13,13N, 3N, 123.23, and 23N which were found in Kenya, Germany and Austria (Muiru et al. 2010). TLB was also found in Uganda (Castiano et al. 2012), Thailand (Wathaneeyawech et al. 2015), Argentina (Sartori et al. 2015) as well as other countries in Asia, Africa, Europe, Australia and America. In Indonesia, TLB was first discovered in North Sumatra in 1917 (Van Hall 1929). At this moment the disease has been widespreaded throughout Indonesia (Semangun 2008). Specific research had been conducted in South Sulawesi (Surtikanti 2009) and Batu, Malang (Latifahani et al. 2014).

TLB is potential in areas where the air temperature drops at night while the air humidity is high. The fungus releases many conidia at noon after a warm night with a relative humidity above 90%. The optimum temperature for the formation of conidia is 20-26^{o}C. Infection takes 6-18 hours at a temperature of 18-17°C. As known, this disease can infect plants from germination to harvest time. Losses due to yield decrease will be greater if the plants were infected during the flowering and grain filling phase (Semangun 2008). TLB damages or even kills the leaf tissue, and it will decrease the amount of chlorophyll where the carbohydrate, fat and protein are produced in plants. It was reported about 91% reduction in the rate of photosynthesis when severity of turcicum leaf blight incidence in maize exceeded 50% (Reddy et al. 2014). When the leaf area that die from this disease is quite large, yield will decrease. As a result of the breadth of green leaves die, the formation of starch will be retarded and the grains produced will be empty (chaffy). The leaves which withered as a result of this disease are not eligible to be used as animal feed (fodder) because it has lost all of the containing nutrients (Semangun 2008; Reddy et al. 2013). The degree of infection is determined by the disease resistance of the plants, because plant resistance can reduce the number of patches that cause chlorotic and necrotic (Semangun 2008). Growing resistant varieties is recommended because of it most effective way to control

this disease and safe for the environment (Pattaky 1992; Semangun 2008).

To determine the resistance of a certain cultivar to this disease can be carried by research. The research can be established in the field, in the greenhouse or utilizing molecular marker (Inghelandt et al. 2012). Research regarding TLB had been done in Indonesia. These researchs were purposed to determine the resistance of existing cultivars on the market of South Sulawesi Province and East Java Province. Both researchs were conducted in the field and the laboratory. Isolates used were common TLB isolates which were taken from the farmer fields (uncharacterized/ unknown races). The results showed higher virulence and decreased yield (Surtikanti 2009 and Latifahani et al. 2014).Therefore, the resistance research of maize varieties against TLB is a mandatory.

The purpose of this research was to determine the resistance level of 11 (eleven) prospective genotypes (tested genotypes) to TLB infection. Prospective genotypes which had resistance level equal to or more superior than BISI 18 would be included in multi-location trials in order to release national new superior varieties.

MATERIALS AND METHODS

Research materials

This research used 13 materials. The materials of this research consisted of 11 new prospective hybrid corn varieties (genotypes) with two control varieties that had already existed in the market, namely BISI 18 and Sukmaraga. BISI 18 was representing less resistant (LR) and hybrid cultivars, while Sukmaraga was representing the resistant (R) ones (Ministry of Agriculture 2013). Sukmaraga also represented OPV cultivars due to its progenitor random cross pollination during the production of the seed.

The above mentioned 11 prospective genotypes consisted of six threeway cross hybrids and five single cross ones. These prospective genotypes were the outcome of the author breeding program which was began in 1997. The prospective genotype progenitors were inbred lines which were extracted from landrace populations introduced from 7 countries (USA, Mexico, Colombia, India, Thailand, Malaysia, Philippines) and some indigenous landraces of some areas in Indonesia. Based on their progenitors resilience, these 11 prospective genotypes were expected to be classified as resistant (R) or very resitant (VR) cultivars according to CIMMYT (1999) scoring system which is being adopted by The Variety Assessment and Release Team of The Republic of Indonesia. The complete data on 11 new prospective genotypes and the control ones, is presented in Table 1.

Methods

The research used randomized complete block design with three replications. Each plot size of 5 m x 2.8 m was tillaged with a complete tillage system (first plowing, second plowing after 14 days interval and harrowing 14 days after second plowing). Each plot consisted of 4 (four) rows with a spacing of 70 cm x 20 cm. Research material were planted in the plot with 2 (two) seeds per hole, therefore 200 plants per plot were expected at planting time (50 seeds per row x 4 rows). The first thinning was done before the first fertilization by cutting unwanted plants especially at holes which consisted 2 plants. At this time 120 plants remained per plot (30 plants per row x 4 rows) regardless plant-count per hole. Second thinning was done before the second (last) fertilization by same method with the first thinning. At this time until the time of observation 100 plants had to be remained in one plot (25 plants per row x 4 rows) regardless plant-count per hole.

Fertilization were done two times during planting period. The first fertilization was done 14 days after planting (DAP), using Urea, SP-36 and KCl at a dose per hectare 250 kg, 100 kg and 50 kg respectively. Second fertilization is done when the plants were 30 DAP, using urea at a dose of 100 kg per hectare. The dose of fertilization was adapted from local farmers who experienced growing hybrids corn. Weeding was done right after fertilization, while irrigation was utilizing rainfall.

Table 1. Research materials

Code of Genotypes	Cross	Pedigree		TLB resistance		Expected F_1 TLB resistance	Remark
		Female parent	Male parent	Female parent	Male parent		
SSU3X17782	Threeway	SSU3X17782FF	SSUSX02791M	S	R	R	Tested
SSU3X28871	Threeway	SSU3X28871FF	SSUSX76844M	R	VR	VR	Tested
SSU3X29131	Threeway	SSU3X29131FF	SSUSX68849M	R	VR	VR	Tested
SSU3X30735	Threeway	SSU3X30735FF	SSUSX48274M	R	R	R	Tested
SSU3X45172	Threeway	SSU3X45172FF	SSUSX06145M	LR	R	R	Tested
SSU3X68276	Threeway	SSU3X68276FF	SSU3X68276M	LR	R	R	Tested
SSUSX02791	Single	SSUSX02791F	SSUSX02791M	R	R	R	Tested
SSUSX06145	Single	SSUSX06145F	SSUSX06145M	R	R	R	Tested
SSUSX48274	Single	SSUSX48274F	SSUSX48274M	LR	R	R	Tested
SSUSX68849	Single	SSUSX68849F	SSUSX68849M	VR	VR	VR	Tested
SSUSX76844	Single	SSUSX76844F	SSUSX76844M	R	VR	VR	Tested
BISI 18	Single	-	-	-	-	LR	Control
Sukmaraga	OPV	-	-	-	-	R	Control

Note: The expected TLB resistance level of BISI 18 and Sukmaraga were based on Ministry of Agriculture (2013)

Innoculation of the disease was utilizing spreader rows (sweet corn) which very susceptible to TLB. These spreader rows were planted 4 weeks prior the planting of research materials. Spreading of disease relied on nature. It was done because the research location had been classified as endemic to TLB and the research had to be conducted in the field/not at laboratory (National Seed Board 2008). The outcome of this research would be included as part of multilocation trial data in order to release new superior hybrid corn cultivars. Innoculation utilized specific race isolates was not possible to be done because characterization on TLB had been never done before this research.

Observation or data collections in this research was done through visual observation at the end of flowering stage by using scoring system (CIMMYT 1999 and National Seed Board 2008). Observations were made on the entire plot and all plants in the plot (100 plants) individually. It meant that every single plant in the plot was examinated for TLB infection and the score was given. The plot score was the average of all plants score in the respective plot. Score 1 was the best (VR) while score 5 was the worst one (VS). Scoring was based on TLB degree of infection with the guidelines presented in Figure 1 (CIMMYT 1999).

Figure 1 can be explained as follows: (i) Score 1 (very resistant/VR): there are no infections on any leaves, (ii) Score 2 (resistant/R): 2-3 leaves under ear are infected, (iii) Score 3 (less resistant/LR): infectious disease reaching 2-3 leaves upper the ear, (iv) Score 4 (susceptible/S): infection reached almost all the leaves except the 2-3 upper leaves of the plant, (v) Score 5 (very susceptible/VS): all the leaves of the plants are infected.

Location and time of research

The research was conducted at experimental field located in The Village of Kuta Kendit, District of Mardingding, Karo Regency, North Sumatra Province, Indonesia. It was conducted in two seasons, the dry season (DS) 2015 and the rainy season (RS) 2015/2016.

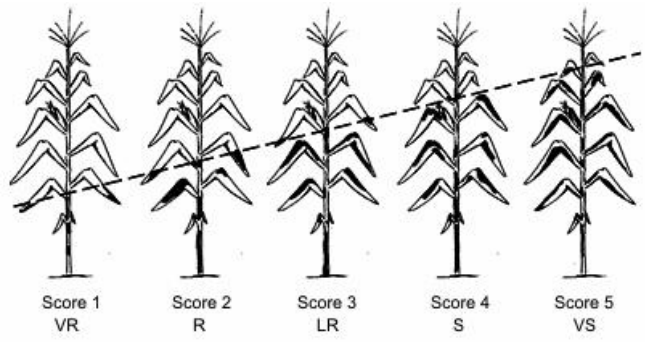

Figure 1. TLB scoring system (CIMMYT 1999)

RESULTS AND DISCUSSION

Transmission of TLB infection on this research had been going well at both season. It could be proven by the actual TLB degree of infection on BISI 18 and Sukmaraga (Table 2) which were lower than their expected level of resistance stated in Table 1 which based on Ministry of Agriculture (2013). This deteroriation was probably due to the TLB virulence level of infection which continued to be more severe over the time. This was in line with the research of Dutta et al. (2005) and Muiru et al. (2010) which stated that new races of TLB is always found all the time. Based on the above facts, it could be ascertained that the occurence of stress escape phenomenon could be avoided in this research.

Unfortunately, unlike downy mildew, the characterization of TLB had been never conducted in Indonesia, therefore specific race isolates were not available yet. Four major genes (Ht1, Ht2, Ht3 and HtN) are responsible for the resistant of TLB in maize plant. Actualy, 1 gene (HtNB) of which responsible for resistant of TLB Race 1 were discovered from Indonesian landrace named "Bramadi" (Wang et al. 2012), but the isolate of TLB Race 1 was not taken from Indonesia. It provided by the Plant Pathology Laboratory of Huazhong Agriculture University.

At the beginning of this research, authors tried to find "Bramadi" for resistant cultivar control, but beside it was not registered in Ministry of Agriculture (2013), it also could not be found throughout Indonesia. It might be confused with "Permadi", an OPV cultivar which was registered in Ministry of Agriculture (2013). But "Permadi" which was released in 1966 (Ministry of Agriculture 2013), was not available anymore. Base on the above mentioned facts, data of this research was base on general (unspecific) TLB resistant.

Analysis of variance stated that both control genotypes showed uniform genetic stability in both seasons. In the dry season the rainy season, control genotype Sukmaraga had P-value = 1.83594 and 0.46738 repectively. Control genotype BISI 18 possesed P-value = 1.18633 in the dry season, while in the rainy season P-value = 1.49958. Both genotype controls also showed uniform genetic stability of inter-block in every season. Meanwhile, most of all prospective genotypes in the rainy season showed ununiform genetic stability except prospective genotype SSUSX68849 (P-value = 0.49228). In the dry season, the genetic stability relatively uniform except on the prospective genotypes SSUSX76844 (P-value = 1.59782), SSUSX02791 (P-value = 0.00005) and SSU3X17782 (P-value = 0.01220).

Base on the data presented in Table 2 and Figure 2, it also could be stated that there were no variation among replications in the dry season (P-value = 0.142883). In the rainy season variation among replications were significant (P-value = 0.040443). It probably happened due to ununiformity of soil fertility as a result of the movement of nutrients from the higher plots to the lower ones. This movement was mainly caused by rainfall which often

exceed 250 mL per day during the rainy season. In the dry season, rainfall was rarely exceeds 100 mL per day so it was not strong enough to move nutrients from the upper plots. The research location was a hilly area on the plateau (990 meters above sea level) with average slope more than 6%. This phenomenon was in line with Carson (1995) and Treikale et al. (2014) that the development of TLB was strongly influenced by the resistance of varieties, cultivation systems and the weather/climate.

Table 2. TLB degree of infection

Code of genotypes	Dry season				Rainy season				Aggregate	Resistance level*
	Rep 1	Rep 2	Rep 3	Average	Rep 1	Rep 2	Rep 3	Average		
SSU3X17782	3.69	3.79	3.52	3.67^{b}	4.01	4.09	4.14	4.08	3.87	S
SSU3X28871	1.41	1.48	1.53	1.47^{ab}	1.34	1.42	1.40	1.39^{ab}	1.43^{ab}	R
SSU3X29131	1.55	1.49	1.58	1.54^{ab}	1.38	1.40	1.29	1.36^{ab}	1.45^{ab}	R
SSU3X30735	1.77	1.83	1.68	1.76^{ab}	1.31	1.40	1.46	1.39^{ab}	1.58^{ab}	R
SSU3X45172	1.86	1.96	1.83	1.88^{ab}	1.45	1.39	1.38	1.14^{ab}	1.65^{ab}	R
SSU3X68276	2.74	2.91	3.07	2.90^{b}	2.93	3.05	2.84	2.94^{b}	2.92^{b}	LR
SSUSX02791	1.85	1.92	1.70	1.82^{ab}	1.38	1.52	1.37	1.42^{ab}	1.62^{ab}	R
SSUSX06145	1.68	1.64	1.79	1.70^{ab}	1.62	1.74	1.73	1.70^{ab}	1.70^{ab}	R
SSUSX48274	1.67	1.86	1.52	1.68^{ab}	1.42	1.44	1.34	1.40^{ab}	1.54^{ab}	R
SSUSX68849	1.00	1.00	1.00	1.00^{ab}	1.01	1.00	1.00	1.00^{ab}	1.00^{ab}	VR
SSUSX76844	1.10	1.30	1.29	1.23^{ab}	1.34	1.33	1.36	1.34^{ab}	1.29^{ab}	R
BISI 18	3.96	3.87	4.07	0.97	4.04	3.82	3.97	3.94	3.96	S
Sukmaraga	2.75	2.92	2.99	2.89^{b}	2.91	3.03	2.94	2.96^{b}	2.92^{b}	LR

Note: a = significantly better than Sukmarga at LSD 5%, b = significantly better than BISI 18 at LSD 5%, * = according to CIMMYT (1999).

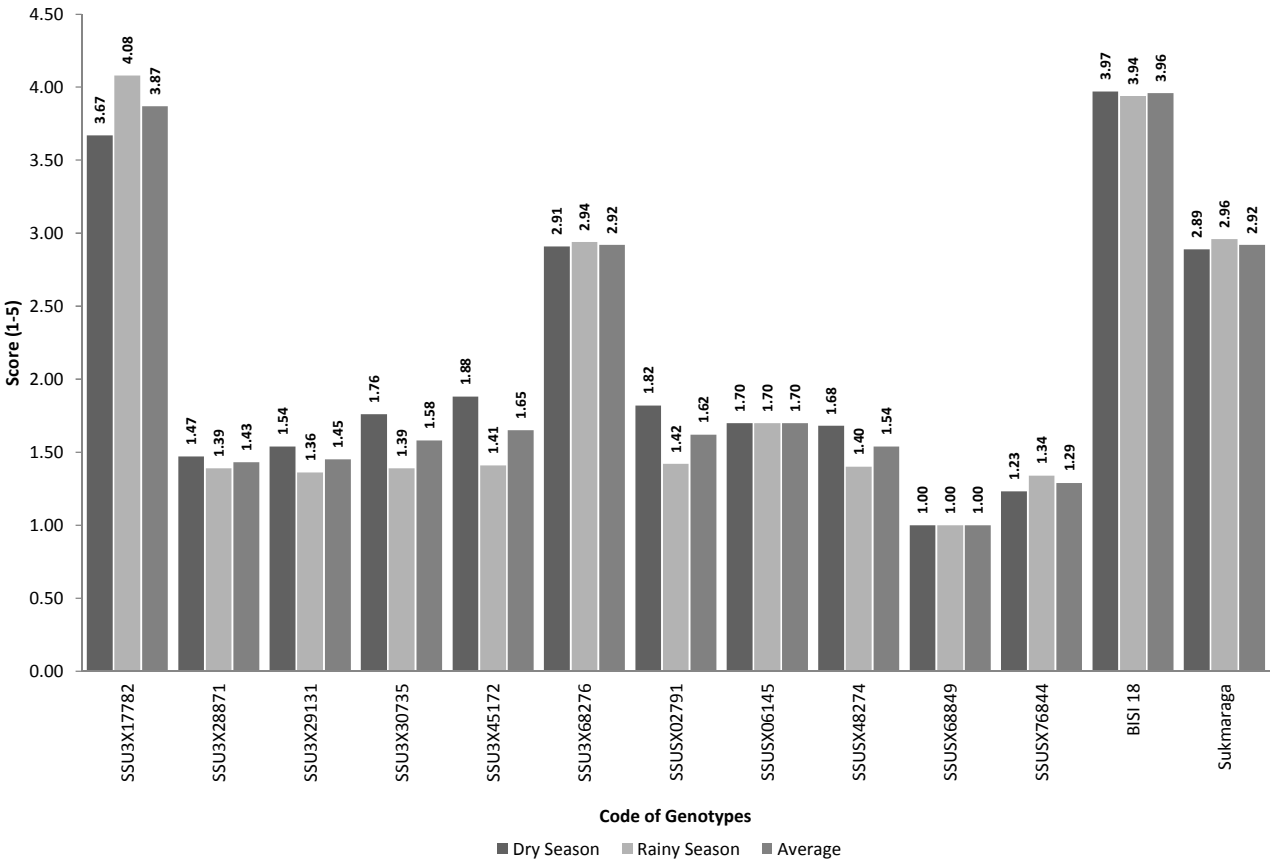

Figure 2. TLB degree of infection

In general, most of the prospective genotypes (90.9%) have genetic stability againt seasons alteration, except SSU3X17782. This prospective genotype was more susceptible to the TLB during the rainy season. In the dry season, resistance level of SSU3X17782 was equal to Sukmaraga and significantly better compared to BISI 18 at LSD 5% (α=0.05). Otherwise, in the rainy season resistance level of SSU3X17782 was significantly lower than BISI 18 and Sukmaraga. This trait was probably inherited from its male parent SSUSX02791M. Prospective genotype SSUSX02791 which shared same male parent with this prospective genotype, showed similar genetic instabilitity during both season.

Besides SSU3X17782, in the dry season, resistance level of prospective genotype SSU3X68276 was equal to resistance level of Sukmaraga and significantly better than BISI 18 at LSD 5%. However, in contrast to SSU3X17782 this prospective genotype was genetically remained stable and still had the same resistance level during the rainy season. The remaining 9 prospective genotypes (81.8%) were significantly better than Sukmaraga and BISI 18 at LSD 5% in the both season.

Based on CIMMYT (1999) scoring system, 7 prospective genotypes (63.6%), which were SSU3X30735, SSU3X45172, SSU3X68276, SSUSX02791, SSUSX06145, SSUSX48274 and SSUSX68849 had actual resistance level (Table 2) equal to their expected resistance level (Table 1). Prospective genotypes SSUSX68849 was classified in the range of very resistant (VR), while the other 6 prospective genotypes (54.5%) were classified in the range of resistant (R). Five prospective genotypes (45.5%) which were SSU3X17782, SSU3X28871, SSU3X29131 and SSUSX76844 had actual resistance level (Table 2) lower than their expected resistance level (Table 1). This deteriorization was probably due to epistasis phenomenon. Resilience to TLB is controlled by many genes (polygenic), so that many genes interact each other during the crossing between the progenitors (Muiru et al. 2010; Castiano et al. 2012; Wathaneeyawech et al. 2015 and Sartori et al. 2015).

Prospective genotype SSUSX68849 was very resistant (VR) to TLB. It was proven by 299 plants out of 300 plants on all plots and all replications, were not infected at all by the fungus E. turcicum. Score 2 (2-3 leaf below the ear infection) occured only 1 time in this prospective genotype. Score 2 was found in replication-1, line 4, plant number 21. This phenomenon was probably caused by outcrossing from susceptible line during the crossing of prosopecrive genotype SSUSX68849 or natural mutation in one or both its parents. The both parents of this prospective genotype possesed excellent resistance to the TLB. It was similar with Hurni et al. (2015) that showed mutant cultivars were more susceptible than their progenitors.

Based on statistical tests and CIMMYT (1999) scoring system, it could be concluded that 10 prospective genotypes (90.9%) which were SSU3X28871, SSU3X29131, SSU3X30735, SSU3X45172, SSU3X68276, SSUSX02791, SSUSX06145, SSUSX48274, SSUSX68849 and SSUSX76844 had passed the preliminary examination of the TLB infection. Therefore, these 10 prospective genotypes could be included in multi-location researchs in order in order to release national new superior varieties.

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Characterization of soybean genotypes for Asian soybean rust reaction under screen house condition

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Abstract. *Inayati A, Yusnawan E. 2016. Characterization of soybean genotypes for Asian soybean rust reaction under screen house condition. Biodiversitas 17: 609-613.* Asian soybean rust (ASR) caused by *Phakopsora pachyrhizi* is one of the major diseases limiting soybean yield. This disease has widely spread on soybean crops in Indonesia. The use of resistant cultivars is one of the economical approaches to control ASR. The objectives of this study were to assess the resistance of soybean lines derived from crossing two large-seeded cultivars (Baluran and Grobogan) with a broad adaptive cultivar (Kaba) and to identify resistant genotype characteristics under screen house conditions. All genotypes were artificially inoculated with *P. pachyrhizi* uredospores. Number of pustules per leaf, the development of ASR, and yield components including number of intact pods per plant, number of empty pods, and weight of pods per plant were observed. Thirteen lines of Baluran pedigrees had higher resistant response to ASR compared to Grobogan pedigrees. Fewer numbers of pustules (8 pustules cm^{-2}), lower value of area under the disease progress curve (AUDPC), and redish brown (RB) lesion type were observed in resistant lines. In contrast, susceptible lines had more pustules (> 21 pustules cm^{-2}), higher AUDPC value, and had mixed lesion type (RB and Tan). ASR reduced seed size and yield. The average weight of 100 seeds of resistant lines was 10.2 g while on susceptible lines, the 100-seed weight ranged from 8.7 to 12.6 g. The average yield per plant varied from 2.7 to 6.1 g. Baluran/Grobogan pedigrees were more susceptible to ASR than Baluran/Kaba pedigrees, however, those pedigrees showed better yield per plant and were supposedly more tolerant to ASR.

Keywords: Asian soybean rust, genotype, resistant, soybean, susceptible

INTRODUCTION

Asian soybean rust (ASR) caused by *Phakopsora pachyrhizi* is one of the important pathogens which reduces soybean yield (Semangun 1993). This pathogen has widely spread on soybean crops from subtropics to tropical regions. In Indonesia, ASR infection had been reported since 1991 (Semangun 1993). This disease emerges in every season, especially at the end of rainy season (T > 28°C, RH > 95%) (Sumartini 2010). ASR is considered the most destructive soybean foliar disease (Li and Young 2009, Miles et al. 2003). Heavily infected crops result premature defoliation which effect on pod filling (Kumudini et al. 2008; Ribeiro et al. 2009) and reduce number of pods as well as seed size (Diaz et al. 2007). Significant yield loss occurs on early infected soybean crops than late infection. Severe infection significantly reduces the yield up to 80% (Twizeyimana et al. 2008).

The main symptom of infected soybean crops is the lesions on leaves, which consist of pustules containing large numbers of uredospores. In sub tropical regions, the first symptom appears after flowering (R1 to R3) on the leaves in the lower canopy (Faske et al. 2014). However, in tropical areas such as in Indonesia, rust disease appears at three or four weeks after planting (V3 to V4) (Sumartini 2010). Fungicide application, the use of resistant cultivars, and cultural practice are basic management for reducing soybean rust epidemics (Rupe and Sconyers 2008). Fungicide applications are an effective control for short period, however, not effective for long term management. Several fungicides effective to control soybean rust are from groups of chloronitriles, strobilurins, and triazoles (Muller 2007; Rupe and Sconyers 2008). The use of resistant cultivars and good cultural practices are considered more promising.

Recently, soybean resistant cultivars to all isolates of *P. pachyrhizi* have not been available yet (Bonde et al. 2006, Goellner et al. 2010). Factors affecting the susceptibility of resistant cultivars are durability of the resistance to ASR which is easily broken due to pathogen variability (Oliveira et al. 2005), the complexity of genes that controls resistance to ASR, and environmental factors (Garcia et al. 2008). Thus, tolerant cultivars are more reasonable for ASR integrated control (Twizeyimana et al. 2008). Tolerance can be defined as plant capacity to resist pathogen infection and development, without significant reduction in yield or quality of the product (Schafer 1970). Three different reaction types may occur on soybean in response to *P. pachyrhizi* infection based on lesions types, i.e. (i) immune reaction (IM) without visible lesions, (ii) a resistant reaction with reddish-brown (RB) lesions, and (iii) a susceptible reaction with tan (TAN) lesions (Bromfield 1984; Goellner et al. 2010).

The best possible techniques to develop new cultivars resistant to rust diseases are to screen soybean germplasm and to create pedigree derived from the existing cultivars and lines. Studies to estimate yield losses caused by this disease are also important, so future cultivation of such

cultivars suffering from huge yield losses could be avoided. Cultivars with larger seed size, early maturity, high yield and having broad adaptation have become a main goal of Indonesian breeder program to reduce soybean production gap and the national demand. Until 2011, Grobogan is the only superior cultivar in Indonesia which has those ideal characteristics: large-seeded, early maturity and high yield. However, Grobogan has limitations due to less adaptation to large area and only specific to certain locations. Crossing between Grobogan with broad adaptive cultivars, namely Kaba and Malabar is promising to develop new cultivars which have large-seeded, early maturity, high yield and broad adaptation. This study therefore, aimed to assess the resistance of soybean breeding lines derived from crossing of two large-seeded cultivars (Baluran and Grobogan) with a broad adaptive cultivar (Kaba), and to identify the characteristics of resistant lines under screen house conditions.

MATERIALS AND METHODS

Plant materials and experimental design

Thirteen soybean genotypes derived from crossing Baluran, Grobogan, Kaba, and Malabar cultivars were planted in Indonesian Legumes and Tuber Crops Research Institute (ILETRI) screen house at Kendalpayak, Malang, East Java, Indonesia. The experiment was arranged in completely randomized design (CRD) with triplicate. Each pot consisted of two plants and every soybean genotype was planted on six pots.

Pathogen isolate and inoculation procedure

Uredospores of *P. pachyrhizi* were used as inoculum. The uredospores were harvested from susceptible cultivar (Ringgit) which was cultivated in a screen house as a source of inoculum. Ringgit cultivar was planted a month prior to the study. The average temperature during study was maintained at 25-28°C, and the relative humidity was 80-85%. Prior to inoculation, infected leaves of Ringgit were harvested and placed on plastic trays and incubated for 24 h. Uredospores were removed from the leaves with a paintbrush. Inoculum was prepared by suspending uredospores in water and 20 µL Tween 20. The suspension was mixed well, and filtered through cheese cloth. Uredospore concentration was adjusted to 10^4 spores mL^{-1}. The uredospores were sprayed on healthy soybean breeding line leaf surface in the evening at 4 pm with a hand sprayer. Inoculation was conducted twice at three and four weeks after planting (WAP) (Sumartini 2010; Inayati and Yusnawan 2016).

Table 1. The resistance level of soybean lines to ASR (Shanmugasundaram 1977)

Resistance criteria	IWGSR Score
Immune (I)	111
Resistant (R)	122, 123, 132, 133, 222, 223
Moderately resistant (MR)	142, 143, 232, 233, 242, 243, 322, 323
Moderately susceptible (MS)	332, 333
Susceptible (S)	343

Disease rating and data analysis

Response of soybean lines to ASR was evaluated starting from seven days after inoculation (DAI). ASR infection level and the resistance of soybean lines were rated using modified three digits of IWGSR (International Working Group on Soybean Rust) (Shanmugasundaram 1977) (Table 1). The first digit denotes the upper position of the most diseased leaves in the leaf canopy of the plant, where 1 = bottom third of the leaf canopy, 2 = middle third of the leaf canopy, and 3 = upper third of the leaf canopy). The second digit denotes the density of rust lesion on the most diseased leaves, where 1 = no pustules, 2 = light pustules density (1-8 pustules cm^{-2}), 3 = medium pustules density (9-16 pustules cm^{-2}) and 4 = heavy pustules density (> 16 pustules cm^{-2}). The third digit denotes the infection type on the most diseased leaves, where 1 = no pustule, 2 = no sporulating pustules, 3 = sporulating pustules. Disease progress was quantified by calculating the Area Under Disease Progress Curve (AUDPC) according to Simco and Piepho (2012). Yield components consisting of filling pods, empty pods, and weight of 100 seeds were observed to evaluate the effect of ASR to the yield. Analysis of variance followed by least significant different test (LSD, $p < 0.05$) was performed to determine the difference among genotypes.

RESULTS AND DISCUSSION

Results

Soybean genotype response to ASR varied in relations to the number of pustules, lesion types, and the disease progress (AUDPC). The number of pustule increased in line with the plant age and the duration of infection (Table 2). Significant difference ($p < 0.01$) in the number of pustules at 42 and 49 DAI and AUDPC of lesion density of soybean lines was observed. In general, majority of Baluran/Grobogan pedigrees showed higher number of pustules than those of Baluran/Kaba. From the data collected at 21 DAI, 11 lines had light pustule density (1-8 pustules cm^{-2}) and 2 lines had medium pustule density (9-16 pustules cm^{-2}). The average number of pustules at 49 DAI ranged from 8 to 24 pustules cm^{-2} and *P. pachyrhizi* had covered almost the whole plants. Nine of thirteen lines had the average number of pustules more than 16 pustules cm^{-2} and were categorized as susceptible (S). The positive correlation between AUDPC and pustule number ($r = 0.73$, equation not shown) was observed related to rapid advance to the disease progress. This caused by the increased number of pustule as a source of inoculums which resulted more secondary infections. Three different lesion types as a response to ASR were observed in this study (Figure 1). Most of the genotypes showed RB lesion type (Table 2), and only one genotype showed T and mixed (RB/T).

Phakopsora pachyrhizi infection reduced yield, yield components, and seed size (Table 3). Even though there was no significant difference on the number of intact pods between the susceptible and resistance genotypes, the average number of intact pods on susceptible genotypes was fewer than the resistant breeding lines. The intact pod

Table 2. Number of pustule cm^{-2}, score, resistant criteria, lesion type, and AUDPC values on large-seeded soybean lines

Large-seeded soybean lines	Number of pustules per cm^2			IWGSR score	Resistant criteria	Lesion type	AUDPC
	21 DAI	42 DAI	49 DAI				
Bl/Kb1	9.00^{a}	14.00^{a}	14.83^{cd}	333	MS	RB	131.88^{ab}
Bl/Kb2	10.17^{a}	12.67^{ab}	14.17^{cd}	333	MS	RB	112.04^{ab}
Bl/Kb3	7.33^{a}	11. 67^{ab}	8.17^{d}	223	R	RB	55.21^{b}
Bl/Gr15	7.50^{a}	9.83^{ab}	20.83^{abc}	343	S	RB	159.50^{ab}
Gr/Bl16	8.33^{a}	12.83^{ab}	24.67^{a}	343	S	RB	227.00^{a}
Bl/Gr19	8.67^{a}	11.00^{ab}	19.33^{abc}	343	S	T	103.33^{ab}
Bl/Gr21	5.67^{a}	10.50^{ab}	21.00^{abc}	343	S	RB	150.54^{ab}
Bl/Gr23	8.50^{a}	8.50^{b}	20.17^{abc}	343	S	RB	91.04^{ab}
Bl/Gr51	5.17^{a}	10.33^{ab}	18.00^{abc}	343	S	RB	88.59^{ab}
Bl/Gr58	5.17^{a}	14.00^{a}	16.00^{bcd}	333	MS	RB	177.50^{ab}
Bl/Kb66	6.00^{a}	10.17^{ab}	23.33^{ab}	343	S	RB/T	202.67^{ab}
Bl/Gr70	6.67^{a}	13.00^{ab}	21.50^{abc}	343	S	RB	179.34^{ab}
Bl/Kb71	6.83^{a}	11.00^{ab}	21.17^{abc}	343	S	RB	187.13^{ab}

Note: R: resistant, MS: moderately susceptible, S: susceptible, RB: reddish brown, T: tan, RB/T: mixed reddish brown and tan. Number at the same column followed by the same notation was not significantly different based on LSD test ($p < 0.05$).

A **B** **C**

Figure 1. Lesion type of ASR on large-seeded soybean lines. A. Reddish Brown, B. Tan, and C. Mixture of Reddish Brown and Tan

Table 3. Number of intact pods, number of empty pods, weight of 100 seeds and yield of large-seeded soybean lines infected by *P. pachyrhizi*.

Large-seeded soybean lines	Number of intact pods	Number of empty pods	Weight of 100 seeds (g)	Yield per plant (g)
Bl/Kb1	18.58 ^{a}	5.50 ^{a}	9.97^{bc}	3.07^{b}
Bl/Kb2	19.42 ^{a}	5.25 ^{a}	8.74 ^{c}	2.79^{b}
Bl/Kb3	22.83 ^{a}	6.83 ^{a}	10.22^{bc}	4.37^{ab}
Bl/Gr15	19.42 ^{a}	5.42 ^{a}	11.61^{ab}	4.43^{ab}
Gr/Bl16	24.00 ^{a}	3.58 ^{a}	11.16^{ab}	5.10^{ab}
Bl/Gr19	25.08 ^{a}	5.00 ^{a}	11.67 ^{a}	6.11^{a}
Bl/Gr21	22.00 ^{a}	4.92 ^{a}	11.66 ^{c}	5.22^{ab}
Bl/Gr23	17.33 ^{a}	2.00 ^{a}	10.18^{bc}	3.49^{ab}
Bl/Gr51	21.67 ^{a}	6.33 ^{a}	9.77 ^{c}	4.15^{ab}
Bl/Gr58	18.92 ^{a}	4.92 ^{a}	12.52^{a}	4.63^{ab}
Bl/Kb66	25.67 ^{a}	5.83 ^{a}	10.25^{bc}	4.29^{ab}
Bl/Gr70	19.50 ^{a}	4.25 ^{a}	12.64^{a}	4.89^{ab}
Bl/Kb71	23.75 ^{a}	3.50 ^{a}	10.52^{ab}	4.92^{ab}

Note: Number at the same column followed by the same notation was not significantly different based on LSD test ($p < 0.05$)

numbers varied from 17 to 25 pods. In contrast, the number of empty pods on resistant genotypes was the highest. However, there was no significant difference on the number of empty pods between the susceptible and resistant genotypes. The average yield per plant was low due to the ASR infection. The yield varied from 2 to 6 g and the average weight of 100 seeds on resistant lines was 10.2 g while on susceptible lines, that variable ranged from 8 to 12 g (Table 3).

Discussion

The genotype characteristics resistant to ASR in the present study had lower number of pustules, lower AUDPC value, and reddish brown (RB) lesion type, in contrast to susceptible genotypes which presented severe symptoms and more rapid disease progress. The intensity of uredospore sporulation and periodical severity assessment were important parameters for classification of genotypes into tolerant or susceptible to ASR (de Araujo and Vello 2010). Large variation was observed in the AUDPC, even

though lack association was noticed between AUDPC value and the resistance of large-seeded soybean genotypes to ASR. The AUDPC value was influenced by genotype x environment interactions as explained by Steffenson and Webster (1992) and Cherif et al. (2010). They reported that relationship between the final disease severity and the AUDPC was highly influenced by the environment. In addition, they noted that high values of apparent infection rate could occur sometimes on genotypes with reduced disease severities when there was rapid increase of pathogen infection from a low to a moderate level within a short period. A positive correlation between number of pustule and AUPDC value suggested that genotypes which had plenty of pustules possessed faster disease progress. This could imply that the resistance mechanism present in these genotypes respond rapidly once the rust pathogen established in the host cells as observed in the present study.

RB lesions are formed because of the hypersensitive response of the soybean crops to *P. pachyrhizi*. This reaction will inhibit the fungus development. Lesion color is known to be controlled by resistance genes of Rpp2 and Rpp4, thus it should be considered when selecting resistant genotypes (Yamanaka et al. 2010; 2013). On susceptible lines, lesion was clearer (Tan) and some were mixture between RB and Tan, even though most of the lesion type in this study was RB. However, the development of the pathogen which represented by the number of pustules was still high, resulting variation of lesion color. Variation among genotypes makes the difficulty for grouping all phenotypes into a limited number of lesion types, such as RB (Resistant) and TAN (Susceptible) (Kato and Yorinori 2008).

ASR reduced yield, yield components, and seed size since *P. pachyrhizi* infection initiated early defoliation which effected on pod filling (Kumudini et al. 2008; Ribeiro et al. 2009) and reduced number of pods and seed size (Dias et al. 2007). Foliar pathogens were not only impairing the healthy green leaf area of crops, but also influencing the photosynthetic activity of the healthy (green) parts of the leaves (Kumudini et al. 2008). The seed size represented by the average weight of 100 seeds was categorized as medium seeds. In this study, the average weight of 100 seeds on resistant lines was 10.2 g while on susceptible lines, that parameter ranged from 8.7 to 12.6 g.

A study conducted by Ahmad et al. (2010) on leaf rust infected wheat showed that cultivars or genotypes in which AUDPC was maximum, the yield losses were also maximum. Cultivars which performed lower AUDPC value, these cultivars suffered from less yield losses (Ahmad et al. 2010). Dissimilar response was observed on ASR, the increase value of AUDPC was not linearly followed by the maximum reduction of the yield. In the present study, AUDPC value is possibly highly influenced by genotype factor. As explain by Pham et al. (2010), the response of the genotypes which were controlled by Rpp genes to soybean rust was dependent on the experiment and the time of the trial were conducted.

In conclusion, response of soybean breeding lines to ASR showed that Baluran/Grobogan pedigrees were more susceptible to ASR than Baluran/Kaba pedigrees. On the other hand, Baluran/Grobogan pedigrees showed better yield per plant, and were categorized as tolerant lines.

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Diversity and phylogenetic relationship of cellulolytic bacteria from the feces of Bali Cattle in South Central Timor, East Nusa Tenggara, Indonesia

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Abstract. *Missa H, Susilowati A, Setyaningsih R. 2016. Diversity and phylogenetic relationship of cellulolytic bacteria from the feces of Bali Cattle in South Central Timor, East Nusa Tenggara, Indonesia, Indonesia. Biodiversitas 17: 614-619.* There are three types of cattle farms with different kinds of feed in South Central Timor, that are maintained around Supul Lake, quarantined and left in the wild. This research aims to isolate, identify and determine the genetic relationship among of cellulolytic bacteria from the feces of Bali Cattle (*Bos javanicus javanicus* D´Alton, 1823, syn. *Bos javanicus sondaicus* Temminck, 1839/Blyth, 1842) in South Central Timor. The isolation of cellulolytic bacteria was done by using spread plate method on Carboxymethyl Cellulose (CMC) media. Cellulolytic activities were determined by the clear zone visibility using 0.1% congo red indicator. 16S rRNA encoding genes amplification was conducted using Polymerase Chain Reaction (PCR) using 63F and 1387r primers. Sequences of the 16S rRNA encoding genes were analyzed by bioinformatics using Nucleotide BLAST on NCBI website to determine the species of bacteria based on sequence similarity. The construction of the phylogenetic tree of cellulolytic bacteria was done using MEGA 7.0 software. The results were 48 isolates showing cellulase activity. There were 12 isolates from Supul Lake which have high cellulase activity namely: S1H6, S2H5 S2H7 S3H; in quarantine location: K1H6, K2H3, L2H7, K2H4, K1H2, K3H2; and in wild care system: L1H4 and L1H5. These activities were presented in clear zone about 7.08 to 1.47 cm. Based on the analysis of 16S rRNA encoding genes, there were 5 different genera found in 12 isolates with high cellulolytic activity. The isolates possessed similarity with *Pseudomonas* sp. 96%, *Acinetobacter* sp. 95%, *Bacillus* 97%, *Stenotrophomonas* 88%, and *Brachybacterium* sp. 97%. There were seven bacterial isolates having the potential to be declared as new bacterial species with <97% similarity percentage that are SIH6, S2H5, K2H3, K2H4, LIH4, LIH5, L2H7. Based on the phylogenetic tree cellulolytic bacteria showed the closest genetic relationship of 0.0% and farthest of 19.3% L2H7 isolate with Brachybacterium sp. S21F1

Keywords: 16S rRNA encoding gene, Bali Cattle, *Bos sondaicus*, cellulolytic bacteria, diversity, phylogenetic relationship

INTRODUCTION

Cellulolytic bacteria are bacteria that can hydrolyze cellulose complex into smaller oligosaccharides and ultimately into glucose (Lamid et al. 2011). Cellulolytic bacteria are naturally very common in agricultural soil, fertilizer or in the plant tissues, the rumen of ruminant animals and also on cattle feces. Cattle feces is the waste products of digestion secreted from the body in the form of solids (Hidayah et al. 2012). The availability of farm wastes is abundant, such as manure which is rarely used by the community of South Central Timor, therefore it becomes one of the causes of environmental pollution.

The problem of Bali Cattle (*Bos javanicus javanicus* D´Alton, 1823, syn. *Bos javanicus sondaicus* Temminck, 1839/Blyth, 1842) or livestock breeding in South Central Timor, East Nusa Tenggara is the shortage of the main fodder i.e. king grass, so as to maintain the potential of dairy farms that are part of Timor people's lives, the cattle breeders select one of three alternatives i.e. firstly, raising cattle extensively by means of cattle grazing in the field, orchard or yard, with the kind of food that are commonly consumed: agati leaves, banyan leaves, cottonwood leaves, banana stems and leguminous leaves; or secondly, breeding cattle in semi-intensive manner in the way that the cattle are kept in the shed at night, then grazed during the day around Lake Supul. The third is the fodder for cows is grazed from around Lake Supul, in the form of marshes grass and the water is from the lake. While producing Bali Cattle in South Central Timor, the breeders prefer an alternative technique by quarantining the cattle for fattening process since by being quarantined the feeding of the cattle is more regulated. The type of feed that is usually given is banana stems, as well as several grasses such as *Panicum maximum*, *Pennisetum purpureum*, and *Pennisetum purpureum*, and the drinking is water mixed with salt.

Alternative fodder has a quite high content of cellulose to affect the microbial enzymes in digesting the nutrients in the rumen which is ideally suited for a number of microorganisms. Cellulolytic bacterial isolates has a specific activity as a producer of cellulase enzymes, so it has the use of certain commercial functions such as garbage sewage treatment and is often used in the textile industrial field. Additionally, cellulase is also used in the pharmaceutical industry as an agent to help the digestive

system e.g. fiber material for diet purposes. Cellulase is also used in the fermentation process of biomass into biofuels, such as ethanol. Cellulase-producing cellulolytic bacteria are often found from the genus of *Pseudomonas*, *Cellulomonas*, *Bacillus*, *Micrococcus*, *Cellvibrio*, and *Cytophaga* (Lamid et al. 2011).

MATERIALS AND METHODS

Sampling

Sampling was from the feces of Bali Cattle in the South Central Timor, East Nusa Tenggara, Indonesia based on the predetermined sampling points, i.e., in semi intentions cattle raising around Lake Supul, in cattle quarantine and in extensive cattle care (wild) locations. Determination of sampling points used purposive sampling method. Sampling was determined by the difference in fodder. Feces of Bali Cattle were taken in wet condition for as much as 5-10 grams, and then they were inserted into the sample bottle and labeled with information of the place they were taken. Each sampling was feces of three different cattle which were taken from a nearby location.

Isolation of cellulolytic bacteria

Isolation of cellulolytic bacteria was carried on by diluting 1 g of sample into 9 mL of sterile distilled water aseptically. Then, serial dilution was made into 10^{-6}, and from serial dilution of 10^{-4} to 10^{-6}, it was taken 0.1 mL and it was distributed to CMC media using rod spreader. This technique was called spread plate technique. Finally, it was incubated at the temperature of 37°C for 4 x 24 hours (Syulasmi et al. 2009). Each colony of bacteria showing different morphology was taken and was considered as a pure culture.

Selection and cellulase activity test

Pure culture bacterial isolates on CMC slanted media were spotted on a petri dish containing CMC media, then incubated for 48 hours and at the end of incubation period, 0.1% congo red staining was conducted, being settled for 15 minutes and rinsed with 1M NaCl solution. If there were a clear zone around the colony, it indicated cellulose hydrolysis activity by cellulase enzymes (Jalgaonwala et al. 2011).

Genomic DNA extraction

The first stage to isolate the genomic DNA of bacteria was to grow the bacteria in CMC liquid medium and incubate it in the bacteria incubator shaker for 24 hours. A total of 1.5 mL of culture was centrifuged at 14.000 rpm speed. Then the DNA was extracted using Presto™ Mini gDNA bacterial kit (Geneaid).

16S rRNA encoding gene amplification by PCR

16S rRNA encoding genes were amplified using the polymerase chain reaction (Veriti Thermal Cycler). The reaction was conducted by mixing 12.5 µL of Kapa 2G Fast Ready Mix (Kapa Biosystem), 1.25 µL of 63 forward primers (63F: 5' CAGGCCTAACACATGCAAGTC 3'). 1.25 µL of 1387 reverse primer (1387r: 5' GGGCGGCGTGTACAAGGC 3') (Marchesi et al. 1998), 2 µL of DNA template and 8 µL of ddH_2O. PCR run with the following profiles: predenaturation at a temperature of 94°C for 2 min, followed by 30 cycles of denaturation stages at a temperature of 94°C for 30 seconds, annealing at a temperature of 55°C for 30 seconds, elongation at 72°C for 1 minute, and finalizing at a temperature of 72°C for 5 minutes. Then it was stored at 4°C for use at proper time and checked with electrophoresis (Marchesi et al. 1998). The DNA underwent sequencing in 1st Base Laboratory in Singapore. The DNA sequences were analyzed bioinformatically using Nucleotide BLAST on NCBI website (Waturangi et al. 2008) to determine similarity of the isolates compared to species existing in the database.

Phylogenetic relationship analysis

Genetic relationship among cellulolytic bacteria species from the feces of Bali Cattle around Lake Supul, in quarantine, and in wild care location that have been found in previous studies were analyzed using MEGA (Molecular Evolutionary Genetics Analysis) 7.0 software on a computer device (Kumar et al. 2015).

RESULTS AND DISCUSSION

Population of cellulolytic bacteria from Bali Cattle feces in South Central Timor

The population number of cellulolytic bacteria contained in the samples was determined randomly by multiplying the number of colonies being formed by liquidation factor on the concerned spread plate. The average populations of cellulolytic bacteria by plate count method on the feces of Bali Cattle in South Central Timor, especially in three cattle care sites around Lake Supul, cattle quarantine, and wild care areas, were eligible for the calculation of the bacterial population at dilution of $1x10^{-6}$. The highest cellulolytic bacteria population was on the feces of Bali Cattle in cattle quarantine which was equal to 1.68×10^8, while the lowest average population was on the feces of Bali Cattle around Lake Supul i.e. 1.05×10^8 (Figure 1).

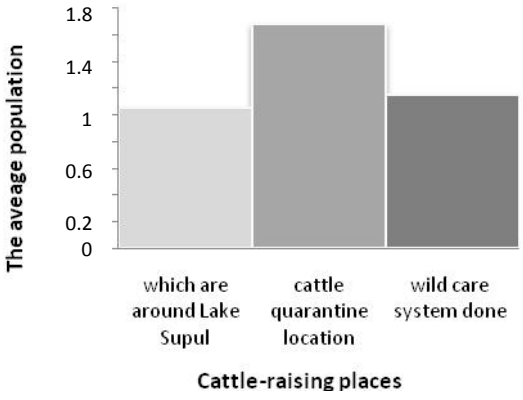

Figure 1. The average population number (10^6) of cellulolytic bacteria in the feces of Bali Cattle in South Central Timor based on the spread plate method on CMC media

The average population of cellulolytic bacteria per gram of Bali cattle feces in South Central Timor was considered high when compared to the population of cellulolytic bacteria per gram of rumen contents in the sheep rumen which was 2.98 x 10^7, was 5.12 x 10^7 in goat rumen, was 2.80 x 10^7 in deer rumen, was 4.76 x 10^7 in cattle rumen, was 7.58 x 10^7 in buffalo rumen (Thalib et al. 2000). Total population of cellulolytic bacteria varies depending on the feed consumed, the sampling time after feeding, different animal species, season and the availability of forage.

Cellulolytic activity of bacteria isolates

The isolation of cellulolytic bacteria from three sampling sites found 70 isolates. Each isolates was obtained from nine research samples. After being tested for its cellulase activity using 0.1% congo red staining, 48 isolates of positive cellulolytic bacteria were acquired. Therefore 12 bacterial isolates were selected to represent 48 isolates which were found for further research. The spread plate showing positive results of cellulolytic bacteria was presented in Figure 2.

Cellulose was hydrolyzed on CMC agar and if it was added with congo red staining, it would clear zone due to the reaction between congo red and bonding β-1,4-glycosidic contained in the cellulose polymer. Cellulose itself was hydrolyzed due to the activity of cellulase enzymes produced by the bacteria (Steensma 2001). With 1 mL of NaCl solution, it could be used to dilute the congo red dye around the colony, so that the clear zone was more visible (Sumardi 2004).

Cellulolytic bacterial isolates produced different clear zone diameter from one isolates to another. It can be caused by the size of the colonies that vary from one isolate to another. Cellulolytic activity index of cellulolytic bacteria from the feces of Bali Cattle in South Central Timor in three sites namely around Lake Supul, in cattle quarantine and in wild care were shown in the following diagram (Figure 3, 4, 5).

The highest index of clear zone on three cellulolytic bacterial populations in the feces of Bali Cattle in South Central Timor was produced by K1H6 isolates while the lowest ratio was produced by K1H3 isolates. When compared to the research conducted by Gusmailina et al. (2002) who obtained the highest index of clear zone around 5.8 and the research conducted by Hidayah et al. (2012) who obtained the highest index of clear zone around 3.7 then this K1H6 isolates was a cellulolytic bacteria that had high activity in degrading cellulose. Some microbes, mainly the bacteria types, had the ability to hydrolyze cellulose naturally through its cellulase activity. Although many microorganisms were able to degrade cellulose, only a few microorganisms could produce cellulase in a significant amount that was capable of hydrolyzing crystalline cellulose. Based on the graphic on figure 3, 4 and 5, it was selected 12 high activity cellulolytic bacterial isolates representing three sampling sites of Bali Cattle feces in South Central Timor for molecular identification. The selected isolates were SIH6, S2H5, S2H7, S3H1, K1H2, K1H6, K2H3, K2H4, K3H2, L1H4, L1H5, L2H7.

Amplification of 16S rRNA genes encoding

Isolation of DNA generally consists of four stages: cell lysis process, DNA binding, washing, and precipitating. After DNA isolation stage was completed, DNA concentration measurement was done to obtain DNA purity so as to qualify for the molecular analysis. DNA isolation results show that the DNA of 12 high activity cellulolytic bacteria was isolated properly. Isolation can be said to be pure and qualified to proceed to the molecular analysis if the value ratios of A260/280 were from 1.8 to 2.0 (Sambrook et al. 1989).

The results of the amplification of 16S rRNA genes by PCR was analyzed using 0.5% (w/v) agarose gel electrophoresis for 45 minutes at a voltage of 85 volts and a current of 300 mA to see the size of the DNA as the amplification product. Profile amplicons of 16S rRNA gene amplification by PCR can be seen in Figure 6.

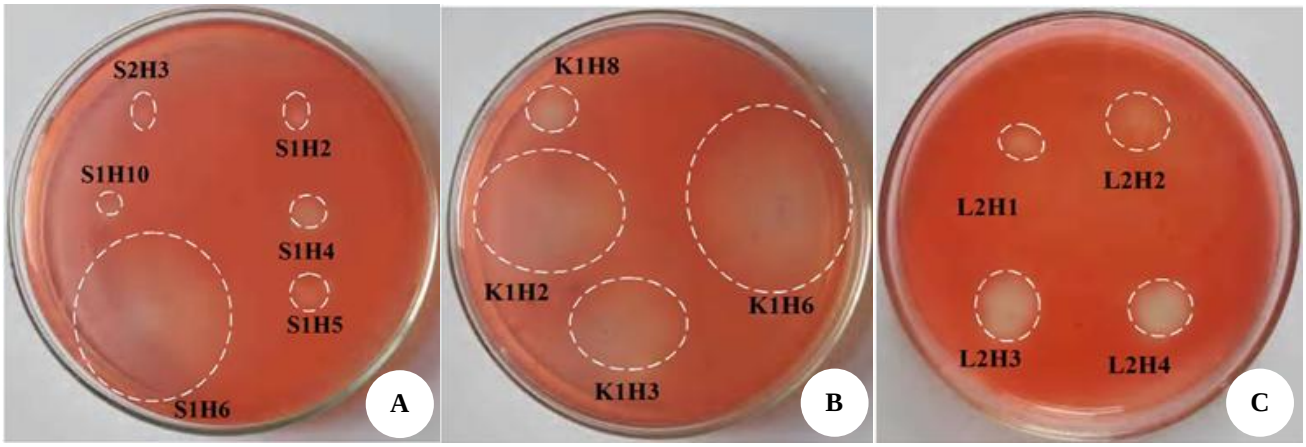

Figure 2. Clear zone showed cellulase activity of bacteria isolates in CMC media with congo red staining from Bali Cattle feces in South Central Timor. A. Around Lake Supul, B. Cattle quarantine, C. Wild care area

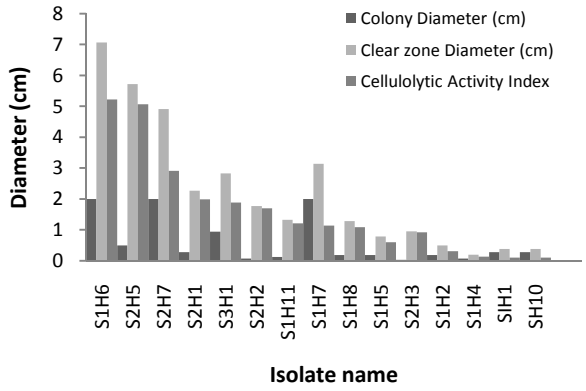

Figure 3. Cellulolytic activity index of the bacteria from Bali feces around Lake Supul represented by clear zone diameter reduced by diameter of the bacteria colony

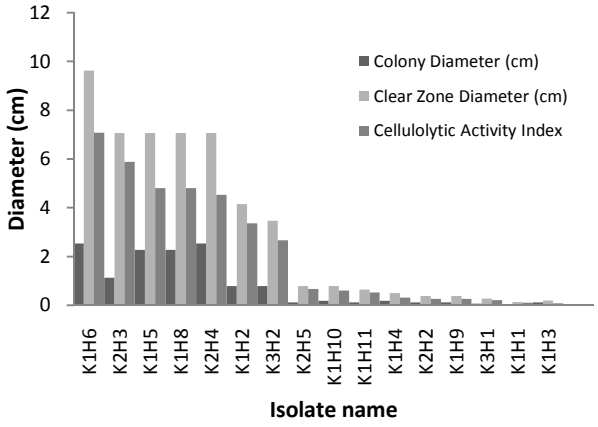

Figure 4. Cellulolytic activity index of the bacteria from Bali Cattle feces in quarantine represented by clear zone diameter reduced by diameter of the bacteria colony

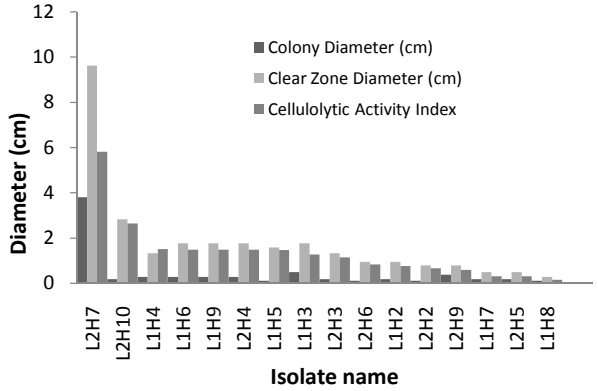

Figure 5. Cellulolytic activity index of the bacteria from Bali Cattle feces in wild care represented by clear zone diameter reduced by diameter of the bacteria colony

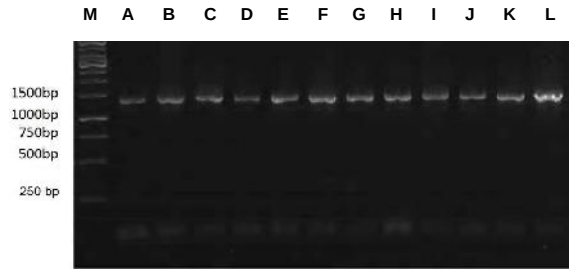

Figure 6. Electropherogram amplicon of 16S rRNA encoding genes cellulolytic bacteria isolated from Bali Cattle feces; M. Marker DNA, A. S1H6, B. S2H5, C. S2H7, D. S3H1, E. K1H2, F. K1H6, G. K2H3, H. K2H4, I. K3H2, J. L1H4, K. L1H5, L. L2H9

Table 1. Cellulolytic bacteria similarities by 16S rRNA encoding genes using the BLAST program

Isolate codes	Most similar species	Access number	Similarity (%)
S1H6	*Pseudomonas* sp. 53 (2015)	KU321296.1	96%
S2H5	*Acinetobacter* sp. XT-40	KR063566.1	95%
S2H7	*Pseudomonas* sp. C25	KT361090.1	97%
S3H1	*Bacillus cereus* ASDS9	KF256128.1	98%
K1H2	*Uncultured Bacterium* Clone QXJ-18	KJ957714.1	97%
K1H6	*Brachybacterium* sp. MCCC IA09822	KU560279.1	97%
K2H3	*Bacillus Cereus* MER 35	KT719615.1	95%
K2H4	*Pseudomonas* sp. DGM MH46	JF923454.1	96%
K3H2	*Bacillus Cereus* YBNY-1	KU363977.1	97%
L1H4	*Pseudomonas* sp. UIWRF 1386	KR189244.1	96%
L1H5	*Pseudomonas* sp. HJX22	KP979553.1	88%
L2H7	*Stenotrophomonas* BAB 5314	KT254651.1	80%

It is shown in the picture that the gene coding for 16S rRNA bacteria is amplified properly. This was shown by the existence of bands which were bright and bold as well as parallel to one another. This indicates that the primers being used are attached to the DNA template on the optimum temperature for primer annealing (Utami et al. 2012). The emergence of single band shows that the primers pair being used are specific and only stick to the expected position (Ratnayani et al. 2009).

Identity of cellulolytic bacteria based on 16S rRNA encoding genes sequences

The identification of bacteria using a gene encoding for 16S rRNA sequencing involves a comparison between the results and the reference sequences stored in the GenBank database. The results of the analysis of 16S rRNA gene using BLAST can be seen in Table 1.

The similarity percentage of ≥ 99% indicated that the species being compared were the same species, whereas by 97-99% of similarity percentage, it can be stated that the isolates being compared were in the same genus and by <97% of similarity percentage, it can be stated that the isolates had the potential to be declared as a new species of bacteria (Drancourt et al. 2000). Based on this research, the

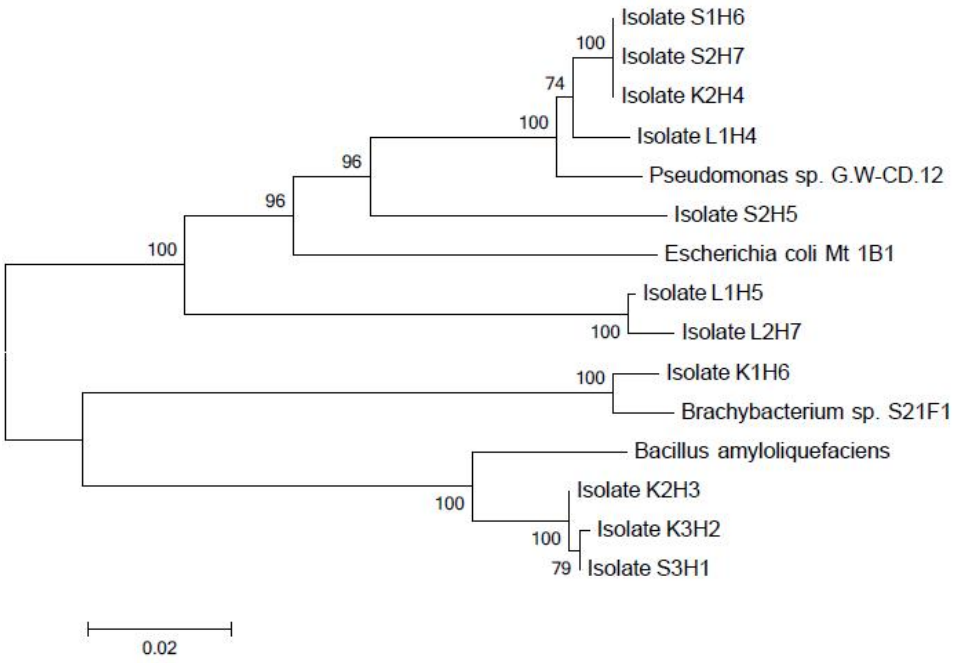

Figure 7. Phylogenetic relationships on the basis of 16S rRNA encoding gene. Based on the distance matrix, a neighbor-joining tree was reconstructed using MEGA software version 7.0. The bootstrap was performed with 1000 replicates. The bar indicated a 25-nucleotide difference. Genetic relationship of each of these bacteria isolates from each location of raising cattle i.e. around Supul Lake (S), in quarantine (K) and wild care system (L) was not in the same group. The cellulolytic bacterial isolates clustered and had a close relationship with the bacteria in the same genus with reference genus (*Pseudomonas, Bacillus, Brachybacterium*)

bacterial isolates that showed genus similarity were S2H7, S3H1, K1H2, K1H6, and K3H2 isolates, and there were seven bacterial isolates that have the potential to be declared as new bacterial species that are S1H6, S2H5, K2H3, K2H4, L1H4, L1H5, and L2H7 isolates.

Phylogenetic relationship of high activity cellulolytic bacteria

Phylogenetic relationship of cellulolytic bacteria among the isolates and some bacteria that have been found in this research was discoverable using a phylogenetic tree by looking at the genetic distance (Figure 7). Based on the phylogenetic tree, each of these species which were found in each location formed a group together, but they spread across all groups. This suggests that the bacteria isolated from each location have non-adjacent phylogenetic relationship. They formed groups based on the proximity of the genus. Despite being on the different location and different kind of food, the isolates of high activity bacteria joined the groups that has similar bacterial genus and those that show close relationship.

The results of the analysis of genetic distance between cellulolytic bacteria isolates were as follows: the closest genetic distance was 0.0% on S2H7 isolates with K2H4 isolates, S1H6 isolates with K2H4 isolates, and S2H7 isolates with S1H6 isolates. While the farthest genetic distance was 19.3%, i.e. L2H7 isolates with *Brachybacterium* sp. S21F1. The scale of 0.02 referred to the evolutionary distance on the length of the branch.

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Choosing native tree species for establishing man-made forest: A new perspective for sustainable forest management in changing world

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Abstract. *Subiakto A, Rachmat HH, Sakai C. 2016. Choosing native tree species for establishing man-made forest: A new perspective for sustainable forest management in changing world. Biodiversitas 17: 620-625.* Establishment of tree plantation on degraded lands and forest clearly favored some exotic species such as *Gmelina arborea*, *Acacia,* and *Eucalyptus.* High productivity, less harvesting time, and deeper silvicultural knowledge are the beneficial factor for choosing those exotics species. However, the use of a wide variety of native tree species becomes more significantly important in reforestation projects due to the greater biodiversity benefits and wider environmental services. This research was carried out as a multiyear observation and continuous experiment to value how native tree species can be prospective alternatives in providing and supporting human need. The performances of two native Indonesian *Shorea* species, *Shorea leprosula* and *Shorea selanica*, were evaluated at a dipterocarp planting trial in two different sites in Indonesia. Growth data was obtained from 15 and 17 years old plots, twelve 100 m X 100 m square plots on mineral soils (Gunung Dahu Experimental Forest/GDEF, Bogor) and eight resembled plots on frequently flooded peat land (PT. Arara Abadi/PT. AA, Riau). Survival rates were varied, ranged from 36-77%, diameter at breast height from 13.7-24.9 cm, tree height from 10.8-16.9 m, mean volume from 0.119 m^3/tree-0.567 m^3/tree, and total volume from 79.420 m^3/ha-215.412 m^3/ha. Growth rates of planted saplings were affected by species, site and spacing distance. The development of man-made dipterocarps forest in the tropic, especially in South East Asia can be as prospective as developing an exotic fast growing plantation. Eventhough *in situ* conservation would give the most benefit in conserving genetic resources of native tree species, establishing man-made dipterocarp forest still have higher environmental value than using exotic tree species such as acacia. Moreover, establishing man-made dipterocarp forest is considered more environmental friendly and possess lower to no risk of species invasion compare to those of developing exotic trees plantation.

Key words: exotic species, native tree, plantation, *Shorea leprosula*, *Shorea selanica*
Abbreviations: GDEF = Gunung Dahu Experimental Forest, PT. AA = PT. Arara Abadi, *S. leprosula* = *Shorea leprosula*, *S. selanica* = *Shorea selanica*.

INTRODUCTION

Dipterocarpaceae, a large family of dominant tree species in the tropical rain forests of Malaysia and Indonesia, is ecologically dominant and its timber is economically significant. Conservation of the dipterocarps was not an important issue in the past as the family was seen common and none were presumably threatened. However, fast dwindling natural forest resources is a general trend in Southeast Asia. Bradshaw et al. (2009) determined that Southeast Asia forests are the fastest disappearing among all other tropical region. Phat et al. (2004) is also estimated that Southeast Asia contributes 29% of the global total from deforestation. Most lowland dipterocarp forests in the area are either converted into oil palm plantations or are heavily logged in production forest of concession forest area. In such condition, it may take many decades to recover the commercial volume. Hence, there is an urgent need not to only decrease the current rate of deforestation, conserve the remaining forest cover, and restore the degraded land, but also to begin of growing new forests.

Establishment of tree plantation on degraded lands and forest initiates the recovery of native forest communities, but most of the reforestation projects favored some exotic species (e.g. *Gmelina arborea*, *Acacia*, *Eucalyptus*). There is a persisting myth for most tropical regions that exotic monocultures would significantly ease the pressure on remaining natural forests because it supplies timber needs in relatively short time. However, beyond these silvicultural aspects there are other issues in the debate on exotic vs. native tree species which deserve a deeper review. Barlow et al. (2007) reported that plantation of exotic species in the tropics have considerably lower diversity value than secondary forest and conversion of secondary forest to exotic plantation will lead to further biodiversity losses. Furthermore, reforestation is not only about restoring productive capacity of forest for future timber production but it has principal objective of recovering biodiversity and other environmental services that could be provided by forest itself. This is why the use of a wide variety of native tree species becomes more significantly important in reforestation projects. Establishment of commercial plantations of valuable

dipterocarp species has been focus of research in Southeast Asia for nearly a century. The use of native tree species for plantation is likely to have greater biodiversity benefits than either exotic plantations or conversion to other land uses such as oil palm.

However, the constraint of less economic benefit and silvicultural knowledge on most of native tree species has led the establishment of native tree plantation becomes more complicated than that of established exotic plantation species. Furthermore, past studies have also been found that the native tree species of dipterocarps showed poor survival and grow much slower than those of pioneers or exotic trees in open grasslands (Otsamo et al. 1996, 1997; Tolentino 2008).

The purpose of the study was to examine the feasibility of growing dipterocarps plantation forest in Indonesia by evaluating the performance of the species in different spacing distance and sites. This will provide how dipterocarps plantation can be potentially established in the tropics.

MATERIALS AND METHODS

Study area

There were two experimental sites chosen for establishing a dipterocarp forest plantation trial. First is Gunung Dahu Research Forest (GDRF) which is located in Bogor, and second is plots at PT. Arara Abadi (PT. AA), Riau. GDRF trial plot lies around 40 km from Bogor city. Total area of the site is about 250 ha, where around 160 ha of the area had been successfully planted with dipterocarps with different objectives. The oldest stands were planted in 1997 while the youngest were planted in 2010. At the beginning of the project, most area was abandoned farmlands or secondary forests with few residual trees. GDRF consists of red-yellow latosol (inceptisol) with hilly terrain topography (elevation: 550-700 m asl.) and represented a mineral soil type. The hilly site was surrounded by villages, farm land, and mixed gardens. While plots at GDRF represented plantation area in mineral soils, plots at PT. AA represented dipterocarps plantation in drained shallow peat swamp soils, this area is frequently flooded at certain times. PT. AA is a forest plantation concession in Riau establishing *Acacia* and *Eucalyptus* plantation. The dipterocarps experimental plots at PT AA were established in 1998 with total area of 64 Ha with remaining planted area was about 8 ha.

Planting design, data collection and analysis

Planting stocks at both sites were originated either from seed and cutting propagules. Propagation by cutting was done by applying a mass propagation technique of cutting which was developed by The Forestry Research and Development Agency-Komatsu Project (Sakai et al. 2002). The technique and facilities for producing cutting propagules of dipterocarps is known as KOFFCO system (Subiakto et al. 2005). Planting stocks were raised at KOFFCO greenhouse and nursery, Conservation and Rehabilitation Research and Development Center (CRRDC), Bogor. The average size of planting stocks at the time of planting was 0.5 cm in diameter and 60 cm in height. Prior to this study, measurement at GDFR only carried out to plots those planted by *Shorea leprosula* and *Shorea selanica* each at spacing distance of 2 x 2 m, 3 x 3 m, and 4 x 4 m with total planting system. There were 6 combinations of the treatments, each represented by one-hectare (100 m x 100 m) plot with two replicates.

Measurement on plots at PT. AA conducted to plots those planted by *S. leprosula* and *S. selanica* each at spacing distance of 3 x 2.5 m and 3 x 5 m. There were 4 combinations of the treatments, each treatment was represented by one-hectare plots with two replicate. In total there were 8-ha plots measured at PT. AA. We tabulated the available data measurement for each the plot in both sites including survival rate, tree diameter, tree height, and standing stock per ha.

Diameter at breast height (DBH) and tree height (H) were calculated for both species and sites. Volume per hectare was calculated based on formulas developed by Wahyono and Soemarna (1984). One-way analyses of variance (ANOVA) were performed on DBH and H values to test the significance of species differences and spacing distance. A Duncan test was further executed to DBH and H values to determine statistical differences between treatments. No consecutive annual data were available for each of the plot; however, data collection were carried out at the time of planting, eight years after planting and fifteen and seventeenth year after planting (in about half cutting cycle of the dipterocarps).

RESULTS AND DISCUSSION

Establishment of man-made dipterocarp forest in GDRF

Dipterocarp plantation at GDRF was established for various purposes. The initial objective of planting dipterocarps in this area were as follow: (i) to test the adaptability of various dipterocarp to be planted outside their natural habitat; (ii) to determine the growth rate increment; (iii) to reveal its silvicultural technique both in nursery and field; and (iv) to conduct ex-situ conservation strategies of the species in a reliable and secure location (Subiakto et al. 2001). However, this study focused only to those block planting system plots of the two *Shorea* species with different spacing distance. Direct planting of dipterocarps and other primary forest species especially on *Imperata* grasslands have largely been unsuccessful because of slow growth and high mortality arising from their shade and moisture requirements (Otsamo et al. 2001).

At seventeenth year after planting, the survival rates were varied between 30.5%-77% depends on species and spacing distance. Survival rate within the same species showed that *S. leprosula* and *S. selanica* had their highest survival rate at 3 x 3 m spacing distance and the value went down when planted at more spacious distance of 4 x 4 m (Table 1). Hai et al. (1996) studied that shade together with terrain were the factor that significantly affect the survival

rate. At the early stage of dipterocarps growth, denser population in semi-open area would create more shade environment and support higher survival rate compare to those planted on more spacious distance. In addition, Widiyatno et al. (2013) reported that *S. leprosula* were more resistant on availability of sunlight and hence they could be easily established in open planting that has high heat and temperature stress in the early plantation establishment.

Diameter at breast height varied among species and spacing distance (Table 1). In contrast to survival rate, both species showed similar tendency that they gained their highest DBH at more spacious distance (4 x 4 m > 3 x 3 m > 2 x 2 m). In further developmental stages, denser population would increase competition among individual and hence limit the availability of nutrient, light, and water for each tree. Comparison among two studied species showed that *S. leprosula* had higher DBH compare to *S. selanica* except for 2 x 2 m spacing distance when both species has no significant differences (Table 1). Similar to those DBH, height growth also increased along with more spacious distance except for *S. selanica* at 3 x 3 m spacing distance that resulted in shorter height compare to its 2 x 2 m spacing distance. Comparison between two studied species also showed that *S. leprosula* gave better height performance compare to *S. selanica* (Table 1). The estimation of standing stock per hectare based on numbers of tree survived indicated that *S. leprosula* gave the highest result at 3 x 3 m spacing distance, whereas for *S. selanica* the highest was 2 x 2 m. Both species showed similar result that more spacious distance would yield in highest DBH and height growth but lowest volume per hectare related to less number of trees per hectare.

In the primary forest at tropical rain forest the wood volume was estimated 211.75 m^3/ha where dipterocarps was 86,9% of total volume (Bischoff et al. 2005). The current dipterocarps standing stock potency of logged over area (LOA) in Indonesia range between 35 to 40 m^3/ha (Van Gardingen et al. 2003). This value is not economically profitable and attractive for the forest companies and thus many forest companies converted the area to exotic monoculture plantation. Therefore, several silviculture managements are needed to establish more economically attractive native tree plantation with the stressing on improving the yield. Standing stock value in GDRF showed that *S. leprosula* (2 x 2 and 3 x 3 m), could reach 195.855 m^3, 215.412 m^3 respectively, whereas *S. selanica* (2 x 2) could reach 181.395 m^3at around their half rotation cycle. These values are prospective considering that GDRF is not an optimum habitat for lowland dipterocarps and hence contain several major growth limitations.

In many cases exotics have been favored for plantation and reforestation projects due to the assumption that they grow faster. In contrast to widely believed that dipterocarps and others native trees perform poorly when planted in open and degraded lands (Suzuki and Jacalne 1986; Otsamo et al. 1996, 1997; Tolentino 2008), our results revealed that dipterocarps can be a promising alternative to those of exotic species to be used for reforestation projects and establishing tropical plantation. Considering both experimental sites have their major limitation for the suitability of optimum growth, our study showed that *S. leprosula* and *S. selanica* still grow well under their peripheral site condition. For GDRF, altitude seems to be the major limitation, since both species are the lowland dipterocarps components. The elevation of GDRF is about 700 m a.s.l. may haltered the optimum growth and performance of the species. While plots in PT. AA did not meet its criteria for optimum growth because once in a time it still has frequent flooded because the sites were originated from drained peat swamp forest. Hence, the growth of the two dipterocarps still performed well in this location. Other critical factor for growth of dipterocarps is precipitation. The average rainfall of GDRF and PT AA were 2500 mm/year and 2700 mm/year respectively, which are considered suitable for both tested species. The good growth performance of those species on both less suitable sites may be due to intensive maintenance of the weeding activity once every three months considered as reducing competition against weeds during early growth stage.

Our result further support those of Shono et al. (2007) and Santos Martin et al. (2010) who determined that native trees, including some Dipterocarpaceae, can survive and perform well even in open degraded sites. The very important native dipterocarps merit further research into developed for commercial plantation when all silvicultural management and site-species matching are determined so that survival rate and growth performance are increased (Langenberger 2006; Wishnie et al. 2007; Millet et al. 2013) to similar or nearly similar of that exotics species. In fact, our result supported previous study that *S. leprosula* showed faster growth and are potential to be established into commercial plantation especially in well-drained sites (Soekotjo 2009; Adjers et al. 1995).

The growth rates provided by this study help improve understanding of the potential prospect of the dipterocarps for establishing commercial plantation in the tropics. For most of dipterocarps, most of the studies focus on recently established plantings. The growth and performance review only to those of early stage and there are very few data points for plantations of more than 10 years, or for smallholder-managed sites. Thus, this study helps build knowledge about dipterocarps species management, and provides a comparison point for relative species and site performance. However, several important factors that influence tree performance such as site management and frequency and intensity of weeding around planted trees does not take into detailed consideration in this study.

Accordingly, site care and maintenance varied among two of experimental sites and so did for the spacing distance. Our study indicated that even the very frequent weeding activity conducted to every tree in PT. AA sites, the result for survival rates does not show extreme differences to those of GDRF. However, intensive weeding in PT. AA might give a positive impact to maintain the survival rate value. Similar to that of survival rate, even though diameter showed more consistent value among treatment in PT. AA, its increment also did not show an extreme difference to that of GDRF. In spite of intensive

and frequent maintenances, the result seemed to indicate that several species do better in certain soil types and site conditions (Shono et al. 2007; Yamada et al. 2012; Scheneider et al. 2013; Dong et al. 2014); however swampy area might need more intensive nurturing than those of mineral soil for a dipterocarp lowland dry species to reach the similar growth performance and so as the vice versa.

More recent research in Singapore has also shown that native species, including certain dipterocarps such as *S. leprosula*, *S. acuminata*, *H. nutans* and *D. caudatus*, can successfully be planted in open lands (Shono et al. 2007), while other studies demonstrate that native dipterocarps in Sri Lankan such as *D. zeylanicus*, *S. disticha*, *S. migisttophylla* and *S. trapezifolia*need for nurse trees (Ashton et al. 1997, 1998), and others showed that dipterocarps performed well in enrichment plantings in semi-open selectively logged plantations (Millet et al. 2013).

This study demonstrated that in similar environment of hilly semi-open area, *S. leprosula* and *S. selanica* vary in their tolerance during their growth stages. However, *S. leprosula* showed higher growth both for diameter and height compare to those of *S. selanica* those resulted in higher mean volume/tree and total volume/ha. *S. leprosula* is known to be capable of fast initial growth and benefit from full open conditions (Appanah and Weinland 1990; Symington 2004; Shono et al. 2007). *S. leprosula* has also been shown to be the most suitable *Shorea* species for reforestation in several studies (Adjers et al. 1996; Howlet and Davidson 1996; Otsamo et al. 1996). Among four species of twelve years old dipterocarps studied by Hai et al. (1996), *S. leprosula* gave the best result in height and diameter for both locations of terrain and hill tops. Its better performance compare to *S. selanica* in this study somehow was not surprising and hence support the previous studies.

The performance of man-made dipterocarps forest in peat swamp areas of PT. AA

Similar to those in GDRF, establishment of initial man-mad forest in swampy areas showed a potential prospect. Survival rate for both *S. leprosula* and *S. selanica* showed higher value at denser population and that *S. leprosula* gave higher vol/ha to those of *S. selanica* (Table 2). Tables 2 also showed that DBH at denser population had lower value compare to those at more spacious distance, however tree height did not show similar tendency.

Mean volume per tree showed higher value at more spacious distance (Table 2). However, total volume/ha has not showed similar tendency, those depend on numbers of survived trees/ha. Both species showed prospective standing stock at their half rotation cycle when planted with 3 x 2.5 m spacing distance, with the wood volume per/ha were 208.448m^3 and 183.688 m^3 for *S. leprosula* and *S. selanica*, respectively. Considering the target volume for dipterocarp plantation in their optimum habitat considered to yield 174-400 m^3 at 30 years (Appanah and Weinland 1993; Soekotjo 2009), our study plots in PT. AA showed a prospective result for further development of dipterocarp plantation even in swampy peat land.

Table 1. Data measurement for a 17-years old dipterocarp plantation forest in Gunung Dahu Research Forest, Bogor

Species	Spacing (m x m)	DBH		Height		Survival rate		∑ trees survived/ha (ind)	Mean volume/tree		Vol/ha (m3)
		(cm)	sd	(m)	sd	(%)	sd		(m^3)	sd	
S. leprosula	2 x 2	13.7^{c}	2.35	12.5^{d}	1.65	66^{ba}	0.13	1650	0.119^{c}	0.05	195.855
	3 x 3	19.7^{b}	2.47	14.9^{cb}	1.77	69^{ba}	0.12	767	0.281^{cb}	0.08	215.412
	4 x 4	24.9^{a}	2.44	16.9^{a}	1.23	36^{c}	0.17	225	0.567^{a}	0.13	127.602
S. selanica	2 x 2	13.9^{c}	2.06	13.3^{dc}	0.48	58^{b}	0.17	1450	0.125^{c}	0.04	181.395
	3 x 3	15.2^{c}	2.22	10.8^{e}	1.12	77^{a}	0.11	855	0.124^{c}	0.05	105.993
	4 x 4	19.6^{b}	2.45	16.2^{ba}	1.85	45^{c}	0.17	281	0.306^{b}	0.13	85.992

Note: Numbers followed with one or more similar letter in the same column showed that the result not significantly different based on Duncan test; DBH = diameter at breast height; sd = standard deviation

Table 2. Data measurement for a 15-year old dipterocarp plantation forest in swampy area of PT. AA, Kampar, Riau

Species	Spacing (m x m)	DBH		Height		Survival rate		∑ trees survived/ha	Mean volume/tree		Vol/ha (m^3)
		(cm)	sd	(m)	sd	(%)	sd	(ind)	(m3)	sd	
S. leprosula	3 x 2.5	18.53^{c}	2.46	13.30a	2.22	69.5^{a}	0.13	926	0.225^{c}	0.08	208.448
	3 x 5	22.96^{a}	2.49	14.49a	2.29	59.5^{b}	0.17	397	0.367^{a}	0.10	145.650
S. selanica	3 x 2.5	19.34^{c}	1.79	14.33a	2.61	52.0^{b}	0.15	693	0.265^{cb}	0.11	183.688
	3 x 5	21.09^{b}	1.31	13.44a	2.92	40.5^{c}	0.12	270	0.294^{b}	0.10	79.420

Note: Numbers followed with one or more similar letter in the same column showed that the result not significantly different based on Duncan test; sd = standard deviation

Shorea leprosula maintained higher volume/ha at more spacious distance, but *S. selanica* decreased almost 40% of its volume when the species planted at 3 x 5 m spacing distance (Table 2). This condition indicates the possibility of different tolerance to exposure during their growth development with *S. selanica* is more sensitive to exposure than *S. leprosula*.

Both *S. leprosula* and *S. selanica* are naturally grown at mineral soil. However, this study demonstrates that establishing man-made dipterocarp forest in swampy area is surely possible. Yet, some notes are needed to be considered. Species and spacing distance were determined to affect DBH, survival rate, and mean volume/tree, thus resulting in different gain for total volume/ha. Site-species matching is a factor that needs to be considered properly through trial and experiments. Hence, it is needed to screen and select the most appropriate species growing in lowland swampy area and establish native tree plantation as an alternative to those of most exotic species planted in Indonesia (e.g. *Acacia, Eucalyptus*). While *S. leprosula* and *S. parvifolia* actually will reach their optimum grow in well drained sites, other species such as *S. macrophylla* was shown the best choices to be planted in frequently flooded sites (Mohd Nawar 2012).

The significant difference between plots on hilly mineral soils and lowland swampy area descriptively could be seen in their generative ability in which a 15-years old dipterocarp forest in PT. AA have been reported for three times fruiting but not for a 17-years old dipterocarps in GDRF. Monsoon climate as characterized by distinct separation between wet and dry months is widely proposed as one of cues for triggering generative reproductive of the many dipterocarps. Most area in Java Island are affected by monsoon, however Bogor is among the few areas that has tropical wet climate without any influence by monsoon. Having similar Koppen *Af* (tropical rainforest climate) climatic group as those of PT. AA in Perawang Riau, monsoon climate seems not to be the plausible factors for determining why GDRF has not experienced flowering season like those in PT. AA-Riau. Another environmental factor that is possible in determining the flowering pattern is precipitation. However, both GDRF and PT. AA are characterized by showing almost similar data of precipitation with total annual rainfall > 2500 mm/year and total rainy days >160 days/year (Meteorology and Geophysics Bureau; www.bmkg.go.id). Altitude is remaining as the only one that showed rather extreme differences in which PT. AA is located in < 100 m a s l, while GDRF is located at nearly 700 m asl. In this case, we proposed that the differences for flowering phenology of *S. leprosula* and S. *selanica* in two sites mainly caused by the altitude. This is also supported by the fact that *S. platyclados*, a hill dipterocarps species, have been experiencing flowering events several times in GDRF. Since this is the phenomena that we found along the establishment of the plot, it will need further observation and more comprehensive analysis in determining the most possible factor responsible for the differences of the generative reproduction of the two studied *Shorea*.

It is generally believed that the limitation of domestication efforts of the native trees still exists probably due to problems with germplasm multiplication, distribution, and availability. However, as the series of research on the prospect of the native tree species recently increased progressively, the important findings began to shed a light. As the germplasm multiplication generatively is a problem in dipterocarps due to its irregular fruiting and their recalcitrant seed characteristics, a mass vegetative propagation by means of cutting and known as KOFFCO technique showed a significant progress.

Considering changing wood processing technology, producing timber of lower dimension may be currently acceptable. Depending on site and management intensity, rotation cycles of 30 years or less with a target diameter of 30-50 cm seem to be feasible for some of the faster growing dipterocarps. Based on our result, it is determined that planting dipterocarps in more spacious distance would be suitable to fulfill demand of timber for the purpose of construction and furniture (yield in bigger DBH), while planting dipterocarps in denser population would result in higher volume/ha with smaller DBH in which will be suitable for carbon stock and pulp industry.

In the long term, the need for the establishment of man-made native tree plantation will increase. Timber product will not be available to be supplied only from natural forest due to their rapid loss. Future forest products must be fulfilled from commercial forest plantation. Considering the environmental issues emerge along with the development of the exotic species in the tropics, the development and establishment native tree commercial forest plantation will be the only choice left to ease pressure of the remaining natural forest. Native tree plantation, especially dipterocarps in SE Asian tropical forest, would gain several benefit started from carbon sinks, gene pools, source for biologically active compound/medicinal benefit, water regime and maintaining biodiversity. The need to establish dipterocarp plantation with proper silvicultural management technique however is a must.

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Long-term variability of zooplankton community under climate warming in tropical eutrophic man-made lake

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Abstract. *Sunardi, Yoshimatsu T, Junianto N, Istiqamah N, DeWeber T. 2016. Long-term variability of zooplankton community under climate warming in tropical eutrophic man-made lake. Biodiversitas 17: 626-633.* The climate warming is increasingly acknowledged as an important driver of lake ecosystems. However, there are no generic patterns of how the aquatic species/community responds the warming climate; instead the changes are complicated by interactions of many factors. To regard the important role of zooplankton in the lake ecosystems, this paper questions whether the climate warming affects their community structure in tropical eutrophic man-made lake. We analyzed a series of data resulted from a long water quality monitoring activities in the Cirata Lake, Indonesia. We anticipated that there would be a strong association between the climates warming with the response of zooplankton community after 19 years. Our result suggested that the lake has been becoming slightly warmer following the atmospheric temperature. Instead of decreasing, the shifting water temperature tend promotes a greater species richness, density, and diversity of the zooplankton. Relevant changes in species composition have been observed. It seems that the magnitude of the shift of the temperature, and the eutrophication status played an important role in shaping the changes of the zooplankton community structure.

Keywords: Cirata Lake, climate warming, eutrophic, tropics, zooplankton

INTRODUCTION

Climate change is a challenge for species survival and ecosystems sustainability. Globally, temperature and precipitation have changed dramatically and are predicted to change even more (Meehl et al. 2007), thus, threats of the climate change to all life-forms are believed to remain in the next few decades. Researches have reported that freshwater ecosystems, such as lakes, rivers, streams, and wetlands, are vulnerable to climate change (eg. Magnuson et al. 1997; Sahoo and Schladow 2008; Sunardi and Wiegleb 2016). Climate warming will likely affect inland waters more than ocean (Christensen et al. 2007), as warming over land is expected to be greater than global annual warming due to the smaller thermal inertia and less available water for evaporative cooling on land.

Climate change is increasingly acknowledged as an important driver of lake ecosystems (Adrian et al. 1995; IPCC 2014), but the understanding of the mechanisms by which climate affects lakes is still patchy (Keller 2007). The complexity of the issue of lake responses to climate change arises from the fact that the various climatic components act on lake physical, chemical, and biological characteristics through many interconnected pathways (Battarbee 2000; Leavitt et al. 2009). Various components of the climate system have been shown to relate to temporal dynamics of natural plankton communities on time scales varying from days (diel periodicity) to years (seasonal periodicity). With our environment changing at an unprecedented rate, an important challenge is to assess the impact of climate change on the temporal plankton dynamics of lake ecosystems (Christensen et al. 2007).

The community structure and ecological role of zooplankton in natural and man-made lakes are issues of fundamental concern to aquatic productivity and/or ecosystem stability. Zooplankton communities are highly diverse and thus perform a variety of ecosystem functions. Arguably, the most important role of zooplankton is as the major grazers in aquatic foodwebs, providing the principal pathway for energy from primary producers to consumers at higher trophic levels, such as planktivorous fish, macroinvertebrate, and turtles. In view of their grazing activities and role in nutrient recycling, zooplankton actually or potentially exert both subtle and gross effects on phytoplankton populations, which in turn have a prime bearing on water quality (Mavuti1990).

A possible effect of climate warming is that the water temperatures would increase to levels that are suboptimal or lethal for aquatic organisms, particularly to those with limited dispersal ability like zooplankton. Temperature affects nearly all biological process rates, from biochemical kinetics to species generation time, with higher temperatures typically resulting in higher rates until an optimum is reached, above which rate processes usually decrease rapidly (Kingsolver 2009). Life history parameters (e.g., growth, development, reproduction), respiration, behavior, and survival of aquatic poikilotherms are affected by temperature (Goss and Bunting 1983).

It is proven that water temperature plays a key factor in species distribution and richness along elevational and altitudinal gradients (Ward 1985; Reyjol et al. 2001). Climate warming will consequently, to some extent, shape the structure of zooplankton community of lakes, rivers and other water bodies. On one hand, zooplanktons are poikilotherms whose development rate at each life stage is determined by temperature. Nevertheless, locality of the climate shows that increase in temperature will not always be so extreme or a deadly levels. As a matter of fact, the tropical ecosystems experience relatively less variability in temperature compared to those in temperate areas. On the other hand, zooplankton inhabiting the eutrophic lakes may respond differently to the climate warming compared to those living in oligotrophic lakes. This might be due to differences of resource availability and environmental factor complexity. Research suggested that eutrophic waters are generally characterized by high nutrient concentration, organic matters, and turbidity, and hence attenuation of solar radiation (e.g. Wunderlich and Elder 1973; Imai et al. 2001; Parikesit et al. 2005). Alric et al. (2013) stated that lake vulnerability and responses to climate warming are modulated by lake trophic status. Therefore, such variabilities of the environment make prediction of the climate warming effects on zooplankton community more difficult because the components interconnect one another.

Indeed, research on how climate warming affects the zooplankton community in tropical eutrophic lakes will enrich our knowledge of ecological processes in tropical ecosystems, which left behind the temperate areas. In this paper, we question if the climate warming in the eutrophied Cirata Lake shows a clear link to the variability of the zooplankton community in a long perspective. We anticipate that the long-term increase of temperature will pose a significant threat to the zooplankton community.

MATERIALS AND METHODS

Study site

The study was carried out in Cirata man-made lake situated at West Java Province, Indonesia. The lake covers about 62 km^2 of inundated area which is a part of Bandung Barat, Cianjur, and Purwakarta District. The lake has average depth of about 40 m with maximum temperature difference between water layers is 4^{o}C. Permanent sampling stations are distributed throughout the lake as established during the Environmental Impact Assessment (EIA) of the project (Figure 1). Cirata was built in 1987 with main purpose of generating electricity to supply Java and Bali by hydropower. The lake receives water discharge from twelve rivers, and the catchment is characterized by a very dense-populated city (Bandung Metropolitan) and intensive agricultural practices. As the time has passed, the lake function has expanded to include wider uses. Beside energy production, Cirata also serves for aquaculture, irrigation, and as a tourism site. There is a fast growing number of floating net cage aquaculture in the lake, which has increased from less than 1,000 unit in the early time of its development (1988) to 53,000 units in 2011 (PT. PJB 2012). Recently, the lake has been experiencing severe eutropication, organic pollution, and high levels of other chemical contaminants (Institute of Ecology 2013).

Data source and sampling protocols

A database of plankton and environmental quality parameters from quarterly water monitoring activities was used in this study. Samples collections were generally conducted in February, May, August, and November each year. The Institute of Ecology-Padjadjaran University organized the monitoring activities from 1995 to 2013 in service to PT. PJB (state electricity company), the authority of the Cirata Lake. During that period, some sampling was carried out by two different institutions and the data generated by the institutions were verified before use to ensure the uniformity of the data, and thus, the analysis.

Over the entire study period, the Institute of Ecology and the other two laboratories employed the same procedures and techniques. Data on biology, the zooplankton, and environmental parameters, water temperature and transparency, were obtained from regular cruises on a speed-boat. Samplings normally started from about 11.00 AM and finished at 14.00 PM. Water temperature and transparency were measured on-site using an Hg thermometer and a Secchi-disc, respectively (APHA 1989). Water samples were collected from 20 cm water depth for other physico-chemical analysis. Meanwhile, the biological samples were collected using plankton net no. 25 (mesh size 0.55 μm). The zooplankton were identified to the highest taxonomic separation possible and counted in a Sedgwick Rafter under a light microscope. Following determinants of plankton community structure were obtained: species richness, density, Shannon-Wiener diversity index, and functional groups.

In addition, the data on air temperature was collected from the nearby source, i.e. Cirata weather station. The air temperature was collected from hourly record every day, and is presented as daily temperature corresponding to that obtained from water measurement.

Data analysis

The data on the zooplankton community structure were correlated to the data on the water temperature and transparency. The linear regression and correlation analysis were done using the R statistical environment (R Core Development Team 2015). Meanwhile, the changes of zooplankton community structure were described through species and functional group composition.

RESULTS AND DISCUSSION

Results

Our study found that the average daily atmospheric temperatures were lower than the midday water temperatures. In the Cirata lake area, however, inter-annual water and air temperature exhibited a clear warming tendency over time regardless the magnitude. In fact, the phenomenon of climate warming has determined the water

temperature of that tropical aquatic ecosystem (Figure 2). Over 19 years, the average increase in water temperature was 0.53°C which corresponds to 0.03°C per year. However, there was also a remarkable seasonal variation of water temperature in the lake; in dry-season the water temperature could rise up to 3°C higher than that in rainy-season. Therefore, the shift in water temperature in the long run was clearly within the range of seasonal temperature variability.

The water transparency decreased over time (Figure 3) showing a continuous augmentation of load of particulate matters. Such particulate matters might consist of organic and non-organic materials which could have either direct (such as food recruitment, survival, mobility) or indirect (such as growth and community structure) effects on zooplankton responses.

With regard to community metrics, our results showed that the elevated water temperature had a positive association with species richness, density, and diversity index (Temperature vs Species Richness: *y = 2.497x-56.295, r = 0.32*; Temperature vs Density: *y = 14282x-324133, r = 0.12*; Temperature vs Heterogenity: *y = 0,03706x + 0,39162, r = 0.07*) (see Figure 4a, b, and c). The correlations between water temperature and the community indicators seemed to be poor (as indicated by *r* values); but nonetheless there was a slight increased tendency of the three parameters were observed. The long-term increase in lake water temperature had increased the species richness, density, and diversity. It seems that the warming water have promoted the zooplankton species to develop better than the previous environments.

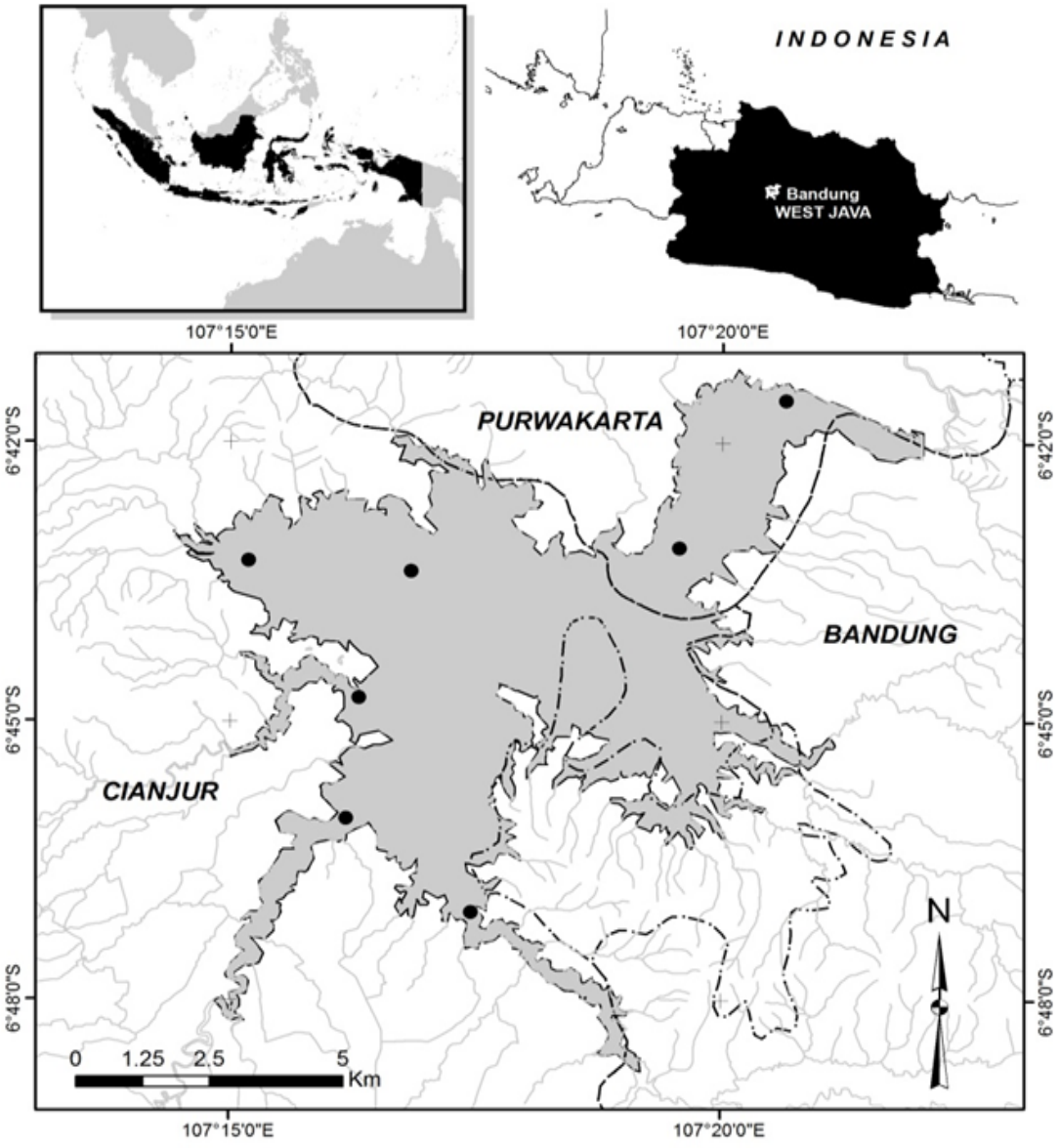


Figure 1. The study site: the Cirata Lake in West Java Province-Indonesia (solid dots show the water sampling sites)

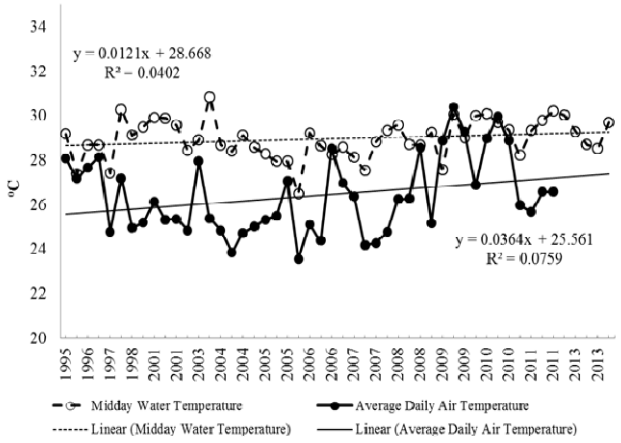


Figure 2. Trend in air and water temperature in the Cirata lake

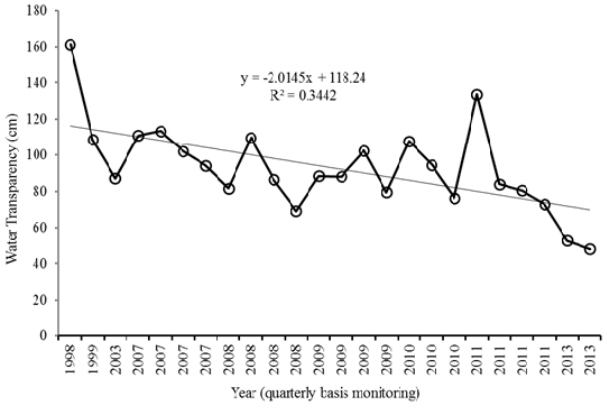
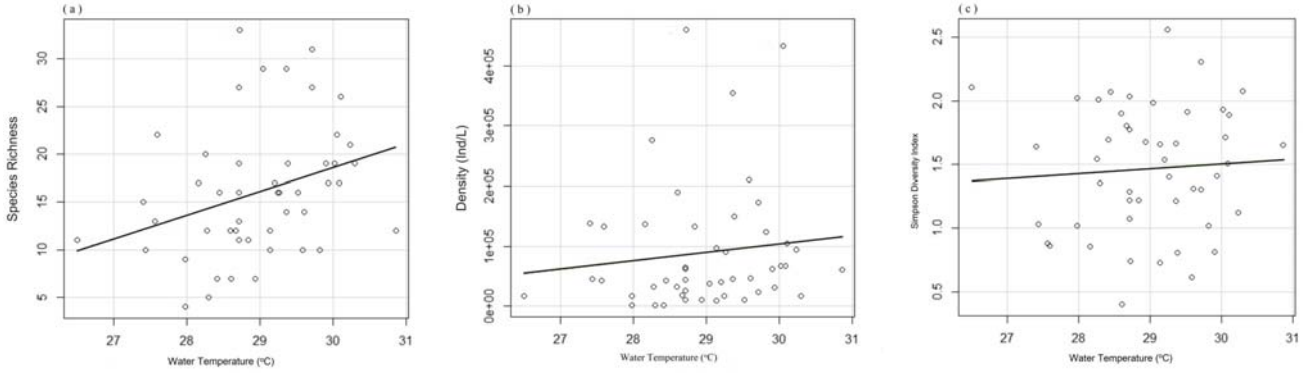


Figure 3. Trend in water transparency in the Cirata lake



A **B** **C**

Figure 4. The relation between water temperature and zooplankton community metrics: A. Species richness, B. Density, and D. Diversity index

The number of zooplankton species fluctuated over the study period showing that species composition changed dynamically. Species alternately dominated the community over time; in term of abundance there was no single species remained at the top of the community (Figure 5a). Eight species observed to be predominant in the Cirata Lake were *Cyclops* sp., *Brachionus* sp., *Macrothrix* sp., Nauplii, *Bosmina* sp., *Filinia* sp., *Moina* sp. and *Moinodaphnia* sp. The result showed that some species (such as *Cyclops* sp., *Brachionus* sp., and *Macrothrix* sp.) remained in higher number of individuals compared to the other species. They seemed to be more adaptable to changes of the water temperature and of the eutrophication status. There was also a short period where some species (*Moinodaphnia* sp., *Filinia* sp.) occasionally dominated the community, but again disappeared in the other years.

Interestingly, if we refer to higher taxonomic groups the structure and variability of the zooplankton community was more visible. Cladocerans, copepods, and rotifers seemed to be the main components of the eutrophic lake (Figure 5b). It was expected that the three groups would be more common in term of biomass. In the early years, copepods were well developed and dominated the community structure, but in the later years their population decreased gradually. In contrast to that, cladocerans were found less abundant in the early time but then it tends to be dominant in the later years. Meanwhile, the populations of rotifers were generally constant over the time even though in the most recent period it was found less frequently.

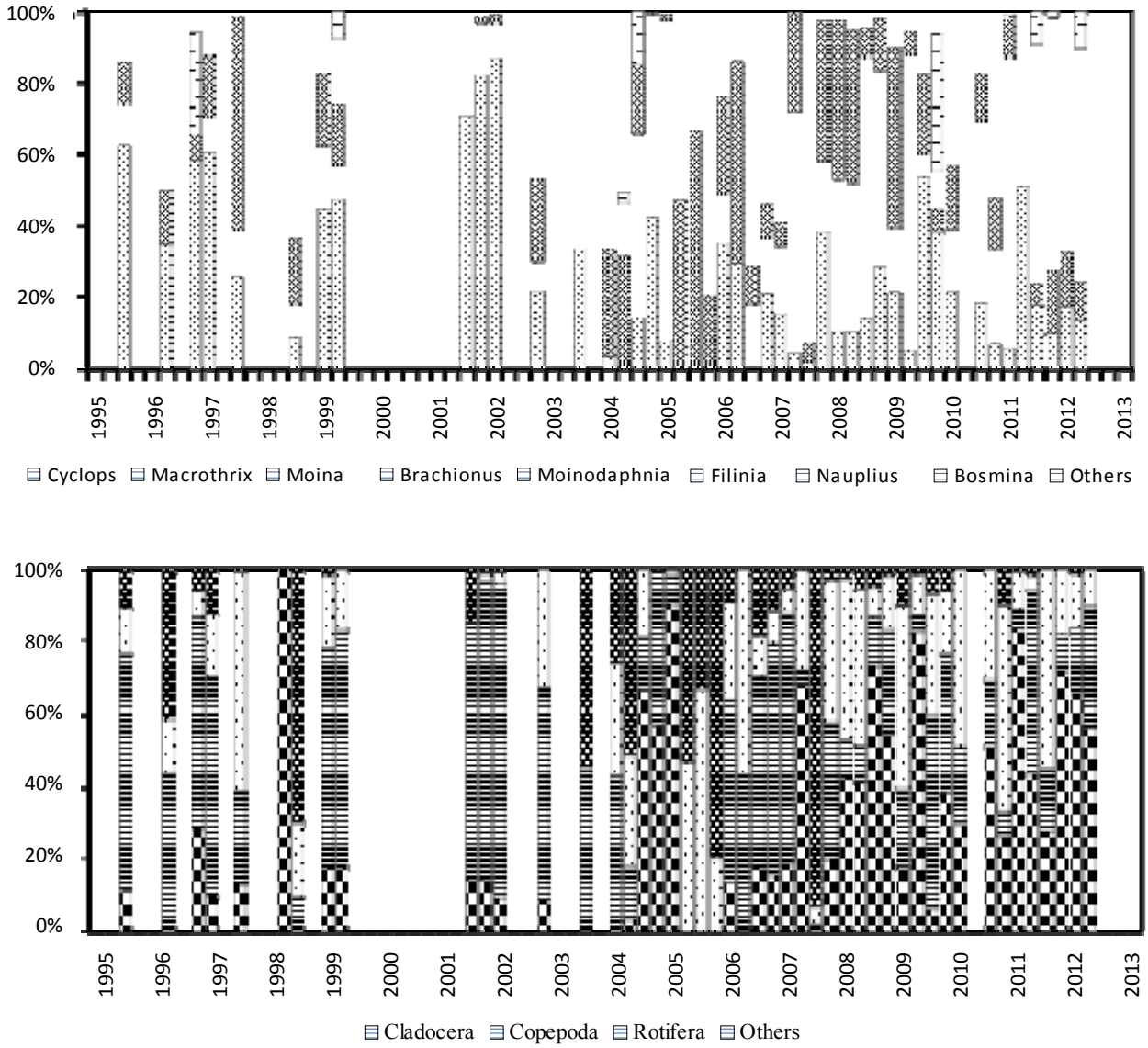


Figure 5. Long-term variability in species composition (above) and functional groups (below) of zooplankton in the Cirata Lake (Blank spots between bars stand for missing data on the relevant periods).

Discussion

The climate warming is truly influence the global environments, no exception of the tropical aquatic ecosystems. The water of the Cirata Lake has been warming following the increase in global atmospheric temperature. Nevertheless, the shift of the water temperature after a long period was very low (< 1^{o}C) confirming the present research which demonstrated that tropical areas have lower variabilities in temperature (Lowe-McConnell 1987). The magnitude of amplifying thermal regime in water system depends on many environmental factors. It has been reported that the level of suspended matters contributes to the heating process of the natural water systems (Dwivedi 2014; Bonalumi et al. 2012; Paaijmans et al. 2008). Water turbidity affects water temperature, as suspended particles in a water column absorb and scatter sunlight and hence determine the extinction of solar radiation. When the water is turbid, sunlight will warm it more efficiently. The warming contribution of suspended particles in water may strengthen the threats of already increased temperature to water-inhabiting organisms such as phytoplankton, zooplankton, fish, etc.

In case of eutrophied lakes, levels of eutrophication and water transparency/turbidity may pose additional forcing to climate warming effects on aquatic organisms as heating can be substantially facilitated. The suspended particles may be either inorganic or organic. Research reported that eutrophic waters usually associate with higher suspended matters loads originated from high siltation and organic

pollution (e.g. Wunderlich and Elder 1973; Imai et al. 2001; Parikesit et al. 2005). That is truly the case of the Cirata Lake (Institute of Ecology 2013). High amount of organic matters and or high assemblage of phytoplankton in eutrophic waters benefit and may help the zooplankton compensate the stress posed by elevated temperature. In such case, it is more difficult to predict whether increased temperature will exclude or promote the zooplankton species, thereby difficult to expect generic trajectories of the community changes driven by climate warming.

It is commonly discovered that climate warming has negative effects on zooplankton species diversity and aquatic ecosystem stability (Bonecker et al. 2013; Deksne et al. 2011). Drake (2005) pointed out that temperature variability has a major effect on zooplankton growth rate and generation times. Existing evidence showed that climate warming has decreased the total abundance of zooplankton and species diversity (Deksne et al. 2011), trophic structure and the dynamic of the population (Wrona et al. 2006), and likely affect the survival, reproduction and growth, distribution, persistence and diversity of species (Leveque et al. 2005). Purvina et al. (2009) suggested that the increasing water temperature can lead to decrease in food assimilation and the filtration rate by the zooplankton grown-up representatives, as well as to the eggs' degeneration and even its abortion.

Interestingly, instead of declining, the zooplankton species richness, density, and diversity index climbed up over the time as the water temperature elevated. In line to this, a research confirmed that temperature variability promotes greater richness of zooplankton species (Shurin et al. 2010). In case of the Cirata Lake, the better condition of the environment (in view of ecosystem concept) may be attributed by two factors: (i) the magnitude of the temperature shift, which was insubstantial (i.e. within the range of tolerance limit), and (ii) high organic resource availability. As it has been noted that the seasonal variabilities of temperature in the Cirata Lake were greater than those in a long run period. Such experience may benefit the zooplankton species to cope with the shifting thermal regime in the water. According to Almén et al. (2014), zooplankton experience widely varying conditions in their physicochemical environment on a diurnal basis. The amplitude of the fluctuations may affect the zooplankton species' ability to respond to climate change.

High productivity of phytoplankton in nutrient-rich waters or suspended organics facilitates more zooplankton species to develop. In the Cirata Lake, the total density of zooplankton exceeded the producers indicating that zooplankton do not consume merely on phytoplankton but also on suspended organics (Institute of Ecology 2013). There are many zooplanktons which feed on organic particulate matters (Hebert 1977). Allochtonous organic carbon often dominates the carbon pools of tropic lakes, and may represent a significant resource for zooplankton consumers (Karlsson et al. 2002; Cole et al. 2006; Jonsson et al. 2007). Strong evidence suggests that zooplankton assimilate significant amounts of terrestrial carbon in some lakes (Carpenter et al. 2005; Cole et al. 2006, 2011). Even though there are some droughts on the designation of terrestrial organic carbon as a resource subsidy (Brett et al. 2009; Jones et al. 2012) due to low nutritional quality. However, it seems that this was not the case of the Cirata Lake. "Moderate" increase in temperature over a long-term period combined with high organic matters seems to promote more zooplankton species to grow, and thereby increase the species richness, density, and diversity.

Zooplankton in lakes is composed mainly of rotifers, cladocerans, and copepods. Perturbation in lake ecosystems by climate warming will significantly affect their community structure. In species level, the trajectory of changes of species composition is hard to generalize. Research suggests that the nature of the plankton responses is diverse as species exhibit complex life-history traits (Adrian et al. 2006). But interestingly, grouping using higher taxa (such as using the dominant taxa: copepods, rotifers, and cladocerans) provide a visible trend. In the early years of the study period, copepods dominated the zooplankton community, but their populations declined over time. On the contrary, cladocerans became dominant in the later years, while rotifers were more or less stable. These changes may be due to the eutrophication process and input level of allochtonous organic contaminants in the lake. Cladocerans are likely the best survivors of the warmer temperature as well as of the eutrophied and high organic contaminated waters. Most cladocerans remove particulate organic matter from water by filtration, have the ability to ingest food of a wider size range, and have higher filtering rates, which could give them competitiveness over the rotifers (Allan 1976; Lynch 1980), and perhaps over the copepods as well.

The greater species richness, total density, species diversity, and overall changes in community structure of the zooplankton in the Cirata Lake may be attributed to the climate warming. However, instead of inhibiting or excluding species, the warmer climate favors zooplankton species to develop or to grow. The magnitude of temperature variability in long-term and the amplitude of short-term (diel and seasonal) temperature variability seems to play an important role in the definition of forces posed to the zooplankton community. The wealth of resources (such as high phytoplankton density and suspended organic matters) in aquatic ecosystems may support the zooplankton harness the "moderate" shift of temperature, or in opposite manner, combat the negative effects of climate warming.

To summary, warming of the water of the Cirata Lake has been observed over 19 years, which was clearly driven by the climate warming. The effects of the climate warming, however, would not be necessarily negative to organisms. Greater species richness, density and diversity of zooplankton indicated that zooplankton species could harness the changing climate. Beside the range of long-term variability of temperature, the eutrophic status of the aquatic ecosystem may concurrently determine the types of their responses. This result confirms the existing paradigm suggesting that the impact of climate change on zooplankton community structure is not straight forward and potentially highly complex. Tropics or temperate, eutrophic status, and other environmental factors work as

concomitant forcing which may amplify or hide their individual affects on the studied community.

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Short Communication: Fish diversity of the Batang Toru River System, South Tapanuli, North Sumatra

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Abstract. *Roesma DI, Chornelia A, Mursyd A, Kamsi M. 2016. Short Communication: Fish diversity of the Batang Toru River System, South Tapanuli, North Sumatra. Biodiversitas 17: 634-641.* A rapid survey on fish diversity was carried in the Batang Toru river system. The survey was carried out in the wet season between 8-14 February 2015 and 15-21 of March 2015 along various tributaries on the east (10) and west (1) side of the main Batang Toru river system. We obtained 427 individuals fish samples consisting of 24 species, from 10 families. These consist of Cyprinidae (11 species), Balitoridae (2), Channidae (2), Gobiidae (2), Nemacheilidae (2), Aplocheilidae (1), Bagridae (1), Cichlidae (1), Mastacembelidae (1), and Sisoridae (1). Four Sumatra fish species were encountered during the surveys, namely *Neolissochilus sumatranus*, *Nemacheilus pfeifferae*, *Homaloptera gymnogaster* and *H. heterolepis*. *N. sumatranus* and *Puntius binotatus* were the most frequently found in all of sampling sites.

Keywords: Diversity, endemism, *Neolissochilus sumatranus*

INTRODUCTION

Freshwater ecosystem may be the most endangered ecosystems in the world. Fish diversity represents as much as one third of all vertebrate species, and declines in freshwater fish is occurring at a greater rate than species loss in the most affected terrestrial ecosystems (Sala et al. 2000; Dudgeon et al. 2006). Our knowledge of freshwater diversity is woefully incomplete; especially in tropical latitudes that supports the greatest proportion of species diversity (Stiassny 2002). In the Indo-Pacific region, Indonesia has the highest freshwater fish species richness (Allen 1991; Kottelat and Whitten 1993) and is a mega-biodiversity country along with Brazil. Indonesian is home to approximately 1000 species of freshwater fishes (Suwelo 2004) relative to. 50,000 fish species worldwide (Vida and Kotai 2006). Of these about 22,000-25,000 species have been named with valid description (Allen 2000; Gilbert and Williams 2002) and new species are being discovered or recognized at a rate of approximately 200 species per year of which 40% are freshwater fishes (Nelson 1994).

According to Zakaria-Ismail (1994), the distribution pattern of Southeast Asian freshwater fishes can be divided into five zoogeographic regions. The island of Sumatra, Borneo and Java are the fourth zoogeographic area on the fish distribution and characterized by a high degree of endemism. Sumatra has a number of major rivers, with the Batang Toru River being one of them. It is located in Batang Toru forest. According to Khakim (2011), Batang Toru forest covers some 136.000 ha of primary forest. The forest is located in North Sumatra. It is situated in three sub-districts, North Tapanuli, Central Tapanuli and South Tapanuli. The greater part of the Batang Toru forest is at present allocated as production forest and land to be converted to other uses (81%) and only a small part (19%) is allocated as protected forest.

Batang Toru River systems and its tributaries are critical habitat for abundant freshwater fish resources ranging from socially and economically important species such as Gariang (Mahseer fish), Puntius (barb) and Rasbora (minnow). There is lack of knowledge regarding to species diversity in the Batang Toru while there was an anthropogenic activities, over fishing till human building such as dam are known as critical factor for fish diversity in entire river's length and its tributaries. It is therefore critical to assess species diversity along the stretch of the Batang Toru River in terms of endangered, rare, and endemic species and related threats from anthropogenic activities in order to guide planning of conservation interventions.

Studies of fish diversity are needed to establish an inventory of the fish fauna present in the Batang Toru river systems area. This study aims to develop baseline data that will be valuable to assess the future environmental impacts of development and conservation.

MATERIALS AND METHODS

Study site

We sampled fish in eleven locations in Batang Toru River systems comprises of Aek Malakut, Aek Toras, Aek Sikkut, Aek Batang Toru, Aek Marancar, Aek Sitandiang,

Aek Simajambu, Aek Sihoru-horu, Aek Batang Paya, Aek Na Pot Pot, and Aek Sirabun (Figure 1). Sampling was done in the sub-districts of Batang Toru, Marancar, Sipirok in South Tapanuli District, North Sumatra, Indonesia. We sampled the sites in daylight hours between 8-14 February 2015 and between 15-21 March 2015. The eleven sampling locations comprised each of two sites (upstream and downstream reference). Downstream locations refer to locations towards the main Batang Toru River.

Sampling methods

Sampling was done by following standard procedures according to Cailliet et al. (1986), using cast fishing nets (1st sampling session, 8-14 February 2015) and backpack electrofishing gear (12 Volt) during the 2nd sampling session (15-21 March 2015). Sampling at each location was done for approximately one hour. For each fish sample obtained, we described key characteristics such as body color, color of fins which may get lost or change after death, made measurements of the shape of the body, photographed each sample, and preserved samples with formalin 10%, after which they were taken to the laboratory at Universitas Andalas in Padang, West Sumatra. Not all individual fish caught were taken as a sample. For those species whose sample number was considered sufficient, specimens were released back into the river. All specimens were later preserved in 70% ethanol. Identifications were based on the main keys for freshwater fishes in the region (Weber and Beaufort 1916; Kottelat et al. 1993; Kottelat 2013).

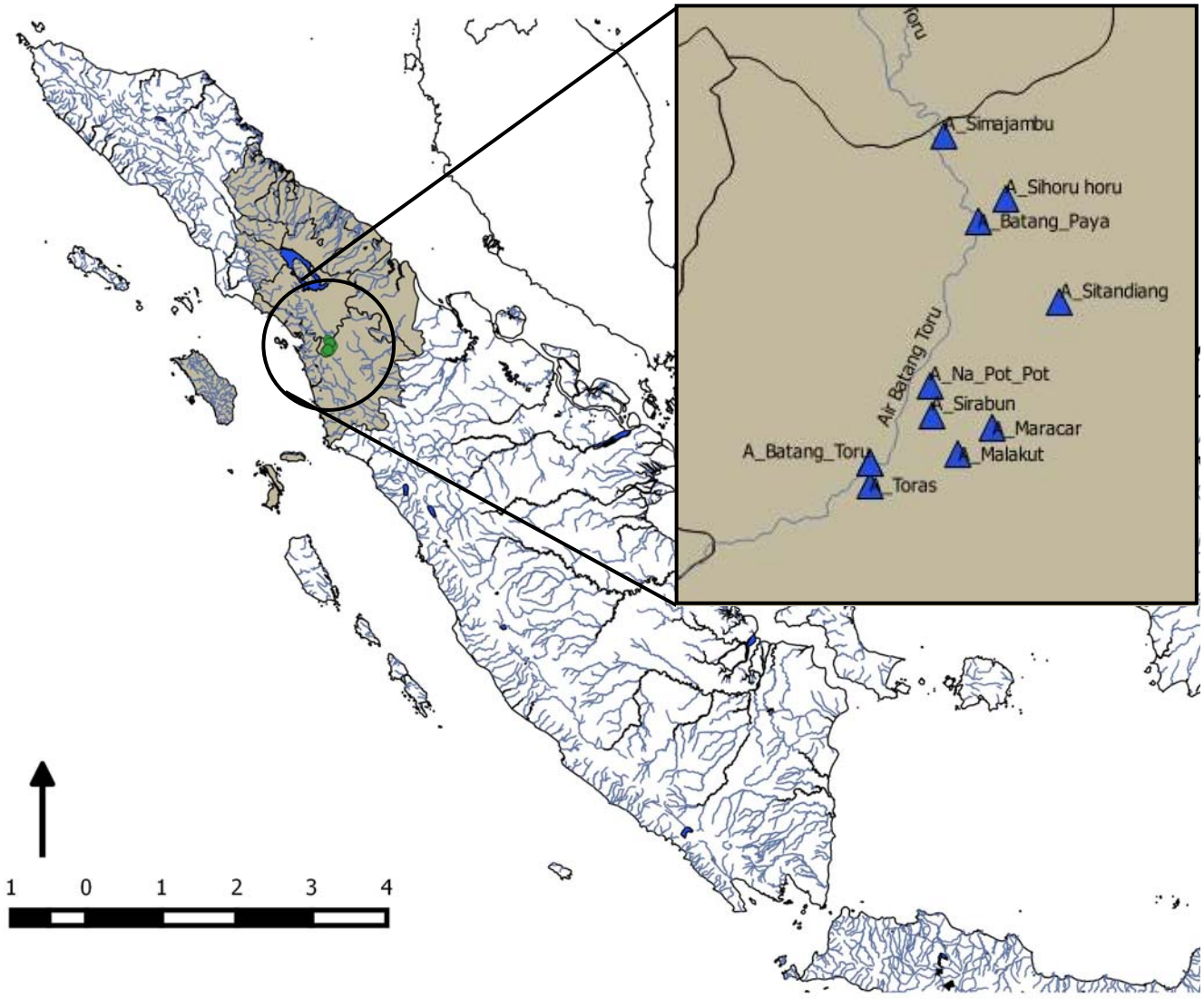

Figure 1. Sampling location in Batang Toru River System, North Sumatra

RESULT AND DISCUSSION

A total of 427 individuals fish samples were collected, consisting of 24 species placed in 10 families (Table 1). Samples were collected at 12 locations on 11 rivers. The species encountered were from the following families: Cyprinidae (11 species), Balitoridae (2), Channidae (2), Gobiidae (2), Nemacheilidae (2), Aplocheilidae (1), Bagridae (1), Cichlidae (1), Mastacembelidae (1) and Sisoridae (1). A juvenile of *Tilapia niloticus*, recorded in Aek Simajambu which predicted as an alien species because the downstream of the sampling sites has been used as an area of "Lubuk Larangan" and according to local people interview, the used they Lubuk Larangan as cages of Tilapia species. Lubuk Larangan is a segment of the river where the public is prohibited to catch fish in the timeframe set together.

We recorded the largest family in Batang Toru is Cyprinidae which consists of eleven species. Those species are *H. macrolepidota, M. marginatus, N. sumatranus, O. hasseltii, O. waandersii, P. binotatus, R. elegans, T. douronensis, T. soro, T. tambra* and *T. tambroides* (Table 1). Cyprinidae is the largest Family of freshwater fish and are spread all over the world except Australia, Madagascar, New Zealand and South America (Kottelat et al. 1993; Nelson 1994). Cyprinids are good source of proteins and as ornamental fish therefore they are economically important (Sharma et al. 2014). *Hampala macrolepidota, Neolissochilus sumatranus*, *Osteochilus hasseltii*, *O. waandersii*, *Tor tambra*, *T. douronensis*, *T. soro,* and *T. tambroides*, are economically important fish, with *Tor spp*. are sold at high local prices. Those fish species are also used in traditional ceremonies in Batak tribe at North Sumatra. *Tor spp*. are also potential species for freshwater sport fishing, like Salmon, because they are strong swimmers. Unfortunately, population of *Tor* in native habitats is becoming rare while their domestication has not been successful, and its systematic still problematic (Ng 2004).

Almost all of Sumatran Tor species has been recorded in Batang Toru. Currently, overfishing and habitat perturbation bringing them into declining population in the wild. Taxonomic chaotic also leave a big question for ichtyologist to make decision about their conservation status. *Tor* spp are interesting both for consumption as well as to be kept as ornamental fish, commonly are known as Mahseer fish. This fish has low population density primarily due to degradation of freshwater habitat, both in quality and quantity. This fish species is very sensitive to the water changes. In addition, uncontrolled harvesting and distortion of the riverine ecosystem and its surrounding habitats have further contributed to the general decline in number of Tambra fish in the world.

Neolissochilus has three species, which two of them noted as endemic Sumatra island, one in Toba Lake, North Sumatra (Kottelat et al. 1993). Most of *Tor* and *Neolissochilus* are threatened especially by forest clearing and overfishing. *N. sumatranus* as an endemic island species named by Weber and Beaufort (1916) as *Lissochilus sumatranus* n. sp. Generally, local villager named both of *N. sumatranus* and *Tor* as "Jurung" because of their morphological similarity.

Puntius binotatus is commonly found in rivers which are also potentially as ornamental fish. Based on molecular (Roesma 2011) and morphological studies (Vitri et al. 2012) the *P. binotatus* from several locations in West Sumatra showed an overview of the complexity, genetic variation and differences in morphological characters between sampling locations. We need to pay attention on this species, The status of *Puntius* is obscure, the delimitation and nomenclatural validity of the genus have remained unsettled, largely owing to the scantiness in knowledge of its inter and intrageneric relationships (Taki et al. 1978). The synonymous name of *Puntius* is *Barbodes* (Kottelat 2013).

Species with high presence were *Glyptothorax platypogonoides* and *Nemacheilus pfeifferae* (54.55%). These two species live in clear and fast-flowing water. According to Kottelat et al. (1993) and Kottelat (2012) *N. pfeifferae* distibuted in Sumatra. Previous study suggested that *N. pfeifferae* in West Sumatra has low variation in morphological characters. Species with low variation in genetic and morphological characters are very vulnerable to extinction. *N. pfeifferae* is considered as an ecological indicator species.

Figure 3. shows the value of Shannon-Wiener (H') species diversity index of all locations sampled in the Batang Toru Rivers Ecosystem. This index gives an illustration on the species diversity, the productivity of ecosystems, the pressures on ecosystems, and the stability of ecosystem. Fish communities respond significantly and predictably to almost all kinds of anthropogenic disturbances, including eutrophication, acidifition, chemical pollution, flow regulation, physical habitat alteration and fragmentation, human exploitation and introduced species (Li et al. 2010). A value of H'<1.0 means low diversity, low productivity as an indication of severe ecological pressures, and unstable ecosystem. A value of 1.0<H'<3.322 means moderate diversity, sufficient productivity, with ecosystem conditions being fairly balanced, and medium ecological pressure. Values of H'>3.322 mean high species diversity, high productivity and stable ecosystem. From the 11 samples sites none of these indicated high diversity. From personal communication with local people living near the sampling areas, we know that years ago some people used a poison to catch fish resulting in mass mortality of fishes in that area. However, we still sampled rivers with moderate H' index value. The highest one is Aek Simajambu (H'= 2.06). We sampled at upper part of a "Lubuk Larangan" on this river.

The next river with a high diversity is Aek Batang Paya (H '= 1.85). The location is very interesting because of all kinds of "Jurung" fish which consisting of *N. sumatranus*, *T. douronensis*, *T. soro*, *T. tambra* and *T. tambroides* can be found in that river. We also recorded the the highest frequency of presence species in this river is *T. douronensis* (42%) from the total number of species collected, followed by *T. soro* (15%), *T. tambra* (3:33%) and *T. tambroides* (1.67%). Furthermore, we found that *N. sumatranus* had absence percentage value 8.33% among other species in this locality. This value also observed in Aek Malakut (H '= 0.59) and Aek Na Pot Pot (H' = 1:16) for *N.*

sumatranus, , therefore we recommend that three of those river as preferred habitat of its species as known by the high number of individuals *N. sumatranus* (30 individuals for each river). In despite of this, we also supposed that Batang Paya (H '= 1.85) and Aek Sirabun (H' = 1.39) are the preferred habitat by *T. douronensis*, as known by the high number of individuals *T. douronensis* (25 and 33 individuals for each river). However, we found that another three species of *Tor* species had less appearance for the rest localities.

There are several factors which contributed to affect the diversity which interacting each other. They are over-exploitation, water pollution, flow modification, destruction or degradation habitat and invasion by exotic species (Allan and Flecker 1993; Jackson et al. 2001; Postel and Richter 2003; Revenga et al. 2005). Environmental changes also contributed which occurring at global scale, for example nitrogen deposition, warming and shifts in precipitation and runoff patterns (Galloway et al. 2004; Dudgeon et al. 2006). The freshwater environment conservation and management are critical to the interest of all nations and governments (Hadwen et al. 2003; Dudgeon et al. 2006). The problem for most part of the ichthyologist is inventories of freshwater biodiversity are incomplete in many part of the world. Conservation of biodiversity is complicated posed by endemism, limited geographic ranges and non-substitutability (Dudgeon et al. 2006).

Table 1. Fish species collected in Batang Toru River System, North Sumatra

Species	Common name	Conservation status (IUCN 2014)	Locality
Aplocheilidae			
Aplocheilus panchax (Hamilton, 1822)	Blue panchax	LC	TRS
Bagridae			
Mystus planiceps (Valenciennes, 1840)	-	NA	BTR, SMJ
Balitoridae			
*Homaloptera gymnogaste*r (Bleeker, 1853)	Balitora sumatranska	NA	MLK, TRS, NPP
Homaloptera heterolepis (Weber & de Beaufort, 1916)	Ray finned fish	NA	NPP
Channidae			
Channa lucius (Cuvier, 1831)	Forest snakehead	LC	SMJ
Channa melasoma (Bleeker, 1851)	Black snakehead	LC	TRS, NPP
Cichlidae			
Tilapia niloticus Linnaeus, 1758	Nile tilapia	NA	SMJ
Cyprinidae			
Hampala macrolepidota (Kuhlt & van Hasselt, 1823)	Hampala barb	NA	BTR, SMJ
Mystacoleucus marginatus (Valenciennes, 1832)	Wader	LC	BTR, BTP
Neolissochilus sumatranus (Weber & de Beaufort, 1916)*	Parmoun sumatransky	NA	MLK, SKT, MRC, STD, SHR, BTP, NPP, SRB
Osteochilus hasseltii (Valenciennes, 1842)	Silver sharkminnow	LC	SKT, BTR, SMJ, BTP
Osteochilus waandersii (Bleeker, 1853)	Waandersii's hard-lipped bard	LC	SKT, BTR, SMJ, BTP
Puntius binotatus (Valenciennes, 1842)	Common barb	LC	TRS, SKT, MRC, STD, SMJ, BTP, NPP, SRB
Rasbora elegans (Volz, 1903)	Twospot rasbora	LC	SMJ
Tor douronensis (Valenciennes, 1842)*	Mahseer	DD	SKT,STD,SHR,SMJ,BTP, NPP, SRB
Tor soro (Valenciennes, 1842)*	Mahseer	DD	MLK, SHR, SMJ, BTP, NPP
Tor tambra (Valenciennes, 1842)*	Mahseer	DD	BTP, SRB
Tor tambroides (Bleeker, 1854)*	Mahseer	DD	BTP
Gobiidae			
Glossogobius sp.1	Gobies	-	BTR
Glossogobius sp.2	Gobies	-	BTR
Mastacembelidae			
Macrognathus maculata (Cuvier, 1832)	Spiny eel	LC	SMJ
Nemacheilidae			
Nemacheilus chrysolaimos (Valenciennes, 1846)	-	NA	TRS
Nemacheilus pfeifferae (Bleeker, 1853)	-	NA	TRS, BTR, STD, SMJ, BTP, SRB
Sisoridae			
Glyptothorax platypogonoides (Bleeker, 1855)	-	NA	BTR, STD, SMJ, BTP, NPP, SRB

Note: MLK= Aek Malakkut; TRS= Aek Toras; SKT= Aek Sikkut; BTR= Aek Batang Toru; MRC= Aek Marancar; STD= Aek Sitandiang; SHR= Aek Sihoru-horu; SMJ= Aek Simajambu; BTP= Aek Batang Paya; NPP= Aek Na Pot-Pot; SRB= Aek Sirabun. LC= Least Concern, DD= Data Deficient, NA= Not Assessed in IUCN Red List, Ni=Could not compare to IUCN; *upstream for spawning migrators species

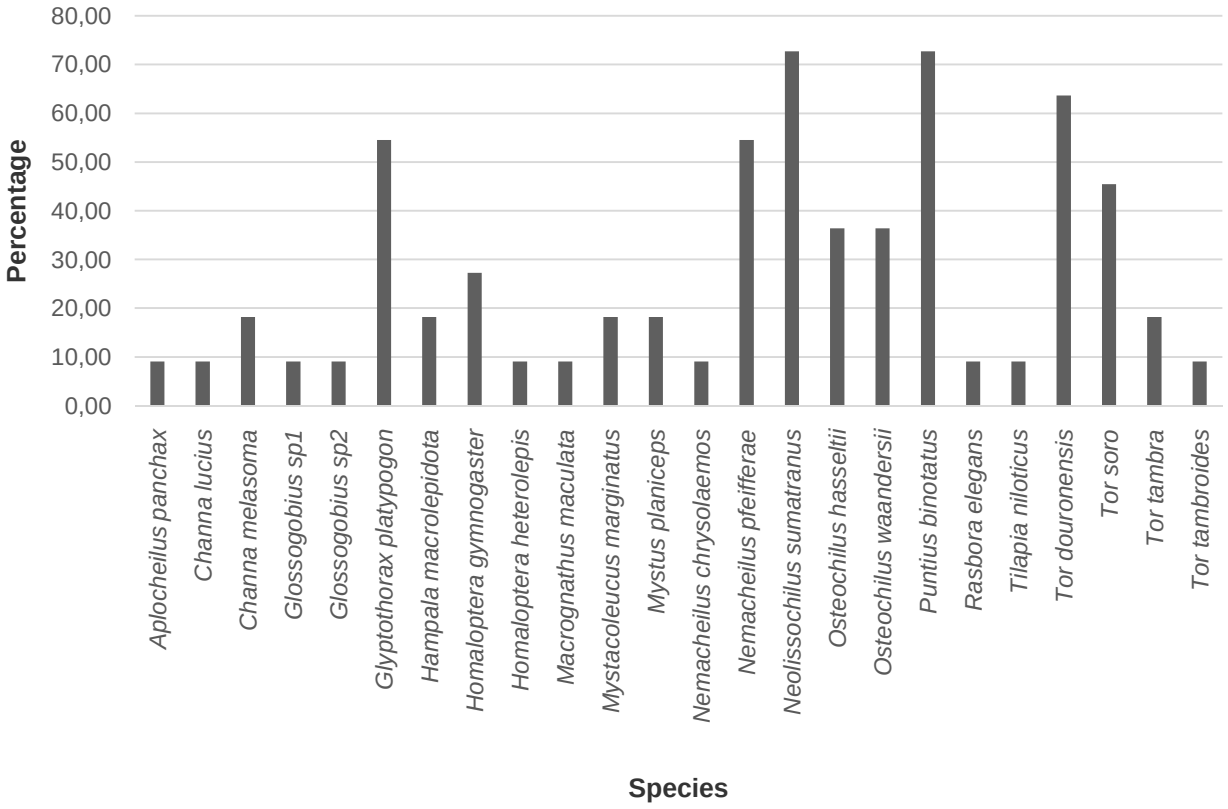

Figure 2. Percentage of fish species detected in Batang Toru River System, North Sumatra

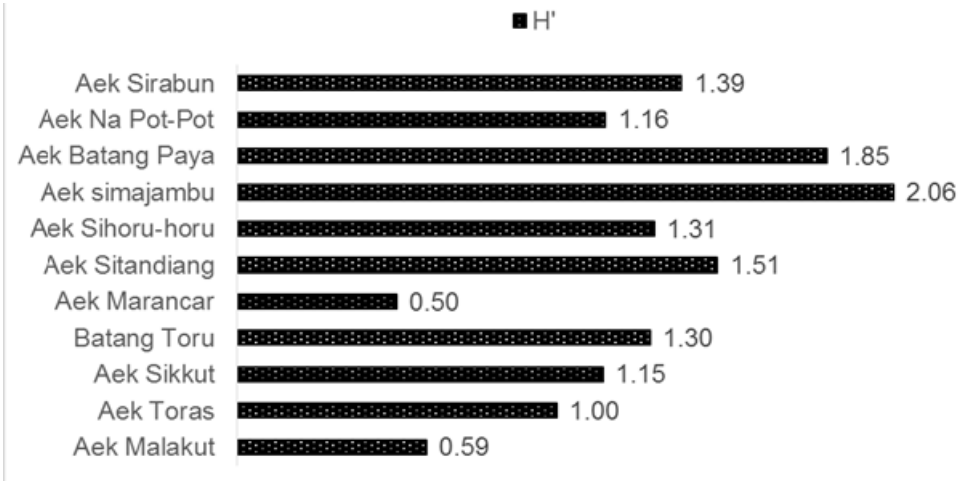

Figure 3. Shannon-Wiener species diversity in the Batang Toru River, North Sumatra

It is recommended to pay attention to the water condition in those rivers because it is important for Jurung fish habitat. As this fish species is a migratory species, heading to the headwaters for spawning, they need clear water and fast flowing rivers. We also found higher densities of *N. pfeifferae* in Aek Simajambu and Aek Sirabun than in other rivers. Both of them have a clear river water, rocks, sand gravel substrate, 0.85 to 0.88 flow velocity m/s and largely shielded by vegetation. We consider this species as an ecological indicator species. An ecological study need to be done to support our prediction.

We also looking at similarity of species richness among the tributaries (Figure 4). Those tree were constructed according to presence and absence species between the river system. Among 11 tributaries, we recorded that Aek Sitandiang and Aek Sirabun shared the same species

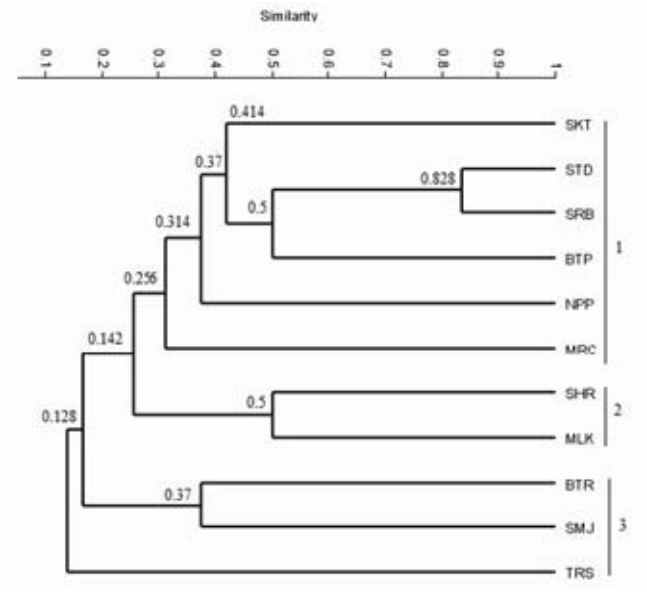

Figure 4. Similarity index of presence and absence species among tributaries

closely. Both of rivers showed the same species such as *N. sumatranus, P. binotatus, T. douronensis, N. pfeifferae, G. platypogon* with similarity index 0.828. There are three groups according to the similarity of species present among eleven rivers. Those are Aek Sikkut, Sitandiang, Sirabun, Batang Paya, Na Pot Pot and Marancar as group 1, Aek Sihoru horu and Malakut as group 2, and Batang Toru, Simajambu and Toras as another group. The rivers in group 1 commolny has the same species recorded such as *N. sumatranus, P. binotatus, T. douronensis, N. pfeifferae,* and *G. platypogon*. Whereas the second group only have two similar species detected *(N. sumatranus* and *T. soro*). Batang Toru and Aek Simajambu have 6 similar species recorded (*M. planiceps, H. macrolepidota, O. hasseltii, O. waandersii, N. pfeifferae, G. platypogon*) but in comparison to group 1, they only have two common species. The similarity value between group and river provided in Figure 4.

Batang Toru River system has a complex water flows systems with variety environmental variables along the tributaries. There was lack evidence of relationship between different environmental affect the species richness in this study. Further research and survey are needed in order to know the factor which might be causing the differences of species assemblages in each locality.

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Antidiabetic screening of some Indonesian marine cyanobacteria collection

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Abstract. *Priatni S, Budiwati TA, Ratnaningrum D, Kosasih W, Andryani R, Susanti H, Susilaningsih D. 2016. Antidiabetic screening of some Indonesian marine cyanobacteria collection. Biodiversitas 17: 642-646.* Cyanobacteria have been known as a potential extracellular-polysaccharide (EPS) producer. The objective of this study was to screen the marine cyanobacteria as potential antidiabetic agents. The present investigation was designed to determine the antidiabetic activity of EPS, intracellular-polysaccharide (IPS) and biomass extracts from marine cyanobacteria isolates. 10 cyanobacteria isolates were cultivated in IMK medium, at 25^{o}C for 21 days. The morphology of cells was identified by a light microscope. EPS and IPS were separated by ethanol precipitation method and their antidiabetic activity was analyzed by the inhibition of α-glucosidase activity method. Results of morphology identification of 10 cyanobacteria isolates consist of *Oscillatoria limnetica, Oscillatoria* sp., *Leptolyngbya* sp., *Pseudanabaena* sp., *Lyngbya* sp. and *Phormidium* sp., *Coelastrella sp., Aphanothece* sp. and *Synechococcus* sp., and *Chroococcus* sp. Almost all of EPS from marine cyanobacteria isolates were potential as inhibitor of α-glucosidase, except for *Oscillatoria limnetica* and *Phormidium* sp. isolates. The highest activity in α-glucosidase inhibition was detected in *Pseudanabaena* sp. (14.02%) and *Chroococcus* sp. (13.0%) isolates.

Keywords: antidiabetic, cyanobacteria, extracellular-polysaccharide, screening

INTRODUCTION

Diabetes mellitus is a common metabolic disease in which the concentration of glucose in the blood is above the standard level. This is due to insulin deficiency or functional disturbance of the receptors, which causes blood glucose to rise and induce disorders in the metabolism of fat and proteins (Yang et al. 2012). Diabetes is a silent disease and generally detected after chronic symptom. Unhealthy lifestyle has an impact on weight gain that can trigger diabetes. Another factor that triggers diabetes is urbanization and lack of activity. Consumption of suitable food for diabetics is indispensable so that the blood sugar levels can be controlled. Nutrition intake for people with diabetes must be maintained through the consumption of functional food product. In Indonesia, in 2013 8.5 million people were diabetic and in 2030 they are expected to be 21.3 million. This will put Indonesia on the fourth rank highest diabetes prevalence in the world (Mulyanti et al. 2010).

A sudden increase in blood glucose levels, which causes hyperglycemia in type 2 diabetes patients, occurs as the result of the hydrolysis of starch by pancreatic α-amylase and glucose uptake due to intestinal α-glucosidase. An effective strategy for the management of type 2 diabetes patients involved the profound inhibition of intestinal α-glucosidase and the mild inhibition of pancreatic α-amylase. Several natural resources have been evaluated for their ability to suppress the production of glucose from carbohydrates in the gut or glucose absorption from the intestine (Lee and Jeon 2013). The study on anti-diabetic compound was conducted with intense interest using a mangrove species *Sonneratia alba* because its close relative terrestrial plant, *Lagerstroemia speciosa* had previously shown many anti-diabetic properties. When subjected to anti-diabetic bioassay using standard Glucometer, data showed that it has significantly high attenuating activity for blood glucose because it reduced blood sugar level by 19.2% during the first 6 hours and reduced further to 66.9% after 12 hours (Morada et al. 2011).

Indonesian as a tropical country has rich of biodiversities, primarily from marine resources. Tropical marine microalgae is an interesting subject for research because its potency as producer of unsaturated fatty acids, carotenoids and polysaccharide. A great diversity in the chemical composition of these organisms, and therefore, this makes them extremely attractive for bio-prospecting and potential exploitation as commercial sources of a wide range of biomolecules. The potential of microalgae as new sources of valuable chemicals and other products recently has regained wide interest (Ko et al. 2000; Borowitzka 2013). Microalgae have the advantage because they can be cultured in a small area, they are very efficient in light capture and their cultivation varies between 7 and 10 days (Kabinawa 2001; Huang et al. 2010). Microalgae can be used to produce a wide range of metabolites such as proteins, lipids, carbohydrates, carotenoids or vitamins for

health, food and feed additives, cosmetics and for energy production (Priyadarshani and Rath 2012). The range of polysaccharides produced by microalgae is large and the polysaccharides of unicellular red algae such as *Porphyridium* and *Rhodella*, as well as many cyanobacteria have long been studied for their properties and potential applications. However, as yet, cyanobacteria have not been significantly accepted in the market, mainly for the existence of cheaper alternatives than macroalgae and higher plants. However, few microalgal polysaccharides have found niche markets, mainly in the area of cosmetics (Borowitzka 2013). Cyanobacteria are a rich source of potentially useful natural products. Some research has been focused on adapting cyanobacterial collections and cyanobacterial-derived compounds for screening the new bioactive compounds (Burja et al. 2001). Extracellular polysaccharide (EPS) from marine bacterial contain the new combination that very important in pharmaceutical industries (Satpute et al. 2010). Cyanobacteria have been known since long as a potential EPS producer. The presence of proteins, uronic acids, pyruvic acid, and *O*-methyl, *O*-acetyl and sulfate groups emphasizes the complex nature of cyanobacterial EPS (Parikh and Madamwar 2006). The information about antidiabetic activity from marine cyanobacteria is still not clear. Therefore, the objective of this study was to screen marine cyanobacter which potential as α-glucosidase inhibitory, so that potential for nutraceutical and functional food. The screening of α-glucosidase inhibitory from marine cyanobacteria was carried out to the extracellular polysaccharide, intracellular polysaccharide and the total extract of biomass.

MATERIALS AND METHODS

Materials

Ten marine cyanobacteria isolates was obtained from culture collection of InaCC Indonesian Institute of Sciences (LIPI). The IMK medium was sea water which enriched with the following additives (per liter): $NaNO_3$ 0.2 g, $Na_2HPO_4.H_2O$ 14 mg, K_2HPO_4 50 mg, NH_4Cl 268 mg, Na-EDTA 372 mg, $ZnSO_4.7H_2O$ 23 µg, $CoSO_4.7H_2O$ 140 µg, $Na_2MoO_4.H_2O$ 7.3 µg, $CuSO_4.5H_2O$ 25 µg, $MnCl_2.4H_2O$ 180 µg, $FeCl_3.6H_2O$ 315 µg. The medium was sterilized (120°C, 20 min) prior to use. Vitamin B_{12} 15 µg, thiamin HCl 2 mg and biotin 15 µg (per liter) was added to the sterile medium.

Methods

Cyanobacteria cultivation

The cultivation of marine cyanobacteria was carried by some steps cultivation as follow: (i) 1 ml stock culture was cultivated in first 5 ml of medium, continued to 10 ml and 20 ml of medium. The cultivation was carried out in incubator at 28°C for 3 days. (ii) The cultivation of marine cyanobacteria was increased to 100 ml of medium. Cyanobacteria in these experiments was cultivated in bottles which connected to an aeration pump (pump output: 70 L per minutes) exposed to 2x10 W white lamp equal to 500-2000 lux at 25°C. The experiments were carried out for 21 days.

Microscopic observation

Morphological observation of cyanobacteria cells was done with the light microscope Olympus BX 53 (x 1000) and image analysis was done with the Sklanlt RE for various Flash 2.4.3 program.

Extracellular polysaccharides (EPS) separation

Extracellular polysaccharide (EPS) was separated by Velea et al. (2011) method. After separation of cyanobacteria biomass by centrifugation, the aqueous solution containing EPS and the remaining dissolved salts in the nutrient medium are concentrates through evaporation, at 50°C up till 25% from the initial volume. The resulting creamy white product was then mixed with ethanol (1:2 v/v) and let in 4°C for overnight. The precipitate was centrifuge at 2500 rpm for 20 minutes. The precipitate was then rinsed and dried at 100°C for 2 hours.

Intracellular polysaccharides (IPS) separation

Intracellular polysaccharide (IPS) was separated by El-Sheekh et al. (2012) method. IPS was separated by homogenizing the biomass in distilled water (50 mL). The homogenates were then heated in water bath at 95°C for 6 hours. The extracts were filtrated through Whatman No.2 filter paper, then precipitated with four volumes of 95% ethanol, stirred vigorously and left overnight at 4°C. The precipitated IPS was recovered by centrifugation at 8.000 rpm for 15 min and the supernatant was discarded.

Biomass extraction

Extraction of cyanobacteria biomass was carried out by modification of Akah et al. (2011) method, using methanol with ratio 1:20, at 60°C, 100 rpm for 2 hours. Methanol extract was separated by centrifuge at 4°C, 10.000 rpm for 10 minutes and the extract was then concentrated.

Analysis of total carbohydrate content

The total carbohydrate content was analyzed by Carbazole method (Frazier et al. 2008) with modification. The anhydrous glucose (Merck) was used as standard. 1 ml of EPS was mixed with 0.5 ml of 1.5% cystein solution and 6 ml of 70% sulfuric acid. The sample solution was then mixed with 0.2 ml of 0.12% carbazole in ethanol 95%. The sample solution was incubated at 60oC for 10 minutes and cooled to room temperature. Total sugar content of sample was measured on a Spectrophotometer UV Vis at 560 nm.

Analysis of inhibition of α-glucosidase activity

In vitro, the method used to analyze the inhibition of α-glucosidase activity was that of Yang et al. (2012) using ρNPG as a substrate and modifying the dosage. The assay mixture contained 0.25 mL of 0.1 M phosphate buffer (pH 7.0), 0.25 mL of substrate solution (2.5 mM ρNPG in 0.1 M phosphate buffer) and 0.1 mL of sample solution in DMSO. The mixture of solution was incubated at 37 ◦C for 5 minutes, 0.25 mL of enzyme solution (0.2 U/mL α-

glucosidase in 0.01 M phosphate buffer containing 0.2% BSA) was added, and the reaction mixture was incubated for 15 min at 37 ◦C. The reaction was stopped by adding 0.1 mL of 0.2 M Na_2CO_3. The amount of ρNP released was measured on a Spectrophotometer UV Vis at 400 nm. The results were expressed as % inhibition calculated using the formula:

$$\%\,inhibition = \frac{\text{Abs (Control)} - \text{Abs (Sample)}}{\text{Abs (Control)}} \times 100$$

RESULTS AND DISCUSSION

Cultivation of marine cyanobacteria has been carried out to 10 isolates using IMK medium in processed of sea water. On the preliminary study, the cultivation was carried out in a shaker incubator (28^{o}C, 150 rpm) and in bottles connected to an aeration pump. The growth of cyanobacteria was monitored for 10-21 days. Results shown that the cultivation in shaker incubator was much slower than cultivation in bottles with aeration. The simple design of small scale cyanobacteria cultivation was shown on Figure 1. Aeration pump system can improved the mixing of microalgae during its growth. Mixing is necessary to prevent sedimentation of the algae, to ensure that all cells of the population are equally exposed to the light and nutrients, to avoid thermal stratification (*e.g.* in outdoor cultures) and to improve gas exchange between the culture medium and the air. Depending on the scale of the culture system, mixing is achieved by stirring daily by hand (test tubes, erlenmeyers), aerating (bags, tanks), or using paddle wheels and jet pumps (Lavens and Sorgeloos 1996). The light intensity also is important in microalgae culture growth. Doubling the irradiance level has led to 25% higher yields in algal mass. Also, the irradiance proves to be a determining factor in the biosynthesis and accumulation of EPS (Velea et al. 2011).

The characteristic growth of marine cyanobacterial isolates was evaluated by monitoring the biomass color, and cultivation time when harvested. Based on the data on Table 1, it is shown that almost all marine cyanobacteria isolates have green color except the isolate of *Chroococcus* sp. The growth of marine cyanobacteria was monitored until 21 days. *Oscillatoria limnetica, Coelastrella* sp. and *Leptolyngbya* sp. were shown grew faster compared to other isolates. These cultures grown well and can harvested after 10 days. The dark green color of these cultures is probably due to its high chlorophyll content. Cyanobacteria contain chlorophyll *a* as a major pigment for harvesting light and conducting photosynthesis. They also contain other pigments that harvest light in the green, yellow and orange part of the spectrum (500-650 nm), which is hardly used by other phytoplankton species (Luuc et al. 1999). Meanwhile, *Chroococcus* sp., *Pseudanabaena* sp., *Phormidium* sp. and *Synechococcus* sp. grew very slowly at the selected experimental conditions. Presumably, the salinity values and the presence of some trace elements in medium influenced the growth of these strains. The growth of cyanobacteria is influenced by a number of factors, therefore it is necessary to evaluate the optimum condition for their mass culture. Due to their complicated requirements for salts, pH, light, temperature, vitamins, organic carbon, nitrogen and trace elements, marine cyanobacteria are difficult to isolate and culture. These requirements may differ from species to species. The successful cultivation and growth of cyanobacteria require modification in the media composition as well as other physico-chemicals parameters (Bano and Siddiqui 2004). The growth rate of cyanobacteria is usually much lower than that of many alga species. Slow growth rates require long water retention time to enable cyanobacteria to bloom. Therefore cyanobacteria do not bloom in water with short retention times (Luuc et al. 1999).

The morphology cell of marine cyanobacteria culture was identified by a light microscope (Olympus BX 53). Cell images of these isolates are shown in Figure 2. Some isolates are filamentous cyanobacteria such as *Oscillatoria limnetica, Oscillatoria* sp., *Leptolyngbya* sp., *Pseudanabaena* sp., *Lyngbya* sp. and *Phormidium* sp. Species in the order Oscillatoriales, with unseriated and unbranched trichomes, are composed of essentially identical cells (Luuc et al. 1999). Other isolates such as *Coelastrella* sp., *Aphanothece* sp. and *Synechococcus* sp. are conidial forms. *Chroococcus* sp. has a unique morphology with its unicellular forms. The unicellular cells may aggregate in irregular colonies, being held together by the slimy matrix secreted during the growth of the colony. By means of a more or less regular series of cell division, combined with sheath secretions, more ordered colonies may be produced (Luuc et al. 1999).

Figure 1. Small scale cultivation of marine cyanobacteria in bottles with aeration

Table 1. Characteristic growth of marine cyanobacteria culture

Isolates	Biomass color	Cultivation time (day)
Oscillatoria limnetica	Dark green	10
Coelastrella sp.	Dark green	10
Oscillatoria sp.	Green	16
Chroococcus sp.	Black	21
Leptolyngbya sp.	Dark green	10
Pseudanabaena sp.	Green	21
Lyngbya sp.	Green	16
Aphanothece sp.	Green	16
Phormidium sp.	Dark green	21
Synechococcus sp.	Green	21

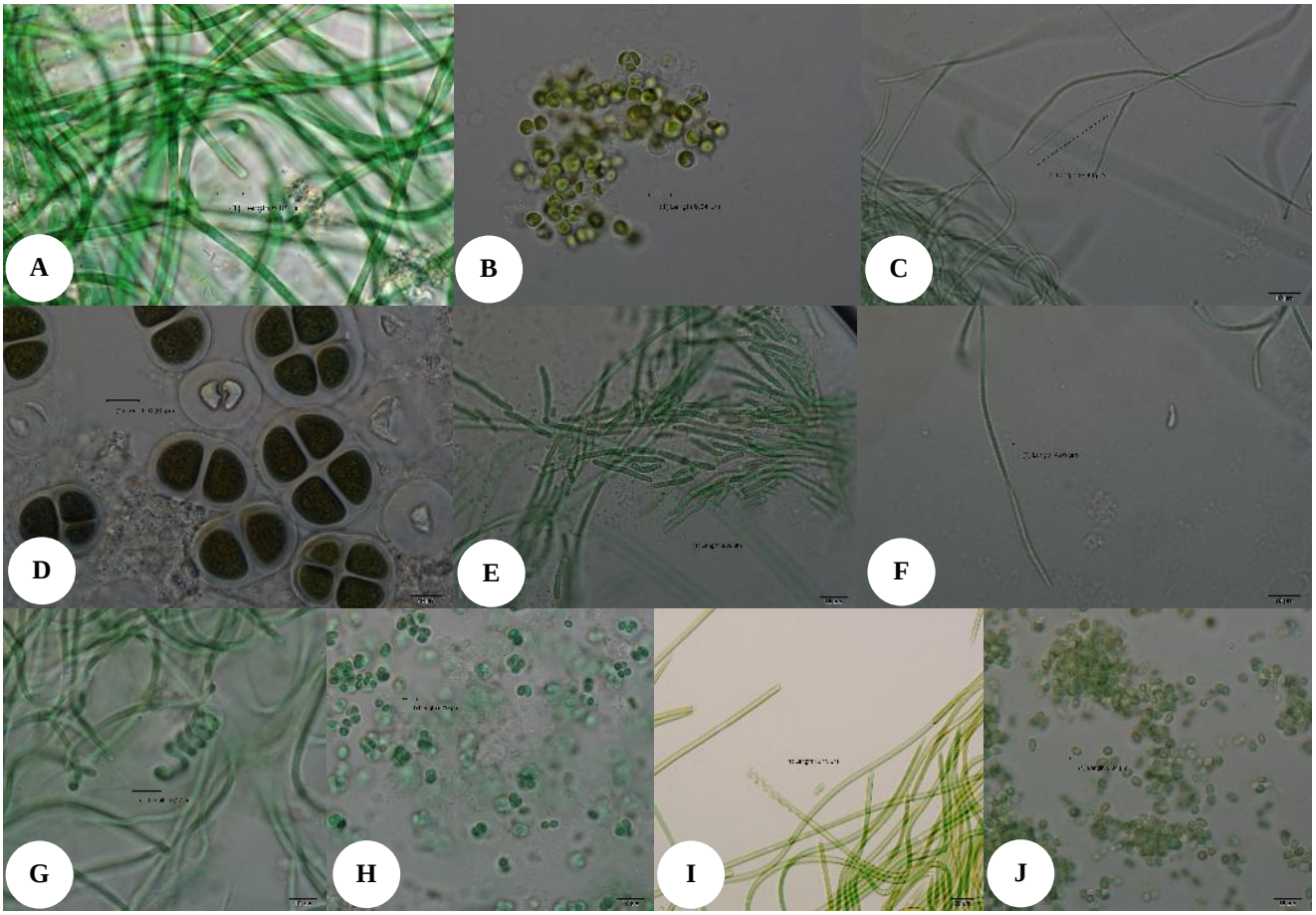

Figure 2. Cell images of marine cyanobacteria isolates; A. *Oscillatoria limnetica*, B. *Coelastrella* sp., C. *Oscillatoria* sp., D. *Chroococcus* sp., E. *Leptolyngbya* sp., F. *Pseudanabaena* sp., G. *Lyngbya* sp., H. *Aphanothece* sp., I. *Phormidium* sp., J. *Synechococcus* sp.

Table 2. The yield data of biomass, EPS and total carbohydrate content of EPS from 100 ml culture of marine cyanobacteria

Isolates	Biomass (g)	EPS (g)	Total carbohydrate (mg/mL)
Oscillatoria limnetica	1.4010	0.3737	0.0651
Coelastrella sp.	0.9825	0.3798	0.0976
Oscillatoria sp.	1.0272	0.3706	0.0849
Chroococcus sp.	0.3971	0.6318	0.5332
Leptolyngbya sp.	1.1241	0.1690	0.0665
Pseudanabaena sp.	0.2132	0.6175	0.0523
Lyngbya sp.	0.4246	0.3842	0.0863
Aphanothece sp.	2.1796	0.0462	0.0651
Phormidium sp.	1.2266	0.3096	0.0778
Synechococcus sp.	0.3806	0.6015	0.0693

Table 3. Antidiabetic activity of extracellular polysaccharide (EPS), intracellular polysaccharide (IPS) and methanol extract of marine cyanobacterial strains

Isolates	Inhibition of α-glucosidase (%)		
	EPS	IPS	methanol extract
Oscillatoria limnetica	-	-	-
Coelastrella sp.	10.93	3.91	-
Oscillatoria sp.	9.27	1.90	-
Chroococcus sp.	13.0	-	-
Leptolyngbya sp.	2.13	-	-
Pseudanabaena sp.	14.02	-	-
Lyngbya sp.	9.19	-	5.48
Aphanothece sp.	8.08	-	-
Phormidium sp.	-	-	-
Synechococcus sp.	3.13	-	-

Note:-= negative

Microalgae enable in the production of polysaccharides or whichever other compounds with similar properties, either chemical or physical. Polysaccharides and sulphated exopolysaccharides are released by many species of microalgae (de Jesus Raposo et al. 2013). EPS are high molecular weight carbohydrate polymers. Many marine microorganisms produce extracellular polymers which form a layer surrounding the cells that helps them to withstand or resist adverse and extreme environmental conditions industries (Satpute et al. 2010). On this study, EPS was separated from concentrated of culture media by precipitated method with ethanol. The yield data of

biomass and EPS, and also total carbohydrate content of EPS was presented in Table 2.

Data on Table 2 shown that *Chroococcus* sp. and *Pseudanabaena* sp. produced higher yields of EPS compared to other marine cyanobacteria culture, although its biomass yields were very low. This indicated the marine cyanobacteria growth rate was not influenced by the production of EPS. The algal cells can release extra-cellular polysaccharides EPS into the environment. The production of polysaccharide by green algal species indicated the involvement of this polysaccharide in protecting the algal cells against toxic species. The increasing in polysaccharides contents in crude microcystins treated cultures in algae indicated that these polysaccharides may be involved in certain defense mechanisms in response to toxin stress (El-Sheekh et al. 2012). We assumed that the production of EPS by *Chroococcus* sp. and *Pseudanabaena* sp. is the response to toxin that contained in culture medium.

EPS and IPS from marine cyanobacter culture were screened for antidiabetic activity by analysis its inhibition to the α-glucosidase activity, the results are shown in Table 3. The data shown that almost all of EPS from marine cyanobacteria culture were potential as inhibitor of α-glucosidase, except *Oscillatoria limnetica* and *Phormidium* sp. isolates were negative or no inhibition to the α-glucosidase activity. The highest activity in inhibition of α-glucosidase was *Pseudanabaena* sp. (14.02%) and *Chroococcus* sp. (13.0%) isolates. This data shown has correlated with EPS production (Table 2), in which these cultures were produced high yields of EPS.

The distribution of *Pseudanabaena* is widespread, although their actual abundance is still to be determined (Acinas et al. 2009). C-phycoerythrin was isolated and purified from marine *Pseudanabaena* sp. to evaluate its fluorescence properties for future applications in biochemical and biomedical research (Mishra et al. 2011). The antidiabetic activity of EPS from *Pseudanabaena* sp. is the first report and potential for further research.

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Data provision of PIK3CA gene diversity and recombinant plasmids preparation for control DNA in developing the trastuzumab predictive response diagnostic kit

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[1]Research Center for Biotechnology, Indonesian Institute of Sciences. Jl. Raya Jakarta-Bogor Km 46, Cibinong, Bogor 16911, West Javat, Indonesia. Tel.: +62-21-87907604/87907636, Fax.: +62-21-87907612, ♥email: desrianilipi@gmail.com, gerodes@yahoo.com

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Abstract. *Desriani, Budiarto BR, Harahap WA, Warisman MA, Ompusunggu AVC, Athariah D, Mirnawati F, Sriyani IY, Alahwani F, Kurniawan AR. 2016. Data provision of PIK3CA gene diversity and recombinant plasmids preparation for control DNA in developing the trastuzumab predictive response diagnostic kit. Biodiversitas 17: 647-652.* HER-2 overexpression is well known as a poor prognostic factor for breast cancer patients. Targeted therapy can be carried out using monoclonal antibody named trastuzumab. Some reports have higlighted the core problem of HER2 positive-breast cancer resitance on trastuzumab due to incorrect in selecting HER2 status patient who will reciept the drug and the emergence of PIK3CA mutations especially in exon 9 and 20 which is the downstream of HER-2 pathway. In this study, data provision of PIK3CA gene and preparation of plasmid to support developing the trastuzumab predictive response diagnostic kits will be reported. Based on direct DNA sequencing result, two samples of 68 breast cancer patients exhibited mutation at exon 20 H1047R, while another three samples showed silent mutation (T1025T) at the same exon. On the other hand, careful strategy should be considered for exon 9 analysis, since we found that almost 68 samples sequenced none of them were exon 9 positive (pesudogene). Two prepared plasmids, pGEMT-easy PIK3CA exon 9 and 20 will be applied as control PIK3CA gene for qPCR SYBR green I-based PIK3CA genotyping, while pGEMT easy HER-2 will be applied as a reference gene for scoring HER-2 status. The standard curve equation of plasmid-cloned HER2 gene amplification was Y=-3,0472x + 46,465, R^2= 0,99 with qPCR efficiency was 115%, respectively. Inconclusion, data provision and control DNA preparation of predicted factors for breast cancer patients who positively respond to trastuzumab are very fundamental important aspects for the development of trastuzumab response diagnostic kit which is based on Indonesian population genetics profile.

Keywords: Breast cancer, DNA, *PIK3CA*, HER-2, resistance trastuzumab, mutation

INTRODUCTION

Cancer is one of the leading causes of death worldwide. In 2012, it was approximately 8.2 million deaths caused by cancer. Lung cancer, liver, stomach, colorectal, and breast cancer are the biggest cause of cancer deaths each year. Cancer is regulated by many genes, known as oncogenes which express oncoprotein. The emergence of oncogenes could be as results of mutation, amplification and so forth. Some of cancer diagnostic test have been developed not only in the genomic level but also in the proteomic level as well. Oncotype DX test is an example of detection techniques in genomic level. Oncotype DX test has been manufactured and commercialized. With this test, as many as 21 cancer biomarker genes can be detected at the genomic level. These targeted genes were determined based on American Society of Clinical Oncology (ASCO) and National Comprehensive Cancer Network (NCCN)'s guideline and it has widely been applied by Oncologist in therapying and treating their cancer patient. At the protein level, oncoprotein detection in serum can be analyzed using two-dimensional gel electrophoresis (2-DE). Examination of information obtained can be used for the same purposes as the Oncotype test (Wang et al. 2003)

Trastuzumab is a monoclonal antibody for targeted therapy purposes in cancer patients with overexpressed HER-2 status. HER-2 protein regulates the malignancy of cancer through cell proliferation, angiogenesis, migration and invasion. HER-2 and HER-3 interaction mainly effect on metabolism process through PIK3CA pathway. This Dimer formation may be a key determinant for sensitive of breast cancer to HER-2 targeted therapy (Hynes and Dey 2009; Wang et al. 2011; Paplomata and O'Regan 2014). Detailed molecular mechanisme of Trastuzumab recognizes its target was unclear yet, but some evidence highlighted the mechanism where this antibody does its action on breast cancer such as (1) blocking of HER-2 dimerization that effected on the signal transduction pathway, and (2) activation of antibody dependent cell mediated cytotoxicity for tumor cell lysis. The duration of trastuzumab therapy according to NCCN, St Gallent were approximately 12 month duration of orally taken drug therapy. Since the prize of the drug was expensive, it takes careful consideration to decide whether the patients are true

for the targeted drug in term of HER2 status. Some reports have shown the high resistance incidence due to trastuzumab treatment for non-target ones. False interpretation of immunohistochemistry (IHC) results in scoring of HER-2 status as the main factor for that incident. Another factor is phophatidylinositol 3-kinases/ PIK3CA gene mutation. In breast epithelial cells, this gene acts as a regulator of the cellular growth, cell migration, survival, apoptosis and proliferation. This gene located on HER-2 signaling pathway, encodes the p110α catalytic subunit of the PI3K enzyme. These mutations cause the lipid kinase activity increased two times higher, producing an increase of phosphorylated AKT protein and hence inducing oncogenic transformation. These mutations are generally clustered in exon 9 and 20 of PIK3CA gene. Mutations in PIK3CA are also found in other exons but in very rare frequency. Patients harbor simultaneously mutations at E545K and H1047R position have been reported to be more resistant to therapy than other mutant types based on in vitro study. ESMO at 2014 has officially issued that detection of PIK3CA mutation in cancer patients is required to predict their response to trastuzumab. Furthermore, PIK3CA mutations have also been reported as a potential biomarker for predicting prognostic status in breast cancer patients. Indeed, PIK3CA mutations is associated with increased tumor aggressiveness (Kurebayashi et al. 2001; Gallia et al. 2006; Kato et al. 2007; Hale et al. 2008; Zhao et al. 2008). So far, targeted therapy using trastuzumab given to breast cancer patient is solely based on IHC result. The high percentage of resistance to trastuzumab in single used reached over 60-80%, indicating that HER-2 test which is only refer to IHC alone is not enough. Moreover, IHC test is currently known to be subjective due to the factors of operator skill and the type of antibody used which potentially lead to misreading. Based on this finding, the quantifying of HER-2 status using other molecular methods with complement to existed methods is significant to be developed (Clifford and Hudis 2007; Siddig et al. 2008; Breyer 2009; Cremoux et al. 2012; Alaoui-Jamali et al. 2015). In a diagnostic kit for cancer detection, the standard reagent preparation was one of the important things to be well prepared.

Here, the development of trastuzumab predictive response diagnostic kits through PIK3CA gene data provision and plasmids preparation to support the diagnostic kit study will be reported. We provided diversity data of PIK3CA gene exon 9 and 20 detected with Sanger DNA sequencing. We have also prepared three recombinant plasmids as part of manufacture the predictive factor detection kit at genomic level for patient responsiveness against trastuzumab where two of them were applied for PIK3CA genotyping in exon 9 and exon 20 while the other for HER-2 status scoring. The diversity data in term of PIK3CA mutation obtained in this study and the PIK3CA gene-contained recombinant plasmids can be as a basis for creating and validating new cancer detection kit which is unique only for PIK3CA genotypes originated from Indonesian population. Furthermore, HER2-contained recombinant plasmid can be applied as part of standard kit preparation in qPCR to overcome the subjectivity problem of IHC methods, avoiding false interpretation that may occurs. The prepared control plasmids could significantly contribute to the quality controls and quality assurance program of cancer detection kit for predictive factors to trastuzumab treatment.

MATERIALS AND METHODS

Genomic DNA isolation from Breast Cancer Tissue

The research was conducted using ethical clearance issued by Indonesia Ministry of Health. Breast cancer tissues were provided from several hospitals in West Sumatra province, Indonesia. The fresh tissue samples were stored at -80°C. Extraction was done using PureLink® Lysate-Mini Kit from Invitrogen. The Genomic DNA obtained then was confirmed using electrophoresis with 1.5 % agarose, visualized with UV-Transilluminator.

The PIK3CA gene amplification and sequencing

For HER-2 primer were forward Primer (5'-TGA TCT GCC CAC AGA CTC-3 ') and reverse Primer (5'-TCT CAT CGT CCG CTT GTA CC-3'), for *PIK3CA* exon 9 5' AGT AAC AGA CTA GCT AGA GAC AAT 3', reverse primer 5' CTG TGA CTC CAT AGA AAA TCT 3', Primer for *PIK3CA* gene exon 20 were (5'-TTT TTT CCT TCT CCA TCA TTT CTA-3', reverse primer (3'-GTT TCA GGA GAT GTG TTA CAA-5'). PCR composition used are 12.5 µL of DreamTaq Green PCR Polymerase, 0.25 µL of forward primer, 0.25 µL of reverse primer, 5.5 µL of MilliQ nuclease-free water, and 0.25 µL of genomic DNA. The PCR condition used as follows: pre-heat at 95°C for 5 minutes then followed by 35 cycle of denaturation at 95°C for 30 seconds, annealing at 50.9°C for 30 seconds, extension at 72°C for 30 seconds. After the PCR process completed, the gene was confirmed using DNA electrophoresis with 1.5% agarose, visualized with UV-Transilluminator. Gene sequencing was done using Applied Biosystem® 3100 Genetic Analyzer based on Sanger method.

Purification, ligation, transformation

PCR product was purified using Wizard SV® Gel from Promega, then was inserted to pGEM®-T Easy following manufacture instruction. Transformation to *E. coli* DH5α competent cells were done with heat shock method and were spread in LB media containing with 100 ppm of ampicillin, 50mM of X-Gal, and 1 mM of IPTG for selecting targeted and non-targeted *E. coli.* Positive bacteria colonies (containing recombinant plasmids) will be white while the negative bacteria will be blue.

PCR colony for screening targeted *E. coli*

Suspected white colony of *E. coli* may contain targeted gene were screened with colony PCR method. The procedure was as follow: The white competent cell colonies were taken slightly with sterile toothpicks and used as a PCR template. Each tube is labeled in accordance with the number of colonies in the Petri dish, than placed into thermal cycler with PCR condition same as above PCR

methods, except for HER-2 confirmation we used 5'-CCAGCCCTCTGACGTCCAT-3' for forward primer and 5'-CGTGTACTTCCGGATCTTCTGCTG-'3 for reverse primer which recognized inside HER-2 producing 116 bp PCR product. Positive clone than were cultured for overnight in an incubator shaker at the speed of 150 rpm and temperature 37°C.

Plasmid extraction

Plasmid extraction was done using High-Speed Plasmid Mini Kit from Geneaid. The plasmid result then visualized with UV-Transilluminator.

Control curve standardization for HER-2 amplification

pGEMT-easy HER-2 serial dilutions were started from 12.5 ng diluted by two times for each in five spots. Dilutions were used as plasmid copy number standards to generate a standard curve and to quantify *her-2* chromosomal DNA copies. General formula used: (6.02 x 10^{23} copies/mol) x (concentration in g/µL) / (MW in g/mol) = copies/µL (Mendoza et al. 2013).

qPCR experimental conditions

For amplification and data collection we used CFX-96 Real-Time PCR from Biorad. Reactions were carried out in triplicates, SYBR-Green from KappaBiosains®, 1µM of each primer. Cycling conditions were 95˚C for 5 min, 35 cycles; at 95˚C for 30 sec; at 60˚C for 10 sec and at 72˚C for 30 sec.

RESULTS AND DISCUSSION

PIK3CA data provision

There are many theories which trastuzumab resistance problems arise. Some of report showed that mutation in PIK3CA gene especially in the hotspot area such as in exon 9 and 20 contributed to the therapy implication. Patients with mutation in this hotspots area have been shown unresponsive toward trastuzumab therapy.

Based on sequencing method, two samples among 68 breast cancer samples obtained from West Sumatra province of Indonesia showed mutation in exon 20 H1047R (2.94%) and three samples showed T1025T silent mutation (4.41%). While for exon 9 study careful strategy should be considered, since we found almost 68 samples showed mutation at A1634C (E545A) position and a base deletion at nucleotide 1659 which referred as pseduogene (Samuel et al. 2004). Mutation in exon 20 H1047R was reported to have oncogenic capability and it was responsible for trastuzumab resistance problems, while T1025T position has no reports implicate to cancer development. Mutation in PIK3CA gene quite varies around 8%-40% and the contribution of this mutation to prognostic implication is still controversial (Levine et al. 2005; Mangone et al. 2012; Arsenic et al. 2014). According to Li et al. (2006), mutation in exon 20 of PIK3CA gene were predominated in breast cancer. To confirm the data obtained in this study, the large number for PIK3CA study is needed for future works.

Although the percentage of mutations were low in Indonesian breast cancer especially in West Sumatra province, the detection system is important to be prepared since H1047R has oncogenic capabilities. According to Meyer et al. 2013, PIK3CA H1047R was able to induce mammary tumor growth compared to its wild type and also this mutant showed more oncogenic pontency compared with PIK3CA E545K in the transgenic mouse models. Furthermore, PIK3CA mutations was reported associated with lower tumor stages which mean it could be as a biomarker for early tumor detection (Rudd et al. 2011; Dumont et al. 2012). Prepared recombinant plasmids and the results of PIK3CA genotyping could be used for developing and validating a new method for predictive factor therapy with trastuzumab in genomic level in future.

Control DNA preparation for pGEM-T easy HER-2 and PIK3CA gene exons 9 and 20

HER2 gene as a predictive factor of treatment with trastuzumab using was successfully cloned into pGEM-T easy plasmid as a vector. This prepared plasmid was required as a control to support targeted detection kit development. Furthermore, the performance of the kit such as sensitivity, specificity, reproducibility and suitability are prerequisite to be performed before the kit commercially produced. The property of the kit and all the associated reagents should fulfill the sensitive, specificity, fast and easy to interpret the result (Yang and Rothman 2004).

Table 1. Mutations profile in exon 20 of PIK3CA gene

Sample code	Mutation		Amino acid changes		Mutation type	Mutation nomenclature
	1025 codon	1047 codon	1025 codon	1047 codon		
0014		CAT ↓ CGT		Histidin ↓ Arginin	Substitution (*Missense*)	H1047R
0020	ACC ↓ ACT		Threonin ↓ Threonin		Substitution (*Silent*)	T1025T
0022	ACC ↓ ACT		Threonin ↓ Threonin		Substitution (*Silent*)	T1025T
0048		CAT ↓ CGT		Histidin ↓ Arginin	Substitution (*Missense*)	H1047R
0068	ACC ↓ ACT		Threonin ↓ Threonin		Substitution (*Silent*)	T1047T

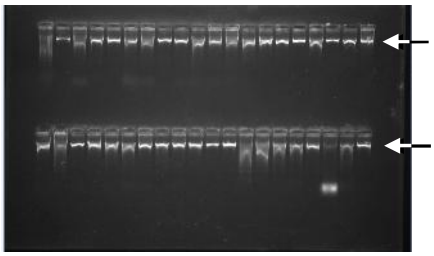

Figure 1. Breast cancer genomic DNA (arrow)

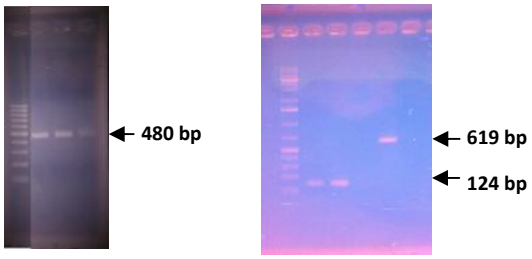

Figure 2. PCR product for HER-2 (480 bp), PIK3CA exon 9 (124 bp) and exon 20 (619 bp). DNA ladder marker: 100 bp DNA ladder (left) and 1Kb plus DNA ladder (right)

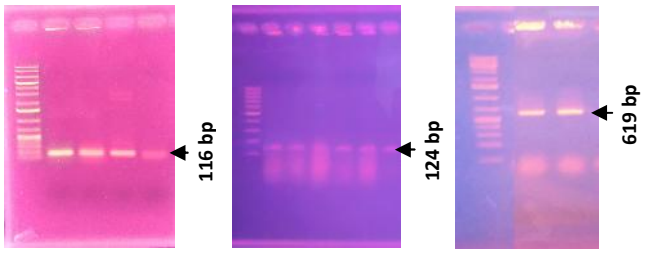

Figure 3. PCR colony of *E.coli* DH5α for HER-2 (116 bp), PIK3CA exon 9 (124 bp) and exon 20 (619 bp). DNA ladder marker: 1 Kb plus DNA ladder.

Genomic DNA was used as template for PCR amplification of exon 9 and 20 of PIK3CA as well as HER-2 gene. The genomic extraction result is shown in the Figure 1. The amplification, insertion and transformation of targeted genes for each were shown in Figure 2.

The results of the preparation of plasmid were sequenced to confirm the targeted gene (data not shown). Three recombinant plasmids prepared then were preserved in *E. coli* DH5α bacterial strain as glycerol stocks for future usage.

pGEM-T easy PIK3Ca exon 9 and 20

Mostly cancer biomarker detection method was based on PCR methods, which were massive, high-trough put and rapid (Kristensen and Hanse 2009). Prepared recombinant plasmids could be used as reference gene in conducting breast cancer genotyping in Indonesia based on PCR methods. The commercial products currently developed and commonly used are ARMS and probe technique. Both of the technique provides a negative and positive control reaction in the detection kit. By using a prepared plasmid not only could be applied as control part of the kit, but also could be used to test and to develop new techniques easier and cheaper avoiding limited sample usage. Here below the plasmid map of pGEMT easy PIK3CA for exon 9 and 20.

pGEM-T easy HER-2 for standard curve calculation in scoring HER-2

post-qPCR data processing can seriously affect the interpretation of the results. If there is no reference gene it is needed the wisdom of researchers in executing the data processing. The standard curve in real time PCR approach may have advantages. The standard curve method simplifies calculations and avoids practical and theoretical problems. (Mendoza et al. 2013). pGEM-T easy HER-2 was successfully tested for a standard curve preparation for HER-2 scoring application.

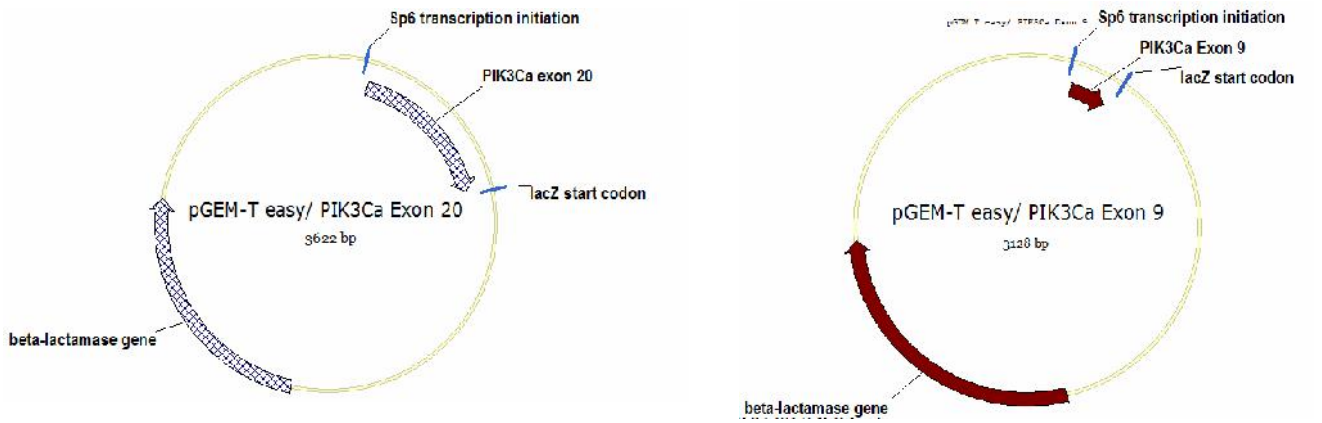

Figure 4. Map pGEM-T easy PIK3Ca exon 9 (*left*) and exon 20 (*right*)

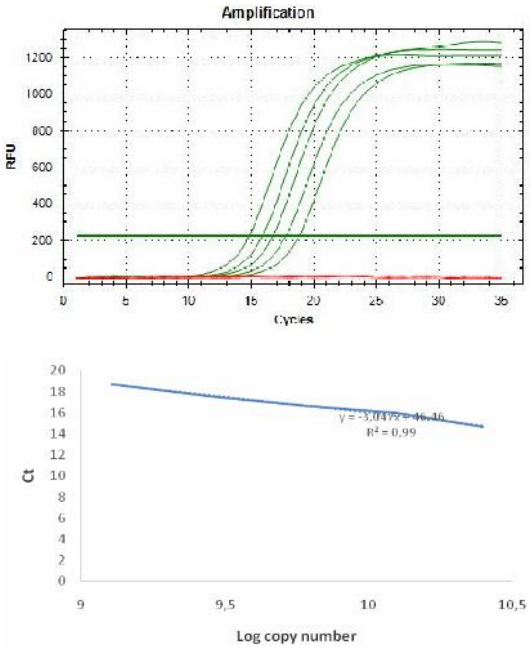

Figure 5. Standard curve of HER-2 amplification calculation

In the figure showed above, the curve is used as reference to calculate the copy number and to score the over expression of HER-2 by comparing between breast cancer and normal patients. The standard curve equation was Y=-3.0472x + 46.465, R^2= 0.99, the efficiency was 115%. This equation meets the requirements in qPCR theory. Quantifying HER-2 satus using qPCR was expected to minimize the subjectivity in selecting HER2 positive candidate among patients tested. HER-2 quantification detection kit is already commercialized, such as by Roche. The kit provides specific primers, hybridization probes, positive and negative control reaction (Beyser et al. 2001). Since the probes frequently used for the DNA labeling process hence it directly causes the kits price becoming slightly expensive. In our future detection kit development, the application of such probe is avoidable in order to minimize the price so that the kit will be more affordable especially for Indonesia market.

PIK3CA data provision and three recombinant plasmids preparation in our study are important aspect as a part of supporting in development of diagnostic kit of predicted factor for patients which is responsive to trastuzumab at genomic level based on Indonesian population. The provided kit could be applied for determination of HER2 amplification status and PIK3CA genotyping that those are becoming the major cause for resistance towards trastuzumab. Furthermore, the availability of these kits is expected helping the oncologist from inappropriate treatment of trastuzumab administrated-patients.

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Short Communication: Using ITS as a molecular marker for *Mangifera* species identification in Central Sumatra

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Abstract. *Fitmawati, Hayati I, Sofiyanti N. 2016. Using ITS as a molecular marker for* Mangifera *species identification in Central Sumatra. Biodiversitas 17: 653-656.* The relationship among *Mangifera* species in Central Sumatra is currently unclear. Previous molecular studies on these taxa using cpDNA were unable to produce well-resolved phylogenetic trees. In this study, we explored the potential of the ITS sequences as molecular markers for *Mangifera* species to better resolve the phylogenetic analysis. Parsimony analysis revealed that the common ancestor *M. quadrifida* as the first species appeared in Central Sumatra. *Mangifera* sp. which assumed as new species had the longest genetic distance among species examined and may assumed as the most primitive species of *Mangifera* in Neighbor-Joining analysis. *M. sumatrana* and *M. torquenda* were closely related as well as *M. foetida* and *M. odorata*. Also, *M. indica* was closely related to *M. kemanga*. This finding and the other marker of cpDNA such as trnL-F and rbcL gene may suggest a possibility to revision in the latest *Mangifera* classification based on morphological character. Our results also revealed and support the genus *Mangifera* is a monophyletic group.

Keywords: Central Sumatra, ITS, *Mangifera*, molecular marker, phylogenetic analyses

INTRODUCTION

The genus *Mangifera* is one of the most important genera from family Anacardiaceae which used for commercial fruit production in the world. The characteristics of *Mangifera* species in Central Sumatra were tolerant to high rainfall, capable of fruiting out of season, high production and flowers resist against wet climate. The species with these traits had a potential as germplasm resources (Fitmawati et al. 2013). Exploration on *Mangifera* species has been done by Fitmawati et al. (2013) in three provinces of Central Sumatra, i.e.: Riau, West Sumatra and Jambi. Ten of *Mangifera* species which typical in Central Sumatra were obtained. *Mangifera* species in Sumatra were divided into three categories such as: wild types, semi cultivated types and cultivated types (Fitmawati et al. 2015). Due to high frequency of forest and land fires in Sumatra, specific types of *Mangifera* Sumatra were threatened in natural habitat, therefore wild germplasm resources must be conserved before it lost in the wild.

The most recent and acceptable classification of *Mangifera* were proposed by Kostermans and Bompard (1993) based on morphological character and divided into two groups based on disc flower characteristic namely sub genus *Limus* and sub genus *Mangifera*. Sub genus *Limus* has narrower disc than the base ovary, stalk-like or even lacking whereas sub genus *Mangifera* has broader disc than the base of the ovary, cushion-like (Kostermans and Bompard 1993). Morphological plasticity and continuity characters were the main problem to define phylogenetic relationship therefore using molecular approach based on DNA sequence which has more informative characters and support morphological characteristics. Molecular Phylogeny of *Mangifera* has been done using nuclear genome marker, ITS region for *Mangifera* in Thailand by Yonemori et al. (2002), as well using chloroplast DNA marker of trnL-F Intergenic spacer on *Mangifera* sp*ecies* in Java and Sulawesi (Fitmawati and Hartana 2010), also matK (Hidayat et al. 2012) and *rbc*L (Suparman et al. 2013) on *Mangifera* mainly in Thailand and a few part of Indonesia.

Internal Transcribed spacer (ITS) of nrDNA has been used for molecular markers at specific level of Angiospermae (Baldwin et al. 1995; Yonemori et al. 2002). Sequences of ITS were also useful because it has conserve region, short size (±700 bp), high evolution rate, informative and universality (Baldwin et al. 1995). Molecular study of specific *Mangifera* in Central Sumatra based on ITS sequences has never been informed, so that tries to reveal relationship among *Mangifera* species in Central Sumatra. Molecular approach has benefit to find the best phylogenetic tree model which useful in conservation and cultivation strategies.

MATERIALS AND METHODS

Plant material and DNA extraction

All samples used in this study (Table 1) were collected in Central Sumatra from exploration in 2012-2013. Two

genera from Anacardiaceae family were used as outgroup obtained from Genbank Data (NCBI) by Yonemori et al. (2002).

Whole genome DNA were isolated from leaves of each plant after soaking in aquadest by the CTAB method of Doyle and Doyle (1987) with a slight modification, by soaking leaf in demineralization water for 24 hours before isolation. In isolation process CIAA solution were substitute by chloroform only. DNAs were then suspended in TE buffer.

Amplification and sequencing

The genomic DNA was amplified using universal primer ITS4 and ITS5 (White et al. 1990) for the entire ITS regions. Reaction mixture (50 µL) contained DreamTaq Buffer 10x, 2mM each dNTP Mix, 25 pmol of each primer, 20-50 ng genomic DNA, 1 units of DreamTaq DNA Polymerase and nuclease free water. Thirty five cycles of PCR were conducted using Thermal Cycle under following profiles: 94^{o}C for 5 m, 94^{o}C for 1 m, 47.4^{o}C for 30 s, 72^{o}C for 1 m 30 s, 72^{o}C for 7 m. PCR products were sent to First Base Laboratories, Malaysia. The amplified products were then purified by PCR Clean-Up or Gel Extraction depend on Visualization results for Single Pass DNA Sequencing. Forward sequencing reactions were performed by a Big Dye Terminator v3. 1 cycle sequencing kit using ITS5 (White et al. 1990) (First Base Laboratories).

Phylogenetic analysis

DNA sequences of ITS regions of *Mangifera* species and outgroup taxa were first alligned by ClustalW Multiple Allignment in Bioedit (Thompson et al. 1997). The boundaries of ITS1 and ITS2 were determined by comparing the aligned sequence with previously published sequences (Yonemori et al. 2002). The 5.8S coding sequence separating the ITS1 and ITS2 regions were also used in phylogenetic analyses, although only few variations were found among species examined.

The data matrix of alligned sequences was analyzed by PAUP 4.0 program (Swofford 2002) for parsimony and neighbor joining method with bootstrap replicate method.

RESULTS AND DISCUSSION

ITS sequence analysis

The length of ITS1 is 264 bp and of ITS2 ranged from 226 to 230 in *Mangifera* species studied. There is not much variation in length for 5.8S gene region having 162 - 163 bp. The G+C content was fairly equivalent between of ITS1 and ITS2, although 5.8S gene has lower content than ITS1 and ITS2 (Table 2).

Allignment of the entire of ITS sequences among *Mangifera* species obtained 660 bp. There were 48 and 98 polymorphic sites in ITS1 and ITS2 respectively, whereas three sites were polymorphic in 5.8S gene region (Table 2). Among these 149 polymorphic sites, 77 sites (33 in ITS1, 42 in ITS2 and two in 5.8S gene region) were supposed to be informative for phylogenetic analysis using parsimony method. However, when the sequences of two outgroup were added to the allignment, it resulted more indels due to short length of outgroup sequences, especially in ITS1. It resulted in 666 bp of the aligned length for the entire sequence in all species including outgroup taxa. The polymorphic sites became 234 in the entire sequence in all species including outgroup taxa, and 90 sites among them were assumed to be informative for parsimony analysis.

Table 1. List of 10 *Mangifera* species collection in 2012-2013 with their distribution and two outgroup taxa used in this study

Species name	Distribution			Accession number
	R	WS	J	
M. kemanga Bl.	√	√	√	KX347955
M. foetida Lour.	√	√	√	KX347956
M. odorata Griff.	√	√	√	KX347957
M. torquenda Kosterm.	√	√	√	KX347958
M. quadrifida Jack.	√	√	√	KX347959
M. indica L.	√	√	√	KX347960
M. sumatrana Miq.	√	√	√	KX347961
M. zeylanica (Bl.) Hooker f.	√	√	√	KX347962
M. laurina Bl.	√	√	√	KX347963
Mangifera sp.	√	-	-	KX347964
Anacardium occidentale L.	√	√	√	AB071690
Bouea macrophylla Griff.	√	√	√	AB071691

Note: R: Riau, WS: West Sumatra, J: Jambi

Table 2. The characteristic features of the ITS region among *Mangifera* species and combination with outgroup taxa

	Length range (nt)	Length mean (nt)	Aligned length (nt)	G+C content (%)	G+C mean (%)	No. of variable sites	No. of informative sites	Tree length	CI	RI
Mangifera spp.										
ITS1	264	264	264	63.6-67.4	65.4	48	33	53	0.92	0.94
5.8s rDNA	162-163	162.9	163	54.6-55.8	55.4	3	2	4	1.00	1.00
ITS2	226-230	228.3	233	55.8-61.4	58.6	98	42	123	0.90	0.80
Entire seq.	652-657	655.2	660	58.9-62.4	60.6	149	77	201	0.81	0.73
Mangifera spp. + 2 outgroup taxa										
ITS1	232-264	261.3	269	62.1-70.6	64.6	90	48	123	0.85	0.83
5.8s rDNA	162-163	162.8	164	54.3-56.7	55.3	14	4	17	0.94	0.80
ITS2	220-230	227.3	233	55.8-69.1	59.9	130	38	200	0.87	0.62
Entire seq.	615-657	651.5	666	58.4-64.4	60.6	234	90	338	0.87	0.76

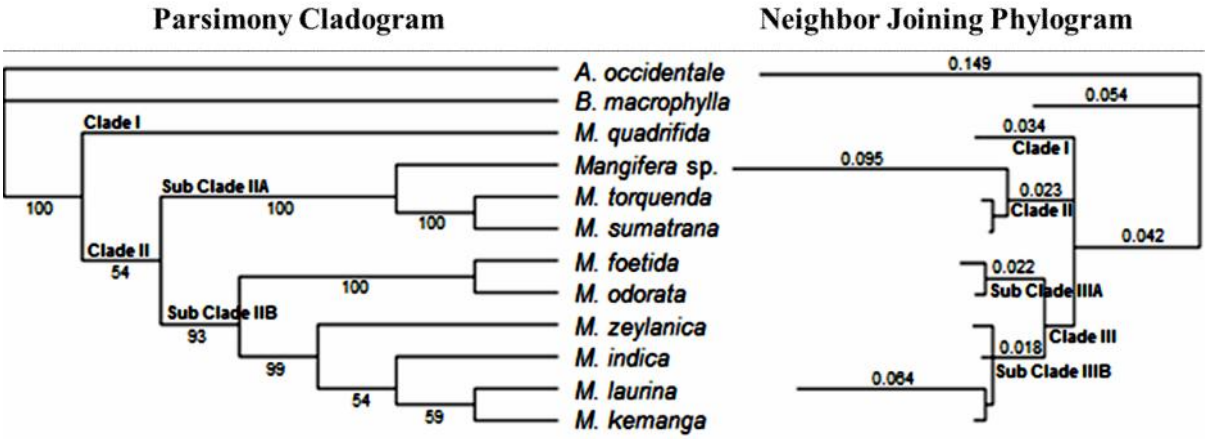

Figure 1. The phylogenetic tree based on the ITS sequences generated from maximum parsimony analysis with bootstrap value below the branch and number on the base of branch showed nodes number (*left side*) and evolution tree from HKY85 model evolution generated from neighbor joining analysis with total branch length 0.55. Branch length (number above line) corresponds to the genetic distance (*right side*).

Phylogenetic analysis of *Mangifera* species in Central Sumatra

The results of parsimony analysis based on the sequence data of ITS region are summarized in Table 2. Based on parsimony criteria, it was obtained a cladogram with CI value 0.87 and RI value 0.76. Monophyletic group of *Mangifera* species were separated from outgroup in the 21st branch with thirty three nucleotide changes (17 different sites in ITS1, 3 base in 5.8S and 13 sites in ITS2). Evolution tree from ten *Mangifera* species formed two clades with bootstrap value 100%. Clade I consists of *M. quadrifida* while Clade II consists of *Mangifera* sp., *M. torquenda, M. sumatrana, M. foetida, M. odorata, M. zeylanica, M. indica, M. laurina* and *M. kemanga.* Clade II evolved and divided into two sub clade. Sub clade IIA consists of *Mangifera* sp., *M. torquenda* and *M. sumatrana* while sub clade IIB consists of two groups were split *M. foetida* and *M. odorata* with *M. zeylanica, M. indica, M. laurina* and *M. kemanga.* (Figure 1 *left side*).

Neighbor joining (NJ) analysis reconstructed three clades. Clade I consisted of *M. quadrifida*, Clade II consists of monophyletic groups of *Mangifera* sp., *M. torquenda* and *M. sumatrana*, and Clade III consists of *M. foetida, M. odorata, M. zeylanica, M. indica, M. laurina* and *M. kemanga* (Figure 1 *right side*). The main contradiction in the NJ tree compared with the parsimonious tree was the place of clade II and clade III. Both clades formed a larger monophyletic group in parsimony analysis whereas in NJ analysis both clades were separate and resulted multifurcating tree.

Discussion

The results of parsimony analysis based on the sequence data of ITS region are noted in Table 2. Based on parsimony analysis *M. quadrifida* became the early wild type founded in lowland rainforest of Central Sumatra (Fitmawati et al. 2015). This finding was supported by fifteen nucleotide base changes in specific sites which separated *M. quadrifida* with the other nine *Mangifera* species. *M. torquenda* was closely related to *M. sumatrana*. They were separated from *Mangifera* sp. with fifty nine different nucleotide base characters. They formed a clade by sharing coriaceous leaf texture.

Mangifera foetida was showed a closely related to *M. odorata*. This theory about *M. odorata* is a hybrid from *M. indica* and *M. foetida* stated by Hou 1978 and also supported by Teo et al. (2002) and Yonemori et al. (2002). This fact does not agree with Kostermans and Bompard (1993) for saying the reticulation of *M. odorata* was definitely different from *M. indica* and *M. foetida* and also the flower was not an intermediate of both *Mangifera*.

Another monophyletic group consisted of *M. zeylanica, M. indica, M. laurina* and *M. kemanga.* The first three species were supported by Kostermans and Bompard (1993) based on morphological character, but we found the contradiction of *M. kemanga* place in this tree (Figure 1 *left side*). It was different with Kostermans and Bompard (1993) which classified *M. kemanga* into sub genus *Limus* but based on this research *M. kemanga* united in one group with *M. zeylanica, M. laurina* and *M. indica,* which is belong to sub genus *Mangifera* according to Kosterman and Bompard 1993. The previous note about relationship among *M. kemanga* and the other three species has never found therefore it became new finding on this study.

Neighbor Joining (NJ) analysis showed that *Mangifera* sp. had the longest evolutionary history from ten *Mangifera* species in this study and it assumed as the most primitive species found in Central Sumatra. *Mangifera* sp. has combination character between sub genus *Limus* and *Mangifera.* This species is included in sub genus *Mangifera* due to cushion-like disc flower while it can be included to sub genus *Limus* by deciduous character. Another important finding is the stomata type of *Mangifera* sp. is cyclocytic whereas the remaining species are anomocytic type (Astuti 2014). The discovery of *Mangifera* sp. in Central Sumatra was assumed as new species by Fitmawati et al. (2013). In this study it was found *Mangifera* sp. has high similarity in morphological

characters with *M. magnifica* Kochummen (Kostermans and Bompard 1993), with slight different found in pear-shape fruit and young bud which deciduous in *Mangifera* sp. but lacking in *M. magnifica*.

Generally, leaf texture is a suitable character to divide genus *Mangifera*. Leaf texture was described by Kostermans and Bompard (1993) where it can be divided in two large groups namely coriaceous type and chartaceous type. Coriaceous type is more primitive in ecological studies (Bews 1927). In case it was synchronize the sequence of ITS, it is assumed that the character of leaf texture was a synapomorph character in *Mangifera* classification. Some species of *Mangifera such as M. quadrifida and M. torquenda* showed transition leaf texture relatively towards coriaceous or chartaceous. It is assumed as biparental inherited from nuclear genome therefore *Mangifera* species which has transition leaf texture is a natural hybrid from different parental such as *M. odorata* hybrid from *M. foetida* and *M. indica*. Hence, ITS marker potentially track origin and evolutionary of polyploidy in plant (Kim and Mabry 1991).

Results of alligned sequence of entire ITS revealed ITS region was flanking conserve 5.8S region (coding region) encoded ribosomal RNA which is important in protein synthesis (add reference). Mutation rate of conserve gene is slower than non coding region. ITS region as non coding region has more variation and higher mutation rate than coding region. Non coding region (intron) has role in gene expression regulation which adaptable with niche/habitat. Most of this non coding region could be observe through phenotypic characters.

Based on this study we found many differentiations between classification based on morphological characteristics by Kostermans and Bompard (1993) and molecular study .This results could be use as strong basis to develop a new system of classification. Classification based on DNA sequence is assumed to produce nature and accurate classification because DNA is a basic unit of information that encode organism.

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The water quality parameters controlling diatoms assemblage in Rawapening Lake, Indonesia

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Manuscript received: 16 May 2016. Revision accepted: 11 August 2016.

Abstract. *Soeprobowati TR, Tandjung SD, Sutikno, Hadisusanto S, Gell P, Hadiyanto. 2016. The water quality parameters controlling diatoms assemblage in Rawapening Lake, Indonesia. Biodiversitas 17: 657-664.* Diatoms are microalgae that have an important role in aquatic ecosystem. The silicious diatoms fossil had been used for paleolimnological analysis. However, data set diatoms of Indonesia does not develop yet. The aims of this research were to assess water quality parameters controlling diatoms assemblage of Rawapening Lake, to assess transfer function, to develop diatoms data set of Rawapening, and to compare Rawapening diatoms data set with European diatoms data set for the reconstruction of total phosphorous and pH. Water samples were taken from 3 sites of Rawapening Lake for water quality analysis. Sediment samples had been taken from 3 sites in Rawapening Lake using hand auger, and were sliced every 0.5 cm for diatoms analysis. Diatoms slides were prepared from about 5 g of dry sediment using 10% of chloride acid followed by 10% of peroxide to remove organic matters and carbonates, respectively.300 diatom valves were calculated on the entire samples, calculation the number of valve's diatom aims to identify the lowest level of taxonomy. Phosphate, temperature, and calcium were the environmental parameters that influence the diatoms assemblage of Rawapening Lake. Phosphate was contributed 50% on diatoms assemblage. Internal based transfer function of diatoms provides more suitable diatoms data set rather than European diatom data set. The Rawapening diatoms data set of total phosphorous is the initial Indonesian diatoms data set for past trophic status, therefore, research has to be continued spatially for other Indonesian lakes and temporary on specific Indonesian lake to develop Indonesia diatoms data set.

Keywords: Diatoms, Indonesia, phosphate, Rawapening Lake, water quality

INTRODUCTION

Global concern on the environmental issue had been focused on the sustainability of the availability, management and conservation of freshwater ecosystem. The phenomenon of increasing population growth, urbanization, wastewater management, will influence water quality problem in the future, particularly in Asia, therefore required an effort to overcome the problem of water quality degradation (Avans et al. 2012).

Freshwater quality problem were also occurred in Indonesia, and become a national problem. In 2009 there was an agreement between 9 Indonesian ministers about 7 lakes priority criteria, regarding to the lake degradations: (i) sedimentation; (ii) pollution, eutrophication, and water quality degradation; (iii) lake usage: power electricity, agriculture, fisheries, drinking water, social and religious life, tourism; (iv) commitment between government and community; (v) lake strategy for national function; (vi) biodiversity (endemic species); and (vii) level of risk disaster. There were 7 programs for 15 lakes priority, set about lake ecosystem management, scientific and technological approach on using lakes resources; the development of lake monitoring, evaluation and information systems; preparation of adaptation and mitigation steps; the impact of environmental changes to the lakes; the development of capacity, regulation and coordination; improvement of community involvement; and sustainable funded. There are 15 lakes national priority in the year 2010-2014 that are Lakes of Toba Maninjau, Singkarak, Kerinci, Tondano, Limboto, Poso, Tempe, Matano, Cascade Mahakam Semayang-Melintang-Jempang, Sentarum, Sentani, Rawa Danau, Batur, and Rawapening (MoE 2010).

Rawapening Lake is located in the S 7°04'-7°30' and E 110°24'46"-110°49'06" in the urban area between of 3 cities in the Central Java, i.e.: Yogyakarta, Solo, and Semarang. Despite of its small size (26.23 km^2), 207,438 people depend their life to Rawapening Lake. The catchment area of the lake is consists of 55.40 km^2 paddy's fields; 1,126.42 $km^2$of plantations; 44.08 km^2 of settlements; and 21.64 km^2 of forests (BPS 2010). Ecologically, Rawapening Lake had been changed particularly because there are plants that grow uncontrollably due the influence of eutrophication, 70% of lake area are covered by water hyacinth. Eutrophication

and sedimentation were huge problem that had induced lake shallowest. This environmental degradation had threatened its function for electricity power, irrigation, fisheries, source of drinking water and recreation area. These were typical problem of Indonesian lakes. Limnological research was 1 out of 6 super priority programs for Save Rawapening Lake have to be developed as a basic national policy (MoE 2011).

It was not doubtly about the potential use of diatoms for bioindicator of water quality, and used diatoms to reconstruct past water quality changes (Gell et al. 2007; Reid and Ogden 2009; Soeprobowati et al. 2012; Adams et al. 2014; Yun et al. 2014). Diatoms are dominant microalgae almost in all aquatic ecosystems, contribute 20-25% primary production, have an important role in the silica and carbon cycle (Soeprobowati et al. 2012). Different taxa have different toleration to the environmental parameters. Therefore, diatoms assemblage reflects water quality effectively. Quantitatively, reconstruction of past condition can be done by 3 approaches namely indicator species approach, involving bioclimatic modeling that was causal relationship between species distribution and climate variable; assemblage approach involving modern analog technique and response surfaces; and multivariate calibration-functional that known as a transfer function have an important role in future quaternary paleoecology (Birks et al. 2010).

The aims of this research were to assess water quality parameters controlling diatoms assemblage of Rawapening Lake Indonesia, to assess transfer function and to develop diatoms data set of Rawapening, and to compare Rawapening diatoms data set with European diatoms data set on reconstruction of total phosphorous and pH.

MATERIALS AND METHODS

Three research sites were chosen based on representative of inlet, outlet, and water body of the Rawapening Lake, Central Java, Indonesia (Figure 1). Hand-auger was used to collect sediment samples, and then slice every 0.5 cm thick for diatoms analysis. 5 gr of dry sediment was digested by heated with 10% of chloride acid followed by 10% of peroxide for 2 hours to remove organic matters and carbonates, respectively (Battarbee et al. 2001). Distilled water was added when the solution almost dry. After settled for at least 4 hours, supernatant was discharged and added with distilled water until 50 mL and allowed to settle for another 4 hours. This was done repeatedly until the pH was neutral (7). The washed frustules were mounted in Naphrax and examined by optical microscope at 1,000 magnifications. In each sample, an average of 300 valves was counted in order to establish the relative abundance of the species (Soeprobowati 2010). Diatom taxonomy followed the guidelines Kramer and Lange-Bertalot (2010a,b (teil 1-2), 2008 (teil 3), 2011 (teil 4) volume 1-4; Guiry and Guiry 2016) and valves were identified to the lowest taxonomic level possible.

The water quality measurements was done in-situ by measuring the temperature, pH, dissolved oxygen, turbidity, conductivity, and water clarity on the depth of 20 cm from surface water. The Rawapening lake depth was measure from the surface water until the bottom of the lake. Water samples were also taken to analyze the concentration of dissolved silicate, total suspended solid (particulate material), biological oxygen demand (BOD), calcium, Fe, Mg, Na, Pb, Cd, Cu, Cr, phosphate, total phosphorous, total nitrogen, nitrite, nitrate, ammonia, and chlorophyll-*a*. BOD was measure by counted of dissolved oxygen on the first day and 5 days. Total phosphorous and total nitrogen was the total of minerals and organics part. 40 data from 2004-2008 had been compiled to develop training set of Rawapening (RP40), that were chosen for the same parameter from the same site as time series. Water quality variables were transformed by log (x+1).

Principle Component Analysis (PCA) followed by Canonical Component Analysis (CCA) was done to determine the water quality parameters that influence the diatoms assemblage and its distribution. CANOCO version 4.56 (ter Braak and Smilauer 2009) was used to do PCA and CCA. CCA was designed to detect the variation trend of diatoms species due to environmental variables, the CCA had combine regular ordination aspect and regression (Kireta et al. 2012). The relationship between diatoms distribution and environmental dataset was assessed by direct gradient technique by CCA with the limited axis ordination as a linier combination of environmental variables (ter Braak and Smilauer 2009). Forward selection was used to reduce redundancy variables and select significantly variables that able to reflect the variation on the diatoms data set by unlimited Monte Carlo permutation. The variables that significantly responsible to the diatoms distribution are when $p < 0.05$ on the 199 permutation. Insignificant variables were not included in the further analysis. Samples of data set as well as environmental parameters were selected. The samples with inflation variant > 20 have a stable data effect, so those samples did not include in the analysis (ter Braak and Smilauer 2009).

Furthermore, transfer function was analyzed with Computer Program C2 1.7.6 (Juggins 2014). The diatoms species with more than 5% of relative abundance had been included in the analysis with 3 significant environmental parameters, i.e. phosphate, calcium, and temperature. *Weighted Averaging* (WA) with inversed and deshrinking was the model that implemented for multivariate calibration function to estimate water quality based on diatoms (Birks et al. 2010). Prediction model was assessed based on correlation coefficient (R^2) between observed diatoms and inference diatoms in the form of Root Mean Square Error (RMSE). The validation for prediction error had assessed with leave-one-out jackknifing (Juggins 2014).

Reconstruction past condition of nutrient status of the lake was done based on the European diatom data set with Computer Program of ERNIE (Environmental Reconstruction using the European Diatom Database (Juggins 2016), compare to Rawapening diatom data set.

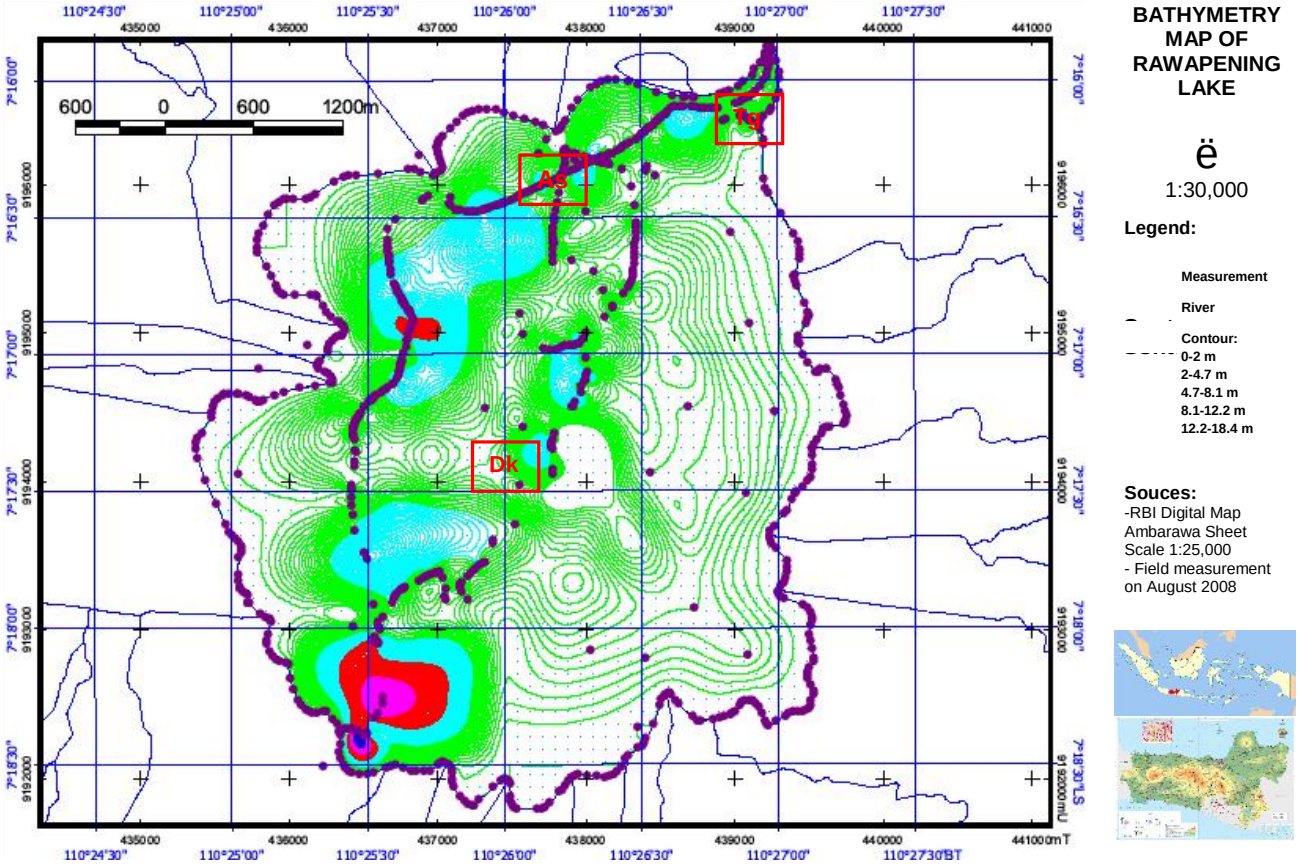

Figure 1. Research site for water quality and diatoms in Rawapening Lake, Central Java, Indonesia

Table 2. Marginal and conditional effect of environmental variables on the diatom variation in Rawapening Lake, Indonesia

Variable	Marginal effects		Conditional effects			
	Var. N	Lambda1	Var. N	LambdaA	P	F
Phosphate	13	0.36	13	0.36	0.005	3.51
Calcium	23	0.32	23	0.3	0.005	3.03
Temperature	3	0.3	3	0.16	0.001	1.7
Turbidity	27	0.35	27	0.13	0.06	1.37
Silica	10	0.3	10	0.12	0.125	1.28
Conductivity	2	0.16	2	0.12	0.175	1.21
Fe	26	0.24	26	0.1	0.365	1.12
Water clarity	4	0.14	4	0.1	0.27	1.1
Depth	5	0.16	5	0.11	0.225	1.2
pH	1	0.32	1	0.1	0.5	1.01
Mg	24	0.3	24	0.09	0.445	1
TSS	17	0.15	17	0.09	0.55	0.92
Pb	6	0.08	6	0.08	0.56	0.9
Nitrite	16	0.18	16	0.08	0.605	0.88
Nitrate	15	0.22	15	0.09	0.56	0.95
Total phosphorous	11	0.14	11	0.08	0.68	0.79
Cd	7	0.09	7	0.07	0.695	0.73
Cr	8	0.08	8	0.12	0.275	1.29
Cu	9	0.08	9	0.12	0.235	1.27
BOD	29	0.19	29	0.06	0.76	0.7
Na	25	0.28	25	0.09	0.57	0.9
Chlorophyll	20	0.27	18	0.08	0.64	0.83
NH_3	14	0.31	14	0.06	0.74	0.67
Dissolved oxygen	28	0.23	28	0.06	0.93	0.55
Total nitrogen	12	0.25	12	0.11	0.405	1.09

RESULTS AND DISCUSSION

The Rawapening diatoms data set was started by Principle Component Analysis (PCA) on the water quality, followed by Canonical Correspondence Analysis (CCA). PCA is a multivariate analysis with an ordination technique which developed theoretical variables by minimized total residual after fitted the strike line and regression (Jongman et al. 2005).Using 21 environmental parameters from 40 sites compiling with previous research (2004-2008) in Rawapening Lake, the Eigen value to axis 1 and 2 were 0.417 and 0.108, which reflected 65.2% environmental variation (Table 1). Axis 1 was dominated by phosphate and temperature, whereas axis 2 was connected with more heterogeneous variables. The environmental variable was significantly influence the diatoms assemblage if $p<0.05$ with the permutation 199. Temperature and phosphate were in thefirst axis, whereas calcium was in the fourth axis (Figure 2). Phosphate explained 41.7% species variation on the Rawapening diatoms data set whereas calcium explained 65.2% species variation in Rawapening (Table 1).

All environmental variables, consisting of temperature, pH, dissolved oxygen, turbidity, conductivity, water clarity, dissolved of silicate, total suspended solid (particulate material), BOD, calcium, Fe, Mg, Na, Pb, Cd, Cu, Cr, phosphate, total phosphorous, total nitrogen, nitrite, nitrate,

ammonia, and diatoms data set from 40 sites were analyzed. Furthermore, based on the Monte Carlo permutation test, the environmental variables from marginal effect with lambda >0.2 were reduced from the data. However, based on conditional effect, 3 variables that responsible to the diatoms assemblage ($p<0.05$) were phosphate, calcium, and temperature (Table 2). Those result related to the allochtonous effect from the inlet.

Based on the CCA, the variation of Rawapening's diatoms was controlled by phosphate, temperature and calcium (Figure2). The diatom species that correlate to the environmental parameters were provided in Table 3. Phosphate was the strongest variable that influenced diatoms assemblage in Rawapening Lake with lambda of 0.36 and $p < 0.005$ (Table 2).

Calcium carbonate is the most contributors for water alkalinity. Alkalinity was buffer capacity for water pH. Bicarbonate was alkali due to the reaction with H^+, meanwhile when hydrolyzed it may produce OH^-. The alkalinity of CO_3^{2-}were stronger than CO_2, therefore in equilibrium condition, OH^-in the bicarbonate solution always exceed H^+. Based on this research, the major cation dominated in Rawapening was calcium. People living surrounding Rawapening were used calcium carbonate on the production of organic fertilizer from water hyacinth. The tailing from fertilizer process diffuses to Rawapening Lake. Diatoms used bicarbonate ion as source of carbon and induced hydroxide accumulation ($CO_3^{2-} + H_2O \leftrightarrow HCO_3^- + OH^-$). In turn, this hydroxide accumulation may induce an increase of pH (9-10). This alkali condition had also indicated by blooming of *Aulacoseira granulata* (Ehr.) Simonsen (Soeprobowati et al. 2012).

Development of transfer function basically was based on the Weighted Averaging (WA) with inversed and deshrinking. WA calibration was more suitable rather than WAT (Weighted Averaging with Tolerance-down weighting) as a predictive model of phosphate. Diatoms assemblage was correlated with phosphate ($R^2= 0.50$, RMSE = 0.22, Table 4). Phosphate contributes 50% on diatom assemblage, and the rest were another factors.

Generally, diatom data set and transfer function was developed based on some lakes numbers. Mills (2009) developed transfer function based on 86 Uganda's lakes. However, Mackay et al. (2003) had successfully developed transfer function based on the internal diatoms of Baikal Lake. The exploration of quantitative correlation between diatom species and environmental variables had been calculated based on the environmental internal gradient of Rawapening Lake and shown a quite good trend.

Table 1. Eigen value 4 axis resulting from PCA

Axis	1	2	3	4	Total variant
Eigen value	0.417	0.108	0.067	0.059	1.0
Correlation species-environment	0.853	0.829	0.793	0.826	
% cumulative species data	41.7	52.6	593	65.2	
Sum of Eigen value = 1					
Sum of canonical Eigen value = 0.681					

The reconstruction of total phosphorous of Rawapening Lake based on the European diatoms data set compare to Rawapening diatoms data set shown a similar pattern for Asinan and Tuntang sites (Figure 3). There was an increase of total phosphorous in between 1984-1990 which was related to the low of rainfall. Total phosphorous from catchment area were entered to Rawapening Lake from16 inlets, whereas the only one outlet did not discharge the water during the dry season to maintain water level for electricity power. This condition had induced a sharp increase of total phosphorus content.

However, the reconstruction from Dangkel site shown a different pattern. There was a total phosphorous concentration decreasing trend in the middle layers with European diatoms data set, but an increase trend with Rawapening diatoms data set (Figure 3). For Dangkel site, total phosphorous content more representatively reconstruct with Rawapening diatoms data set rather than European data set, due to diatoms variation species at Dangkel site which was higher than European data set, which are not represented in European diatoms data set.

The reconstruction of pH of Rawapening lake had differ patter for different sites both using European diatoms data set and Rawapening diatoms data set. For examples, Asinan site shown fluctuate pH when reconstruct by European diatoms data set. However, based on Rawapening diatoms data set, the Rawapening pH tends to increase by year (Figure 4). Many diatoms species found in Rawapening Lake, do not found in European diatoms data set. In Rawapening diatoms data set, those species characterized such specific condition.

The pH of Rawapening Lake was fluctuant. In 1978, pH in Rawapening was neutral about 7.2-7.6 (Goltenboth 1979). In 1999, pH tent to increase, in the range of 7.5-8.8 (BPDL-PPLH Undip 1999). In 2003, pH relatively neutral, in the range of 6.5-7.7 (Wibowo 2004). In 2004 and 2005, pH in the inlet rivers tent to neutral, but in the site near spring and floating island, the pH tent to be highwhich was 9.52 (Soeprobowati et al. 2012). In this research (2009), the pH of Rawapening Lake was neutral, except near the spring and floating island that were 11 and 9.2.Naturally, lake will be more acid by time, but in Rawapening as stated before, the pH tent to increase. This condition is caused by the decomposition of organic materials by microbes in the peat sediment. The other reason is the use of calcium for treat organic fertilizer from water hyacinth. In the rainy day, the calcium leaches and enters to the lake. In recent study, those paleoreconstruction of ecological change in Rawapening was proven a trend of increasing pH. In the measurement in June 2015, pH of Asinan site was 7.8-8.1 (Soeprobowati 2015).

Internal based diatoms of Rawapening Lake developed from different 40 sites and time scale, shown quite good results since it was usually represent the specific diatoms. The diatoms data set of Rawapening was initial Indonesian diatoms data set for reconstruction total phosphorous, therefore, research has to be continued to add and fulfill data of water quality and diatoms from other Indonesian lakes.

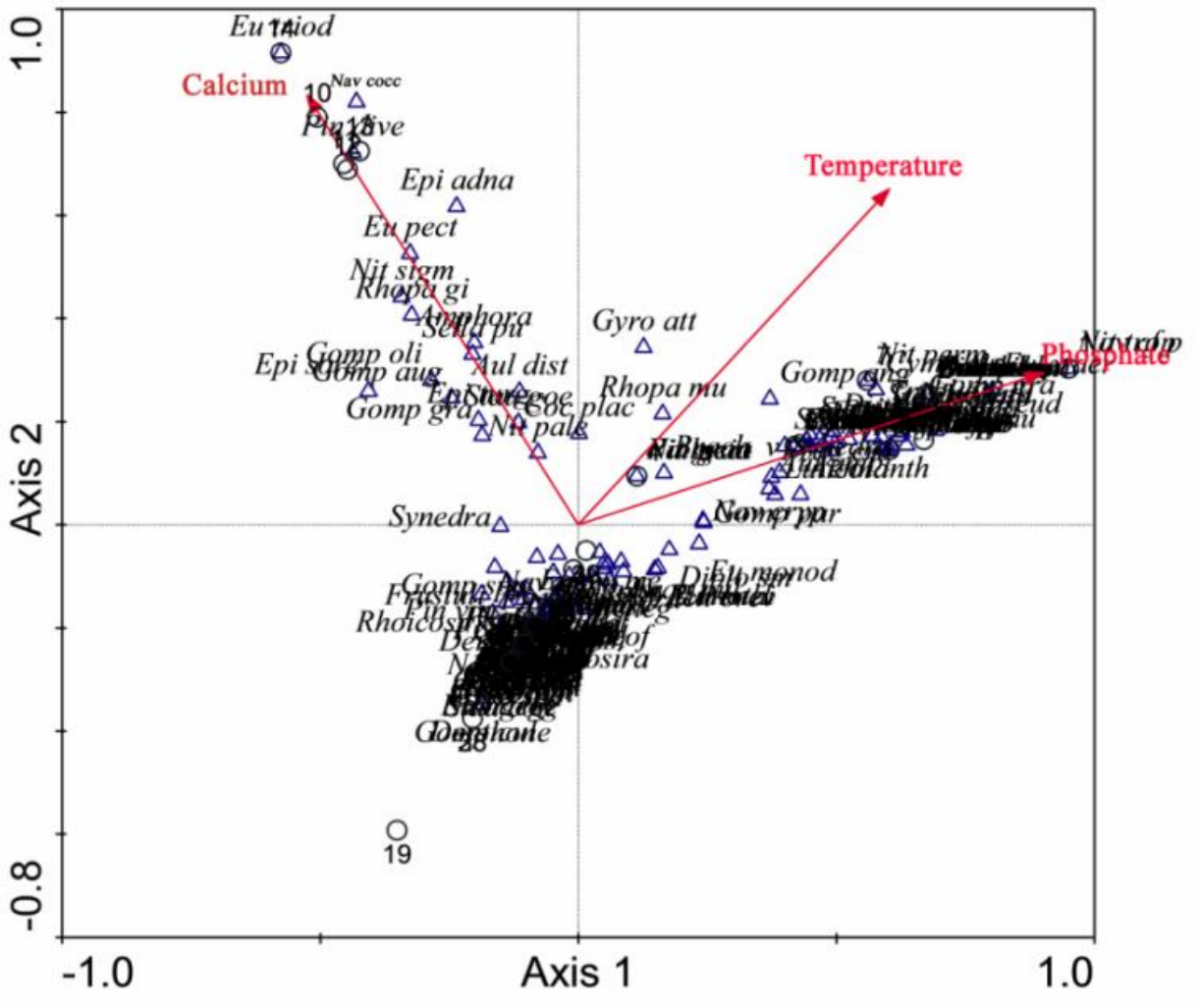

Figure 2. Triplot CCA; Phosphate, temperature and calcium the major of environmental variables which influence diatom variation of Rawapening Lake, Central Java, Indonesia

Table 3. List of diatom species of Rawapening Lake, Central Java, Indonesia in correlation with water quality as mention in Figure 2.

Abbreviation	Name of species
Ach brev	*Achnanthes brevipes* Agardh
Ach exi	*Achnanthidium exiguum* (Grunow)
Ach exi1	*Achnanthidium exiguum1* Grunow
Ach inf	*Achnanthidium inflata* (Kutzing) Grunow
Ach min	*Achnanthidium minutissimum* (Kutzing) Czamecki
Ach neo	*Achnanthidium neomicrocephalum* Kutzing
Amp cop	*Amphora copulata* (Kutzing) Schoeman & R.E.M. Archibald
Amp hol	*Amphora holsatica* W. Smith
Amp ova	*Amphora ovalis* (Kutzing) Kutzing
Amp sp	*Amphora* sp.
Amp ven	*Amphora veneta* Kutzing
Amph ala	*Amphipora alata* (Ehrenberg) Kutzing
Amph cof	*Amphora coffeaeformis* Kutzing
Ano sphae	*Anomoeoneis sphaerophora* Pfitzer
Aul ambi	*Aulacoseira ambigua* (Grunow) Simonsen Manitoba
Aul dis	*Aulacoseira distans* (Ehrenberg) Simonsen
Aul gran	*Aulacoseira granulata* (Ehrenberg) Simonsen
Bac pax	*Bacillaria paxillifer* (O. F. Muller) T. Marsson
Brach breb	*Brachysira brebissonii* Ross
Brach neo	*Brachysira neoexilis* Lange-Bertalot
Brach vit	*Brachysira vitrea* Grunow
Calo ven	*Caloneis ventricosa* (Egrenberg) F. Meister
Camp nor	*Campylodiscus noricus* Ehrenberg ex Kutzing
Coc pla	*Cocconeis placentula* Ehrenberg
Cy atom	*Cyclotella atomus* Hustedt
Cy men	*Cyclotella meneghiniana* Kutzing
Cy pse	*Cyclotella pseudostelligera* Hustedt
Cym pros	*Cymbella prostata* (Berkeley) Cleve
Cym sol	*Cymatopleura solea* (Brebisson) W Smith
Cymb af	*Cymbella affinis* Kutzing
Cymb cae	*Cymbella caespitosum* Kutzing
Cymb cis	*Cymbella cistula* (Ehrenberg) O. Kirchner
Cymb tum	*Cymbella tumida* (Brebisson) Van Heurck
Den ele	*Denticula elegans* Kutzing
Den sp	*Denticula* sp.
Dia cont	*Diadesmis contenta* (Grunow) D.G. Mann
Dia conf	*Diadesmis confervacea* Kutzing
Dia hie	*Diatoma hiemale (*Roth) Heiberg
Dia vul	*Diatoma vulgare f.brevis* (Grunow) Bukhtiyarova
Diplo el	*Diploneis elliptica* (Kutzing) Cleve
Diplo ova	*Diploneis ovalis* (Hilse) Cleve
Diplo smi	*Diploneis smithii* (Brebisson) Cleve
Disc stel	*Discostella stelligera* (Cleve & Grunow) Houk & Klee
Ency mes	*Encyonema mesiana* (Cholnoky) DGMann
Eny min	*Encyonema minutum* (Hilse) DGMann
Epi ad	*Epithemia adnata* (Kutzing)Brebisson
Epi ar	*Epithemia argus* Kutzing
Epi tur	*Epithemia turgida* (Ehrenberg) Kutzing

Eu bi	*Eunotia bilunaris* (Ehrenberg) Schaarschmidt
Eu cam	*Eunotia camelus* Ehrenberg
Eu exi	*Eunotia exigua* (Brebisson ex Kutzing) Rabenhorst
Eu inc	*Eunotia incisa* W. Smith ex W Gregory
Eu mon	*Eunotia monodon* Ehrenberg
Eu nae	*Eunotia naegelli* Migula
Eu par	*Eunotia parallela* Ehrenberg
Eu pec var pec	*Eunotia pectinalis* var. *pectinalis* Kutzing
Eu prae	*Eunotia praerupta* Ehrenberg
Eu sep	*Eunotia septentrionalis* Geniculata .Berg
Eu ten	*Eunotia tenella* (Grunow) Hustedt
Eu trio	*Eunotia triodon* Ehrenberg
Fal pyg	*Fallacia pygmaea* (Kutzing) Stickle & D.G.Mann
Frag cap	*Fragilaria capitata* (Ehrenberg) Lange-Bertalot
Frag capu	*Fragilaria capucina* Desmazieres
Frag capu var rum	*Fragilaria capucina* var. *rumpens* (Kutzing) Lange-Bertalot
Frag croto	*Fragilaria crotonensis* Kitton
Frag int	*Fragilaria intermedia* Grunow
Frag pin	*Fragilaria pinnata* Ehrenberg
Frag pul	*Fragilaria pulchella* (Ralfs ex Kutzing) Lange-Bertalot
Frag vau	*Fragilaria vaucheriae* (Kutzing) J. B. Petersen
Frag vir	*Fragilaria virescens* Ralfs
Frus rhom	*Frustulia rhomboides* (Ehrenberg) De Toni
Frus vul	*Frustulia vulgaris* (Thwaites) De Toni
Gomp acu	*Gomphonema acuminatum* Ehrenberg
Gomp af	*Gomphonema affine* Kutzing
Gomp ang	*Gomphonema angustum* C. Agardh
Gomp aug	*Gomphonema augur* Ehrenberg
Gomp clav	*Gomphonema clavatum* Ehrenberg
Gomp grac	*Gomphonema gracilis* Hustedt
Gomp grac tur	*Gomphonema gracilis turris* Hustedt
Gomp oli	*Gomphonema olivaceum* (Hornemann) Brebisson
Gomp par	*Gomphonema parvulum* (Kutzing) Kutzing
Gomp spp	*Gomphonema* spp.
Gomp trun	*Gomphonema truncatum* Ehrenberg
Gomp ven	*Gomphonema ventricosum* W. Gregory
Gyro acu	*Gyrosigma acuminatum* Kutzing Rabenhorst
Gyro at	*Gyrosigma attenuatum* (Kutzing) Rabenhorst
Gyro obt	*Gyrosigma obtusatum* (Sullivant & Wormley) C.S Boyer
Gyro par	*Gyrosigma parkerii (*M.B. Harrison) Boyer
Gyro scal	*Gyrosigma scalproides* (Rabenhorst) Cleve
Gyro spen	*Gyrosigma spencerii* (Bailey ex Quekett) Griffth & Henfrey
Han arc	*Hannaea arcus* (Ehrenberg) R.M. Patrick
Hantz amp	*Hantzschia amphioxys* (Ehrenberg) Grunow
Lut hal	*Luticola halophila* Grunow
Lut mut	*Luticola mutica* (Kutzing) D.G. Mann
Mas el	*Mastogloiea elliptica* (C. Agardh) Cleve
Mel var	*Melosira varians* C. Agardh
Mer cir	*Meridion circulare* (Greville) C. Agardh
Nav amp	*Navicula amphioxys* Kutzing
Nav at	*Navicula atomus* (Kutzing) Grunow
Nav car	*Navicula cari* Ehrenberg W. Gregory ex Greville
Nav cf pla	*Navicula* cf. *placentula* (Ehrenberg) Kutzing
Nav cf pse	*Navicula* cf. *pseudoscutiformis* (Hustedt)
Nav coc	*Navicula cocconeiformis* W. Gregory ex Greville
Nav cons	*Navicula constans* Hustedt
Nav cryp	*Navicula cryptotenella* Lange-Bertalot
Nav cus	*Navicula cuspidata* (Kutzing) Kutzing
Nav daf	*Navicula distans* W. Smith
Nav elg	*Navicula elginensis* (W. Gregory) Ralfs
Nav greg	*Navicula gregaria* Donkin
Nav has	*Navicula hasta* Pantocsek
Nav rad	*Navicula radiosa* Kutzing
Nav rhyn	*Navicula rhyncocephala* Kutzing
Nav spp	*Navicula* spp.
Nei aff	*Neidium affine* (Ehrenberg) Pfitzer
Nit aci	*Nitzschia acicularis* (Kutzing) W.Smith
Nit cap	*Nitzschia capitellata* Hustedt
Nit clau	*Nitzschia clausii* Hantzch
Nit clos	*Nitzschia closterium* (Ehrenberg) W. Smith
Nit dis	*Nitzschia dissipata* (Kutzing) Rabenhorst
Nit fil	*Nitzschia filiformis* (W. Smith) Van Heurck
Nit gra	*Nitzschia gracilis* Hantzsch
Nit lin	*Nitzschia linearis* W. Smith
Nit pal	*Nitzschia palea* (Kutzing) W. Smith
Nit per	*Nitzschia perminuta* (Grunow) M. Peragallo
Nit sig	*Nitzschia sigmoidea* (Nitzsch) W. Smith
Nit trop	*Nitzschia tropica* Hustedt
Ortho ros	*Orthoseira roseana* (Rabbenh.) O'Meara
Pin bor	*Pinnularia borealis* Ehrenberg
Pin brau	*Pinnularia braunii* Cleve
Pin div	*Pinnularia divergentissima* (Grunow) Cleve
Pin gen	*Pinnularia gentilis* (Donkin) Cleve
Pin gib	*Pinnularia gibba* Ehrenberg
Pin int	*Pinnularia interrupta* W. Smith
Pin meso	*Pinnularia mesolepta* (Ehrenberg) W. Smith
Pin micro	*Pinnularia microstauron* (Ehrenberg) Cleve
Pin neo	*Pinnularia neo-major* Krammer
Pin nob	*Pinnularia nobilis* Ehrenberg (Ehrenberg)
Pin sp	*Pinnularia* sp.
Pin sto	*Pinnularia stomatophora* Grunow
Pin subcap	*Pinnularia subcapitata* W. Gregory
Pin subgib	*Pinnularia subgibba* Krammer
Pin supdiv	*Pinnularia superdivergentissima* Chaumont & H. Germain
Pin vir	*Pinnularia viridis* (Nitzsch) Ehrenberg
Pla del	*Planothidium delicatulum* Kutzing
Pla lan	*Planothidium lanceolatum* Brebisson
Pleu	*Pleurosira* sp.
Psam didy	*Psammothidium didymium* Hustedt
Rhoi abb	*Rhoicosphenia abbreviata* (C. Agardh) Lange-Bertalot)
Rhoi cur	*Rhoicosphenia curvata* (Kutzing) Grunow
Rhop gib	*Rhopalodia gibba* (Ehrenberg) Otto Muller
Rhop mus	*Rhopalodia musculus* (Kutzing) Otto Muller
Sel pup	*Sellaphora pupula* (Kutzing) Mereschkovsky
Sel sem	*Sellaphora seminulum* (Grunow) D.G. Mann
Sel wis	*Sellaphora wislouchii* Poretzky
Stau an var jav	*Stauroneis anceps* var. *javanica* Hustedt
Stau coh	*Stauroneis cohnii* Hilse
Stau cons	*Staurosira construens* Ehrenberg
Stau goep	*Stauroneis goeppertiana* Bleisch(Navmutvartrop)
Stau jav	*Stauroneis javanica* (Grunow) Cleve
Stau phoe	*Stauroneis phoenicenteron* (Nitzsch) Ehrenberg
Sur ang	*Surirella angusta* Kutzing
Sur lin	*Surirella linearis* W. Smith
Sur ova	*Surirella ovalis* Brebisson
Sur rob	*Surirella robusta* Ehrenberg
Sur ten	*Surirella tenera* W. Gregory
Syn ac	*Synedra acus* Kutzing
Syn tab	*Synedra tabulata* (C. Agardh) Kutzing
Syn ul	*Synedra ulna* (Nitzsc) Ehrenberg
Tab fasc	*Tabularia fasciculata* (C. Agardh) D.M. Williams & Round
Tab floc	*Tabellaria flocculosa* (Roth) Kutzing
Try cal	*Tryblionella calida* (Grunow) D.G. Mann

Table 4.WA and *WA* tolerance down-weighted (WAT), RMSE, regression of 40 samples for transfer function development

Code	RMSE	R^2	Ave_Bias	Max_Bias	Jack_R^2	Jack_Ave_Bias	Jack_Max_Bias	RMSEP
WA_Inv	0.22	0.50	0.00	0.75	0.04	-0.01	1.16	0.33
WA_Cla	0.31	0.50	0.00	0.31	0.07	0.00	1.11	0.40
WATOL_Inv	0.23	0.46	0.00	0.95	0.03	0.05	1.66	0.40
WATOL_Cla	0.34	0.46	0.00	0.67	0.01	0.11	1.99	0.58

Note: WA_Inv: Weighted averaging model (inverse deshrinking) for Total P. WA_Cla: Weighted averaging model (classical deshrinking) for Total P. WATOL_Inv: Weighted averaging model (tolerance down weighted, inverse deshrinking) for Total P. WATOL_Cla: Weighted averaging model (tolerance down weighted, classical deshrinking) for Total P

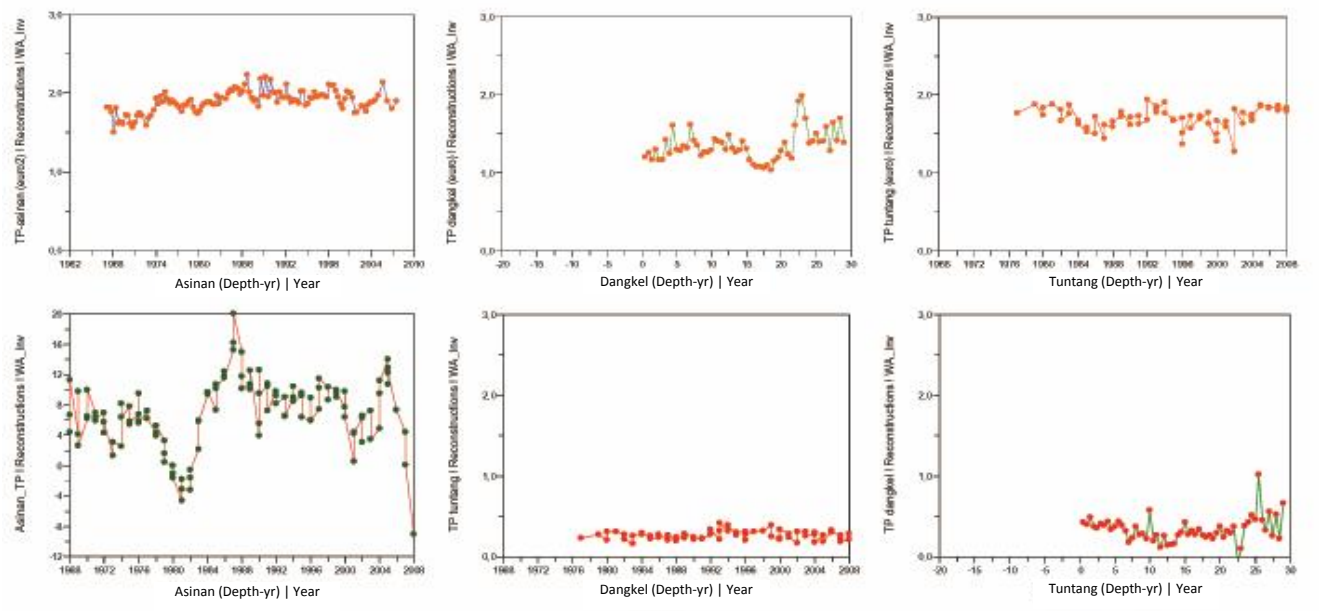

Figure 3. The past total phosphorous reconstruction of Rawapening Lake, Central Java, Indonesia with European diatom data set (above) and Rawapening diatom data set (below)

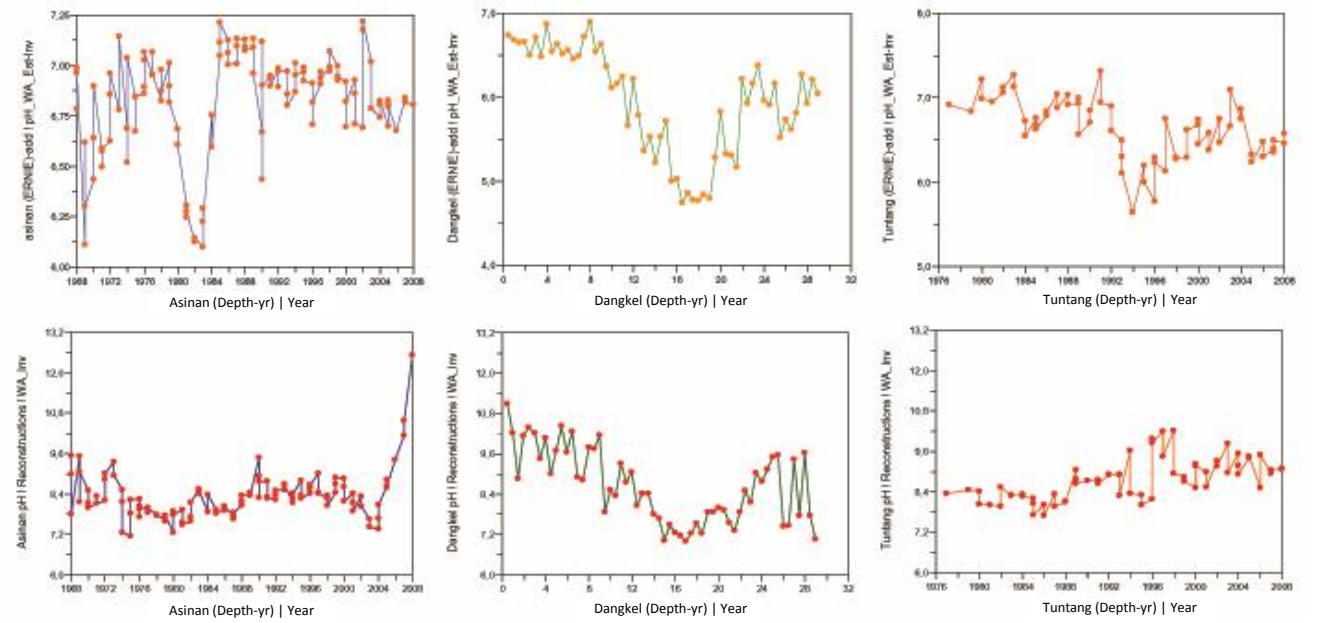

Figure 4. Reconstruction of pH at 3 sites using European data set (above) and Rawapening Lake dataset (below)

The conclusion shows that phosphate, temperature, and calcium were the environmental parameters that influence the diatoms assemblage of Rawapening Lake. Phosphate was contributed 50% on diatoms assemblage. Internal based transfer function of diatoms provides more suitable diatoms data set rather than European diatoms data set. The Rawapening diatom data set of total phosphorous is the initial Indonesian diatom data set for past trophic status, therefore, research has to be continued spatially for other Indonesian lakes and temporary on specific Indonesian lake to develop Indonesia diatoms data set.

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Freshwater fish diversity in an oil palm concession area in Mimika, Papua

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Abstract. *Ohee HL. 2016. Freshwater fish diversity in an oil palm concession area in Mimika, Papua. Biodiversitas 17: 665-672.* New Guinea's freshwater fish diversity may reach 400 species, twice the number of fish recorded in Australia. However, New Guinea's freshwater fishes are facing rapid and poorly-planned social and economic developments, which have accelerated both habitat loss and degradation, impacting its unique biodiversity and threatening natural ecosystems. This study documents freshwater fish diversity and threats due to habitat conservation from oil palm development in the Timika Region, Papua. Fishes were sampled in canals, creeks, streams and rivers in the concession area of Pusaka Agro Lestari Company (PT. PAL) using seine and hand nets and a spear gun. Twenty two freshwater fish species in 15 families and 15 genera were recorded from the area. One of them is an endemic species of Timika (*Glossamia timika*), one rainbowfish species with a restricted Southern New Guinea distribution, and 12 other native fishes. Land clearing leads to increase water turbidity and sedimentation, water temperature, and pollution which are potential threats to native fishes and their habitats. The fact that PAL's concession is part of distribution area of known distribution of *G. timika* in Timika vicinity, habitat conversion to palm oil elevates the threat to this species. Hopefully, PT. PAL will adopt necessary conservation measures to mitigate the potential impact during the land clearing, especially, if they leave riparian buffer regions intact to protect aquatic habitats when clearing land.

Keywords: Freshwater fish diversity, Mimika, oil palm plantation, threats

INTRODUCTION

New Guinea's freshwater fish diversity probably reaches 400 species, twice the number of fishes recorded in Australia (Richards and Suryadi 2002). Many new discoveries in New Guinea (Papua and Papua New Guinea) were made over the last 25 years as Papua's transportation systems were developed, and field research increased (Allen et al. 2008, Allen and Unmack 2008, 2012; Allen and Hadiaty 2011; Kadarusman et al. 2010, 2011, 2012). Papua supports a rich and diverse vertebrate fauna with about 1,240 species known from Papua, but only 250 species (20%) are endemic. In comparison, there are a total of 1,674 vertebrate species found throughout the entire island of New Guinea, about 1,130 (69%) are endemic (Allison 2007). However, the freshwater fishes of New Guinea and Australia are very unique, and are mostly secondary division fishes, having evolved from marine species, whereas other continents' freshwater fishes are primary division fishes (Allen 1991).

As with many parts of Indonesia, fish habitats in Papua are facing rapid habitat degradation and loss as a result of rapid human population growth, large-scale infrastructure development, pollution, domestic waste, and the establishment of new government centers (*kabupaten*). As a result of these threats, some freshwater fishes have been listed in the IUCN Red List with various conservation statuses, and some have become very rare.

The oil palm industry has contributed significantly to Indonesian economic growth, but unfortunately, oil palm also poses potential and direct threats on species and natural habitats, especially in low land areas, where most species richness is found in tropical regions. Lenzen et al. (2008) recorded 30% of global species threats are due to international trade. Consumers in developed countries cause threats on species due to their demands of commodities which are ultimately produced in a certain area in developing countries. It includes oil palm industry from Indonesia which exports its product to some developed countries, and it affects 294 species. Moreover, researchers have shown that, in fact, oil palm plantations harbor far fewer forest-dwelling species of either primary or logged forests (Koh and Wilcove 2012). Field studies have recorded larger scale development activities including logging, mining, plantations, roads and development of new government centers which all have a direct impact on rainbow fish habitat. For instance, 22 plantation companies operated in Papua covering around 540.000 ha and 0.05-21% (100-30.000 ha) with considerable overlap with various rainbow fish distributions (Ohee 2005).

Pusaka Agro Lestari Company (PT. PAL) is an oil palm plantation company operating in Mimika, Papua, with a concession of 35,759 ha. The company plans to establish 5,775 ha of its concession as conservation forest. Five thousand hectares have opened for the plantation and 500 ha of it are set aside to conserve plants and animals. It is very unfortunate this concession area overlaps with the

only known habitat for one endemic species in Timika/Mimika (*Glossamia timika*), thus any company activities will have direct impact on this habitat.

The fact that PT. PAL is willing to set aside some of the concession area for conservation is great news. However, what is needed is greater participation from government and conservation communities to assist the effort for optimal conservation of this important habitat. This study was designed to document fish species diversity in this concession area in relation to freshwater fish diversity in river systems in New Guinea. The goal is to understand the importance of this concession area in the context of Papuan diversity and how PT. PAL can contribute to reduce threats to local species, especially local endemic species which are at greater risk of extinction.

MATERIALS AND METHODS

Field studies were conducted in February and November 2015 in PT. PAL's concession area, Kuala Kencana and Iwaka Districts, Mimika, Papua. Fish samples were collected in 21 sampling sites in four states, PAL 1, PAL 2, PAL 3 and PAL 5 including canals, tributaries, streams, and rivers in the forest and plantation areas (Figure 1, Figure 2).

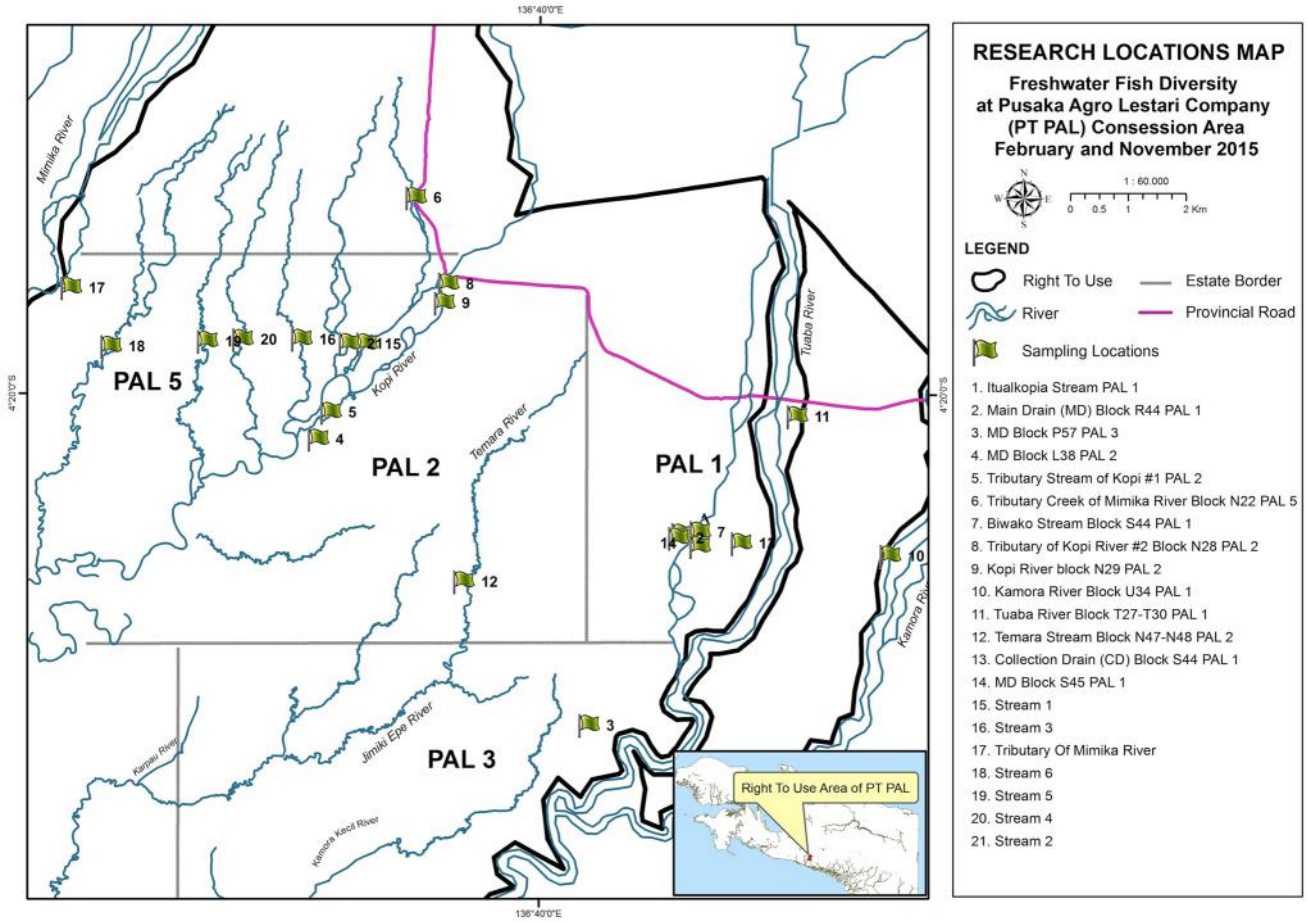

Figure 1. Sampling sites in at least three years old and newly open area plantation area in PT. PAL's concession area, Mimika, Papua

Table 2. Fish collection/observation sites in PT. PAL's concession areas

Location	Description
Site 1	Itualkopia Stream PAL 1; about 3.09 km SW of PT. PAL's Base Camp; S 04° 21' 19.1", E 136° 41' 17.6"; cleared 2 years ago; elevation about 60 m; a clear water stream (3-4 meters wide and 80 cm-2 meters deep); moderately fast flowing over sand and gravel, through open and closed canopy cover; dense wood debris and litter in bottom; 17.02.2015; seine net.
Site 2	Main Drain (MD) Block R44 PAL 1; about 3.5 km SW of PT. PAL's Base Camp; S 40° 21' 20.5", E 136° 41' 16.8"; cleared 2 years ago; elevation about 44 m; a warm and turbid pond over mud (1-1,5 m wide and about 50 cm deep); through open canopy cover; 17. 02.2015; seine net.

Site 3	MD Block P57 PAL 3; about 6.78 km SW of PT. PAL's Base Camp; S 04° 23' 08.5", E 136° 40' 26.1"; cleared 1 year ago; elevation about 28 m; a stagnant turbid water canal over mud (around 1 m wide and 70 cm deep); dense of lichen; through open canopy cover; 17.02.2015; seine net.
Site 4	MD Block L38, PAL 2; about 8.18 km W of PT. PAL's Base Camp; S 04° 20' 23.4", E 136° 37' 56.5"; cleared 1 year ago; elevation about 40 m; a slow clear water canal (about 1-2 m wide and 10 cm-1 m deep) with pools in some points over mud and gravel; dense of wood debris; lichen on gravel; through open canopy cover; 18.02.2015; seine net.
Site 5	Tributary stream of Kopi River #1 PAL 2; about 8.10 km W of PT. PAL's Base Camp; S 04° 20' 15.3', E 136 ° 37' 58.9"; cleared 1 year ago; elevation about 59 m; a clear and slow flowing forest stream (about 2-5 m wide and at least 1 m deep) with more than 1 m pools over sand, gravel, rocks and boulders; wood debris and litter in bottom; through closed canopy cover; seine net, deep net and spear gun.
Site 6	Tributary creek of Mimika River Block N22 PAL 5; about 7.35 km NW of PT. PAL's Base Camp; S 04° 28' 10.5", E 136° 38' 49.9"; newly cleared; elevation about 81 m; a clear slow flowing creek (about 1 m wide and <50 cm deep) over sand, gravel and rocks; water plants and lichen in the creek; through open canopy cover; 19.02.2015; seine net.
Site 7	Biwako Stream Block S44 PAL 1; about 2.67 km SW of PT. PAL's Base Camp; S 04° 21' 11.0", E 136° 41' 29.8"; cleared 2 years ago; elevation about 81 m; a clear and moderately flowing stream (about 1-5 m wide and < 20 cm-4 m deep) with pond around 4 m over silt, sand, gravel and cobble; wood debris and litter in bottom; root of plants along the stream; through closed canopy cover; 19.02.2015; seine net.
Site 8	Tributary of Kopi River #2 Block N28 PAL 2; about 6.3 km E of PT. PAL's Base Camp; S 04° 18' 58.99', E 136° 39"; cleared 1 year ago; elevation about 74 m; a clear and slow flowing tributary (about < 50 cm-3 m wide and < 50 cm-1,5 m deep) with some ponds over sand and gravel; dense of wood debris and litter in bottom; through open and closed canopy cover; 20.02.2015; seine net.
Site 9	Kopi River Block N29 PAL 2; about 6.3 km E of PT. PAL's Base Camp; cleared 1 year ago; elevation about 74 meter; S 04° 18' 58.99", E 136° 39' 06.98"; a clear and moderate fast to very fast flowing river (about 10-50 m wide and less than 1 m deep) over gravel and cobble; through open canopy cover; 20.02.2015; seine net.
Site 10	Kamora River Bloc U34 PAL 1; about 3.47 km arah SE of PT. PAL's Base Camp; S 04° 21' 31.9", E 136° 43'27.4", elevation about 81 meter: a clear and moderate fast to very fast flowing river (at least 100 m wide and 50 cm to at least 2 m deep) with some pools over sand, gravel and cobble; through closed canopy cover; 20.02.2015; seine net.
Site 11	Tuaba River Block T27-T30 PAL 1; about 1.2 km S of PT. PAL's Base Camp; S 04° 20' 33.95, E136° 42' 16.29"; cleared 2 years ago; elevation about 50 m; a clear water river (about 5-15 m wide and less than 1 m deep) over sand, gravel , cobble and boulder; through open canopy cover; 20.02.2015; seine net.
Site 12	Temara Stream Block N47-N48 PAL 2; about 6.5 km E of PT. PAL's Base Camp; S 04° 21' 41.06", E 136° 39' 20.44"; cleared 1 year ago; elevation about 30 m; a moderate fast flowing steam stream (about 1-5 m wide and less than 1 m deep) over sand and gravel; dense pf wood debris; through open and closed canopy cover 21.02.2015; seine net.
Site 13	Collection Drain (CD) Block S44 PAL 1; about 3.15 km SE of PT. PAL's Base Camp; S 04° 21' 20.7", E 136° 41' 16.4"; cleared 2 years ago; elevation about 51 m; a stagnant turbid water canal (about 1-3 m wide and to at least 1,5 m deep) over mud and sand; open canopy cover; 20.02.2015; seine net.
Site 14	MD Block S45 PAL 1; about 3.02 km SW of PT. PAL's Base Camp; S 04° 21' 24.0", E 136° 41' 28.0"; cleared 2 years ago; elevation about 53 m; a clear and slow flowing pond and canal (about 1-3 m wide and 50-100 cm deep) over mud; dense wood debris and lichen on the rocks; through open canopy cover; 23.02.2015; seine net.
Site 15	Stream 1; about 7.6 km W of PT. PAL's Base Camp; S 04°19'31.624"; E 136°38'21.556"; newly cleared; elevation about 54.5 m; a clear water stream (1-8 meters wide and varied deep of water, at least 1 meter); moderate to fast flowing over gravel through open canopy cover; dense wood debris and litter in bottom in some spots; 3.11.2015; seine net.
Site 16	Stream 3; about 8.8 km W of PT. PAL's Base Camp; 4°19'29.658" S; 136°37'44.762"E; newly cleared; elevation about 41.3 m; a clear, slower to moderate flowing water stream over gravel and mud in some spots (1-6 m wide and variety water deep, at least 1 meter, ponds in some spots); through closed canopy cover; dense wood debris and litter in bottom in some spots; 4.11.2015; seine net.
Site 17	Tributary of Mimika River; about 12.8 km W of PT. PAL's Base Camp; 4°19'01.030" S; 136°35'35.497"E; newly cleared; elevation about 46.5 m; a stagnant to slow turbid water tributary over mud and gravel (1-5 m wide and less than 1 meter water deep); through relatively closed canopy cover; wood debris and litter in bottom; seine net.
Site 18	Stream 6; about 12 km W of PT. PAL's Base Camp; 4°19'33.731" S; 136°35'57.849"E; newly cleared; elevation about 46.2 m; a clear water, slow to fast flowing stream (about 1-10 m wide and varied water deep, more than 1 meter) with some deep pools along the stream over gravel; wood debris and litter in some spots; through closed canopy cover; 5.11.2015; seine net, spear gun.
Site 19	Stream 5; about 10.4 km W of PT. PAL's Base Camp; 4°19'30.832" S; 136°36'52.007"E; newly cleared; elevation about 52.3 m; a turbid and slow to moderate flowing forest stream (at least to 6 m wide and variety water deep with pools more than 1 meter deep) over sand, mud, gravel; dense wood debris and litter in bottom; through closed canopy cover; riparian vegetation was clearing along the streams, where some spots till close to the stream edge; 5.1.2015: seine net, spear gun.
Site 20	Stream 4; about 7.9 km W of PT. PAL's Base Camp; 4°19'29.508" S; 136°37'11.627"E; newly cleared; elevation about 41.3 m; a turbid slow to moderate flowing stream (about at least 6 meter wide and 20-30 cm to more than 1 m water deep) over sand and gravel; through closed canopy cover; riparian vegetation was clearing along the stream and only 1-2 meters kept along the stream; 6.11.2015; seine net and spear gun.
Site 21	Stream 2; about 6.3 km W of PT. PAL's Base Camp; S 4°19'32.754" S; 136°38'12.240"E; newly cleared; elevation about 58.1 m; a clear and moderate to fast flowing stream (about to 8 m wide and ± 50 cm to more than 1 m water deep) with some ponds over gravel and white sand in some spots; dense of wood debris and litter in bottom; through closed canopy cover; riparian vegetation was clearing along the stream and only 1-2 meters kept along the stream, some spots still has dense riparian vegetation till 100 meter of the edge of the stream; temperature 24.7°C, pH 7.2; 7.11.2015; seine net.

Fishes were mostly sampled with seine and hand nets, but in few locations, spear fishing was also used. A seine net with 3 m length, 1.23 m height and mesh size less than 1.25 cm was used. The seine net was used in shallow streams with 50-100 cm deep. The net was set up in U-shape by two people and dragged toward certain point, such as under tree roots or across a pond, or the net was put in the certain point location and 2-4 people try to chase fish toward the net. Fishes were identified according to Allen et al. (2000) and Allen (1991) and were preserved in 4% formaldehyde during the survey, and later were transferred to 70% ethanol and were stored in Conservation Department of PT. PAL in Mimika and Biology Department of Cenderawasih University in Jayapura, Papua. Fish diversity is descriptively compared to other freshwater ecosystem in New Guinea and potential threats in the area are discussed.

RESULTS AND DISCUSSION

Fish composition in PT. PAL's concession area

Twenty-two species from 15 families and 15 genera were recorded during the study. At least one species is endemic (*Glossamia timika*) of Timika region, in addition 19 native Papuan species and 2 exotic species were also captured (Table 1). *Glossamia timika* was very rare in the area, only five individuals were recorded from 4 locations: tributary of Mimika River (2 individuals); Bimako Stream (1 individual); tributary of Kopi River#1 (1 individual); and stream 1 at PAL 5 (1 individual). There is no single dominant species in the area. Rainbow fishes and gudgeons were represented by four and three species respectively, while other families were mostly represent by only one species (Table 1). Four unidentified species are common species to Papua, which are two pipefishes, a goby species and a rainbow fish. It needs to further identification to know the species, the distribution area and habitat of the species.

The number of species observed during this study is far less (22 species or 22.4%) than the recorded number of species from this areas (Table 3). Allen et al. (2000) studied this area and recorded 98 freshwater species including 4 endemic species. In addition, Allen et al. (2016) just described a new blue-eye species which is also endemic to this region, bringing the total of 5 endemic species. Thus, it seems likely that the number of species in the PAL concession area should be higher than what was recorded during this single survey. Clearly, the concession area provides important habitat for the survival of at least one of the 5 endemic species of Mimika.

Zoogeographic affinities of fish fauna of PT. PAL's concession area

Fishes in the Mimika region are part of the biogeographic of Southern New Guinea region which has fish fauna quite distinct from the Northern New Guinea region. For example, only *Oxyeleotris fimbriata* (Eleotridae) is broadly distributed on both sides of the Central Dividing Range. Endemic fish of this region is *Glossamia timika* which is known only from Timika vicinity. Three other endemic fishes for this region, a gudgeon (*Oxyeleotris stagnicola)* and two Blue-eyes (*Pseudomugil ivantsoffi* and *P. pelucidus*), were not observed during field studies. Two of five known introduced species in this region which were come from Africa and South Asia are documented in the plantation area (Allen et al. 2000). Thirteen species (59%) recorded in this study are Southern New Guinean species, including two which also occur in northern Australia. Allen et al. (2000) recorded 35 fish species of Southern New Guinea which are also found in Northern Australia, because these areas used to have connections. An endemic and restricted Southern New Guinea species is Ogilby's Rainbowfish (*Melanotaenia ogilbyi*), which is distributed in the Timika region and the Lorentz River (Allen et al. 2000). This species is distributed abundantly in the Mimika area, especially in forest streams in PAL 5.

There are four rainbow fish species recorded in the area: *Melanotaenia* sp., *Melanotaenia ogilbyi*, *M. goldiei*, and *M. rubrostriatus*. Rainbow fishes (Melanotaeniidae family) are a secondary fish family-meaning that their ancestor comes from marine water-(Allen 1991) which are endemic to New Guinea and Australia. Due to the large number of species, rainbow fishes make a major contribution to the unique Papuan freshwater fish diversity and is an important key used to identify important areas for conservation (Polhemus et al. 2004). *Melanotaenia goldiei* and *M. rubrostriatus* have a wide distribution in Southern New Guinea, while *M. ogilbyi* is restricted to the Timika region and Lorentz River (Allen et al. 2000).

In comparison to some river systems in New Guinea, PT. PAL concession area has only one endemic species and fewer species overall (Table 4). However, this area is important for an endemic species *Glossamia timika*, a new species described in 2000 (Allen et al. 2000), which is only known in the vicinity of Timika The Kopi and Mimika River systems, and other rivers closed to the systems in PT. PAL concession area are part of the range of *G. timika*. In addition, the area is also a home for the endemic Southern New Guinea Ogilby's Rainbowfish (*Melanotaenia ogilbyi*). It is abundantly distributed in most of streams and creeks, both in planted and forest's area of PT. PAL concession area. As well as, the region is home for *Pseudomugil luminatus*, a new described blue-eye species (Allen et al. 2016).

Threats and conservation

Oil palm plantations cause habitat degradation and species loss. Habitat degradation is the primary cause of extinction and endangerement globally. Deforestation due to oil palm expansions threatens to drive more species to extinction that did prior episodes of deforestation in countries such as the United States and United Kingdom (Koh and Wilcove 2012). Rubber, coffee, cocoa and palm oil have been affecting 294 species in Indonesia including *Panthera tigris*, the Sumatran serow, *Capricornis sumateraensis*, and Sir David's long beaked echidna, *Zaglossus attenboroughi* (Lenzen et al. 2008). In freshwater ecosystems, agriculture and land clearing have

Table 1. The fish fauna of PT. PAL's concession area, Mimika, Papua

Family/Common name/Species	Locations																				
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
Endemic species																					
Apogonidae																					
Timika Mouth Almighty																					
Glossamia timika					R	R	R								R						
Native species																					
Ambassidae																					
Sailfin Glassfish Perchlet																					
Ambassis agrammus	O	O	O			O						O	O		R						
Ariidae																					
Salmon Catfish																					
Neoarius leptaspis					R													R			
Atherinidae																					
Kubuna Hardyhead																					
Craterocephalus randi	O			O	O	O		O		O					F	O				R	R
Eleotridae																					
Banded Mogurnda																					
Mogurnda cingulata						R	R		R		R			x							
Fimbriate Gudgeon																					
Oxyeleotris fimbriata					R						R										
Giant Gudgeon																					
O. selheimi										R											
Gobiidae																					
Concave Goby																					
Glossogobius concavifrons					O									R	R	R					
Unidentified Goby species						R															
Hemiramphidae																					
Fly River Garfish																					
Zenarchopterus novaeguineae					O										O	R			R	O	
Melanotaenidae																					
Melanotaenia sp.	O	O		O	O	O	O	O							R						
Goldie River Rainbowfish																					
Melanotaenia goldiei														O							
Ogilbyi's Rainbowfish																					
Melanotaenia ogilbyi	O			O	O	O	O			O				O	D	D	D	D	D	D	D
Red-Striped Rainbowfish																					
Melanotaenia splendida rubrostriata						O		O					O	O			R				
Mugilidae																					
Greenback Mullet																					
Chelon subviridis									O												
Plotosidae																					
Papuan eel Catfish																					
Plotosus papuensis									R												
Soleidae																					
Tailed Sole																					
Leptachirus klunzingeri																R					
Syngnathidae																					
Pipefish 1							R														
Pipefish 2							R														
Terapontidae																					
Mountain Grunter																					
Hephaestus habbemai					O	O	O			O		O			O		O	O			O
Introduced species																					
Cichlidae																					
Mozambique Tilapia																					
Oreochromis mossambicus						O				O			O								
Channidae																					
Striped Snakehead																					
Channa striata						R							R								

Note: A = Abundant: very common in area; D = Dominant: the most common species in the area; F = Frequent : many per site, even more than 100; O = Occasionally: 5-30 per site; R = Rare: 3 or fewer per site.

Table 3. Fish community in Timika region, Papua, Indonesia

Family	Common Name	Species	Allen et al. (2000)	This study
Endemic and native species				
Ambassidae-Glassfishes	Sailfin Glassfish Perchlet	*Ambassis agrammus*	x	x
	Giant Glassfish	*Parambassis gulliveri*	x	
Anguilidae-Freshwater Eels	Indonesian Shortfin Eel	*Anguilla bicolor*	x	
	Speckled Longfin Eel	*A. reinhardtii*	x	
Apogonidae-Mouth Almighties	Mouth Almighty	*Glossamia aprion*	x	
	Sande's Mouth Almighty	*G. sandei*	x	
	Timika Mouth Almighty	***G. timika****	x	x
Ariidae-Fork Tailed Catfishes	Daniel's Catfish	*Cochlefelis danielsi*	x	
	Blue Salmon Catfish	*Neoarius graeffei*	x	
	Broad-Snouted Catfish	*N. latirostris*	x	
	Salmon Catfish	*N. leptaspis*	x	x
	Sharp-nosed Catfish	*Potamosilurus macrorhynchus*	x	
	Duckbilled Catfish	*Cochlefelis spatula*	x	
	Taylor's Catfish	*Cathorops taylori*	x	
	Giant Catfish	*Arius* sp.	x	
	Comb-Spined Catfish	*Cinetodus carinatus*	x	
	Thick-Lipped Catfish	*C. crassilabris*	x	
	Smallmouthed Salmon Catfish	*C. froggatti*	x	
	Spoon-Snoted Catfish	*Nedystoma novaeguineae*	x	
	Day's Catfish	*N. dayi*	x	
	Lorentz Catfish	*Cinetodus conorhynchus*	x	
Atherinidae-Hardyheads	Mountain Hardyhead	*Craterocephalus nouhuysi*	x	
	Kubuna Hardyhead	*C. randi*	x	x
Belonidae-Longtoms	Long tom	*Strongylura krefftii*	x	
Centropomidae-Giant Perches	Baramundi	*Lates calcarifer*	x	
Clupeidae-Herrings	Papuan river sprat	*Clupeoides papuensis*	x	
	West Irian River Sprat	*C. venulosus*	x	
	Yamur Bony Bream	*Nematalosa* sp.	x	
Cynoglossidae-Tongue Soles	Freshwater Tongue Sole	*Cynoglossus heterolepis*	x	
Engraulididae-Achovies	New Guinea Thryssa	*Thryssa scratchleyi*	x	
Eleotridae-Gudgeons	Striped-Cheek Gudgeon	*Bostrychus strigogenys*	x	
	Barred Gudgeon	*B. zonatus*	x	
	Greenback Gudgeon	*Bunaka gyrinoides*	x	
	Olive Flathead-Gudgeon	*Butis amboinensis*	x	
	Duckbill Sleeper	*B. butis*	x	
	Snakehead Gudgeon	*Giuris margaritacea*	x	
	Empire Gudgeon	*Hypseleotris compressa*	x	
	Banded Mogurnda	*Mogurnda cingulata*	x	x
	Aru Gudgeon	*Oxyeleotris aruensis*	x	
	Fimbriate Gudgeon	*O. fimbriata*	x	x
	Poreless Gudgeon	*O. nullipora*	x	
	Fewpored Gudgeon	*O. paucipora*	x	
	Giant Gauvina	*O. selheimi*	x	x
	Swamp Gudgeon	***O. stagnicola****	x	
	Paniai Gudgeon	*O. wisselensis*	x	
Gobiidae-Gobies	Golden tank Goby	*Glossogobius aureus*	x	
	Concave Goby	*G. concavifrons*	x	x
	Tank Goby	*G. giuris*	x	
	Munro's Goby	*Glossogobius* sp. 1	x	
	Dwarf Goby	*Glossogobius* sp. 2	x	
	False Celebes Goby	*Glossogobius* sp. 3	x	
	Barred Mudskipper	*Periophthalmus argentilineatus*	x	
	New Guinea Mudskipper	*P. novaeguineaensis*	x	
	Spotfin Goby	*Redigobius chrysosoma*	x	
	Marbled Goby	*Schismatogobius marmoratus*	x	
	Barcheek Goby	*Stenogobius psilosinionus*	x	
	unidentified goby species			x
Hemiramphidae-Garfishes	Long-Jawed River Garfish	*Zenarchopterus caudovittatus*	x	
	Fly River Garfish	*Z. novaeguineae*	x	x
Kurtidae-Nurseryfishes	Nurseryfish	*Kurtus gulliveri*	x	
Megalopidae-Tarpons	Indo-Pacific Tarpon	*Megalops cyprinoides*	x	
Melanotaeniidae-Rainbowfishes	Threadfin Rainbowfish	*Iriatherina werneri*	x	

	Goldie River Rainbowfish	*Melanotaenia goldiei*	x	x
	Ogilby's Rainbowfish	*M. ogilbyi*	x	x
	Red-Striped Rainbowfish	*M. splendida rubrostriata*	x	x
	-	*Melanotaenia* sp.		x
Mugilidae-Mullets	Greenback Mullet	*Chelon subviridis*	x	x
Osteoglossidae-Bony Tongues	Australian Bonytongue	*Scleropages jardini*	x	
Plotosidae-Eel-Tailed Catfishes	Narrowfront Tandan	*Neosilurus ater*	x	
	Shortfin Tandan	*N. brevidorsalis*	x	
	Southern Tandan	*N. equinus*	x	
	Maria's Tandan	*Oloplotosus mariae*	x	
	Papuan Eel Catfish	*Plotosus papuensis*	x	x
	Merauke Tandan	*Porochilus meraukensis*	x	
	Obbe's tcatfish	*P. obbesi*	x	
Pseudomugilidae-Blue-eyes	Inconspicuous Blue-eye	*Pseudomugil inconspicuus*	x	
	Ivantsoff's Blue-eye	***P. ivantsoffi****	x	
	New Guinea Blue-eye	*P. novaeguineae*	x	
	Swamp Blue-eye	*P. paludicola*	x	
	Red Neon Blue-eye	***P. luminatus****	x	
	Transparent Blue-eye	***P. pellucidus****	x	
Soleidae-Soles	Tailed Sole	*Leptachirus klunzingeri*	x	x
	Velvety Sole	*Brachirus villosus*	x	
Sparidae-Breams	Goldsilk sea bream	*Acanthopagrus berda*	x	
Sciaenidae-Croakers	Scale Croaker	*Nibea squamosa*	x	
Synbranchidae-Swamp Eels	Bengal Eel	*Ophisternon bengalense*	x	
Syngnathidae-Pipefishes	Belly Pipefish	*Hippichthys heptagonus*	x	
	Short-Tailed Pipefish	*Microphis brachyurus*	x	
	Barhead Pipefish	*M. leiaspis*	x	
	unidentified pipefish 1	-		x
	unidentified pipefish 2	-		x
Terapontidae-Grunters	Mountain Grunter	*Hephaestus habbemai*	x	x
	Röemer's Grunter	*H. roemeri*	x	
	Lorentz's Grunter	*Pingalla lorentzi*	x	
	Jamur Lake Grunter	*Variichthys jamoerensis*	x	
Toxotidae-Archerfishes	Spotted Archerfish	*Toxotes chatareus*	x	
Introduced species				
Clariidae-Air Breathing Catfishes	Philippine Catfish	*Clarias batrachus*	x	
Aplocheilidae-Killifishes	Blue Panchax	*Aplocheilus panchax*	x	
Cichlidae-Cichlids	Mozambique Tilapia	*Oreochromis mossambicus*	x	x
Anabantidae-Climbing Gouramies	Climbing Perch	*Anabas testudineus*	x	
Channidae-Snakeheads	Striped Snakehead	*Channa striata*	x	x

Note: * endemic species

Table 4. Comparison of fish faunas of various river systems in New Guinea

River system	Total species (excluding introductions)	Endemic species	Percent endemics
Fly, PNG*	103	5	4.8
Kikori, PNG*	100	14	14.0
Aikwa/Iwaka, Papua*	75	2	2.7
Lorentz, Papua*	60	2	3.3
Purari, PNG*	57	6	10.5
Sepik, PNG*	53	3	5.7
Ramu, PNG*	50	2	4.0
Wapoga, Papua*	46	3	6.5
Digul, Papua*	40	0	-
Mamberamo, Papua*	28	6	21.4
Gogol, PNG*	25	0	-
Lakekamu, PNG*	22	1	4.5
PT. PAL Concession Area, Mimika (this study)	**20**	**1**	**5**

Note: *) Allen et al. (2002)

increased sedimentation in wetlands and streams, which in turn leads to an overall loss of these aquatic habitats (Groom et al. 2006). Plantation Service of Papua Province reported 24 large plantations companies in 8 regencies in Papua Province in 2015 with total 477,462.10 ha. Frazier (2007) also documented data of plantations service of Papua and a researcher which showed large size of land reserved for oil palm plantations in Papua, which is 6,115,443 ha in 1999 and 2.8 million in 2003 in only 8 regencies of Papua. There is still possibility to extend oil palm plantation in Papua and to continually increase potential threats to habitats and species. As a result, species extinction especially in freshwater habitats will be difficult to prevent in plantation areas without strong conservation actions. Loss of riparian vegetation along streams and tributaries, the introduction of exotic species and the use of poisons are main threats in PT. PAL's concession area. During the survey in a newly opened area of the company concession, we recorded extensive land clearing along streams, which increases water turbidity and sedimentation,

changes stream discharge and increases water temperature. An additional threat comes from fertilizer and pesticides use in the plantation where it can flow into freshwater systems. This, in turn, will increase nutrients and lead to eutrophication with subsequent reductions of dissolved oxygen in the water. Forest conversion is the main threat not only for aquatic inhabitants, but to all species, as it has been suggested that 80-100% of mammal, reptile and bird species are lost when palm plantations are established on primary forest (Frazier 2007). Deforestation for oil palm resulted in erosion and increased water turbidity in Arang-arang Lake in Muaro Jambi, Sumatera (Asra 2009). Water turbidity decreases light penetration into the lake, which reduces photosynthesis and, thus, phytoplankton growth. Phytoplankton is the primer producer in the lake and very important for aquatic biota such as benthic invertebrates and fishes. Fertilizers and pesticides used in the plantation resulted in water pollution of the lake (Asra 2009). Koh and Wilcove (2012) stated that a prohibition on the conversion of primary or secondary forests to oil palm is urgently needed to take care of tropical biodiversity. Until that happens, oil palm might well be the single most immediate threat to almost all number of species.

The best conservation effort to protect endemic and native fishes is natural habitat protection. Therefore, threats to endemic and native fishes by deforestation and plantation should be minimized to decrease negative impacts. It is recommended that over clearing riparian vegetation, excessive fertilizer use, and fishing with poison should be better managed to conserve diversity of freshwater fishes. For instance, most of the impact from plantations would be eliminated if riparian vegetation buffer zones along any water body were at least 200 m wide from the water body edge. In addition, plantations should limit fertilizer use or use more environmental friendly fertilizers as well as developing waste management systems to control fertilizer loss from of the plantation to prevent them entering water courses. If these conservation efforts were enacted, then plantations would be able to support good habitat for the fishes, which needs clear, clean and fresh water environment.

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The abundance of phytoplankton and its relationship to the N/P ratio in Jakarta Bay, Indonesia

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Abstract. *Sidabutar T, Bengen DG, Wouthuyzen S, Partono T. 2016. The abundance of phytoplankton and its relationship to the N/P ratio in Jakarta Bay, Indonesia. Biodiversitas 17: 673-678.* The occurrence of phytoplankton blooms in Jakarta Bay has increased significantly, and resulted in, the mass mortality of fish and other organisms. Phytoplankton bloom events are indicated by a change in the color of the sea's surface. Generally, phytoplankton growth is influenced by the levels of nutrients in the water, while spatial distribution is influenced by the pattern of the current. In connection with this phenomenon, research was conducted in 2010, 2011 and 2013, to determine the abundance and distribution of phytoplankton and their connection with the N/P ratio. The results showed that the abundance of phytoplankton ranged from $40x10^6$ cells/m^3 up to $1699.1x10^6$ cells/m^3, with the highest recorded data was during the east monsoon in 2010 and the lowest during the first transition period of 2011. The predominant phytoplanktons were frequently diatoms such as *Skeletonema*, *Chaetoceros* and *Thalassiosira*. The distribution of phytoplankton seemingly follows the nutrient concentration ratio where phosphate acted as the limiting factor and nitrogen as the triggering factor. The higher the N/P ratio, the more potentially uncontrolled growth of phytoplankton occurred. When the availability of nutrients increased an increase in total algal biomass occurred, however, the alteration in nutrient composition led to a change in composition of community.

Keywords: Abundance, limiting factor, nutrient ratio, phytoplankton, triggering factor

INTRODUCTION

The abundance of phytoplankton has a close relationship with the availability of nutrients such as nitrates, phosphates and silicates, and these parameters can be used as a benchmark for the productivity of the waters (Weyl 1970; Odum 1971). The continuous discharge of organic material into the waters causes nutrient enrichment, namely eutrophication (Nixon et al. 2008; Nixon 2009). Nutrients such as phosphate, nitrate and silicate play an important role in supporting the life of phytoplankton. However, in excess, they can result in excessive growth of phytoplankton population, or a bloom. The surface water discoloration due to dense phytoplankton cells is known as a red tide or algal bloom, and sometimes, is called an exceptional bloom or noxious bloom and has lately been referred to as a harmful algal bloom (Hallegraeff and Fraga 1998; Reynolds 2006; Heisler et al. 2008).

A phytoplankton bloom may lead to mass mortalities of fish as recently happened in Jakarta Bay. The nature of the relationship between eutrophication and the expansion of algal blooms is still unclear, although in general eutrophication leads to explosions in the phytoplankton population. Increasing phytoplankton blooms coincide with nutrient enrichment, in the South China Sea and Hong Kong (Lam and Ho 1989; Qiu et al. 2010). How eutrophication can stimulate the presence of toxic algal bloom species, is not yet completely understood and is still under debate. Discharge of any organic material to the waters can lead to an overall increase in nutrient availability, and changes in nutrient composition or nutrient ratio. In general, nutrient availability is associated with increased biomass, and can lead to changes in the community. Phytoplankton populations often increase in quantity but decline in quality. The abundance of phytoplankton cells increases significantly, but the number of species does not increase, indicating there is a predominance of certain species.

This observation had been carried out in relation to the increased amount of nutrients, especially nitrate and phosphate, in the waters of Jakarta Bay and is discussed in this paper.

MATERIALS AND METHODS

Study area

This research was conducted in the waters of Jakarta Bay, Indonesia. It is a shallow bay with an average depth of 15 m, its shoreline is about 149 km long and covers an area of approximately 595 km^2. The bay is located in the north of Jakarta, the capital of Indonesia. Thirteen rivers around the bay are known to discharge a large amount of anthropogenic material from land-based sources into it, including industrial effluent, sewage, and agricultural discharges. A map of the bay and sampling stations is shown in Figure 1.

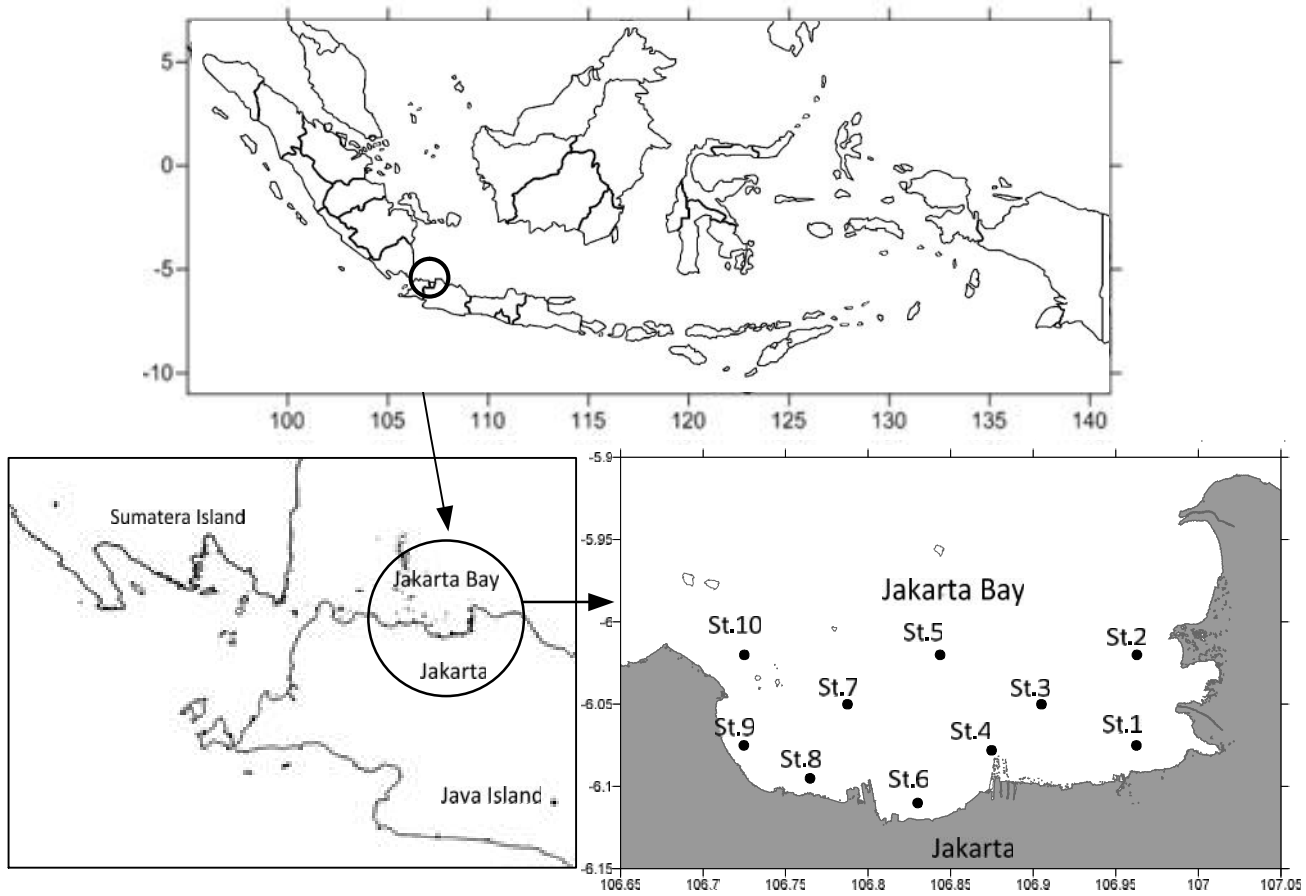

Figure 1. Map of Indonesia and Jakarta Bay with the sampling stations

Sampling period

This study was conducted in 2010, 2011 and 2013 and each period included the first inter-monsoon (MP1) and the east monsoon (MT) when algal blooms mostly appeared. Jakarta Bay is controlled by the annual monsoon pattern where the highest total precipitation falls during the northwest monsoon, and drier conditions occur during the southeast monsoon. The transitional or inter-monsoon generally runs from March to May while the east monsoon is from June to August. Jakarta Bay receives a lot of organic material through the rivers during the rainy seasons and the first inter-monsoon.

Sampling techniques

Sample collection was done using plankton net with mesh size of 20 um, one meter in length and 25 cm diameter of mouth. It was also weighted at the aperture of the net so it could be lowered vertically to a depth of the waters. Samples were then collected in small bottles and put in 2% formaldehide preservative. The volume of filtered water was calculated using formula $v = \pi r^2 \times d$; where v: volume of filtered water; r: radiant of net mouth and d: depth of plankton net lowered into the water. Identification and counting of phytoplankton was performed using Sedgwick-Rafter counting chambers (Sournia 1978) and the results expressed in cells/m^3 (Omori 1991; Michael 1995) through a light microscope at 400x and 1000x magnification. The identification and enumeration of phytoplankton was carried out using references such as Yamaji (1966), Taylor (1976), Newell and Newell (1977), Thomas (1993), Davis (1995), and Hallegraeff (1995). Phytoplankton abundance is calculated by the following formula (Sournia 1978):

$$N = n \times \frac{Vt}{Vs} \times \frac{1}{V}$$

Where,
N : the amount of all phytoplankton
V : volume of filtered water
Vt : the initial sample volume
Vs : sub-sample volume (fraction)
n : number of phytoplankton in sub-sample

Water samples for nutrient analysis were collected from an average depth of 1.0 m using a Kemmerer sampler. Each sample was immediately decanted into an acid-washed bottle and was acidified with 1% v/v HNO3. Nutrients (phosphate, nitrate) were determined based on the colorimetric method (Strickland and Parson 1972; Parson et al. 1984) using a spectrophotometer with wavelengths for phosphate (690 nm) and nitrate (543 nm).

RESULTS AND DISCUSSION

Composition of population

The composition of the phytoplankton populations collected from these waters is presented in Table 1. The amount of phytoplankton recorded was around 35 genera that included approximately 12-25 genera of diatoms and 9-11 genera of dinoflagellates. It seemed that the genera of diatoms and dinoflagellates in these waters were higher in the inter-monsoon (MP1) than in the east monsoon (MT). Generally, the abundance and number of diatom genera in this bay is higher than dinoflagellates (Sidabutar 2006, 2008). During the study, the highest composition of phytoplankton was recorded in the inter-monsoon in the year 2011. About 34 genera were found including 25 diatoms and 9 dinoflagellates. The composition of the species that make up the plankton community can illustrate the diversity of the population or the number of species in a community. This kind of diversity can be increased if the community is more stable or diminish when the environment is unstable or impaired (Krebs 1972; Michael 1995).

Predominant phytoplankton

The predominant phytoplankton in these waters during the study included *Skeletonema*, *Chaetoceros* and *Thalassiosira*. They are almost always found and commonly appear in high numbers in these waters. Therefore, they are classified as a common species. The relative abundance of phytoplankton in these waters during the study is shown in Table 2. The relative abundance of *Skeletonema* ranges between 30-87%. The relative abundance of *Chaetoceros* ranges between 14-57%, being the next most abundant after *Skeletonema*. The *Chaetoceros* was found to be higher in the east monsoon (MT) while *Skeletonema* was found to be higher in inter-monsoon (MP1). The *Skeletonema* predominate in the inter-monsoon (MP1) during high rainfall, while *Chaetoceros* predominate in the east monsoon (MT), when the rainfall is relatively low. However, both of them often found simultaneously as the predominant phytoplankton. It seems that their occurrence is influenced by the season. Therefore those two species seem seasonally dependent, with *Skeletonema* predominant during rainy season and *Chaetoceros* predominant in the dry season.

The predominant phytoplanktons are the members of the population with an abundance of more than 10% and therefore these species are thought to play an important role in the life of the waters. In addition they can usually be used as a biological indicator for the waters (Day et al. 1989). The change in water quality due to climate change and anthropogenic material input into these waters could affect the abundance of planktonic organisms (Hadikusumah 2008)

Other kinds of phytoplankton that sometimes predominant in these waters include *Thalassiosira* with a relative abundance range between 18-26%. The predominance of *Thalassiosira* is seen in the east monsoon (MT) while in the inter-monsoon (MP1) it tends to be lower than 10%. Nevertheless, these three genera of phytoplankton; *Skeletonema*, *Chaetoceros* and *Thalassiosira* play an important role in Jakarta Bay.

Phytoplankton abundance

The results of this study noted that the abundance of phytoplankton in Jakarta Bay during first inter-monsoon (MP1) and east monsoon ranging from 40.90×10^6 up to 1699.10×10^6 cells/m^3. The highest abundance of phytoplankton was recorded in the east monsoon 2010 (MT_2010) and the lowest was in the first inter-monsoon 2011 (MP1_2011). An abundance graph of the phytoplankton during the study in Jakarta Bay is shown in Figure 2. It appears that the abundance of phytoplankton tends to be higher in the east monsoon compared to the first inter-monsoon. The abundance of phytoplankton in the east monsoon period in 2010 was very high and there was a phenomenon categorized as a bloom that caused greenish-brown discoloration in the surface water. The predominant phytoplanktons during this time were *Skeletonema* (30%), *Chaetoceros* (24%), *Thalassiosira* (18%) and others (28%). The resulting color apparently comes from the combination pigments from these three predominant species.

Generally, algal bloom events in Jakarta Bay appear in the first inter-monsoon (MP1) such as in April and May and also in the second inter-monsoon (MP2) such as in September, October and November (Sediadi 2011), while they rarely occur at other times. According to Wouthuyzen et al. (2007), during the period 2004-2007, there were seven cases of fish mass mortality in Jakarta Bay due to algal bloom events. In 2004, there were two cases (May and December), while in 2005, three cases were recorded (April, June and October) and in 2007, two cases were recorded (April and November). In 2006 and 2008, there were no cases of mass fish mortality although algal blooms occurred.

The relation of N/P ratio to algal bloom.

The most important elements for phytoplankton growth are nitrogen and phosphorus, and, particularly for diatoms, silicate (Egge and Askne 1992). Nutrient enrichment in the waters may cause eutrophication where the nutrient nitrogen serves as the primary trigger for the occurrence of blooming (Anderson et al. 2002). Nitrate concentrations in estuarine or coastal waters are generally higher than the level of phosphate, because a lot of input is from organic material discharged through the rivers and run-off from the mainland. The opposite may occur where phosphate levels are even higher than the levels of nitrates in the waters. The consequences of high nutrient availability will increase the total algal biomass, and the change of nutrient composition or N/P ratio can lead to changes in the community composition (species composition) (Gilbert et al. 2005, 2008). Jakarta Bay is unique as the estuaries of 13 rivers bring fresh water and other input materials there, and also the condition of the bay is much influenced by the open sea. Therefore, it is estimated that the N/P ratio is very volatile and dependent on season, whether it is the rainy or dry season. Comparisons of the nitrate and phosphate concentrations in these waters at the time of the study are presented in Figure 3. In general, the increase of

Table 1. The composition of phytoplankton genera in each sampling period

Genera	Sampling period					
	MP1_ 2010	MT_ 2010	MP1_ 2011	MT_ 2011	MP1_ 2013	MT_ 2013
Diatomae						
Amphora	+	+	+			
Asteromphalus			+			
Asterionella			+	+	+	
Bacteriastrum	+	+	+	+	++	+
Coscinodiscus	+	+	+	+	+	+
Chaetoceros	+	++	++	++	+	++
Climacodium			+			
Dytilum	+		+		+	
Eucampia	+		+	+	+	
Guinardia	+		+	+	+	+
Hemiaulus	+	+	+	+	+	
Lauderia	+	+	+	+	+	+
Leptocylindrus	+	+	+	+	+	+
Nitzschia	+	+	+	+	+	+
Navicula			+	+		+
Odontela	+		+		+	+
Pleurosigma	+		+	+	+	
Rhizosolenia	+	+	+	+	+	+
Surirella			+			
Skeletonema	++	++	++	+	++	++
Streptotheca	+		+	+	+	+
Stephanopyxis			+			
Thalassiosira	+	+	+	++	+	+
Thalassiothrix	+	+	+	+	+	+
Dinoflagellate						
Alexandrium	+	+			+	+
Ceratium	+	+	+	+	+	+
Diplopsalis	+	+			+	
Dinophysis	+	+	+	+	+	+
Dictyocha			+	+		
Gonyaulax	+	+	+	+	+	+
Gymnodinium	+	+	+	+	+	+
Noctiluca	+	+	+	+	+	
Prorocentrum	+	+	+	+	+	+
Protoperidinium	+	+	+	+	+	+
Pyrophacus	+	+		+	+	+
Scriepsiella	+	+	+		+	+
Diatomae	19	12	24	17	18	14
Dinoflagellate	11	11	9	9	11	9
Total genera	30	23	33	26	29	23

Note: ++ predominant; MP: transition period; T: east monsoon; 2010, 2011,2013: years

Table 3. The concentration of phosphate, nitrate and N/P ratio

Period	Ortho fosfat (ugA/L)	Nitrate (ugA/L)	Ratio N/P	Status
MP1_2010	0.056	6.134	110.2	P-limiting
MT_2010	0.056	6.134	110.2	P-limiting
MP1_2011	0.68	0.268	0.4	N-limiting
MT_2011	3.135	5.956	1.9	N-limiting
MP1_2013	0.272	5.034	18.5	P-limiting
MT_2013	1.372	15.893	11.6	P-limiting

Note: MP: inter-monsoon, MT: east monsoon

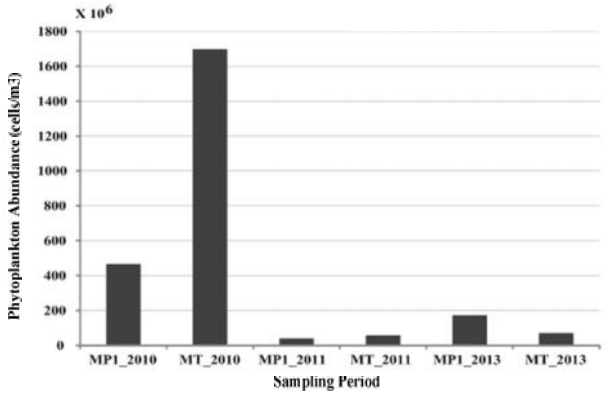

Figure 2. The abundance of phytoplankton in each sampling period

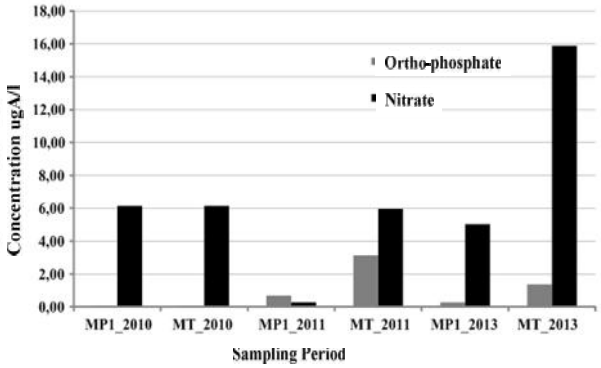

Figure 3. The concentration of nitrate and orthophosphate

Table 2. The relative abundance of predominant genera

Genera	Relative abundance (%)					
	MP1_2010	MT_2010	MP1_2011	MT_2011	MP1_2013	MT_2013
Skeletonema	87	30	43	0	79	67
Chaetoceros	0	24	39	57	14	14
Thalassiosira	0	18	0	26	0	0
Others	13	28	18	17	7	19
Total (10^6 cells/m^3)	465.90	1699.10	40.90	57.90	172.70	70.00

Note**:** 0: percentage <10 %, MP1_2011: first inter-monsoon 2011, MT_2010: east monsoon 2010.

phytoplankton abundance is in accordance with the increase of nutrient composition or N/P ratio. The ratio of nitrate to phosphate during this study was relatively high only in 2010 and 2013, while in 2011 it was relatively low. When the N/P ratio is relatively high, it indicates that nitrate is as a triggering factor and phosphate is as a limiting factor, but in 2011, while the N/P ratio was relatively low, it indicates that phosphate is as a triggering factor and nitrate is as a limiting factor (Table 3).

There is a link between high levels of nitrate or N/P ratio with the abundance of phytoplankton. An N/P ratio above 10, as seen in MP1-2010, MT-2010, MP1-2013 and MT-2013, indicates phosphate is the limiting factor. Phosphate and nitrate are nutritional components that play important role in supporting the life of aquatic organisms. However, if the amount is excessive, it will deviate from providing the normal benefits as a nutrient and an explosion of phytoplankton species may occur. When the ratio of N/P is greater than 10, this indicates phosphate as a factor limiting phytoplankton growth, which means that the shortage of phosphates in the water prevents the further growth of microalgae. Similarly, a ratio of less than 10 indicates nitrogen is the growth limiter. Ortho-phosphates can be a limiting factor for the process of photosynthesis. According to Qiu et al. (2010) eutrophication is one of the main factors that led to the deterioration of the aquatic environment in the estuary. Therefore, understanding the role of nutrients, especially nitrogen and phosphorus as a limiting factor of phytoplankton, is an important aspect for reducing and regulating eutrophication (Paerl 2009). Sometimes, the atomic ratio of dissolved nutrients in the water is very different from those required for the growth of phytoplankton. In normal sea waters, the N/P ratio is 15: 1. The N/P ratio increase will potentially cause an algal bloom where there is uncontrolled growth of phytoplankton.

The spatial distribution of phytoplankton abundance tends to follow the spatial distribution pattern of nutrient ratios. The pattern of algal bloom distribution can be predicted by the pattern of nutrient distribution in a particular location. Nutrient and phytoplankton distribution is determined by the currents, but the growth of phytoplankton cells is highly dependent on the ratio of N/P and solar energy. The results showed that an N/P ratio more than 10 indicates the availability of nitrate to trigger the growth of phytoplankton, rather than the availability of phosphate as limiting factor. The ratio of N/P in estuaries or coastal waters can indicate the patterns of phytoplankton growth as follows: when the N/P ratio ≤ 5, it means that N is limiting factor while if the N/P ratio is between 5-10, it is referred to as intermediate, and when the N/P ratio ≥ 10, phosphate is the limiting factor for growth of phytoplankton (Duarte 2009). At a time when the N/P ratio is high (more than 10), the abundance of phytoplankton tends to be high, whereas when the ratio of N/P is low (less than 5) the abundance of phytoplankton tends to be low.

Phytoplankton, such as *Skeletonem*a, *Chaetoceros* and *Thalassiosira*, plays an important role in Jakarta Bay. They are not only known as common species but also as seasonally dependent species. The explosion of phytoplankton populations in the Jakarta Bay is closely related to the ratio of nitrogen and phosphate in the waters. A bloom will occur at least when the N/P ratio is at higher than 10, indicating that nitrate is acted as a trigger and phosphate is as a limiting factor. Besides that, current patterns might play a role in the distribution and accumulation of phytoplankton cells that lead to the appearance of discoloration in surface waters. In future, it is interesting to study the potentially harmful species and toxigenic phytoplankton in this bay, especially which was designated as the main cause of blooms.

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Biological characteristics on three demersal fish landed in Tegal, north coast of Central Java, Indonesia

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Abstract. *Nugroho D, Patria MP, Supriatna J, Andrianto L. 2016. Biological characteristics on three demersal fish landed in Tegal, north coast of Central Java, Indonesia. Biodiversitas 17: 679-686.* Java Sea has a potential marine biodiversity that has been harvested since years. Demersal fish resources is one of the targeted species by Danish seine fisheries operated in North coast of Java. To support on developing conservation and management measures, an observation on species composition, length frequencies and maturity stages were carried out during August 2014 to July 2015. Sampling took place in landing place of Tegalsari fishing port central Java. A total 129 fish species identified, among them 91 species were targeted as edible fish. Sampling on three dominant species i.e., Purple-spotted bigeye *Priacanthus tayenus* (Richardson, 1846), Lattice monocle bream *Scolopsis taenioptera* (Cuvier, 1830) and goatfish *Upeneus sulphureus* (Cuvier, 1829) were measured regarding length, weight and their maturity stages. The results shows that length-weight relationship for each species were *P. tayenus* $W = 0.0324\ L^{2.7321}$, *S. taenioptera* $W = 0.0366\ L^{2.7262}$ and *U. sulphureus* $W = 0.038\ L^{2.7312}$. Monthly average GSI of *P. tayenus* and *S. taenioptera* indicated that the highest index occurred during SE monsoon while *U. sulphureus* on NW monsoon. Maturity stage analysis indicated that estimated of length at first maturity (L_m) were at 12.9 cmFL (*U. sulphureus*), 16.8 cmFL (*S. taenioptera*) and 19.4 cmFL (*P. tayenus*). Size frequency distribution shows that most of the fish were caught at immature cohorts. The diversity or evenness indices of ichtyofauna is also described as descriptors of community structure and be complemented with information on biological characteristics of those dominant species.

Keywords: Demersal fish, dominant species, biological characteristics, north coast, Java

INTRODUCTION

Knowledge on nature and function on marine fish diversity including its biological characteristics could help to provide baseline information for long-term suitable ecosystem base for fisheries management. Utilization of marine biodiversity is the oldest impact on the ocean environment by humans. Widespread developments of fisheries become the major issues on ecological impact to marine biodiversity. Java Sea is one of vast shallow water (<100 m) that significantly contributed on demersal fish production among fisheries management areas in Indonesia. Broad range of pelagic, demersal and benthic fish community existed in the area. Three dominant demersal of fish species i.e. of purple-spotted bigeye *Priacanthus tayenus* (Richardson, 1846), lattice monocle bream *Scolopsis taenioptera* (Cuvier, 1830) and goatfish *Upeneus sulphureus* (Cuvier, 1829) play a significant role in demersal fish landing in north coast of Java. Those are the common species caught by demersal Danish seine fishery for years. The aim of this study is to describe the diversity index and the biological characteristics of three dominant species. Regular observation was conducted during August 2014 to July 2015 in Tegalsari fishing port, Tegal City, Central Java, Indonesia. The result showed that the catches consisted of 76 families, 101 genus and 129 species in landing place. Among them, 91 species were categorized as edible fish and others were indirectly served as fishmeals. Data on length, weight, stage of maturity of each species were measured on monthly bases. Other measurements on catch composition were also observed to provide a good understanding of ecosystem functioning. Three combine species contributed at around 55-60% of the total demersal fishes. Understanding diversity index and the reproductive biology of major species that under fishing pressures are the critical aspect of providing sound scientific advice for fisheries management. Knowledge on reproductive biology will largely determine the productivity of a stock and its resilience to exploitation.

MATERIALS AND METHODS

Sampling site

The sampling site was in Tegalsari fishing port with a geographical reference of 6°50'58.01" S-109° 7'43.74" E. It located in Tegal City, western part of north coast of Central Java, Indonesia (Figure 1). Regular sampling from commercial drag wooden demersal Danish seiner (trawl like fishing gear) was collected during August 2014 to July 2015. The fleet size ranged from 10-30 GT, completed with fish net of 18-27 m length of head rope and cod end of ¾ inch mesh size. The gear operated at the bottom of the shallow water of the Java Sea. Sample were collected from

selected fleets that landing during the date of observation. Selected samples were taken into the mini field laboratory in polyethylene bags containing ice blocks to prevent deterioration. A total of 2038 fish specimens (688 *P. tayenus*, 587 *S. taenioptera* and 763 *U. sulphureus*) belong to 3 families of (Priacanthidae, Nemipteridae and Mullidae) were systematically observed.

Procedures

Species were identified by using FAO Species catalog (Carpenter and Niem 1999). Sampling protocol was adopted from Sparre and Venema (1998) and Sparre (2000). Measurements were carried out based on one box (25-30 kg) sample of each group of species then subsample in weight around 5 kg or in number of 100 specimens. Individual specimens processed in temporary field laboratory and the following measurements made for fork length (cmFL), body weight (g), gonad weight (g) and sexual determination. Catches from 10 commercial fishing boats (< 6 m LOA) representing fishing activities in near shore fishing areas (< 12 nmi or around 20 m depth) and other 10 boats with > 6 m LOA representing off shore fishing (>12 nmi) were recorded. Catch composition were obtained based on the landing volumes. The catch was first standardized by trip, then dividing the total weight of fish species caught by the total weight of all species. Diversity index were measured through proportion of its catch composition. The species grouped at genera level then sub-sampled into species. Fork length (cm) of each fish was measured from the tip of the mouth (mouth closed) to the middle of caudal fin using a standard measuring paper. Body weight was measured to the nearest gram using top model digital balance. Accuracy of 0.01 g and measured to 0.1 cm were applied. Maturity stages were macroscopically observed.

Maturation and spawning characteristics were observed by conventional methods. Different color levels and percent of abdominal cavity occupied by gonads were used to identify their stage of maturity. The stages were marked as resting, developing, ripe, spawning and spent according generic scale of maturity stages by King (1995). The stages were identified into immature (stage 1, II) and mature (stage III, IV, V) as presented in Table 1.

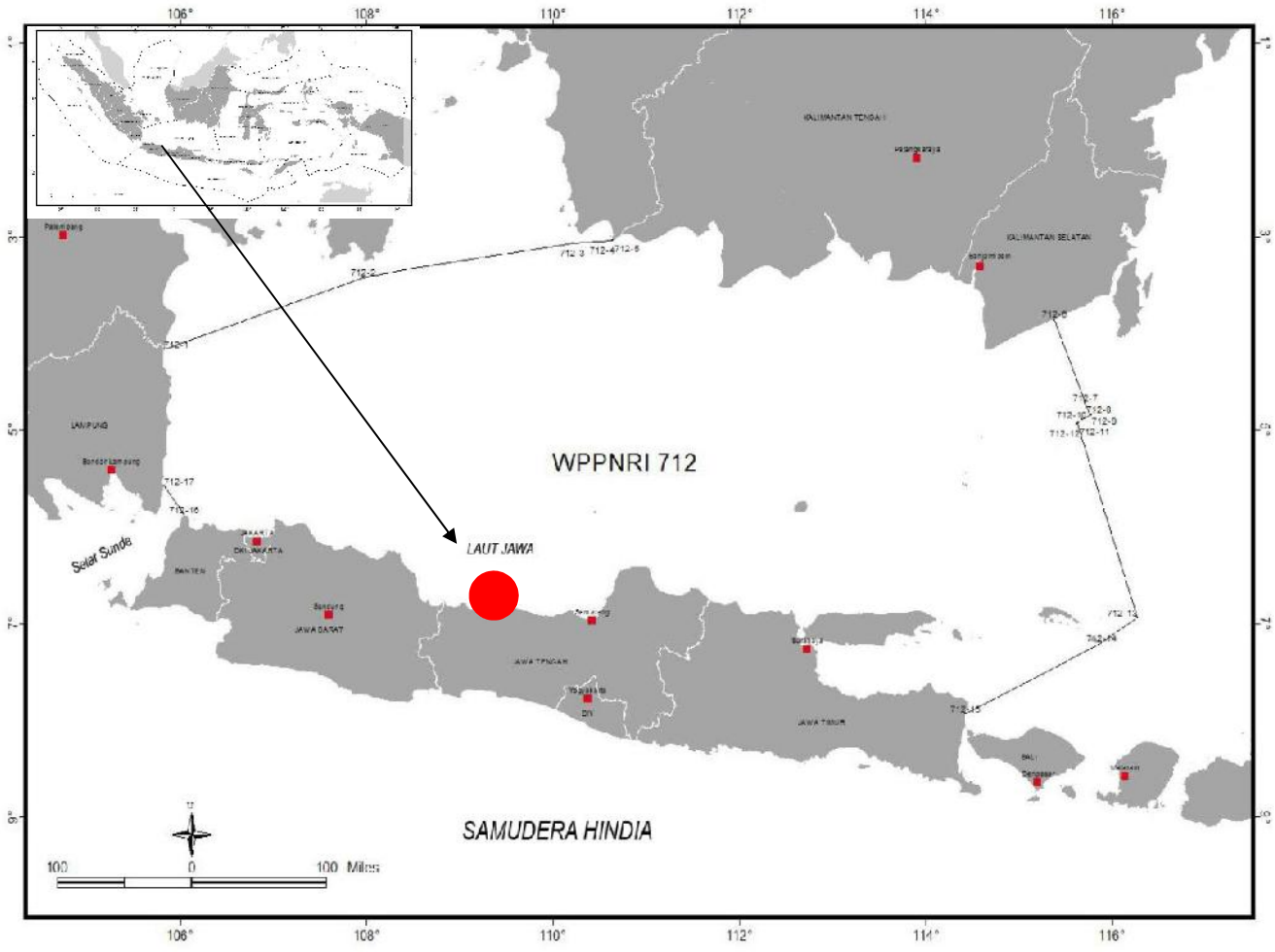

Figure 1. Tegal, north coast of Central Java, Indonesia (6°50'58.01" S-109° 7'43.74" E). Note: ● site location

Table 1. classification on ovary development stage

Stage	Description	Ovary	Eggs
I	Resting	Undeveloped, small, translucent	None visible to naked eye
II	Developing	Opaque, orange color	Visible and opaque
III	Ripe	Fills body cavity	Translucent, large and round
IV	Spawning	Release eggs when pressed	Large, translucent, some free ovary
V	Spent	Shrinking/slack	Some residual eggs

Data analysis

Diversity of fish were estimated based on catch data by group of species. Data standardized into kg per each haul. The presence of taxa in each tow was used to derive species richness (S) that defined as the cumulative number of taxa found in a given number of tows. Species evenness was expressed by Pielou's index (Heath and Speirs 2012) as follows:

$$J = \frac{H'}{\ln(S)} J = \frac{H'}{\ln(S)} \quad \text{..... (1)}$$

Where, H' is the Shannon-Wiener diversity index:

$$H' = \sum_{i=1}^{s} p_i \ln(p_i) H' = \sum_{i=1}^{s} p_i \ln(p_i) \quad \text{..... (2)}$$

and

$$p_i = \frac{B_i}{\sum_{i=1}^{s} B_i} \; p_i = \frac{B_i}{\sum_{i=1}^{s} B_i} \quad \text{..... (3)}$$

Where, B_i is relative biomass of each genera. The indices expressed in terms of biomass (weight) and not in terms of number of individuals.

Data on length and weight by species were graphically plotted in scattered diagram while the relationship (LWR) was estimated using the equation of Ricker (1975).

$$W = aL^3 \quad \text{..... (4)}$$

Where, length (L) in cm and weight (W) in gram. The data were converted into linear equation by using logarithmic expression of Ln W = Ln a + b Ln L. Parameters a and b were calculated by least square regression. The b value for each species is close to 3 in isometric growth and tested by t-test to verify if significantly different from 3 and a is a constant determined empirically.

Fulton condition factors (K) is also the parameters used in fisheries research and closely related to LWR. The value of K is calculated as follows (Froese 2006):

$$K = W/L^3 * 100 \quad \text{..... (5)}$$

Gonado-somatic index (GSI) is often used to follow the reproductive cycle of species over the year. The index, which assumes that an ovary proportionally increases in size and development stages compares the mass of gonad (Wg) with total mass of fish (W) were determined by following equation (King 1995) of:

$$GSI = 100 * (Wg/W) \quad \text{..... (6)}$$

The percentages of each stage were shown in histogram. Stages of reproductive development indicated the appearance of ovary and eggs. Empirical equations were applied to length at sexual maturity of those three interested species. The proportion of length of first maturity was derived following equation (King 1995) of:

$$P = \frac{1}{(1 + \exp(-r(L - L_m)))}$$

$$P = \frac{1}{(1 + \exp(-r(L - L_m)))} \quad \text{..... (7)}$$

Where, P is proportion of sexual mature individual by length (L), r is the slope of the curve; Lm is the mean length at first sexual maturity.

RESULTS AND DISCUSSION

General condition

Observation during August 2014 to July 2015 showed that the species composition landed by demersal Danish seine predominantly by group of small size species. The fleets operated in wide range of depth. Fishing were done during the daylight with 6 to 8 hauls per day. Active fishing day at about 14 to 30 days per trip. In general, the proportion of large fish species was relatively low in number and weight. The landing of group of species with an average weight of more than 200 gram per individual (Losse and Dwiponggo 1977; Beck and Sudradjat 1978) were less than 5%. A group of small size species belong to genera *Priacanthus* spp., *Nemipterus* spp. and *Upeneus* spp. were the major contribution of each daily landing. Among them, purple-spotted bigeye *P. tayenus*, lattice monocle bream *S. taenioptera* and goatfish *U. sulphureus* were the major (55-60% by biomass) species landed in Tegalsari fishing port.

Species diversity

The species caught in different scale of distance from the shore. A simple count of species was applied for measuring diversity to yield species richness value for assemblage in area of interest. Diversity were particularly analyze to the ichtyofauna landed by the demersal Danish seine net. Other group of elasmobranch and cephalopods were also contribute in their harvesting as an unintended species but commercially accepted. The relationship between species landings and its diversity by monthly landing were determined by applying equations of species evenness and its diversity indexes. The result is presented in Table 2.

Table 2. Biodiversity index of demersal fish landed in Tegalsari fishing port (August 2014-July 2015)

Period of observations	2014					2015						
	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
Inshore												
Species richness (S)	17	18	17	21	19	21	16	22	17	15	16	17
S index of diversity (H)	2.13	1.98	2.11	1.54	2.44	2.13	1.86	2.45	1.97	2.17	1.99	2.21
Species eveness (H'/ln (S))	0.75	0.68	0.74	0.51	0.83	0.70	0.67	0.79	0.70	0.80	0.72	0.78
Offshore												
Species richness (S)	30	37	33	34	31	35	34	32	29	27	31	31
S index of diversity (H)	2.39	2.33	2.48	2.50	2.40	2.59	2.55	2.49	2.21	2.39	2.44	2.43
Species eveness (H'/ln (S))	0.70	0.64	0.71	0.71	0.70	0.73	0.72	0.72	0.66	0.72	0.71	0.71

Note: S = Shannon-Wiener index

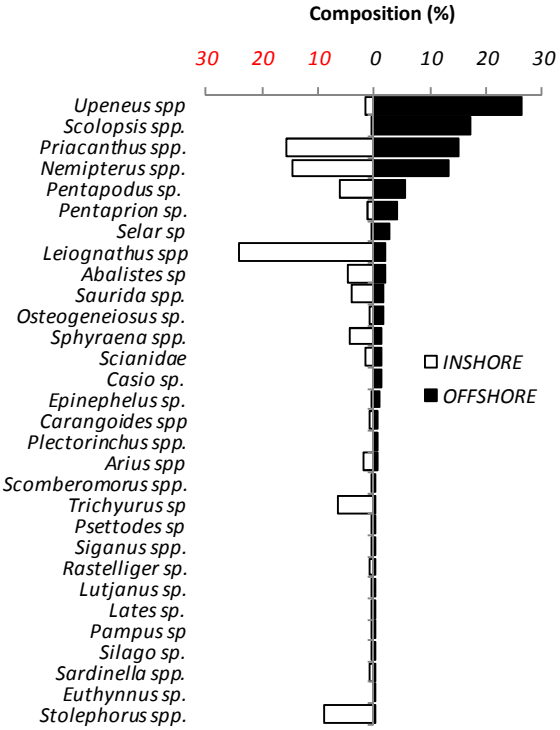

Figure 2. Catch composition of demersal by area inshore (*left*) and offshore (*right*)

The lower species richness (S) and its index of diversity (H) in the inshore waters were presumably due to the significance of fishing mortality in the area. The National capture fisheries data (DGCF 2015) indicated that the estimate fishing mortality were high represented by huge number of trawl and trawl like (demersal Danish seine) fishing boats of around 10,000 units in which 80% of them are harvesting in inshore waters of < 12 nmi or approximately at 20 m isobaths along the coast of north of Java. This results shows that all harvesting activities not only caused a direct impact on target species and species may related to ecological characteristics as stated by Cochrane (2002) but it could also affected the whole marine ecosystem including population structure, habitats, biodiversity and its productivity (Bas 2005).

Catch composition

Fishing affects the demersal fish communities through selective removal of target species, unintended species and habitat modification, resulting overall biomass in species composition and size structures (Bianchi et al. 2000). The catch composition in inshore waters was predominantly composed of small size Leiognathidae, i.e. *Secutor ruconius*, *S. insidiator*, *Leiognathus bindus*, *L. elongatus* and *Gazza minuta* at around 10.4% followed by larger *Leiognathus* spp. such as *L. splendens* and *L. equulus* (8.3%), *Priacanthus* spp. (particularly *P. tayenus*) and *Nemipterus* spp. (*Nemipterus japonicus*) 5%. The offshore waters were dominated by *Upeneus* spp. (particularly *U. sulphureus* at about 21%, *S. taenioptera* 14.7% and *P. tayenus* 12.2% (Figure 2). In relation to spatial distribution of the species, Ibrahim et al. (2003) found that *P. tayenus* and *S. taenioptera* in coastal waters of east Malaysia peninsula were distributed at depth of > 20m, while Silvestre and Pauly (1997) suggested that the three interested species distributed at > 10 m depth at coastal waters of Bangladesh. Uiblein and Heemstra (2010) in coastal waters of western Indian Ocean found that *U. sulphureus* inhabited the water of 20 to 60 m depth.

Size composition

The fish landed by this demersal fishery varied according to species. As many as 12969 specimens were measured for length frequency distribution during August 2014-July 2015 and 2038 specimens of those were observed for bio-reproduction data. Cumulative length frequency distribution during the observation indicated that *U. sulphureus* ranged at 5.5 to 16 cmFL, *S. taenioptera* at 9 to 26 cmFL, and *P. tayenus* at 7 to 30 cmFL with mean length of each subsequent species approximately at 11.2, 18.9 and 17.1 cmFL. These data indicated broad range of size occurred at juvenile to adult stages. Adopting the estimate length at first maturity of each species (see Figure 7), the size composition of catch are a sign of immature cohort except for *S. taenioptera*. The graph (Figure 3) indicates that 68% size distribution of *P. tayenus* and 79% of *U. sulphureus* were belong to immature cohort while for *S, taenioptera* at about 24.7%.

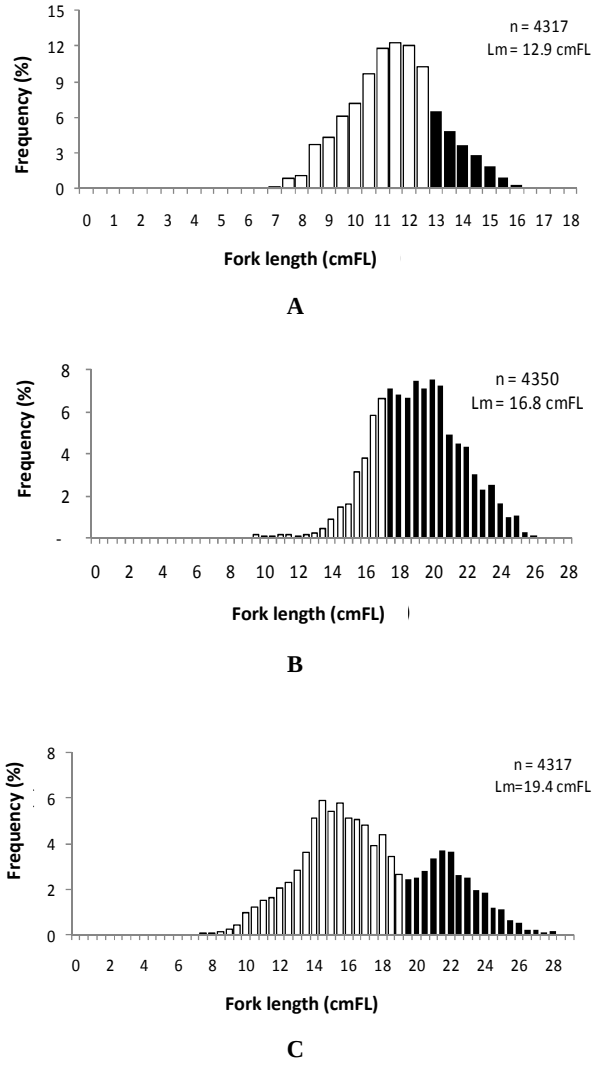

Figure 3. Length frequency distribution of A. *U. sulphureus*; B. *S. taenioptera*; C. *P. tayenus.* Note: Blank bar represented distribution under size of Lm and black bar is larger than Lm. Lm = estimated length at first maturity

Length-weight relationship

Length weight relationship (LWR) is of great importance in fishery assessment (Haimovici and Velasco 2000). Length and weight measurements, in conjunction with age, could contribute information on catch composition, age at maturity, life span, mortality, growth and reproduction (Diaz et al. 2000). A total number of 688 specimens of *P. tayenus*, *S. taenioptera* and *U. sulphureus* were observed during August 2014 to July 2015. Three dominant species revealed significant portion on the exploitation. Fish body size and weight were measured on monthly bases. The fish size was relatively small as 5.5 cm FL (5.0 g) of *U. sulphureus* and as larger size of 29.8 cm FL of *P. tayenus*. All three species exhibited allometric negative (b<3) and the student t-test showed growth coefficient b (ranged of 2.671-2. 884; sb = 0.022-0.073; t-test t = 0.043-0.144; p<0.05) was significantly lower than the theoretical value of 3. This indicates negative allometric growth. Other study on length weight relationship on *P. tayenus* in Malaysian waters shows that $W = 0.0068\ L^{3.3525}$ (Awong et al. 2011), this difference probably due to different environment and its unequal fishing pressures as mentioned by selectivity at high rates of exploitation can radically alter the age/size structure and breeding structure of exploited populations. Overall growth parameters were positive and highly correlated (>0.90). Summary of length-weight relationship is presented in Table 3.

The Fulton condition factors (K)

The condition factor of fish (K) is part of its biological characteristics and it represents a quantitative healthiness parameter of fish in its habitat. It is based on the hypothesis that the heavier fish in a given length would represent the better physiological condition (Froese 2006). Figure 4 illustrates the distribution monthly values of K of each species. The lowest average monthly condition factors (1.3-2.1) were *U. sulphureus*, followed *P. tayenus* and the highest is *S. taenioptera*. Prihatiningsih et al. (2013) indicated that K value of *P. tayenus* in Banten Bay north

Tabel 3. Length and weight relationship for three species of *P. tayenus*, *S. taenioptera* and *U. sulphureus*

Family Species	Sex	n	Length (cmFL)			Body weight (g)			Growth coefficient					
			Min	Max	Mean± SD	Min	Max	Mean± SD	a	b	r^2	sb	t-test	A/I
Priacanthidae	M	318	13.2	30.1	21.7±3.36	43	369	152.9±69.2	0.0270	2.788	0.93	0.044	0.086	A
P. tayenus	F	370	13.2	30.1	21.5±3.57	46	366	153.3±76.6	0.0259	2.808	0.94	0.038	0.074	A
	All	688	13.2	30.1	21.6±3.48	43	366	151.6±73.4	0.0201	2.884	0.93	0.029	0.056	A
Nemipteridae	M	157	14.7	25.4	20.3±2.50	52	263	141.5±47.7	0.0401	2.688	0.95	0.050	0.097	A
S. taeniopterus	F	428	11.5	25.5	18.5±2.42	35	255	108.1±42.1	0.0346	2.735	0.92	0.039	0.077	A
	All	585	9.1	25.5	19.0±2.60	21	263	117.2±46.1	0.0354	2.738	0.94	0.022	0.043	A
Mullidae	M	154	8.2	15.5	12.1±1.54	13	68	36.4±13.3	0.0390	2.722	0.91	0.073	0.144	A
U. sulphureus	F	471	8.2	15.9	12.4±1.47	12	72	37.9±12.1	0.0510	2.617	0.91	0.043	0.084	A
	All	763	5.5	15.9	11.9±1.80	5	72	34.5±13.8	0.0380	2.731	0.94	0.027	0.052	A

Note: M = Male; F = Female; A = allometric; I = isometric

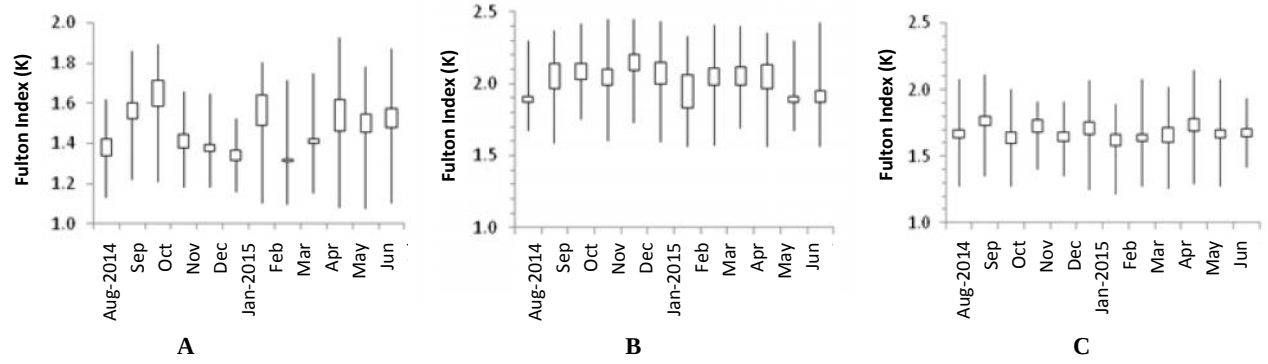

Figure 4. Monthly average of Fulton condition factor index by species. A. *P. tayenus*, B. *S. taenioptera*, C. *U. sulphureus*

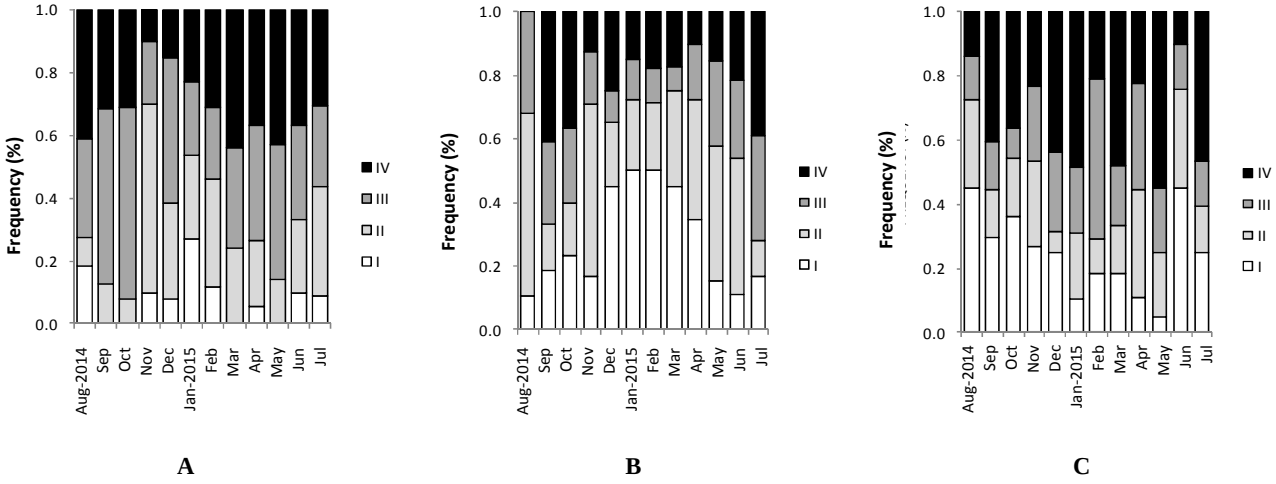

Figure 5. Monthly maturity stages of: A. *P. tayenus*, B. *S. taenioptera*, C. *U. sulphureus*

coast of west java at around 1.26, which is lower than this present observation. The K value is also used as a parameter to estimate fish body structures. In this study, the mean K values of those species varied on monthly basis. Le Cren (1951) and Gilliersa et al. (2006) suggested ecological condition of the habitat or variation in physiology of the animal or both are responsible for growth rate variation in the same species in different month.

Maturity stages

Monthly variation of its maturity indicated that highest proportion of immature specimens (I, II) of *P. tayenus* found in November to May while for *S. taenioptera* on occurred from December to April and for *U. sulphureus* occurred during June to August (Figure 5). The highest proportion of mature stage (III, IV, V) for *P. tayenus* occurred during March to October where as November-January intensity of reproduction is lower. For *S. taenioptera*, July-October is marked out by large number of fish ready for spawning (stage IV), and *U. sulphureus* occurred on December to March and May. These may indicate that the pattern of maturity stages for those species were almost in the same for *P. tayenus* and *S. taenioptera* and opposite pattern for *U. sulphureus.* In general all species have two peaks season for their spawning period (Figure 5). These might be related to typical spawning of tropical fish that regularly batch with peak season in certain period. Observation in east coast of Malaysia peninsula that *P tayenus* spawned throughout the year (Ambak 1987), while in Hongkong, only on a relatively short period from June to July (Lester and Watson 1985). Several general studies on fish maturation indicates that largely depends on a combination of age and length, long-term environmental changes including fisheries activities that alter the environment and population structures (Hunter et al. 2015).

Gonado-somatic index (GSI)

The monthly average GSI showed that two dominant species of *P. tayenus* and *S. taenioptera* relatively have similar trend with fluctuation in between, while *U. sulphureus* has an opposite trend that February to April tend to increase (Figure 6). The highest average GSI of *P. tayenus* found May to October, *S. taenioptera* on May to October and November to April for *U. sulphureus*, Highest index just prior to spawning period and it is relatively similar trends on the occurrence of macroscopic maturity stages (Figure 5).

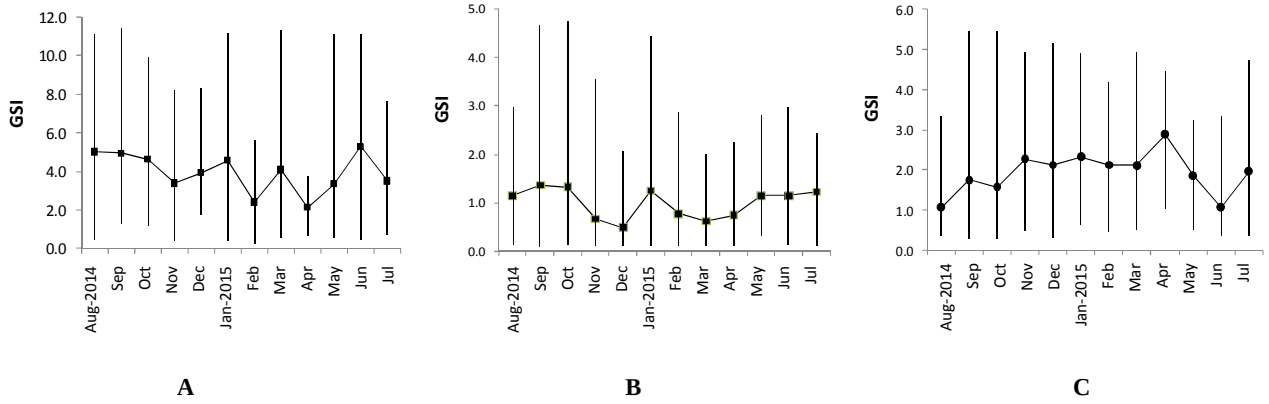

Figure 6. Monthly average GSI of three dominant species. A. *P. tayenus*, B. *S. taenioptera*; C. *U. sulphureus*

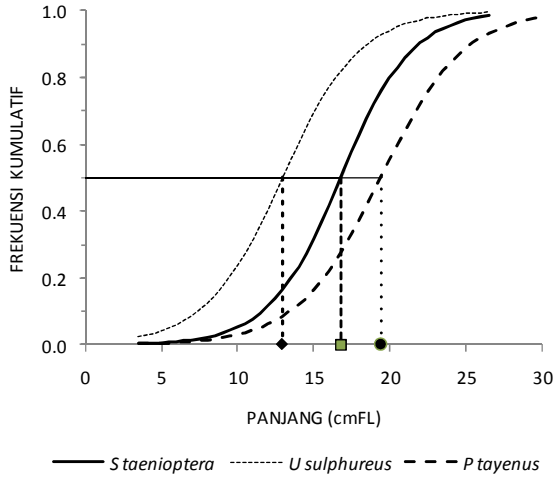

Figure 7. Estimated length at first mature of three species

Due to unclear threshold to distinguish between reproductive and non-reproductive season, there are some probabilities that intensive spawning period of *P. tayenus* occurred in May to October, *S. taenioptera* in May to October and longer period of November to April for *U. sulphureus*, while low-season of spawning occurred in February to April for *P. tayenus*, November to December for *S. taenioptera* and June to August for *U. sulphureus.*

Length at first mature

The percentage distribution of mature ovaries (stages III and above) in relation to length was plotted. This depicted the minimum length during sexual maturity for *U. sulphureus*, *S. taenioptera* and *P. tayenus* was at 12.9, 16.8 and 19.4cmFL (Figure 7). It is interesting that most of the size from these dominant species occurred in size of less than their length of first maturity. Observation on the average size of length combined with the estimated length at first mature indicated that most of the catches were on a stage before their regenerating age. Fishes always have a definite course of life history that often starts from hatching of eggs or life births in some few groups to larva stages, juveniles and the adults stages (Mustapha 2014). Following long historical harvesting on demersal fish in the area, yield of this marine fish species tend to be at high pressures. Nugroho and Atmaja (2014) explain that serial landing data on demersal Danish seine fishery harvesting near overfishing might occurred in the area. Lack of response on regular catch documentation scheme caused some difficulties on establishing the biodiversity impact of this existing harvesting strategy. A fundamental goal on sustaining biodiversity as part of conservation biology is to ensure the long-time survival of species (Meffe and Carol 1997; Primack 2006). However, these indicated that to sustain the long-term utilization of the diversity of the demersal fish, introducing an appropriate harvest strategy by using selective fishing gear should be implemented through government regulation.

Historically, the benefit for human wellbeing of harvesting marine biodiversity recorded since 1970s. Broad range of pelagic demersal and sedentary fish species existed in northern coastal waters of Java. The target species were shifted from large size demersal to small size group of species. These indicated that multispecies harvesting marine biodiversity occurred in the north coast of Java. Landing dominated by small size of fish and low trophic level fisheries. Size compositions were at broad range of juvenile to adult and most of the catches were belong to immature cohorts. Species richness in coastal waters were found at lower level than offshore, this would indicate that coastal ecosystem were already on pressure by fishing activity. Lower species richness remain that are not capable of sustaining abundant commercial catches in these local geographical area. Adopting the precautionary approach and using the bio-exploitation indicators of a general status of this fishery can be suggested at risk level. If further work has to be undertaken, an independent method would be valuable to clarify on rationale of the lower abundance of demersal fish in the area.

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Short Communication: RAPD fingerprinting key and phylogenetic of nine seagrass species from Sanur coastal water, Bali, Indonesia using *matK* sequences

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Abstract. *Pharmawati M, Nurkamila US, Stevanus. 2016. RAPD fingerprinting key and phylogenetic of nine seagrass species from Sanur coastal water, Bali, Indonesia using* matK *sequence. Biodiversitas 17: 687-693.* In Bali, there are nine species of seagrass identified based on morphological characteristics. Development of molecular markers assist identificaton and evolutionary studies of many species including seagrass species. This study aimed to develop a fingerprinting key of nine seagrass species found at Sanur (Sanur Beach and Sindhu Beach), Bali, based on RAPD markers and to analyse their phylogenetic relationships using the *matK* region. Seagrass samples were collected at low tide and DNA was extracted using CTAB buffer. Six RAPD primers were used in the study. Sequences of *matK* were analyzed using MEGA 5.2. The phylogenetic tree was constructed by Maximum Likelihood method with 1000 bootstrap replicates. Based on RAPD banding pattern, a DNA fingerprinting key was successfully developed using only one primer — OPB12. Phylogenetic analysis of *matK* sequences grouped seagrass species by genera. There were five clades identified and the tree recognised that Cymodoceaceae was paraphyletic. This result is in disagreement with a previous study using combined *rbcL* and *matK* sequences which discussed the monophyly of Cymodoceaceae. Recent published paper using ITS sequence showed that cymodoceaceae might not be monophyletic group. This result supported our finding that Cymodoceaceae is not in the monophyletic group. Combined DNA sequences of chloroplast, nuclear and published paper using ITS sequencing showed that Cymodoceaceae mitochondrial DNA will further resolve phylogeny of seagrass species.

Keywords: DNA fingerprinting key, *matK*, phylogenetic, RAPD, seagrass

INTRODUCTION

Seagrasses are flowering plants (Anthophyta) and grow in shallow water of coastal regions. Seagrass vegetation may consist of one species or a mix of two or more species. The seagrass ecosystem has an important role as a primary producer, stabilizer of the sea bed, and a habitat for animals such as the sea cucumber (Holothuridae), shrimp and dugong (*Dugong dugon*) (McKenzie 2003; Short et al. 2007). Seagrass meadows also support coral reef ecosystems by filtering and precipitating pollutants (Larkum et al. 2006).

There are 12 seagrass species in seven genera and two families reported in Indonesia (Kuriandewa et al. 2003). In Bali, seagrass beds are distributed along the south and southeast coast including Nusa Dua, Geger, Serangan and Sanur beaches (Arthana 2004). In other parts of Bali, seagrass grows at Candidasa (Sudiarta and Sudiarta 2011), Menjangan, Nusa Penida, Nusa Lembongan, Nusa Ceningan (Yusup and Asy'ari 2010) and Teluk Gilimanuk (Al Hakim and Wahyuni 2009).

Arthana (2004) reported seven seagrass species found in Sanur coastal waters, from Sanur Beach to Mertasari Beach. Those species are *Enhalus acoroides, Cymodocea rotundata, Cymodocea serrulata, Halophila ovalis, Halodule uninervis, Halodule pinifolia* and *Syringodium isoetifolium.* Another study by Yusup and Asy'ari (2010), recorded eight species in the same region but their descriptions were somewhat different.

Those different results may be because of an identification problem in taxonomy, because several seagrasses have a similar morphological character. Therefore, accurate and more reliable methods of identification are needed. Confirmation of identifications based on morphology can be done using DNA barcoding technique. DNA barcodes employ short DNA fragments (400-800 bp) that accommodate species diversity in wide taxa (Lucas et al. 2012; Selvaraj et al. 2013). Several genes in chloroplast DNA that have been used for identification, biodiversity detection and phylogenetic analysis are *rbc*L, *matK, trnH-psbA* dan *rps16-trnQ* (Lucas et al. 2012).

Random Amplified Polymorphic DNA is a common molecular marker applied to detect genetic diversity at the interspecific level (Pharmawati et al. 2004; Arif et al. 2010) and at the intraspecific level (Pharmawati and Candra 2015; Priya et al. 2015). This genetic marker has been used for species and cultivar identification in numerous plant species (Zhao et al. 2011; Saengprajak and Saensouk 2012).

A study on seagrass species identification and phylogeny in Indonesia, based on molecular markers, has not been reported. This paper reports on the use of DNA barcoding based on *matK* fragments and RAPD markers to identify seagrass species from Sanur coastal waters and determine their genetic relationships. This paper also

demonstrates development of a DNA fingerprinting key for seagrass species based on RAPD markers. Furthermore, a phylogenetic tree of seagrass species from Sanur coastal waters based on *matK* sequences was constructed.

MATERIALS AND METHODS

Sample collection

Seagrass samples were collected from Sanur Beach and Sindhu Beach, Bali (Figure 1). Two samples for each species were collected in Sanur Beach (S 8° 67' 68.8" and E 115° 26' 52.2"), while one sample for each species was collected from Sindhu Beach (S 8° 68' 47.9" and E 115° 26' 65.1"). Morphological identification was conducted following den Hartog and Kuo (2006) and McKenzie and Yoshida (2009).

DNA extraction

DNA was extracted following Doyle and Doyle (1990). A leaf sample (0.1 g) was ground using a mortar and pestle and 1 ml of CTAB buffer (2% CTAB, 100 mM Tris/HCl, pH 8, 1,4 M NaCl, 50 mM EDTA, 2% mercaptoethanol) was added. The slurry was transferred to a microtube. The samples were incubated at 65°C for 30 min and centrifuged at 14,000 rpm for 10 min. Extraction with the same volume of chloroform:isoamyl alcohol (24:1) was done twice and DNA precipitation was conducted by adding 2/3 volume of cold isopropanol. The mixture was stored at -20°C for 16 hours. The DNA pellet was collected by centrifuging for 5 min at 8,000 rpm. The supernatant was discarded and the DNA pellet was washed with 70% ethanol followed by centrifuging for 5 min at 8,000 rpm and the DNA pellet was air dried. As much as 100 µl of sterile H_2O was added to dissolve DNA. RNase A (final concentration 10 µg/ul) was added, and incubated at 37°C for 30 min. Finally centrifuging at 8,000 rpm for 5 min was conducted to remove impurities, and the supernatant was transferred to a new tube.

DNA electrophoresis was done using 1% agarose gel in TAE buffer (Tris acetate-EDTA) stained with ethidium bromide (final concentration 0.5 µg/ml). DNA was visualised with a UV transilluminator. Lambda DNA (MBI Fermentas) at concentrations of 100 ng and 200 ng to compare the estimate of DNA concentration.

PCR-RAPD

PCR-RAPD was conducted in a 20 µl reaction mixture containing 1xPCR buffer (Taq Gold Applied Biosystem), 200 µM dNTP, 3 mM $MgCl_2$, 1.5 µM primer, 1U taq polymerase (Taq Gold Applied Biosystem), 25ng DNA and H_2O to reach 20 µl. The PCR cycles were as follows: initial activation step at 95°C for 1 min, followed by 38 cycles of 1 min at 94°C, 1 min at 37°C and 1.5 min at 72°C, with the final single cycle at 72°C for 10 min. Six RAPD primers (Operon Technology, USA) were used including OPA2 (5'- TGCCGAGCTG-3'), OPA4 (5'- AATCGGGCTG-3'). OPB12 (5'- CCTTGACGCA-3'), OPD11 (5'- TGCCCGTCGT-3'), OPH6 (5'- ACGCATCGCA-3') and UBC127 (5'-ATCTGGCAGC-3'from the University of British Columbia, Canada).

PCR of *matK*

The amplification of the *matK* region was conducted in a 25 µl reaction mixture. The mixture contained 1 x PCR buffer (Taq Gold Applied Biosystem), 200 µM dNTP, 2.5 mM $MgCl_2$, 1.5 µM of a forward primer and a reverse primer, 1U taq polymerase (Taq Gold Applied Biosystem), 25ng DNA and H_2O. For *matK* amplification, the primers used were P646 5'-TAATTTACGATCAATTCATTC-3' and P647 5′-GTTCTAGCACAAGAAAGTCG-3′ (Lucas et al. 2012).

The program of thermal cycling was as follows: initial activation step at 95°C for 5 min, followed by 35 x of denaturation at 95°C for 1 min, annealing at 54.°C for 2 min, elongation at 72°C for 2 min. Final elongation was conducted for one cycle at 72°C for 7 min.

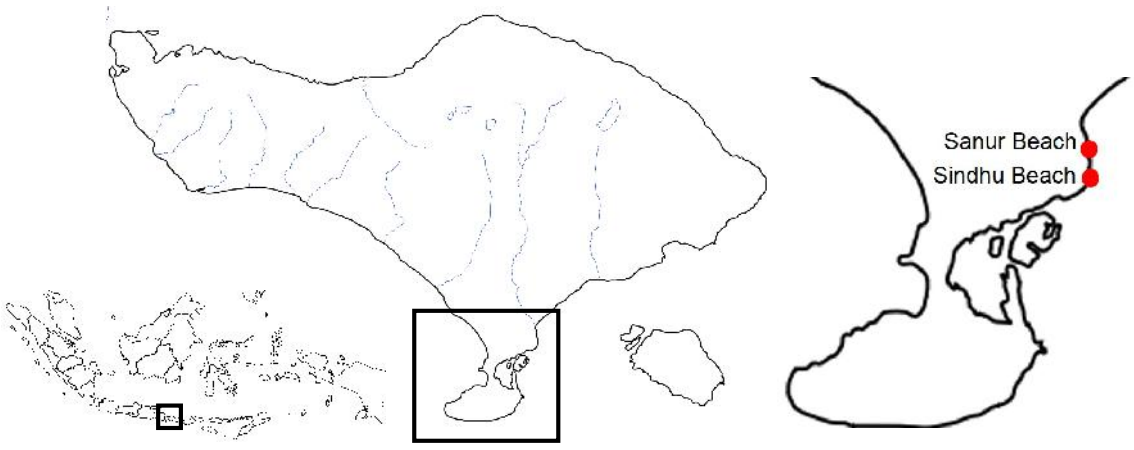

Figure 1. Sites of sample collection at Sanur Beach and Sindhu Beach, Bali, Indonesia

Electrophoresis of PCR products

PCR products of RAPD were visualised using 1.5 % agarose in TAE buffer stained with ethidium bromide, while for *matK* products, electrophoresis used 1% agarose gel. As a size marker, and 100bp DNA ladder (Promega) was included in each gel.

DNA sequencing

DNA sequencing of *matK* was done at forward strand using primer P646. The PCR products were sent to *Berkeley Sequencing Facility* in USA for sequencing,

Data analysis

A RAPD fingerprinting key was developed based on clear, major and reproducible PCR-RAPD bands from three samples in each seagrass species. Sequences of *matK* from all seagrass species were analysed using MEGA 5.2 *(Molecular Evolutionary Genetic Analysis*) and ClustalW was used to determine homology between sequences (Tamura et al. 2011). Phylogenetic reconstruction was conducted using a maximum likelihood General Time Reversible+ Gamma (GTR+G) model with 1000 bootstrap. *Pistia stratiotes* was used as an outgroup. Species determination used Basic Local Alignment Search Tool (BLAST).

RESULTS AND DISCUSSION

Based on morphological characteristics, nine seagrass species were identified from Sanur Beach and Sindhu Beach, Denpasar, Bali, Indonesia. The species were *E. acoroides, T. hemprichi, S. isoetifolium, Th. ciliatum, H. uninervis, H. pinifolia, Ha. ovalis, C. serrulata,* and *C. rotundata* (Figure 2; Table 1). Voucher specimens were deposited at the Herbarium Biologi Udayana (HBU), Universitas Udayana, Bali, Indonesia The morphological characters noted in the seagrass samples matched with those described in den Hartog and Kuo (2006) and McKenzie and Yoshida (2009). Previously, in Sanur water there were seven seagrass species reported (Arthana 2004), while in 2010, there were eight seagrass species found by Yusup and Asy'ari (2010). Other studies reported 10 seagrass species: *Zostera* sp., *H. pinifolia*, *H. uninervis*, *C. rotundata*, *C. serrulata*, *S. isoetifolium*, *Th. ciliatum*, *E. acoroides*, *Ha. ovalis* and *Th. hemprichii* (Sudiarta and Restu 2011). *Zostera* is distributed mainly in temperate coastal waters (den Hartog and Kuo 2006; Short et al. 2007). There are hardly any other reports available on the presence of *Zostera* in Indonesia. Careful identification is important to support a seagrass database for conservation strategies.

The 6 RAPD primers screened amplified multi-band patterns in each seagrass species. Among the six primers tested, OPB12 resulted in the clearest and most reproducible band patterns. PCR-RAPD products using primer OPB12 are shown in Figure 3. Using the PCR-RAPD data from primer OPB12, a DNA fingerprinting key for nine seagrass species was developed (Figure 4).

Identification of seagrass species relied on morphological characteristics. Seagrass has both sexual and asexual reproduction, however the flower, as a distinct morphological trait, is hardly ever found (Papenbrock 2012). This makes identification of seagrass species difficult. Genetic analysis provides a tool to clarify species identity, diversity and distribution (Short et al. 2007). A fingerprinting key based on PCR-RAPD banding pattern has now been generated to identify seagrass species. The success of RAPD as a molecular marker to develop a fingerprinting key has been reported in radish cultivars using five RAPD primers (Pradhan et al. 2004). The RAPD fingerprinting key for seagrass species could be developed using only one primer (OPB12). This is an ideal condition, because the low number of primers will reduce time and cost. Therefore, this finding provides guidance for seagrass identification for further purposes such as single species identification, and distribution and conservation studies.

Fragments of *matK* were amplified from 18 seagrass samples (nine species each from Sanur Beach and from Sindhu Beach). Electrophoresis of PCR products resulted in 950 bp fragments in all species. There was no length polymorphism detected. Analysis of each seagrass sequence using BLAST (*Basic Local Alignment Search Tool*) showed high similarities (92%-100%). BLAST analysis does not confirm all seagrass species that were identified morphologically Three out of nine seagrass species could not be confirmed (Table 2). This could be because there are limited sequence variations in *S. isoetifolium*, *Th. ciliatum* and *H. pinifolia* available at the NCBI database. In addition, according to Arif et al. (2010) the failure of sequence base analysis to differentiate between the species is caused by the high similarity between the DNA sequences of amplified regions.

Phylogenetic analysis based on a Maximum Likelihood GTR+R model is shown in Figure 5. The seagrass species were grouped based on their genera. Each seagrass species from Sanur Beach and Sindhu Beach was grouped at the same branch. This indicates a low variation of seagrass species from the two locations. Sindhu Beach is located next to Sanur Beach, it is predicted that there is gene flow between the two locations. The pollination system of seagrass is hydrophily (Papenbrock 2012) which supports the hypothesis of gene flow where pollen may be distributed by sea currents.

The tree recognised that the Cymodoceaceae were not monophyletic (Figure 5). The phylogenetic using the partial *matK* gene in this study is in disagreement with the phylogenetic of seagrass published previously (Lucas et al. 2012) which combined data of *rbcL* and *matK* sequences. According to Lucas et al. (2012) the concatenated *rbcL* and *matK* sequences resulted in major clades which represented Hydrocharitaceae, Zosteraceae and Cymodoceaceae families. The clade containing *Enhalus*, *Thalassia* and *Halophila* was well supported. Similarly, the clade with *Halodule, Sringodium* and *Cymodocea* had a high support value (Lucas et al. 2012).

The discordance of our results with published findings could be because of the use of partial *matK* fragments in our analysis. In our study, unidirectional sequencing was conducted for 18 seagrass samples representing nine species using forward primer. Complete *matK* sequence

from bidirectional sequencing and other DNA fragments may better resolve phylogenetic of seagrass species from Sanur Beach and Sindhu Beach. Sequence of *rbcL* gene is a major locus gene that is considered to be used in plant DNA barcoding (CBOL Plant Working Group 2009; Vijayan and Tsou 2010; Hollingsworth 2011; Lucas et al. 2012).

Recent study using ITS sequence reported that Cymodoceaceae might be a non-monophyletic group (Nguyen et al. 2015). Using ITS sequences, *Halodule* and *Cymodocea* were grouped in different clades. This seem to be in concordance with our finding. Peterson et al. (2014) also suggested that Cymodoceaceae is non monophyletic group. Sequences from nuclear, chloroplast and mitochondrial DNA need to be carefully combined to further clarify whether Cymodoceaceae is a monophyletic or non monophyletic group (Nguyen et al. 2015).

Figure 2. Seagrass species found at Sanur Beach and Sindhu Beach, Denpasar, Bali, Indonesia. Note: A. *Enhalus acoroides*, B. *Thalassia hemprichii*, C. *Syringodium isoetifolium*, D. *Thalassodendron ciliatum*, E. *Halodule uninervis*, F. *Halodule pinifolia*, G. *Halophila ovalis*, H. *Cymodocea serrulata*, I. *Cymodocea rotundata*. Bar = 5 cm

Table 1. Morphological characters of seagrass species from Sanur Beach and Sindhu Beach, Bali, Indonesia

Sample code	Morphology of rhizome	Leaf	Species
A_1, A_2, 1	Herbaceous, covered with bristles, diameter 1.5 cm-2.5 cm, short branches	Ribbon like, leaf tip rounded, L=30-60 cm, W=2-3 cm, margin rolled slightly, parallel longitudinal vein, vein number 15-20	*E. acoroides*
B_1, B_2, 9	Herbaceous, internode 2-5 mm, 1 short branch from each node	Ribbon like, round apex, L=3-10 cm, W= 05-1 cm, longitudinal vein, vein number=10-15	*T. hemprichii*
C_1, C_2, 2	Herbaceous, internode distance 1-1.5 cm, 1 branch from each node	Erect shoot, cylindrical, L=4-18 cm, W=1-2 mm	*S. isoetifolium*
D_1, D_2, 3	Strong/robust, internode 0.6-1.5 cm, 1 branch from each node	Ribbon like, linier, falcate, L=2-8 cm, W= 0.5-1 cm, leaf tip rounded, margin with teeth	*Th. ciliatum*
E_1, E_2, 5	Herbaceous, internode 5-9 mm, 1 branch from each node	Ribbon like, L=3-8 cm, W=3-5 mm, leaf apex tridentate	*H. uninervis*
F_1, F_2, 4	Herbaceous, internode 2-5 mm, 1 branch from each node	Ribbon like, L=5-14 cm, W=1-2 mm, rounded leaf tip with serrations	*H. pinifolia*
G_1, G_2, 6	Herbaceous, internode 1-1.5 cm, 2 short branches from each node	Leaf shape oblong, ovate, smooth leaf surface, L=0.5-1.5 cm, W=4-10 mm, branched cross vein, vein number=12-16	*Ha. ovalis*
H_1, H_2, 7	Strong/robust, internode 1-2 cm, 1 branch from each node	Ribbon-like, narrow at the base, serrate to dentate apex, L=4-8 cm, W= 0,5-1 cm, longitudinal vein, vein number=13-17	*C. serrulata*
I_1, I_2, 8	Herbaceous, internode 1,5-3 cm, 1 branch from each node	Ribbon like, apex rounded, L=2-10 cm, W=4 mm-0.8 cm, longitudinal vein, vein number=10-15	*C. rotundata*

Note: A-I: Seagrass species from Sanur Beach, 1-9: Seagrass species from Sindhu Beach, L: Length of leaf, W: Width of leaf

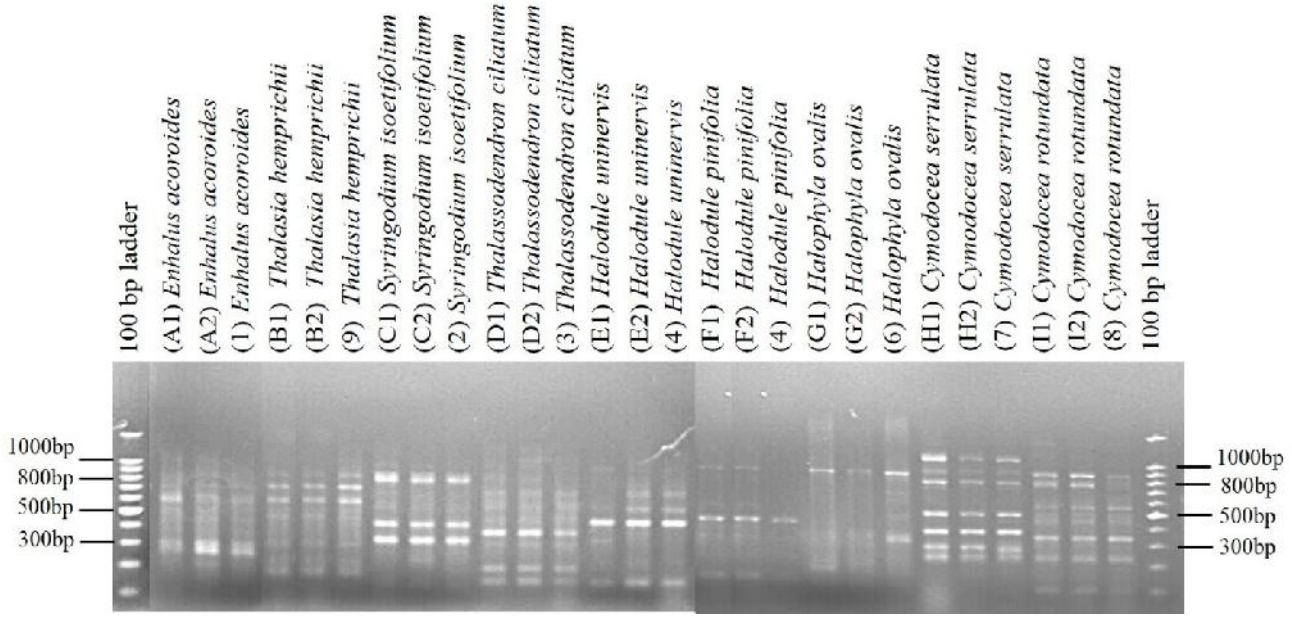

Figure 3. PCR-RAPD amplification of nine seagrass species using primer OPB12. A-I: Seagrass species from Sanur Beach, 1-9: Seagrass species from Sindhu Beach, Bali, Indonesia

1. OPB12$_{(800)}$	P	*T. hemprichii*, *S. isoetifolium*, *C. serrulata*, *C. rotundata*
1.1. OPB12$_{(1160)}$	P	***C. serrulata***
1.2. OPB12$_{(1160)}$	A	*T. hemprichii*, *S. isoetifolium*, *C. rotundata*
1.2.1. OPB12$_{(310)}$	P	***S. isoetifolium***
1.2.2 OPB12$_{(310)}$	A	*T. hemprichii*, *C. rotundata*
1.2.2.1..OPB12$_{(695)}$	P	***T. hemprichii***
1.2.2.2. OPB12$_{(695)}$	A	***C. rotundata***
2. OPB12$_{(260)}$	P	*E. acoroides*, *C. serrulata*
2.1. OPB12$_{(590)}$	P	***E. acoroides***
2.2. OPB12$_{(590)}$	A	***C. Serrulata***
3. OPB12(355)	P	*Th. ciliatum*, *C. rotundata*
3.1. OPB12$_{(172)}$	P	***Th. ciliatum***
3.2. OPB12$_{(172)}$	A	***C. rotundata***
4. OPB12$_{(900)}$	P	*H. pinifolia*, *Ha. ovalis*, *C. serrulata*
4.1. OPB12$_{(400)}$	P	***H. pinifolia***
4.1.1. OPB12$_{(378)}$	P	***C. serrulata***
4.1.2. OPB12$_{(378)}$	A	***Ha. ovalis***
4.2. OPB12$_{(400)}$	A	*Ha. ovalis*, *C. serrulata*
5. OPB12$_{(122)}$	P	*Th. ciliatum*, *H. uninervis*
5.1. OPB12$_{(400)}$	P	***H. uninervis***
5.2. OPB12$_{(400)}$	A	***Th. ciliatum***

Figure 4. PCR-RAPD fingerprinting key for seagrass species from amplification products using primer OPB12. RAPD bands are designed as primer name followed by the fragment size in bp. The presence (P) and absence (A) of bands resulted in identification of seagrass species listed in Table 1 (bold text)

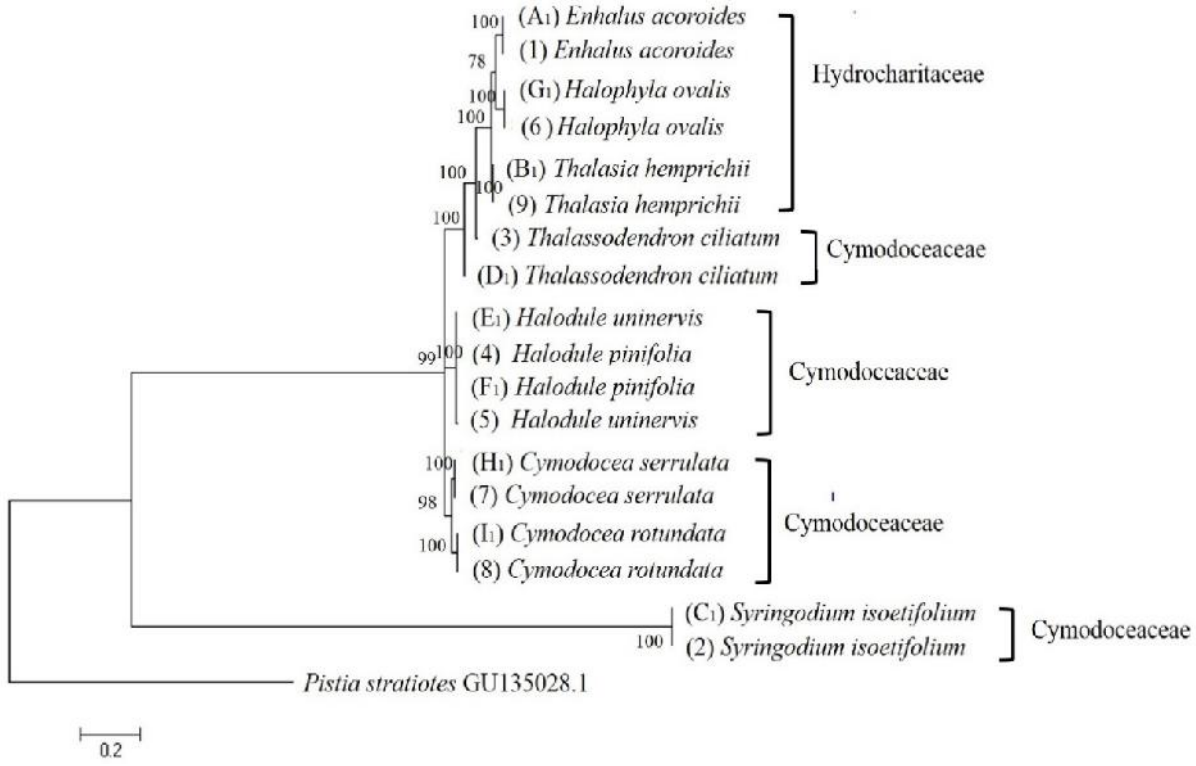

Figure 5. Phylogenetic analysis of seagrass species collected from Sanur Beach and Sindhu Beach, Bali, Indonesia using *matK*. Numbers at branch line demonstrate bootstrap support with 1000 replicates. A-I: Seagrass species from Sanur Beach, 1-9: Seagrass species from Sindhu Beach

Table 2. Characteristics of the *matK* sequences from seagrass species collected from Sanur Beach and Sindhu Beach, Bali, Indonesia

Sample code	Species name	Size* (bp)	Readable sequence (bp)	Accession number	Organism	Sequence identity	E-value
A_1	*E. acoroides*	950	856	AB002569.1	*E. acoroides*	100	99
B_1	*T. hemprichii*	950	856	AB002577.1	*T. hemprichii*	99	99
C_1	*S. isoetifolium*	950	849	KF488511.1	*S. filiforme*	99	95
D_1	*Th. ciliatum*	950	856	AB002577.1	*T. hemprichii*	92	99
E_1	*H. uninervis*	950	856	JN225379.1	*H. wrightii*	99	99
F_1	*H. pinifolia*	950	855	JN225379.1	*H. wrightii*	100	98
G_1	*Ha. ovalis*	950	856	AB002570.1	*Ha. ovalis*	99	99
H_1	*C. serrulata*	950	855	JN225359.1	*C. serrulata*	99	98
I_1	*C. rotundata*	950	556	KF488504.1	*C. rotundata*	100	97
1	*E. acoroides*	950	855	AB002569.1	*E. acoroides*	99	99
2	*S. isoetifolium*	950	846	KF488511.1	*S. filiforme*	99	96
3	*Th. ciliatum*	950	856	AB002577.1	*Th. hemprichii*	94	99
4	*H. pinifolia*	950	855	JN225379.1	*H. wrightii*	100	98
5	*H. uninervis*	950	856	JN225379.1	*H. wrightii*	98	99
6	*Ha. ovalis*	950	856	AB002570.1	*Ha. ovalis*	99	99
7	*C. serrulata*	950	856	JN225359.1	*C. serrulata*	99	98
8	*C. rotundata*	950	856	KF488504.1	*C. rotundata*	100	97
9	*T. hemprichii*	950	856	AB002577.1	*T. hemprichii*	99	99

Note: * = Determine by gel electrophoresis. A-I: Seagrass species from Sanur Beach, 1-9: Seagrass species from Sindhu Beach

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Ethnoastronomy-The Baduy agricultural calendar and prediction of environmental perturbations

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Abstract. *Iskandar J, Iskandar BS 2016. Ethnoastronomy-The Baduy agricultural calendar and prediction of environmental perturbations. Biodiversitas 17: 694-703.* In the past, the village farmers of Java and other islands owned extensive the traditional ecological knowledge (TEK) on climate or *pranata mangsa*. It had culturally practiced as guidance to various agricultural activities, such as planting rice which is considered and fixed with dynamic climate conditions. Nowadays, however, the *pranata mangsa* has eroded and neglected by the majority irrigated rice (*sawah*) farmers. Unlike the *sawah* farmers, the Baduy people have culturally maintained the *pranata mangsa* (called by Baduy as *pananggalan*) for annual practicing the swidden farming (*ngahuma*). This paper discusses the way in which cultural practices of Baduy swidden farming based on traditional calendar. Method used in this study qualitative which is based on ethnoecology or ethnoastronomy approach. The result of study shows that the Baduy rice farming cycle is fixed annually with reference to an agricultural calendar. It has slightly affected by the various environmental perturbations, because the Baduy people have developed some strategies, such as by organizing the traditional calendar and applying the traditional agroforestry that productions can be used for both subsistence and commercial purposes.

Keywords: Baduy, ethnoastronomy, environmental perturbations, traditional calendar, swidden farming

INTRODUCTION

A farm system is a very complex that is generally influenced by biophysical factors and socio-economic and cultural factors, including the rural society's value, knowledge, skills, and technologies (Rambo and Sajise 1984; Lovelace 1984; Beets 1990; Reijntjes et al. 1992). In the past, the traditional rice farming system of West Java and Banten was managed by local knowledge (*corpus*) and cosmos/belief and local institution (cf. Mustapa 1996; Toledo 2000). For example, both the swidden farmers (*petani ladang*) and the wet-rice farmers (*petani sawah*) had plentiful local knowledge on climate because their farming systems are annually influenced by the various climate conditions. As a result, it has widely been recognized that farmers in cross-cultural in different areas of Indonesia had traditionally applied an agricultural calendar which is called *pranoto mongso* or *pranata mangsa* (van den Bosch 1980; Daldjoeni 1984; Arsana et al. 2003; Hidayat 2011; Wiramihardja 2013; Retnowati et al. 2014; Ammarell and Tsing 2015). The *pranata mangsa* system is not less complex with the calendar of ancient Egypt, China, Maya, and Burma (Sindhunata 2011). In the *pranata mangsa* system has a complex and close interaction between cosmography and bioclimatology which is as a fundamental farmer live society (Daldjoeni 1984; Hidayat 2011). On the basis of environmental or ecological history, *pranata mangsa* has been recognized as the traditional ecological knowledge (TEK) which is inherited by the oral through inter generations for a long ago. The *pranata mangsa* as a TEK has some characteristics, such as inherited by oral, teaching through doing, holistic, subjective and experiential based on trial and error in the agricultural system, based on intensive interrelationship between farmers and their local environment (cf. Ellen and Harris 2000; Silitoe 2002).

Before introduction of the green revolution, before earlier 1970s, rice farmers in Java and Bali had traditionally managed planting rice with the same time in simultaneity. They had used water in efficient, and management of pest based on the traditional ecological knowledge (TEK), such as implementing the *pranata mangsa* and cosmos or belief (cf. Gelpke 1986; Lansing 1991). However, by introduction of the green revolution, the *sawah* cultivation system had dramatically changed. The green revolution had affected both positive and negative aspects.

The main positive or benefit of the green revolution has been increase in rice farming productivity in macro level. Some negative aspects, however, have also occurred. For example, the new rice cultivation system is more dependent on subsidies from outside. In addition, ecologically damage such as pest outbreaks, loss of local genetic diversity of rice, and environmental pollution have also occurred (cf. Fox 1991; Lansing 1991). Indeed, the traditional ecological knowledge of the *pranata mangsa* has seriously eroded.

Unlike the rice farmers, the Baduy people who reside in South Banten, have traditionally maintained the traditional

calendar (*pananggalan* or *pranata mangsa*) and embedded with cosmos to manage their swidden farming in sustainable system despite various environmental perturbations, such as long drought and an abnormal high rainfall have frequently occurred and affected the Baduy swidden farming system.

This paper discusses the way in which cultural practices of Baduy swidden farming based on traditional calendar and tended to able to adapt to the environmental perturbations, particularly anomaly climate caused of the global warning.

MATERIALS AND METHODS

Study sites

The Baduy area was recognized as the communal land (*Tanah Ulayat*) decided by the local regulation of District of Lebak No. 32 year of 2001, with has size 5.136,58 hectares. Geographically Baduy village is located at $6^0$27'27''-6^0 30' South and longitude $106^0$3'9"-106^0 4' 5" East. On the basis of the culture, the Baduy area can be divided into two main groups, i.e. Inner Baduy (*Baduy Jero, Baduy Dalam*) and Outer Baduy (*Baduy Luar, Baduy Panamping*). Inner Baduy consist of 3 permanent hamlets, Cikeusik, Cikartawarna, and Cibeo, while the Outer Baduy composes of 59 hamlets, such as Kampung Kaduketug Gede, Kaduketug I, Kadukaso, Cipondok, Cihulu, Marengo, Gajeboh, Cibalingbing, Cigula, Kadujangkung, and Karahkal located in the north of Inner Baduy. The hamlets are scattered along valleys near the Ciujung river and its tributaries, or near other water resources, at altitude of between 170 m and 410 m asl. In addition, initially there are Dangka area (*Kawasan Dangka*) consists of 7 areas, namely Cihandam, Kamancing, and Kompol located in the Muslim enclave and lead by the informal leader representative (*Jaro Dangka*). Today, however, several *Dangka* areas, such as Cihandam and Kamancing have been taken over by the Muslim community and the *Jaro Dangka* have been moved to Kaduketug. However, an interim of the government administrative, the Baduy area is legalized as a village (*desa*) of Kanekes, sub-district (*kecamatan*) of Leuwidamar, district (*kabupaten*) of Lebak, province (*provins*i) of Banten, Indonesia (Figure 1).

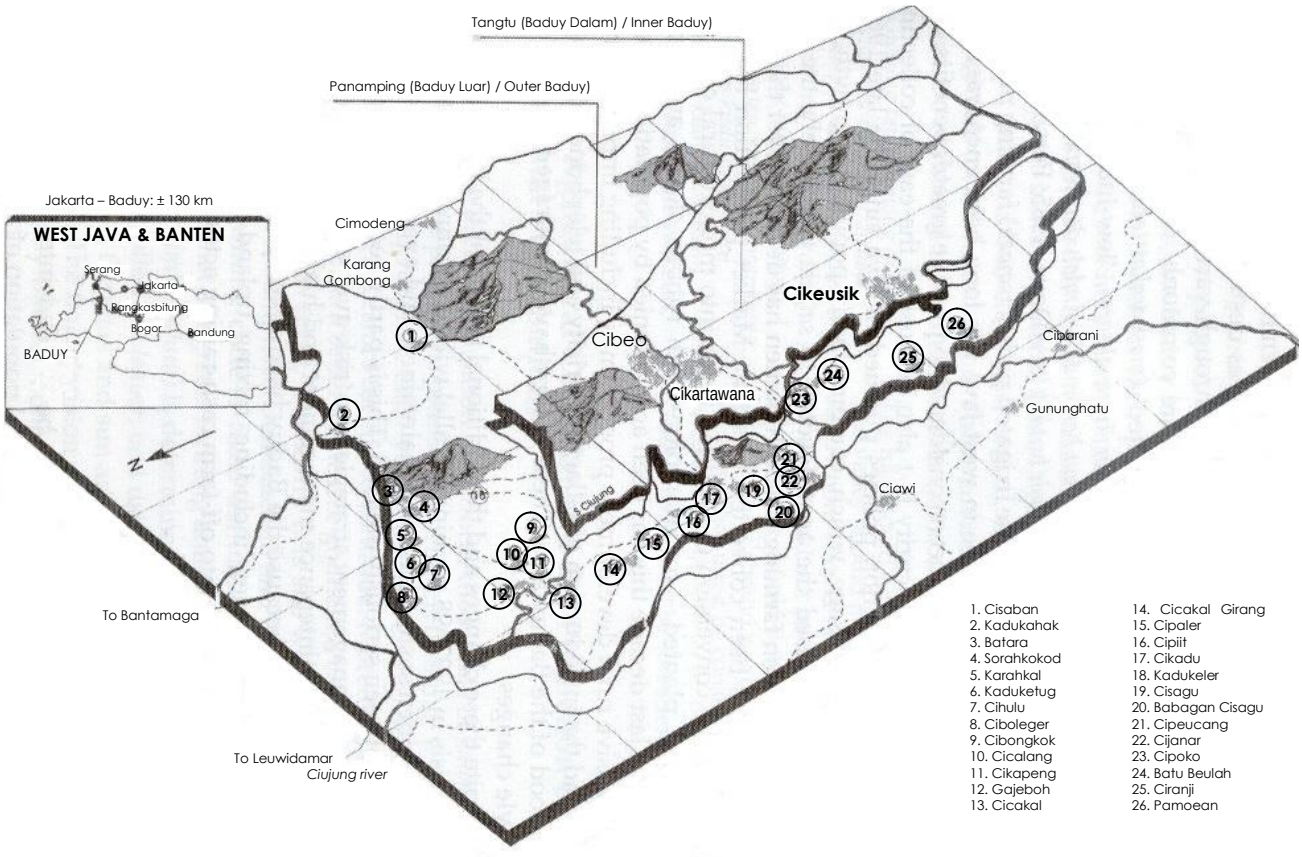

Figure 1. Study site Baduy area of Kanekes village, Leuwidamar sub-district, Lebak district, Banten province (adapted from Rangkuti 1988)

In 2010, population of Baduy was recorded 11,172 people, representing 2,948 households consisting of Inner Baduy 1,170 people (10.48 %) and Outer Baduy 10,002 people (89.52 %). While in 2015, the total population of Baduy increased to 11,620 people representing 3,395 households. Swidden cultivation is the main source of Baduy subsistence. However, Baduy women are also involved in making traditional woven cloth (*kain tenun*). Some Baduy men also involved in making traditional bags from bark cloth; *koja* and *jarog* which are produced for personal use, as well as being sold to visitors or to small shops (*warung*) in their village or in the neighboring area. Trade in non-rice crops, such as durian (*Durio zibethinus* Murr), petai (*Parkia speciosa* Hassk), banana (*Musa paradisiaca* L), and brown sugar of aren (*Arenga pinnata* (Wurmb) Merr) is also important for some Baduy people.

Procedure

This study was applied qualitative method and used of the ethnoecology and ethnobiology approach (cf. Newing et al. 2011; Iskandar 2012; Albuquerque et al. 2014). To collect some primary data, the observation, participant observation and deep interview with competent informants were applied. Observation was conducted to observe conditions of human settlements, swidden fields, and forests. Participant observation was applied by involving the researchers in some swidden farming activities of informants, including cutting shrubs and trees (*nyacar* and *nuar*), planting rice (*ngaseuk*), weeding (*ngored*), harvesting rice (*panen pare*) and various traditional rituals. The first author had completely involved in almost every stage of swidden farming due to intensively conducted the field research for master thesis program in 1984/1985 (Iskandar 1991) and in 1994/1995 for the dissertation program (Iskandar 1998). In addition, between 2000 and 2015 we took every possible opportunity to visit Baduy village to accompany students to conduct the field research in this area. While, deep interview was applied with some competent informants which was purposively selected and heterogeneity or categorization are considered. Some informants were mainly selected, mainly village formal leader (*jaro pamarentah*), village secretary (*carik desa*), hamlet informal leaders (*kokoklot lembur*) of Outer Baduy, *Jaro Tanggungan Dua Belas* of Outer Baduy, informal leader staff of Inner Baduy (*Jaro Tangtu*), several old farmers of Inner and Outer Baduy. In deed, in terms of the study on ethnoastronomy, we undertaken deeply interviewed with local experts on the traditional calendar, including informal leader staff and old farmers, and some time we participated in observing the position of *bentang kidang* (the belt of Orion) on the horizon at dawn, and observed and identified of the biological indicators for predicting the annual seasonal changes of the traditional Baduy calendar, including various flowering plants and soil spider.

Data analysis

Analysis data involved cross-checking, summarizing and synthesizing from different sources, including observation, participant observation, semi structure or deep interview, and secondary data, and built up a narrative accounts as a descriptive analysis which is focused on the annual Baduy swidden system management which is based on the traditional calendar (*pranata mangsa* or *pananggalan*) (cf. Iskandar 2012; Newing et al. 2011).While various species of plants that are culturally used as indicator for predicting the annual season changes of the traditional Baduy calendar were identified in the Herbarium Bogoriense, Indonesian Institute of Sciences (LIPI), Cibinong, Bogor, West Java, Indonesia.

RESULT AND DISCUSSION

The swidden cultivation as an obligation of Baduy

Since the early nineteenth century various names have been given by outsiders to the traditional Sundanese minority who live in the area of 'Desa Kanekes': *urang Baduy* (Baduy people), *urang Rawayan* (Rawayan people), *urang Kanekes* (Kanekes people), and *urang Parahiang* (Parahiang people). Although the name 'Baduy' is now established among outsiders, it is strongly rejected by Baduy themselves. They prefer to call themselves *urang Kanekes* (Danasasmita and Djatisunda 1986, Iskandar 1998). According to some scholars (e.g. Ekadjati 1985; Iskandar 1998; Wessing and Barendreght 2005) there are three main theories concerning their origin. The first is that they are descendant of people who managed to escape from the Hindu kingdom of Pajajaran, near present day Bogor, before Islamic forces from the Sultanate of Banten destroyed it AD 1579. The second is that they are descendant of Hindu people who originally lived in Banten but who fled to present day Kanekes from Islamic forces of Sultanate Banten. The third theory, based on old Sundanese text, is that Baduy are descendants of an ascetic group living in sacred parts of the forest in pre-Islamic times. Such places and people were usually called *mandala* areas and *mandala* communities.

On the basis of the culture, unlike the Sundanese ordinary, in their daily lives, the Baduy have tried to keep their original culture as pure as possible based on their ancestry (*karuhun*), particularly in the practicing of swidden farming (*ngahuma* or *berladang*). They would like to live in harmony with their environment, the forest. So Baduy life is regulated by many prohibitions, such as planting rice in wet-rice-fields (*sawah*), using chemical fertilizers, pesticides, selling swidden rice, rearranging buffalo and sheep, growing monoculture commercial crops, such as rubber, teak, and clove; digging wells, and poisoning wild animals and fish (Iskandar 1998; Ichwandi and Shinohara 2007). Baduy life is regulated many prohibitions related to their concept of sacred place. It is central to their belief that they maintain a simple way of life (*hidup sederhana*), faitfull to their ancestry regulated by asceticism (*tapa*); in contrast to the hectic life of the modern world (*ngaramekeun negara*). They prefer honesty (*bener*) to cleverness (*pinterteu bener*), honoring the various obligations they have to their ancestors.

Culturally, Baduy respect six main obligations in their daily lives: (i) *ngersakeun Sasasaka Pusaka Buana* (they should keep the holly place of *Sasaka Buana*); (ii)

ngersakeun Sasaka Domas (they should keep the holly place of *Sasaka Domas*); (iii) *ngasuh ratu ngajayak menak* (they should take care of King or Sultanate or President and noble families in the present time); (iv) *ngabaratakeun nusa telu-puluh-telu,bagawan sawidak lima, pancer salwe nagara* (they should ascetic rituals for thirty tree hamlets, sixty five sub-rivers, and twenty five country centers); (v) *kalanjakan kapundayan* (they should hunt animals and catch fish for *kawalu*; and (vi) *ngukus kawalu muja ngalaksa* (they should burn incense in conducting ascetic, to perform ritual of *kawalu* and *ngalaksa*) (Danasmita and Djatusunda 1986; Iskandar 1998; Wessing and Barendreget 2005).

On the basis of these duties, for Baduy, swidden cultivation is necessary part of the annual obligation to perform *kawalu* and *ngalaksa*. In other words, rituals such as *kawalu* and *ngalaksa* firmly unite of swidden cultivation with their religion, *Sunda Wiwitan*. Each year swidden rice (*pare huma*) must be offered to the ancestor (*karuhun*) for life to continue. Swidden cultivation is necessary, therefore, for the effective practice of Baduy religion; it is not simply a matter of subsistence economics. Moreover, practicing swidden cultivation is a religious, even when it appears to be economically irrational. Although Baduy traditional farming had been opposed and given negative stereotype by the government since the Dutch (cf. Kools 1935; Dove 1985; Li 2000), nowadays, it has still widely practiced due to considered by Baduy as their religion obligation.

Six types of swiddens (*huma*) recognized in Baduy: (i) *huma serang*, swidden belonging to enter community, both Inner and Outer Baduy, consist of *huma serang* of Cibeo, Cikartawarna, and Cikeusik, located in the southern hamlet area of Inner Baduy (and never overlapping with plot types, considered very sacred); (ii) *huma puun*, swiddens belonging to and managed by families of the religious leaders (*puun*), consists of *huma puun* of Cibeo, Cikartawarna, and Cikeusik obtained through inheriting from their ancestral, located in special place in the southernmost part of each Inner Baduy hamlet; (iii) *huma girang seurat*, swiddens belonging to and managed by families of the informal leader who assist the *puun* (*girang seurat*), located in a special place attached to the *huma serang*; (iv) *huma jarodangka* or *huma tauladan*, consists of 7 *huma tauladan*, swidden belonging to and managed by informal leader staff or assistants of *puun*, called *jaro dangka* living in *dangka* area. These are considered less sacred than *huma serang*, and used as a model swidden in Outer Baduy. Some activities, particularly planting rice, involve cooperative ritual; (v) *huma tangtu*, swiddens belonging to each household of Inner Baduy, mostly in the north of each Inner Baduy hamlet. Obtained through ancestral felling mature forest; and (vi) *huma panamping*, swiddens belonging to Outer Baduy households, obtained on loan and through inheritance, rent share-cropping or exchange for labor from neighboring non-Baduy. On the basis of annual cultivation practice, the Baduy swidden types, i.e. *huma serang*, *huma puun*, and *huma masyarakat* are always sequentially cultivated, which is culturally based on specific traditional calendar of Baduy (*pananggalan Baduy* or *pranata mangsa*).

Traditional calendar and working activities of the swidden farming system

For the Baduy, the annual obligation to perform the rituals of *kawalu* and *ngalaksa* is an integral part of the practice of swidden farming, which thus unites agricultural and religion. *Kawalu* derives from *walu* meaning *bali*, *balik*, *kabali* or *kembali* (comeback). The ritual is undertaken after harvesting rice of *huma serang* that is considered very sacred. The rice has been carried back from the swidden plot to the hamlet and placed in the rice barn (*leuit*). At that time, rice is considered to have 'comeback' to the rice barn after staying with her husband on earth (*bumi=pertiwi* or swidden plot). Because based on Baduy culture, it is believed that in sowing rice, the rice goddess, *Nyi Pohaci* (*Dewi Sri* in Javanese) becomes engaged (*direremokeun*) to the earth, *pertiwi* (Danasasmita and Djatisunda 1986; Iskandar 1998). According to Baduy beliefs, the fast must be conducted during *kawalu* to fulfill an obligation and continue the ritual work of their ancestors. The *kawalu* ritual is considered very important and must offer the cooked of new rice harvested from the *huma serang* to their ancestors and can determine the beginning of the first day of the new years (*tindak tahun* or *tunggul tahun*). In other words, although various methods, such as environmental indicators, including the appearance and disappearance of certain stars, and appearance of certain flowering plants and animals are used to determine *tindak tahun*, the most important factor determine *tindak tahun* is the harvesting time of the *huma serang*, as three months before *tindak taun*, the ritual *kawalu* must be performed. Therefore, if there is a delay in harvesting rice from the *huma serang*, the *kawalu*, *ngalaksa*, and *seba* ritual will also be delayed, as will the next *tindak tahun* (Iskandar 1998; Iskandar 2007).Because the *kawalu* can be considered very important in determining the Baduy calendar, therefore, the names of Baduy month are now rather confusing, particularly compared to the original names of the Javanese calendar (*pranata mangsa*). For example, the first month of the Baduy Calendar on *Sapar* or *Kapat* instead of *Kasa* of the traditional Javanese calendar, while *Kasa* of Baduy calendar is known as the first *Kawalu* or *huma serang* harvesting time. The completed Baduy calendar can be seen in Table 1.

On the basis of Baduy calendar, one year is divided into 12 months which each month always constant consists of 30 days instead of between 23 and 43 days of Javanese calendars (cf. van den Bosch 1980; Ammarell 2005; Ammarell and Tsing 2015). Various methods such as *kolenyer* and environmental indicators, namely position of stars, fruiting certain kind of plant and animal are culturally used to determine *tindak tahun*, and the final decision is usually made by the traditional leader (*puun*) of Cibeo. The *kolenyer* is traditionally used to calculate *naptu* which is made by wood of 6 cm x 25 cm. The various symbols written in the both front and back of *kolenyer*, such cross line, empty, one dot and four dots. These symbols have special meaning namely, cross line, empty, one dote, and four dotes means *pati* (unlucky), *neutral*, *milik leutik* (little luck), and *milik gede* (much luck), respectively. In the front of *kolenyer* written symbols are used to locate auspicious

times for engaging in special works. For example, on Sunday *isuk-isuk* (5.00-9.00), *tengah naek* (9.00-12.00), *tangange* (12.00-13.00), *lingsir* (13.00-14.00) and *burit* (14.00-16.00) is considered as unlucky, little luck, much luck, neutral, and much luck, respectively. Therefore, if, for example, someone wants to hunt animals or to press for payment of a debt on Sunday, the auspices time must be chosen in the afternoon; during *tangange* and *burit* because these times are considered to be more auspicious. While, various symbols in the back are used to locate auspicious direction. For example, direction of arrival on a Sunday should be to the west, east and south, while direction of departure must be to the north east.

Arranging the annual calendar is usually done by expert, *dukun*. Today, however, there remain few *dukun* who are recognized as experts who can assist the *puun*. Environmental indicators, *kikandayan tani*, are used to decide the beginning of the farming year. Both *bentang kidang* (the belt of Orion) and *bentang kartika* (the Pleiades) are usually observed on the horizon at dawn. Particularly, the Baduy people regulate various phases of the swidden farming cycles with reference to the position, appear and disappear of *kidang* (Kools 1935; Iskandar 1998, 2007). This was described by informants as follow:

Tanggal kidang turun kujang	When *kidang* first appears, a chopping knife should be used
Kidang ngarangsang kudu ngahuru	When *kidang* appears in a position similar to that of the sun at 8.00-10.00 a.m., vegetation should be burned
Kidang nyuhun atawa condong ka barat kudu ngaseuk	When *kidang* appear overhead or sideways to the west, rice should be planted
Kidang marem turun kungkang, ulah melak pare	When *kidang* disappears, insect pests will appear, and rice planting should stop

On the basis of various position of *kidang*, it can be integrated with various phases of the annual swidden farming cycle. Normally *kidang* is observed appearing on the east horizon at dawn corresponds to *Sapar* or *Kapat* (April-May) of the Baduy calendar. At this month the vegetation must be cut for preparing the swidden (*ladang* or *huma*) plot, particularly for the *huma serang* (Table 2). The *kidang* position is observed in a position similar to that of the sun at 8.00-10.00 above Eastern horizon at dawn corresponds to *Kanem* (June-July) and burning vegetation of the *huma serang*. The next month, the *kidang* position is observed overhead (zenith) or sideways, usually corresponds to *Kapitu* (July-August), considered to commence to planting rice of the *huma serang*, the burning vegetation (*ngaduruk*) of *huma puun*, and the cutting shrubs (*nyacar*) of *huma masyarakat*. While the *kidang* has could not be seen or disappeared on west horizon at dawn corresponds to *Hapit Kayu* (December-January), rice planting should stop. It is considered to be inappropriate to plant rice, because the soil is too 'hot' and insect pests (*kungkang*) come to *buana tengah*. According to Baduy cosmology, the world can be divided into three parts: *buana tengah* (the presence world), *buana handap* (the world where the human body is buried after death), and *buana luhur* (the hereafter). Other environmental indicators, such as flowering certain plants and animal behaviors are also used as indicator to commence to undertake certain phase of the swidden farming cycle. For example, based on information of the informants, besides traditional calculations used *Kolenyer*, observing the belt of Orion (*kidang*), the times when plant flower and fruit can also be used as indicators to determine certain month of the Baduy calendar. For example, *kanyere* (*Bridelia monoica* (Lour.) Merr), *jampang kidang* (*Centotheca lappacea* (L.) Desv.) and *jampang kerti* (*Centotheca* sp.), the flowering and fruiting of which is usually synchronizes with the appearance *bintang kidang* on Eastern horizon or the dry season. In addition, *lancah kidang* (the soil spider) indicates the time when people should start planting rice. The *lancah kidang* usually make its nest on grasses growing in swidden fields. If her web has a hole in the middle, and she stays most of time on the edge of the nest, rice planting in the swidden should start.

Table 1. Comparison of the Baduy calendar and Javanese *pranata mangsa*

Baduy calendar*)				Javanese calendar**)		
Month	**Month name**	**Duration in days**	**Corresponds to**	**Mangsa**	**Duration in days**	**First day civil calendar**
1	Sapar or Kapat	30	April-May	Kasa (1st)	41	21 or 22 June
2	Kalima	30	May-June	Karo or Kalih (2nd)	23	1 or 2 August
3	Kanem	30	June-July	Katelu or Katiga (3rd)	24	24 or 25 August
4	Kapitu	30	July-August	Kapat or Kasakawan (4th)	25	17 or 18 Sept.
5	Kadalapan	30	August-September	Kalima or Gangsal (5th)	27	12 or 13 October
6	Kasalapan	30	September-October	Kanem (6th)	43	8 or 9 November
7	Kasapuluh	30	October-November	Kapitu (7th)	43	21 or 22 December
8	Hapit lemah	30	November-December	Kawolu (8th)	26 or 27	2 or 3 February
9	Hapit kayu	30	December-January	Kasanga (9th)	25	ult.Feb. or March
10	Kasa	30	January-February	Kasepuluh or Kasadasa (10th)	24	25 or 26 March
11	Karo	30	February-March	Desta (11th)	23	18 or 19 April
12	Katiga	30	March-April	Sada (12th)	41	11 or 12 May

Note: *) The field research (Iskandar 1991, 1998, 2007). **) Adapted from van den Bosch (1980); Ammarell (2005); Ammarell and Tsing (2015)

About five months after sowing, or in *Kasa* (January-February), *huma serang* rice matures and is ready to be harvested. Moreover, the new rice must be offered to their ancestors in the ritual of the first *kawalu* (*kawalu kahiji* or *kawalu tembey*).Thus, to conduct the first *kawalu ritual* (*kawalu kahiji*) in Inner Baduy, the new rice harvested in *huma serang* must be used. In addition, the new rice of *huma serang* can be used to conduct other rituals, namely *ngalaksa* in *Katiga* (March-April) and *seba* in *Sapar* (April-May). Because the rituals of *kawalu*, *ngalaksa* and *seba* must use new rice of *huma serang*. Therefore, if there is a delay in harvesting rice from *huma serang*, the *kawalu*, *ngalaksa* and *seba* will also delayed, as will the *next tindak tahun*, as happened in the farming years of 1994/95 and 1997/98 due to long drought. In normal circumstances, *huma serang* rice must be harvested during *Kasa* (January-February) (Table 2). However, because in 1994 there had been a drought, the *huma serang* rice was harvested during *Katiga* (March-April). Consequently, the first *kawalu* was performed during *Katiga* (March-April) instead of *Kasa* (January-February), the second *kawalu* during *Sapar* (April-May) instead of *Karo* (February-March) and the third *kawalu* during Kalima (May-June) instead of *Katiga* (March-April). In addition, *tindak tahun* was fixed for *Kanem* (June-July) instead of *Sapar* (April-May). Therefore, for re-adjustment with the dynamic of climate conditions, particularly rainfall conditions, in every month and in each year this calendar by a ritual specialist (namely *puun Cikeusik*), who resynchronizes the calendar with the rotation of the *bentang kidang* and with the flowering and fruiting season of particular species (Iskandar 1998, 2007). To determine the beginning of annual new year is determined by the harvesting of *huma serang*, the various position of *bentang kidang*, the flowering and fruiting particular plant species, animal behavior (soil spider), and auspicious day (*hari bagus*), and is used standard that the total number of day in each year 360 days instead of 364/365 (Table 2).In other words, the total number of day in the Baduy's calendar is 360 instead of 364/365 days of BC calendar (*masehi*) or Muslim calendar (*hijrah*). It is caused of between 4 and 5 days are usually excluded in the traditional calendar Baduy due to *puun* perceptions considered as not appropriate. Therefore, the *puun* is an important role in setting a date for the new year of the Baduy's calendar and can determine to the success and failure of practicing swidden farming. For example, it is commonly expressed by informants as; " one of main tasks of informal leader (*puun*) is to calculate time of the traditional calendar for the benefit of all of the Baduy community (*tugas puun ngitung waktu ngeja bulan kalender keur sarerea Urang Baduy*)".

On the basis of the Baduy calendar (Table 2) it can be seen the Baduy informal leaders (*puun*) and his staff organize their swidden agriculture activities, including various rituals which are appropriately adapted to seasonal monsoon cycle of the Baduy for centuries. For example, cutting vegetation of the *huma serang*, *huma puun* and *huma masyarakat* is normally undertaken in *Sapar* (April-May), *Kalima* (May-June) and *Kanem* (June-July) during the low rainfall or dry season. This time is considered appropriate time to cut vegetation and is properly burned dry biomasses to produce ash of composing nutrients for growing rice and other crops. Planting rice of the *huma serang*, *huma puun*, and *huma masyarakat* is undertaken on *Kapitu* (July-August), *Kadalapan* (August-September), and *Kasalapan* (September-October), respectively that is considered as suitable time for planting rice due to starting rainy season or increasing rainfall. While the harvesting rice of *huma serang*, *huma puun*, and *huma masyarakat* is done on *Kasa* (January-February), *Karo* (February-March), and *Katiga* (March-April), respectively in correspond to decrease rainfall or starting the dry season that is considered as right time to dry new harvested rice. Given this example, it can be inferred that Baduy people aware that are surrounded by seasonal rhythms including climatologically changes, such as rainfall, humidity, and winds that are closely related with phenological changes in flora, fauna, position of stars and sun; as a result, they must variably adapted to the climate changes manifested in agricultural calendar. Baduy believe that if they depart from the traditional seasonal pattern of their calendar (*pananggalan* or called *pranata mangsa* in non-Baduy), their work will fail either totally or partly (cf. van den Bosch 1980; Daldjoeni 1984; Brossius et al. 1986; Ammarell 2005; Hidayat 2011; Ammarell and Tsing 2015).

Environmental perturbations

In the past, the village farmers of Java, Bali, Borneo, Sulawesi, and other parts of Indonesia, traditionally cultivated rice based on local knowledge, belief or cosmos, and local institution (cf. Wessing 1978; van den Bosch 1980; Gelpke 1986; Adimihardja 1992; Mustapa 1996; Wisnubroto 1999; Lahajir 2001; Arsana et al. 2003; Ammarell 2005; Sani and Nuhaedar 2007; Iskandar and Iskandar 2011; Ammarell and Tsing 2015). One of the set traditional knowledge (TK) or traditional ecological knowledge (TEK) is the agricultural calendar or *pranata mangsa*. Unlike Western knowledge, the TEK is recognized as" a cumulative body of knowledge, practice, and belief, evolving by adaptive processes and handed down through generations by cultural transmission, about the relationship of living being (including humans) with one another and with environment" (cf. Berkes 1999, Alexander et al. 2011). Thus, unlike the scientific Western knowledge, the TEK has some characteristics, namely holistic, subjective, communicated by oral, teaching through doing and inherited by oral, and its value for environmental conservation and sustainable use and analysis and monitoring of long-term ecological changes (cf. Ellen and Harris 2000; Silitoe 2002; Maffi 2004).

On the basis of the ecological history, the *pranata mangsa* system has been an important role in contributing to achieve to the success and glory of the old Mataram Kingdom, Pajang, and Mataram Muslim or Mataram Sultanate. The *pranata mangsa* was intensively applied by the kingdom family members to practice of farming, trading, traveling, and running the royal government (Sindhunata 2011). Similarly, before introduction of the green revolution, before earlier 1970s, farmers in Java and Bali had traditionally managed planting rice with the same

time in simultaneity. They had used water in efficient, and management of pest based on the traditional ecological knowledge (TEK), such as implementing the *pranata mangsa* and cosmos or belief (cf. van den Bosch 1980; Gelpke 1986; Lansing 1991).Four seasons are traditionally recognized by the Sundanese people, namely wet season (*usum ngijih*), dry season (*usum katiga* or *usum halodo*), transition season from dry wet season to dry season (*usum dangdangrat*), and transition season from dry season to wet season (*usum mamareng*). To adapt with local environment, particularly the rain fall conditions, the wet-rice field (*sawah*) had been traditionally cultivated by farmers twice in each year. The main rice cultivation which is called *nyawah gede* (literally 'big rice cultivation' or 'main rice cultivation') or *ngawuluku* (literally 'plowing land') during the wet season, while the second cultivation or re-cultivation, called *nyawah leutik* (literally little rice cultivation), *morekat* (re-rice cultivation) or *malik jarami* (flip straws), had been carried out in the dry season. The astronomical indicators, the belt of Orion (*bentang kidang* or *bentang wuluku*) and the Pleiades (*bentang kerti*, *bentang ranggeuy*, *kartika* or *gumarang*) are the most important factor determination planting time of the wet-rice cultivation (cf. Wiramihardja 2013). In the recent past, before the impact of rice intensification, farmers had often been able to use efficient water availability. For example, after harvesting rice in the main season, if the water supply is still plentiful, before re-planting rice in the second rice planting, the wet-rice field had been reared various fishes, such as *ikan mas* or common carp (*Cyprinus carpio* L), tilapia or nila (*Osteochilus hasselti* Valenciennes) and *mujair* (*Oreochromis mossambicus* Peters) for only between 3 and 4 months. In addition, the rearing fishes had been mixed with rice crops (*sistem mina padi*) in the early planting season of rice cultivation only for about 3 months. By practicing of *mina padi* system had provided some ecological and socio-economic benefits, such as pest controlling, creating soil fertility, and providing fish production. Conversely, if the lack of water supply, after the rice harvesting, the wet-rice filed had been cultivated by non-rice crops (*palawija*), including corn (*Zea mays* L), cucumber (*Cucumis sativus* L), sweet potato (*Ipomoea batatas* (L) L) and peanuts (*Arachis hypogaea* L) (cf. Iskandar 2007). As a result, the requirement for water was low compared with that needed for the continuous and intensive planting of rice alone. Moreover, pests could usually be contained more easily as their population tended to decrease after the rice harvest with the dramatic reduction in their food supply and destruction of their habitat, as rice was replaced with other crops.

Table 2. Baduy agricultural calendar and associated with various works in swidden farming and ritual activities

Months	**Rainfall**	***Huma serang**)**	***Huma puun* **)**	***Huma masyarakat* ***)**	**Rituals**
Sapar (April-May)	200-250 mm	Cutting shrubs (*nyacar*)	Fallowed	Fallowed	*Seba*
Kalima (May-June)	100-150 mm	Felling trees (*nuar*)	Cutting shrubs (*nyacar*)	Fallowed	*Puun ziarah* to sacred place, and *hajatan;* circumcision (*sunatan*); wedding (*kawinan*)
Kanem (June-July)	100-150 mm	Burning vegetation(*ngaduruk*)	Felling trees (*nuar*)	Fallowed	Wedding (*kawinan*)
Kapitu (July-August)	100-150 mm	Reburning (*ngahuru*) and planting rice (*ngaseuk*) in *huma serang*	Burning vegetation (*ngaduruk*)	Cutting shrubs (*nyacar*)	*Ngaseuk humaserang;* circumcision (*sunatan*); wedding (*kawinan*)
Kadalapan (August-September)	120-160 mm	First weeding (*ngored munggaran*)	Reburning vegetation (*ngahuru*) and planting rice (*ngaseuk*)	Felling trees (*nuar*), burning	*Narawas* and *Nukuh* of *huma masyarakat*
Kasalapan (September-October)	200-250 mm	Second weeding (*ngored ngarambas*)	First weeding (*ngored munggaran*)	Reburning, and planting rice	*Ngaseuk* of *huma masyarakat*
Kasapuluh (October-November)	250-350 mm		Second weeding (*ngored ngarambas*)	First weeding (*ngored munggaran*)	*Ngirab sawan* of *huma masyarakat*
Hapit lemah (November-Desember)	350-400 mm			Second weeding (*ngored ngarambas*)	*Ngirab sawan* of *huma masyarakat*
Hapit Kayu (December-January)	350-450 mm				
Kasa (January-February)		Harvesting (*panen*) *huma serang*			*Kawalu kahiji*
Karo (February-March)			Harvesting (*panen*) *huma puun*		*Kawalu tengah*
Katiga (March-April)				Harvesting (*panen*) *huma masyarakat*	*Kawalu tutug* and *ngalaksa*

Note: *) *Huma serang*-three of communal sacred swiddens located in Cibeo, Cikartawarna and Cikeusik of Inner Baduy. **) *Hum puun*-three of swiddens owned by Baduy informal leader located in Cibeo, Cikartawarna and Cikeusik of Inner Baduy. ***) *Huma masyarakat*-swiddens of ordinary Baduy household (*huma masyarakat*) of Inner and Outer Baduy.

However, by introduction of the green revolution in early 1970's consists of the application of a package of inputs through had top down and homogenous the five endeavors of rice intensification programs (*panca usaha tani*), i.e. introduction of the new high-yielding rice varieties (HYVs), such as IR-8, IR-5, IR-22 and IR-24 mainly produced by the International Rice Research Institute (IRRI) in Los Banos, the Philippines; using synthesis inorganic fertilizer produced by factories; using intensive pesticides; improvement cultivation practice; and development and improvement of irrigation systems—the sawah cultivation system had dramatically changed (Iskandar 2001; 2014). The green revolution had affected both positive and negative aspects. The main positive or benefit of the green revolution has been increase in rice farming productivity in macro level. Some negative aspects, however, have also occurred. For example, the new rice cultivation system is more dependent on subsidies from outside. In addition, ecologically damage such as pest outbreaks, loss of local genetic diversity of rice, and environmental pollution have also occurred. The homogenous rice crop varieties planted continuously rice-rice-rice in the same fields three times in one year, and not simultaneous plant and harvest rice have become vulnerable to diseases and insect destruction, particularly the brown plant hopper (*wereng coklat*, *Nilaparvata lugens* Stal) and water deficit in the dry season (cf. Fox 1991; Lansing 1991; Bardini 1994). Indeed, the traditional ecological knowledge of the *pranata mangsa* has seriously eroded. As a result, at the present time the anomaly climate due to global warming has frequently occurred and its impact has been globally felt (cf. Rosenzweig et al. 2000; Orlove et al. 2002; Nabegu 2009; Alexander et al. 2011; Boillet et al. 2011), the rice farmers in different areas of Indonesia have become vulnerable to fail rice harvesting (*puso*).For example, in 1997 a drought was reported as due to the El Niño and about 426, 000 hectares of wet-rice field (*sawah*) in Indonesia was disturbed (PEACE 2007). In more recently, in 2012 it was reported 2,345 hectares of sawah in West Java were not harvested (*puso*) (Saturi 2012). In general, the rice crops due to long drought are more serius than the high rainfall and flood (cf. Surmaini and Susanti 2009). However, there are indications that the sawah affected by flood in the previous season are most likely explosion of brown planthoppers (Boer 2010). Therefore, with increasingly frequent climate anomaly and exploitation of pests can disturb rice production and food insecurity (Syaukat 2011). In addition, the farmers have tended to powerless against increasing unpredictable climate anomaly among others caused by the erosion or loss of the traditional ecological knowledge (TEK) of the *pranata mangsa*. Whereas in the past farmers used very carefully observed natural phenomena and able to predict and to a certain extent able to predict perturbations, such as climate anomaly. In other words, by application of *pranata mangsa*, farmers had obtained benefit because they had encouraged recognized the natural character of each place (cf. Sriyanto 2009). Therefore, on the basis of the experiences in last decade, climate anomaly events such as long drought caused of El Niño and flood due to La Niña, have caused fail rice harvesting and have a high more risk for the future (cf. Bardini 1994; Sani and Nuhaedar 2007; Naylor et al. 2007; Keil et al. 2009; Surmaini and Susanti 2009; Boer 2010; Kusnanto 2011; Syaukat 2011; Solichah 2014) because of various factors, including the framers have lost of the traditional ecology knowledge (TEK) of *pranata mangsa* to predict and to adapt with dynamic climate changes.

Unlike sawah, the swidden farming traditionally practiced by the Baduy had only slightly affected by the drought as well as pest in the last decades. Because they have tried to adapt to climate changes by application of the traditional calendar (*pananggalan* or *pranata mangsa*), synchronously rice plant and rice harvest, burning of field before planting rice, fallow land, implementation of the traditional agroforestry systems, and development of diversification of off-farm jobs (cf. Rambo 1984; Altieri and Liebman 1986; Altieri 1993; Iskandar 2007; Turner 2012; Iskandar and Iskandar 2015). Although swidden rice (*pare huma*) did not harvest well due to drought, non-rice crops, such as fruit, vegetables and industrial crops farmed in the traditional agroforestry of swidden fallow (*reuma*) and in hamlet forests (*dukuh lembur* or *leuweung lembur*), were not seriously affected. Perennial fruit trees even tended to increase their yields in the year following the dry periods, because of the sunshine to which they were exposed at a critical period (Iskandar 2007).

On the basis of ecological history, unlike the wet-rice farmers, the Baduy have rejected the green revolution. They have managed the swidden system by applying organic farming and LEISA (Low External Inputs and Sustainable Agriculture) that modern inputs, such as chemical fertilizers, pesticide and modern rice varieties are prohibited to apply (cf. Reijntjes et al 1992). However, Baduy have not avoided with the environmental changes, such as weather and climate, but they have culturally adapted with these natural changes, namely by harmonizing the traditional calendar, *Pananggalan*. Thus, the Baduy's calendar is associated with the swidden cycle (cf. Iskandar 2007; Ellen 2016). It is arranged based on carefully observed natural phenomena changes of various indicators, such as the annual cycle of appearance and disappearance of the configuration the belt of Orion (*bentang kidang*), the flowering time of certain kind of plants, and animal behavior. The Baduy traditional calendar has also closely related to human welfare due to may influence to successful and fail of practicing swidden agriculture and moreover impact on abundance or scarcity of food, and to maintain the harmonization between human with the environment, and social harmony, some ritual performances are integrated in the traditional calendar. Therefore, the Baduy calendar has been very complex, integrated traditional ecological knowledge of atmospheric parameters, biological phenomena, and socio-cultural factors of local people (cf. Daldjoeni 1984; Ammarell 2005; Hidayat 2011). In other words, the swidden agricultural cycle and the ritual life integrated in the traditional ecological knowledge (TEK) of Baduy's calendar has been an important role in management local ecology to adapt with environmental disturbances, such as

climate changes. Therefore, due to climate change is a global phenomenon and its impacts have been felt globally and the studies have widely been undertaken globally across culture (Rosenzweig et al. 2000; Orlove 2002; Nabegu 2009; Kristoko et al. 2012; Boillet and Berkes 2013; Aryal and Choudury 2015), adaptation strategies must be specific to given location and needed of the local community participants (cf. Crate and Nuttall 2009; Boiessere et al. 2013). Indeed, collaboration between TEK of the local community and the Western scientists knowledge has been an important role in the context of development programs, such as to adaptation of agriculture on climate changes (cf. Warren et al. 1995; Silitoe 2002; Carlson and Maffi 2004). In general, the Baduy swidden farming has slightly affected by the various environmental perturbations, such as drought and flood disaster because the Baduy people have developed some strategies, including organizing a flexible traditional calendar, applying agroforestry traditional, and developing diversification of off-farm jobs (cf. Ichwandi and Shinohara 2007; Iskandar 2007). In conclusion, it can be said that with regard to development process, we further suggest that, rather than ignoring or attempting to replace or overcome the complex traditional ecological knowledge (TEK) systems which are embedded by cosmos or belief, it may be more useful to consider how these systems of ideas can be usefully incorporated into process of development and modernization to strengthen the sustainable agriculture in Indonesia.

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The diversity of secondary metabolites in Indonesian soybean genotypes

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Abstract. *Yusnawan E. 2016. The diversity of secondary metabolites in Indonesian soybean genotypes. Biodiversitas 17: 704-710.* Soybean secondary metabolites protect the crop from pathogen infection. These secondary metabolites especially phenolic compounds also have functional properties.This study aimed to determine total phenolic and flavonoid contents as well as antioxidant activity of Indonesian soybean genotypes. A total of 63 soybean genotypes with four different seed coat colors (green yellow, light yellow, yellow, and black) and different seed sizes (small, medium and large) was used in this study. Total phenolic content was measured using Folin-Ciocalteu's reagent and antioxidant activity was determined with 2,2-diphenyl-1-picrylhydrazyl. All six genotypes with black seed coat color had higher total phenolic and flavonoid contents as well as antioxidant activity than those in other colors' genotypes. Total phenolic contents of those six black soybeans ranged from 7.19 to 14.72 mg gallic acid equivalent per gram and total flavonoid contents varied from 1.91 to 5.30 mg catechin equivalent per gram. Antioxidant activities of these genotypes ranged from 10.99 to 20.38 µmol trolox equivalent per gram. An estimation of the total phenolic contents as well as antioxidant activities particularly in black soybeans was valuable and important for seeking soybeans with high antioxidant properties.

Keywords: Antioxidant, flavonoid, phenolic, soybean genotype
Abbreviations: AA = antioxidant activity, DPPH = 2,2-diphenyl-1-picrylhydrazyl, GAE = gallic acid equivalent, CE = catechin equivalent, TE = Trolox equivalent.

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is one of the legume crops which is rich in phytochemical compounds such as flavonoids, phenolic acids, saponins, and triterpenoids (Lee et al. 2009, 2011; Lee and Cho 2012; Zilic et al. 2013). These plant secondary metabolites protect soybean crops from pathogen infections, such as *Macrophomina phaseolina*, *Phytophthora sojae*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani* and *Phakopsora pachyrhizi* (Lygin et al. 2009; 2013; Bellaloui et al. 2012). Phenylpropanoids are known to be responsible during plant-pathogen interactions and have toxic properties or inhibitory to pathogens when either constitutively formed (phytoanticipins) or induced (phytoalexins) (Lygin et al. 2013).

Apart from inhibiting the plant pathogens in chemical defense mechanisms, the secondary metabolites in soybean especially soluble phenolic compounds of isoflavones and anthocyanins have functional and nutritional properties. In most cases, the availability of soluble phenolic compounds is dominant, therefore, contributing higher antioxidant properties than insoluble forms (John and Shahidi 2010; Cho et al. 2013). Flavonoids in soybean particularly isoflavones possess health benefits such as antioxidant, anticancer, antiosteoporosis, antibacterial, and anti-mutagenic (Messina 1999; Gallagher 2001; Kumar et al. 2010; Dixit et al. 2012). As antioxidant, soybean seed extract, genistein and daidzein show scavenging activity against 2,2-diphenyl-1 picrylhydrazyl (DPPH) (Malencic et al. 2007; Riedl et al. 2007). Anthocyanins as a dominant compound in black seed coat of soybean also contribute significant functional properties including antioxidant properties, anticancer, anti-inflammatory as well as cardiovascular disease prevention (He and Giusti 2010; Zhang et al. 2011; Lee and Cho 2012).

Concentration and composition of phytochemicals in legumes vary significantly based on the seed coat colors (Lee et al. 2010; Segev et al. 2010; Zilic et al. 2013). Several seed coat colors of green, brown, black, and yellow are found in soybeans with different hylum color and seed size (Messina 1999; PVT 2007). The role of phenolics in the seed coat is to provide resistance against fungal pathogens and pest insects, to regulate impermeability to water, and to maintain cell integrity (Moise et al. 2005; Bellaloui et al. 2012).

Overall, soluble phenolic compounds function as initial plant defense against pathogen infection as well as contribute to health benefits (Messina 1999; Lee et al. 2011; Ng et al. 2011; Lygin et al. 2013). This study therefore, focused on the soluble phenolics in soybean seeds. Several studies have investigated soluble phenolics and antioxidant properties of soybean genotypes with varying seed coat colors (Kumar et al. 2010; Zhang et al. 2011; Malencic et al. 2012; Cho et al. 2013; Zilic et al. 2013), however, only few employing different seed sizes. A study on quantifying several secondary metabolites including total phenolic, total flavonoid as well as antioxidant activity in Indonesian soybean germplasm have not been conducted yet. Nine different seed coat colors are described in Indonesian soybean genotypes (PVT 2007). However, only four different seed coat colors were studied since genotypes with those seed coat colors were

commonly cultivated. This study aimed to determine and to compare total phenolic and flavonoid contents as well as antioxidant activity of 63 Indonesian soybean genotypes with different seed coat colors and seed sizes.

MATERIALS AND METHODS

Plant materials

Soybean seeds (*G. max*) as many as 63 genotypes with four different seed coat colors (green yellow, light yellow, yellow, and black) and seed sizes (small, medium and large) were obtained from germplasm collection of Indonesian Legumes and Tuber Crops Research Institute (ILETRI). As many as 50 g of each genotype was taken from the collection (moisture content < 9%). Each soybean genotype characteristic is presented in Table 1.

Chemicals

Gallic acid, catechin, trolox, Folin-Ciocalteu, and 2,2-diphenyl-1-picrylhydrazyl were purchased from Sigma Aldrich (St. Louis, MO, USA). Aluminum chloride, sodium nitrite, sodium hydroxide, sodium carbonate, acetone, ethanol were pro analysis grade from Merck (Darmstadt, Germany).

Sample preparation and extraction

Intact dried soybean (50 g) were ground using a sample mill (Cyclotec sample mill, Sweden) into fine particles (< 80 mesh) of soybean flour. The flour was kept in sealed plastic bags and stored at -20 ^{o}C before used. Moisture content was determined before secondary metabolite analysis to estimate dry weight of the sample.

Extraction was conducted according to Xu and Chang (2007, 2008a) with slight modification. Briefly, the soybean flour was dissolved in 50% acetone (1:10 v v^{-1}) for estimating total flavonoid and phenolic contents and in 70% ethanol (1:10 v v^{-1}) for studying antioxidant activity. All extraction was conducted in triplicate. Samples were placed on an orbital shaker at 200 rpm (Stuart Scientific, UK) for 2 h at room temperature. After incubation for 18 h in the dark room, the samples were centrifuged (Beckman Allegra 21R, US) at 3000 rpm for 10 min. The same procedure was repeated and the supernatants were combined (total volume ± 10 mL) and stored in amber vials at 4^{o}C.

Determination of total phenolic content

Folin-Ciocalteu assay according to Singleton et al. (1999); Xu and Chang (2007) was used for estimating total phenolic content in the soybean flour extract. A certain volume of soybean extract was added to distilled water (1:60 v v^{-1}), 250 µL of Folin-Ciocalteu's reagent, and 750 µL of sodium carbonate. The mixture was vortexed and incubated for 8 min at room temperature before 950 µL of distilled water was added. The final mixture was incubated for 2 h in the dark at room temperature. The absorbance values were read using a spectrophotometer (Genesys 10s, US) at 765 nm. A linear concentration of gallic acid at 12.5 to 800 µg mL^{-1} was used as a standard (r = 0.999). The

Table 1. Seed coat color, 100 seed weight, and seed size of 63 Indonesian soybean genotypes (ILETRI 2004, 2015)

Genotype ID	Seed coat color	Hylum color	Weight/ 100 seeds (g)	Seed size category [a]
MLGG 0029	Yellow	Dark brown	13.0	Medium
MLGG 0030	Yellow	Light brown	12.5	Medium
MLGG 0031	Yellow	Dark brown	8.0	Small
MLGG 0032	Yellow	Brown	10.5	Medium
MLGG 0033	Yellow	Brown	10.5	Medium
MLGG 0096	Yellow	Dark brown	10.0	Medium
MLGG 0099	Yellow	Dark brown	10.0	Medium
MLGG 0100	Black	Black	7.5	Small
MLGG 0101	Yellow	Dark brown	8.0	Small
MLGG 0102	Black	Black	8.0	Small
MLGG 0111	Light yellow	Light brown	12.0	Medium
MLGG 0464	Green yellow	Dark brown	7.0	Small
MLGG 0533	Yellow	Brown	13.0	Medium
MLGG 0534	Green yellow	Dark brown	7.5	Small
MLGG 0669	Yellow	Brown	13.5	Medium
MLGG 0681	Green yellow	Dark brown	7.0	Small
MLGG 07xx	Light yellow	Light brown	13.0	Medium
MLGG 0747	Light yellow	Brown	8.3	Small
MLGG 0795	Yellow	Brown	11.0	Medium
MLGG 0796	Yellow	Brown	10.0	Medium
MLGG 0801	Yellow	Black	10.0	Medium
MLGG 0805	Light yellow	Brown	15.0	Large
MLGG 1053	Green yellow	Brown	10.0	Medium
MLGG 1054	Yellow	Dark brown	9.6	Small
MLGG 1058	Light yellow	Dark brown	8.5	Small
MLGG 1059	Yellow	Brown	8.0	Small
MLGG 1061	Black	White	11.5	Medium
MLGG 1062	Yellow	Dark brown	10.0	Medium
MLGG 1063	Yellow	Brown	12.0	Medium
MLGG 1064	Yellow	Light brown	12.0	Medium
MLGG 1065	Yellow	Black	12.0	Medium
MLGG 1066	Yellow	Brown	12.5	Medium
MLGG 1067	Yellow	Brown	10.0	Medium
MLGG 1068	Yellow	Brown	10.5	Medium
MLGG 1069	Yellow	Light brown	12.0	Medium
MLGG 1070	Yellow	Brown	10.6	Medium
MLGG 1071	Yellow	White	16.0	Large
MLGG 1073	Yellow	Yellow	17.0	Large
MLGG 1075	Yellow	Brown	10.7	Medium
MLGG 1076	Yellow	Brown	10.4	Medium
MLGG 1077	Yellow	Dark brown	11.0	Medium
MLGG 1078	Yellow	Brown	11.5	Medium
MLGG 1078	Yellow	Brown	12.5	Medium
MLGG 1080	Yellow	Light brown	16.8	Large
MLGG 1081	Yellow	Light brown	15.1	Large
MLGG 1082	Yellow	Light brown	10.5	Medium
MLGG 1083	Green yellow	Light brown	9.1	Small
MLGG 1085	Yellow	Light brown	16.0	Large
MLGG 1086	Yellow	Brown	11.2	Medium
MLGG 1087	Light yellow	Dark brown	18.5	Large
MLGG 1088	Green yellow	Dark brown	9.5	Small
MLGG 1089	Green yellow	Dark brown	10.5	Medium
MLGG 1091	Green yellow	Brown	15.8	Large
MLGG 1092	Yellow	Light brown	17.8	Large
MLGG 1093	Yellow	Black	23.2	Large
MLGG 1094	Green yellow	Brown	6.8	Small
MLGG 1095	Green yellow	Brown	8.3	Small
MLGG 1096	Light yellow	Brown	11.9	Medium
MLGG 1097	Black	White	14.8	Large
MLGG 1098	Black	Brown	13.5	Medium
MLGG 1099	Yellow	Brown	18.0	Large
MLGG 1100	Black	Light brown	9.5	Small
MLGG 1103	Yellow	Dark brown	10.7	Medium

Note: [a] seeds size category was grouped according to PVT (2007)

total phenolic content was expressed as gallic acid equivalents per gram of sample (mg GAE g^{-1} sample) based on dry basis.

Determination of total flavonoid content

Total flavonoid content was estimated according to Heimler et al. (2005); Xu and Chang (2007). To 2500 µL of distilled water, aliquot of 500 µL of soybean extract was added in a glass tube and thoroughly mixed using a vortex. Then, 150 µL of 5% $NaNO_2$ solution was added and incubated for 6 min at room temperature. Another 5 min incubation was conducted after adding 300 µL of aluminum chloride into the mixed solution. A solution of 1 M sodium hydroxide as many as 1000 µL was added into the mixture. The final volume was brought to 5000 µL with distilled water and mixed thoroughly. The absorbance values against blank were read at 510 nm using a spectrophotometer. The results were expressed as catechin equivalents per gram of sample (mg CE g^{-1} sample) based on dry basis using the calibration curve of the catechin. A linear range of 12.5 to 400 µg mL^{-1} was used as a calibration curve (r = 0.999).

DPPH free radical scavenging activity

DPPH scavenging activity or antioxidant activity of soybean extract was determined according to Xu and Chang (2008a, b). Aliquot of soybean extract was mixed with 0.1 mM ethanolic DPPH solution (1:19 v v^{-1}). The solution was vortexed and incubated in the dark room for 30 min at room temperature. The absorbance values of sample (A_{sample}) and blank ($A_{control}$) were recorded using spectrophotometer at 515 nm. The radical scavenging activity as reflected by the percent DPPH discoloration was calculated as follows: percent discoloration = [1-($A_{sample}/A_{control}$)] x 100. $A_{control}$ was recorded after the DPPH solution was reacted with the extraction solvent. The DPPH scavenging activity of each sample was expressed as micromoles of Trolox equivalent per gram of sample (µmol TE g^{-1} sample).

RESULTS AND DISCUSSION

Total phenolic content in soybean genotypes

Total phenolic contents of soybean genotypes between four different seed coat color groups were significantly different ($p < 0.05$), showing seed coat color based genotypic variation among them (Table 2). However, no significant difference was observed in three seed size groups (small, medium and large) ($p > 0.05$). Black soybean genotypes showed higher total phenolic contents than those in green yellow, light yellow and yellow soybeans. Total phenolic contents among 63 soybean genotypes varied from 3.49 to 14.72 mg GAE g^{-1} (Figure 1). The genotype of MLGG 0030 with yellow seed coat had the lowest (3.49 ± 0.03 mg GAE g^{-1}), whereas MLGG 1098 with black seed coat had the highest phenolic contents (14.72 ± 0.07 mg GAE g^{-1}). Significant genotypic variations ($p < 0.05$) of soybeans within different seed coat color groups were also investigated by Xu and Chang (2007, 2008c) as well as Kumar et al. (2010), in which black soybean genotypes expressed the highest phenolic contents.

Six genotypes with black seed coat color, i.e. MLGG 0100, MLGG 0102, MLGG 1061, MLGG 1097, MLGG 1098, and MLGG 1100 were rich in total phenolic contents (from 7.19 to 14.72 mg GAE g^{-1}). Results suggested that seed coat contributed higher phenolic contents than those in embryonic axis and cotyledon (Jeng et al. 2010). Higher phenolic contents in black soybean compared to yellow soybeans were also reported by Xu and Chang (2007, 2008a), where the variability among Indonesian soybeans was 2.05-fold in those six genotypes. However, more variation among genotypes up to 7.27-fold was observed in six Indian black soybeans (Kumar et al. 2010). Genotypic variations of black soybeans were also found in three Taiwan black soybean varieties, where these varieties contained total phenolics ranged from 4.38 to 7.49 mg GAE g^{-1} (Jeng et al. 2010). The two varieties containing 7.05 ± 0.50 mg GAE g^{-1} and 7.49 ± 0.39 mg GAE g^{-1} of total phenolics (Jeng et al. 2010) were similar to MLGG 1097 (7.19 ± 0.19 mg GAE g^{-1}) and MLGG 1100 (8.22 ± 0.64 mg GAE g^{-1}) determined in this study. Different extraction and quantification methods may attribute to different phenolic measured, apart from different soybean genotypes used in the study as reported by Zilic et al. (2013).

Of the green yellow, light yellow and yellow seed color genotype groups, no difference of total phenolic contents was observed (Table 2). The ranges of total phenolic contents of these three seed coat classes were 3.89 – 5.17, 3.70 – 5.02 and 3.49 – 5.42 mg GAE g^{-1}, respectively. Higher range of total phenolic contents of yellow soybeans was found in Indonesian genotypes compared to that of Indian genotypes, which was 1.06 – 1.54 mg GAE g^{-1} (Kumar et al. 2010). Average of total phenolic contents of green yellow soybean genotypes (4.70 ± 0.38 mg GAE g^{-1}) in this study was also higher than total phenolic content of green soybean (3.46 ± 0.09 mg GAE g^{-1}) as observed by Malencic et al. (2012).

Table 2. Average values of total phenolic, flavonoid contents, and DPPH scavenging activity of 63 soybeans with different seed coat colors and different seed size

Seed coat color	Total phenolic content (mg GAE g^{-1})	Total flavonoid content (mg CE g^{-1})	Antioxidant activity (µmol TE g^{-1})
Black (n = 6)	11.11 ± 3.27 a	3.47 ± 1.50 a	15.58 ± 4.53 a
Green yellow (n = 10)	4.70 ± 0.38 b	0.34 ± 0.05 b	6.68 ± 0.75 b
Light yellow (n = 7)	4.29 ± 0.43 b	0.36 ± 0.04 b	7.21 ± 1.01 b
Yellow (n = 40)	4.39 ± 0.46 b	0.34 ± 0.06 b	6.78 ± 0.77 b

Note: n = number of genotypes with the same seed coat color. Values followed by the same number in the same column were not significantly different based on the Duncan test ($p < 0.05$).

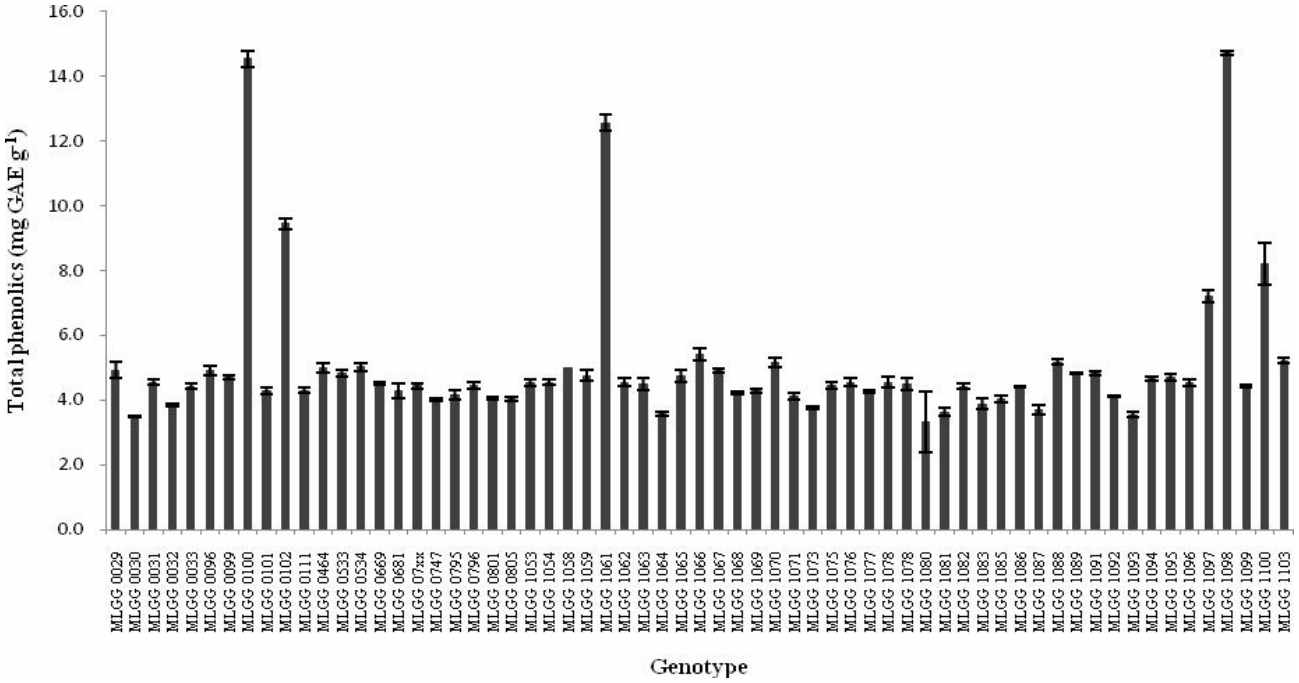

Figure 1. Total phenolic contents of 63 soybean genotypes with different seed coat colors and seed sizes. Error bars represent standard deviation from measurements in triplicate.

Total flavonoid content in soybean genotypes

All soybean genotypes with black seed coat accumulated more flavonoid contents than green yellow, light yellow and yellow genotypes and the difference among these groups was significant ($p < 0.05$). However, no difference in total flavonoids among green yellow, light yellow and yellow soybean genotypes was found (Table 2). No difference was also observed in the three seed size groups ($p > 0.05$). The average total flavonoid contents in Indonesian black soybeans was higher than that in black soybeans (2.57 ± 0.03 mg CE g^{-1}) as investigated by Xu and Chang (2007). However, the yellow soybeans in this present study did not exhibit so high total flavonoid value (1.47-fold) as reported by Xu and Chang (2007). This finding clearly showed that genotypic variation within seed coat color group may be responsible for different flavonoid contents. Environmental factors such as geographic condition, light, temperature, planting year, and soil moisture also contributed to the difference of the distribution and concentration of bioactive compounds (Lee et al. 2003; Lee and Cho 2012).

Major groups of flavonoids accumulated in seeds were anthocyanins, proanthocyanidins, and isoflavones (Lepiniec et al. 2006). Anthocyanins and proanthocyanidins were more concentrated in black seed coat of soybeans (Xu and Chang 2008b; Lee et al. 2009; Jeng et al. 2010). However, anthocyanins were not detected in yellow and green seed coats of many soybean genotypes (Cho et al. 2013). In whole green and yellow soybeans, isoflavones are a group of compounds which are responsible for the high total flavonoid contents (Cho et al. 2013; Zilic et al. 2013). Total isoflavones in different seed coat color of soybeans from the highest to the lowest followed the order of green > yellow > black > brown soybean genotypes (Cho et al. 2013). However, no variation among green yellow and yellow soybean genotypes was observed in the present study, suggesting other compounds than isoflavones may also contribute to the total flavonoid contents. In fact, carotenoids and luteins were reported high in green and yellow soybeans (monma et al. 1994; Kumar et al. 2010).

Total flavonoids of 63 soybean genotypes were in the range from 0.22 to 5.30 mg CE g^{-1} (Figure 2). As observed in total phenolic contents, black soybean genotypes consistently also contained high flavonoid compared to yellow soybeans. MLGG 0100, MLGG 0102, MLGG 1061, MLGG 1097, MLGG 1098, and MLGG 1100 with black seed coat had 1.91 to 5.30 mg CE g^{-1} of total flavonoid contents. Unlike black soybeans, higher flavonoid contents in yellow, light yellow and green yellow soybean genotypes did not always contain higher phenolic contents. Total flavonoid contents of the yellow, light yellow, and green yellow seed color were from 0.22 to 0.48 mg CE g^{-1}. It is difficult to compare the flavonoid contents in this study to the previous studies since a different flavonoid standard was utilized to estimate total flavonoid equivalent (Malencic et al. 2012). However, a trend of black soybeans contained more flavonoids than those in yellow soybeans were in agreement.

DPPH scavenging activity in soybean genotypes

Genotypes with black seed coat possessed significantly higher DPPH scavenging activity than yellow soybean groups ($p < 0.05$) (Table 2). However, no significant

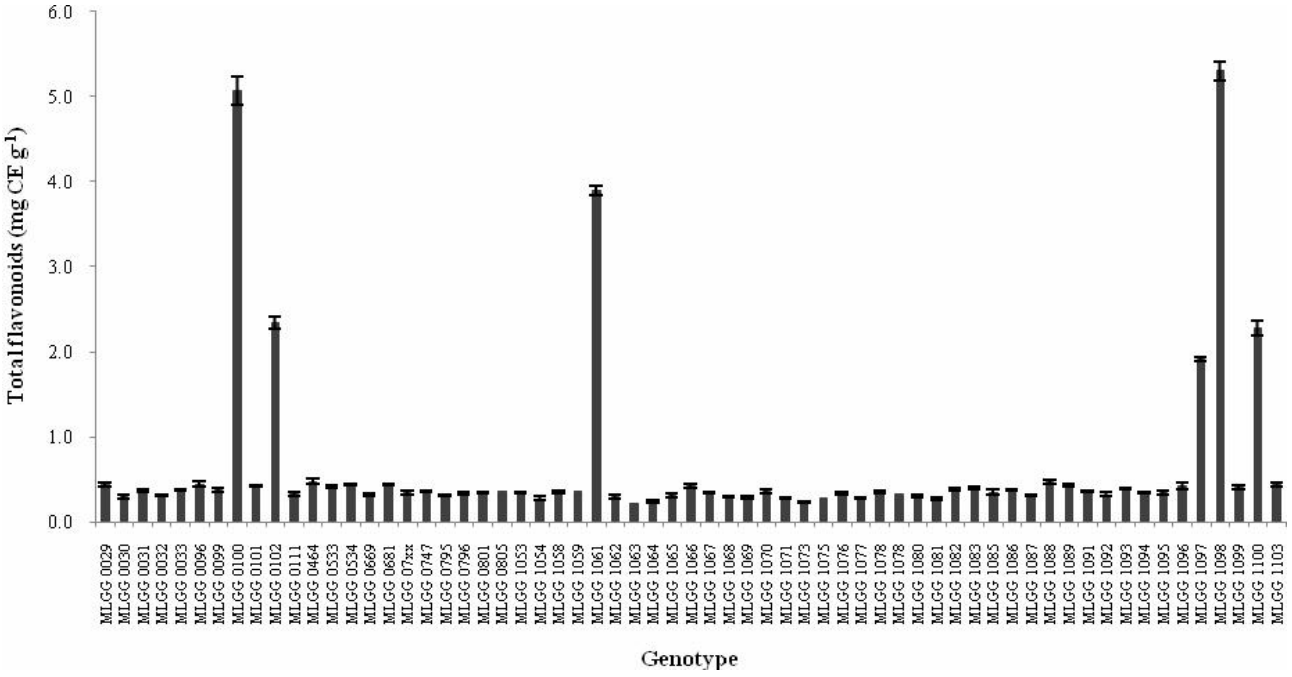

Figure 2. Total flavonoid contents of 63 soybean genotypes with different seed coat colors and seed sizes. Error bars represent standard deviation from measurements in triplicate.

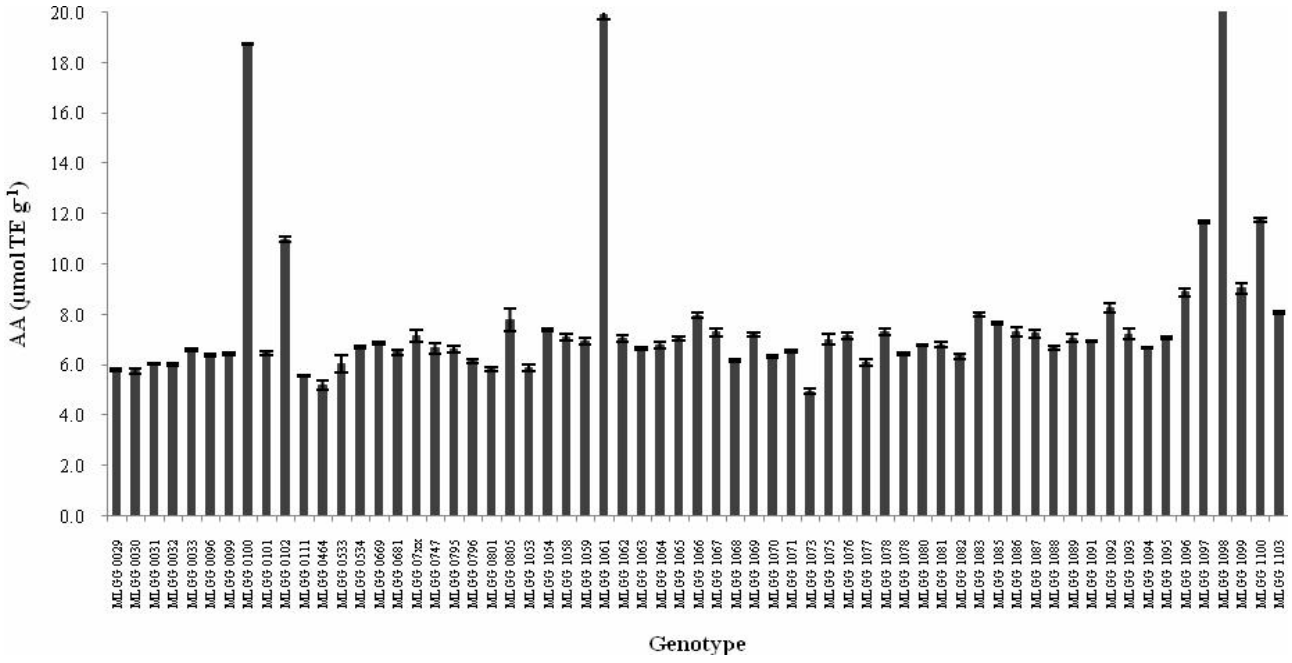

Figure 3. Antioxidant activity (AA) of 63 soybean genotypes with different seed coat colors and seed sizes. Error bars represent standard deviation from measurements in triplicate.

difference was found within three seed sizes ($p > 0.05$). The DPPH values of the antioxidant extracts of black soybean genotypes ranged from 10.99 to 20.38 µmol TE g^{-1} for MLGG 0100, MLGG 0102, MLGG 1061, MLGG 1097, MLGG 1098, and MLGG 1100 (Figure 3). The average DPPH value of black soybean genotypes (15.58 ± 4.53 µmol TE g^{-1}) was lower than in another study (Xu and Chang 2007), which was 17.93 ± 0.03 µmol TE g^{-1}.

However, higher antioxidant activity of black soybeans in comparison to other seed color groups was in agreement with a study conducted by Zhang et al. (2011). In their study, high antioxidant activities were noted with DPPH, oxygen radical absorbance capacity (ORAC) as well as ferric reducing antioxidant power (FRAP) methods (Zhang et al. 2011). This high antioxidant activity in dark seed coat of soybeans could possibly due to high polymerized procyanidin and anthocyanin contents (Takahata et al. 2001).

The green yellow, light yellow and yellow soybean genotypes possessed DPPH scavenging activities from 4.97 to 9.04 µmol TE g^{-1}. The average antioxidant activity of the yellow soybeans was 3.5-fold higher than that DPPH scavenging activity observed in Xu and Chang (2007) study. The reason may be that phenolic compounds such as isoflavones and ferulic acid were dominant in cotyledons and embryo contributing to high antioxidant activity as observed by Zilic et al. (2013).

Another contributing reason could be that the DPPH scavenging activity of the four groups of soybean with different seed coat colors may strongly correlate with isoflavones and anthocyanins as previously described (Cho et al. 2013). However, the scavenging activity against DPPH of isoflavones was less than procyanidins and anthocyanins (Takahata et al. 2001; Jeng et al. 2010).

Correlations between total phenolic, flavonoid contents and DPPH scavenging activity

Correlation analyses between total phenolics, total flavonoids and DPPH scavenging activity among all soybean genotypes were also determined (Table 3). Black soybeans exhibited significant ($p < 0.01$) linear correlations between total phenolic content and total flavonoid content ($r = 0.99$), total phenolic content and DPPH scavenging activity ($r = 0.92$), and total flavonoid content and DPPH scavenging activity ($r = 0.93$). These results confirmed the previous findings that the three parameters were significantly correlated (Xu and Chang 2007). Linear correlations among total phenolics, flavonoid contents and DPPH activity of black soybeans were also investigated by Jeng et al. (2010) and Kumar et al. (2010).

Unlike black soybeans, a significant linear correlation ($p < 0.01$) in yellow soybeans was only observed in total phenolic contents and total flavonoid contents ($r = 0.57$). This correlation was not surprising because flavonoids are a large group of phenolic compounds (Jeng et al. 2010). However, this finding was not in agreement with results reported by Xu and Chang (2007). These authors mentioned that linear correlations among the three parameters were not only observed in black soybeans, but also noted in yellow soybeans. Other different antioxidant activity assays such as ORAC and FRAP could possibly be conducted to obtain better understanding of the correlations among the three parameters in this present study.

Flavonoids including anthocyanins, proanthocyanidins and isoflavones found in soybeans are most likely responsible for the antioxidant activities in the tested samples (Lee et al. 2005; McGhie and Walton 2007; Jeng et al. 2010). Significant linear correlations among total phenolics, total flavonoids and DPPH scavenging activity determined in those black soybeans were consistent with other reports in which total phenolics contributed to a high antioxidant activity found in colored legumes (Segev et al. 2010; Zhao et al. 2014). Therefore, it can be assumed that antioxidant activities could be estimated based on the degree of total phenolic contents, especially for the black soybeans (Jeng et al. 2010; Segev et al. 2010).

Table 3. Correlation coefficient values observed among total phenolic content, total flavonoid content, and antioxidant activity of varying seed coat color soybean genotypes.

Parameters	Correlation coefficient (r) of genotype			
	Black	Green yellow	Light yellow	Yellow
TPC and TFC	0.987^{**}	0.392^{ns}	0.391^{ns}	0.567^{**}
TPC and AA	0.924^{**}	0.481^{ns}	0.106^{ns}	0.246^{ns}
TFC and AA	0.929^{**}	0.285^{ns}	0.750^{*}	0.211^{ns}

Note: * significance at $p < 0.05$, ** significance at $p < 0.01$, ns = non significance. TPC = total phenolic content, TFC = total flavonoid content, AA = DPPH scavenging activity

In conclusion, this study revealed that significant radical scavenging activities in soybean genotypes may be influenced by high phenolic contents. Results presented in this study suggest that estimation of phenolic contents as well as antioxidant activities in soybean genotypes may be useful for seeking soybeans with high antioxidant properties.

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Short Communication: Identification of Growth Hormone gene polymorphism for beef cattle in Pesisir Selatan District, West Sumatra, Indonesia

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Abstract. *Putra DE, Sumadi, Kanazawa T, Hartatik T. 2016. Short Communication: Identification of Growth Hormone gene polymorphism for beef cattle in Pesisir Selatan District, West Sumatra, Indonesia. Biodiversitas 17: 711-715.* This study aimed to determine gene polymorphism of growth hormone of domestic cattle in Pesisir Selatan District of West Sumatra. The research was conducted at Laboratory of Animal Genetics and Breeding Faculty of Animal Science, UGM from August 2013 to January 2014. Blood samples were collected from 66 individuals consist of 15 Pesisir cattle, 15 SimPes cattle, 15 SimPO cattle, 15 Bali cattle and 6 PO cattle. DNA was extracted from each blood samples after SDS-proteinase K digestion, and used for PCR-amplification for a region of growth hormone gene (211 bp), and then the PCR products were analyzed for restriction fragment length polymorphism (RFLP) using *Alu*I restriction enzyme. The results showed that GH gene of Pesisir, PO and Bali cattle are monomorphic, which frequencies of L allele was 1.00 and V allele was 0.000 while these LL genotype was 1.000. Frequency of L and V alleles in SimPO and SimPes cattle were 0.634, 0.366 and 0.700, 0.300, respectively. SimPO and SimPes cattle were polymorphic, LL and LV of SimPO cattle was 0.733 and 0.267 as well as SimPes cattle which LL and LV was 0.600 and 0.400, respectively. The correlation between genotype and the performance (body weight and body size) was not significant. The present study indicates that polymorphism of growth hormone gene in *Alu*I site could not yet be used as a molecular marker for body weight and body size of beef cattle.

Keywords: Domestic cattle, Growth Hormone gene, polymorphism

INTRODUCTION

Pesisir Selatan District was known as a place for developing the Pesisir cattle as local cattle. The populations of Pesisir cattle in Pesisir Selatan District reach 78.322 head at year 2011 (Statistic of Pesisir Selatan 2012). There are also Bali cattle as one of cattle of Indonesia origin and the other local cattle of Indonesia and the product of crossbreeding with exotic bull such as such as PO, SimPO, Limpo and SimPes. The development of cross breed cattle as resources of beef cattle in Indonesia was very potential in this area. However the study about genetic resources is still face the limitation of the information. The development of molecular genetics has opened up opportunities to determine the level of genetic diversity at the DNA level. It can be used to identify the genetic potential of livestock. Pesisir cattle are unique in body size, very small but have higher value of carcass than those of Bali cattle. The increasing number of crossbreed cattle such as SimPO, Limpo and SimPes were being one of basic reason on molecular studies to growth hormone (GH) gene which predicted to be one of major gene plays an important role in the growth process (Etherton and Bauman 1998).

The polymorphism in exon V has been observed when digested by the *Alu*I enzyme (GH-*Alu*I), and the 2 alleles called L (*leucine* in the 127th codon) and V (*valine* in the 127th codon) are distinguished (Zhang et al. 1993). The diversity of genes in cattle can be identified by the method restriction fragment length polymorphism (RFLP). The PCR-RFLP technique is an easier way and is efficient to identify the nucleotide sequence variation in DNA gene fragments of the cattle. This technique has been successfully employed to identify the growth hormone gene polymorphism in local cattle of Indonesia such as Madura cattle and its crossbreed with Limousin (Hartatik et al. 2013; Volkandari et al. 2013). The role of GH gene in appearance in cattle gives very obvious effect so that allegedly there are differences in GH gene between Pesisir cattle and Bali cattle and others cattle. Reis et al. (2001), Dario et al. (2005), Sutarno (2010), Akis Akad et al. (2012), Moravcikova et al. (2012), Deepika and Salar (2013), Korkmazagaoglu and Akyuz (2013), Sari et al. (2013), and Akcay et al. (2015) has conducted studies on the effects of GH gene polymorphism on productivity of beef cattle. Study about GH 211 bp in buffalo also reported by Hussain et al. (2014). Akcay (2015) also investigated that GH is a candidate gene for selection program in beef cattle.

The present study aimed to identify the polymorphism of growth hormone gene in local beef cattle in Indonesia and to study the association with body weight and body size. These basic data can be used as a potential marker assisted selection in the future.

MATERIALS AND METHODS

Samples collection

Sixty six blood samples were collected from Indonesia beef cattle that consisted of Pesisir cattle (15), Simmental x Pesisir or SimPes cattle (15), Bali cattle (15), Simmental x PO or SimPO (15) and PO (6). These cattle distributed to three villages at Pesisir Selatan District, West Sumatra Province, and were managed by farmers by their traditional ways. Three milliliter of blood samples were collected from jugular vein into vacuum test tubes, which contained K_3EDTA as an anticoagulant. Blood samples were stored at -20C until use.

DNA Extraction

DNA was extracted from blood samples using an SDS-PK (sodium dodecyl sulfate-proteinase K) method described by Sambrook et al. (1989) and Sulandari and Zein (2003), with modifications. The blood sample (approx. 200 µL) was mixed with 800 µL buffer A solution in an Eppendorf tube for 1.5 mL, then centrifuged at 10,000 rpm for five minutes. After removal of the supernatant, the pellet was resuspended with 300 µL buffer A solution, then centrifuged again at 10,000 rpm for five minutes. This step was repeated until the pellet color ~~is~~ become pale. The pellet was ~~a~~dded with 270 µL of buffer B and further added with 30 µL buffer C, then the mixture were incubated at 50°C for overnight. The next day, the mixture was added with 71 µL of 5 M NaCl solution, shacked vigorously for 15 second, and then was centrifuged at 10,000 rpm for 10 minutes. The top layer (approx. 300 µL) was transferred into a new 1.5 mL Eppendorf tube, added with 600 µL of 96% Ethanol, and mixed slowly. After emergence of DNA, then the tube was centrifuged at 12.000 rpm for 10 minutes. The supernatant was carefully deposed, and the DNA pellet was washed by addition of 100 µL of 70% ethanol and subsequent centrifugation at 12.000 rpm for 5 minutes. The supernatant was discarded and the DNA pellet was air-dried until it became semi-transparent. The dried DNA was added with 100 µL of TE (Tris-EDTA) solution (pH 7.4) or aquabidest sterile (Otsuka) then left for overnight to dissolve DNA.

Polymerase Chain Reaction (PCR)

Amplification of DNA fragments of 211 bp of a specific region of growth hormone gene was performed by polymerase chain reaction technique. The following set of primers was used according to Reis et al. (2001): *GH-forward*, 5' GCT GCT CCT GAG GGC CCT TC 3'; and *GH-reverse*, 5' CAT GAC CCT CAG GTA CGT CTC CG 3'. The amplification was performed using a final volume of 30 µL containing 15 µL PCR Kit KAPA (KAPA Biosystems), 11.25 µL aquabidest, *forward* and *reverse* primer 1.5 µL (10 pmol/µL) respectively, and 0.75 µL DNA template (20-100 ng). PCR was performed in thermocycler (PEQLAP Primus 25 Advance). The amplification condition for GH gene were an initial denaturation at 97°C for 1 minute 30 seconds, denaturation at 94°C for 1 minute, annealing at 62°C for 1 minute, elongation at 72°C for 1 minute. Steps two to four were repeated for 30 cycles and the reaction ended with a final extension at 72°C for 10 minutes (Mu'in 2008). The PCR products were separated on a 0.8% agarose gel, stained with ethidium bromide, and visualized under UV light in UV Transilluminator. Photographs were taken using digital camera (Canon EOS 600D).

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP)

Growth hormone gene variants were identified by a PCR-RFLP method. *Alu*I restriction enzyme (5'-AG | CT-3') was used in this analysis. The total volume of PCR-RFLP mixture was 15 µL containing 5 µL of PCR product, 0.5 µL of *Alu*I restriction enzyme (x U/µL, Fermentas, Life Science), 1.5 µL of 10x buffer tango, and 8 µL *aquabidest* that incubated at 37°C for 3 hours in multiheater (EYELA). PCR-restriction fragments were separated by electrophoresis on 2% agarose gel in 1x TBE buffer at 50 V for 2 hours, and visualized under UV light after staining with ethidium bromide. Photographs were taken as described above.

Statistics analysis

The frequency of alleles and genotypes were estimated following the method describe by Falconer and Mackay (1989):

$$p^2+2pq+q^2=1$$

Where:
p^2 = frequency of LL
$2pq$ = frequency of LV
q^2 = frequency of VV

Chi-square (X^2) analysis was used for finding the genetic equilibrium in population.

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

Association analysis between genotype and quantitative traits (body weight and body measurement) used SPSS version 20.0 program

RESULTS AND DISCUSSION

In the present study, 66 individuals from 5 Indonesian beef cattle breeds (Pesisir, PO, Bali, SimPO (Simmental x PO), and SimPes (Simmental x Pesisir)) were genotyped for GH-*Alu*I locus using PCR-RFLP technique. A pair of primer for GH gene (Reis et al. 2001) was used for amplifying 211 bp DNA fragment (see, Figure 1, Lane 2). Single Nucleotide Polymorphism (SNP) in exon 5 (at codon 127) of the bovine GH gene was located in the PCR product. The SNP has been found in the all of beef cattle populations and caused changed amino acids Leucine to Valine (GTC to GTG). It is a point mutation (a transversion mutation) in position 2141 of GH gene (GenBank accession Number M57764) (Lucy et al. 1993; Zhang et al. 1993).

Two patterns of restriction fragments were observed in the present study using *Alu*I restriction enzyme (restriction site 5'-AG | CT-3'). There were LL and LV genotypes (see, Figure 1 Lanes 3-6). A point mutation at position 52 that loses *Alu*I restriction site, could not find of restriction site (5'-AG | CT-3) that was known as V allele whereas L allele indicated of absence of mutation. So, LL genotype has one restriction site, yielding two fragments of 52 and 159 bp, LV genotype reveals 211, 159 and 52 bp fragments whereas VV genotype was undigested fragment and yields only one fragment of 211 bp.

Genotype and allele frequencies

The genotype and allele frequencies of Growth Hormone gene of beef cattle in Pesisir Selatan District were summarized in Table 1. Polymorphisms of Growth Hormone gene were found in SimPO (Simmental x PO) and SimPes (Simmental x Pesisir) breeds while Pesisir, PO, and Bali cattle were monomorphic populations. Monomorphic of GH-AluI in this study are similar to those reported in earlier studies (Mu'in 2008). It indicated that native cattle breeds (*Bos indicus*) have one variant allele in GH locus. Migration or introducing the other variant allele was not showed in this study.

Based on previous studies, *Bos indicus* cattle have 0.99-1.00 of L allele frequency, which were found in Gyr cattle (Kemenes et al. 1999; Pawar et al. 2007), Nelore cattle (Kemenes et al. 1999), Sahiwal cattle (Biswas et al. 2003), Kankrej cattle (Pawar et al. 2007), Madura cattle (Hartatik et al. 2013), Bali cattle/*Bos sondaicus* (Mu'in 2008). While its predominance in the taurine breed cattle with 0.642 – 0.80 of L allele frequencies such as Simmental cattle (Dybus et al. 2002), Limousine cattle (Hartatik et al. 2013), Charolais cattle (Kemenes et al. 1999), and Holstein cattle (Moravcikova et al. 2012; Arango et al. 2014; Hartatik et al. 2015). *Bos taurus x Bos indicus* crosses yielded L allele dominant in GH locus but lower than *Bos taurus*. It means that migration or introduction of V allele has been happened in population. Chanchim cattle, is synthetic cattle with 5/8 Charolais (*Bos taurus*) and 3/8 Nelore (*Bos indicus*), have L allele frequency (0.99) (Silveira et al. 2008).

LL genotype frequencies of GH gene in all of breeds were higher than LV genotype. The GH L allele was predominantly found in both of cattle populations This is same with previous studies in the Growth Hormone gene in cattle, Reis et al. 2001; Silveira et al. 2008; Mu'in et al. 2007; Hartatik et al. 2013; Volkandari et al. 2013; Moravcikova et al. 2012; Akcay et al. 2015).

Based on the observed versus expected genotype frequencies, the SimPO and LimPO population were in Hardy-Weinberg genetic equilibrium (see, Table 2).

Genotype effects

Growth Hormone acts directly by binding its receptors on the cells of bone, muscle, and fat tissue, and induces cell proliferation in these tissues. GH increases muscle protein synthesis and affects mammary growth in mammalians. Moreover, it plays an indirect role in cell growth (Ardiyanti et al. 2009; Akcay et al. 2015). Based on previous studies, polymorphism in GH locus affected weight gain, carcass weight, birth weight and marbling in cattle (Biswas et al. 2003; Tatsuda et al. 2008; Ardiyanti et al. 2009).

Genotypes of GH gene in SimPO and SimPes population showed no significant differences regarding any variables (Body weight, Body Length, High Shoulder, Hip Height and Heart girth). Production traits were controlled by many genes (polygenes) and interaction both of environment and genetic (Warwick et al. 1983).

Pereira et al. (2005) found that GH-LV genotype had positive effects on Yearling Weight in Chanchim cattle (synthetic cattle). The other hand, Aruna Pal et al. (2004) reported that genotype of GH-LL genotype had a significant effect on the average birth weight, 3 months body weight and daily body weight gains in Karan Fries bull populations.

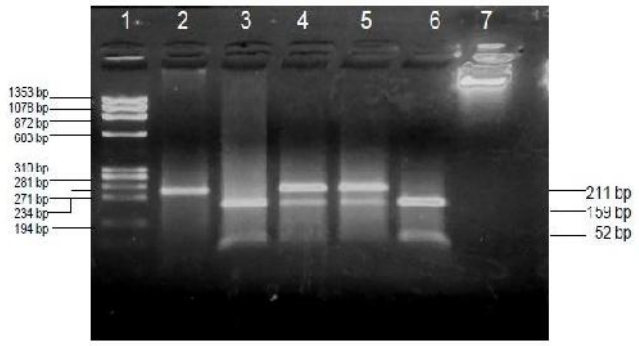

Figure 1. Representative result. Note: (1) Marker, (2) PCR product, (3-6) RFLP product, (7) Extracted DNA

Table 1. Allele and genotype frequencies of GH *AluI* loci

Breed	N	Genotype			Allele	
		LL (46)	LV (20)	VV (0)	L (57)	V (9)
Pesisir	15	1.000 (15)	0.000 (0)	0.000	1.000	0.000
PO	6	1.000 (6)	0.000 (0)	0.000	1.000	0.000
Bali	15	1.000 (15)	0.000 (0)	0.000	1.000	0.000
SimPO	15	0.267 (4)	0.733 (11)	0.000	0.634	0.366
SimPes	15	0.400 (6)	0.600 (9)	0.000	0.700	0.300

Table 2. Expected (He), observed (Ho) and HWE value for GH-*Alu*I

Breeds		Genotype *GH*			Allele frequencies		χ^2
		LL	LV	VV	L	V	
SimPO	*Observed*	4	11	0	0.6334	0.3666	5.029
	Expected	6.018	6.966	2.016			
SimPes	*Observed*	6	9	0	0.700	0.300	2.755
	Expected	7.350	6.300	1.350			

Note: $\chi^2_{0.05;2}$ = 5.99; *GH* = Growth Hormone; HWE = Hardy-Weinberg Equilibrium

Table 3. Body weight and body size of SimPO cattle and SimPes cattle based on genotype LL and LV by 18 months of age

Variables	Breeds			
	SimPO (LL)	SimPO (LV)	SimPes (LL)	SimPes (LV)
N	4	11	6	9
BW (kg)	270.50+63.07	260.00+49.26	176.17+34.89	176.61+26.73
BL (cm)	112.00+4.32	107.91+9.33	96.33+7.58	96.67+5.24
HS (cm)	117.00+12.36	111.36+7.57	98.50+6.35	99.11+3.66
HH (cm)	121.00+11.83	116.27+7.95	104.00+6.23	106.44+6.39
HG (cm)	155.5+22.23	148.55+12.41	130.00+6.78	131.33+8.12

Note: BW = Body weight; BL = Body Length; HS = High Shoulder; HH = Hip Height; CS = Heart Girth

In conclusion, by PCR-RFLP technique has been detected genotypes in the 5 Indonesian beef cattle. Polymorphisms were found in SimPO and SimPes populations. GH-L allele is dominant allele in GH locus. Non-significant effects of growth traits were found in this study.

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Review: Persistent pioneers; *Borassus* L. and *Corypha* L. in Malesia

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Abstract. *Eagleton G.E. 2016. Review: Persistent Pioneers;* Borassus *L. and* Corypha *L. in Malesia. Biodiversitas 17: 716-732.* This review traces advances in taxonomic and ethnobotanic understanding of the genera *Corypha* L. and *Borassus* L. gained from research since the time of publication of "Harvest of the Palm; Ecological Change in Eastern Indonesia'' by James J. Fox in 1977. It posits testable hypotheses arising from the literature: firstly, that both genera were present in the furthest parts of island Southeast Asia prior to a definitive Indianized cultural expansion in the first millennium CE.; secondly, that two of their species − "lontar" *Borassus flabellifer* L. and "gewang" *Corypha utan* Lam. − were significant components of pre-agricultural economies of the archipelago, but that their full economic exploitation benefited from later cultural stimuli from the Indian subcontinent. To test these hypotheses, lines of research with potential benefits for local economies in semi-arid Indonesia are proposed.

Keywords: *Borassus*, *Corypha*, origins, proto-agricultural uses, Southeast Asia

INTRODUCTION

It is forty years since James Fox's "Harvest of the Palm" gathered together written and oral traditions concerning the pivotal role of two palm species, *Borassus flabellifer* L. and *Corypha utan* L., in certain subsistence economies of Nusa Tenggara Timur, Indonesia, and other parts of the semi-arid tropics (Fox 1977). In the intervening years, research has accumulated providing a clearer picture of the taxonomic status of the two palms and of their economic significance, past, present and potential. The purpose of this paper is to re-examine the findings of Fox (1977) in the light of this more recent research and to proffer avenues of useful new investigation.

NAMES

"Lontar" (*Borassus flabellifer* L.) and "gewang" (*Corypha utan* Lam.) are Indonesian names for two palm species concentrated mainly in the drier parts of the archipelago. In colonial India, *B. flabellifer* was known as "palmyra" and the majestic *Corypha umbraculifera* L. as "talipot".

Lontar and gewang are fan-leafed palms in contrast to feather-leafed palms like coconut and oil palm, and can be mistaken for one another at first glance. Nevertheless, they are very different in growth habit, especially reproductive biology (Figure1). Gewang is hermaphroditic (its flowers having both stamens and gynoecium) and monocarpic (the palm dies after a single spectacular flowering and fruiting season). Lontar is dioecious (staminate flowers and pistillate flowers are borne in separate inflorescences, on different plants) and pleonanthic (the palms flower repeatedly, potentially over several seasons; not dying after flowering; Uhl and Dransfield 1987).

There are other local Indonesian names for the lontar, for example: "siwalan", "tal", "ental" and "rontal" in Java; "kori" or "koli" in Flores; "tua" in Roti; and "duwe" in Savu (Heyne 1927; Tjitrosoepoma and Pudjoarinto 1983). Of particular relevance to the question of origins, are the cognates of the Sanskrit name "tala" that are to be found in India and Sri Lanka, Java and Madura, to at least as far east as Sumbawa and Sulawesi (Burkill 1966; Fox 1977).

The close ecological association and similarities in form between *Corypha* species and the lontar gave rise to parallels in the names attributed to the two. Rumphius applied the Latin name *Lontarus silvestris* (common name 'Lontar Utan') to the gewang (see Figure1), and *Lontarus domestica* to the lontar, while recognizing their striking differences in reproductive structures (Rumphius 1741). Similarly, according to Burkill (1966), the Sanskrit word "tala" was not confined to *B. flabellifer*, but was also used to refer to *Corypha* species and even to other common Indian palms. For Sanskrit literati and later Buddhist scholars, the fact that leaves of *B. flabellifer* could substitute for the leaves of *Corypha umbraculifera* L. as the writing medium for their sacred scriptures was no doubt a significant reason for the parallelism in nomenclature.

Nevertheless, traditional taxonomies recognized clear difference between the two genera, and in the Malesian archipelago the names attached to *Corypha utan*, for example "ibus" in Aceh and North Sumatra; "gebang" in parts of Java and Bali; "pocok" in Madura; "silar" in Sulwesi; "tula" in Roti; and "buri" in parts of the Philippines, suggest negligible reference to Indianized influences (Heyne 1927; Burkill 1966).

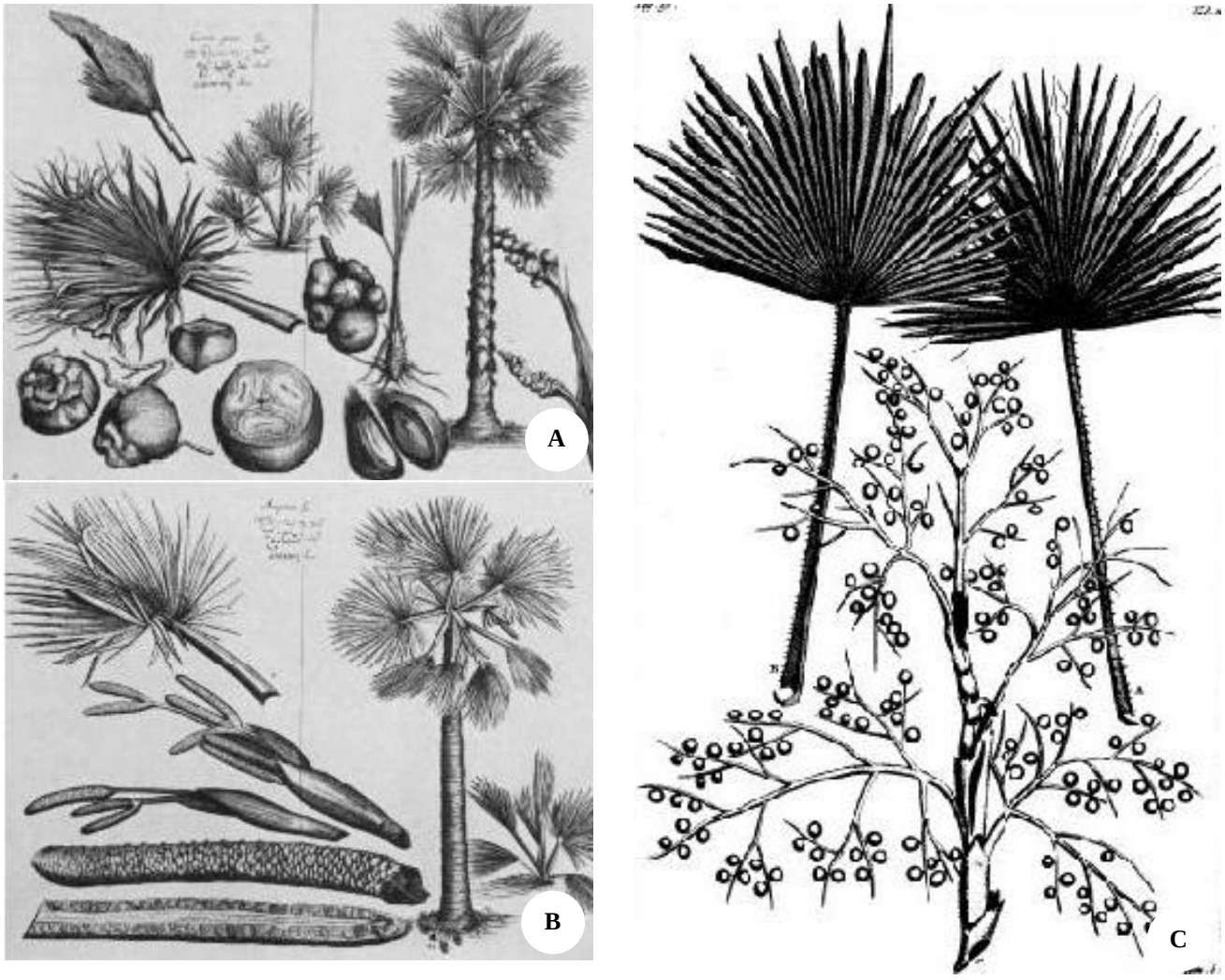

Figure 1. Early records. **A.** The lectotype for *Borassus flabellifer* L. − The pistillate form − *Ampana*; Rheede (1678-1703), Hortus Indicus Malabaricus 1:13-14, *pl.*10. **B.** The staminate form − *Carimpana*; Rheede (1678-1703), Hortus Indicus Malabaricus 1: 11-12, *pl.* 9. **C.** The lectotype for *Corypha utan* Lam. − *Lontarus silvestris*; Rumphius (1741), Herbarium Amboinense 1: 53-56, *pl.* 11

TAXONOMY

Borassus L.

Up until Beccari's revision of the genus *Borassus* in 1914 and more substantially in Beccari (1924; published after his death), the consensus had been that the genus in Asia was represented by a single widely distributed species, *B. flabellifer*, described by Rheede tot Drakenstein (1678) (see Figure 1) and by others in the seventeenth century and recognized as a distinct species by Linnaeus (1753). In Beccari (1924), the distinction first drawn by Martius (1838) between the Asian *B. flabellifer* and African members of the genus was elaborated. In addition, two new species were defined for the Asian region; namely *B. heineanus* Becc., from the northern coastal regions of the island of New Guinea, and *B. sundaicus* Becc., located by Beccari in the Indonesian archipelago. Subsequently, *B. heineanus* was accepted as a separate species by other botanists in the field. However, the distinction drawn by Beccari (and later supported by Fox 1977) between *B. flabellifer* and *B. sundaicus* did not gain wide acceptance (see for example Heyne 1927; Burkill 1966). More detailed anatomical observation of a much wider sampling in Indonesia than was available to Beccari, removed support for the concept of lontar as a separate, Indonesian, species of *Borassus* (Pudjoarinto 1982; Sastrapradja and Davis 1983; Tjitrosoepomo and Pudjoarinto 1983).

In recent times, advances in molecular identification have provided a more secure basis for the classification of flowering plants (APG IV 2016), including the Arecaceae in particular (Asmussen and Chase 2001; Bayton 2005; Dransfield et al. 2005; Asmussen et al. 2006; Dransfield et al. 2008). Based on these new insights, Bayton (2007) has revised the taxonomy of *Borassus* L., the first comprehensive review of the genus since Beccari (1924). He recognizes five species in contrast to Beccari's seven: they are, from east to west in distribution, *B. heineanus* Becc.; *B. flabellifer* L.; *B. madagascariensis* (Jum. & Perrier) Bojer ex Jum. & Perrier; *B. aethiopum* Mart.; and *B. akeassii* Bayton, Ouedraogo & Guinko.

Borassus heineanus Becc. is enigmatic. Nuclear and chloroplast DNA sequencing (Bayton 2005) indicate that *Borassus* including *B. heineanus* is "monophyletic in its current circumspection", nevertheless this New Guinea species resembles members of the genus *Borassodendron* Becc. in its ecology and several morphological features. Knowledge of *B. heineanus* (based on limited data from seven locations in the coastal hinterland of northern New Guinea) indicates a tropical rainforest adaptation in contrast to the semi-arid savannah adaptation of the other *Borassus* species. In keeping with this tropical forest adaptation is the dorsi-ventral differentiation of the tissue layers in the leaf lamina (which it shares with *Borassodendron* species and most other palm species of the moist tropics) in contrast to the iso-lateral leaf anatomy of the other *Borassus* species adapted to the semi-arid tropics (Tomlinson 1961; Bayton 2007; Horn et al. 2009; Tomlinson et al. 2011). Other morphological characteristics which *B. heineanus* shares with *Borassodendron* include unarmed petioles with a comparatively high length to width ratio (Dransfield 1972; Bayton 2007); staminate inflorescences in the male plants that branch to one order only; and in the fruit of the female palms, pyrenes that are longer than they are wide and with internal flanges perpendicular to the main endocarp walls. Nevertheless, in several other respects − for example, in its pollen morphology (Ferguson et al. 1986), its costapalmate leaf lamina and most importantly its molecular characteristics (Bayton 2005) − *B. heineanus* resembles other members of *Borassus* rather than *Borassodendron*.

Geographically, the member of *Borassus* most closely connected with *B. heineanus* is *B. flabellifer*. It is to this latter species that the lontar belongs. Outliers for *B. flabellifer* are found in Southern China and the Western side of the Arabian Gulf but it is likely that these palms are not part of the species' natural distribution but are the result of dispersion by humans. The manifest material benefits from all species of the genus have led to their utilization across the full range of its distribution. Indeed, the morphological resemblances as well as similarities in the way different human societies have used the palms led early authors to group the African and Asian members under the single species label, *B. flabellifer*. It was only after reviews of the genus, by Beccari (1914) and later on by Kovoor and Hussein (1983), Dransfield and Beentje (1995), Ake Assi and Guinko (1996) and Bayton et al. (2006), that the African populations of the genus were considered sufficiently distinct to justify separate species status for the three African species recognized in Bayton's (2007) recent review; namely, *B. aethiopum* throughout much of equatorial Africa, *B. madagascariensis* confined to Madagascar, and *B. akeassii* in West Africa.

***Corypha* L.**

As for *Borassus*, the genus *Corypha* straddles the Wallace line with a distribution stretching from Southern India to Northern Australia. But unlike the case for *Borassus*, there are no African species of *Corypha*, and though greatly influenced by human usage, the distribution for *Corypha* clearly reflects an underlying natural distribution. Beccari (1933) recognized eight species in his posthumously published review of the genus, but most recent authors maintain no more than six.

The gewang, *Corypha utan* Lam., has been variously named in its different locations. Fox (1977) used *C. elata* Roxb., following early authors in India; other synonyms include *C. gebang* Mart., *C. gembanga* (Blume) Blume, *C. griffithiana* Becc., and *C. macropoda* Kurz ex Linden. However, in most modern treatments, *C. utan* has been accepted as a single species dispersed from southern India, the Andaman Islands and the Myanmar/Thai peninsular through to Southern New Guinea and Arnhem Land and Cape York in Northern Australia (Henderson 2009; Dowe 2010).

A striking member of the genus, the "talipot" of Southern India and Sri Lanka, *Corypha umbraculifera* L., can readily be distinguished from *C. utan* (Roxburgh 1832); the base of the leaf petiole in *C. umbraculifera* has a very distinct pair of auricles on the outer edge (Griffith 1850) that is not present in *C. utan*. Moreover, the petioles of *C. utan (*synonym *C. elata*) are much narrower than in the talipot and armed with teeth that are much larger. Roxburgh (1832) observed that *C. utan* has flowers with stamens longer than the petals, and an inflorescence that is more compact in structure than that of *C. umbraculifera*. This was confirmed in careful observations by Douglas and Bimantoro (1957) at Bogor Botanic Gardens. For all *Corypha* species, what appears to be a single enormous, pyramidal inflorescence atop the crown of leaves at the final phase of life is a structure of "*separate inflorescences … each one emerging from the axil of a reduced leaf*" (Henderson 2009). Douglas and Bimantoro (1957) confirmed Beccari's observation that in *C. umbraculifera* the primary inflorescence branches split through the subtending leaf sheaths, in contrast to the inflorescences of *C. utan* (synonym *C. elata*) that emerge from the mouths of the leaf sheaths.

The genus as a whole is in need of revision. *Corypha taliera* Roxb., which shares some of the characteristics of *C. umbraculifera* (e.g. auricles at the base of the leaf petioles, and inflorescences piercing the subtending leaf sheaths), was nevertheless considered a distinct species by Roxburgh (1820). Its separate species status has been maintained by subsequent authors. Recorded in the nineteenth century as being endemic to the Bay of Bengal, the species is currently listed on the IUCN Red List as extinct in the wild and in recent decades appears only to have been recorded in cultivated garden settings in India (Indian Botanic Garden, Kolkata), USA (Fairchild Tropical Garden, Florida) and perhaps Bangladesh (IUCN 2016). Another species, *Corypha microclada* Becc., is listed as vulnerable (IUCN 2016) and has only ever been recorded from the small island of Biliran in the Philippines. It remains to be seen whether separate species status for the Biliran populations can be maintained, given the widespread distribution of *C. utan* in the Philippines.

On the other hand, the species *Corypha lecomtei* Becc. ex Lecomte is extant in the wild and quite distinct from *C. utan* (Lecomtei 1917; Rukan and Suwanwaree 2010; Rukan et al. 2010) despite sharing with it narrow, non-

auricular petioles and having inflorescences that emerge from rather than breaking through the subtending leaf sheathes (Henderson 2009). The petioles have distinctive margins with much finer armament than *C. utan*. The maximum height of *C. lecomtei* at flowering is significantly shorter (5-15 m) than is the case for *C. utan* (20-30 m) but its inflorescence is larger and less compact than in *C utan.* There is very little overlap in the geographical distribution of the two, with *C. lecomtei* being distributed from eastern Thailand through Cambodia and Laos into Vietnam while *C. utan* has a more southerly coastal and riverine distribution.

DISTRIBUTION AND ECOLOGY

Fox (1977), like others before him (Banks 1771; Cook 1773), was deeply impressed with the lontar's adaptation and productivity in the harsh, rocky semi-arid environment of the island of Savu. Ormeling (1956) writing about the alluvial coastal plains of west Timor observed that lontar and gewang are pioneer species on seasonally burned lands, forming palm savannahs often in the wake of swidden cultivation.

Throughout the tropics of South and Southeast Asia, *B. flabellifer* L. and species of the genus *Corypha* L. occupy niches on semi-arid riverine plains and nearby foothills that most other Arecaceae with their wet-tropical origins eschew. Nevertheless, neither *Corypha* nor *Borassus* quite escape their humid tropical evolutionary past for they rarely thrive far from riverine plains or underground water sources. In the particular case of *B. heineanus* Becc., signs of its tropical forest adaptation are apparent in such a characteristic as the dorsi-ventral differentiation of its leaf lamina anatomy that contrasts with the isolateral leaf anatomy of other *Borassus* species adapted to the higher light intensity of their usual savannah habitats.

Borassus L.

It is an intriguing fact that the genus *Borassus* appears to be almost entirely absent from the Philippine archipelago. From past literature and feedback from researchers around the world, Kovoor (1983) assembled an approximate distribution for the genus which correlates quite closely with the map produced by Bayton (2007) based on herbarium specimens. Table 1 summarizes this distribution; apart from a few outliers, *Borassus* has a tropical distribution extending from West Africa through South Asia to Mainland Southeast Asia and eastern Indonesia but not to the Philippines.

As was noted above, Fox (1977) followed Beccari in maintaining separate species status, *B. sundaicus* Becc., for the lontar palm of Indonesia, a viewpoint that was later overturned by the taxonomic studies of Tjitrosoepomo and Pudjoarinto (1983). However, Fox was not inclined to attribute independent cultural origins for many of the practices he observed in the utilization of the *Borassus* palm in south-eastern Indonesia. While recording certain unique features in the technology employed in exploiting the palms on the islands of Roti and Savu, he nevertheless observed the overall resemblance of this technology to that in other significant centres of *Borassus*-use such as on the island of Madura off Java and in Tamil Nadu, India. According to Fox, Rotinese oral traditions maintained that the technique of tapping the lontar palm for its sugar had not originally been part of their own practices but had been brought to them via the islands of Ndao or Savu islands by legendary figures from western regions of Indonesia. Fox reckoned that "on balance, there is no conclusive evidence of origin but present appearances point clearly to India and Ceylon."

To the north of Indonesia, in Cambodia, the Indian elements in the *Borassus*-tapping traditions of the Khmer kingdoms are unmistakable. Likewise, *Borassus* planting and tapping remains today a significant part of the rural economy of Buddhist Pagan in Myanmar (Figures 3 and 6). On the other hand, the practice of tapping *Borassus* palms for sugar has never been a significant part of Philippine traditions. It is surely no coincidence that the Philippines for most of their history remained largely outside the sphere of influence of the Indianized kingdoms.

Kovoor (1983) had few reservations in asserting that the distribution of *Borassus* in Southeast Asia is a reflection of the dispersion of Indian cultural influence in the region. Based on biochemical studies (Kovoor and Hussein, 1983), he acknowledged a distinct biological difference between Asian *B. flabellifer* and *B. aethiopum*, but nevertheless appeared to imply that the designation of the two branches of *Borassus* as separate species was more for pragmatic reasons than reflecting a discontinuity with deep prehistorical roots.

On the Ivory Coast of West Africa, a long term study of an area of *Borassus aethiopum* savanna in a protected forest reserve has revealed much of the dynamics of the *B. aethiopum* life cycle in the absence of normal pressure from human exploitation (Gignoux et al. 2007). In common with other *Borassus*, but in stark contrast to species of *Corypha*, the fruits of the palm are large and heavy (0.5-1.5 kg), falling to the ground in relatively small numbers of 50-100 fruit per tree in any single year (Barot et al. 1999). In the protected forest, free from large animals (baboons and elephants for example), the majority of *B. aethiopum* fruits (normally carrying 3 seed each) are dispersed no more than 10 metres away from the mother tree. There is negligible seed dormancy and seed germination is remote-tubular. The hyperphyll (i.e. cotyledonary petiole) grows quickly to a length of up to 30-50 cm, burying the sprouting embryo well underground where it largely escapes the effects of normal seasonal grass fires (Uhl and Dransfield 1987; Tomlinson and Jeffrey 1990). The same is almost certainly the case for *B. flabellifer* in natural savannas such as those arising in fired lands in West Timor, Indonesia (Figure 3).

Fire plays a major part in contributing to the dominance of *Borassus* in such savanna lands (Ormeling 1956; Gignoux et al. 2007). In each of the life stages of the developing palm, there are features which confer a competitive advantage in the presence of fire. When the first entire leaves of *B. aethiopum* seedlings emerge from the ground, no part of the stem is visible. During the subsequent succession of one or two split leaves that

slowly develop for as long as ten years, the stem widens to attain its full juvenile girth but with its terminal bud still remaining below ground level, safe from the effects of fire. At the time when the first fully expanded costapalmate leaves appear, the apical bud remains relatively well protected from fire by a widening crown of green leaves (Tomlinson and Jeffrey 1990; Barot and Gignoux 1999). However, it is at this stage, before its stem elongates to lift the crown above the height of the predominant grass species that the palm is at its most vulnerable. As the trunks of juveniles develop and the initial leaf laminas begin to age and drop off, their petioles remain attached to the stem conferring another mechanism of protection from fire; it is the old dead leaf bases that draw the flame rather than the green crown with its protected apical bud. These petioles remain until the stem is about ten metres high. As the palms reaches sexual maturity, the dead leaves fall off as entire units, leaving the trunks bare (Tomlinson and Jeffrey 1990).

Palms at the onset of maturity are about 10 metres high and initially bear 10 to 25 living leaves, but by the final stages of life can attain 25-30 metres in height. In the case of *B. aethiopum* and the other two African species, *B. madagascariensis* and *B. akeassii*, the onset of sexual maturity is almost always heralded by a characteristic swelling of the trunk both in male and female palms (Dransfield and Beentje 1995; Bayton, 2007). In *B. flabellifer*, on the other hand, such swelling of the trunk is not observed either in the Indian subcontinent or in Southeast Asia.

Adult palms of *B. aethiopum* have a high root density within 3 metres of the main trunk (in which zone 75% of the mature fruit fall) and appear to be very effective scavengers of scarce soil nutrients. This presents considerable competitive pressures for their seedlings. In the absence of large animal dispersion of fruits, seedlings emerge within ten metres of the mother palm. However, Barot et al. (1999) suggest that *B. aethiopum* has evolved its big fleshy fruits to attract dispersion by large herbivores. This raises questions about the evolutionary connections between the African and Asian branches of *Borassus* and indeed about the differences in evolutionary pressures that produced the markedly different reproductive behaviors of *Borassus* and *Corypha*.

***Corypha* L.**

Tracing the natural distributions of the two genera is difficult because both have wide human usage and many of their habitats have been transformed by the agricultural revolution (Table 1). Thus the species *C. taliera* last observed growing in "natural" circumstances in 1979 (in Birbhum, West Bengal) is now declared to be extinct in the wild (IUCN 2016). Moreover, even for the closely related species *C. umbraculifera*, its apparent natural distribution in Kerala and places like Kumta and the Yellapur Ghats in Karnataka of South West India, as well as in northern Sri Lanka, are greatly influenced by human usage. The occasional plants observed in Myanmar and Thailand are most commonly associated with Buddhist temple compounds. Already by the late 19th Century, Joseph Hooker was writing of *C. umbraculifera*: "*This must be a native palm* [of Ceylon] *but I have never seen it in original jungle. Of the vast number of seedlings which come up near the parent tree, very few arrive at maturity, the young leaves being continually cut. Beddome* [of the Madras Forest Department] *remarks that he has never seen it wild in S. India.*" (Hooker, cited in Blatter 1926).

In contrast to *C. taliera* and *C. umbraculifera*, the gewang palm, *C. utan*, was almost certainly part of the indigenous flora of Eastern India and Southeast Asia (Table 1). In Northern Australia, there are significant pockets of *C. utan* in east Arnhem Land (Liddle et al. 1994) and *Corypha*-dominated riverine forest strips occupying hundreds of hectares in Cape York Peninsula (Frith and Frith 1995). Dowe (2010) records: "*in the Northern Territory it occurs on the floodplains of the Tomkinson and Liverpool Rivers and the Arafura Swamp, and in Queensland in Cape York Peninsula from the lower reaches of Mitchell River in the west, throughout the peninsula (except the far north) to Normanby River in the east. It occurs in groves, small groups or scattered individuals associated with floodplain channels, anabranches, billabongs and seasonally wet depressions, and as a riparian element on the banks of seasonally flooded streams and rivers, as the dominant tree or as a canopy element where large trees are present*" (see Figure 6).

In the small, conserved riverine forest of Bipolo, West Timor, Indonesia, giant gewang trees in the final stage of life are found in flower in all months of the year (Figures 2 and 6). They are prolific seeders and beneath open gaps in the forest canopy, vast numbers of seedlings emerge. It is likely that germination of seeds is inhibited by modified light conditions beneath the canopy proper, as is found in several other palm species of tropical forests (Latifah et al. 2014). Under natural conditions very few of the seedlings survive to form mature palms within the vicinity of the mother palm, but near human habitations large uniform groves of the single species are observed, which are almost certainly artifacts of human exploitation (Figure 2). The fruit walls are food for birds, bats, and small animals which can disperse the small but very hard seeds far and wide (Dowe 2010).

Brown and Merrill (1919) noted that *C. utan* also occurs very widely dispersed in the Philippines extending from northern Luzon, southern Mindanao, Palawan, to the Sulu Archipelago as scattered palms and occasionally planted groves. In some places it is "exceedingly abundant, gregarious, and locally the dominant species" amounting to thousands of hectares: "*in the Rio Chico region, Pampanga Province, Luzon … there is … a buri forest covering approximately 5,000 hectares …. with 9,205,710 buri palms mostly over 2 meters in height but without clear trunks. Of such sizes, there were 6,368,432 palms on the area. Buri is especially abundant in the provinces of Pangasinan, Pampanga, Tayabas, Camarines, and Sorsogon in Luzon, and in parts of the islands of Palawan, Mindoro, Panay, Neeros, Masbate, Cebu, Bohol, and Mindanao*".

UTILIZATION

Borassus L.

As is the case in Africa, the most significant use of the *Borassus* palm in Asia is for its sugary sap that can be tapped and processed into a range of end-products: a sugary drink, ('sweet toddy' known as 'nira' in some parts of Indonesia); palm wine ('tuak'); vinegar ('cuka'); distilled spirits ('arak'); and a variety of evaporated products ranging from raw brown sugar, through treacle, to crystallized and candied sugar (Batter 1926; Davis 1988). In eastern Indonesia, Fox (1977) estimated that at the height of the tapping season, a mature lontar palm could yields up to 6.7 litres of juice (or 1 kg of evaporated sugar

Table 1. The core distributions for the genera *Corypha* L. and *Borassu*s L. according to a selection of published records.

Species	Country	Sources of information [1]
Corypha		
utan	**Australia** (North Queensland,Northern Territory); **Papua New Guinea** (Western Province); **Indonesia** (Maluku, Sulawesi, Lesser Sunda Islands, Kalimantan, Java; Sumatra); **Philippines** (Luzon, Mindoro, Palawan, Cebu, Mindanao, Sulu Archipelago); **Cambodia**; **Vietnam**; **Laos**; **Thailand** (Peninsula); **Malaysia** (Sabah, Kedah); **Myanmar** (Tanintharyi); **Bangladesh**; **India** (Andaman Is; West Bengal, Assam).	Van Rheede Drakenstein (1678): *Cum* Rumphius (1741): *Cut* Lamarck (1786): *Cut* Roxburgh (1820): *Ct*; *Cum* Roxburgh (1832): *Cut; Cum* Griffith (1850): *Cut;Cum*
microclada	**Philippines** (Biliran Island only).	Lecomte (1917): *Cl*
lecomtei	**Vietnam** (Cochinchina); **Cambodia**; **Laos**; **Thailand** (Prachinburi Province).	Brown & Merrill (1919): *Cut* Blatter (1926): *Cut*; Ct; *Cum*
taliera	**Extinct in the wild**: **Bangladesh** (Dhaka, only in cultivation); **India** (West Bengal, only in cultivation).	Heyne (1927): *Cut* Beccari (1933): Cut; Cm; Cl; Ct; Cum Burkill (1966): *Cut*
umbraculifera	**Sri Lanka**; **India** (Tamil Nadu, Kerala, Karnataka, Maharashtra); C**ambodia**, **Thailand**, and **Myanmar** (only in cultivation).	Johnson IUCN (1998): *Ct* Basu et al. (1987) Cut; *Ct*; Cum Barfod et al. (2001): *Cut* Henderson (2009): *Cut*; *Cl*; *Ct; Cum* Dowe (2010): *Cut* Rukan et al. (2010): *Cut*; *Cl*
***Borassu*s**		
heineanus	**Papua New Guinea** (East Sepik and West Sepik provinces); **Indonesia** (Papua Province: Jayapura and Sarmi regencies).	Rheede tot Drakenstein (1678): *Bf* Rumphius (1741): *Bf*
flabellifer	**Indonesia** (Papua, Maluku, Sulawesi, Lesser Sunda Islands, Madura, Java, Sumatra); **Vietnam**; **Cambodia** (Kandal, Takeo, Kompong Chhnang, Kompong Speu); **Laos**; **China** (South Central); **Thailand** (Bangkok, Nonthaburi, Phichit, Rayong and Songkhla); **Malaysia** (Kelantan state); **Myanmar** (Mandalay, Magwe); **Bangladesh** (Chittagong, Chittagong Hill Tracts and Dhaka); **Sri Lanka** (Eastern, North Western, Northern); **India** (West Bengal, Odisha, Tamil Nadu, Uttar Pradesh, Karnataka; Maharashtra); **Yemen** (Socotra Island).	Martius (1838): *Bf*; *Bae* Ferguson (1850): *Bf* Jumelle & Perrier (1913): *Bm* Beccari (1914): *Bh*; *Bf*; *Bm*; *Bae* Beccari (1924): *Bh; Bf; Bm; Bae* Heyne (1927): *Bf* Chevalier (1949): *Bae* Portères (1964): *Bae*
madagascariensis	**Madagascar** (Mahajanga and Toliara).	Burkill (1966): *Bf*
aethiopum	**Madagascar** (Antsiranana, Nosy Be); **Comoros**: **Mozambique**; **South Africa** (Limpopo); **Zimbabwe** (Masvingo); **Zambia** (Southern province); **Malawi**; **Tanzania** (Mara, Pemba South, Pemba Island, Tanga); **Kenya** (Coast province); **Ethiopia** (Benishangul-Gumaz, Gambela); **South Sudan** (Junqali, Upper Nile); **Uganda**; **Democratic Republic of the Congo** (Kassai-Occidental and Sud-Kivu);**Central African Republic**; **Chad**; **Cameroon**; **Gabon**;**Nigeria** (Delta and Niger states); **Niger** (Dosso department); **Benin**; **Togo**; **Ghana** (Ashanti, Greater Accra regions); **Burkina-Faso** (Ganzourgou, Kompienga and Tapoa provinces); **Ivory Coast** (Bas-Sassandra, Lagunes and Sud-Comoe regions); **Mali** (Kayes, Djenné); **Guinea**; Guinea-Bissau; **Senegal** (Matam, Tambacounda, Thiès, Cap-Vert); **The Gambia; Mauritania** (Trarza).	Fox (1977): *Bf* Lubeigt (1982): *Bf* Kovoor (1983): *Bf*; *Bae* Kovoor & Hussein (1983): *Bae* Paulas & Muthukrishnan (1983): *Bf* Tjitrosoepomo & Pudjoarinto (1983): *Bf* Uhl & Dransfield (1987): *Bf*; *Bae* Davis and Johnson (1987): *Bf* Dransfield & Beentje (1995): *Bm; Bae* Aké Assi & Guinko (1996): *Bak* Barfod et al. (2001): *Bh* Sambou et al. (2002): *Bae* Bayton et al. (2006): *Bm*
akeassii	**Democratic Republic of the Congo** (Kassai-Occidental province); **Central African Republic**; **Nigeria**; **Niger**; **Benin**;**Burkina Faso** (Comoé, Houet, Kadiogo); **Ivory Coast**; **Mali**; **Senegal** (Kaolack, Louga, Thiès)	Bayton (2007): *Bh*; *Bf*; *Bm*; *Bae; Bak*

[1]Note: Meanings for the codes following the author citations are: *Cl – C. lecomtei*; *Cm – C. microclada*; *Ct – C. taliera*; *Cum*– C. *umbraculifera*; *Cut – C. utan*; *Bae – B. aethiopum*; *Bak – B. akeassii*; *Bf – B. flabellifer*; *Bm – B. madagascariensis; Bh*– B. *heineanus.*

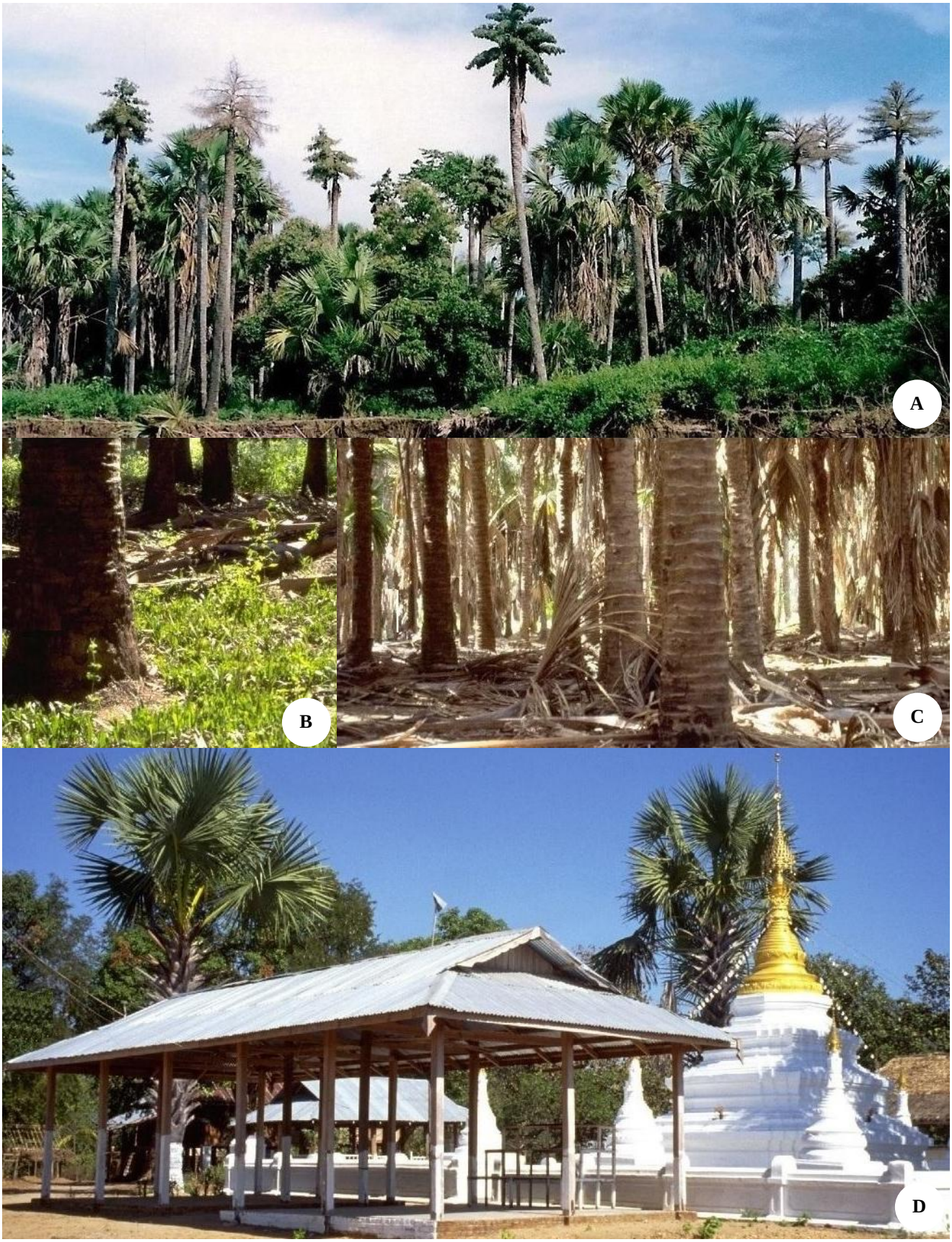

Figure 2. *Corypha* spp. and their habitats. A. Mature riparian forest dominated by *Corypha utan* Lam; B. Abundant *C. utan* seedling germination, at forest edge; C. Exclusion of competing vegetation in a 'managed' self-seeded *C. utan* 'mono-crop' nearby (A-C: Bipolo, West Timor, East Nusa Tenggara, Indonesia; 1995); D. *Corypha* sp. planted and maintained in a temple complex (Kyaukme, Shan State, Myanmar, 1998)

Figure 3. *Borassus flabellifer* L. and its diverse habitats. A. Typical leaf and fruit of a pistillate lontar palm; B. Mature palm grove (Sumenep, Madura Island, East Java, Indonesia, 2016); C. Minimum care lontar grove in savannah foothills (Kupang, West Timor, East Nusa Tenggara, Indonesia, 1995); D. Palm monocrop (Nyaung-U District, Mandalay Division, Myanmar, 1998).

syrup) per day. Khieu Borin (1996) has reported similar yields in Cambodia. Fox described in detail the technology employed in Roti and Savu to extract and utilize the sugary sap, and then compared these techniques with published accounts of those in Madura and Southern India. Kovoor (1983) summarized the variation in practices found in India, Sri Lanka and Southeast Asia. He noted that the Asian method for extracting the sap from the rachillae of inflorescences (of either male or female palms) has distinct advantages over the technique employed in much of Africa where the sap is usually extracted from the terminal growing point leading to a quick end to the productive life of the palm (Sambou et al. 2002). Dalibard (1999) has compared the sugar producing capacity of *Borassus flabellifer* with that of a number of other palm genera including the other important Indonesian sugar palm *Arenga pinnata* (Wurmb) Merr. that is better adapted to higher rainfall ecosystems than is the lontar (Mogea et al. 1991).

The scientific literature of the colonial era recognized that tapping the palm for its sugary sap was just one of the wide range of technologies traditional societies had developed to exploit the versatility of the hardy *Borassus* palm. Ferguson's (1850) classic account suggested that

exploitation of the palm in India could be traced back thousands of years and referred to the 801 uses for the palm catalogued for example in the "Tala Vilasam", a famous Tamil poem extolling its virtues. Among the products the 'Tree of Life' was listed as providing were: the edible, jelly-like, immature endosperm of the seed (which in modern times in some countries is canned, preserved in syrup); the sweet, mesocarp pulp enveloping the pyrenes of the mature fruits that can be sun dried or roasted (Rumphius 1741); the tender apical bud known as the palm 'heart'; the underground seedling (Figure 4) with its starch-filled, geotropic, apocole (cotyledonary sheath) and upward growing hyperphyll (cotyledonary petiole of the first bladeless leaf) (Padmanabhan et al. 1978); mature palm trunks used as pillars and posts; and narrow planks split from tough outer layers of the mature trunk used as rafters for roofing supports (Blatter, 1926); strong fibres extracted from the leaf bases and used for a wide variety of purposes including cordage and as bristles in scrubbing brushes (Davis and Johnson 1987); whole leaf laminas or their leaflets that can be used for thatch, and to make fans, hats, mats, sails, as well as a diversity of plaited containers (Figure 4); and individual leaflet portions of the laminas that were used in the past in great numbers as writing media to communicate Hindu and/or Buddhist sacred teachings (Bhoi 2010).

One example only of the complexity of this technology and the depth of the literature describing it is quoted here from the venerable account of the lontar written by Rumphius (1741). Here, he is describing the utilisation of the pulpy endocarp of the mature fruit to produce a foodstuff known in Sri Lanka as 'punatoo': "*after the ripe fruits … have been collected, the stalk and the cups* [presumably, the persistent tepals] *are twisted off with the hands, the outer rind is stripped off, and the peeled fruit are washed … then pressed out … until all the yellow juice has been drawn out; this is … repeated twice, thrice … and it assumes a thick consistency; … they spread large mats … on which they then pour out the liquor … then leave it to dry for one day and on the next day they pour fresh juice, which is again left to dry, repeating the same labour until this cake has acquired the thickness of three fingers … When this becomes a hard as cheese, it is cut into square pieces … placed in baskets, and sprinkled … with water in which salt has been dissolved. … And these baskets … are … smoked for several days; but not too much lest the Punata become bitter. … The people of Makassar prepare the fruits in a much more convenient manner, nor do they spend so much labor. They merely press out the juice, and then pour it into large platters and mix it with the rice-meal, and prepare many kinds of foods with it."* (Rumphius 1741).

Fox (1977) noted that many of the uses listed by Ferguson and others in India (including the making of punatoo) were not practiced in the palm cultures of eastern Indonesia. On the other hand, there were a number of uses of *Borassus* unique to these islands not only for making the iconic headwear of the Rotinese and their unique musical instrument the 'sesandu', but also innovations in the tapping and processing of sugar. The sap with a soluble sugar content exceeding 10% provides not only a direct energy input in the diet of palm-tapping communities, but also sustains the important small livestock component of the economy (Khieu Borin 1996; Dalibard 1999). Fox observed that on Roti the lontar is so plentiful that rarely is there a need to plant it out. However, in Savu where there is greater dependence on the palm, it is often carefully planted, sometimes spaced out in walled off groves, sometimes in rows on rice bunds, at other times as wind breaks and boundary markers around plots of sorghum, annual crops and fruit trees (Figure 6).

In Tamil Nadu, the number of palmyra palms has been estimated to be as many as 40 million (Kovoor 1983), which traditionally has sustained the livelihoods of thousands of poor village communities. In modern times there has been great social change in the region and the palm economy is often viewed as a legacy of the past (Hardgrave 1969; Depommier 2003).

Perhaps nowhere is the present-day utilization of *Borassus* more sophisticated than in the thousands of hectares of neatly planted rows distributed along the eastern bank of the Ayeyarwady River, south of Pagan in Myanmar (Figures 4 and 6). Lubeigt (1982) has used the term "Palm Civilizations" to describe the kind of economy that evolved in the Indianized centres of Southeast Asia, for example in the Buddhist principalities of Thailand and the Khmer kingdom of Cambodia.

South of Pagan, the *Borassus* palms are planted out in a checkerboard fashion as wind breaks around annual crops like maize, sorghum, pigeon pea, sesame and chilli (Figure 4). Occasionally, there are orchard-like blocks of *Borassus* palms (Figure 3). Generally, it is the land-owning farmer who possesses the palms. These are contracted out to palm-climbing specialists who live a hard and sometimes dangerous life tapping the sugary sap of about 30 palms a day; this is boiled, evaporated and processed by the

climber's family into a range of marketable sugar end-products. The important study of Khieu Borin (1996) in Cambodia suggests that producing crystallized sugar from the sap is becoming increasingly unsustainable because of the quantities of scarce woody fuel required in boiling down the syrup. However, the study also showed that using the sugary sap directly as the principle energy in livestock rations results in a much higher economic return with fewer negative consequences for the environment.

In the island of Madura, off the Northeast east of Java, another locality of intensive Borassus use, palms were traditionally planted close together in rows with their canopies touching so that bamboo scaffolding could enable tappers to pass from one palm to another without the need to ascend and descend individual trees in the process of collecting the sugary sap from the tapped inflorescence rachillae (Gebius and Abdul Kadir 1929). The legacy of this can be seen today (Figure 3) even though in modern times sugar tapping is a less mainstream activity than it was in the past. Harvesting the leaf blade for a wide range of uses continues today on Madura as in Tamil Nadu, India, but in the age of plastic is of less commercial importance than it assumed in the 1920s.

In most parts of this "Palm Civilization", the life of the climber and his family has often been a hand-to-mouth existence. In Tamil Nadu, for example, the social complexity of the palm-tapping way of life is a hotly contested political arena (Vannan 2011). Nevertheless, the palm-based economy is often less precarious than the alternatives that might be available. Fox (1977) determined that the palm-tapping of the Savunese and Rotinese has provided a more secure subsistence platform from which to launch into other economic pursuit than is possible for shifting cultivator communities living on the proceeds of rain-fed annual crops and livestock grazing in much of semi-arid Nusa Tenggara Timur. On dry, rocky Savu it is lontar alone that has been pivotal to this stability, but in the better watered Roti gewang has been almost as important.

Figure 4. Economic use of *Borassus flabellifer* L. A. Inflorescence rachillae tapped for palm sugar. Pagan, Myanmar, 1998. B. Ingenious use of palm leaves as seen here in the vessel used to catch the sugary sap. Waingapu, East Sumba, 2013. C. Harvested leaf parts are sold for a wide range of uses. Sumenep, Madura, 2016. D. The palm fruit can be eaten fresh and or even canned, but the seeds from the fruit can be sprouted by burying them in pits. The large sprouted seeds are sold as seen here in Thiri Mingalar Market in Yangon, 1998.

***Corypha* L.**

It is easy to underestimate the contribution that *C. utan* has made to the way of life of the eastern end of the Malesian archipelago. Even in modern times the leaf petioles of the gewang are still used as an excellent simple building material more than 50% of traditional housing in lowland Timor and surrounding islands (Figure 5). Unlike the timber of the main trunk and the leaf lamina which are of mediocre value as building materials compared to that of the lontar, the long, straight, tough leaf petioles of the gewang allow for the construction of cheap wall-panelling that compares favorably with other traditional panelling.

In the Philippines, a substantial export industry has long existed in products crafted from the leaves of the palm they call 'buri' (Brown and Merrill 1919). Calapis et al. (2011) record that in the year 2000 the size of this export amounted to $53 million. Of greatest value is the fine fibre bundles called 'buntal' extracted from *C. utan* leaf petioles and converted into such things as the fashionable "Lucban" hats and hand bags (The Buri Bag Project 2016). But there are several other leaf materials with different properties ('buri' from the mature leaves and 'raffia' from the unfolded immature leaves) that have niche uses for cordage, basketry, matting, brushes and wrapping materials of export quality, but also for humbler local purposes (fencing, fuel, carrying bags and the like).

There is a complementarity in the use of *Borassus* and *Corypha* in locations such as the island of Roti, Indonesia. In the Indian subcontinent, the superiority of *Corypha* leaves over *Borassus* was recognized from ancient times for some purposes (e.g. as a preferred writing material on which to record their sacred texts), but the sheer versatility of the *Borassus* trees appears to have led to the demise of *Corypha* in places where perhaps it was once more abundant. Nevertheless, this has come at a cost; for in Tamil Nadu where heavy use of *Borassus flabellifer* is made for its leaves, cannibalism of the leaf crown is believed to reduce sugar yields, weaken the trees and even lead to their demise (Davis and Johnson, 1987).

Similarly, in the case of Madura, the exploitation of Borassus for its leaves in the 1920s was believed to be having deleterious effects on the commercial production of palm sugar from the island (Gebuis and Abdul Kadir, 1929). This situation might have been worse if there hadn't been a relative abundance of gewang palms ('pocok') on the island. At that time, the leaves of the *Corypha* palms on Madura were woven into a high quality matting ('agel') which was widely used as packaging for the export of agricultural produce including coffee from Java and Sumatra to overseas markets (Heyne 1927).

Pith from the mature gewang's trunk, known in North Sulawesi as 'gumbar' and in West Timor as 'putak' (Heyne 1927; Bamualin et al. 1990; Umar et al. 1991), is processed into sago. Even today, use is made of the gewang's 'putak' as animal food (Ginting-Moenthe et al. 2002) and of its sago for occasional human food (Figure 5). The gewang can also be tapped to produce palm sugar (Figure 5), although this is rarely done in locations where lontar is plentiful (Rumphius 1741; Heyne 1927, Dalibard1999).

ORIGINS

***Borassus* L.**

The Coryphoid tribe Borasseae is classified into two subtribes each with four genera; namely subtribe Lataniinae (consisting of genera *Borassus* L., *Borassodendron* Becc., *Lodoicea* Comm. ex DC, and *Latania* Comm. ex Juss.); and subtribe Hyphaeninae (consisting of *Bismarckia* Hildebr. & H. Wendl., *Satranala* J. Dransf. & Beentje, *Medemia* Wurttenbe ex H. Wendle., and *Hyphaene* Gaertn.). The tribe is centered on the Indian Ocean, with its westernmost margin in West Africa and easternmost in Papua New Guinea.

Bayton (2005) carried out a phylogenetic analysis of the Borasseae and its outgroups, based on the nucleotide sequencing of five well characterized chloroplast regions and two low copy nuclear genes. The analysis provided broad support for the taxonomic consensus summarized by Dransfield et al. (2008); the Borasseae, its subtribes Hyphaeninae and Lataniinae, as well as the genus *Borassus* L. were each confirmed to be monophylous.

Bayton (2005) went on to examine the issue of whether the nucleotide sequencing data could provide an estimate of just when it was that the constituent genera of the Borasseae began to diverge from one another. Initial attempts to calibrate the dating for the evolution of the tribe based on the estimate of 7.8 Mya for the volcanic uplifting and formation of the Mascarene island chain where the endemic genus *Latania* currently grows, produced unrealistically late estimates for the timing of key evolutionary events. A more plausible scenario was obtained by calibrating the evolutionary tree obtained from the sequencing data with well characterized events in the fossil record, such as the occurrence of the Coryphoid fossil, *Sabalites magothiensis* (Berry) Berry, estimated by independent methods (Berry 1914) to be from the Upper Cretaceous, around 80 Mya.

Based on this admittedly preliminary analysis, Bayton (2005) estimated that the two subtribes comprising the Borasseae diverged from one another about 47 Mya in the Eocene, that *Borassus* diverged from its sister genus *Borassodendron* about 35 Mya at the end of the Eocene, and that the widely dispersed semi-arid adapted species of *Borassus* (*B. flabellifer* and *B. aethiopum* for example) diverged about 26 Mya from their sister *Borassus heineanus*, confined in modern times to the humid fringes of tropical northern New Guinea.

The recent phylogenetic analyses have also produced two surprises: (i) The taxon that appears to be phylogenetically closest to the Borasseae-*Corypha* clade is the Caryoteae (Uhl et al. 1995; Hahn 2002; Dransfield et al. 2005; Asmussen et al. 2006) a monophyletic tribe so distinct in its morphology from the rest of the Coryphoid subfamily that in previous taxonomic treatments of the Arecaceae it had been placed in the subfamily Arecoideae (Uhl and Dransfield 1987). (ii) On the basis of Bayton's (2005) analysis, the Borasseae diverged from their apparent sister clade, the genus *Corypha*, only 57 Mya; long after the initial break up of Gondwanaland, an event which some

authors had previously speculated to be the triggering mechanism for the dispersal and evolution of the Borasseae.

***Corypha* L.**

In not all published phylogenies does *Corypha* emerge unequivocally as sister to the Borasseae (e.g. only 66% bootstrap support in the study of Asmussen et al. 2006). However, the monophyly of a clade incorporating the Caryoteae along with *Corypha* and Borasseae receives high bootstrap support (e.g. 91%, in Asmussen et al. 2006). Like *Corypha*, the tribe Caryoteae is distributed from south eastern Asia, through Melanesia (including Vanuatu in the case of the genus *Caryota*) to north eastern Australia.

According to Bayton's (2005) analysis, the clade 'Borasseae + *Corypha*' separated from the Caryoteae about 67 Mya at the end of the Cretaceous. This raises the counter-intuitive possibility that in the breakup of Gondwana, the ancestral line for this clade may have rafted on the Australian/New Guinea shard of the ancient southern continent rather than having arisen on the north-western fringes of the Indian Ocean.

On balance, this seems unlikely. The genus *Caryota* L. straddles the Wallace Line, with more species West of Sulawesi than East (Dransfield 1981). The same is true for the other Caryoteae genera, *Arenga* Labill. ex DC (Dransfield 1981; Mogea 2004) and *Wallichia* Roxb. (Henderson 2009) and for the next most closely related Coryphoid genera, *Kerriodoxa* J. Dransf., *Chuniophoenix* Burret and *Nannorrhops* H. Wendl. (Bayton 2005; Henderson 2009). Moreover, as we have seen, the genus *Corypha* has a distribution that spans the whole of south eastern Asia from Sri Lanka and the Andaman Islands through Indonesia to the Philippines. Is it remotely plausible that *Corypha* or its immediate evolutionary precursors could have evolved on the Australasian/New Guinea Plate?

There is evidence that the core monocot families may have had their beginnings in Gondwana in a nexus between what is now South America, Antarctica and Australasia during the favorable climatic period of the mid Cretaceous around 100 Mya (Janssen and Bremer 2004; Bremer and Janssen 2006). The earliest undisputed palm fossils are north American; *Sabalites* spp.- costapalmate leaves with apparent Coryphoid attributes-from the late mid Cretaceous to early Upper Cretaceous (Berry 1914). During the later favorable climates of the Palaeocene-Eocene (around 65-35 Mya), the palms underwent a significant radiation giving rise to most of the genera recognized today (Harley 2006; Dransfield et al. 2008).

The apparent triggering event in this process of evolution was the break-up of the super continent of Pangaea, beginning with the gradual separation of the land masses that would eventually become North America and Eurasia away from the Gondwana continent. A rift in Gondwana itself began opening up in the Lower Jurassic Epoch, 180-165 Mya, between the west coast of the African land mass and the east coast of the South American (McLoughlin 2001). The Madagascar/India shard broke away from Antarctica in the Lower Cretaceous (around 130 Mya), later coming into collision with the Eurasian plate during the Palaeocene (by 65-55 Mya) giving rise to the Himalayas in the Eocene about 45 Mya (Briggs 2003).

Meanwhile, South America and Australasia remained connected through Antarctica until well into the Paleogene Period and the breakup of this connection did not take place until as late as the Eocene around 50-35 Mya (Veevers et al. 1991; McLoughlin 2001). Once breaking its connection, New Guinea/Australasia /New Caledonia drifted north, until about 35 Mya, when New Guinea-the leading edge of the Australian Plate-began colliding with the south western part of the Pacific Plate in the Miocene (15 Mya) an event which pushed up the high mountains of the New Guinea range, and created the rain shadow that triggered increasing aridity in Australia from the late Miocene onwards (Metcalf 2002).

The extreme desiccation of the Australian continent has resulted in a comparatively impoverished modern day palm flora (54 species in 17 genera, compared with 280 species in 31 genera for the island of New Guinea; Dowe 2010), but fossil evidence suggests that palms were more widespread in Australasia in the past. The most diverse of the of Australia's modern day genera is *Livistona* R. Br., a genus in the Coryphoideae but one that is somewhat distantly related to *Corypha* and *Borassus* (Bayton 2005; Dransfield et al. 2005). There are currently 18 Australian species recognized in this genus and according to preliminary molecular investigations (Dowe 2001) these form a monophyletic clade that is sister to the other modern day *Livistona* species in Asia and further west. Some remote Australian outliers of the genus have been regarded as relics of a former more favorable climate left stranded by aridification; however recent research has suggested, at least in the case of *L. mariae* F. Muell., that isolation in Central Australia is more likely to have been the result of active dispersal, very possibly by Aboriginal people, in millennia past (Kondo et al. 2012).

Based on the molecular evidence, Dowe considers that, despite its diversity and well established presence, the Australian branch of *Livistona* is more likely to have evolved from a single ancient introduction from a Eurasian source rather than having arisen from an authotochonous element (Dowe 2001, 2010). The same, he believes, is likely to be the case for the gewang, *Corypha utan*. In the case of *C. utan*, he notes that there was a land bridge joining southern New Guinea to Cape York prior to 10,000 year ago and from south-western New Guinea to eastern Arnhem prior to 18,000 years ago (Chivas et al. 2001). Palms were present on the land-bridges as indicated by (unidentified) palm fossil pollen (Prebble et al. 2005). Today, *C. utan* populations of significant extent in Cape York and eastern Arnhem Land are cut off by the Torres Strait Sea from the populations in similar habitats on flood plains and riparian environments in southern New Guinea. The massive fruiting potential of the terminal hapaxanthic inflorescence producing seed small enough to be widely disseminated by southward migrating frugivorous birds (Dingle 2004) indicates that dispersal into tropical northern Australia is very likely an on-going phenomenon today despite the Torres Strait gap.

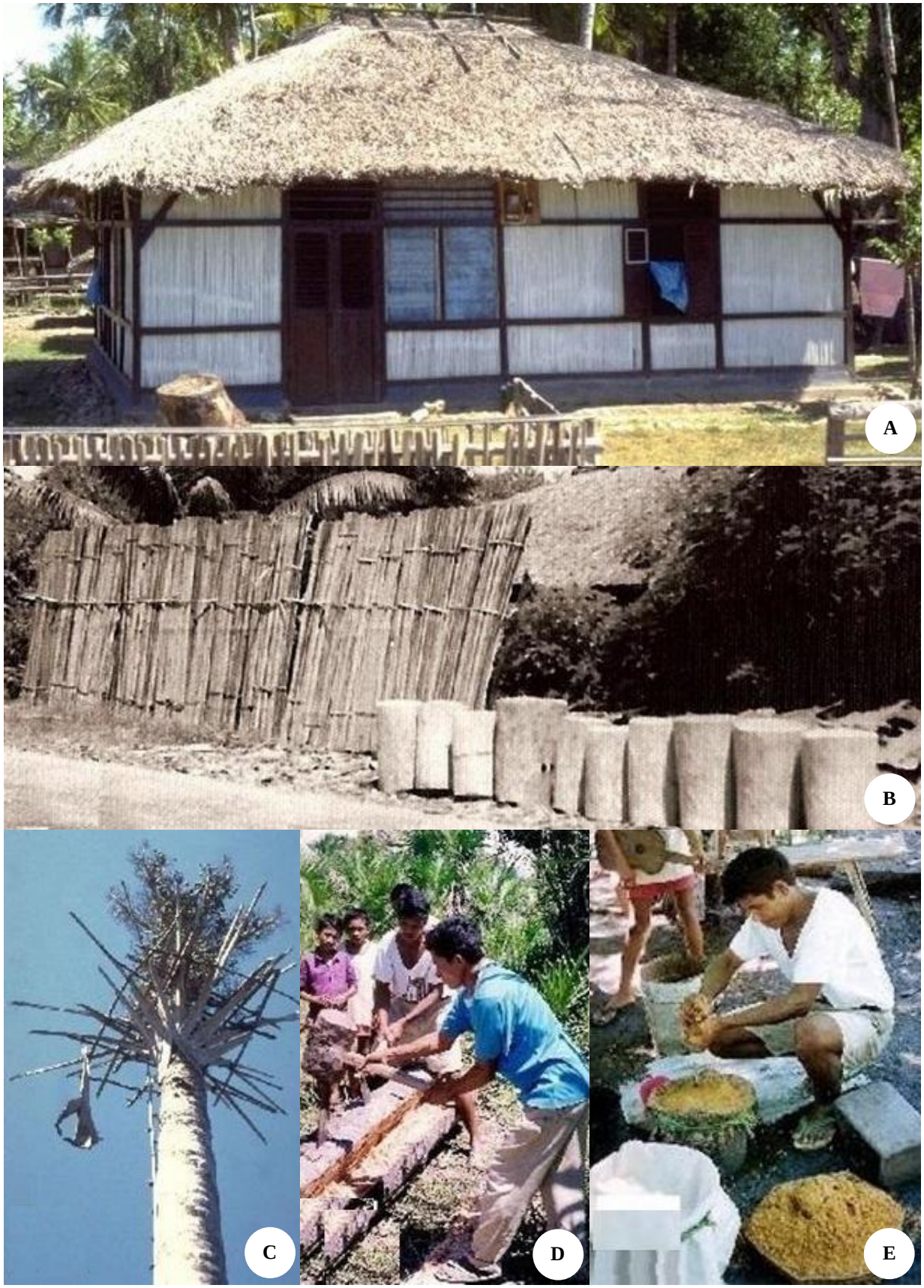

Figure 5. Economic uses of *Corypha utan* Lam. A. Wall paneling, roofing and fencing; B. Road-side sale of harvested leaf petioles (used in wall panels) and palm pith sections for animal feed; C. Platform high in a late stage palm from which inflorescence rachillae are tapped for palm sugar. D. Trunk of a felled palm being split for its sago; E. Palm pith ground, soaked and filtered to produce sago (Babau, West Timor, East Nusa Tenggara, Indonesia; 1995)

THREE HYPOTHESES

This review of recent advances in our taxonomic understanding of the pivotal role of *Borassus* and *Corypha* in the ecology and traditional economies of semi-humid parts of Malesia gives rise to three testable hypotheses. These are: (i) The gewang (*C. utan*) was a significant component of pre-agricultural economies of the Malesian archipelago in the periods prior to and during the peopling of continental Australia and well before the arrival of the definitively Indianising cultural influences of the last two millennia. (ii) The lontar (*B. flabellifer*) was present and utilized in the archipelago prior to its full flowering during the Indianized kingdoms of the Shailendras beginning around the 8th century CE. (iii) The palm family as a whole has been of greater importance in the sustenance of pre-agricultural peoples than has often been recognized, and that (in the words of Fox (1977): "*whole cultures can legitimately be described as adaptations to certain species of palms …. There is the doom palm (*Hyphaene thebaica*) of ancient Egypt and the date palm (*Phoenix dactylifera*) of the Middle East; the coconut palm (*Cocos nucifera*) and the oil palm (*Elaeis guineensis*)-commercially, the world's most exploited. Among sap-producing palms, there is the wild date palm (*Phoenix sylvestris*) of India, the sugar palm (*Arenga pinnata*) and the Nipa palm (*Nypa fruticans*) of Indonesia and the Philippines, and the African wine palm (*Raphia vinifera*). In a class by itself is the sago palm (*Metroxylon sagu*) and its related segregates. A single felled trunk of this species can actually yield up to 1,200 pounds of edible crude starch.*"

Proto-agricultural origins

It is quite possible that *C. utan* pre-dated the arrival of man into the archipelago and was among the earliest of plant species to be used there. During the last ice age, which ended about 12,000 years ago, the climate of the region was more arid than it is now and large areas of the existing sea-bed between Southeast Asia and Australia was exposed (Chivas et al. 2001), providing a wider habitat for semi-arid species like the gewang. It is probable that the utilization of the gewang belonged originally to a foraging lifestyle and only later was it incorporated, virtually without domestication (in the genetic sense of the word) into the fringes of agricultural lifestyles. Sago processed

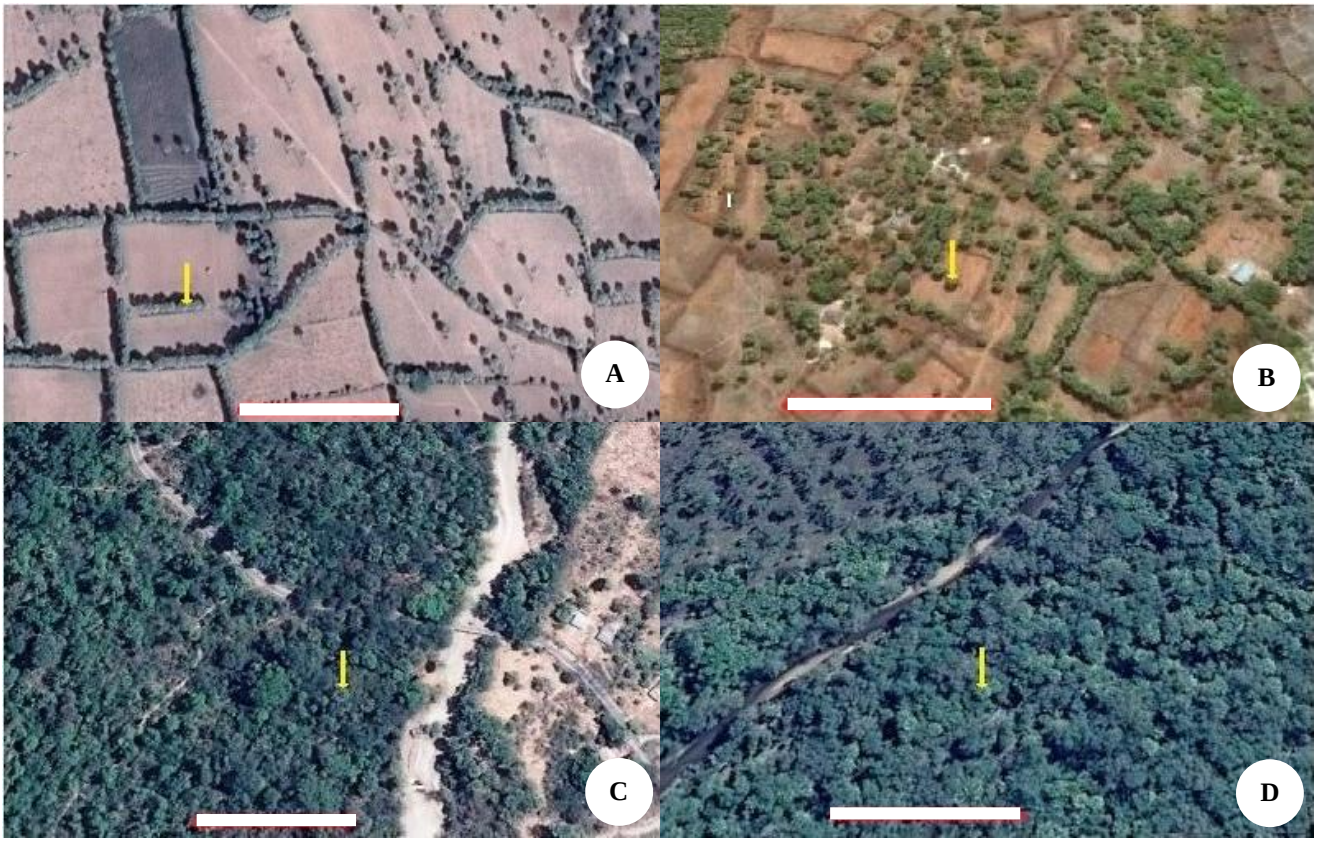

Figure 6. Aerial views of contrasting palm ecosystems (A-B *Borassus flabellifer* L.; C-D *Corypha utan* Lam) based on Google Earth© images [accessed late 2015]. A. Nyaung-U District, Mandalay Division, Myanmar (21^{0}04' N, 94^{0}57' E; 23/9/2015); B. Seba, Sabu Raijua Regency, NTT, Indonesia (10^{0}27' S, 121^0 53' E; 8/10/2012) C. Bipolo, Kupang Regency, NTT, Indonesia (10^{0}1' S, 123^{0}4' E; 23/9/2013); D. Normanby River, Lakefield National Park, Queensland, Australia (15^0 15' S, 144^{0}32' E; 8/8/2013). The length of the red line is equivalent to 100 m on the ground. Each yellow arrow points to a single mature palm. Bar = 1 km

from the mature gewang's trunk may have assumed greater significance in human nutrition in the past (Figure 5), before the arrival into the semi-arid parts of Southeast Asia of sorghum (from the west), domesticated rice (from the north) and maize, cassava and sweet potato (from the east); at a time when the principle carbohydrate sources would have been yams, cycads, water lilies, taro, water chestnut, wild millets, Job's Tears (*Coix* spp.) and wild rice species (Chang 1976; Fox 1977; Glover 1986; Jones and Meehan 1989).

In Sri Lanka, the sago of the talipot (*C. umbraculifera*) was prepared and used in a similar way to the gewang (*C. utan*) in Timor (Blatter, 1926). Interestingly, Jones and Meehan (1989) have recorded that the Gidjingali people in the Blyth River area of Arnhem Land, Australia, make use of the trunk pith of the gewang palm in a similar way to the sand palm, *Livistona humilis* R.Br., as an occasional food particularly in times of impending famine.

It is precisely in famine-avoidance that the great palms of the Arecaceae have provided such pivotal stability in the ecology and economy of Malesia. On the island of Roti, lontar trees are not planted; natural groves are manipulated (e.g. by thinning) and tapped to provide a year round supply of palm sugar, but as Fox (1977) determined, during the annual 'musim lapar' or 'paceklik' (literally, 'season of hunger'), the lontar provides for a substantial component of the total food energy requirements of the economy. In the islands of Roti and Savu, the traditional economies have supported human population densities in the order of 100 people/km (Figure 6) in contrast with densities of 50 people/km in the nearby non-lontar economies of Sumba and Timor.

Nevertheless, the local origins of lontar (*B. flabellifer*) are obscure. Kovoor (1983), following the taxonomic insights of Pudjoarinto (1982) and Tjitrosoepomo and Pudjoarinto (1983), proposed that lontar had its origins in India and perhaps even further west in Africa, and was carried to the east by human hands-paralleling the origins for crops like sorghum, sesame, cowpea, coffee and oil palm. There are undoubted Indian elements in the use of lontar, as is attested for example in the temple reliefs at Borobudur and the palm leaf writings of Bali (Hinzler 1993) mirroring those of India (Bhoi 2010) and the Southeast Asian kingdoms. However, it would be premature to assume that the existence of *B. flabellifer* in far eastern Indonesia is purely the result of its dispersal during the Indianising cultural waves of the archipelago's history.

CONCLUDING REMARKS

Species of *Borassus* L. and *Corypha* L. have long been part of the biological landscape of the semi-arid tropics in Asia and Africa, and pioneers in underpinning the stability of foraging and agricultural ways of life More targeted research into the two genera would not only cast new light on the proto-agricultural phase of the human story, but be of material benefit to communities who are dependent, though less so than in the past, on the many products of the palms.

Four particular lines of investigation would provide answers to some of the questions raised in this review: (i) a morphological and molecular-based taxonomic revision of the genus *Corypha*, (ii) a broad survey of the current ecological status of the two genera, of the kind that was carried out for *Borassus* by Kovoor (1983) thirty years ago. The IUCN assessment for *Corypha* in particular is in need of review, (iii) an economic and environmental evaluation of utilizing the sugary sap of the lontar palm in various livestock rations, (iv) an investigation into tissue-culture and cell-hybridization techniques in both genera. Large palm species have been difficult to handle using traditional taxonomic and genetic procedures; new lab-based technologies hold promise for intractable plant species like these.

However, the really important unanswered question is what the future holds for economies that have relied on these palms in the past. At the time when Davis and Johnson (1987) examined this issue for the State of Tamil Nadu, in India, there was at Srivilliputhur a well-established Palmyra Research Station with a germplasm collection representing a range of diversity for the species. However, since then the palmyra research program has slipped away, along with the search for quicker maturing palms with shorter more manageable plant height and for sap-harvesting technologies that could make the lot of the palm-tappers less hazardous and more profitable.

Wherever the exploitation of *Borassus* for its sugar and plentiful supply of useful leaf parts has existed it has been regarded as a subsistence industry. Palm-tappers have often belonged to a landless and poorly rewarded class of workers (Hardgrave 1969). Nevertheless, such persons do not go away. They persist, and the dependence on their precious palms continues (Vannan 2011; Walter Scott 2014).

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Syzygium diversity in Gunung Baung, East Java, Indonesia

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Abstract. *Mudiana D. 2016.* Syzygium *diversity in Gunung Baung, East Java, Indonesia*. *Biodiversitas 17: 733-740. Syzygium* (Myrtaceae) consists of a lot of species which are widely distributed. One of the distribution areas is the Natural Park of Gunung Baung (TWA Gunung Baung) in Pasuruan, East Java. The results of exploration and characterization of known species show that there are six species of *Syzygium* known to grow in this region namely *S. cumini, S. littorale, S. pycnanthum, S. polyanthum, S. racemosum*, and *S. samarangense. S. pycnanthum* is the most frequently found in Gunung Baung. *S. polyanthum* and *S. samarangense* are the only species that are known to be cultivated. Four other species are wild and have not been explored for their potential utilization.

Keywords: Diversity, East Java, Gunung Baung, Pasuruan, *Syzygium*

INTRODUCTION

Syzygium is widespread in a variety of habitat types. There are approximately 1,200 recorded species of *Syzygium*, which are widely spread in South Asia, Southeast Asia, Australia, and New Caledonia. Some species are also found in Africa, Malagasy and southwestern region of the Pacific Islands, Hawaii and New Zealand. Species spread across Asia are in several areas as follows: the Indo-China (70 species), Thailand (80 sp.), the Malay Peninsula (190 sp.), Java (50 sp.), Borneo (165 sp.), the Philippines (180 sp.), and New Guinea (140 sp.). The Malay Peninsula and Borneo are the two main areas of endemicity of this group (Haron et al. 1995). *Syzygium* generally grows in the rain forest, but grows well in nearly all types of vegetation, such as coastal forest, swamp forest, resembled monsoons, bamboo forests, peat swamp, lowland heath forest, savanna, montane forest and shrub vegetation in the sub-alpine region (Parnell et al. 2007). Some species are able to grow in conditions of extreme habitats such as limestone and ultramafic (Partomihardjo and Ismail 2008; Mustain 2009).

The large amount of genus *Syzygium* makes species classification become complicated; therefore many taxonomists have done some studies to classify these species and the most recent study was done by using a phylogenetic approach (Lucas et al. 2005; Craven et al. 2006; Craven and Biffin 2010). Although the number of species is quite high, still very few species of this genus are known by the public. Species that are known by the public at large are species that have been cultivated for their benefits and uses, such as *Syzygium aqueum, S. samarangense, S. malaccensis, S. aromaticum*, and *S. polyanthum*. The mainly usages of this genus are raw material for medicine, fruit-bearing, ornamental plants as well as a source of lumber and carpentry (Coronel 1992; Panggabean 1992; van Lingen 1992; Haron et al. 1995; Sardjono 1999; Verheij and Snijders 1999).

As for wild species that grow naturally in the forest or non-cultivated areas, not much is known of the *Syzygium* existence. If information about species diversity and its existence is unknown, it is feared that wild species can be neglected even become scarce before functions and uses can be understood. Flora expedition occasionally reveals important information about floristic diversity of certain area. Shenoy (2015) stated that *S. kanarense* was re-discovered after 67 years of its last existence's record.

Environmental conditions of habitat and human activity can affect the existence of a plant species. Ultimately this will affect the level of threat and the conservation status in the wild. Raju (2014), suggested that the reproductive capacity of *Syzygium alternifolium* was limited by a variety of environmental conditions. The factors that inhibit them are: low ability to produce fruit from the fertilization process, short viability of seeds, high mortality of seedling due to water stress, the pressure of dry climate in the dry season and fruit utilization by the community.

Some studies suggest that in addition to having the functions and benefits of direct usefulness to humans, *Syzygium* species have ecological functions for the sustainability of an ecosystem. Hasanbahri et al. (1996) suggest that there are at least 33 species of plants that become food for the *Macaca fascicularis* in hardwood forest regions. The most general type is of the genus *Ficus* and *Syzygium*. Alikodra (1997) recorded species of *Syzygium lineatum* and *Syzygium* sp. as species of plants that grow on the banks of the river of Kuala Samboja, which became fodder for proboscis (*Nasalis larvatus*). Besides this, they also used them to perform daily activities, such as exploring, sleeping, and more.

Syzygium plays an important role in the forest ecosystem to maintain the balance of the components inside. This could mean the relationship complementary and mutually beneficial among components in ecosystem. Crome (1985) stated that one form of this relationship is the system of pollination and fertilization of *Syzygium cormiflorum* with bats, birds, and insects as pollinator agents.

In addition, some species of *Syzygium* have an important role in the stabilization of the region along the banks of the river. This is mainly due to the nature and character of roots that can withstand the river flow, as well as hold up or slows down the rate of river flow. Root systems jon strong pedestal stems, making it an excellent plant to prevent erosion of the banks of the river (Wiriadinata and Setyowati 2003). Riswan et al (2004), revealed that along the banks of the Ciliwung and Cisadane rivers, there are five species of *Syzygium*, namely: *S. aqueum*, *S. aromaticum*, *S. malaccensis*, *S. polycephalum*, and *S. pycnanthum*. Only *S. pycnanthum* was intended to grow naturally, while the other four species were intentionally grown for various purposes. This species has the potential of plants as barriers to erosion by the river flow. In another study, Waryono (2001), states that *S. polyanthum* is quite often found in the Jakarta area. This species has a considerable number of individuals encountered, both at tree level and seedling, along the banks of the river. One of its roles in the riparian ecosystem is as a source of food for various species of birds that live in that area.

In general, the region of Natural Recreation Park of Gunung Baung (TWA Gunung Baung) has the ecosystem characteristics of a lowland monsoon forest. Flora species that are quite often found in these areas include: *Ficus benjamina*, *F. variegata*, *Sterculia foetida*, *Artocarpus elastica* and bamboo (Bambusoideae). Some parts of the region are dominated by bamboo forest. Chess (2008) mentions as many as 9 species from 4 genera of bamboo grow in the area of TWA Gunung Baung, namely: *Bambusa arundinacea*, *B. blumeana*, *B. spinosa*, *B. vulgaris*, *Dendrocalamus asper*, *D. blumei*, *Gigantochloa lear*, *G. atter*, and *Schizostachyum blumei*. Mudiana (2009) argues that there are four *Syzygium* species that found growing along the Welang river in this area, namely: *S. samarangense* (fruit greenish white), *S. javanicum*, *S. pycnanthum*, and *Syzygium* sp. This study aims to determine *Syzygium* species diversity, field character and distribution in the area of TWA Gunung Baung, East Java, Indonesia.

MATERIALS AND METHODS

Study area

Natural Recreation Park of Gunung Baung (TWA Gunung Baung) was established by decree of Minister of Agriculture 657/Kpts/Um/12/1981, dated January 1, 1981, covering an area of 195.50 hectares. TWA Gunung Baung regional government is administratively located in the Village of Cowek, Purwosari, Pasuruan District, East Java, Indonesia. Located about 68 km from the city of Surabaya towards Malang city. Geographically, TWA Gunung Baung is located at 07 ° 46 '09'-07°47 '23" South and 112°16' 23"-112°17'17" East. This region has the following boundaries: North side is adjacent to the Kertosari Village, Purwosari District, bordering the East is Lebakrejo Village, Purwodadi District, southern border is Cowek Village, Purwosari District, and western border is Purwodadi Botanical Gardens (Figure 1) (BBKSDA 2008).

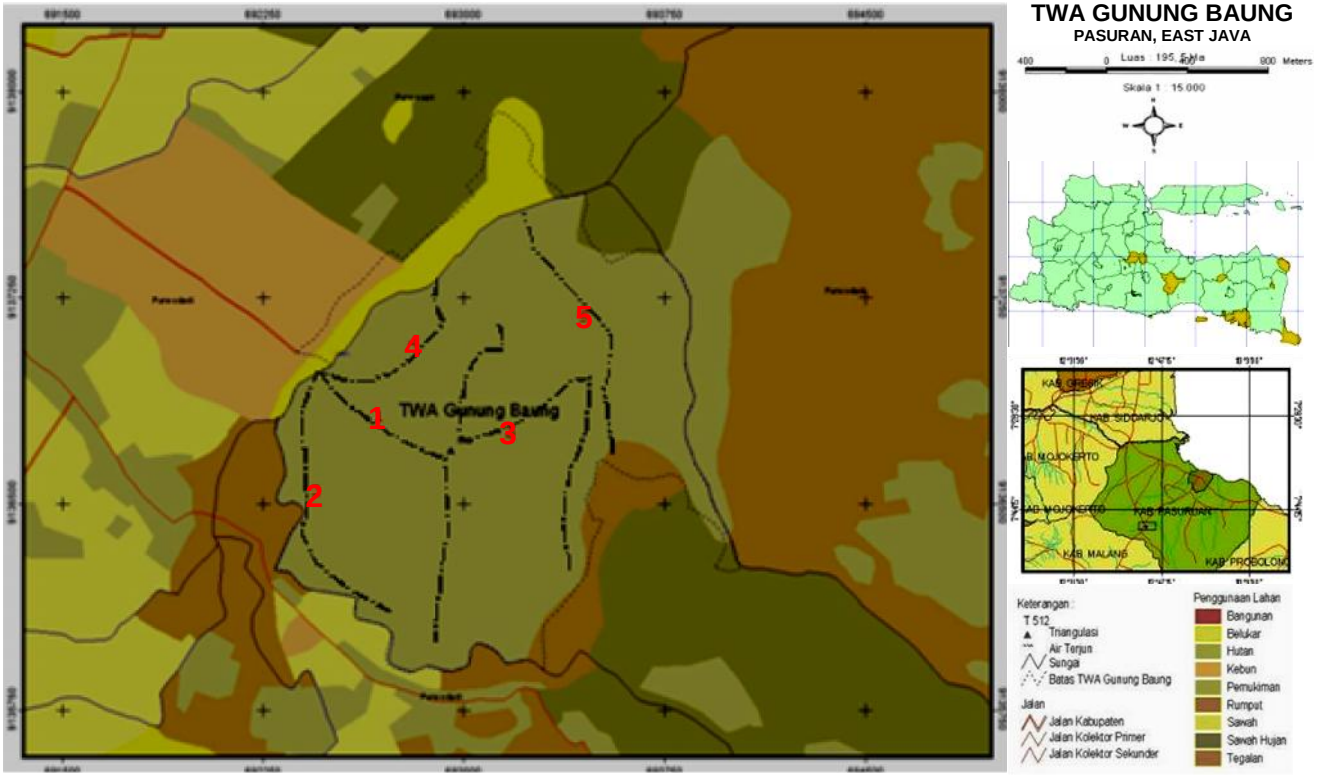

Figure 1. Regions of Gunung Baung Nature Park in Pasuruan, East Java. No. 1-5 indicate five explorative route to encounter *Syzygium*

The topography is generally undulating with steep slopes. Few have flat topography. The highest peak is around 501 m above sea level. The soil is made up of yellow and red mediterranean component of latosols quarter rocks formed from old metamorphic sediments. Climatic conditions of the area including the TWA Gunung Baung belong into type D, with a value of Q = 76.47%. There is an average rainfall of 2571.5 mm with the annual number of rainy days per year of 144.20. Daily temperatures range from 20^{o}C to 23^{o}C. The rainy season with rainfall ≥ 100 mm/month, generally occurs between November to April, while the dry season (with rainfall ≤ 60 mm/month) occurs between May and October.

Procedures

The method used in this study was a survey method, which explores the research areas, and records the encountered *Syzygium*. Explorative exploration was done wherever possible to examine most of the area. A total of five trails were conducted in this research (Figure 1).

Data collection was mainly carried out in the core of TWA Gunung Baung region. Data collected includes: morphological characters of *Syzygium*, location coordinates, general vegetation conditions, altitude, temperature and humidity. Voucher of herbarium specimens were collected for identification and determination of species. Identification of herbarium specimens was done in the Herbarium Bogoriense (BO) and the Hortus Botanicus Purwodadiensis Herbarium. The data were analyzed descriptively by an identifier of *Syzygium* morphological characters that can be easily recognized in the field and that can be made using a simple identification key. The characters include: habit, bark, leaves, flowers and fruits.

Then, based on the data obtained by morphological characters, we made dendogram to figure out the close relationship between species *Syzygium* in Gunung Baung. Present method is absent on some morphological characters which is used for species grouping. Data analysis was performed using the method of cluster analysis using Minitab 14 software.

RESULTS AND DISCUSSION

As many as 347 individuals of *Syzygium* from six species are recorded in this study. These six species are: *Syzygium cumini* (duwet), *S. littorale* (kopo laut), *S. polyanthum* (salam), *S. pycnanthum* (klampok, jambu hutan), *S. racemosum* (kopo mangut), and *S. samarangense* (jambu semarang). *S. pycnanthum* is the species mostly found in the area of TWA Gunung Baung followed by *S. racemosum* (Mudiana 2012) (Figure 2).

Of the six species of *Syzygium* encountered, *S. samarangense* and S. polyanthum are species that have been commonly recognized and cultivated/planted by the community. Although *S. cumini* has been known by the public, it is not commonly grown as a cultivated plant. *S. littorale*, *S. pycnanthum*, and *S. racemosum* are *Syzygium* species that still grow wild and are not cultivated by the community yet.

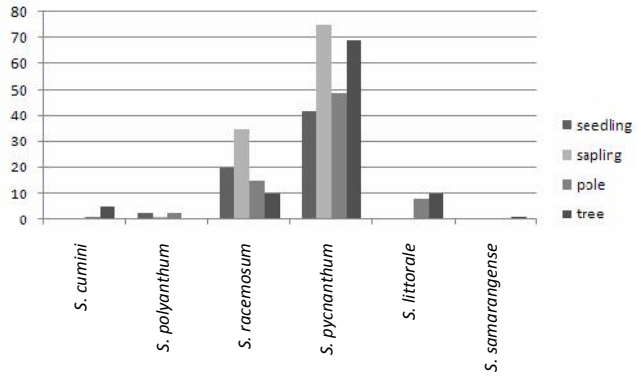

Figure 2. The number of individuals of *Syzygium* in TWA Gunung Baung, East Java

***Syzygium cumini* (L.) Skeels.**

Syn.: *Calyptranthes capitellata* Buch.-Ham. ex Wall, *Calyptranthes caryophyllifolia* Willd, *Calyptranthes cumini* (L.) Pers, *Calyptranthes cuminodora* Stokes, *Calyptranthes jambolana* (Lam.) Willd, *Calyptranthes jambolifera* Stokes, *Calyptranthes oneillii* Lundell, *Caryophyllus corticosus* Stokes, *Caryophyllus jambos* Stokes, *Eugenia calyptrata* Roxb. ex Wight & Arn, *Eugenia caryophyllifolia* Lam, *Eugenia cumini* (L.) Druce, *Eugenia jambolana* Lam, *Eugenia jambolifera* Roxb. ex Wight & Arn, *Eugenia obovata* Poir, *Eugenia obtusifolia* Roxb, *Eugenia tsoi* Merr. & Chun, *Jambolifera chinensis* Spreng, *Jambolifera coromandelica* Houtt, *Jambolifera pedunculata* Houtt, *Myrtus corticosa* Spreng, *Myrtus cumini* L, *Myrtus obovata* (Poir.) Spreng, *Syzygium caryophyllifolium* (Lam.) DC, *Syzygium jambolanum* (Lam.) DC, *Syzygium obovatum* (Poir.) DC, *Syzygium obtusifolium* (Roxb.) Kostel.

A short-truncate tree that can reach 20 m high and has no buttresses. Branching is grey or yellowish brown. It has single leaves arranged opposite, oval to oval, green-dark green, and has flat leaf edges. Size of leaves is about 7-15 cm x 5-9 cm and has a long apetiole 1 to 3.5 cm. Flowers are small (4-7 mm diameter) and are arranged in a single inflorescence. It has white to yellowish flowers, arranged in inflorescences that appear in axillary panicles at the ends of twigs and branches. Berry fruit of the seed is oval-shaped, dark red and purple when ripe, with a sweet taste of kelat (Figure 3.A, B, C). According to Backer and Bakhuizen van den Brink (1963), in Java, in teak forests, this species grows at altitude of below 500 m asl. and is widely cultivated for its fruit. This species is found in open places; a location with no bamboo and with relatively flat topography. A total of 6 individual observations are recorded in plots consisting of individual levels of pole 1 and 5 individual tree levels.

***Syzygium littorale* (Bl.) Amshoff**

Syn.: *Eugenia littoralis* (Bl.) Meijer Drees; *Eugenia subglauca* Koord. & Valeton; *Jambosa littoralis* Bl.

Tall stature of trees can reach 10-20 m. Single leaves are arranged opposite and are lanceolate-oblong-shaped

with a pointed tip. Leaf length is three times the size of its width. The inflorescence terminal appears on the petiole twigs as the former falls. Some flowers are arranged in a single inflorescence. There are white flowers with a size of about 1.5 to 2 cm (Figure 3.D, E, F). Fruit is round, campanulate (bell-shaped), green and yellow with a diameter of about 2.5-3.5 cm. Backer and Bakhuizen van den Brink (1963) states that this species is a native species in Java. They grow in forests, especially along the river bank.

In the area of TWA Gunung Baung, they are found growing in places where there is no bamboo, in bush areas with trees that are not too tight. There were a total of 18 individuals recorded in the observation plots, consisting of 8 individual poles and 10 individual trees.

***Syzygium polyanthum* (Wight.) Walp.**

Syn.: *Eugenia atropunctata* C.B.Rob., *Eugenia holmanii* Elmer, *Eugenia junghuhniana* Miq., *Eugenia lambii* Elmer, *Eugenia lucidula* Miq., *Eugenia microbotrya* Miq., *Eugenia nitida* Duthie, *Eugenia pamatensis* Miq., *Eugenia polyantha* Wight, *Eugenia resinosa* Gagnep., *Myrtus cymosa* Bl., *Syzygium cymosum* Korth., *Syzygium micranthum* Bl. ex Miq., *Syzygium microbotryum* (Miq.) Masam., *Syzygium pamatense* (Miq.) Masam.

A tree with a single trunk and clear, dense canopy shape, and can reach 25 meters high. It has dark brown, rough grooved bark. Single leaves are arranged opposite elliptic-round shaped or obovate (obovate) with a pointed tip. Leaf size is 5-15 x 3.5 to 6.5 cm with petiole length between 5-12 mm. Meeting at the end of the inflorescence branches or armpit. Compact white flowers are fragrant and reddish. Sweet fruit is round with a diameter of 8-9 mm and red to dark red (Figure 3.G, H, I). Its natural habitat is the forest area at an altitude of 5-1000 m asl. This species is often planted in home gardens for the leaves and fruit (Backer and Bakhuizen van den Brink 1963).

Seven individuals are recorded in the observation plots consisting of: 3 individual seedlings, saplings and 1 individual, 3 pole individual levels. They grow in places that are not dominated by bamboo and not too dense thickets of trees, in hillside areas.

***Syzygium pycnanthum* Merr. & L.M. Perry**

Syn.: *Eugenia corymbosa* Roxb., *Eugenia densiflora* (Bl.) DC., *Eugenia densiflora* (Bl.) Duthie, E. Axillary Auct. Non Willd., *Jambosa densiflora* (Bl.) DC., *Myrtus densiflora* Bl.

Syzygium pycnanthum is a small tree, up to 15 m high. Trunk diameter can reach 30 cm with no buttresses. Single leaves are oppositely arranged, dark green on the upper surface and pale green on the lower surface. Leaf shape is ovate-oblong-lanceolate (elongate-ovate-oblong), average leaf edge, acute-acuminate leaf tip (pointy-tapered). Leaf size ranges from 12.5 to 37 cm x 3-10 cm, has an intramarginal vein at a distance of 8-10 mm from the edge of the leaf. Inflorescence appears at the end of twigs. Compact flowers with short flower stalks 3-4 mm, crown-purplish white flowers, white-colored greenish-purple petals, have many stamens. The fruit is berry which is round, light green, dark purplish red or reddish-green with a diameter of 2.5-3.5 cm (Figure 3.J, K, L).

Two variants of *S. pycnanthum* are found in Gunung Baung TWA, which have purplish-red and green fruit. Stature and other characteristics are relatively the same, the only difference is the color of the fruit. Comparison of the amount between the two is unknown. This is because the time of the study does not coincide with the time of flowering or bearing fruit.

This species has a wide range of habitats. It can grow from lowlands to highlands with various types of environmental conditions. According to Backer and Bakhuizen van den Brink (1963), in Java, this species grows naturally in the underbrush, open woods or edges of rivers, at an altitude of 5-1500 m asl. Mustian (2009) found *S. pycnanthum* along with several other *Syzygium* species in a nickel mining region in Sorowako, South Sulawesi, on ultramafic soils. This type is found growing naturally on the banks of the river flow (Mudiana 2009; 2011). Sunarti et al. (2008) recorded this species habitat at an altitude of 750-850 m asl. in the Polara forests of Waworete Mountains, Wawonii Island, Southeast Sulawesi.

This species of *Syzygium* is the most often found species in TWA Gunung Baung. A total of 235 individuals were recorded in the observation plots, consisting of 42 individual seedlings, 75 saplings, 49 individual levels and 69 depressed pole level tree individuals. This species grows in a variety of conditions, such as the location of the dominance of bamboo groves *B. blumeana*, in an open space, where there is dominance of shrubs and trees, or in the hillsides.

***Syzygium racemosum* (Bl.) DC.**

Syn.: *Calyptranthus racemosa* Bl., *Eugenia brunneoramea* Merr., *Eugenia cerasiformis* (Bl.) DC., *Eugenia evansii* Ridl., *Eugenia expansa* Duthie, Eugenia jamboloides Koord. & Valeton, *Eugenia robinsoniana* Ridl., *Jambosa cerasiformis* (Bl.) Hassk., *Myrtus cerasiformis* Bl., *Syzygium brunneorameum* (Merr.) Masam., *Syzygium cerasiforme* (Bl.) Merr. & L.M.Perry, *Syzygium costatum* Miq., *Syzygium javanicum* Miq.

Tree's height can reach 3-20 meters, generally in the form of a small tree with dense branching. Bark is light gray-brown. Ellipse-shaped leaf face round with a tapered tip, leaf size is about 8-15 x 3.5-5 cm, petiole 0.5-1.5 cm size. Young leaves are reddish-copper. Inflorescence terminal or axillary panicles appear on the end of the branch. Yellowish-white flowers, with a crown like a small calyptras. Fruit is yellowish green, rounded bell-shaped with a diameter of 2-3 cm (Figure 3.M, N, O).

Backer and Bakhuizen van den Brink (1963) stated that this species is found growing in Java, in a mixed forest and a teak forest at an altitude of 10-1200 m asl. In TWA Gunung Baung, it is often found growing mainly in areas with a predominance of bamboo, *B. blumeana*, on the local hillsides. A total of 77 individuals were recorded in this study, consisting of 20 individual seedlings, 35 individuals, 14 individuals saplings and 8 pole level individual trees level.

***Syzygium samarangense* (Bl.) Merr. &L.M.Perry**

Syn.: *Eugenia javanica* Lam. non *Syzygium javanicum* Miq., *Eugenia samarangensis* (Bl.) O. Berg, *Jambosa javanica* (Lam.) K. Schum. & Lauterb., *Jambosa samarangensis* (Bl.) DC., *Myrtus javanica* (Lam.) Bl., *Myrtus samarangensis* Bl.

Stature of *S. samarangense* is a small tree with a lot of dense branching, height can reach 10 meters. Leaves are arranged opposite and are oval or oblong, bright green young leaves with leaf size of 6 to 11.5cm x 12-24cm and petiole length of 3-5 mm. Inflorescence appears in the former leaves that have fallen. Yellowish-white flowers with a diameter of 3-4 cm (Figure 3.P, Q, R). The fruit is bell-shaped and green and yellow. Its existence has been common and planted by residents in the garden and yard for some uses. Only found in 1 individual tree level recorded in the observation plots. It grows in the open area, in areas of bamboo bush.

Based on the fact that morphological characters are easily recognizable in the field, it is simple to identify. Key for *Syzygium* species in TWA Gunung Baung is as follows:

Identification key

I.A. Habit of a big tree

I.A.1. elliptic-oblong leaf shape; flowers are small, clustered; perianth white-reddish flowers; sweet, small round, red-colored or dark red fruit ..... ***S. polyanthum***

I.A.2. elliptical-obovate leaf shape; flowers are small, clustered; white-yellowish flowers; sweet, oval, purple-dark purple fruit ..... ***S. cumini***

I.B. Habit of a small tree

I.B.1. light gray or light brown sunny bark

I.B.1.1. elliptic-ovate leaf shape, thick leaf; purplish white flowers; round green or purplish green fruit ....***S. pycnanthum***

I.B.1.2. elliptical-oblong leaf shape, leaf thin copper-colored young leaves; small round white flowers ..... ***S. racemosum***

I.B.2. dark gray or dark brown bark

I.B.2.1. elliptic-ovate bright green leaf; axillary inflorescence; white flowers; bell shaped fruit ..... ***S. samarangense***

I.B.2.2. elliptic-ovate dark green leaf; axillary inflorescences on the top branches; white flowers; round shaped fruit ..... ***S. littorale***

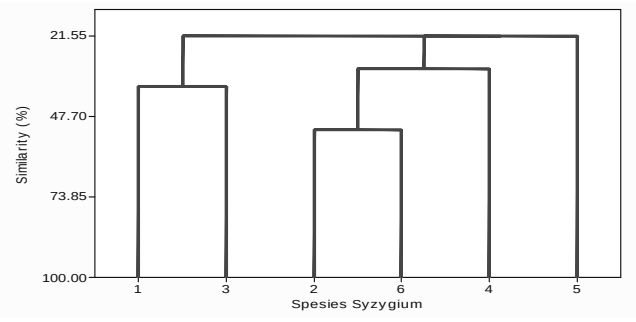

Figure 4. Dendogram of *Syzygium* in Gunung Baung based on morphological character practically recognized in the field. Note: 1. *S. cumini*, 2. *S. littorale*, 3. *S. polyanthum*, 4. *S. pycnanthum*, 5. *S. racemosum*, 6. *S. samarangense*

Based on the identification key and dendogram in Figure 4, it can be seen that in general there are three groups of *Syzygium* growing on Gunung Baung. The first group consists of *S cumini* and *S. polyanthum*, while the second group is *S. littorale*, *S. samarangense* and *S. pycnanthum*, and the third group is *S. racemosum*. Differences among the three are primarily on two morphological characters that are easily seen in the field, namely: flower size and habit. The first group has the small flowers with a large tree habit. The second group has a large flower size with a small tree habit. While the third group has a small flower size and small tree habit. *S. cumini* and *S. polyanthum* have the same character with a small flower size and habit of a large tree (the first group). Despite having a small flower size, *S. racemosum* has a tree with a small size habit (third group). *S. littorale*, *S. samarangense* and *S. pycnanthum* is classified into second group.

In addition, the character of the surface of the bark can also be a distinguishing character between *Syzygium* species in the field. *S. pycnanthum* and *S. racemosum* is very easily recognizable in the field because of the bright color of the bark (brown-light gray) with a relatively smooth surface. Other species have a dark color of bark with a rough surface.

Potential and utilization *Syzygium*

Syzygium cumini, *S. polyanthum*, and *S. samarangense* are *Syzygium* species that had been commonly recognized and utilized by the local community. It is traditionally and mainly used for fruit consumption, as a seasoning, as traditional medicine or timber used for household furniture and buildings.

As a producer of fruit, *S. samarangense* is one that is undergoing a process of "most advanced" cultivation techniques compared to other species. Today, many cultivars of this species have been produced. Even, sometimes, it leads to new species as a result of human intervention. Widodo (2007) stated that the activity of hybridization to produce new varieties is one speciation process that occurred in the genus *Syzygium*. There are at least 9 *Syzygium samarangense* cultivars that have been recognized and developed by the community (Cahyono 2010).

Traditionally, the potential use of *S. cumini* includes fruit for jam making or as material consumption of fruit; the wood is used as raw material for home furnishings and building materials, as well as leaves and seeds for traditional medicine. Intensive studies on the potential of the active substance content in this species suggest that there are many medical benefits provided by this species. One is as a producer of raw materials of diabetes mellitus drug. The content of oleanolic acid in this plant (the stem bark, leaves, and especially in the seeds) is an efficacious material for lowering blood glucose levels (hypoglycemic) and acts as an anti-diabetic (Tjitrosoepomo 1994; Dalimartha 2003; Mas'udah et al. 2010). Lestario (2003) suggested that a *S. cumini* fruit is a source of antioxidants which is beneficial to health. These substances are needed by the body to prevent degenerative diseases. In fact, the leaf extract of *S. cumini* contains a substance (i.e. methanol) that potentially developed as growth inhibitor of bacteria or anti bacteria (Gowri and Vasantha 2010).


A
B
C
D
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K
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Figure 3. Flowers (A), fruit (B), and tree physique of *Syzygium cumini* (C); Flowers (D), flower buds and leaves (E), and tree physique of *Syzygium littorale* (F); Flower (G), leaf (H), and tree physique of *Syzygium polyanthum* (I); Variant of young purplish red fruit (J), green fruit variant (K), and tree physique of *Syzygium pycnanthum* (L); Flowers (M), leaf (N), and tree physique of *Syzygium racemosum* (O); Flower buds (P), leaf (Q), and tree physique of *Syzygium samarangense* (R)

Several studies on the chemical content owned by *S. polyanthum* suggest that this species has the potential as a producer of tannins, flavonoids and essential oils (at 0.05%). Citric acid and eugenol are also produced by this species (Sumarno and Agustin 2008). Bay leaves (*S. polyanthum*) contain chemicals that could potentially be used as anti-diarrheal drugs as proposed by Wiryawan et al (200). His research notes that the chemicals in the leaves can suppress populations of the bacterium *Escherichia coli* that cause diarrhea in chickens. Chemical substances contained in the leaves include: essential oils, triterpenoids, saponins, tannins and flavonoids.

The three species, *S. littorale, S. pycnanthum* and *S. racemosum* are not widely known and neither has been utilized their potential for specific uses. All three of these species are wild and not cultivated. Traditionally, people use their wood as firewood. Not many studies have been done to explore the potential of these species. One was done by Wahidi (2001), which suggests that *S. pycnanthum* contains 15 essential oil components. From the results of these studies, it can be concluded that this species could be a source of α-farnesen and eugenol. Mudiana (2008) suggested that *S. pycnanthum* has the potential to be developed as an ornamental out door plant because it has characters of small-boned medium tree, leafy canopy forms, attractive colors and shapes of flowers and also attractive fruit.

To conclude, a total of six species of *Syzygium* are found growing in the area of TWA Gunung Baung. These six species are *S. cumini, S. littorale, S. polyanthum, S. pycnanthum, S. racemosum*, and *S. samarangense*. Based on morphological characters that are easily recognizable in the field in Gunung Baung, *Syzygium* species can be classified into 3 groups. The first group consists of *S. cumini* and *S. polyanthum*. The second group consists of *S. littorale, S. samarangense* and *S. pycnanthum*. The third group is *S. racemosum*. Flower size, habit, and the surface of the stem are character identifiers that are easily recognizable in distinguishing species of *Syzygium* in Mt. Baung. Of the six species of *Syzygium*, only *S. polyanthum* and *S. samarangense* have been commonly recognized and cultivated by people, while four other species grow wild in nature.

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Short Communication: The survival rate and one-year growth of *Shorea javanica, Shorea macrobalanos* and *Hopea mengarawan* in coal mined land in Central Bengkulu, Indonesia

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[1]Department of Forestry, Faculty of Agriculture, Universitas Bengkulu. Jl. WR Supratman, Bengkulu 38371A, Indonesia. Tel./Fax. +62-736-21170, [♥]email: wiryonogood@yahoo.com, wiryonogood@unib.ac.id

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Manuscript received: 8 July 2016. Revision accepted: 6 September 2016.

Abstract. *Wiryono, Suhartoyo H, Munawar A. 2016. Short Communication: The survival rate and one-year growth of* Shorea javanica, Shorea macrobalanos *and* Hopea mengarawan *in coal mined land in Central Bengkulu, Indonesia. Biodiversitas 17: 741-745.* Dipterocarp trees used to dominate the lowland forest of Sumatra and Kalimantan. Currently, however, dipterocarp trees are rare due to deforestation of natural forest. One major cause of deforestation in Sumatra and Kalimantan is coal mining. Rehabilitation of coal mined soil is usually done using fast-growing alien species. We tried to restore a piece of mined land in Central Bengkulu, using commercially valuable species of Dipterocarpaceae, namely *Shorea javanica* Koord. & Valet., *Shorea macrobalanos* Ashton and *Hopea mengarawan* Miq. In this article, we presented the survival rate and growth of the one year old seedlings of these three species within one year of observation. Of the three species, *S. macrobalanos* had the highest survival rate (93%), followed by *S. javanica* (80%) and *H. mengarawan* (77%). Within a year, the one year old seedlings of *S. macrobalanos* grew 452% in height, significantly higher than that of *S. javanica* (221%) and of *H. mengarawan* (119%). *Shorea macrobalanos* also had the highest growth in diameter within a year, namely 337%, followed by *S. javanica* (145%) and *H. mengarawan* (135%). It can be concluded that within a year of observation, the three species of dipterocarps could grow relatively well in mined land. It is therefore recommended that in the restoration of mined land in Sumatra economically valuable native species of dipterocarps should be used instead of fast-growing alien species.

Keywords: Coal mined soil, Dipterocarpaceae, restoration

INTRODUCTION

Trees of the Dipterocarpaceae family used to dominate the lowland forest of Indonesia (Whitmore 1984), but most of them are now threatened with the disappearing of lowland forest in Indonesia. One of industries contributing to the deforestation is mining. Between 2000 and 2010, mining industries caused 0.3 million hectares of forest loss, resulting in 3.6-4.4 Mega ton of carbon emission (Abood et al. 2015). Mining activities not only remove the vegetation, but also drastically destroy the ecosystem. Topsoil was removed and the materials previously buried deep underground were brought to surface. Mine soil, is generally unfavorable for plant growth and must be amended before revegetation (Bradshaw 1997; Lottermoser 2010).

The Indonesian government regulations require that the mining companies rehabilitate the mined land in order to restore the ecosystem functions. Usually, exotic fast growing species, such *Acacia mangium* (Suhartoyo et al. 2012), *Paraserianthes falcataria*, *Sesbania grandiflora* (Munawar et al. 2011) are used for the revegetation of mined land. While these species can restore some ecosystem functions of the degraded forest, the establishment of these species changes the species composition of the forest. According to the Society of Ecological Restoration (SER) one of nine ecosystem attributes as the criteria of restoration success is that indigenous species must be present in the area (Clewel and Aronson 2007). Several researchers have tried to use local fast growing species for revegetation of mined land in Kalimantan (Adman et al. 2012) and found that some local fast growing species were able to survive and grow in mined land. Those species, however, had low economical value.

Considering the lack of study of coal mined land restoration using Dipterocarpaceae, we tried to plant three species of Dipterocarpaceae in coal mined land in Central Bengkulu District where raintree (*Samanea saman*) had already been planted. The three species were *Shorea javanica* Koord. & Valet, *Shorea macrobalanos* Ashton and *Hopea mengarawan* Miq. In some literature *H. mengarawan* is written as *H. mengarawan* Miq. *Shorea javanica* is naturally found in primary and secondary forest in Sumatra and Central Java, but it has been planted in many countries of Southeast Asia (Orwa et al. 2009). *Shorea macrobalanos* used to be endemic to Sarawak and East Kalimantan, and it is categorized as critically endangered species in IUCN Redlist (Ashton 1998a; IUCN 2016). *Hopea mengarawan* is native to Indonesia and Malaysia (Fern 2016) and it is also categorized as critically endangered in IUCN Redlist (IUCN 2016). The objective

of this study was to report the survival rate and the growth of one year old seedlings of the three species within a year of observation.

MATERIALS AND METHODS

Study site

This study was conducted in the mining concession area of PT Inti Bara Perdana in Taba Penanjung Sub-district, Central Bengkulu District, Bengkulu, Indonesia (Figure 1). The site was approximately at 300 m altitude, with annual rainfall above 3000 mm. The site was revegetated with raintree (*Samanea saman*) four years earlier. The average canopy opening of the four-year old raintree stand was 40%, so there was some light reaching the forest floor. The light intensity on forest floor under the shade at noon varied between 800 to 3000 lux. The temperature at noon was 28-30^{o}C and the average relative humidity was 82%. During the driest month of 2015, namely October, the raintrees shed most of their leaves, so the canopy was open, and consequently the temperature and light intensity increased while the relative humidity dropped.

The land of this site was not leveled, but consisted of many mounds and depressions. The seedlings were planted on mounds in order to prevent water logging and to avoid compacted soil. Seedlings of three species of dipterocarps were planted, namely *Shorea macrobalanos* Ashton, *Shorea javanica* Koord. & Valet. and *Hopea mengarawan* Miq. The seedlings were approximately one year old. At the beginning of planting, a kilogram of compost was given for each planting hole. Afterward, every two months NPK fertilizer was given to each plant. During the months of July-September 2015, the seedlings were watered every two weeks, and during October they were watered every three days.

Every month, the height and diameter of seedlings were measured. Soil chemical and physical properties were analyzed in Soil Laboratory of the Faculty of Agriculture, Universitas Bengkulu, Indonesia.

Data analysis

The data of height growth and diameter growth were analyzed using ANOVA to know whether there were growth differences among species. If there were significant differences, further analyses were done using Least Square Differences (LSD) to know which species had higher growth than the others.

RESULTS AND DISCUSSION

Shorea macrobalanos had the highest survival rate among the three species (Table 1). Only two out of 30 plants died during the first year of planting. *Shorea javanica* and *Hopea mengarawan* had similar survival rate. *Shorea macrobalanos* also had the highest growth of diameter and height within a year of observation, and there was no statistically significant difference between *S. javanica* and *H. mengarawan* in diameter and height growth (Table 2). Within a year, *S. macrobalanos* grew 452% in height and 337% in diameter, while *S. javanica* 221% and 245%, and *H. mengarawan* 119% and 135%. In general, the results showed that the three dipterocarp species grew relatively well in the study site (Figures 2, 3 and 4).

The success of terrestrial ecosystem restoration is influenced by the plant-soil interactions (Eviner and Hawkes 2008). Plant growth in mined land is sometimes constrained by soil compaction (Sheoran et al. 2010). Soil compaction may impede root penetration, reduce aeration, slow down movement of nutrients and water, and cause the buildup of toxic gases in the rhizosphere (Brady and Weil. 2002). In the United States, the passing of Surface Mining Control and Reclamation Act, which requires the mined land to be returned to its original contour, had resulted in soil compaction due to the use of heavy equipment. As a result, the reclaimed mined land was mostly grown with grasses and herbaceous vegetation, while tree establishment was impeded by the high soil density (Fields-Johnson et al. 2014). To improve the establishment of trees, the soil compaction must be reduced, for example through ripping (Ashby 1997; Fields-Johnson et al. 2014). This problem of soil compaction, however, was not found in our study site, because the mining company did not level the soil. The dipterocarp seedlings were planted in piles of soil which were not compacted. The bulk density of the soil samples ranged between 1.49 and 1.60 g cm^{-3} and the soil particle density was 1.84-1.88 g cm^{-3} (Table 3). The bulk density in this study site was considered normal, because the mineral soil may have bulk density of 1.0-2.0 g cm^{-3} (Chapin et al. 2011) and the soil particle density was lower than the average particle density of mineral soil, which is 2.5-2.7 g cm^{-3}. With normal soil density, the roots of dipterocarp seedlings would not have problem in penetrating the soil. Also, because the seedlings were planted on the mounds, there was no problem of water saturation during rainy day.

The chemical properties of soil in mined land are in general unfavorable for plant growth. Mine soil which sometimes contains high concentration of sulfur can have a pH of 2.3-3.5 which lead to higher availability of toxic metal (Sheoran et al. 2010). In our study site, the chemical soil was very acidic with a pH of 4.0-4.2 (Table 4), but the pH was not extremely low. Even in primary tropical rain forest, the soil is usually also acidic. In Southwest China, Li et al. (2012) found the pH of primary tropical rain was 4.53.

In mine soil, the nutrient availability is usually low, and so it was in our study site (Table 4). However, at the beginning of planting, manure was given for each seedling and subsequently, every two months, NPK fertilizer at the amount of 15 gram was given for each seedling. So, the nutrient content of the soil in the rhizosphere of the dipterocarp seedlings was certainly higher than that in the surrounding. Soil amendment could increase nutrient availability for plants in mine soil (Asensio et al. 2014).

Beside soil properties, light intensity also influences the establishment of plants in reclaimed mined land. Every species requires different need of light intensity for germination and establishment. Ashton (1998b) said that dipterocarps species could be broadly classified as shade-

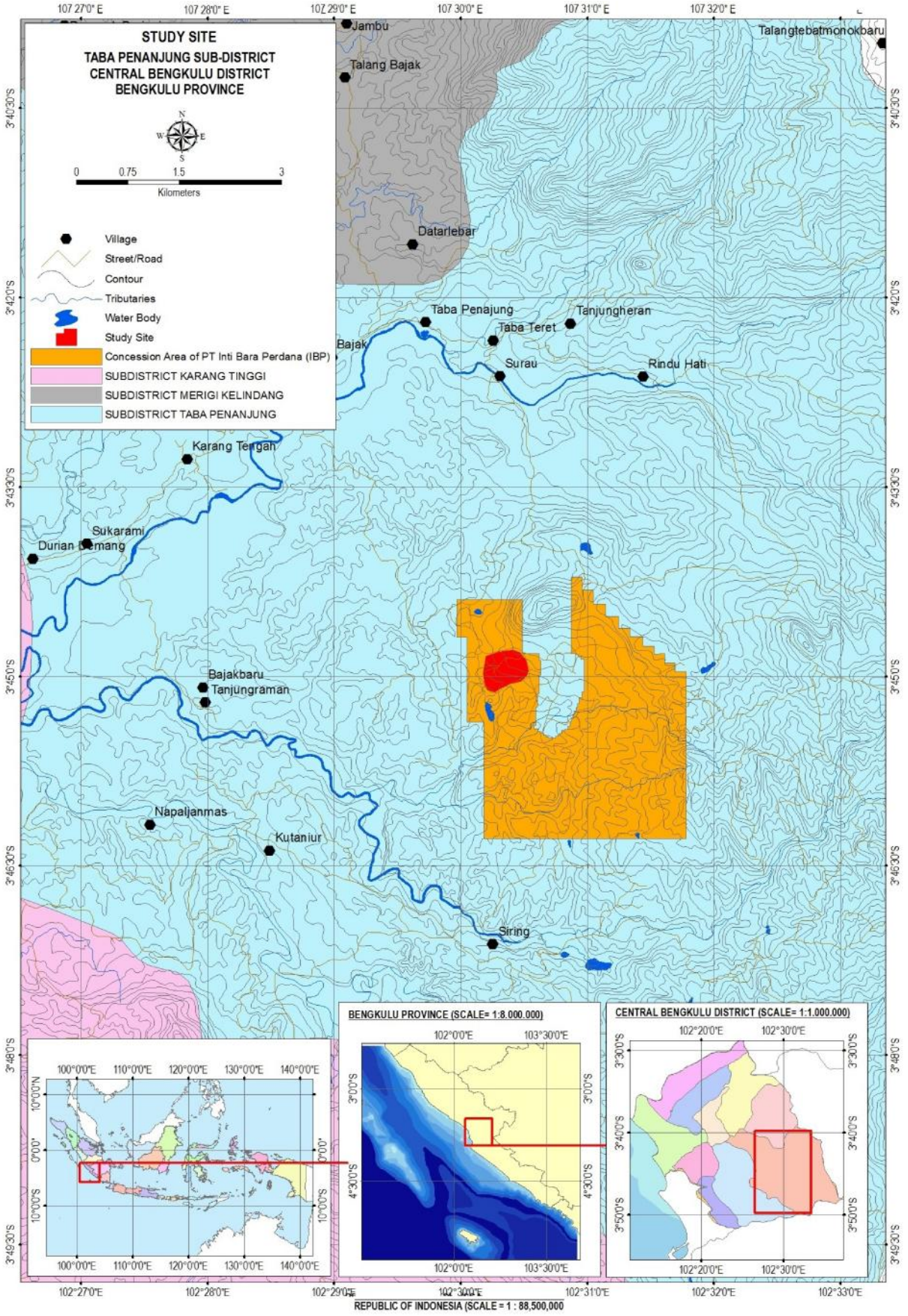

Figure 1. Study site in Taba Penanjung Sub-district, Central Bengkulu District, Bengkulu Province, Indonesia

intolerant, but some species were shade-tolerant. Generally, the seeds require partial shade for germination and early survival, but the seedlings require an increase in light for establishment and growth. Bawalsyah et al. (2015) in their study found in West Sumatra that there was no difference in the growth of *H. mengarawan* in different light intensity, which was 100% and 40%. In our study, the canopy of raintree was not fully closed. The light intensity on the forest floor, under the shade at noon varied between 800-3500 lux.

The plant growth and survival are also influenced by its interaction with other organisms. Attack from pathogen to the dipterocarp seedlings was not found in our study, while minor herbivory was found but not lethal. Some plants died because they were uprooted by wild pigs. Fortunately, the leaves of the three dipterocarps were not palatable to large herbivore, so depredation from large herbivores was absent.

The most important threat came from competition with weeds, such as *Mikania micrantha*, *Synedrella nodiflora*, *Chromolaena odorata*, *Nephrolepis biserrata* and *Mimosa pudica*. These weeds can easily overgrow and enclose the dipterocarps seedlings, depriving the seedlings of the light. The worst weed was *Mikania micrantha*. Native to America (Holm et al. 1977), this twinning perennial weed would twin its stem around the seedlings' stem and branches. To ensure the establishment of the dipterocarps, every two weeks we manually removed the weeds surrounding the dipterocarps. In the US, Ashby (1997) found that the use of herbicide helped improve tree establishment in mined land.

It can be concluded that with proper care, *Shorea javanica*, *Shorea macrobalanos* and *Hopea mengarawan* could grow relatively well in coal mined under the rain tree stand in Central Bengkulu. It is recommended that dipterocarp trees be used to revegetate the mined land in Sumatra.

Table 1. The survival rate of three dipterocarp species in coal mined land during a year of observation

Species	Number of plants alive	Survival rate (%)
Shorea macrobalanos	28	93
Shorea javanica	24	80
Hopea mengarawan	23	77

Table 2. The growth of height and diameter of three dipterocarps species in coal mined land during a year of observation

Species	Height increase in a year (%)	Diameter increase in a year (%)
Shorea macrobalanos	452a	337a
Shorea javanica	221b	145b
Hopea mengarawan	119b	135b

Note: numbers followed by the same letter are not significantly different at p of 5% level

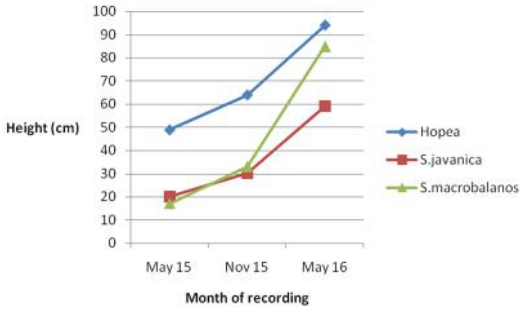

Figure 2. The plant height of three dipterocarps species in coal mined land during a year of observation

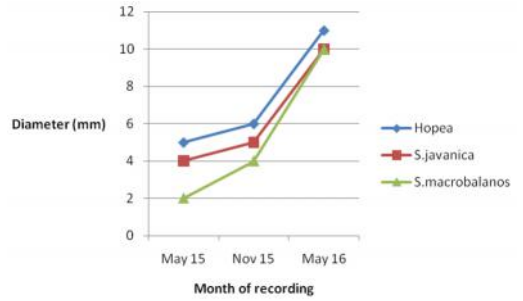

Figure 3. The plant diameter (in mm) of three dipterocarps species in coal mined land during a year of observation

Table 3. Physical properties of soil samples of coal mined land

	Particle density	Bulk density	Texture (hygrometer)			
		g cm^{-3}	Sand (%)	Silt (%)	Clay (%)	Class
Sample 1	1.84	1.49	45.51	24.36	30.13	Sandy clay loam-clay loam
Sample 2	1.88	1.60	76.24	8.68	15.08	Sandy loam

Table 4. The chemical properties of soil samples of mined land

Soil samples	pH (H_2O)	C	N	P_2O_5	K	Ca	Mg	CEC
			%	(mg.kg^{-1})		(cmol kg^{-1})		
Sample 1	4.0 (very acidic)	1.22 (low)	0.05 (very low)	Un-detected	0.35 (medium)	3.34 (low)	5.02 (low)	15.44 (low)
Sample 2	4.2 (very acidic)	1.52 (low)	0.07 (very low)	Un-detected	0.29 (medium)	2.14 (low)	5.13 (low)	14.92 (low)

Figure 4. *Hopea mengarawan*, A. 1 month old, B. 11 months old

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Insect pollinator diversity along a habitat quality gradient on Mount Slamet, Central Java, Indonesia

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Abstract. *Widhiono I, Sudiana E, Sucianto ET. 2016. Insect pollinator diversity along a habitat quality gradient on Mount Slamet, Central Java, Indonesia. Biodiversitas 17: 746-752.* The diversity of wild bees and wasps in seven habitat types (natural forest, teak forest, pine forest, Agathis forest, community forest, gardens, and agricultural areas) representing the habitat quality gradient of Mount Slamet and adjacent areas in Central Java, Indonesia, was studied from April to June 2012. We examined whether habitat quality affected the diversity of wild bees and wasp pollinators. In total, 938 wild bee and wasp specimens representing 13 species of bees and 2 species of wasps were collected using kite netting. Wild bee diversity differed significantly among the habitat types ($F_{6,281} = 1.2$ $p < 0.05$). The Spearman's correlation coefficients confirmed that wild bee diversity was correlated with habitat quality ($r^2 = 0.67$ $p < 0.05$). Habitats that included all of the major wild plant species supported the highest wild bee diversity.

Keywords: Kite netting, major wild plant species, species richness, wasp, wild bee

INTRODUCTION

Mount Slamet (3,428 m asl.) is the highest mountain in Java and is located in the southwest of Central Java province. Previously, this area was covered with plantations and natural forest under the management of Perum Perhutani (The State Forest Agency). Following political and economic instability in Indonesia in 1998, a forested area on the lower portion of this mountain was converted into agricultural land dominated by vegetables crops that require insect pollinators to produce better fruits. A recent study by Widhiono and Sudiana (2015) recorded 17 insect pollinators, predominantly wild bees, visiting vegetable crops. The agricultural area is surrounded by a mosaic of other land-use types that can act as habitats for wild bees, especially forested habitat. Conversion of forested areas to agricultural habitats results in habitat simplification, which can affect the diversity and abundance of insect pollinators due to changes in wild plant diversity and abundance. These differences, in turn, affect the availability of pollen and nectar, which are vital resources for insect pollinators.

Habitat quality is usually measured as plant species richness or abundance. Plants are needed by insects for food and reproduction, and their species richness and abundance significantly affect the diversity of insect pollinators (Potts et al. 2003; Kleintjes et al. 2006; Campbell and Husband 2007). Natural and seminatural (forested) habitats are often critical to the overall species richness of insect pollinators (Hendrickx et al. 2007; Billeter et al. 2008). Wild bee species richness was found to be higher in disturbed forests than in primary forests in tropical Southeast Asia (Liow et al. 2001; Steffan-Dewenter and Tscharntke 2001; Thomas 2001). Comparative studies of a broad range of habitats along a land-use intensification gradient from primary forest to plantation forest, community forest, gardens, and agricultural areas, have generally focused on plant density or flower abundance, and to the best of our knowledge, no studies have examined local wild plant species acting as key factors in supporting the diversity of wild bees.

Furthermore, especially in Indonesia, no studies have addressed the quality of natural, semi-natural, or non-crop habitats and their relationships with insect pollinator diversity, despite the fact that wild bees are responsible for the majority of the pollination of cultivated plants in the region. Widhiono and Sudiana (2015) identified 42 species of wild plants on Mount Slamet and in adjacent areas, 24 of which are visited by wild bees and wasps. Of these 24 species, 8 species (hereafter referred to as the "major wild plant species") were visited by more than one wild bee species: *Cleome rutidosperma* (Cleomeaceae), *Borreria laevicaulis* (Rubiaceae), *Barleria elegans* (Acanthaceae), *Euphorbia heterophylla* (Euphorbiaceae), *Rubus parviflorus* (Rosaceae), *Salomonia cantoniensis* (Polygalaceae), *Tridax procumbens* (Asteraceae) and *Vero cinerea* (Asteraceae). In this study, we defined habitat quality as the species richness and the density of the major wild plant species used for food by insect pollinators. We examined whether habitat quality affects the species richness and abundance of wild bees and wasp pollinators in different habitat types on Mount Slamet, and hypothesized that the habitat types with the highest species richness and density of the major wild plant species support the highest diversity and abundance of wild bees and wasps.

MATERIALS AND METHODS

Study area

The study was conducted from April to June 2012 in the East Banyumas Forest Management Unit of the State Forest Agency (Perum Perhutani), on the southern and northern slopes of Mount Slamet, Central Java, Indonesia. The area lies at approximately 7°18'23.72"S, 109°14'06.51"E at 600-800 m asl. We surveyed seven different habitat types in our study area encompassing a range of wild plant species richness and abundance. The total size of study area was 17 ha, and the habitat types were classified as natural forest (NF, 5 ha), teak forest (TF, 2.5 ha), pine forest (PF, 2.5 ha), *Agathis* forest (2.5 ha), community forest (2.5 ha), gardens (G, 0.5 ha), and agricultural areas (Ag, 1.5 ha).

Sampling protocol

Each habitat type was divided into five random transects, each 5 m wide and 100 m long. The number of wild bees and wasps (Hymenoptera) was recorded during the morning between 6:00 a.m. and 12:00 p.m. in a standardized manner along transects. Sampling was conducted by sweep-netting in the herbaceous layers and understoreys of the plots, twice a month (total sampling six times/transect). Where possible, all bees observed were captured, and the plant visited by each bee was noted. Following each insect survey, the wild plant species richness and density were recorded in each subplot. Because some of our data were collected by nonexpert insect enthusiasts, we were limited in our taxonomic resolution. Some insects could be identified to species level with the help of a Hymenopteran taxonomist from the Indonesian Academy of Sciences, Bogor. Samples of the wild plants were stored for identification in the Plant Taxonomy Laboratory, Faculty of Biology, Universitas Jenderal Soedirman, Purwokerto, Banyumas, Central Java, Indonesia; used standard literatures for Java plants such as Backer and Bakhuizen van den Brink (1963-1968) and Steenis (1972).

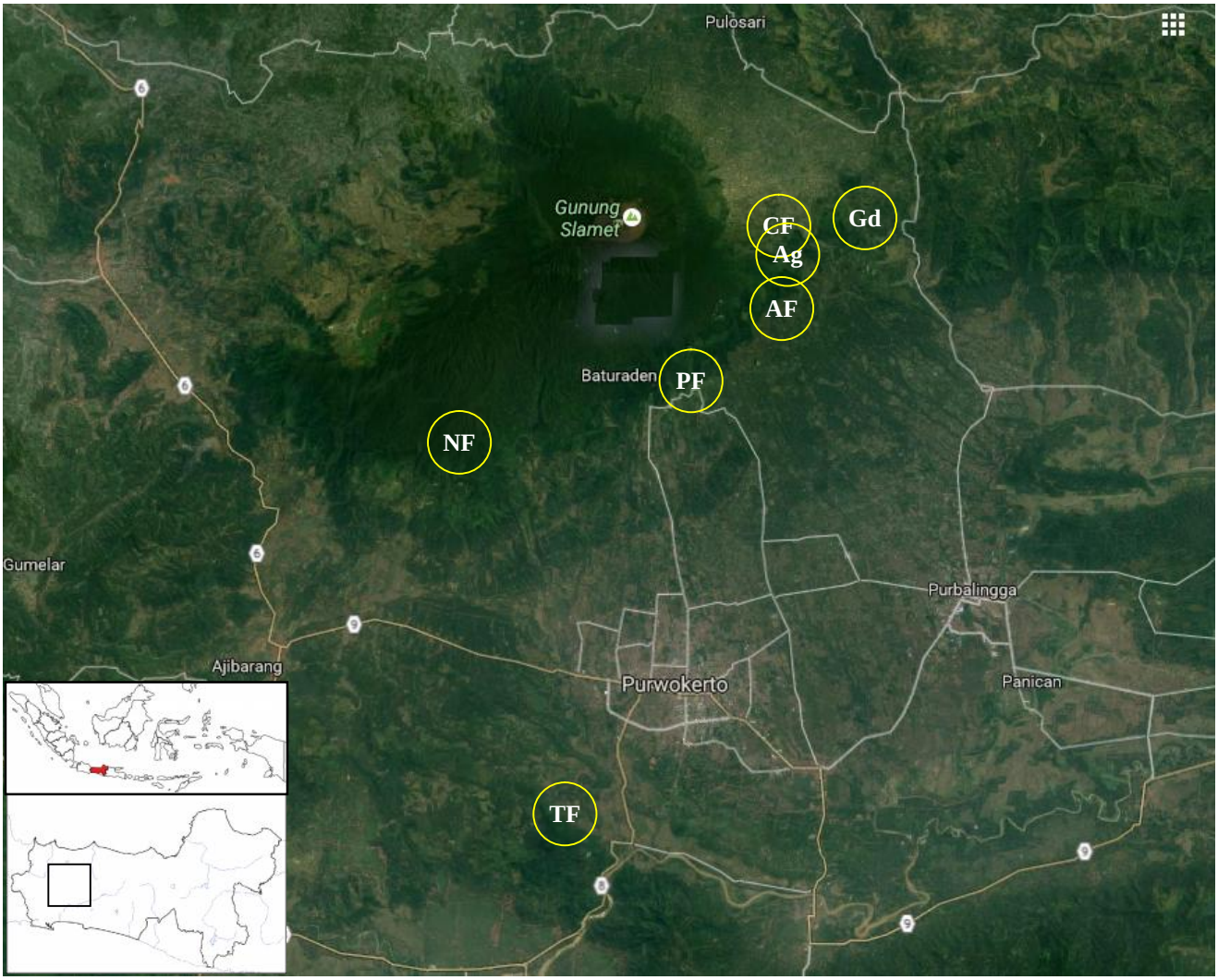

Figure 1. Study site, seven habitat types on Mount Slamet, Banyumas and Purbalingga regencies, Central Java

Table 1. Description of seven habitat types on Mount Slamet, Central Java, Indonesia

Habitat	Location	Main vegetation	Wild plant species	Wild plant densities (stdev)
Natural Forest (NF)	7°20'13.54"S, 109°08'01.06"E, 719 m asl.	19-20 tropical tree species	36 species	70.52 ± 39.88 ind/sp.
Teak Forest (TF)	7°29'11.56"S, 109°11'34.85"E, 239 m asl.	*Tectona grandis*	36 species	67.80 ± 32.88 ind/sp.
Pine Forest (PF)	7°16'52.23"S, 109°15'56.44"E, 929 m asl.	*Pinus merkusii*	27 species	42.69 ± 47.33 ind/sp.
Agathis Forest (AF)	7°17'53.19"S, 109°14'54.62"E, 834 m asl.	*Agathis dammara*	23 species	42.69 ± 47.33 ind/sp.
Community Forest (CF)	7°14'34.86"S, 109°18'12.46"E, 1081 m asl.	*Albazia cinensis*	33 species	45.69 ± 29.93 ind/sp.
Gardens (Gd)	7°14'13.11"S, 109°18'12.15"E, 954 m asl.	Ornamental plants	25 species	45.69 ± 29.93 ind/sp
Agricultural Areas(Ag)	7°15'01.12"S, 109°17'58.53"E, 954 m asl.	Cash crops	26 species	15.83 ± 22.69 ind/sp

Data analysis

To compare the overall community structure of the insect and wild plant taxa among the habitats, we calculated the accumulated species richness, total abundance, and alpha diversity (Shannon H', Simpson D, and Evenness [E]) for the seven habitats after pooling of data set. We calculated the alpha diversity using the Shannon-Wiener diversity index, a measure that takes into account the proportional abundance of each species (Margurran 1988). Comparisons of the species compositions among the different forest habitats were performed using single linkage cluster analysis based on the Bray-Curtis similarity. Diversity parameters were calculated using the Biodiversity Pro2 software (McAleece et al. 1997). We used the Spearman's rank correlation coefficients to determine whether insect species richness and abundance were significantly correlated with wild plant diversity and abundance.

RESULTS AND DISCUSSION

Herbaceous wild plant species richness and density varied among the examined habitat types (Figure 1), ranging from 23 species in AF to 36 species in NF and CF, and from 114 individuals/100 m^2 in Ag to 507 individuals/100 m^2 in NF, respectively. The distribution, species richness, and abundance of the major wild plant species showed significant differences among the habitat types. The most abundant family was Asteraceae, with 11 species, followed by Fabaceae, with 3 species. Seven families were represented by two species each, and nine families were represented by one species each. All of the eight major wild plant species occurred in NF, TF, and CF, whereas only three of these species were found in PF and AF.

Differences in the species richness of the major wild plant species among habitats are caused by the habitat preferences of these plants. Almost all of these wild plants are abundant in sunny or slightly shaded habitats, generally corresponding to young secondary vegetation (Nwaogaranya and Mbaekwe 2015). The forest understory is a heterogeneous and dynamic habitat, within which the bulk of species contribute to ecosystem functioning and sustenance (Sharma 2013). The observed differences in plant species richness can be explained by the responses to environmental variables in the habitats. The major environmental factors that influence the growth of vegetation are sunlight, water, and nutrients, which are the primary drivers of plant species richness at the local scale (Pausas and Austin 2001). Among the forested habitats, the lowest richness of herbaceous wild plant species was found in AF and PF, in agreement with the results reported by Nahdi (2014), who observed low herbaceous species richness in PF and AF in Yogyakarta. The low abundance of wild plants in AF may due to land-management practices, including the application of fertilizer, mowing, and weed control, that lead to greater availability of nutrients, which benefits only a few plant species. The use of mowing and weed control practices to exclude outcompeted plant species changes plant species assemblages, reducing plant diversity and resulting in reduced plant species richness and an altered plant community composition (Williams et al. 2010).

Wild bee and wasp abundances differed significantly among the habitats ($F_{6,281}$ = 1.2, $p < 0.05$). The highest abundance was observed in NF, with 229 individuals (24.41%), followed by CF, with 171 individuals (18.2%), Gd, with 162 individuals (17.2%), TF, with 161 individuals (17.16%), Ag, with 91 individuals (9.7 %), and AF, with 66 individuals (7.03%). The lowest abundance occurred in PF, with only 58 individuals (6.1%). From Tukey HSD test showed that CF was the best habitats comparing to all others forest types (sig-0,015 <0.05) (Table 2). The Shannon-Wiener diversity index ($H^{'}$) indicated that CF had the highest diversity of wild bees ($H^{'}$ = 2.049) (Table 3).

These findings are in agreement with those of Liow et al. (2001), who reported that bee abundance particularly that of Apidae, was significantly higher in CF than in other forested habitat types. However, our results were inconsistent with the findings of Hegland et al. (2009), who reported that local bee densities and diversities were highest in open land, followed by agroforestry systems, and were lowest in primary forests. Forested habitats offer nesting sites for many bee species (Klein et al. 2003; Brosi et al. 2007, 2008). This is demonstrated by the occurrence of *Apis dorsata* in only NF and TF habitats because this bee species prefers to build nests in the very tall trees found in these forest types (Starr et al. 1987; Tan 2007). Although open land provides better food resources in the herbaceous layer, bees often occur across different habitats that provide different resources (Tscharntke et al. 2005).

The intermediate abundance of wild bees observed in Gd and Ag indicates that mass-flowering crop species enhance wild plant populations, which provide floral

resources for pollinators in these habitats (Steffan-Dewenter and Westpal 2008) and that the dispersal ability of wild bees limits their abundance in agricultural fields (Carvalheiro 2010). These positive effects on bee populations occur in areas where agriculture increases the heterogeneity of habitat within the range of foraging bees (Kremen et al. 2007). The fact that the highest wild bee abundance was found in NF can be explained by the correlation between herbaceous vegetation density and habitat structure in NF.

The highest species richness was recorded in CF. The vegetation structure in CF was irregular, which resulted in a diverse canopy cover with a combination of forest and open land structures. This produced higher flower density and therefore a better food supply in the understory than is available in natural habitats (Potts et al. 2003; Bruna and Ribeiro 2005), resulting in greater bee richness and density. However, this result contrasts with the findings of Winfree (2007), who reported that open-land habitats exhibited the highest bee species richness and abundance compared with agroforestry and forest habitats.

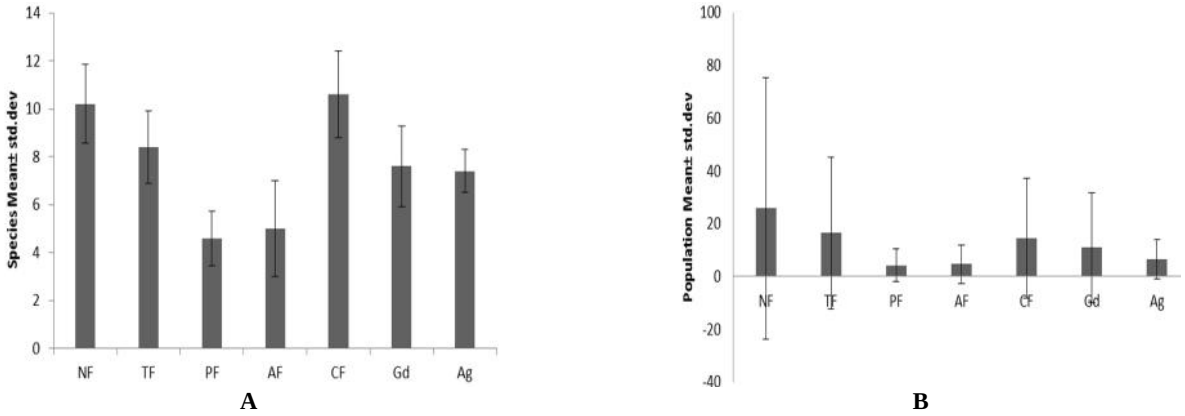

Figure 1. Wild plant species (A) and Wild plant populations (B) in seven habitat types on Mount Slamet (mean ± SD)

Table 2. Total abundance of wild bees and wasps determined from a survey performed using insect nets (n = 15) in seven habitat types on Mount Slamet, Central Java, Indonesia

Family	Pollinator	Habitat type							Total	Relative abundance (%)
		NF	TF	PF	AF	CF	Gd	Ag		
Apidae	*Apis cerana*	78	50	19	21	50	66	27	311	33.1
	Apis dorsata	37	12	0	0	0	0	0	49	5.2
	Trigona laeviceps	60	47	0	0	47	43	0	197	21
	Amegilla cingulata	4	4	0	4	7	4	6	29	3
	Amegilla zonata	8	7	4	3	6	1	0	29	3
	Nomia melanderi	3	3	2	5	5	3	6	27	2.8
	Ceratina nigrolateralis	5	3	0	9	4	9	14	44	4.6
Megachilidae	*Megachile relativa*	15	16	11	11	12	6	4	75	7.9
Halictidae	*Lasioglossum malachurum*	0	0	7	0	6	11	6	30	3,.1
	Lasioglossum leucozonium	0	0	8	0	13	7	10	38	4,.05
	Ropalidia fasciata	0	0	0	3	3	7	1	14	1.4
Colletidae	*Hylaeus modestus*	2	0	0	0	0	0	0	2	0.02
Anthophoridae	*Xylocopa confusa*	0	0	0	0	11	0	12	23	2.4
Vespidae	*Delta campaniforme*	10	9	0	4	1	0	0	24	2.5
	Polistes fuscata	7	10	7	6	6	5	5	46	4.9

Table 3. Diversity parameters of wild bees in seven habitat types on Mount Slamet, Central Java

Parameter	Habitat type						
	NF	TF	PF	AF	CF	GD	AG
Species	11	10	7	9	13	11	10
Shannon H'	1.804	1.856	1.765	1.969	2.049	1.759	2.042
Simpsons (D)	0.217	0.202	0.183	0.160	0.178	0.247	0.150
Evenness (E = H'/ln s)	0.752	0.806	0.907	0.896	0.799	0.733	0.887

Our results show that CF is an important secondary habitat for wild bees (H' = 2.049). The Spearman's correlation coefficients showed that insect pollinators species richness and abundances were correlated with wild plant species richness ($r^2 = 0.67$, $p < 0.05$ and $r^2 = 0.63$, $p < 0.05$, respectively). Our results highlight the importance of wild plant species richness in the habitat for supporting wild bee species richness and abundance; however, this is only true for habitats with low species diversity, such as AF, PF, Gd, and Ag. The positive impact of wild plant richness on pollinator species richness can be explained by increased floral resource heterogeneity, which increases the attractiveness for many pollinator species seeking both single and multiple resources (Tscharntke et al. 1998; Potts et al. 2003). In forested habitats (NF and TF), we also found that wild bee diversity was affected by wild plant abundance ($r^2 = 0.68$, $p < 0.05$), supporting the hypothesis that high floral density within a habitat increases wild bee diversity because greater floral abundance means higher resource availability for pollinators. Previous studies have reported that wild plant abundance is one of two key variables structuring pollinator communities (Potts et al. 2003, 2010).

Our results were consistent with earlier studies performed by Steffan-Dewenter and Tscharntke (2001) in successional fallows and wheat fields (Holzschuh et al. 2007), where bee abundance was reported to increase with plant species richness and abundance (Buri et al. 2014). However, the results from our study are inconsistent with those of Hegland and Boeke (2006), who reported no significant effects of plant species richness on pollinator species richness.

The abundance of the major wild plant species did not affect wild bee diversity ($r^2 = 0.44$, $p < 0.05$). This finding can be explained by floral density's being more important than species richness because the availability of the main food resources (nectar and pollen) has a greater impact on the pollinators, as was reported by Hegland and Boeke (2006). Habitats with a high density of flowering plants are more attractive to pollinators than are those with a high diversity of flowering plants due to the lower travel time between multiple sparse patches in the former. Pollinators also tend to change their foraging behavior in response to flowering plant density to maximize nectar or pollen acquisition (Elliott and Irwin 2009).

Apis cerana was the most abundant and dominant species in all of the examined habitat types, with a total of 331 individuals (33.1%), followed by *Trigona laeviceps*, with 197 individuals (21.0%), whereas *Hylaeus modestus* had the lowest abundance, with only 2 individuals (0.02%). The most numerous family of pollinating insects in the investigated habitats was Apidae, with seven species, followed by Halictidae, with three species, and Vespidae, with two species. The least numerous were Megachilidae, Colletidae, and Anthophoridae, with only one species each. Only three species were found in all of the studied habitats. All of the pollinator taxa recorded in this study is categorized as generalist pollinators, i.e., they visit several plant species, and these pollinator species exhibit a wide range of floral choices and nesting requirements, which is advantageous for switching to alternative resources (Maldonado et al. 2013). Native bees, which are generally specialists and can be solitary, are present in smaller numbers in nature. Comparison of the solitary bee communities observed in this study showed that *Amegilla cingulata*, *Nomia melanderi*, *Ceratina* sp., *Lasioglossum malachurum*, *L. leucozonium*, and *Xylocopa latipes* were present in the highest numbers in artificial habitats (CF, Gd, and Ag) due to their habit of nesting in the soft mortar of building walls, whereas the genera *Apis* and *Trigona*, as well as genera of solitary bees with some tendency toward communalism (Xylocopinae) and subsocial behavior (Family Halictidae, tribes Augochlorini and Halictini), nest in pre-existing cavities in tree trunks or decomposing wood, or on the ground in banks or flat areas (Souza and Campos 2008). Multidimensional scaling supported this finding; bee communities in habitats with high wild plant species richness and abundance (NF, TF, CF, and Gd) included a wider variety of species compositions, whereas those in habitats with low wild plant species richness and abundance exhibited low species diversity. CF and Gd maintain high regional species richness due to diverse management practices and moderate disturbance intensity, which enhances floral species abundance and spatio-temporal habitat heterogeneity

The similarity of the wild bee species among the habitats based on the Bray-Curtis index ranged from 31.5% to 80.56%. We observed an 80.56% overlap between NF and TF and an 80.54% overlap between PF and AF. TF had 79% similarity with CF. These results indicate high similarity in the respective wild plant communities. Clustering of the similarities of wild bees among the habitat types showed that the wild bee community could be divided into the following three groups: NF and TF; Gd and CF; and AG, AF, and PF (Figure 2).

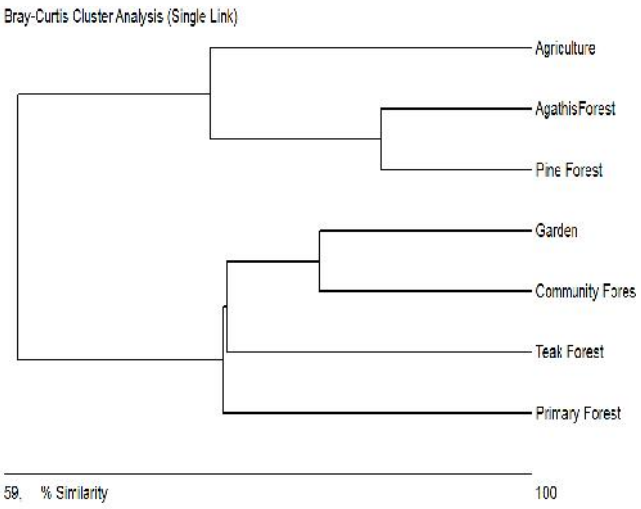

Figure 2. Similarity of wild bee species composition among habitats using Bray-Curtis analysis (single linkage)

In conclusion, the quality of the investigated habitats differed in terms of their relative contributions to wild bee diversity. The wild bee diversity in habitats with few wild plant species was strongly correlated with wild plant diversity, whereas in habitats with high wild plant species richness, flowering plant abundance was more important. The number of wild bee taxa recorded in the studied habitats showed that the diversity of wild plants species in these areas was fairly high and that the quality of the habitats in terms of plant species richness was important in maintaining pollinator diversity, both for solitary wild bee species and for eusocial wild bees.

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Markers-traits association for iron toxicity tolerance in selected Indonesian rice varieties

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[1]Indonesian Agency for Agricultural Research and Development. Jl. Pasar Minggu, Jakarta Selatan 12540, Jakarta, Indonesia. Tel. +62-21-7806202, Fax. +62-21-7800644, ♥email: yudhistira.nugraha@gmail.com

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Abstract. *Nugraha Y, Utami DW, Rosdianti I, Ardie SW, Ghulammahdi M, Suwarno, Aswidinnoor H. 2016. Markers-traits association for iron toxicity tolerance in selected Indonesian rice varieties. Biodiversitas 17: 753-763.* Ferrous iron toxicity is a mineral disorder frequently occurring under flooded soils condition where rice is cultivated. Here we study identification the Single Nucleotide Polymorphism (SNPs) markers associated with iron toxicity tolerance characters. The phenotypical data was collected from exploiting of twenty-four rice genotypes that were grown under Yoshida + 0.2% agar solution with treatment of 400 mg. L^{-1} Fe^{2+} and control conditions. The same genotypes were grown in iron toxicity acute and control sites at Taman Bogo, Lampung Province, Indonesia. The Principle Component Analysis (PCA) of the phenotypic data showed that 18 rice genotypes were selected representing grouping of related characters to iron toxicity condition. The genotyping of selected genotypes was carried out using multiplexes of 384 SNPs Golden Gate Illumina© assay. We identified, TBGI380435 which located on 14.45 Mbp of chromosome 9 was associated to leaf bronzing and relative shoot weight characters in the greenhouse experiment. The marker was associated with heavy metal transport detoxification (HTDT). The results are expected to assist in locating the potential candidate genes or Fe toxicity tolerance and to allow for precise marker-assisted selection. This research will serve for rice improvement through marker-assisted breeding and genomic selection in Indonesia.

Keywords: Golden gate assay, grain yield, leaf bronzing scores, principle component analysis, single nucleotide polymorphism
Abbreviations: SNPs = Single Nucleotide Polymorphisms, AM = Association Mapping, LD= Linkage Disequilibrium, GG= Golden Gate Assay, NGS= Next Generation sequencing ,QTL = Quantitative Trait Loci, LBS = Leaf Bronzing Score, PCA = Principle Component Analysis, SES = Standard Evaluation System For Rice, GLM = General Linear Model, PC = Principle Component, HTDT = Heavy Metal Transport Detoxification

INTRODUCTION

Iron (Fe) toxicity is one of the most important yield-limiting abiotic stresses in flooded lowland rice of humid-tropic areas (Becker and Asch 2005). In Indonesia iron toxicity in rice mostly can be found in swampy-land of acid sulfate soil and acid-clay soil which was occupied about 1 million ha (Ismunadji 1991). The typical symptoms associated with iron toxicity is leaf discoloration (bronzing) and reddish spots (Ponnamperuma et al. 1955). Yield losses associated with iron toxicity commonly range from 15% to 30%. However, in the case of severe toxicity occurs at the seedling stage, total crop failure can happen (Audebert and Sahrawat 2000). While some cultural practices have been suggested to counteract negative effects of Fe excess in soil solution such as water (Prasetyo et al. 2013), soil (Fageria et al. 2008), and nutrient (Ramirez et al. 2002) management strategies, however the most promising approach is to use tolerant genotypes.

Some rice genotypes have been identified as tolerant to iron toxicity, most of them were land races or local varieties which characterized as a photoperiod sensitive, taller plant high and low grain yield (Onaga et al. 2013; Suhartini and Makarim 2009). Introducing the traits of tolerant to iron toxicity from those varieties into the high yield popular varieties is the way to improved rice productivity in iron toxicity environment. Several study have been mapped on the rice genome related with traits involved in tolerance to Fe toxicity, under various environmental conditions and using different segregating populations issued from intra-specific populations (Dufey et al. 2009, 2012a; Shimizu 2009; Shimizu et al. 2005; Wan et al. 2003a, b; Wu et al. 1997, 1998; Wu et al 2014) or interspecific (Dufey et al. 2012b) crosses. These QTLs for traits directly or indirectly linked to iron toxicity tolerance have been localized but challenges of confident genomic localization remain huge, and with several hundred genes involved, their use in breeding programs is difficult. The method for narrowing the QTL via the production of a very large recombinant population, but this method is time consuming, costly and, for small-effect QTLs with low heritability, difficult in practice (Northon et al. 2008).

Marker-traits association is an alternative approach, to identify DNA-markers which are located in or in the neighborhood of the genes of interest. The strategies to identify marker-trait association could be used natural

(unknown ancestry) or breeding population (known ancestry) (Thomson 2014). Association analysis/ association mapping (AM) (= linkage disequilibrium mapping) is a population-based survey used to identify trait-marker relationships based on linkage disequilibrium (LD). LD is defined as the nonrandom association of alleles at different loci in a population (Flint-Garcia et al. 2003). It is measured as the strength of correlation between polymorphisms (i.e., SNPs) caused by their shared history of recombination with phenotypic variations. More recently, AM studies have also been facilitated by the availability of high-throughput and low-cost next generation sequencing (NGS) platforms, so that much of the genotyping work can now be easily outsourced in a cost-effective manner. These NGS platforms are being extensively utilized for de novo development of markers and also for genotyping. In addition to single nucleotide polymorphisms (SNPs) has been discovered in a number of crops (Edwards and Gupta 2013). For SNP genotyping, different methods have been developed, one of the method is the Golden Gate (GG) assays which is allow simultaneous genotyping of 96 to 3072 SNP loci in a fairly large collection of samples (up to 384 samples) in parallel (Gupta et al 2014). This assays are now becoming available in all major cereals including for rice (Utami et al 2013). To date, however has no report in regard exploring SNP using GG for development marker assisted selection in iron toxicity tolerance. Here we study the association analysis based on the SNPs marker developed using GG assay genotype data and phenotype data of the different level of Fe toxicity tolerance rice genotypes under the green house and the field experiment.

MATERIALS AND METHODS

Phenotypic data in the greenhouse experiment

The experiment was conducted in green house experimental station of Indonesian Center for Rice Research, Bogor from May to June 2014. Twenty-four rice genotypes of known degree of tolerance of iron toxicity were used in this study. The germinated seeds were transferred to sheet-holed styrofoam, sized 24 cm x 36 cm x 2 cm that fitted with 10-L plastic tray. Each sheet was consisted 100 holes with 2 cm x 3 cm spacing and each hole was used for growing one seedling. The plastic trays were filled with pre-culture solution using 1 L of 8×strength stock nutrient solution (Yoshida solution) followed 7 L of deionized water. After 14 days the pre-culture media solutions were replaced by new Yoshida solution with addition 400 mg L^{-1} of Fe^{2+} supplied as $FeSO_4$ and a 0.2 % agar. Addition of agar was given to minimize oxidation of ferrous iron (Nugraha et al. 2016). The initial pH was adjusted at 5.5 (±0.2). The nutrient solution of control was the same as well as the first experiment. We did not replace nutrient solution until 10 days for final leaf bronzing scored and samples were harvested for further analysis. The leaf bronzing score were determined using scoring index scale, 1 (no bronzing symptom on the leaf) to 7 (the whole leaves were bronzing) (Shimizu et al. 2005). Ten sample plants were harvested. The shoot length was measured from the longest leaf to base of the shoot. The root length was measured from the longest root to base of the root. These samples were oven-dried at 70 ^{o} C for at least 3-days, for dry matter determinations and separated the root from the shoot. The relative value of shoot and root dry weight were determined by (dw under normal - dw under iron stress)/dw under normal.

The rest of samples were harvested for measuring iron root plaque and shoot iron content. The fresh root of entire roots was incubated in 2 M HCl in 50 mL plastic flask for 60 minutes. The extract was filtered and transferred into new flask for analysis. The shoot samples then were separated with the root and oven dried at 70^{o} C for 3 days. The oven-dried shoot samples were ground and weighed 0.5 g into digestion tube. The sample were digested using 5 mL concentrate acid (HNO_3:$HClO_4$ = 3:1). On the following days, samples were heated on digestion block at 120^{o}C for 24 hours. After the tube had cooled, the digest was transferred to 25 mL flask with deionized water. Iron plaque and shoot concentration were measured by atomic absorption spectrophotometry.

Phenotypic data in the field experiment

The experiment was conducted in experimental station of Indonesian Soil Research Institute Taman Bogo, Lampung Indonesia (05^{o}02 S, 105^{o}50E), using the same 24 genotypes in the first experiment. Two plots were used for acute iron toxicity site and control iron toxicity site. Each plot was set out in the plots of 1 x 3 m^2 at a spacing 20 cm x 20 cm in a randomized complete block design with three replications. The average total of Fe in the soil concentration was 2030 mg.kg^{-1} and 765 mg.kg^{-1} for the acute and control, respectively. Standard agronomic practices for rice cultivation were followed, including plowing, harrowing, and flooding the soil throughout the season. No insecticide or pesticide was used; however, manual weeding was done at 3 and 5 weeks after transplanting. LBS was scored non-destructively at 4 and 6 weeks after transplanting for leaf bronzing using the SES developed by IRRI (IRRI 1996). The yield attributes were determined by randomly sampling 10 hills from each plot. Panicles were hand-threshed and the filled and unfilled spikelet were separated after drying them thoroughly under the sun. The subsamples were then oven-dried at 70^{o} C to constant weights for determining 1000-grain weight and spikelet number per panicle. Grain yield was measured at maturity from 1 m^2 subplots, with area under missing hills subtracted from harvest area. The yield then was adjusted to a moisture content of 14% fresh weight and converted to t. ha^{-1}. Percentage of reduction was measured as trait performance under normal - trait performance under stress to iron toxicity divided with normal condition.

DNA extraction

We selected 18 rice genotypes representing the tolerance level and morphological features based on the principle component analysis. Rice leaves were collected from a single plant of derived genotypes. The samples of

fresh leaf 21-days-old rice seedling were placed in bead and grounded in a tissue-lyser following the manufacture instruction (Qiagen, Venlo, Netherlands). A minimum of 15 µl genomic DNA (50 ng µL-1) was required for the Golden Gate assay. DNA was stored in TE buffer (10 mM Tris, pH 7.5: 1 mM EDTA). DNA purity was determined by using the A260/A280 ratio of 1.8-2.0 (Sambrook dan Russell 2001).

Custom design 384 SNP-chip

The 384 SNP-chip was designed based on the genetic map several genes/ QTL associated with Fe toxicity tolerant character that has been characterized by a previous study (Utami et al. 2014). The SNPs set of GS0014316-OPA was selected for this study based on Golden Gate Vera Code oligo pool assay (OPA) sets for the Illumina Bead Xpress Reader. This SNPs multiplex previously was validated by Thomson et al (2011) using population within *indica* and *aus* germplasm, and also informative for *Indica*/*Indica* populations which it has no call threshold of less than 0.25 and nearly more than >90% call frequency and <10% minor allele frequency.

The SNP sets were designed for the Illumina Golden Gate assay using multiplexes of 384 custom SNP panels. These custom Oligo Pool Assay (OPA) sets were then run on the Illumina platform which consists of an iSCAN reader with autoloader and Genome Studio analysis software which can be used with a variety of chemistries for genotyping based on Illumina Product Guide. The Golden Gate assay is an allele-specific oligo hybridization, ligation and extension assay followed by universal PCR amplification, allowing that no amplification bias can occur. These amplification products were then bond to the 3 µM microbeads and alleles were read by fluorescent readout using the iSCAN reader. The Genome Studio software from Illumina was used for allele clustering based on the ratio of the cy3/cy5 signal intensities to call the three genotype classes.

Data analysis

Statistical analyses were performed with SAS® version 9.1. For continuous data, we used analysis of variance (ANOVA) after verifying that the residuals met the criterion of normal distribution. When comparing up to three pre-determined means, we analyzed differences between means by Least Square Different. The green house and field experiment data were analyzed using principal component analysis (PCA) on the covariance matrix of traits. PCA analysis were performed using software tool Minitab 15. Association analysis between SNP markers and phenotypical data was tested using the General Linear Model (GLM) in the Tassel v. 3.0 software program (Bradbury et al. 2007). Values of the Q matrix obtained in Structure were presented as covariates. The P value determines whether a marker was associated with the marker and R^2 for a marker evaluates the magnitude of QTL effect to phenotypes. Further, dendrogram for clustering among genotypes were done using Tassel v. 3.0 using the selected-identified SNPs marker with probability more than 0.001.

RESULTS AND DISCUSSION

Phenotypic variation of rice genotypes under nutrient solution with iron-toxic stress

In the green house experiment, we identified *IPB107-5-1-1* and *IR64* had the highest bronzing scores 5.3 and 5.2, respectively (Figure 1 and 2). The rice genotypes *Siam Saba* (2.8), *Cilamaya Muncul* (2.9) and *Pokkali* (3.0) were the lowest bronzing scores among all the tested genotypes (Figure 2). Exceed iron also inhibited growth and development of roots and shoot, which was indicated with greatly reduced the sensitive genotypes, *IR64*, by 75% and 48% (Table 1). Less reduction of shoot length and root length was observed in the tolerant genotypes like *Siam Saba*, by 82% and 68%. This genotypes also had less reduction of shoot dry weight along with *Margasari*, by 87% compared to the lowest loss of shoot dry weight *IR64* and *IPB107-5-1-1* (58%). All genotypes also showed reduction in root dry weight, but pronounced in *IR64* by 30% from the normal condition. The less reduction of root dry weight was found in *B13144-1-MR-2* (77%).

Phenotypic variation of rice genotypes under natural iron-toxic stress

The same 20 genotypes (four genotypes could not be planted because of poor germination in nursery) that screened in nutrient solution culture were grown and evaluated for iron-toxicity tolerance in a field in Taman Bogo Lampung (Indonesia), during the 2013 wet season. In the field, plants were not immediately subjected to iron toxicity upon transplanting, in contrast to plants grown in the greenhouse, where iron toxicity was imposed 5 days after they were established in nutrient solution. We observed leaf bronzing scores appearing at the 4-week stage in the field ranged from 3.0 to 7.5 under acute site and 3 to 6 under normal site (Figure 5). *Siam Saba* and *Mahsuri* had the least leaf bronzing symptoms while *IR64, Inpara 5, Fatmawati*, and *IPB107-27* had the most leaf bronzing symptoms. During 6-week stage in the field leaf bronzing score of the most bronzing symptom genotypes became higher from the existing scores, suggesting more accumulation of iron during plant growth. Although *Siam Saba* was the less bronzing score, however we had no data for grain yield and its attributes due to photoperiod sensitive. This cultivar only can be flowering during August-September in the origin where this cultivar is grown in South Kalimantan.

Iron toxicity affected grain yield tiller number, 1000-grain weight and spikelet number and had interactive effect between genotypes and the iron site (Table 2). Significant different reduced of tiller number was also presented in this study. The sensitive cultivar, *IR64* and *Inpara5* had high tiller number under normal iron toxicity site but highly reduced up to 44% and 47% respectively under acute iron toxicity. We observed that there was no consistency in average of percentage reduced in 1000-grain weight and spikelet number among sensitive, tolerant, and normal genotypes. For example, *Batu Tegi*, a sensitive cultivar, showed less reduced 1000-grain weight (2%) while inpara2, a tolerant cultivar, had more reduced 1000-grain

weight (13%) under acute iron toxicity condition. The grain yield of sensitive genotypes was most affected by iron toxicity. *Limboto* was the most less reduced the grain yield (0.5%) but under normal condition this cultivar was quit low also compared to average total genotypes both in stressed iron and normal condition.

Table 1. LBS, Relative plant height, root length, shoot dry weight, and root dry weight of the rice genotypes under 400 mg. L^{-1} of Fe^{2+} for 10 days

Genotypes	Relative Shoot length		Relative Root length		Relative Shoot dw		Relative Root dw	
IR64	0.75	e-g	0.48	d-f	0.58	i	0.30	h
Inpara5	0.75	e-g	0.45	ef	0.64	ih	0.34	gh
Fatmawati	0.78	c-g	0.43	f	0.66	g-i	0.37	gh
Batu Tegi	0.77	d-g	0.48	d-f	0.69	gh	0.58	bc
Limboto	0.86	a-c	0.59	a-d	0.85	ab	0.52	b-d
Margasari	0.82	a-f	0.68	a	0.87	a	0.57	bc
Indragiri	0.79	c-g	0.53	b-f	0.75	b-f	0.52	b-d
A. Tenggulang	0.77	d-g	0.57	a-d	0.74	d-g	0.50	b-e
Siam Saba	0.82	a-f	0.68	a	0.87	a	0.68	ab
Inpara 2	0.78	c-g	0.66	ab	0.84	ab	0.61	b
Inpara 3	0.83	a-e	0.51	c-f	0.79	a-e	0.47	b-f
IPB Dadahp 1R	0.79	c-g	0.54	b-f	0.75	b-e	0.41	d-g
IPB Batola 5R	0.79	c-g	0.49	d-f	0.66	g-i	0.40	d-g
IPB Batola 6R	0.80	c-f	0.50	d-f	0.70	f-h	0.42	d-g
IPBKapuas 7R	0.76	e-g	0.43	f	0.72	e-g	0.40	d-g
IPB107F-5-1-1	0.77	d-g	0.48	d-f	0.58	i	0.34	gh
Pokkali	0.80	c-f	0.60	a-d	0.83	a-c	0.51	b-e
Mahsuri	0.80	c-f	0.62	a-d	0.82	a-c	0.51	b-e
B13144-1-MR-2	0.86	a-c	0.56	a-e	0.84	ab	0.77	a
B13100-2-MR-2	0.77	d-g	0.51	c-f	0.69	gh	0.42	d-g
Cilamaya M	0.88	a	0.58	a-c	0.86	ab	0.73	a
Awan Kuning	0.81	a-f	0.65	ab	0.79	a-e	0.51	b-d
Mesir	0.81	a-f	0.62	a-d	0.80	a-c	0.53	b-d
Kapuas	0.81	a-f	0.63	a-c	0.81	a-c	0.45	c-g
CV (%)	9.4		10.9		13.6		14.4	

Note: Means followed by the same letters are not significantly different at 0.05 probability error of Duncan Multiple Range Test

Figure 1. Appearance of leaf bronzing of rice seedling after exposure by 400 mg. L of Fe^{2+} in nutrient media cultures for 10 days**.**

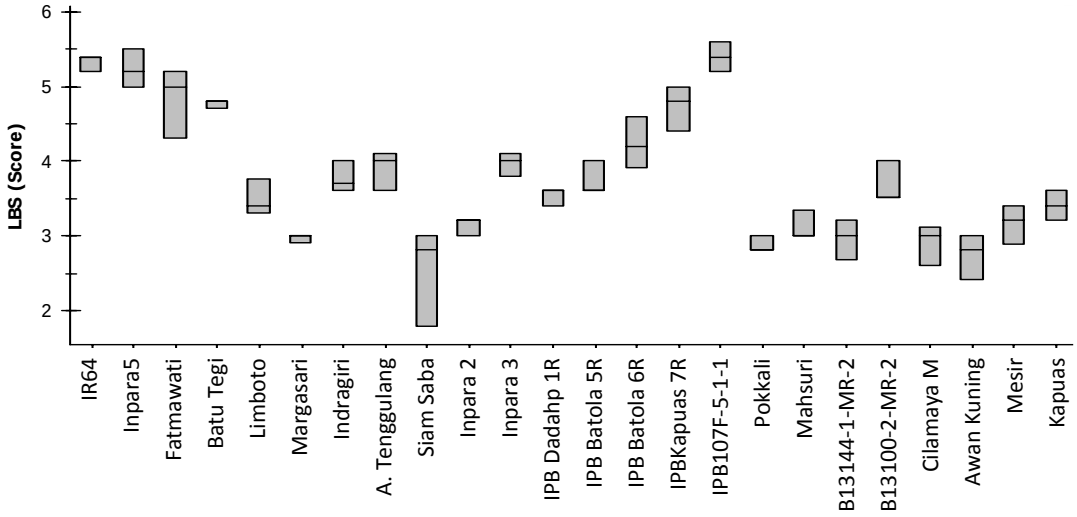

Figure 2. Severity of leaf bronzing of rice seedling (scores) exhibited by 24 genotypes of rice after exposure to 400 mg. L^{-1} of Fe^{2+} in Yoshida with 0.2% agar nutrient cultures

Table 2. Yield and agronomic performance of 20 genotypes under acute iron toxicity and normal iron toxicity stress Taman Bogo in the WS 2013.

Genotypes	Tiller number (no.)			1000-grain weight (g)			Filled grain number (no.)			Grain yield (t. ha^{-1})		
	A	N	%	A	N	%	A	N	%	A	N	%
IR64	8.7 a-c	16.3 ab	44	22.3 e-g	24.1 e	7	70 g	84 f	17	1.43 c	3.94 a-d	64
Inpara5	8.9 a-c	16.7 a	47	22.8 c-f	24.4 e	5	74 g	85 f	11	1.73 c	4.16 a-c	58
Fatmawati	7.2 c	9.0 f-h	22	25.1 a-e	27.3 ab	8	199 a	227 a	12	1.67 c	3.92 a-d	57
Batu Tegi	7.3 c	8.7 gh	22	27.6 a	28.2 a	2	185 a	222 a	17	2.19 bc	3.08 c-e	29
Limboto	7.7 bc	8.6 gh	11	23.6 c-f	25.2 e	6	81fg	92 ef	12	2.05 bc	2.06 e	0.5
Margasari	8.6 a-c	12.0 c-f	25	17.6 i	18.4 h	4	162 bc	180 b	10	2.24 bc	3.09 c-e	28
Indragiri	9.8 a	14.3 a-c	29	24.0 c-e	26.1 cd	8	99 e	113 d	12	2.58 ab	4.90 a	47
A. Tenggulang	7.1 c	11.0 e-h	36	23.2 e-g	25.6 d	9	98 e	109 de	10	2.56 ab	3.50 b-d	27
S. Saba[a]	7.5 c	9.3 e-h	11	-	-		-	-		-	-	
Inpara 2	8.7 a-c	11.7 c-g	25	23.7 c-f	24.2 e	13	102 e	116 d	12	3.51 a	4.14 a-d	15
Inpara 3	9.2 a-c	13.3 cd	31	27.9 a	29.0 a	4	105 e	118 d	11	2.67 ab	4.60 ab	42
IPB1 R	8.7 a-c	12.3 c-f	25	18.7 hi	19.9 g	6	160 bc	177 b	10	2.49 a-c	4.29 a-c	42
IPB Batola 5R	8.0 bc	13.3 cd	38	18.4 hi	19.1 gh	4	153 dc	180 b	15	2.40 a-c	4.71 a	49
IPB Batola 6R	8.6 a-c	12.2 c-f	25	19.2 f-h	19.8 g	3	148 dc	165 cb	10	2.29 bc	3.87 a-d	41
IPB Kapuas 7R	9.2 a-c	13.0 cd	31	18.7 hi	20.9 f	11	166 bc	186 b	11	2.06 bc	4.19 a-c	54
IPB107-27	9.0 a-c	12.0 cd	25	20.5 gh	21.1 f	6	138 d	155 c	11	2.02 bc	4.04 a-d	50
Pokkali	7.6 c	9.3 e-h	11	24.9 b-e	26.2 cd	5	93 ef	105 de	11	2.50 ab	2.89 de	13
Mahsuri	9.7 a	13.0 cd	23	18.2 ih	19.0 gh	4	184 a	202 ab	9	2.54 ab	4.14 a-d	39
B13144-1	8.3 a-c	13.7 a-d	43	26.3 a-c	27.0 bc	3	96 ef	108 de	11	2.53 ab	4.54 ab	44
B13100-2	9.8 a	13.3 cd	23	25.8 a-e	26.4 cd	2	104 e	124 a	16	2.59 ab	4.46 ab	42
Means	8.6	12.1	27	22.6	23.8	5	127	145	12	2.3	3.9	39
LSD (0.05) (within columns)	1.6			1.3			11.0			1.9		

Note: *A*, the site with acute iron toxicity stress; *N*, the site with normal; % reduction = (normal iron toxicity stress - stress to iron toxicity)/ normal x 100; [a] no data because of photoperiod sensitive; *LSD*, least significant difference test at 5% level with a column; The means separation in a column by Duncan Multiple Range Test at 5% level; ** and *** significant different of F-test at 0.01 and 0.001 level, respectively

Principal component analysis described the phenotypic variation of the used genotypes for marker-traits association

Principal component analysis indicated that four principal components accounted for most of the variation of the genotypes and observed traits. The first four principal components accounted for 72 % of the total variation. The principle component 1 (31.2%) had strong association with bronzing scores, meaning that the genotypes with high value of bronzing scores were in the same group which indicated as sensitive genotypes (*IR64, Inpara 5* and *IPB107-5-1-1*) (Figure 3). The spikelet per panicles and 1000-grain weight were the most important contributors to PC2 (17.8%), which enabled grouping the genotypes of *Fatmawati, IPB Dadahup 1R, IPB Batola 5R, IPB Batola 6R, IPB Kapuas 7R,* and *Batu Tegi.*

The major traits that contributed to PC3 were different direction from PC1, indicating that those traits had strong association with tolerance to iron toxicity such as, total dry weight, shoot dry weight, root dry weight relative shoot dry weight, relative root dry weight, relative tiller number and grain yield. The genotypes were in the same direction with PC3 were the tolerant genotypes with high biomass accumulation such as *Pokkali, Inpara 2, B13144-1*, and *Cilamaya Muncul*. Meanwhile the lower-right of quadrangle was the position of PC4, which indicated the tolerant genotypes with lower biomass accumulation in seedling stage (*Mahsuri, Siam Saba,* and *Margasari*). The other genotypes could not specify which assumed account about 28% of the total variation.

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Association marker-traits using 384 SNPs

The genotype profile of these samples was showed in FLAPJACK1.15.03.02 software (http://ics.hutton.ac.uk/flapjack) overview (Figure 4). Genotype determination of 18 rice varieties was done by PCA analysis using the phenotypical data. We identified 7 and 4 SNPs markers which were significantly associated (P ≤0.0001) with the phenotype data (Table 3). TBGI380435 (P=0.00054) and id8001543 (P=0.00055) were associated with the leaf bronzing and relative shoot dry weight. While the SNP marker id1000223 (P=0.00022) was found associated with leaf bronzing both in the green house and field experiment. The power of this association also is described using Manhattan plot and quartile-quartile plot (QQ plot) which shows the same trend of expected value. The others SNPs markers were associated solely with phenotypical performance TBGI272458 (P=0.00075) and TBGI427500 (P=0.00075) in the greenhouse experiment for leaf bronzing and relative root length respectively and TBGI272458, TBGI427500 (P=0.00075) and id4010825 (P=0.00077) for leaf bronzing in the field. The list of selected significance SNPs markers was showed in Table 3.

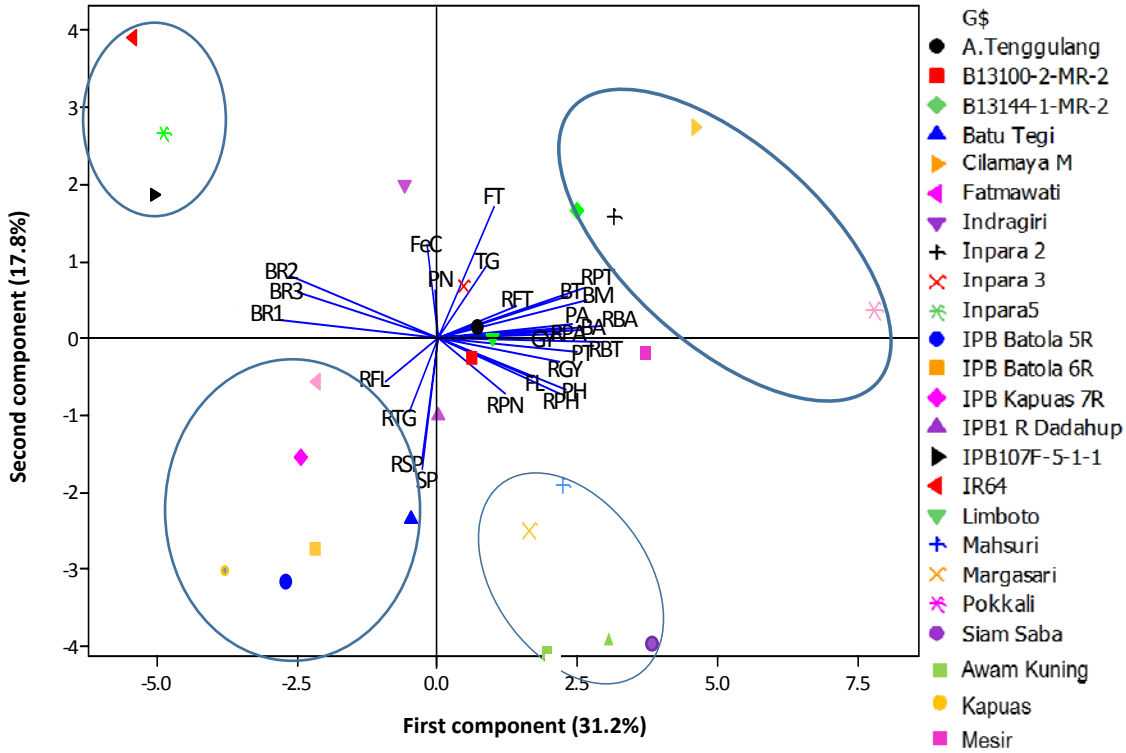

Figure 3. The first two components in principle component analysis for determining the rice genotypes and traits to be used for associated marker-traits analysis. Note: BR1=Bronzing Score in the greenhouse; BR1=Bronzing Score in the field at 4-weeks-after planting; BR3= Bronzing Score in the field at 6-weeks-after planting; FeC= Shoot Fe concentration; PN=Panicle Number (Fe-tox in field); FT=Fertility (Fe-tox in field); TG=1000-grain weight (Fe-tox in field); RFT= Relative Fertility; RPT=Relative shoot length, BM= total dw; BA= Root dw; RBA=Relative root dw; PA=Root length; RPA=Relative root length; GY=grain yield; RGY=Relative grain yield; PT=Shoot length; FL=day to flowering; RFL=Relative day to flowering; SP=Spikelet number; RSP=Relative spikelet number; RTG=Relative 1000-grain weight.

Figure 4. The genotype profile of eighteenth rice lines samples, particularly in one of the significant SNP locus, TBGU313277 which associated with leaf bronzing character and related with the Proline transporter candidate gene.

Table 3. SNP marker significantly associated with selected phenotypic character under Fe stress in the green house and field experiment

Characters	Associated marker	CR[a]	Position Mbp[b] (cM)	Marker probability	R^2 (%)	Candidate gene
Greenhouse experiment						
Leaf bronzing	TBGI380435	9	14.45	$5.46.10^{-4}$	73	Heavy metal transport detoxification
Relative shoot dw	TBGI380435	9	14.45	$2.41.10^{-4}$	67	Heavy metal transport detoxification
Leaf bronzing	id1000223	1	4.21	$2.28.10^{-4}$	66	Expressed protein
Relative shoot dw	id8001543	8	4.70	$5.51.10^{-4}$	63	ATP Binding protein
Leaf bronzing	TBGI272458	6	2.99	$7.46.10^{-4}$	52	Nuclear protein in pre-mRNA
Relative root length	TBGI427500	11	0.90	$7.70.10^{-4}$	69	F-Box domain
Field experiment						
Leaf bronzing	id8001543	8	4.70	$5.51.10^{-4}$	63	ATP Binding protein
Leaf bronzing	TBGU313277	7	0.47	$1.62.10^{-4}$	68	Proline transporter
Relative plant height	id4010825	4	32.30	$7.30.10^{-4}$	64	Unknown protein
Leaf bronzing	id1000223	1	4.21	$2.28.10^{-4}$	66	Expressed protein
Leaf bronzing	TBGI132654	3	5.59	$9.60.10^{-4}$	68	Unknown protein

Note: [a], Chromosome number, [b],Position of SNP marker in the chromosome

Based on the significant SNPs markers, candidate gene (s) could predicted which may contribute to Fe toxicity tolerance. Predicting the candidate gene (s) were done based on MSU IPGRS v.6 genome browser (http://oryzasnp.plantbiology.msu.edu/) and the results was showed on Table 3. The identified genes were Heavy metal transport detoxification, ATP Binding protein, Nuclear protein in pre-mRNA, F-Box domain, Proline transporter, and others expressed protein. The predicted positions of SNPs were distributed in the chromosome 1, 3, 4, 6, 7 and 9 (Figure 6). Those markers also confirm with previous study using QTLs analysis.

Discussion

Evaluation of rice genotypes against iron toxicity provided an insight into the genotypic differences associated with iron toxicity tolerance. Based on the analysis of study in greenhouse revealed that promising genotypes were *Siam Saba, Cilamaya Muncul, Awan Kuning, B13144-1-MR-2, Margasari, Pokkali, Mahsuri*, and *Inpara 2* (Figure 2). The field experiment also indicated that the low-score LBS genotypes not always had high grain yield under normal condition, except for *Inpara2, Cilamaya Muncul* and *Mahsuri.* Those out yielded genotypes in normal condition mostly are improved rice varieties, which have been released and tested in many locations including in iron toxic sites. The genotypes have been described tolerance in one site did not always had same result in the other iron toxicity site due to complexes environmental condition, such as low pH, nutrient starvation (Yamauchi 1989) and others nutrient toxicity such as Al, Mn, and Cd (Shamshuddin et al. 2013;

Muhrizal et al. 2006). Breeding approaches to address iron toxicity are generally favor as they are high yield, tolerance to others biotic and abiotic stresses and accepted to farmer's preferences. This finding also indicated that the improved grain yield through improvement of tolerance to iron toxicity still hampered.

The PCA plot (Figure 3) showed a clear separation of highly susceptible accessions (*IR64* and *Inpara 5*) from tolerant genotypes suggesting efficiency of the screening procedure in discriminating between the tolerance and sensitive genotypes. This method also clearly separating the genotypes based on the biomass, yield and its components. The high yield with tolerance to normal reaction to iron excess were located in the PC3, while tolerant local genotypes were located in the PC4. The position of iron concentration was in the upper-left quadrangle which was also near to tolerant genotypes like *B13144* and *Inpara2*, meaning that the iron concentration relatively high in those genotypes. Meanwhile, in the opposite direction and farther from the iron concentration PC line was *Pokkali*, *Mahsuri*, *Siam Saba*, and *Margasari*. This result indicated that some tolerant genotypes able stored the iron in the shoot, while the others tolerance excluded on the root surface. Other researchers reported the total amount of Fe accumulated in aboveground plant parts was not always related to leaf-symptom scores (Onaga et al. 2013). While other reported that vigorous growth genotypes, *Pokkali* has ability to dilute iron in the shoot minimizing detrimental effect of excess iron (Engle et al. 2012b).

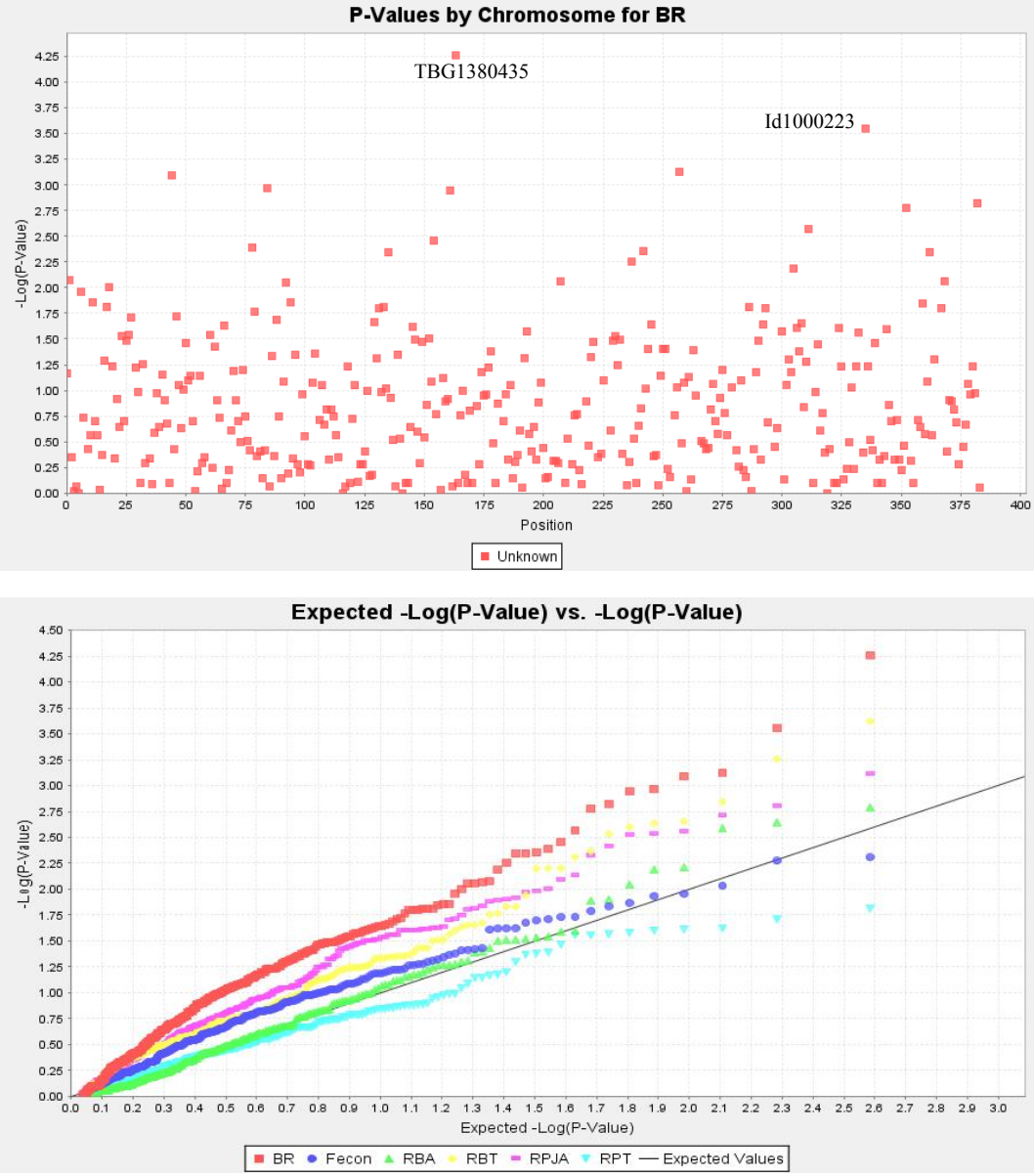

Figure 5. Manhattan plot, log P-values of leaf bronzing in greenhouse experiment are plotted against physical map position of SNPs (A) and Quartile-quartile plot (QQ plot) determines how marker-traits association in greenhouse results compare to the expected results.

Choice of germplasm is critical to the success of association analysis (Flint-Garcia et al. 2003). Generally, plant populations amenable for association studies can be classified into five groups (Yu and Buckler 2006): (i) ideal sample with subtle population structure and familial relatedness, (ii) multi-family sample, (iii) sample with population structure, (iv) sample with both population structure and familial relationships, and (v) sample with severe population structure and familial relationships. In this study, we used 18 selected Indonesian rice genotypes, represented different features morphological as described in the PCA analysis both under greenhouse and field experiment to meet criteria plant populations above.

Seven characters were associated with the SNP markers ($p<0.0001$). Some of them over-lap with different markers for instance TBGI380435 and id8001543 SNPs over-lap with leaf bronzing and relative shoot dry weight and id1000223 was over-lap in different set experiments for leaf bronzing character. In the Table 1 presents the result of the greenhouse experiment, which are tolerant genotypes with high relative shoot dry weight (e.g. *Mahsuri, Siam Saba, Cilamaya,* and *B13144-1-MR-2*). Relationship between leaf bronzing and relative shoot dry weight was also reported by (Onaga et al. 2013). This relationship was also confirmed with strong association with the same SNPs markers. The SNP marker, TBGI380435 which located in chromosome 9 at 14.45 Mbp was mapped on the same position of *heavy metal transport detoxification (HTDT)* gene based on MSU rice SNPs data based (www.http://oryzasnp.plantbiology.msu.edu/TIGR Pseudomolecules v5). High probability of this gene is described with Manhattan Plot and QQ Plot (Figure 5).

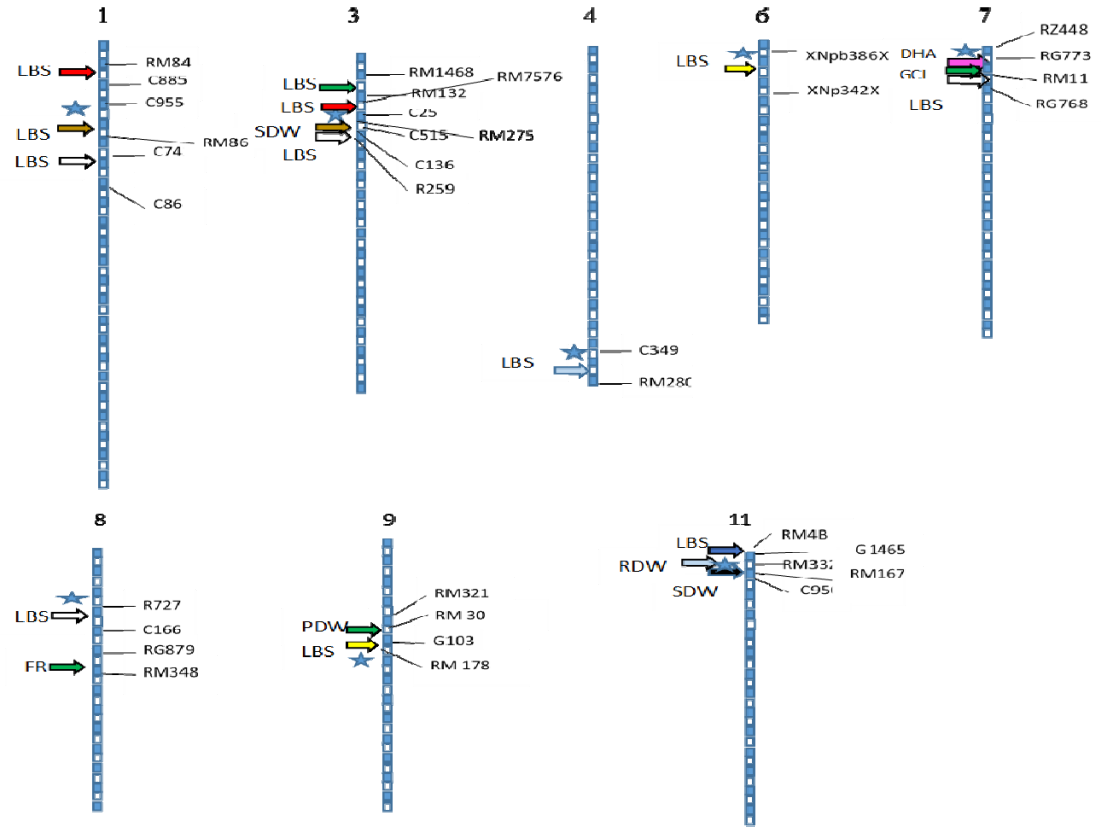

Figure 6. Co-localization analysis of markers-traits association reported in this study with previously reported QTLs for leaf bronzing under Fe toxic condition in rice. QTLs were located on chromosomes based on the physical positions of flanking markers. One quadrate (in blue or white) represents 1 Mb. Stars represent the QTLs mapped in this study and arrows represent the QTLs from other previous reports. *LBS*, leaf bronzing, *SDW*, shoot dry weight, *DHA*, dehydrate ascorbate activity, *RDW*, root dry weight, *PDW*, panicle dry weight, *GCL*, growth cycle length, *FR*, fertility.

Note:

- 135 DH lines from *Azucena/IR64* -nutrient solution, greenhouse at Zeijang, China (Wu *et al.* 1998)
- 96 BC_1F_9 lines from *Nipponbare/Kasalath//Nipponbare* - nutrient solution, greenhouse at Nanjing, China (Wan et al. 2003a)
- 66 CSSLs from *Asominori/IR24* - nutrient solution, greenhouse at Nanjing, China, (Wan et al. 2003b)
- F3 lines from *Gimbozu/Kasalath*- nutrient solution, greenhouse at Tokyo, Japan, (Shimizu 2009)
- 164 RILs from *Azucena/IR64* -fields, Burkina Faso (Dufey et al. 2012a)
- 164 RILs from *Azucena/IR64* - nutrient solution, phytotron, (Dufey et al. 2009)
- 40 RILs from *Azucena/IR64* - nutrient solution, greenhouse in Belgium (Dufey et al. 2012a)
- 220 BC_3DH lines from interspecific cross *MG12/Caiapo//Caiapo* - nutrient solution, (Dufey et al. 2015).
- 121 RILs from *IR29/Pokkali* - nutrient solution, greenhouse (Wu et al. 2014)
- Location of associated SNPs marker using 18 rice genotypes under hydroponic and field.

Previous study also reported some major QTL were located close to the *HTDT* position (Wan et al 2003a; Dufey et al 2012). The other gene that might be related to iron toxicity stress was *proline transporter gene* which was detected by SNPs marker TBGU313277 associated with leaf bronzing character in the field test experiment (Table 3). Majerus et al (2007) reported that high iron treatment causing significant decreasing water potential in the lamina and increasing of proline concentrations in the iron-sensitive but not affected in tolerant genotypes. This suggested that there was the inability of the roots from sensitive genotypes to perform osmotic adjustment while the tolerant genotypes perform more efficient using proline to adjust the water deficit disturbance.

The information about the underlying gene expression under iron toxicity is lacking comparing with iron deficiency-related genes (Ishimaru et al. 2006; Lee et al. 2009; Nozoye et al. 2011; Kobayashi et al. 2012). Ricachenevky et al (2010) reported using cDNA-RDA technique to isolate sequences up-regulated by Fe-excess in shoots of rice plants and found that *OsWRKY80* was up-regulated by Fe excess. Majerus et al. (2009) reported using mRNA accumulation of *OsFer*1induced as early as 24 h after the beginning of the Fe treatment in sheaths. Stein et al (2009) found that excess iron treatment led to accumulate mRNA of *OsFer2*. A micro-array analysis was performed by Quinet et al. (2012) indicating differential gene regulation between short- and long-term responses to excess Fe, and between genes of the same family, highlighted the complexity of plant response and the multi-genic nature of this trait. Recently, Utami and Hanarida (2014) reported based on association analysis, among the three SNP markers, *OsIRT1* was the most significant SNP marker (P value = 0.01) which correlated to Fe toxicity tolerant on vegetative stage. Hence, this study is the first report that the iron toxicity tolerance in rice was associated to HTDT gene with high ($P < 0.0001$).

The rest of the identified genes in this study were not been elucidated or related directly in the tolerance of iron toxicity (eg. ATP Binding protein, Nuclear protein in pre-mRNA, F-Box domain). However, the position of QTLs of iron toxicity tolerance which were reported previously was coincidently near to this trait-marker association study (Figure 6). SNP markers id1000223 was located in chromosome no 1 between 4 Mbp (Wan et al. 2003a) and 5 Mbp (Dufey et al. 2012). TBGI132654 was located on 5.59 Mbp near to QTLs reported by Dufey et al. (2012b). On the top of chromosome seven and eleven there were three markers related to some QTLs studies (Wu et al. 1998, Dufey et al. 2012a, Wu et al 2014) which were near to SNPs marker TBGU313277 and others QTLs studies (Shimizu 2009; Dufey et al. 2012) near to SNPs marker TBGI427500. Two marker of QTLs studies (Wan et al. 2003b; Dufey et al. 2012a) were closed with TBGI380435 on the chromosome no 9. This study was also related to the fact that the gene controlling to iron toxicity were very complexes involving multiple tolerance mechanism, for example excluder-type versus Includer-type (Engel et al 2012). A meta-analysis QTLs study involving 11 scientific journals identified more than 40 candidate genes based on their known function distributed along all rice chromosomes using (Dufey et al. 2015). This study also supported with other reports there are multitude of small effect QTLs underlines the concept of multiple tolerance mechanisms. Furthermore, highlighting the positions of reliable QTLs and association mapping helping to narrow the target candidate regions for marker-assisted selection.

We conclude that the phenotypical performance in the greenhouse and field experiment based on Principle Component analysis were clearly discriminated by PCA analysis. These variations were associated with SNPs marker using illumina bead chip array©, resulting a number of genes related to tolerance of Fe stresses. Some of these gene co-localized with previously reported QTL that were mapped under various crossing population and Fe stress. SNPs markers, TBGI380435 was related to heavy metal transport detoxification and TBGU313277 was related proline transporter, probably associate with tolerance to iron toxicity in rice.

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Prospect of indigenous plant species for revegetation in the tailings area of ex community gold mine

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Abstract. *Ekyastuti W, Astiani D, Roslinda E. 2016. Prospect of indigenous plant species for revegetation in the tailings area of ex community gold mining. Biodiversitas 17: 764-768.* One of the reclamation activities in the tailings area of ex community gold mine is revegetation. The success of revegetation is strongly influenced by the selection of suitable plant species. The purpose of this research was to determine the prospects of indigenous plant species for revegetation in the tailings area of ex community gold mine. Research has been conducted at the tailings area of ex community gold mining in Menjalin subdistricts Landak District West Kalimantan, using an experiment method with randomized complete block design (RCBD). Used ss basic for blocking is the difference of tailings area age: 2 years old and 10 years old after mining activities. In both locations, seven indigenous plant species were planted, i.e: *Dillenia suffruticosa, Vitex pinnata*, *Archidendron pauciflorum*, *Anacardium occidentale*, *Shorea leprosula*, *Alstonia scholaris* and *Hevea brasiliensis*. The results showed that the seven indigenous plant species are tolerant to mercury and can grow well in the tailing areas of ex community gold mine of both 2-years and 10-years after mining. Five indigenous plant species use phytostabilization to remediate mercury in the plant tissue, while two others species use phytoextraction. Therefore, the prospect of using indigenous plant species for revegetation in tailings area of ex community gold mine is very well.

Keywords: Ex community gold mining, indigenous plant species, tailing

INTRODUCTION

In West Kalimantan, small-scale gold mining activities (community gold mining) can be found in almost all districts, with the exception of Pontianak. As a result, tailing areas of ex community gold mining are spread throughout the province. One of the districts in West Kalimantan with quite extensive tailings area is the district of Landak. Particularly in Menjalin Sub District of Landak, data from 2013 showed a vast tailings area of ex community gold mining of 3,209 hectares (Romana et al. 2013). Many of the community gold mining activities are still going on to date. Tailings area of ex community gold mining is a critical land that is barren, arid, nutrient poor, tends to be acidic, and contains heavy metal of mercury (Ekyastuti 2013). Such condition requires proper reclamation to allow the survival of living things around it.

Part of the activities for land reclamation in ex-gold mine is revegetation. Since tailings area of ex-gold mine has limitations as medium for planting, a successful revegetation would be quite challenging. However, these constraints could be minimized by improving the physical, chemical and biological of tailings, i.e: by adding organic matters such as compost, top soil, and inoculation of arbuscular mycorrhizal fungi (Ekamawanti and Ekyastuti 2010, Ekyastuti 2013; Ekyastuti et al. 2016a). Moreover, the success of revegetation is also strongly influenced by the suitable selection of plants. The characteristics of plants suitable for revegetation in the tailings area are: easy to grow, intolerant, catalytic and phytoremediator of pollutants (Setiadi 2003; Mansur 2010; Sarma 2011). This means that besides being able to grow well in the tailings area, the species should also be able to facilitate other species to grow in these places. Furthermore, as written by Setiadi (2003), the catalytic species grow rapidly, generating much litter that decompose quickly, produce fruits which could invite animals who play a role in dispersing seed, which in turn results in fast colonization of plants on the area. Some indigenous plant species are considered having such characteristics, as the indigenous plant species are more adaptable to local environment. Astiani (2016) stated forest degradation has caused the loss of more than 50% of important species, yet based on research results by Ekyastuti and Roslinda (2015) in Menjalin sub district, the availability of seedlings of indigenous plant species for revegetation are still very good. This is made possible because in the surrounding of tailings area in Menjalin sub district, we could still find some secondary forest with a healthy condition. Therefore, it is necessary to study the utilization of indigenous plant species in revegetation activities. In this case, the study is focused on the field (on the tailings area).

Previous research proved that the planting of four indigenous species using the media of tailings ex-gold mine in the greenhouse run well (Ekyastuti et al. 2016b). The study also revealed that planting collectively (together) a number of species in the same location was better than planting one species only. However, testing of planting has not been done in the field. Planting tests in the original areas is very necessary to prove that indigenous species can grow well in the tailings area of ex-community gold mine, which will then enable it for revegetation. This study is

therefore aims to explore the prospects of indigenous species for revegetation on tailings area of ex-community gold mine through field tests.

MATERIALS AND METHODS

Research has been conducted at the tailings area of ex-community gold mine in Menjalin sub districts, using an experimental method with randomized complete block design (RCBD). Used as basic for blocking is the difference of tailings area age: 2 years old and 10 years old after mining activities. The location of tailings area of 2-year after mining is in Lamoanak village, and tailings area of 10-year after mining is in Sepahat village (Figure 1). The treatment in this study was the planting of seven indigenous species, namely: *Dillenia suffruticosa, Vitex pinnata, Archidendron pauciflorum, Anacardium occidentale, Shorea leprosula, Alstonia shcolaris* and *Hevea brasiliensis* in both sites. Planting was carried out on a planting hole of 40 cm x 40 cm, with holes of 40 cm deep each. In order to obtain the optimal conditions of planting medium, each hole was improved in its physical and chemical condition, using a mixture of top soil + compost 1: 1 (v / v) (Ekamawanti and Ekyastuti 2010; Ekyastuti 2013). Planting activity was carried out in the afternoon to avoid excessive evaporation. Planting activity is presented in Figure 2.

Analysis of variance was performed using the software program SAS 13. The data analyzed covered: percentage of plant growth, plant height increment (cm), and plant diameter increment (cm). Analysis of total mercury content in the roots, shoots and media (ppm) at the end of the study were conducted at the Laboratory of Baristand Pontianak, using standard SNI 06-6992.2 2004. The data would be used as baseline data to calculate: bioconcentration factor and translocation factor following the technique of Rabie (2005).

RESULTS AND DISCUSSION

The results showed that in both planting site, tailings area of 2-year after mining and 10-year after mining, the seven indigenous species planted could grow well and normal. No signs of mercury poisoning found on the plants. Results of observation and calculation are described separately below.

Percentage of plant growth

The mean of plant growth percentage at both sites are high, 91.42% at the tailings area of 10-year after mining (with a range between 80.00-97.60%) and 70.23% at the tailings area of 2-year after mining (with a range between 56.0-93.0%). Based on test results, percentage of plant growth at the tailings area of 10-year after mining was

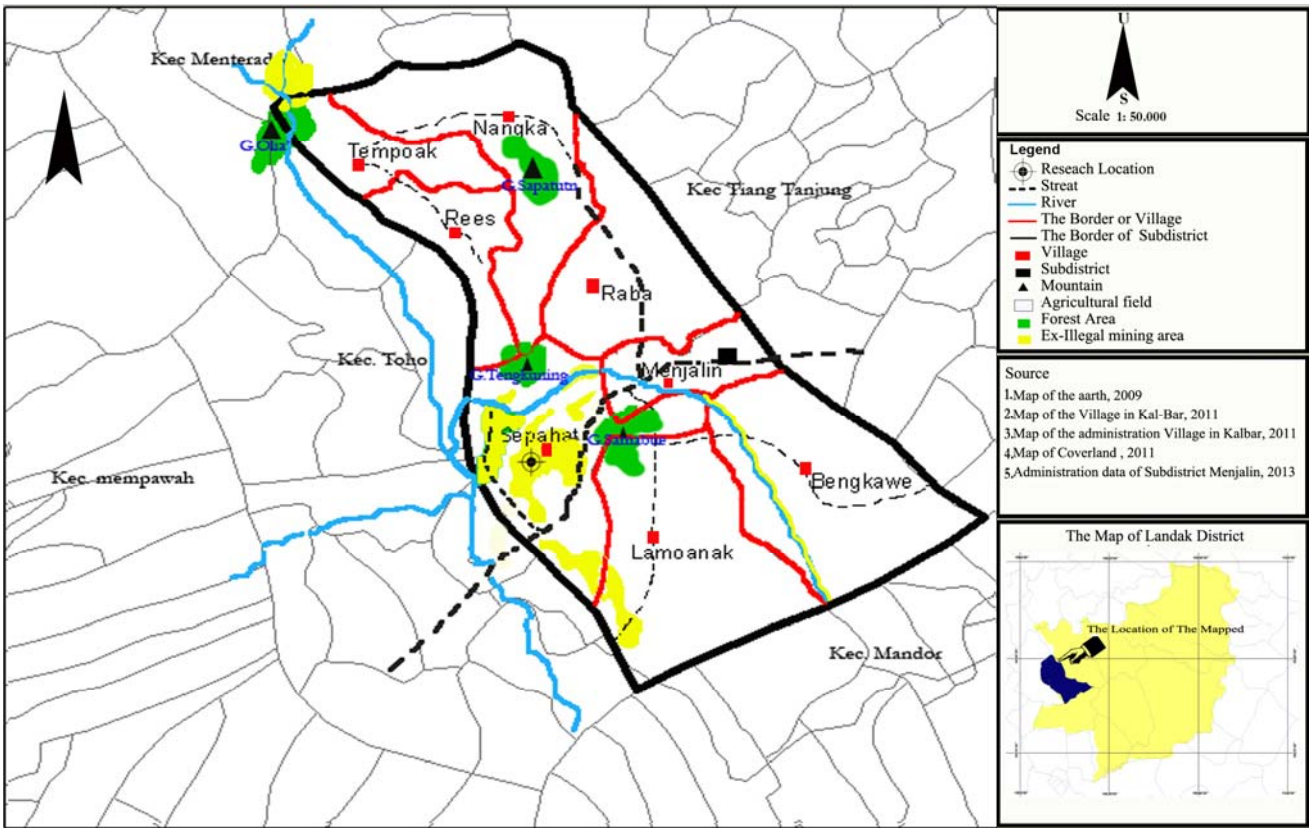

Figure 1. A map of the study site: Menjalin Sub district, Landak District, West Kalimantan, Indonesia. Map source: GPS data processing in the Laboratory of Forest Planning, Faculty of Forestry, Universitas Tanjungpura, Pontianak, Indonesia (2016)

Figure 2. Planting activities at tailing areas of ex community gold mining (A-C) transporting seedlings to the field and (D-F) planting activities

significantly higher than tailings area with the tailings area of 2-year after mining (Table 1). Meanwhile in each planting site, the growth percentage of seven indigenous species was not significantly different. However, there was a tendency that the percentage of plant growth in the tailings area of 2-year after mining slightly fluctuated (Figure 3).

Plant growth response

Plant growth responses in the field are measured through both their height and diameter, which were measured every month. The results showed that neither in tailings area of 2-year after mining nor 10-year after mining showed significant difference in average plant height and plant diameter increase (between seven indigenous species) (Table 2). Although insignificant, four indigenous species, *H. brasiliensis*, *A. pauciflorum*, *S. leprosula* and *V. pinnata,* showed the tendency of lower plant height and plant diameter increase in the tailings area of 2-year after mining compared to those in the tailings area of 10-year after mining (Figure 4). The other three indigenous species showed relatively similar increase. This is in line with the results of physical-chemical analysis of tailings. Physical-chemical conditions of tailings area of 10-year after mining had already begun to improve. Likewise, the content of mercury has declined from 0.5 ppm (tailings area of 2-year after mining) to 0.02 ppm (tailings area of 10-year after mining).

Bioconcentration factor (BF) and translocation factor (TF)

The result showed that the seven indigenous species have a bioconcentration factor > 1. This means that the accumulation of mercury generally occurs in the plant tissue, not in the media (Rabie 2005). It also means the seven indigenous plant species are tolerant to mercury. Based on the translocation factor, it is understood that five indigenous plant species accumulated mercury in the roots (TF < 1). The five indigenous species are: *A. occidentale*, *H. brasiliensis*, *A. pauciflorum*, *S. leprosula* and *V. pinnata*. Meanwhile, two other indigenous plant species, *A. scholaris* and *D. suffruticosa,* accumulated mercury in the shoots (TF > 1). According to Fulekar et al. (2009) and Sarma (2011) the process of mercury remediation is phytostabilization if TF < 1 and phytoextraction if TF > 1.

Tabel 1. DMRT of the percentage of plants growth

Tailing ages	The average percentage of plant growth
2 years after mining	70.23 a
10 years after mining	91.42 b

Table 2. Anova of increments of height and diameter

Treatments	Plants height increment	Plants diameter increment
A. scholaris	13.930 a	0.415 a
A. occidentale	10.550 a	0.305 a
D. suffruticosa	9.650 a	0.195 a
H. brasiliensis	14.320 a	0.245 a
A. pauciflorum	5.880 a	0.290 a
S. leprosula	4.730 a	0.205 a
V. pinnata	6.910 a	0.215 a
P-value	3.279	2.215
F-value	4.280	4.280

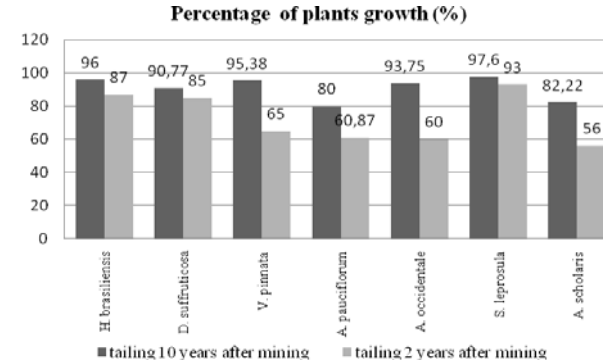

Figure 3. Percentage of plant growth in tailing areas

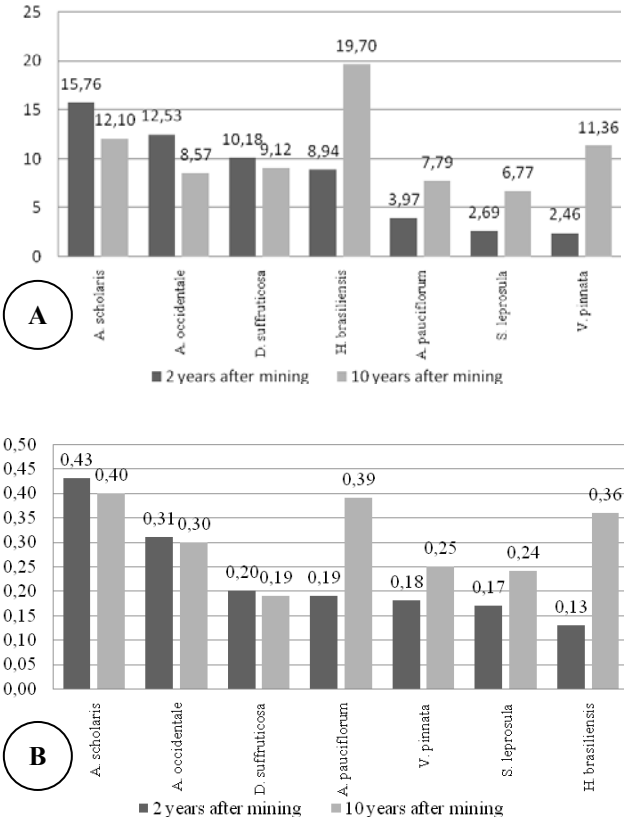

Figure 4. Average of incremets (cm) of (A) height and (B) diameter

Tabel 3. Bioconcentration Factor (BF) and Translocation Factor (TF)

Indigenous plant species	BF	TF
A. scholaris	45.0	1.3
A. occidentale	6.5	0.3
D. suffruticosa	15.5	3.0
H. brasiliensis	13.5	0.1
A. pauciflorum	11.0	0.1
S. leprosula	7.5	0.5
V. pinnata	40.0	0.6

Discussion

The percentage of plants growth at the tailings area of 10-year after mining is very high (91.42%), whereas at the tailings area of 2-year after mining is moderate (70.23%). The difference in percentage growth of plants is suspected due to the difference in tailings' fertility rate as the medium for growing plants. It is evident that based on the results of physical-chemical analysis, tailings area of 2-year after mining is worse than the tailings area of 10-year after mining. At the tailings area of 2-year after mining, we found a fairly high level of mercury (0.5 ppm), low macro nutrient content, low pH (acidic), low cation exchange capacity (CEC) and the soil texture was dominated by sand (89.6%). Fauziah research's results (2009) showed that the tailings area as plant medium is bad or unworthy. However, the condition could be improved by adding humic acid and compost. The opinion is echoed by Sembiring (2008) and Ekyastuti (2013), stating that the tailings area of less than 2-year after mining has not showed any improvement, physically and chemically. Therefore, it is not suitable for plants' growth.

Meanwhile, at the tailings area of 10-year after mining, the success of growing plants is affected by the improvement in tailings' physical-chemical conditions. Based on the analysis on tailings area of 10-year after mining, it is revealed that the nutrient availability has increased, along with normal pH, moderate level of cattion exchange capacity, and mercury content down to 0.02 ppm. Such condition would provide a much better site for plants' growth, so the percentage of plant growth will be very good. Some parts of the tailings area of 10-year after mining in Sepahat village have been used for cultivation of – not only timber – but also horticultural crops.

Based on the analysis, the mercury content in the tailings area is 0.02 ppm - 0.5 ppm. We found that the older the tailings, the lower the mercury content. On the other hand, based on the response of plant growth, the seven indigenous plant species can grow well not only at the tailings area of 10-year after mining, but also at tailings area of 2-year after mining. The conclusion proved by ANOVA is not significantly different. It also proves that the seven indigenous plant species are relatively tolerant to mercury. The results of this study (field study) is in line with previous studies conducted in the greenhouse (Ekyastuti et al. 2016). Such condition is achieved not only due to the physical-chemical improvement of tailings area, including lower mercury content, but also due to the selection of indigenous species, which are more adaptable to local environment compared to other exotic species (Adman et al. 2012). The results of this study are very useful to support plant breeding and preservation program of indigenous species (Uji 2007).

Based on data from bioconcentration factor, it is revealed that the planting of seven indigenous plant species in this study led to the accumulation of mercury in plant tissue instead of the planting medium, because the seventh has a value of FB> 1. The results showed further that the seven indigenous plant species are tolerant to mercury (Rabie 2005). While based on the value of translocation factor, the results are varied. Five indigenous plant species,

A. occidentale, *H. brasiliensis*, *A. pauciflorum*, *S. leprosula* and *V. pinnata,* use phytostabilization to remediate mercury in the plant tissue. As for the other two species, *A. scholaris* and *D. suffruticosa,* use phytoextraction to remediate mercury in the plant tissue. In remediation process using phytostabilization, the mercury content is accumulated in the vacuole of roots, and not distributed to the leaves. Therefore, the photosynthesis process is not disturbed and the plants grow normally. Meanwhile, in the phytoextraction process, mercury is distributed to the leaves (Patra & Sharma 2000; Wang 2004). As a result, if the plant uses phytoextraction processbut the growth is still normal, it is suspected that mercury is stored in the vacuole of leaf, resulting in undisturbed photosynthesis process. However, the theory was not further examined in this study.

Based on the results of plant growth in the field, namely in the tailings area of 2-year after miningand 10-year after mining, it can be concluded that the prospects of using indigenous species as the selected plants for revegetation at the ex-community gold mine is very good. Furthermore, the improvement of physical-chemical properties of the tailings by adding compost and other beneficial microorganisms (in this case is arbuscular mycorrhizal fungi) is also important. In the future, these activities should also involve the communities surrounding the tailings of ex-gold mining to optimize the results.

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Comparison of *Neurospora crassa* and *Neurospora sitophila* for phytase production at various fermentation temperatures

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Abstract. *Kanti A, Sudiana IM. 2016. Comparison of* Neurospora crassa *and* Neurospora sitophila *for phytase production at various fermentation temperatures. Biodiversitas 17: 769-775.* There is general consensus that the presence of phytate in poultry negatively influences protein in and energy utilization by poultry, and these influences would be mitigated by augmentation of hydrolytic enzymes. The objective of this study was to evaluate phytase production by *Neurospora crassa* and *Neurospora sitophila* on solid state fermentation. The isolates were isolated from *oncom* Bogor. Phytase production ability was first determined on submerge fermentation (SmF) using glucose as the main C-sources, and on solid state fermentation (SSF) on media containing maize 30%, soybean 30%, and rice brand 40% w/w. Maximum enzyme activities were observed at 96 h incubation. SSF produce higher phytase than SmF. Optimum temperature for phytase production was 35°C. Highest phytase production by *Neurospora crassa* was 45.25 Unit per g substrate, while 40.78 Unit per g substrate was produced by *Neurospora sitophila.* Peptone and yeast extract were good N-sources for both isolates. Starch supplement increased phytase activity. Increased amylase activity was also observed when starch supplement was added on SSF. This study proves that *Neurospora crassa* and *Neurospora sitophila* can be used to produce phytase for better poultry nutrition.

Keywords: *Neurospora crassa*, *Neurospora sitophila*, phytase, solid state fermentation

INTRODUCTION

Morphological observations, physiological studies, ecology and genetics of the occurrence of *Neurospora* on natural and artificial substrates were reported (Steele and Trinci 1975). The organism is ubiquitous in moist tropical or subtropical climates (Tian et al. 2009). Because dormant ascospores are activated by heat, blooms occur on burnt vegetation (Galagan et al. 2003). The most important finding is that *Neurospora* is generally recognized as safe. Never, in more than a century of observation and experimentation has the genus been implicated in human disease or observed to cause disease in animals or plants (Perkins and Davis 2000; Znameroski et al. 2012). *Neurospora* is a common fungi, it has been used in many experiments, and vast genetic information has been obtained through DNA sequencing (Galagan et al. 2003). Several isolates have been found in traditional fermented food in Indonesia (Perkins and Davis 2000; Liu 2003). More than 1000 loci have been sequenced and mapped on the chromosome (Powell et al. 2007). *Neurospora* have been studied since 1843, and the species *N. crassa* has been a focus of intensive research on traditional fermented food fermentation, and enzymes production (Springer and Yanofsky 1989). There are five important species of conidiating *Neurospora* which include *N. crassa, N. sitophila, N. intermedia, N. tetrasperma,* and *N. discreta.* They can be determined by their distinctive orange color, rapid growth, and profuse production of powdery conidia (Jacobson 1992). *Neurospora* are also important fungi for bioprocess based industry include enzyme for feed (Zhou et al. 2006).

Reduction of feed cost for poultry is the main interest of many scientists. Inclusion of phytase in poultry diet has increased remarkably during the past decade. This is due to a high concentration of phytate in cereal (barley, maize, sorghum and wheat) ranging from 1.86-2.89 ($g.kg^{-1}$). Higher phytate are found in oilseed meals (4.0-9.11 $g.kg^{-1}$), and the highest 8.79-24.20 Phytate-P ($g.kg^{-1}$) are found in rice brand and wheat brand (Maga 1982; Heaney et al. 1991; Haraldsson et al. 2005). Phytase hydrolyzed phytate which eliminate the intense of phytate bound on mineral, carbohydrate and protein (Liebert and Portz 2005). Thus augmentation of phytase will reduce feed costs and increase the efficiency of utilization of phosphate and other nutrients in cereal based feed ingredients (Leytem et al. 2008). It is expected that inclusion of phytase will result in economic and environmental benefits. Not only phytase, but other hydrolytic enzymes (amylase and cellulase) are important components of feed ingredients (Kim et al. 2007). Up to now, inclusion of hydrolytic enzymes in feed ingredients is mostly focused on phytase production, and *Aspergillus niger* is the most popular phytase producer. Several other fungi such as *N. crassa*, *N. sitophila, Rhizopus oryzae,* and *Rhizopus oligosporus* could be important microbes for production of hydrolytic enzymes (Zhou et al. 2006). Those fungi are well known to play a major role in traditional fermented food in Indonesia such as *oncom* and *tempeh*. Solid state fermentation offers higher enzyme production, and less expensive and easier process control. Solid state fermentation has been effectively used to produce phytase. Temperature and

carbon nitrogen sources could be important factors influencing phytase production. The objective of this study was to assess the effect of temperature, and the augmentation of nitrogen and starch on phytase production by *Neurospora crassa* and *N. sitophila* isolated from *oncom*.

MATERIALS AND METHODS

Isolation and identification of *Neurospora*

Isolation of fungi from *oncom* was performed following methods described by Choi et al. 1999. The *oncom* was obtained from the local market in Bogor, West Java, Indonesia. To isolate the fungi from the sample, 1.0 g of the sample was diluted in 9 mL sterilized water and vortex-mixed. One-tenth of a milliliter of successive decimal dilutions was spread on acidified Dichloran Rose Bengal agar chloramphenicol agar (OXOID, Cat.1076012). This selective medium was used because bacteria growth is prevented, and spreading of molds is suppressed.

Plates were incubated for 5 days at room temperature. Strain purification was done at least twice by selecting one of each type of fungi colony and purified twice for single colonies. The plates were incubated at 27°C for 3 days. Representative colonies were picked, purified and maintained on Potato Dextrose Agar (PDA)(OXOID, Cat.CM 0139). Morphological observation of fungi was conducted following Jacobson (1992).

rDNA sequence determination

Fungi DNA template was prepared from freshly-grown cells on the Potato Dextrose Broth and used for extracting the DNA (Butinar et al. 2005). PCR amplification of the partial Internal Transcribed Spacer (ITS) ribosomal subunit with primers ITS 4: 5`– TCC TCC GCT TAT TGA TAT GC – 3`and Primer ITS 5: 5`– GGA AGT AAA AGT CGT AAC AAG G –3` (White et al. 1990) using GoTaq master mix (Promega, M7122). PCR products were visualized on 2% agarose and sequenced with both primers using Big Dye terminator v3.1. Cycle Sequencing Ready Reaction Kit (Applied Biosystems) following the manufacturer's instructions. The partial 26S sequences determined in this study were compared to those in the EMBL/GenBank/ DDBJ databases using the nucleotide Basic Local Alignment Search Tool (BLASTn) (Altschul et al. 1997).

Culture maintenance

The isolated strains of *Neurospora* were first grown in PDA (Potato Dextrose Agar) medium for 120 h, at 30°C. These strains were then evaluated for their ability to produce phytase under submerge culture and solid state fermentation at various temperatures and media composition.

Screening for phytase production

The isolated strains of *Neurospora*-were grown in PDA medium. Enrichment culture media containing 0.5% sodium phytate as the sole phosphorus and glucose were used for the primary screening of phytase producers. The method was based on estimation of phosphate solubilization from sodium phytate in aqueous media. The strains were grown under shaking conditions at 150 rpm, at 30°C for 96 hours. The culture was grown as submerge fermentation (SmF). After 96 hours of incubation time the fungal biomassa was discarded by centrifugation at 8000 rpm for 20 minutes and the supernatant was then used for estimation of phytase production using the method described by Liu et al. (1999).

Inoculum preparation for SSF

The culture was grown and maintained on potato dextrose agar (PDA) slants. The slants were stored at 4°C and sub-cultured for 4 days. Five-day-old fully sporulated slant was used for inoculant preparation. For this, 10 mL sterile distilled water containing 0.1% Tween-80 was added to the slant and spores were scraped with a sterile needle. The inoculant obtained contained 4.7 x 10^7 spores per mL.

Substrates preparation for SSF

The media composition for phytase production were maize 30%, soybean 30%, rice bran 40%, which were obtained from a local market. Ten grams of the dried mixed substrate taken in a cotton plugged 250 mL Erlenmeyer flask were supplemented with 6.0 mL of salt solution containing (%) 0.5, $MgSO_4$.7H2O 0.1 and NaCl.

The effect N-sources

To study the effect of nitrogen sources, the media of SSF was added with either sodium nitrate 0.1%, urea 0.5%, yeast extract 0.5%, and peptone 0.5%. The incubation temperature was maintained at 30°C, 35°C and 40°C. The activity of phytase was determined at 3, 4 and 5-days. The activity was expressed in units defined as µmol phosphate release by 1.0 mL enzymes per minute at 37°C.

The effect of additional carbon source

Starch as additional carbon source was selected to study the effect of additional carbon on phytase production. Additional carbon sources were evaluated at concentration of 0-4%.

Enzyme extraction

Enzyme extraction was carried out using distilled water with 0.1% of Tween-80. Known quantities of fermented substrates were mixed thoroughly with the required volume of distilled water (so that the final extraction volume was 100 mL) by keeping the flasks on a rotary shaker at 180 rpm for one hour. The suspension was centrifuged at 8000 · g for 20 min at 4ºC and the clear supernatant obtained was assayed for phytase activity.

Phytase assay

Phytase activity was assayed by measuring the amount of inorganic phosphorus released from sodium phytate solution using the method of Singh et al. 2013. One unit of enzyme activity was defined as the amount of phytase required to release one micromole of inorganic phosphorus per minute under the assay conditions.

Amylase assay

Alpha amylase was assayed by adding 0.5 mL of enzyme to 0.5 mL soluble starch (1%, w: v) in 0.1 M phosphate buffer, pH 6.0, for 30 min at 40 °C. The reaction was stopped and reducing sugar was determined with dinitrosalicylic acid according to the method of Bernfeld (Saqib and Whitney 2011). An enzyme unit is defined as the amount of enzyme releasing 1 μMol of glucose equivalents from the substrate per hour at 40°C.

Protein estimation

Protein was determined using a UV spectrophotometer (UV mini 1240 UV/VIS Shimadzu) taking readings at 280 nm. Water or buffer was used as a blank. A standard curve for the conversion of OD readings to mg protein was obtained using a series of dilutions of bovine serum albumin.

Biomass estimation

Fungal biomass estimation was carried out by determining the N-acetyl glucosamine released by the acid hydrolysis of chitin present in the cell wall of the fungi (Jost et al. 2011). For this, 0.5 g (dry weight) of fermented matter was mixed with concentrated sulphuric acid (2 mL) and the reaction mixture was kept for 24 h at room temperature (30°C). This mixture was diluted with distilled water to make a 1 N solution, autoclaved for 1 h, neutralized with 1 N NaOH and the final volume was made up to 100 mL with distilled water. The solution (1 mL) was mixed with 1 mL acetyl acetone reagent and incubated in a boiling water bath for 20 min. After cooling, ethanol (6 mL) was added followed by the addition of 1 mL Ehrlich reagent and the resulting mixture was incubated at 65°C for 10 min. Once cooled the optical density of the reaction mixture was read at 540 nm against a reagent blank. Glucosamine (Sigma) was used as the standard. The results obtained are expressed as mg glucosamine per gram dry substrate (gds).

RESULTS AND DISCUSSION

Isolation and identification of *Neurospora*

Morphological observation on hyphal morphology of *Neurospora* colonies grown on PDA plate showed that the hyphal can be divided into two regions: the periphery and interior of the colony. The morphology of *Neurospora* are shown in Figure 1. In the colony periphery, the leading hyphae grew relatively straight and had a subapical branching pattern with primary hyphae exhibiting apical dominance over its branches. This is a wide, fast-growing hypha located at the colony periphery. It consists of an apical, tip-growing hyphal compartment interconnected with subapical compartments separated by perforated septa that provide continuity by allowing the passage of nuclei, other organelles, and cytoplasm. The leader hyphae undergo subapical branching and their growth contributes to the increase in colony diameter. Trunk hypha is located in the colony interior. It is wide and composed of hyphal compartments that typically become highly vacuolate (Riquelme et al. 2011). These hyphal compartments are typically shorter than those of leader hyphae because of the greater frequency of septa. The septal pores of trunk hyphae are frequently occluded.

Fusion hypha is typically a narrow, dichotomously branching hypha that arises from a trunk hypha. It exhibits positive tropisms, homing towards other fusion or trunk hyphae and anastomosing with them. In contrast to the hyphae described above, aerial hyphae grow in or on the surface of solid or liquid media, and grow away from the medium surface into the air. This is believed to be a result of forming hydrophobic hydrophobin proteins on their surfaces (Steele and Trinci 1975; Kasuga and Glass 2008; Wallrath and Elgin 2012).

Macroconidiophore is the specialized hypha that gives rise to macroconidia. Macroconidium (blastoconidium) is large, multinucleate (typically 3-6 nuclei) asexual spore that grows and develops by repeated budding of the apical cell of the conidiophore. The primary hyphae and their branches usually grew in such a way that they actively avoided neighboring hyphae (negative autotropism). Nevertheless, these hyphae occasionally made contact with each other but this did not result in hyphal fusion. In these cases, two alternative behaviors were observed: (i) the growth vector of the intersecting hypha would change, usually beginning just prior to contact, and resulting in subsequent parallel growth of this hypha along the side of a resident hypha or (ii) the growth vector of the intersecting hypha did not change, resulting in the contact of the hyphal tip with a resident hypha.

Molecular analyses of the ITS region confirmed that the two isolates belonged to *Neurospora crassa* and *N. sitophila*. The phylogenetic affiliation of *Neurospora crassa* and *N. sitophila* is shown in Figure 2.

Production of phytase by fungi

N. crassa and N. sitophila originated from *oncom* produced phytase on submerged culture from 2.1 to 7.4 unit (Figure 2). Fermentation time affected enzyme production profile. Maximum phytase production was achieved after 4 days fermentation. The activity of phytase produced by *Neurospora crassa and N. sitophila* was much higher than that produced by *Pichia anomala* (Olstorpe et al. 2009).

Activity of phytase in formulated media

Nitrogen sources affect phytase activity (Figure 3). In the case of *N. sitophila*, yeast extract and peptone supplement increased phytase production by 25%, but urea and sodium nitrate was less effective. However *N. crassa* appeared to use various nitrogen sources (sodium nitrate, yeast extract and peptone). N-supplement increased phytase production by 30%. Various influences of N-supplement were observed by several scientists. Yeast extract were good N-sources for phytase producing *Bacillus subtilis* (Singh et al. 2013), whose introduced nitrogen sources at the 0.5% yeast extract, sodium nitrate, ammonium sulphate, urea and ammonium acetate. This study reveals that *N. crassa* and *N. sitophila* used more variable nitrogen sources than *B. subtilis*.

Effect of incubation temperature

Temperature affected phytase production by *N. crassa* and *N. sitophila* (Figure 4,5 and 6). The highest temperature for phytase production was at 35°C. The optimum temperature for *N. crassa* and *N. sitophila* is lower than *B. subtilis* (Singh et al. 2013).

Effect of starch supplement

Starch supplement increased phytase production, optimum starch supplement was 3% (Figure 7). Starch will be used by fungi after hydrolysis by amylase (Juanpere et al. 2005; Lim et al. 2008). This indicates that *N. crassa* and *N. sitophila* produced amylase (Murthy et al. 2009). Both phytase and amylase are used for feed supplement of monogastric animal (Cowieson and Adeola 2005).

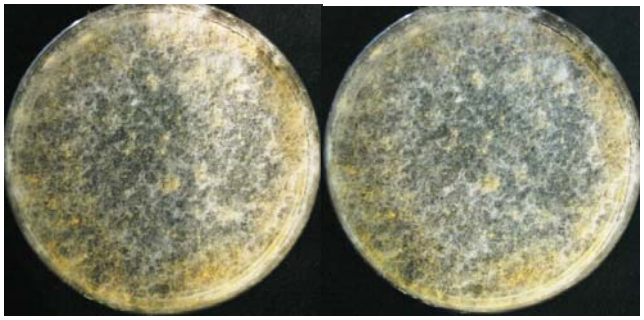
Alpha amylase activity

We observed an increase of phytase activity due to the addition of starch, and therefore we determine the activity of alpha amylase (Figure 8). The activity of alpha amylase increased in both cultures, and maximum enzyme activity was observed at 72 hour fermentation. This implies that the two isolates were able to use starch as carbon sources. The ability of fungi to produce amylase is variable. Higher alpha amylase production was observed on *Aspergillus niger* using sorghum than starch as a media (Abu et al. 2005). Starch supplementation increased carbon sources and finally trigger higher phytase production by the two isolates.

Biomass and protein

Biomass increased and then stabilize after 72 hour incubation (Figure 9 and 10). The biomass production of *Neurospora sitophila* is slightly higher than *N. crassa*. Protein also increased during fermentation (Figure 9 and 10). Increasing protein content in SSF was also observed on raw starch inoculated with *Bacillus* sp. (Hamilton et al. 1999), on sorghum and starch by *Aspergillus niger* (Viniegra et al. 2003; Pothiraj et al. 2006). Increased protein will be good for feed nutrition (Cao et al. 2007). *Neurospora crassa* and *N. sitophila* are important fungi for traditional fermented food. The increase of protein is also good for increasing the nutritional quality of traditional fermented food like *tempeh* and *oncom*.

Animal feed contain bound phosphorus from 18- 88% of total phosphorus content, as phytate. This phytate phosphorus cannot be directly utilized by monogastric animals like poultry and pigs due to a lack of intrinsic phytase in their gastrointestinal tracts. Phytate behave as an antinutrient through chelating various cations such as Ca^{2+}, Fe^{2+}, Zn^{2+}, and Mg^{2+} thereby reducing their bioavailability. Supplementation of phytase into animal feed will break down phytate into inositol and phosphate. Our finding revealed that *N. crassa* and *N. sitophila* produce phytase under submerge fermentation as well as under solid state fermentation. Production of phytase under solid state fermentation was higher than SmF which implies that SSF is more favorable for phytase production. Development of SSF technology to optimize phytase is then necessary. In addition to temperature, substrate composition influences phytase production. Those parameters will be optimized for future study.



A **B**

Figure 1. Colony morphology of *Neurospora crassa* (A) and *Neurospora sitophila* (B) grown in PDA after 3 days incubation at 30ºC

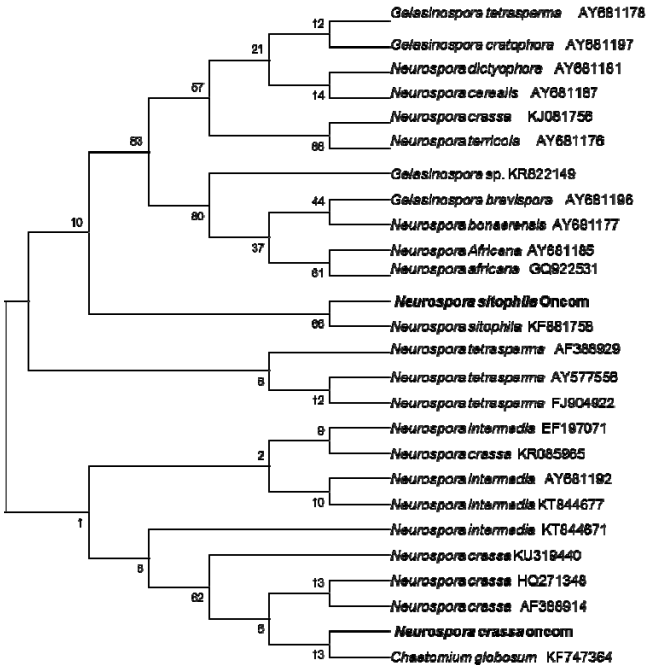


Figure 2. A rDNA-ITS-based phylogenetic tree of *Neurospora* showing the position of isolate. The numbers after the species name represent the accession numbers of isolates in GenBank. The numbers in each branch points denote the percentages supported by bootstrap. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Neim 1993). The bootstrap consensus tree inferred from 1000 replicates (Felsenstein 1985) is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches . Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The analysis involved 26 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 510 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016).

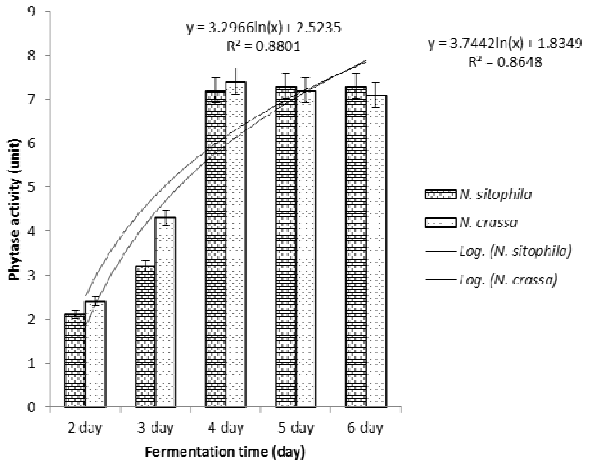


Figure 3. Phytase activity of *N. crassa* and *N. sitophila* in submerge fermentation at 30°C

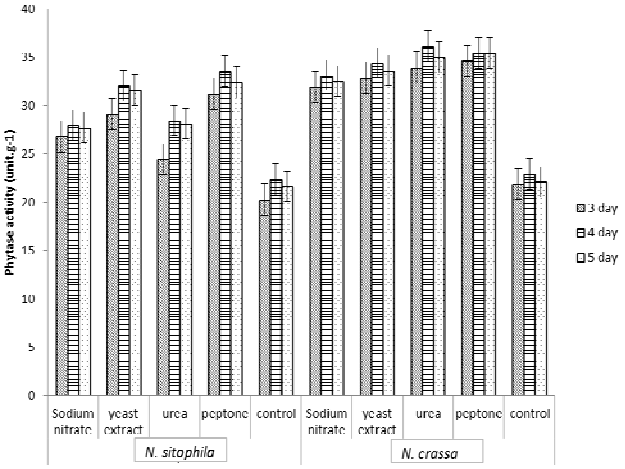


Figure 6. The effect of N-sources on activity of phytase at 40°C on various fermentation times

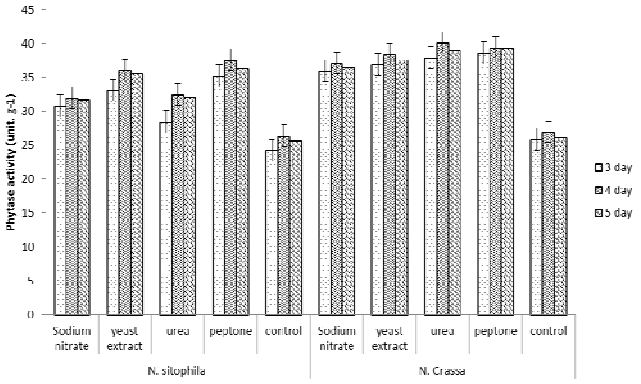


Figure 4. The effect of N-sources on the activity of phytase at 30°C on various fermentation times

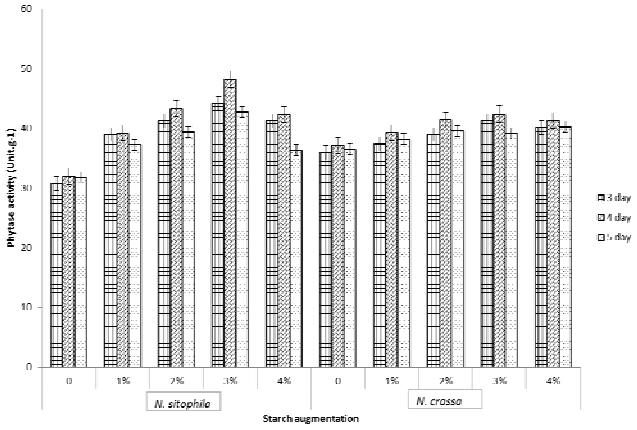


Figure 7. The effect of starch supplement on phytase activity at 35°C

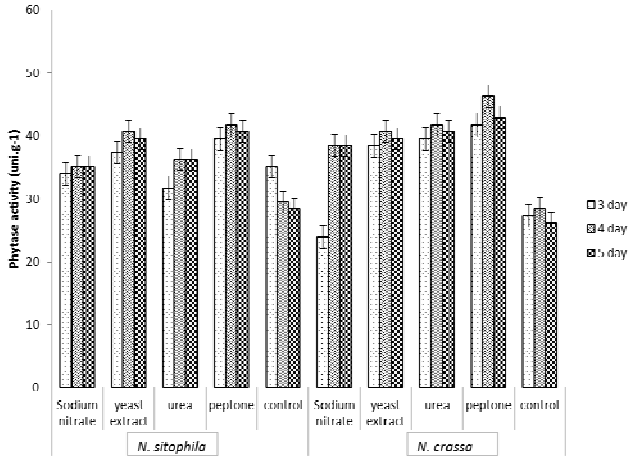


Figure 5. The effect of N-sources on the activity of phytase at 35°C on various fermentation times

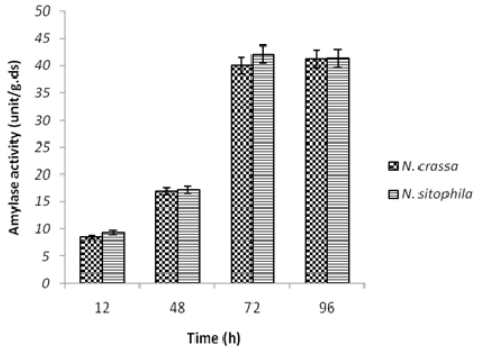


Figure 8. Profile of amylase during solid state fermentation inoculated with either *Neurospora crassa* or *Neurospora sitophila*

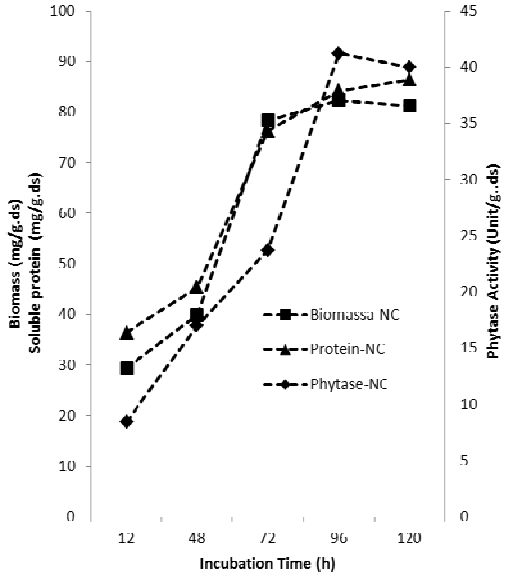


Figure 9. Profile of phytase, biomass and protein during fermentation by *Neurospora crassa*

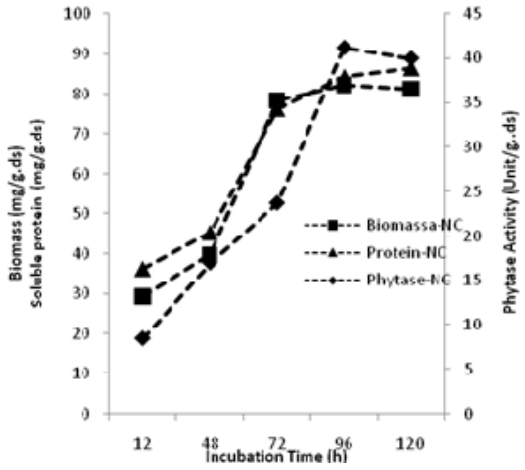


Figure 10. Profile of phytase, biomass and protein during fermentation by *Neurospora sitophila*

Neurospora crassa and *N. sitophila* produced phytase in high amounts (45.25 unit). The phytase production was influenced by media formula especially carbon and nutrient sources. Optimal temperature for phytase production is 35°C on solid state fermentation after 4-days. The ability of *N. crassa* and *N. sitophila* to use starch as carbon sources implies that these isolates also produce other hydrolytic enzymes. *N. crassa* and *N. sitophila* are potential isolates for phytase production which finally can be used as good supplement for monosgastric animals.

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Four new varieties of *Begonia* from interspecific hybridization *Begonia natunaensis* C.W.Lin & C.I.Peng × *Begonia puspitae* Ardi

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Abstract. *Siregar HM. 2016.* Four new varieties of *Begonia* from interspecific hybridization *Begonia natunaensis* C.W.Lin & C.I.Peng × *Begonia puspitae* Ardi*. Biodiversitas 17: 776-782.* Increased phenotypic diversity of ornamental plants (e.g. *Begonia*) can often be achieved by hybridization and selection in the F1 generation. This study aimed to produce new F1 varieties which it was hoped would display improved phenotypic characteristics compared to the contributing parents. The study was conducted in the green house of Bogor Botanic Gardens. Two native species of Indonesia, *Begonia natunaensis* C.W.Lin & C.I.Peng and *B. puspitae* Ardi, were used as parent plants. The mature F1 seeds were sown and selections were made among the plants produced. The result of the selection were four new accessions of F1 plants (X1, X3, X10, X22). The selected F1 plants were then propagated vegetatively with leaf cuttings. These were named, described and registered at the Center for Plant Variety Protection (PPVTPP). This resulted in the registration of the following F1 cultivars: X1 named *Begonia* Blirik which is registered as No. 347/PVHP/2015 and is characterized by purplish peltate leaves with bright whitish green veins, and white flowers; X3 named *Begonia* Fiandani, registered as No. 348/PVHP/2015 and characterized by dark green leaf color, corrugated leaf surface, and pink flowers; X10 named *Begonia* Green Peltate, registered as No. 345/PVHP/2015 and characterized by large green peltate leaves, and red flowers; and X22 named *Begonia* Natunapangean, registered as No. 346/PVHP/2015, and characterized by eccentrically shaped, green basifixed leaves, with ovate shape, and cordate leaf base. Thus the new varieties have been registered in PPVTPP, in accordance with legislation in force. These new varieties are beautiful ornamental *Begonia* hybrids with exotic leaves, which will be developed as commercial ornamental plants.

Keywords: *Begonia natunaensis*, *Begonia puspitae*, *Begonia* Blirik, *Begonia* Green peltate, *Begonia* Natunapangean, *Begonia* Fiandani, interspecific hybridization

INTRODUCTION

Indonesia is a country with among the richest biodiversity in the world. More than about 280,000 species of plants are known to be native to the country. Some of the wild species have good potential as ornamental plants that can be cultivated and produced as important commercial commodities, contributing to increased national revenue. Wild ornamental plants are important genetic resources that can be used for developing a new plant variety. Therefore, an inventory of Indonesian native ornamental plants needs to be developed for selecting potential species to be used as mother plants for artificial hybridization. The aims of a cross-breeding program is to improve the quality of the plants both genotypically and phenotypically.

Begoniaceae species diversity in the world is estimated at more than 1,825 species spread in the tropics and subtropics of Asia, America and Africa (Hughes et al. 2016, Kiew 2005). Indonesia is one of the important centers of *Begonia* germplasm resources in Southeast Asia. Take Java Island for example, Java has at least 15 species of wild *Begonia*. Begonias are also found in other islands of Indonesia, such as 52 species recorded in Sumatra, 8 species in Kalimantan, 44 species in Celebes and 70 species in Irian (Smith et al. 1986). Wild Begonias generally occur in wet tropical rain forest, from lowland areas up to mountainous areas 2,400 m above sea level.

Bogor Botanical Gardens as a Center for Plant Conservation in Indonesia has the second largest *Begonia* collection in the country, second only to Bali Botanic Gardens. Bogor BG has 134 species of *Begonia*, consisting of 37 exotic species and 97 native species, while Bali BG has 313 species, consisting of 213 exotic and 100 native species. All the native species were collected from the wild during plant expeditions. Hoover (2006) declared that Bali Botanic Gardens has the most comprehensive *Begonia* collection in the world.

Horticulturally, Begoniaceae is separated into two ornamental types; flowering Begonias and beautiful leafy Begonias. The uniqueness and beauty of their leaves (i.e. their shape and color), the compactness of the plants and their vigor, and the resistance of the plant to diseases are basic characters that have to be given priority when developing a new variety of ornamental leaf *Begonia.*

Several new varieties of *Begonia* have already been created from artificial hybridization in the Botanical Gardens. The first one is *Begonia* "Lovely Jo" from the hybrid *B. puspitae x B. pasamanensis*. This variety has been registered and certified by the Center for Plant Variety Protection and Licensing Agriculture (PPVTPP), the Ministry of Agriculture with the registration number 00237/PPVT/S/2013. The second variety is *Begonia* "Tuti Siregar" (*B. listada x B. acetosa*) with the number 00 275/PPVT/S/2014: this has also been registered as a new

cultivar of the American Begonia Society with registration number 1001 (Salisbury 2008).

The next step in this research and development project is the morphological observation and characterization of additional wild species to provide a base of support for new variety development. In particular, the research should emphasize selection of parent materials that will be useful in artificial hybridization. Thus the research reported in this paper aims to identify improved quality of leaves characteristics, such as shape and coloration, in order to produce attractive and economically valuable new varieties that can be registered for plant variety protection from the PPVTPP in the Ministry of Agriculture.

MATERIALS AND METHODS

The study was conducted in the Greenhouse Nursery of Bogor Botanical Gardens, West Java, Indonesia at an altitude of 250 m asl., with a daily temperature range 28-33°C and relative humidity between 60-90% in the greenhouse. The hybridization research depended on crosses between two contrasting species (genetic disassortative mating), *viz.* with parent plants that had healthy growth and were capable of flowering to produce fertile, normal flowers (Syukur et al. 2012). The genetic materials used were from the Bogor Botanic Gardens collections. They consisted of *Begonia natunaensis* C.W.Lin & C.I.Peng as pollen recipient and *B. puspitae* Ardi as pollen donor. *B. natunaensis*, a recently discovered species from Natuna Island, Indonesia, has been described by Lin and Peng (2014). *B. puspitae*, an endemic of West Sumatra, was first described by Hughes et al. (2009). From crosses between the two parents, four F1 accession numbers of the interspecies *B. natunaensis* × *B. puspitae* were obtained by the following method.

Female flowers used were receptive flowers of *B. natunaensis*, at a time of blooming when the head of the stigma was ready to receive pollen. Pollen from fresh *B. puspitae* flowers were obtained at the time of anthesis. The pollen was transferred either using cotton buds or else directly from the anthers of the flowers, by smearing it onto the stigma of the recipient flowers. The recipient flowers were tagged and covered with small plastic bags to protect them from pollen contamination of unwanted species. The artificial hybridization was carried out on January 5, 2011, by crosses involving ten recipient flowers. From the ten attempted hybridizations, only one succeeded. The fruit of that one flower was harvested on February 23, 2011 and seeds were planted on 25 February. The seeds began to germinate ten days after planting. Due to the small size and uncountable number of the seedlings, the seedlings were selected four to five months after germination and transplanted into a mixed medium of rice husk charcoal and compost (2: 1). From the selection, 44 accessions were identified.

Superior F1 plant individuals were selected. Then vegetative propagation was carried out on leaf cuttings to propagate clones of these selections. Observations were made on the vegetative characteristics of the clones; by measuring plant height, crown width, leaf length, leaf width, leaf thickness, and length and diameter of petiole; observation of leaf shape, leaf base, leaf tip, leaf nicks, stipule shape, leaf color, color distribution, leaf hairs, etc.. Generative characteristics were also recorded based on observations of inflorescence, flower type, flower color, etc. Determination of color was done using a color map (color chart). F1 selection results were specified according to the guide books of the Ministry of Agriculture of the Republic of Indonesia, Center for Plant Variety Protection (2014), Hindarwati (2006) and MAFF (2010, 2011).

RESULT AND DISCUSSION

Begonia has been the subject of significant plant breeding effort in the past, as indicated by the existence of new varieties of *Begonia* both within and outside the country. Kiew (2005) and Kiew et al. (2015) have estimated that more than 10,000 hybrids of *Begonia* are known world-wide, some with good recording and others without any records. Thus efforts to produce new varieties of *Begonia* through plant breeding programs such as interspecies hybridization must have clear aims if they are to enhance the *Begonia* diversity relative to the source breeding material. The specific characters that the breeder wants to improve are sought from another variety, wild relative or species, or from the recombination of these in controlled hybridization programs. The availability of sources of genetic diversity determine the potential success of such a breeding program (Yunianti et al. 2007).

Begonia breeding programs at the Bogor Botanical Garden *Begonia* have focused on lowland species for the development of new ornamental leaf Begonias. The study reported here is one in the research program described previously by Hartutiningsih et al. (2014).

The female parent used in this study is *B. natunaensis*, a lowland species, morphologically similar to *B. goegoensis* (Tebbitt 2005). It is characterized by rhizomatous and creeping stems; green/reddish, glabrous petioles, 10-21 cm long; laminas peltate, 9-20 cm in diameter, with the upper surface bullate and brownish green in colour between bright green veins, and with red leaf margins; flowers red and small in size (Lin and Peng 2014). Figure 1 illustrates this species.

The male plant is *B. puspitae* a species endemic to West Sumatra, and only found in the karst hills of Nature Reserve Trunk Pangean I, Nagari Solok, Sawah Lunto, West Sumatra. It is characterized by a rhizomatous stem and by basifixed, green, broadly ovate leaves, with dense hairs on lower and upper surface. Its flowers are many, white and small (Hughes et al. 2009).

Hybridization requires cross-pollination, and fertilization to occur. It is indicated by wilted perianth or tepals, that drop off within three days after pollination; then followed by a swollen ovary. The fruit will ripen, change its color from green to brown, and dry within 30-45 days. The ripened fruit should be harvested as soon as possible, before the capsule opens, and should be stored in an envelope, and then seed should be extracted from it (Hartutiningsih 2008).

The results of the hybridization showed that the particular qualitative characters that are conserved (i.e. remaining similar to both parents), are type of stem, leaf type, leaf tips, edges of leaves, stipules, petiole, and flower type (Table 1). A qualitatively different character that arises is the shape of the leaves; i.e. in *Begonia* Natunapangean the leaf shape is much more similar to the male parent (*B. puspitae*), with basifixed and broadly ovate leaves. This character is different in the other three new varieties.

Another distinctive character is the color of the leaves, *Begonia* Blirik has two colors on the upper surface, while the other varieties have only one. Compared to the three other varieties, *B*. Blirik has a brighter color which is a blend of green (G137) and red green (RP 70). The primary color of the lower surface of the leaves is green reddish (RP 70 B) and the flowers are white W 155D (Table 1).

***Begonia* Blirik**

Rhizomatous plant type, plant height (24.5 cm). Single leaf blade, peltate, the leaf base cordate, leaf shape ovate, upper surface of the leaf color green G 137 A, secondary color on upper side red RP 70 B, the color of the leaf surface green, bottom reddish RP 70 C. Flowers single, white color W 155D. Plant stature strong, suitable for beautiful leafy ornamental plants in pots. Different characteristics from the parent are the peltate leaf shape, coloration of veins on upper side bright green, and white flowers. First flowered in January 2012. The derivation of the name comes from the color green leaf with bright colouration of veins, resulting in colored stripes or "blirik". *B.* Blirik is registered by letter as No. 347/PVHP/2015 (Figure 2.A-C).

Table 1. The result of morphological characters on four new varieties of *Begonia* (*B*. Blirik, *B.* Fiandani, *B.* Green Peltate dan *B*. Natunapangean)

Characteristics plant	**Expression of *Begonia***			
	***B*. Blirik (X1)**	***B*. Fiandani (X3)**	***B*. Green Peltate (X10)**	***B*. Natunapangean (X22)**
Plant: stem	Rhizomatous	Rhizomatous	Rhizomatous	Rhizomatous
Plant: height (cm)	Short (24.50)	Short (25.50)	Short (31.50)	Short (29.00)
Plant: width (cm)	Medium (28.00)	Medium (44.00)	Medium (36.00)	Medium (28.00)
Leaf blade: type	Single	Single	Single	Single
Leaf blade: Leaf blade size (cm)	Medium (11.50-16.50: 9.00-13.50)	Medium (14.50-17.50: 12.50-13.00)	Medium (16.00-22.00: 12.00-16.00)	Medium (17.00-20.00: 14.00-16.00)
Leaf: thickness (cm)	Thin (0.35)	Thin (0.55)	Thin (0.41)	Thin (0.21)
Varieties with single leaf only: Position of petiolar attachment	Peltate	Peltate	Peltate	Basifixed
Leaf blade: shape	Ovate	Ovate	Ovate	Ovate
Leaf blade: base	Absent	Absent	Absent	Cordate
Leaf blade: apex	Acuminate	Acuminate	Acuminate	Acuminate
Leaf blade: lobation	Absent	Absent	Absent	Absent
Leaf blade: margin	Crenate	Crenate	Crenate	Crenate
Stipula	Triangular	Triangular	Triangular	Triangular
Leaf: number of colors on upper side	Two	One	One	One
Leaf blade: main color of upper side surface	Green G 137 A	Green N 137 B	Green G 137 A (glossy)	Green G137 C
Leaf blade: secondary color on upper side	Red RP 70 B	Absent	Absent	Absent
Leaf blade: distribution of secondary color on upper side	Whole	Absent	Absent	Absent
Leaf: Variegation on upper side	Absent	Absent	Absent	Absent
Leaf blade: coloration of veins on upper side	Different	Different	Different	Different
Leaf blade: distribution of coloration along veins on upper side	Entire	Entire	Entire	Entire
Leaf blade: width of coloration along veins on upper side	Narrow	Narrow	Narrow	Narrow
Leaf blade: intensity of hair on upper side	Sparse	Sparse	Sparse	Sparse
Leaf blade: main color of lower side	Reddish green RP 70 B	Yellow green YG 144 C	Reddish green RP 65 C	Reddish green RP 65 C
Leaf blade: secundary color of lower side	Absent	Absent	Absent	Absent
Petiole: lenght (cm)	Short (14.50)	Medium (22.83)	Short (14.50)	Medium (20.60)
Petiole: color	Reddish green	Reddish green	Reddish green	Green
Petiole: hair	Sparse	Sparse	Sparse	Sparse
Inflorescense: pedicel/peduncle length (cm)	Medium (27.50)	Medium (21.50)	Medium (36.00)	Medium (21.50)
Inflorescense: pedicel/peduncle color	Reddish green	Reddish green	Reddish green	Reddish green
Flower: type	Single	Single	Single	Single
Flower: color	White W 155D	Pink RP 69 A	Red RP 62 B	Red RP 62 C

Breeding (2011)

♀ *Begonia natunaensis* C.W.Lin & C.I.Peng (B20100416/HT 16) **x** ♂ *Begonia puspitae* Ardi (B2006116/DM)

First season

F1 germination, selection

Second season (2012-2014)

44 selected accessions of F1, individual selection and propagation

Characterization on 4 selected F1 accessions. Observation done on vegetative characters (plant height, plant width, leaf size, thicknes, petioles length) and generative characters

PPVTPP Registration (2015)

4 new registered *Begonia* varieties

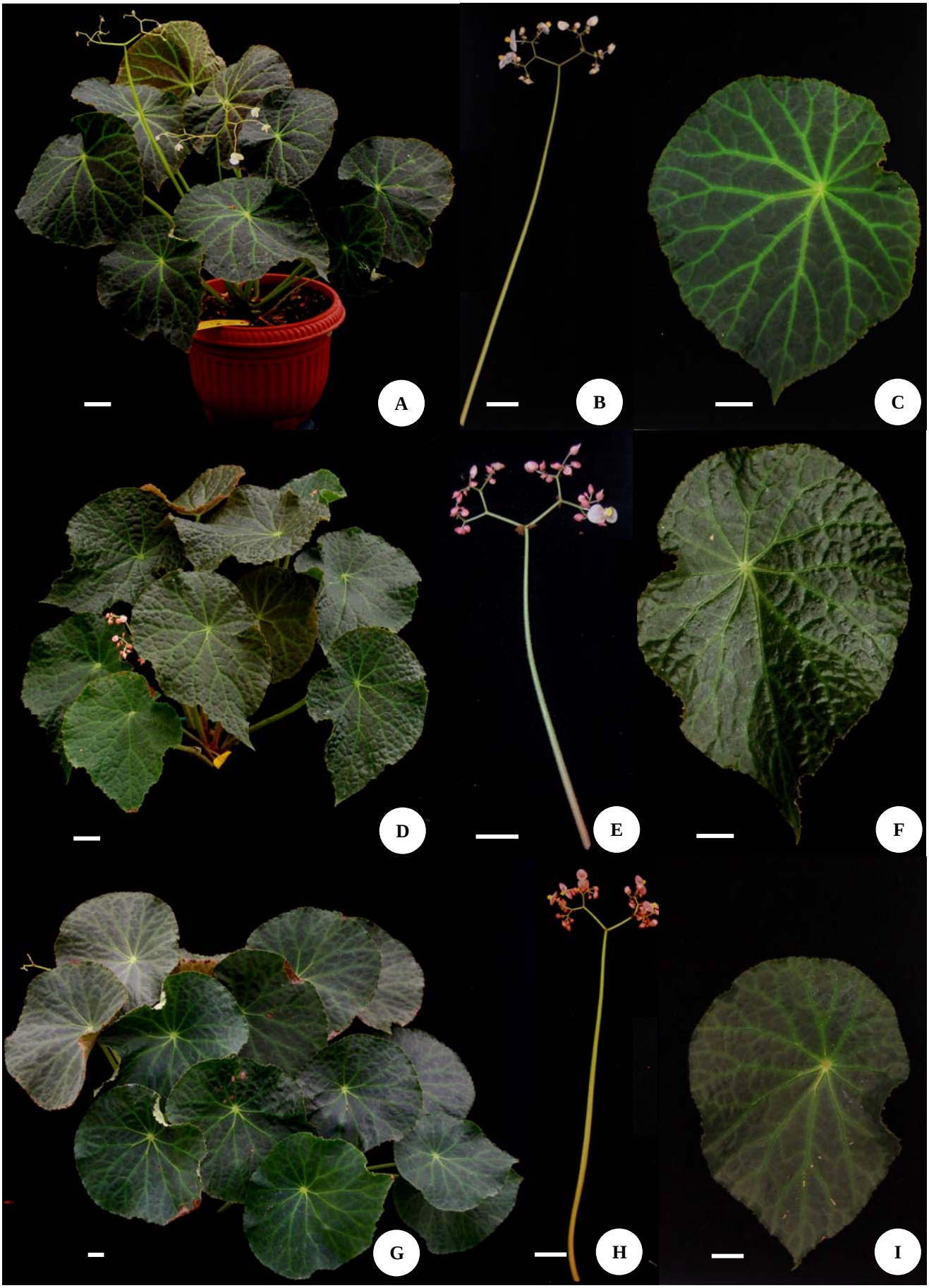
Begonia Blirik

Begonia Fiandani

Begonia Green Peltate

Begonia Natunapangean

Figure 1. Four new *Begonia* hybrid varieties and the breeding scheme that produced them


A
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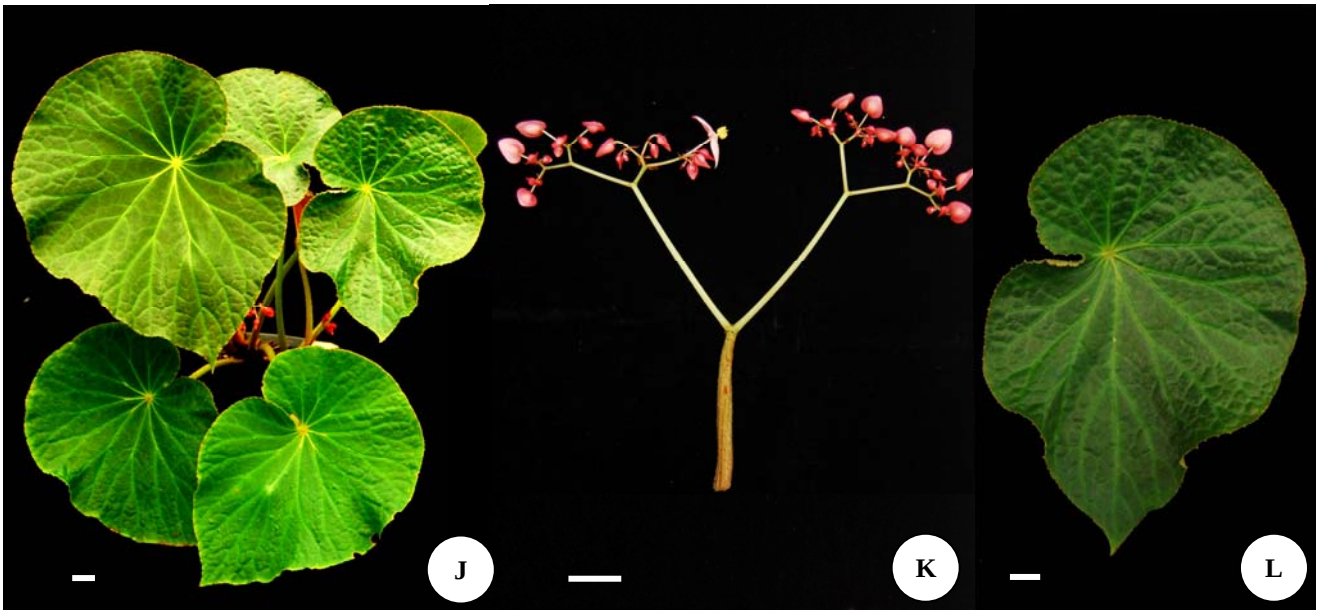

Figure 2.A-C. The morphological features of *B.* Blirik leaves and inflorescence. E-F. Morphological features of *B.* Fiandani leaves and inflorescence. G-I. Morphological features of *B.* Green Peltate leaves and inflorescence. J-L. Morphological features of *B.* Natunapangean leaves and inflorescence. Bar = 2 cm

***Begonia* Fiandani**

Rhizome plant type, plant height (25.50 cm). Single leaf blade, peltate, leaf shape ovate, upper surface of leaf color green G 137 B, the color of the bottom surface of the leaves yellowish green YG 144 C. Single flower, pink color RP 69 A. Stature strong and sturdy plants suitable as beautiful leafy ornamental plants in pots. Different characteristics from the parent are the green leaf color, corrugated surface, pink flowers. First flowered in January 2012. The derivation of the name comes from the name of the breeder's wife. *B.* Fiandani is registered by letter as No. 348/PVHP/2015, characterized by dark green leaf color, corrugated surface, pink flowers (Figure 2.D-F).

***Begonia* Green Peltate**

Rhizome plant type, plant height (31.5 cm). Single leaf blade, peltate, the base corrugated, leaf shape ovate, upper surface of the leaf color green G 137 A, glossy, leaf color green bottom surface red RP 65 C, single flower, red color RP 62 B. Different characteristics from the parent are peltate leaves, the base corrugated, large leaf size, leaf surface is curved, red flower. Plant stature strong and suitable as an ornamental beautiful leafy potted flowering plant. It flowered for the first time in January 2012. The derivation of the name is based on the unique peltate leaf form with dominant green colour. *Begonia* Green Peltate is registered by letter as No. 345/PVHP/2015 (Figure 2.G-I).

***Begonia* Natunapangean**

Rhizome plant type, plant height (29.00 cm). Single leaf blade, position of petiolar attachment basifixed; leaf shape ovate, the leaf base cordate, leaf color upper surface of the green G 137 C, the color of the leaf surface green, bottom reddish RP 65 C. Single flower, red color RP 62 C. Different characteristics from the parent are the ovate leaves, green color, position of petiolar attachment basifixed, the leaf base cordate, red flower. Plant first flowered January 2012. The plant stature is strong, fit as beautiful leafy ornamental plants in pots. Derivation of the name comes from the name of the location of the parents, coming from Natuna Island and Batang Pangean (West Sumatra). *B.* Natunapangean is registered by letter as No. 346/PVHP/2015 (Figure 2.J-L).

In conclusion, hybridization between different species *Begonia* produced new varieties that are more interesting, unique and better than their parents. The four new varieties are registered on PPVTPP as *B. Blirik*, *B.* Fiandani, *B.* Green Peltate and *B.* Natunapangean. These new varieties are ornamental leaf *Begonia* that have potential economic value.

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Human-Leopard Conflict in Girimukti Village, Sukabumi, Indonesia

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Abstract. *Partasasmita R, Shanida SS, Iskandar J, Megantara EN, Husodo T, Malone N. 2016. Human-Leopard Conflict in Girimukti Village, Sukabumi, Indonesia. Biodiversitas 17: 783-790.* Populations of leopards continue to decrease over time. This decline is caused by many factors, such as decreasing animal prey and habitat loss. Due to a lack of animal prey, leopards frequently enter villages to find food, including livestock. Therefore, some conflicts between human-leopard have frequently occurred, and in many cases the leopard has been hunted by the villager. Consequently, the abundance of leopard in some areas of West Java have decreased. The aim of this research is to investigate: (i) local knowledge of the Girimukti Village on morphological variation of leopard; (ii) conflict between leopard and the people of Girimukti Village based on local knowledge; (iii) local knowledge on the hunting of leopard; and (iv) utilization of leopard resulting from human-leopard conflict in Girimukti Village, Sukabumi, West Java, Indonesia. Mixed methods and field observation were applied in this study. The result of this study shows that the village people of Girimukti recognize variations of leopard and their behavior; conflict between humans and leopard has increased; hunting leopard is been undertaken by both traps and shotgun; and leopard are used for various purposes, such as trading skin and other body parts, food, traditional medicines, and as amulets. Based on this study, it can be inferred that many drivers of environmental changes that impact faunal and floral communities are social in origin and strongly related with peoples' activities. As a result, in addition to biological properties, the social, economic and political systems must be considered and integrated into the conservation program of Javan leopards.

Keywords: Girimukti Village, human-leopard conflict, leopard

INTRODUCTION

Human social systems, which consist of populations, technologies, social structure, knowledge, value and economic factors are closely interrelated with ecosystem components such as soil, water, climate, flora and fauna (Iskandar 2014). The ecosystem provides services by mobilizing materials, energy and information for the social system to meet various needs of people. Information, in this case, refers to any signs or indicators about the past, present or future state of individual components of an ecosystem, or to the system as a whole. Various information is received, processed, analyzed, and selected to shape appropriate responses to the environmental information that continually flows into the organism's receptors (Rambo 1984; Marten 2001). With regard to human ecology, local people's environmental information is culturally transmitted from the older to the younger generations as local knowledge or indigenous knowledge. Unlike Western scientific knowledge, local knowledge is predominantly communicated by oral transmission using the local or mother language and teaching through holistic, subjective, and experiential practices (Warren et al. 1995; Sillitoe 2002).

On the basis the local knowledge, the villagers of West Java recognize three races of big cats, namely *macan loreng*, *macan tutul*, and *macan kumbang*. However, based on the biological taxonomy, the diversity of big cats is categorized as two species - tigers (*Panthera tigris)* and leopard (*Panthera pardus)* (Iskandar 2014). Historically, tigers *(macam loreng)* in Java and Bali had two sub-species: *Panthera tigris sondaica* in Java and *Panthera tigris balica* in Bali. However, the Bali tiger was recorded as extinct before the middle of the twentieth century, and the Javan tiger is also determined to be extinct since the 1980s (Whitten et al. 1999). Leopards (*Panthera pardus)* are widely recognized by the local people as comprising two varieties based on dominant coloration with spotted variety being referred to as *macan tutul* and the black morph referred to as *macan kumbang*.

Javan leopards are identified as an identity species of West Java based on decree of the governor of West Java No.27 year 2005, as a form of conservation for this animal. The current population of Javan leopard has been estimated at 700 individuals within the conservation areas in Java (Santiapillai and Ramono 1992). The Javan leopard relative density value based a previous survey conducted at Bodogol, Gunung Gede-Pangrango National Park is one individual per 6 km^2 (Ario 2006), and at Mount Salak is one individual per 6.5 km^2 (Ario 2007). The density value at Gunung Halimun National Park is approximately one individual per 6.67 km^2, based on the primary and secondary forest categorization (Syahrial dan Sakaguchi, 2003). However, Javan leopard populations have recently

decreased in certain areas of Java has been estimated in 2010 between 491-546 individuals (Ario 2010). The factors implicated in the declining leopard populations are illegal hunting, decreasing diversity of prey species, and habitat conversion. Although the leopard had been protected by Indonesian law based on law no.5 of 1990 and listed as Appendix 1 of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora), this animal continues to be illegally hunted (cf. Noerdjito and Maryanto 2001; Suhartono and Mardiastuti 2003; Iskandar 2015). Captured Javan leopard are usually sold in the illegal live animal trade, but some people have also consume leopard meat due to the belief that it has medicinal properties (Larisha et al. 2015). Another factor disturbing the Javan leopard population in nature is the loss of leopard habitat throughout West Java due to the conversion of forested areas into agricultural systems. Finally, the various species that leopard predate upon, such as wild boar and deer have decreased due to illegal hunting by rural communities (Iskandar 2014).

Because the natural balance system related to Javan panther life has been disturbed, namely due to illegal hunting, decreasing prey diversity, and decreasing or loss of forest habitats, the remaining Javan leopards have, particularly in the dry season, frequently sought feed in areas of human settlement. They have usually killed domesticated animals including dogs, sheep and calves. As a result, human-leopard conflict is increasing, particularly if individual animals have entered settlements to kill the various domestic animals of the villagers (Iskandar 2014). For example, in 2001, there was only one recorded case of human-leopard conflict, but in 2011 the number increased to 16 cases (Dipa 2016).

Because populations of Javan leopard are declining and human-leopard conflicts have increased, understanding the local knowledge of Javan leopards is considered very important. As such, this study, undertaken in the village of Girimukti, District of Sukabumi, West Java, documents local knowledge of Javan leopards and conflicts between village people and the focal animal. Additionally, this study has practical implications for the conservation program for Javan leopards. Specifically, the aims of this research are: (i) study the local knowledge of villagers with respect to the Javan leopard; (ii) study the local knowledge of hunting Javan leopards within the Girimukti village, Sukabumi; and (iii) study on the utilization of Javan leopards as related to the causes of conflict between village people and Javan leopard in the focal village.

MATERIALS AND METHODS

Study area

The study was carried out within the village of Girimukti, Sub-district of Ciemas, District of Sukabumi, in the province of West Java, Indonesia (Figure 1). The hamlets of Cingaleng and Cipicung were selected for this study. These hamlets were chosen based on their location close to forests occupied by Javan leopards and a known history of conflicts between Javan leopards and village people.

Procedures

The mixed methods approach used in this study is based on the disciplines of ethnoecology and ethnobiology and their tradition of combining qualitative and quantitative data collection techniques (Newing et al. 2011; Iskandar 2012; Albuquerque et al. 2014). Ethnoecology is defined as the study of how different cultural groups conceptualize the environment, including fauna and flora (cf. Lovelace 1984; Rambo and Percy 1984). Similarly, ethnobiology investigates dynamic human-nature systems through the incorporation of diverse perspectives. The qualitative research components were carried out by field observation and in-depth, semi-structured interviews with informants and local experts. In-depth interviews were completed with competent informants, and sampling across a variety of village members was conducted by snowball techniques. Some informants were intentionally selected namely, formal and informal leaders (*Kades* and staff), village elders (*sesepuh*), animal hunters (*pemburu binatang*), shamans (*dukun*), and animal traders (*pedagang binatang*). Additional field observations were conducted to observe the general condition of settlements, agricultural systems, and forests. In addition, dedicated field observations were undertaken to observe the presence of Javan leopards through the identification of footprints or tracks, prey animal carcasses,, scratches on the trees, and feces.

Quantitative methods comprised structured interview with respondents using questionnaires. Respondents were selected by *proportional random sampling*. Two of the five total hamlets were chosen within the Girimukti Village based upon their location close to forested Javan leopard habitat. Total households were randomly selected using statistical formula of Lynch et al. (1974) as described below:

$$n = \frac{N.Z^2.P.(1-P)}{N.d^2 + Z^2 (1-P),}$$

Where,
n= sample number (respondent) = 84 households
N = total population of households= 645
Z=normal variable value (1.96)
P=largest possible proportion (0.50)
d=error (0.10)

On the basis of statistical formula calculating, a total of 84 households were randomly sampled as respondent. Moreover, structure interviews were undertaken to each head of the household or respondent using questionnaire (cf. Newing et al. 2011; Iskandar 2012).

Data analysis

Qualitative data were analyzed mainly by cross-checking, summarizing, synthesizing, and descriptive characterization (cf. Newing et al. 2011). While quantitative data were analyzed using descriptive statistics, such as percentages of respondents' answers.

Figure 1. Location of Study Area in Girimukti Village (7° 8'54.01"S, 106°29'58.75"T), Sub-district of Ciemas, District of Sukabumi, Province of West Java, Indonesia. A. Research location; B. Map of sub-district of Ciemas; C. Leopard habitat in Balewer Hamlet; D. Cimarinjung and Dogdog waterfall of leopard habitat in Girimukti Village

RESULTS AND DISCUSSION

Local name of Javan leopard

Javan leopard is called locally by people of village of Girimukti as *selang* or *meong*. According to informants, it has been given name the *selang* to demonstrate both respect and fear of this animal. There are three named variants of Javan leopard namely *meong totol*, *sancang manik*, and *macan kumbang*. *Meong totol* is described as has having a distinct skin color pattern without dense spots, while *sancang manik* has distinctly dense spots with grey color with a short mane. *Macan kumbang*, as it is known more commonly, has black hair. The local perception of village people (emic view) on the variants of Javan leopard is different from that of Western, biological taxonomy (ethic view). For example, according Paripurno dan Raharyono (2001) all variations of this animal represents diversity within a single species, while based on van der Zon (1979) the Javan leopard is biologically classified as a single species (*Panthera pardus* Linnaeus 1758, Family Felidae) with sub-specific distinctions (Cuvier 1809) dispersed throughout Java.

On basis of informant perceptions, it can be inferred that local people of Girimurti, Sukabumi, West Java, has

well recognized animal, in this case Javan leopard, in level species and subspecies or variant in terms of biological classification. This result is similar to that has been revealed by other scholars, such as Diamond and Bishop (2000) and Iskandar et al. (2016) that generally local people are well known animals in level species (specific) and variants (varietal) instead of upper levels, such as folk genus and lifeform based on general principles of folk biological classification as presented in the work of Berlin et al. (1973) and Berlin (1992).

Characteristic of Javan leopard

Javan leopards, with respect to skin coloration, have a distinct pattern of spotted flowers (*rosette*). According to informants, and based on their experiences of hunting Javan leopard, the Javan leopard can be readily identified as either male or female based on the form of its feces. The feces form of male Javan leopard is intact, whilst conversely, the feces form of Javan leopard females is mushy, not smoothly shaped, and unevenly dispersed. These differences in the form of feces are caused by differences in how Javan leopards defecate and urinate. The defecation habit of female animals usually coincides with urination. The typical evacuation of urine by male animals is by forward projection of the urine stream. As a result, male feces are form a dry, smooth deposit due to not being mixed with urine.

In addition to the form of feces, the footprint is said to identify the sex and body weight of the Javan leopard. The footprint of male has a single, large rounded bulge with both nail and tread markings on the ground. The footprint of female animal is a wider smaller bulge with triangular form and the absence of distinct nail impressions.

In addition, age determination of Javan leopard can be identified by the characteristics of the animal's hair and nails. Adult Javan leopard have a small body, big feet, large nail projection, larger footprints, and blackish canine tooth color in the middle. Individual juvenile Javan leopard is more proportionate between the size of body and its feet, and pure white coloration of the canine teeth.

Generally, animal hunters active in the surrounding forest have a thorough understanding of the characteristics of individual Javan leopard they have caught and encountered. Because the village people who reside near to the forest have given various local names to the Javan leopard, it can inferred that they reliable observers of these animals. The Javan leopard classification is mainly based on morphological characteristics (Paripurno and Raharyono 2001), while the folk classification is based on a more diverse set of criteria, including behavioral characteristics and feces form between male and female animals. Indeed, as mentioned by Maffi (2004), local knowledge of the environment often proves to be more in-depth than scientific knowledge, and points the value of local knowledge for environmental conservation.

Conflict between Javan leopard and the village people of Girimukti

On the basis of ecological history, the Javan leopard initially began entering into the human settlements of Girimukti Village in 1960's and has continued to do so until the present time (2016). It was noted that the occurrence of Javan leopards entering into the human settlement most frequently occurred in the year 2013. This was confirmed by the recorded killing of many livestock animals by the Javan leopard at that time. Livestock, such as sheep, have usually grazed in close proximity to forest, a location slightly distant from the area of permanent human settlement. The village people consider that grazing livestock in a location far from the village results in increased security against livestock theft by other people.

One of the main factors related to the entering of Javan leopard into human settlement areas is the decreasing extent of forested areas. Many forests located to close to human settlements have been converted to agricultural areas through a process of forest cutting and forest burning. As result, the Javan leopards have frequently entered into the human settlements. Indeed, the destruction of forests in this manner has occurred for generations, and as a result, it has seriously affected both the Javan leopard and its prey animals, including primates and ungulate animals (with the exception of wild boar). According to respondents, Javan leopards have entered human settlements because of hunger (6%), to find livestock (36%), due to the loss of forest (15%), and for unknown reasons (43%). Moreover, based on the respondents, leopards have entered into settlements with the following frequency: one time per year (31%), three times per year (3%), only sometimes (14%), and never entering settlement (52%). Generally, the most respondents (84%) did not know the ecological role of Javan leopards in nature (forest), while others identified them as hunters of wild boar (11%), and consumers of livestock of (5%).

Hunting Javan leopard has been considered as a form of revenge by the village people and express a desire to decrease the population this animal because this animal has frequently entered the human settlements and killed livestock. In a period of six years (2010-2016), there were 14 individuals that were captured as a result of human-leopard conflict. Additional, there were five individuals that were captured, but we didn't know precisely when the conflict happened. For three years (2011-2013) people in Pasir Muncang have been hunting Javan leopard with gun or *bedil*. On the basis of the conflict between Javan leopard and local people of Pasir Muncang encountered at least one individual Javan leopard was captured by hunters in the condition of live.

Techniques of hunting Javan leopard

The hunting Javan leopard is typically carried out if there is evidence that the animal has killed livestock from the village of Girimukti. For the villagers, any animal, including wild boar, that disrupts their economic wellbeing will be killed. However, if there is a Javan leopard has just visited the human settlement without killing livestock, the leopard will not be killed by the village people.

Various techniques are used to hunt Javan leopards. First, the hunters look for the presence of the Javan leopard in the forest using the indicators of footprints or direct visual contact with the animal. This search is important for

estimating the size of Javan leopard. As a result, appropriate traps which are called *bakukung* can be prepared. After the Javan leopard has been located and the size known, the hunters leave the forest and conduct a meeting with community members to make *bakukung*. The *bakukung* is usually made by five people. It is typically made of bamboo due to this material being both durable and easily obtained. The bamboo is tied with the rope from either *kioray* (*sambiloto* plant) or *dolo* (*secang* wood tree). *Bakukung* has a length of between 4-5 meters with a width of 30 cm so that the Javan leopard cannot freely move and remain turned facing backward away from the door of the *bakukung*. The door of the *bakukung* must be heavy so it is difficult to be opened by the Javan leopard.

Moreover, a bait comprising white chicken and the remaining carcass of livestock that was eaten by the Javan leopard is put inside of the *bakukung.* The white chicken is usually used because it can be easily seen by Javan leopard at night. The bait is tied or connected to the door *bakukung* with rope and supported by a twig. As a result, if the Javan leopard enters and eats bait, the door of the *bakukung* will close and subsequently trap the Javan leopard. While inside the *bakukung* a Javan leopard will continuously roar in the presence of people. As a result, a blanket that smells of human sweat is used to cover the *bakukung* in order to silence the leopard. If the village people cannot restrain the leopard long enough inside the *bakukung*, then the animal will be killed by a shotgun (*bedil*).

If Javan leopard is to be evacuated to a rehabilitation center, then the animal is transferred from a *bakukung* made of bamboo to *bakungung* made of iron that is provided by the government authority. The transfer is completed by attaching the iron *bakukung* to the mouth of bamboo *bakukung.* Without removing the blanket cover, the Javan leopard will enter into the iron *bakukung*.

If the Javan leopard hunter will not use a *bakukung*, then a hunter from Girimukti will usually perform a traditional ritual. The hunt is to be done three days after the birthday of the hunter. In addition, some traditional prohibitions or taboos are applied to people who want to hunt the Javan leopard. For example, they are not allowed to use fragrances, to tell to anybody that they want to hunt the Javan leopard, to tell 'dead' (*paeh*), 'successfully caugh' (*beunang*).

While still at home, the hunter begins by conducting prayers to various gods prayers are directed to Brahma, as well as the god of wind (*dewa angin*), the god of water (*dewa air*), the god of trees (*dewa pohon*), and the god of sea (*dewa laut*) in order to ask permission to hunt a Javan leopard. After that, he takes a bath at midnight and prays again. At this point the, hunter enters the forest. On the basis of their tradition, they go to forest to hunt Javan leopard are not allowed to bring fish or shrimp paste (*terasi*) due to the animal can smell such goods and attract to come to the hunters and may be dangerous situation for the hunters. The hunter is not accompanied by a dog due to the potential to be killed by the Javan leopard. The hunter, however, has brought a shot gun, but not allowed to bring a machete (*golok*) to forest. The cutting trees have not been approved by god of trees (*dewa pohon*). After he has been in the forest, the hunter must undress and conduct prayers facing in an eastward direction. The prayers are directed to *Nabi Sulaeman* who keeps animals on earth. Thus, traditionally the local people to hunt the Javan leopard has been strongly embedded by cosmos or belief and traditional ecological knowledge (cf. Toledo 2002). However, today the traditional believe and traditional local knowledge of local people has dramatically changed due to influence of various socio-economic and cultural changes, including formal education, technological and market economic development (cf. Carlson and Maffi 2004).

In 2011, seven individual sheep were killed and eaten by Javan leopard. As a result, the village people made *bakukung* that is a kind of beat made of leftover beef has been eaten by the Javan leopard and is mixed with poison. The *bakukung* was placed in the same place when the Javan leopard had killed and eaten sheep. Generally the local people believe that Javan leopard always returns to that place for eating the rest of the sheep meat. After one night of waiting, a single Javan leopard individual was trapped by the *bakukung* and killed with a gun. The Javan leopard that was killed weighed approximately 45 kilograms, and was burned and consumed by some of the village's young people. Between 40 and 50 people report to having tasted Javan leopard meat. In addition, in 2012 one Javan leopard was hunted by the village due to having killed livestock. Hunting Javan leopard is predominantly undertaken by the village people due to an increased demand for animal skin and other valuable body parts. For example, one skin complete with the head and feet was sold 600,000 rupiah. Another case, in 2013 one individual Javan leopard was killed by the village people in Puncak Darma of Girimukti village.

In 2014, one perspon from Pasir Muncang found a footprint of a leopard in front of his neighbor's house. The footprint was bigger than the typical domesticated cat footprint. However, at that time there was no news about missing livestock due to leopard predation. In 2015 there was a case about a sheep that had been eaten by a leopard, but the villagers were unsuccessful in catching the leopard even after using the traditional trap, or *bakukung*.

Another case of killing a Javan leopard was recorded in 2014. We know that one leopard comes from Tonjong Forest in Ciangsana and has been evacuated by the authorities like people from the Center for Conservation of Natural Resources of West Java (BBKSDA-Jabar), Cikananga Wildlife Center (CWS), Indonesian Safari Park (TSI), and with the help of Perbakin (Indonesian Shooter Community). This leopard was evacuated to Cisarua Safari Park in Bogor. Another evacuation happened in July 2014. They found one leopard that comes from Balewer Village forest. This leopard is evacuated to CWS, Sukabumi. On the basis of the conflict between the Javan leopard and the village people, only two individuals have been successfully evacuated by the government authority because it has difficulty being managed by the government, while the village people are hesitant to keep the leopard in the *bakukung* for an extended time period.

From media reports there was news about two Javan leopard that had been caught in Girimukti. One leopard came from Ciangsana and was caught on 12 October 2013.

The sex of the leopard was male, and its age is approximately 7-8 years old with 50 kg body weight. This leopard was evacuated to Bogor Safari Park. Another leopard was caught in Balewer after eating eight sheep from a villager's ranch. After they caught the leopard they gave it to BBKSDA-Jabar, and then they evacuated it to CWS Sukabumi. This sex of that leopard was male with an age of about ten years old (RadarSukabumi.com 2014).

Another case happened in Cikeueus. In this instance there was three individual leopards that were killed. One individual was killed in 2014, and we don't know when specifically the other two deaths occurred. In yet another case, three individual Javan leopards were killed in 2012, and prior to that villagers of Margamukti/Balewer also killed two individual animals. Other than that, in June 2015, in forest near Margamukti/Balewer, a leopard was shot and killed. Additionally, two individual Javan leopards were killed in Pasir Salam hamlet and killed in 2010. Finally, one individual Javan leopard in Cibuti was killed in 1980.

Not all the people in the village of Girimukti village have a gun (*bedil*) to kill Javan leopard. Another alternative which can be used to kill this animal is the poison cyanide. This poison has been obtained from people working the gold mines in Ciletuh, Sukabumi. The cyanide poison is usually inserted into bait. Once the leopard has consumed the poison bait, it typically travels some distance from the site of consumption. As such, the village people generally unable to locate the animal after eating the poison bait. Between October 2015 and March 2016, two individual Javan leopards were killed due to the consumption of cyanide poisoned bait.

On the basis of the information of informants, in the last decades many individuals of Javan leopard have frequently entered into the human settlements and created continuous conflict between the Javan leopard and village people. For example, in February 2015, one of village people encountered an adult male Javan leopard in *Curug Dogdog*, Jembatan Cimarinjung. Nearby, a cave was found that was used as a habitat for this animal. Previously, in 2014, other villagers found the footprint of a Javan leopard in the front of cave which indicated that the animal had been present in this location. At the same year (2014) one of the village people of Pasir Muncang village found the footprint of the Javan leopard in the front of a villagers house. This footprint has a larger size compared to that of domestic cat. At that time, however, it has not been reported the livestock which are eaten by this animal. One year later, in June 2015, one adult Javan leopard was located in the forest near to Margamukti/Balewer hamlet.

Since 1960s, village people of Girimukti have directly or not directly encountered Javan leopard based on dead and live animals caught by *bakukung*. Only one individual Javan leopard identified as *macan kumbang* has been killed in this way. Until the present time, no village people have encountered *macan kumbang* within the forest near the village of Girimukti.

Almost half of the human-leopard encounters have happened during the rainy season (Table 1). This fact is consistent with the information from interviews that leopards often come to the village during the rainy season because they can't smell the leopard, and the leopards movements are less detectable during the rainy season. In the dry season, the sound of leopard footsteps is easily heard because of the dry leaves thereby making the leopard easily detectable. Also, in the dry season, leopards won't travel as far and may avoid hot places. Another cause of human-leopard encounters is also the movement of leopard prey, especially wild boar. In the rainy season, increased activity around villagers' farms attract wild boar. Frequent foraging by wild boar often damages or destroys. Gunawan (2014) states that leopards increase activity patterns in the rainy season because of increased prey activity and abundance. As we see from the movement of the wild boar in the dry season, the villagers alter farm activity so the wild boar won't enter the village. However, villagers increase activity near the river because of its comfort and suitability. This matches obtained information that suggested leopard won't go near the village during the dry season. Instead, they won't go far from the core area of their range and focus a lot of activity in watery areas such as those nearest to the river.

In addition, another case of killing Javan leopard was recorded in 2014. At that time two individual animals were hunted by the villagers. One individual leopard inhabited the forest of Tonyong Balewer and the other leopard came from the forest adjacent to Balewer hamlet. Both individuals have been evacuated by stakeholders, the BBKSDA-Jabar and TSI, and assisted by the Perbakin. One of the hunted Javan leopard individuals has been rehabilitated in the CWS, while the other one has been sent to TSI.

Table 1. Ecological history of encounter Javan leopard and humans at Girimukti village, Sukabumi, West Java, Indonesia

Year	Season/ Month	Location (hamlets/rivers)	Number of ind.	Condition of the animal
2010	-	Pasir Salam	2	Dead
2011	-	Pasir Muncang	1	Dead
2012	-	Balewer	2	Dead
2012	-	Pasir Muncang	1	Dead
2012	-	Cikeueus	1	Dead
2013	-	Pasir Muncang	1	Dead
2013	October	Ciangsana	1	Live
2014	July	Balewer	1	Live
2014	Wet season	Pasir Muncang	1	Live (footprint)
2015	-	Dogdog river-Cimarinjung bridge	1	Live
2015	January	Pasir Muncang	1	Live
2015	Juny	Balewer	1	Live
2015/2016	Oct-Mar	Cingaleng	2	Dead
-	-	Cikeueus	2	Dead
-	-	Cisaar	1	Dead

Figure 2. Skins (A) and facial hairs (B) of a Javan leopard hunted in 2013

Utilization of Javan leopard

It is historically known that a villager's sheep was killed by a Javan leopard in 1964. The resulting anger resulted in the Javan leopard being killed and burned by the villagers. In addition, some village people used the ash of the burned animal as traditional medicine. The ash was rubbed onto diseased skin, and the disease was cured. Since that time, the village people have believed that the skin of Javan leopard holds medicinal benefits with antibiotic properties. The purpose of hunting the Javan leopard, however, is mainly the result of having entered into a human settlement to predate upon livestock and not motive by obtaining animal parts for traditional medicine (Figure 2).

Contrastingly**,** Javan leopard hunters of Girimukti village have also been known to trade various parts of these captured animals. Additionally, their meat is freely distributed to people both the within the village of Girimukti and also to people outside of the village. According to some informants, the meat of Javan leopard has a spicy taste causes sweating. In addition, there are some parts of a Javan leopard's body, such as its bone, has been sold for 250,000 rupiah/kg for traditional medicinal purposes. Indeed, according to Negi and Veerendra (2007), the meat of Javan leopard is believed to enhance the strength and virility of men, and its bone can be used as an aphrodisiac. The ash of burnt Javan leopard hair can also be used as a treatment of foot and mouth disease.

In addition, the teeth of Javan leopard have traditionally been used by the village people as an amulet. As a result, nails of Javan leopard are usually decorated with gold and worn as a necklace. Additionally, the liver and bile are used as an antibiotic medicine, the brain for lung and heart disease, and the heart for treatment of asthma. The skin of Javan leopards have been sold for as much as 2.5-6 million rupiah which can be used for amulet and home decoration. In addition, the tongue of a Javan leopard can be sold for about 1 million rupiah. To process the tongue, it is inserted into wood and dried with in an upright position. For the religious leaders (*kiyayi*), the tongue of Javan leopard is wrapped in a cloth containing Arabic writing for an amulet that is called as *ajimah*.

Based on this study, it can be inferred that many drivers of environmental changes, including those impacting fauna and flora, are social derived and strongly related with human activities. As a result, in addition to biological properties, the social, economic and political systems must be considered and integrated into the conservation program of Javan leopards.

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The value of secondary forest patches for bird conservation in palm oil landscapes of Riau, Sumatra

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Abstract. *Erniwati, Zuhud E, Santosa Y, Anas I. 2016. The value of secondary forest patches for bird conservation in palm oil landscapes of Riau, Sumatra. Biodiversitas 17: 791-798.* Land use change due to palm oil expansion is considered to be one of the key drivers of biodiversity loss in the tropics, particularly in Indonesia, which is the biggest producer of palm oil in the world. In the last three decades, large scale plantations and smallholdings of palm oil have come to dominate the agricultural landscape, leaving small secondary forest patches surrounded by plantations. We currently have only limited current knowledge about the value of secondary forest patches for bird conservation in the palm oil landscape. The aim of this study was to contribute to our understanding of the value of remnant forest patches, smallholdings, and large scale plantations for bird conservation in the palm oil plantation landscape. We also examined the influence of the age of the palm oil plantations on bird diversity. We conducted the survey from March to April 2016. We surveyed 40 line transects in palm oil landscape in Riau Province, eight transects in secondary forest patches, 16 transects in smallholdings and 16 in a large scale plantation. Seventy three bird species, 41families and 1579 individuals were recorded; 16 species being protected in all sites. Our result showed that secondary forest has higher bird diversity than the palm oil plantations; large scale plantation support higher bird species abundance than smallholdings, while old age stands (>19 year) have higher species abundance within large scale palm oil plantation. An important management implication arising out of our results is that preserving natural forest patches in a landscape dominated by palm oil plantations is one of the strategies to conserve avifauna diversity.

Keywords: Bird, conservation, diversity, palm oil, Riau, secondary forest patches

INTRODUCTION

Land use change due to agricultural expansion is widely known as one of the key drivers of biodiversity loss in the tropics (Lamb et al. 2005; Laurance 2014; Newbold et al. 2015). The most rapidly expanding agricultural crop in Southeast Asia in the last three decades is palm oil. In Indonesia, the palm oil plantation area has increased from approximately 1.1 million hectares in 1995 to 11.4 million hectares in 2015 (Director general of plantation 2014). The production of crude palm oil (CPO) contributes 51 % of palm oil globally, making Indonesia the biggest palm oil producer in the world. Currently, the palm oil sector plays an important role in rural development and in economic growth. It is estimated that about 25 million people in Indonesia depend directly or indirectly on the cultivation of palm oil (WWF 2011). However, palm oil development has been blamed as a major environmental problem, leading to biodiversity loss in tropical countries (Donald 2004; Basiron 2007; Koh and Wilcove 2007; Koh and Wilcove 2008; Fitzherbert et al. 2008).

The establishment of palm oil plantations has a direct relationship to the deforestation in Indonesia (Clay 2004). Over 56% % of total area of palm oil plantations in Indonesia which were established between 1995-2005 expense of natural forest (Koh and Wilcove 2008). Compared to natural forest, palm oil plantation supports fewer forest-dependent species (Aratrakorn et al. 2006). Most of the plants and animals observed in palm oil are generalist species with low conservation importance (Danielsen et al. 2008). Some studies have shown the effect of palm oil plantation on biodiversity, such as studies on arthropods (Turner and Foster 2009), forest ants (Bruhl and Eltz 2009), arboreal ants (Pfeiffer et al. 2008), butterflies (Koh 2008; Koh and Wilcove 2008), orangutan *Pongo* spp. (Nantha and Tisdell 2008), mammals (Kartono AP 2015) and birds (Koh 2008a; Koh and Wilcove 2008; Edwards et al. 2010; Koh et al. 2011; Azhar 2011; Teucher 2015).

Birds are good bio-indicators for environmental changes resulting from landuse changes. Birds are very sensitive to changes in the ecosystem so they can be a strong indicator of the species richness and of the presence of certain plant species. The use of birds as bio-indicators can explain to what extent human activities have changed habitat quality and how this change has affected biodiversity. Moreover, birds play important roles in the

ecosystem due to their contribution to seed dispersal and pollination (Birdlife International 2010, Donald et al. 2001, Burgess et al. 2002, Bibby et al. 1992). In palm oil plantation, a previous study found that insectivorious birds help control leaf-eating pests (Koh 2008). Barn owls (*Tyto alba*) can control crop pests (Heru 2008). Birds also have beneficial impacts on agroforestry systems because they can suppress arthropod density, thus increasing the yield of palm oil (Maas et al. 2013).

There is limited knowledge concerning the conservation value of secondary forest patches and palm oil plantation indifferent management types and age classes, especially for tropical birds. To date, only a few studies have emphasized the impact on bird communities associated with age, class and management type of plantations, as factors affecting biodiversity in palm oil (Azhar et al. 2011). This knowledge would help us formulate conservation biodiversity strategies in palm oil plantation, which has become the dominant agricultural landscape in Indonesia in recent years The objective of this study was to compare the diversity of birds in secondary forest patches and smallholdings and in large palm oil plantations of different age class. The research questions were: (i) Does the richness and abundance of birds differ between smallholdings, large scale plantations and secondary forests around the plantations, (ii) Does the richness and abundance of birds differ between age classes of palm oil plantations?

MATERIALS AND METHODS

Study area

The study was conducted in four districts of Riau Province, Indonesia, namely; Kampar, Pelalawan, Siak and Kuantan Singingi. The field works were focused on eight large-scale palm oil plantations, 16 independent smallholding palm oil plantations and eight sites of secondary forest patches adjacent to palm oil plantation companies. The average area of a smallholding was 2 ha, while that of a large-scale palm oil plantation owned by both state and private companies was more than 10.000 ha. The secondary forests were either forest patches within large scale plantations conserved as high conservation area or secondary forest patches outside plantations. The palm oil plantations were established between 1986 and 2005. Most of the large-scale plantations were formerly lowland forest concession areas, while the smalholdings were previously rubber plantations or young secondary forest patches. Large scale palm oil plantations constitute 40% of the total palm oil plantation area in Riau, while smalholdings constitute 60%.

Procedures

Data were collected from March to April 2016 using the line transect method. We walked along a 1 km-long transect line recording the birds found within 50 m to the left and right side of the transect line. All transects were visited in the morning from 6.00 to 9.00 and in the afternoon from 16.00 to 18.00. We repeated the observations for three days at the same plots to maximize the number of bird species recorded. Rainy days were avoided. We used binoculars and camera to observe the birds and a MacKinnon and Philips (1993) field guide to identify them.

In total we had 40 transects. Eight transects were in secondary forests, 16 transects in smallholdings and 16 in large-scale palm oil plantations. The palm oil plantations were classified into three age classes; young (1-8 years old), mature (9-18 years old) and old (19-30 years old). Each class was recognized by its height and frond coverage. Young palm oil habitat was an open habitat, with ground cover crops planted to avoid exposure of the soil surface. Mature trees can grow up to 20 m with longer fronds that touch the fronds of neighboring palms. Old palm oil trees can reach up to 30 m. Both visual and acoustic survey methods were used to identify the species, count the numbers and determine the locations of the birds. To describe the characteristic of the habitat in secondary forest fragments, we assessed the vegetation using plots measuring 113 m x100 m on each site. In total we had 12 plots and our sampling area totaled 1,536 hectares. We classified plant species into stage of growth (tree, sapling, pole, and seedling).

Data analysis

We determined the total number of species that was actually recorded in the research sites (Clarke and Warwick 2001). To estimate species diversity, we used the Shannon-Wiener Index (H'), the formula for which takes into account the number of species in the study sites and their relative abundance. To estimate species richness, we used the Margalef (Dmg) Index. Margalef Index has the ability to respond to differences in species and has high sensitivity (Magurran 1988). The similarity of bird communities among different sites was estimated using the Sørensen Index. To draw the relationship of species richness among sites, we used species accumulation curves. The true species richness was estimated by including the mean of the abundance based estimator (ACE, CHAO1, JACK1 and BOOTSTRAP) to produce the species accumulation curves (Barlow et al. 2007). Except for the Margalef Index, all the calculations were done using EstimateS version 9.1 (Colwell 2013). To determine the evenness of species richness between site types, we used an Evenness Index (E'). E' = H'/Ln S (Pieulou 1975). The differences in the mean of species richness and abundance on sites, management regimes, distances to forest, and locations were determined using t tests. To determine the influence of stand age on bird species richness and abundance, we used analysis of variance (ANOVA). For these statistical calculations, we used Minitab 16 software. Our data were distributed normally. We completed analyses of variance only for our bird data from palm oil transects. We excluded the data from the eight secondary forest transects from these analyses because that information was used only for comparisons with palm oil cultivation areas.

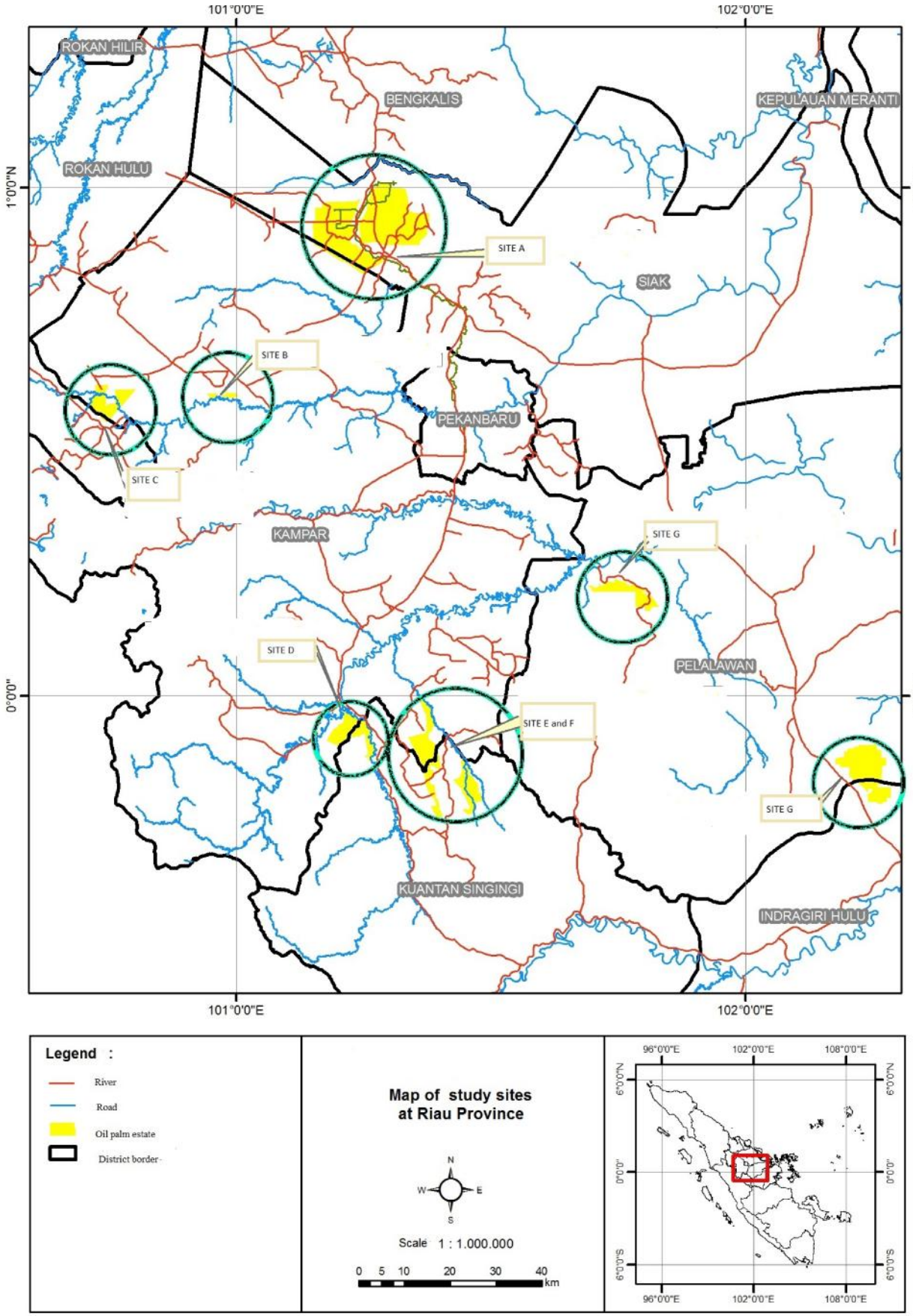

Figure 1. Location of 8 estate plantations, 16 smallholdings and 9 sites of secondary forest patches surrounding the large scale plantation

RESULTS AND DISCUSSION

Vegetation structure in secondary forest patches

In all 15,360,000 $m^2$of plots of secondary forest fragments, we recorded 363 species of plants belonging to 75 families, dominated by Euphobiaceae (6.6 %), Leguminosae (5.5 %) and Myrtaceae (4.75 %). The density of vegetation for each stage of growth was as follows: trees 1.89 individuals/ha, poles10.61 ind/ha, saplings 201.28 ind/ha and seedling 35,555.56ind/ha.

Bird species diversity among sites

We recorded 1579 birds from 73 species and 38 families from all sites. We found 55 species with 469 individuals in secondary forests, 32 species with 277 individuals in smallholdings, 43 species with 511 individuals in large-scale plantations (Table 2). Glossy Swiftlet (*Collocalia esculenta*) was found to be abundant in secondary forests (19 %), while Bar-winged Prinia (*Prinia familiaris*) was abundant in smallholdings (14.4%). Yellow-vented Bulbul (*Pycnonotus goiavier*) was found to be the most abundant species in all sampling sites, comprising 12% of the total number of birds. This species is not endemic to Riau Province and is a non-forest species. This species was also the most abundant species in young and mature large-scale palm oil plantations and the second most abundant species in smallholdings (Table 1).

The species accumulation curves, with the x-axis showing the number of individuals sampled and the y-axis the species richness, reveals the relationship between secondary forest patches and palm oil plantation. Bird species richness was higher in secondary forest patches than in the palm oil plantations. Palm oil plantations of different management were plotted separately, but their species accumulation curves overlapped (Figure 2). Analysis by t-test (Table 3) revealed that the means for species richness and for species abundance in secondary forests and in palm oil plantations were highly significantly different ($p<0.05$).The data in Table 2 demonstrates that in all cases, the birds in the secondary forest were considerably more diverse than those in the smallholdings and large-scale palm oil plantations. Margalef Index of species richness of bird in secondary forests (Dmg 8.78) was higher than that in large-scale plantations (Dmg=6.71) and smallholding plantations (Dmg=5.51). Shannon Index showed that secondary forest had slightly higher diversity (H'=3.24) than large-scale plantations, (H'=3.11). Smallholding palm oil plantation had the lowest bird species diversity (H' = 2.73). There is a significant difference between large-scale palm oil plantations and smallholdings in species abundance, however there are no differences in species richness (Table 3). The evenness of bird species index among habitats was about 0.8.According to the concept of evenness, if the index of evenness is close to 1, the species are distributed evenly. The data in Table 2indicates that the bird species were distributed almost evenly among sites.

Diversity indices show that the bird species in old age palm stands was the most diverse. Based on Margalef Index, species richness in old age palm oil plantations is higher than in young age palm oil plantations. Shannon Index also confirms that the bird diversity in the old age palm oil stands was slightly higher (H'=2.98) than in young and mature palm oil (H ' = 2.7 and 2.6). Table 2 confirms that old stands in smallholding palm oil plantation have higher value Shannon Index (H'= 2.61) than mature and young age. However Margalef Index shows that mature age stands are more diverse than either old age or young age stands in the smallholding plantations. Shannon Evenness Index revealed the same evenness across the age stands. Analysis of variance showed that the age classes were significantly different only for species abundance in the large-scale plantations, while smallholding stands of different age were not significantly different either for species richness or species abundance. The distance of palm oil to the secondary forest patches influenced bird diversity, indicating that the existence of forest patches surrounded palm oil plantation can increase bird species diversity. Furthermore, the bird diversity also differed among different districts in Riau Province especially between Kampar and Pelalawan, Kampar and Kuantan Singingi, Kampar and Siak (Table 3).

The similarity of the bird communities in different sites

The index of bird community similarity assesses the similarity in species composition between habitats. The results show that there is a low similarity in the bird communities of the different habitats. The estimates obtained for the Sorensen Index, (a measure of the similarity between two communities) was 0.39 between secondary forest and large scale palm oil plantations; 0.26 between secondary forests and smallholding palm oil plantations; and 0.28 between smallholding and large-scale palm oil plantations. The results indicate that the types of birds to be found in each of the three habitats tended to be different. Some species found in the secondary forest are categorized as specialists highly dependent on the kinds of food resources only available in the forest, while the bird species found in the palm oil plantations could best be classified as generalists. Habitat characteristics is correlated with the richness and diversity of bird species; improvement in the vertical and horizontal structures of a habitat, increases habitat diversity which in turn increases richness in bird species (Greenberg et al. 1995).

Conservation status

Most of the bird species recorded in our survey can be regarded as of little concern from the viewpoint of conservation i.e. fall into the species category of 'Least Concern' according to IUCN criteria. There were four species classified as 'Near Threatened': namely, the Long-tailed Parakeet (*Psittacula longicauda*) found both in large scale plantations and secondary forest; the Black Magpie (*Platysmurus leucopterus*) found only in secondary forest; the White-crowned Hornbill (*Aceros comatus*) found only in large scale plantations; and the Rhinoceros Hornbill (*Buceros rhinoceros*) detected in secondary forest as well as in smallholdings (Table 4).Moreover, we detected that

there were a total of thirteen different bird species that are protected by Indonesian law and nine different species listed in Appendix II of CITES. Three species in Appendix II and ten species protected by Indonesian law were found in the secondary forest; six species in Appendix II and seven species protected by Indonesian law were found in large scale palm oil plantations; three species in Appendix II, and seven species protected by Indonesian law were recorded in smallholding palm oil. The bird species along with their conservation status and their distribution by habitat are presented in Table 3.

Table 1. The proportion (%) of the most abundant bird species found in secondary forest patches, smallholdings and large-scale palm oil plantations

Species	Scientific name	Family	Percentage %
Secondary forest patches			
Glossy Swiftlet	*Collocalia esculenta*	Apodidae	19.2
Yellow-vented Bulbul	*Pycnonotus goiavier*	Pycnonotidae	12.4
Long-tailed Parakeet	*Psittacula longicauda*	Psittacidae	9.38
Sooty-headed Bisulbul	*Pycnonotus aurigaster*	Pycnonotidae	6.82
Blue-throated Bee-eater	*Merops viridis*	Meropidae	6.18
Spotted Dove	*Streptopelia chinensis*	Columbidae	4.05
Smallholding palm oil			
Bar-winged Prinia	*Prinia familiaris*	Sylviidae	14.4
Yellow-vented Bulbul	*Pycnonotus goiavier*	Pycnonotidae	12.3
Sooty-headed Bulbul	*Pycnonotus aurigaster*	Pycnonotidae	11.2
Spotted Dove	*Streptopelia chinensis*	Columbidae	10.5
Ashy Tailorbird	*Orthotomus ruficeps*	Sylviidae	10.1
White-throated Kingfisher	*Halcyon smyrnensis*	Alcedinidae	8.3
Lesser Coucal	*Centropus bengalensis*	Cuculidae	6.5
Zebra Dove	*Geopelia striata*	Columbidae	4.33
Large scale plantataion			
Yellow-vented Bulbul	*Pycnonotus goiavier*	Pycnonotidae	12.3
Bar-winged Prinia	*Prinia familiaris*	Sylviidae	9.20
Spotted Dove	*Streptopelia chinensis*	Columbidae	8.81
Glossy Swiftlet	*Collocalia esculenta*	Apodidae	7.44
Sooty-headed Bulbul	*Pycnonotus aurigaster*	Pycnonotidae	6.07
Pacific Swallow	*Hirundo tahitica*	Hirundinidae	5.87
White-throated Kingfisher	*Halcyon smyrnensis*	Alcedinidae	5.48
Ashy Tailorbird	*Orthotomus ruficeps*	Sylviidae	5.28
Lesser Coucal	*Centropus bengalensis*	Cuculidae	4.50
Zebra Dove	*Geopelia striata*	Columbidae	4.31

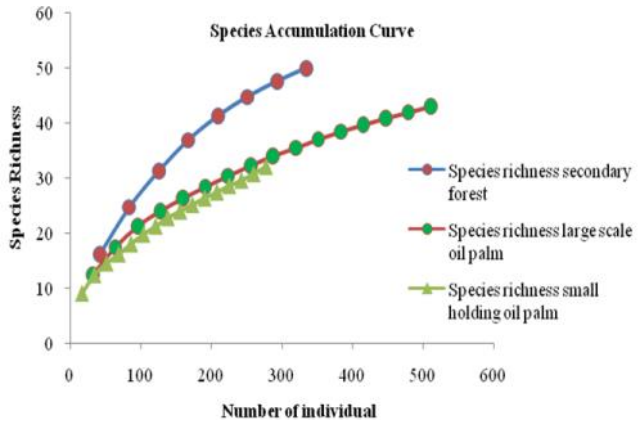

Figure 2. Species accumulation curves, with the x-axis showing the number of individuals sampled. Bird species richness was higher in secondary forest patches than in palm oil landscapes.

Table 2. Bird Diversity Index, Species Richness Index, and Evenness Index in different locations

Site	Ind.	N	S	Dmg	E
Secondary forest patched	469	55	3.2	8.8	0.8
Large scale plantation all	511	43	3.1	6.7	0.8
Young (1-8 year old)	197	25	2.8	4.5	0.9
Mature (9-17 year old)	92	22	2.7	4.6	0.8
Old (>18 year old)	225	31	3.0	5.5	0.9
Smallholding plantation all	277	32	2.7	5.5	0.8
Young (1-8 year old)	49	15	2.4	3.6	0.9
Mature (9-17 year old)	110	21	2.6	4.3	0.6
Old (>18 year old)	118	21	2.6	4.2	0.9

Note: Ind. = Individual, N = Number of species, S = Shannon Index, Dmg = Margalef Index, E = Evenness Index

Discussion

Overall bird species richness was higher in the secondary forest than in palm oil plantation. Higher bird species richness in secondary forest related to the vegetation structure which consisted of different plant growth forms that contributed to canopy stratification in this habitat, in contrast to palm oil plantations which only have one canopy layer. The vertical and horizontal structures of the forest were heterogonous; the plants in the forest had different crown heights and were not distributed evenly. This structural heterogeneity and high diversity of plant species provide niches for many species of birds, and therefore can support a great number of bird species sharing the same habitat. The diversity of vegetation is an important factor affecting bird species diversity in the secondary forests. More complex habitats are known to increase the diversity of species (MacArthur 1961). Forest conversion to palm oil plantation has changed the plant species composition from a heterogeneous to homogeneous one, therefore it has altered the food availability and habitat conditions for potential bird species.

Most of the bird species present in palm oil plantations were non forest-dependent. Conversion of forest land into palm oil plantations has replaced species that are forest-dependent with species that are generalists, and replaced species of high conservation concern with species that are of low conservation concern. Aratrakorn (2006) stated that the replacement of species-rich communities by species-poor communities, and the replacement of threatened and range-restricted species by species of lower conservation concern and with extensive ranges, following forest conversion to palm oil plantation a severe threat to biodiversity. Our results are consistent with previous studies in Malaysia and Thailand showing that bird species richness decreases due to land clearing for palm oil plantations (Peh et al. 2005; Aratratkorn et al. 2006; Azhar et al. 2011).

Old palm oil stands in large-scale plantations provide for more bird species diversity than do young stands. This suggests that planting a mixture of age groups would have positive effects on species richness in palm oil landscapes. Old palm oil stands have more locally complex habitat structures than do young oil plantation. The variations in species composition and species diversity of birds in different palm oil age classes is associated with differences in local vegetation characteristics within the plantation (Clough et al. 2007). Most of the trunks of old palm oil tree in our study area have epiphytic plants attached to them. Even though we did not take into account this variable in our statistical analysis, nevertheless, the existence of these

Table 3. Summary of statistical analysis of mean species richness and abundance using t-tests of significance between Sites, between Management Types, between Distances to secondary forest patches and between Locations; and using ANOVA for testing among the three Age classes

Variables	Probability value	
	Species abundance	Species richness
Site		
Secondary forest patches ~ Palm oil plantation	0.000	0.004
Management type		
Large scale palm oil plantation and Smallholding palm oil plantation	0.000	0.148
Age classes		
Large palm oil (1-8 years old, 9-18 years old, 19-30 years old)	0.021	0.814
Distance to forest		
(< 100 m and >2 km)	0.000	0.189
Location		
Kampar ~ Pelalawan	0.000	0.000
Kampar ~ Kuantan Singingi	0.000	0.000
Kampar ~ Siak	0.000	0.000

Note: Significant differences if *P* value <0.05

Tabel 4. Conservation status of birds in secondary forest, smallholding palm oil and large scale palm oil plantations at Riau

Species	Scientific name	Conservation status			Secondary forest	Small-holding	Large-scale
		IUCN	CITES	Indonesian Law			
Long-tailed parakeet	*Psittacula longicauda*	NT	II		√		√
Woolly-necked tork	*Ciconia episcopus*	LC		P	√		
Crimson sunbird	*Aethopyga siparaja*	LC		P	√		
Olive-acked sunbird	*Nectarinia jugularis*	LC		P		√	
White throated kingfisher	*Halcyon smyrnensis*	LC		P	√	√	√
Collared kingfisher	*Halcyon chloris*	LC		P	√	√	
Crested hawk-eagle	*Spizaetus cirrhatus*	LC	II	p	√		√
Black-winged kite	*Elanus caeruleus*	LC	II	P	√		√
Crested serpent eagle	*Spilornis cheela*	LC	II	P	√	√	
White crowned hornbill	*Berenicornis comatus*	NT	II	P			√
Oriental pied hornbill	*Anthracoceros albirostris*	LC	II	P	√		
Cattle egret	*Bubulcus ibis*	LC	II	P		√	
Stork-billed kingfisher	*Pelargopsis capensis*	LC		P	√		
Rhinoceros hornbill	*Buceros rhinoceros*	NT	II	P		√	√
Barn owl	*Tyto alba*	LC	II			√	√
Black magpie	*Platysmurus leucopterus*	NT			√		
Total					11	7	7

Note: Near threatened 4 species, Appendix II 9 species, Indonesian law 13 species. P= protected according to wild life Indonesian regulation No 7/1999, NT= near threatened, LC= Least Concern, II= appendix II according to CITES, X= species found

epiphytes might be related to bird species abundance. Epiphytes provide food for insect-eating birds because they are well known as habitats for arthropods. The abundance of epiphytes is related to the abundance of other species embedded in these plants (Ellwood and Foster 2004). Bobo and Waltert (2011) found that arthropod richness and density attracts many understory forest birds in agricultural areas. Compositional and structural heterogeneity in palm oil plantation is related to the different ages of palm oil stands. Mixed age plantations can support greater biodiversity (Luskin and Pott 2011; Azhar 2013). Thus, retaining some mature or old stands in a newly replanted plantation is likely to have benefits for conservation of bird biodiversity (Sheldon et al. 2010).

Large scale palm oil plantations have higher species diversity than smallholding palm oil plantations. Mostly, large palm oil plantations in our research location were surrounded by secondary forest patches, while smallholding palm oil plantations were mostly adjacent to roads and housing. Such conditions are likely to affect the outcomes for biodiversity because of the edge effects. Edge effect theory refers to the influence of ecotone (the transition between adjacent biomes) for increasing diversity and density of species. The conjunction of the boundary between natural habitats, especially forests, and adjacent disturbed or developed land is one example commonly used to explain the edge effects (Odum 1971). The few forest bird species and individuals present in large scale palm oil plantations very likely draw upon food resources obtained from distant forest habitats (Luck and Daily 2003).

The presence of near-threatened forest-dependent species in mature and old palm oil plantations may be related to the occurrence of some epiphytes and secondary forest patches surrounding palm oil plantations. Secondary forest patches serve as refugia for some forest birds, including rare bird species. The existence of forest growth near the plantation and the presence of epiphytic plants contribute to the high bird species richness (Hughes et al. 2002). Moreover, our results are supported by another study reporting that primary forest fragments in the vicinity of palm oil plantation in southern peninsular Malaysia influence the occurrence of relatively high forest bird species richness in the palm oil plantation (Peh et al. 2006). Riau province is categorized as a lowland tropical area which is suitable for palm oil monoculture but also supports habitat for valuable bird species such as the Long-tailed Parakeet (*Psittacula longicauda*) and White-crowned Hornbill (*Aceros comatus*). The occurrence of these near-threatened bird species in the palm oil plantations in our study area, indicate the importance of retaining secondary forest patches along with the palm oil plantation.

Based on our study in Riau, we conclude that species diversity of birds differs among the vegetation types and the age of palm oil stands. Secondary forest has the highest bird species diversity, while smallholding palm oil plantation has the lowest diversity. Furthermore, old age stands in large scale plantations have more diversity of bird species than have mature and young age stands, while in smallholding palm oil plantation, the mature age stands have the highest species richness and young age stands have the lowest species richness. The implications of our results for management are that preserving natural forest patches in a landscape dominated by palm oil plantations is one of the strategies that could conserve avifauna diversity. This is in accordance with Indonesian government regulations concerning the obligation of large-scale plantation to maintain areas of high conservation value (HCV).

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Short Communication: Spiders of Sabah: Fifty new records including the description of a new *Leucauge* species

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Abstract. *Nasir DM, Wong CX, Bakri A, Rahim F, Yusoff NR. 2016. Short Communication: Spiders of Sabah: Fifty new records including the description of a new* Leucauge *species. Biodiversitas 17: 799-807.* This paper is the second part of a continuing series, with the main objective of compiling and recording the spider species that can be found in Sabah, Malaysia. Based on the specimens collected during this field trip, a total of 50 new records of spider species from 11 families and 37 genera have been found. This includes one newly discovered spider species, the *Leucauge sabahan* sp. nov which is described based on a female specimen. It is hoped that this inventory can be used to assist in the knowledge about the spider species for this stage. In summary, an increment of 18% from the total number of spider species has contributed to a total of 272 recognized spider species recorded in Sabah, Malaysia.

Keywords: Araneae, checklist, diversity, distribution, Borneo

INTRODUCTION

Spiders are one of the most diverse orders that can be found in almost all types of habitats, ranging from forests to human settlements, including buildings and gardens (Jongkar 2004). These predatory arachnids have the capability to successfully adapt and thrive in a wide range of temperatures and environmental conditions**.** Different spider species can co-exist simultaneously in the same habitat. Unfortunately, despite their widespread existence, proper documentation of our local spider species is still lacking in Malaysia. Currently, only 644 spider species from Peninsular Malaysia (Norma-Rashid and Li, 2009; Dzulhelmi et al. 2014a), 247 spider species from Sarawak (Koh et al. 2013; Dzulhelmi et al. 2016), and 222 spider species from Sabah (Dzulhelmi et al. 2014b) have been formally recognized and documented. However, these records are compilations of commonly found species only. Deeleman-Reinhold et al. (2016) mentioned an approximately 749 morpho-species in 36 families with many of the undescribed species are found in Sabah. In addition, newly described spider species such as *Cebrenninus berau* and *Crockeria kinabalu* (Benjamin 2016), *Depreissia decipiens* (Deeleman-Reinhold et al. 2016) and *Myrmarachne* species (Yamasaki and Ahmad 2013) warrants the need to nurture interest in the spider study in Sabah state.

Sabah is the second largest state in Malaysia and one of the two Malaysian states located on the Island of Borneo. With more than 50% of its land mass under forest cover, more attention should be given to exploring and researching the rich and diverse spider species present in this large state. As far as can be determined, knowledge about the different types of the spider fauna that can be found in Sabah is very limited. This paper is the second report based on a series of field research conducted. Its long-term objective is to discover and record the myriad of unique spider species presenting the state of Sabah. This research is a concerted effort to improve the lack of knowledge and to provide an in-depth and broader view about the spider diversity of Sabah, Malaysia.

MATERIALS AND METHODS

Spider specimens were collected by hand-picking and stored in 75% ethanol during fieldtrips to Sabah in January and May 2015 in selected localities in Sabah (Figure 1). The specimens were examined and categorized according to the following classification: gender (male: ♂, female: ♀), date collected, condition of the spider, and location. Specimens collected were viewed under SMZ-U stereo microscope (Nikon, Japan) or under 50x dissecting microscope (AmScope, USA). Species identification was carried out using the following literatures and references therein where applicable: Koh (1989), Barrion and Litsinger (1995), Sebastian and Peter (1999), Song et al. (1999), Murphy and Murphy (2000), Anonymous (2011), Lau et al. (2011), Koh and Ming (2013), Dzulhelmi and Suriyanti (2015) and World Spider Catalog (2015). The following abbreviations are used throughout the text: anterior lateral eyes (ALE), anterior median eyes (AME),

posterior lateral eyes (PLE), posterior median eyes (PME). All morphological measurements are in millimeters (mm).

RESULTS AND DISCUSSION

A total of 50 species from 11 families and 37 genera were successfully collected and identified. Most of the collected specimens were from the family Araneidae (15 species from nine genera), Tetragnathidae (eight species from eight genera) and Salticidae (eight species from eight genera). Family Oxyopidae, Pholcidae, Scytodidae, Uloboridae and Zodariidae were represented by a single species each. About 18% of the newly recorded species were found with male specimen, while others were based on female specimens. Although these specimens collected are categorized as very common spider species, they have never been formally recorded in the inventory list of spider species of Sabah. In addition, the first spider inventory list was based on documented findings from previous field studies which included a few areas from the southern part of the Sabah state. This study investigates on the spider fauna collected from the northern and western regions of Sabah.

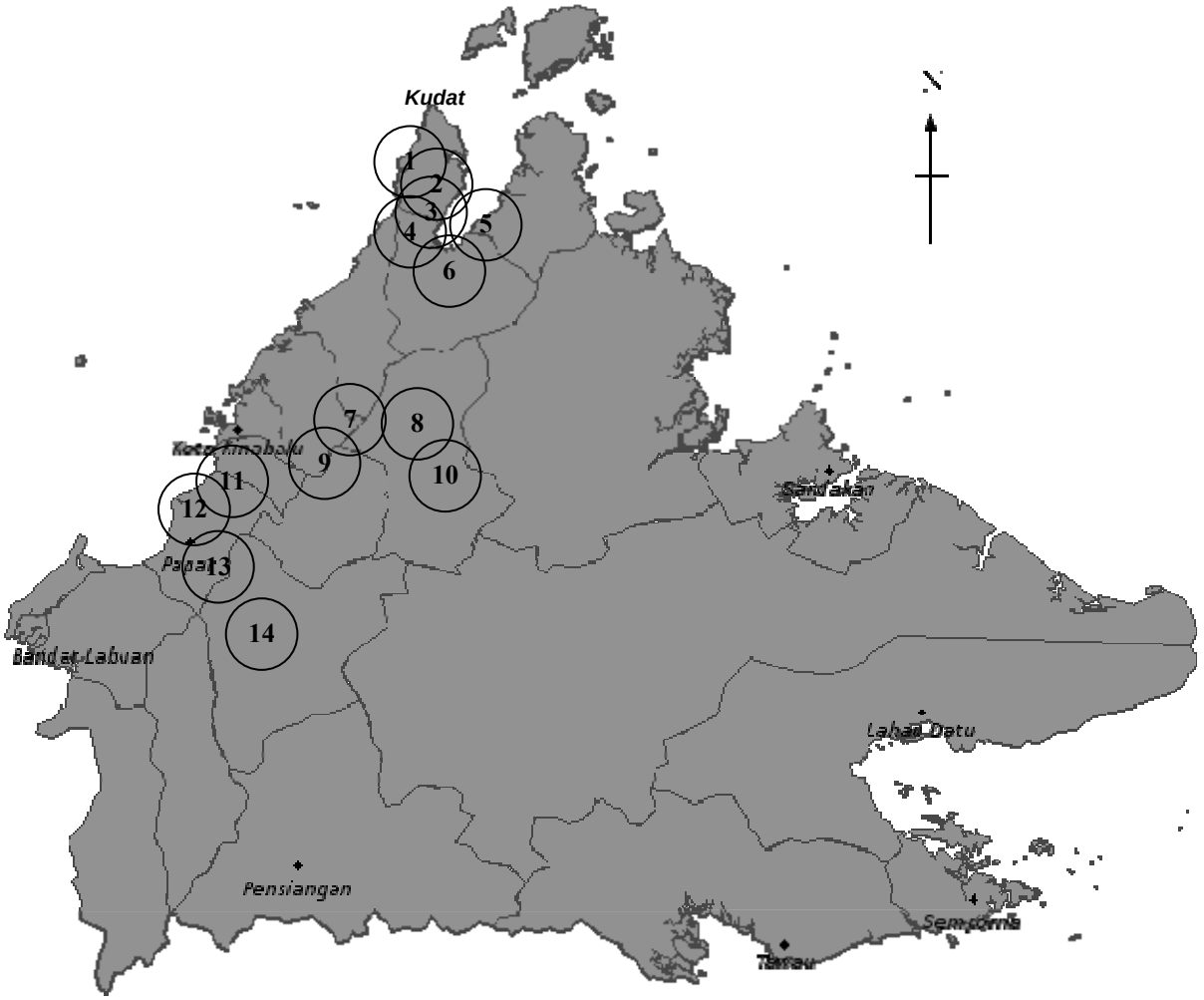


Figure 1. Study sites in Sabah state where spider specimens were collected. 1, Simpang Mengayau at Tips of Borneo, Kudat, 7°01'16"N, 116°44'34"E, beach forest, 13-15 February 2015; 2, Pantai Bak Bak, Kudat, 6°56'50"N, 116°50'22"E, beach forest, 16-18 February 2015; 3, Kudat Golf and Country Resort, Kudat, 6°53'21"N, 116°51'22"E, garden, 19-21 February 2015; 4, Esplanade Kudat, Kudat, 6°52'44"N, 116°51'18"E, building, 22-24 February 2015; 5, Kampung Bangkau-bangkau, Pitas, 6°43'14"N, 116°59'50"E, rubber plantation, 10-12 January 2015; 6, Kampung Korongkom Laut, Kota Marudu, 6°30'26"N, 116°46'50"E, oil palm plantation, 7-9 January 2015; 7, Mesilau Resort Nature Reserve, Ranau, 6°02'5"N, 116°34'55"E, dipterocarp forest, 16-18 May 2015; 8, Poring Hot Spring Nature Reserve, Ranau, 6°2'35"N, 116°42'7"E, dipterocarp forest, 22-24 May 2015; 9, Kinabalu National Park, Ranau, 6°01'16"N, 116°32'43"E, montane forest, 19-21 May 2015; 10, Sabah Tea Garden, Ranau, 5°56'2"N, 116°48'5"E, tea plantation, 13-15 May 2015; 11, Kota Kinabalu Wetland Centre, Kota Kinabalu, 5°59'14"N, 116°5'20"E, mangrove forest, 7-9 April 2015; 12, Signal Hill, Kota Kinabalu, 5°59'3"N, 116°4'44"E, disturbed forest, 13-15 April 2015; 13, Sri Kinabalu Resort, Kota Kinabalu, 5°57'43"N, 116°36'14"E, garden, 10-12 April 2015; 14, Crocker Range National Park, Tambunan, 5°58'5"N, 116°08'2"E, montane forest, 25-27 May 2015.

List of newly recorded spiders in Sabah

ARANEIDAE

Acusilas coccineus Simon, 1895

Material examined: ♀, 17.02.2015, inside rolled leaf, Pantai Bak Bak.

Notes: This spider normally builds its web inside rolled leaves of plants.

Anepsion depressum (Thorell, 1877)

Material examined: ♀, 18.05.2015, spiders were found at the chalet, Mesilau Resort Nature Reserve.

Notes: This small size spider with flat abdomen constructs very small webs in-between the leaves of small shrubs.

Araneus inustus (Koch, 1871)

Material examined: ♀, 20.02.2015, the spider was found at the center of its orb-web, in a shrub in the gardens of Kudat Golf and Country Resort.

Notes: This spider normally constructs an orb-web in garden shrubs.

Araneus mitificus (Simon, 1886)

Material examined: ♀, 10.04.2015, the spider was found inside their silken retreat, Sri Kinabalu Resort.

Notes: They always rest inside their silken retreat and will only crawl out to their web when a prey is trapped.

Argiope reinwardti (Doleschall, 1859)

Material examined: ♀, 20.05.2015, the spider was found under the ceiling of a hut situated along the trail, Kinabalu National Park.

Notes: This spider will drop from their web when disturbed and will pretend to be lifeless.

Argiope perforata Schenkel, 1963

Material examined: ♀, 22.05.2015, the spiders were found at the center of their webs, Poring Hot Spring Nature Reserve.

Notes: Orb-web is decorated with various patterns of silk stabilimentum.

Argiope pulchella Thorell, 1881

Material examined: ♀, 18.05.2015, the spiders were found at the chalet, Mesilau Resort Nature Reserve.

Notes: This spider will escape to the opposite side of the web if disturbed.

Cyrtophora cylindroides (Walckenaer, 1841)

Material examined: ♀, 18.05.2015, the spiders were found building their webs on a tree near a chalet, Mesilau Resort Nature Reserve (Figure 3.B).

Notes: This spider builds large webs that are constructed to face direct sunlight during the day. They are normally found in heath forests and in cooler areas at higher elevation.

Cyrtophora moluccensis (Doleschall, 1857)

Material examined: ♀, 22.05.2015, the spiders were found at the center of its web, Poring Hot Spring Nature Reserve.

Notes: This spider can be found in dipterocarp forests and in gardens.

Lipocrea fusiformis (Thorell, 1877)

Material examined: ♀, 25.05.2015, the spider was found crawling on a fine thread amongst the shrubs, Crocker Range National Park.

Notes: They are commonly found in agricultural sites, but can also be found in secondary forests.

Neoscona nautica (Koch, 1875)

Material examined: ♀, 16.02.2015, the spider was found constructing its web in a small bush, Pantai Bak Bak; ♀, 15.05.2015, the spider was found resting under tea leaves, Sabah Tea Garden.

Notes: This common species is found in gardens, mangrove forests, human settlements and also inside buildings. They prey on the Homoptera (i.e. Cicada).

Neoscona theisi (Walckenaer, 1841)

Material examined: ♀, 23.02. 2015, the spider was found eating its prey, Esplanade Kudat; ♀, 18.05.2015, the spider was found building its web in a shrub near a chalet, Mesilau Resort Nature Reserve.

Notes: Common species found in agricultural and garden areas.

Neoscona vigilans (Blackwall, 1865)

Material examined: ♀, 21.05.2015, the spider was found resting at the center of its web in a shrub, Kinabalu National Park.

Notes: This common species is found in gardens, the forest fringe and human settlements. It hides in rolled leaves during the day (Yong 2009).

Parawixia dehaani (Doleschall, 1859)

Material examined: ♀, 25.05.2015, the spider was resting at the center of the orb-web in a shrub, Crocker Range National Park.

Notes: The spider will free-fall if disturbed, and will then climb back to the center of the web. It can be found in mangrove forests, dipterocarp forests, disturbed habitats and rural settlements.

Zygiella calyptrata (Workman & Workman 1894)

Material examined: ♀, 13.02.2015, the spider was found walking on its fine silk thread in a shrub, Simpang Mengayau

Notes: The spider usually waits under the tip of the leaves at the edge of its web

LYCOSIDAE

Pardosa pseudoannulata (Bosenberg & Strand, 1906)

Material examined: ♀, 27.05.2015, the spider was found walking on sand, Crocker Range National Park.

Notes: This common spider species is found near water in agricultural areas and ponds.

Pardosa pusiola (Thorell, 1891)

Material examined: ♀, 25.05.2015, the spider was found resting in a shrub amongst the undergrowth, Crocker Range National Park.

Notes: Sometimes, this spider retreats from threats by running on the water surface. This common spider is found in gardens and human settlements.

OXYOPIDAE

Oxyopes birmanicus Thorell, 1887

Material examined: ♂♀, 17.02.2015, female mount on the back of the male, Pantai Bak Bak.

Notes: The female was mounting on the back of the male during the night time. There was also another female of conspecific about 20 cm away on the same branch.

PHOLCIDAE

Physocyclus globosus (Taczanowski, 1874)

Material examined: ♀, 12.04.2015, ceiling corner, Sri Kinabalu Resort.

Notes: Commonly found resting in their tangled webs in ceiling corner.

SALTICIDAE

Bavia sexpunctata (Doleschall, 1859)

Material examined: ♀, 09.01.2015, on shrubs, Kampung Kerongkom Laut; ♀, 10.01.2015, on shrubs, Kampung Bangkau; ♀, 22.05.2015, spiders was found on chalet wall, Poring Hot Spring Nature Reserve.

Notes: This common spider is found inside buildings, human settlements and gardens.

Epocilla calcarata (Karsch, 1880)

Material examined: ♀, 23.05.2015, on leaves, Poring Hot Spring Nature Reserve.

Notes: This spider is normally found in gardens, dipterocarp forests, and human settlements.

Hyllus lacertosus (Lucas, 1858)

Material examined: ♀, 09.04.2015, on railing, Kota Kinabalu Wetland Centre.

Notes: It will jump onto nearby fingers or camera lens due to its curiosity and braveness.

Mantisatta trucidans Warburton, 1900

Material examined: ♀, 10.01.2015, on leaves, Kampung Bangkau; ♀, 10.04.2015, on leaves, Sri Kinabalu Resort.

Notes: This species can also be found resting inside its constructed silk nest on mango trees.

Orsima ichneumon (Simon, 1901)

Material examined: ♀, 14.04.2015, crawling on trail railings, Signal Hill.

Notes: Can be found resting on the upper part of a leaf of shrubs.

Parabathippus petrae (Proszynski & Deeleman-Reinhold, 2012)

Material examined: ♀, 19.05.2015, walking on rock, Kinabalu National Park.

Notes: The spider sleeps under leaves in shelter made of silk during the night (Koh and Ming 2013).

Portia labiata (Thorell, 1887)

Material examined: ♂, 24.05.2015, on rock, Poring Hot Spring Nature Reserve.

Notes: Can be found near webs of other spiders waiting to prey on the web owners.

Viciria praemandibularis (Hasselt, 1893)

Material examined: ♀, 14.04.2015, walking on rock, Signal Hill.

Notes: Rather than retreat, this spider often observes with curiosity at the collector when found.

SCYTODIDAE

Scytodes fusca Walckenaer, 1837

Material examined: ♀, 15.05.2015, resting under house, Sabah Tea Garden (Figure 3.C).

Notes: This spider is found inside buildings and human settlements.

TETRAGNATHIDAE

Leucauge argentina (Hasselt, 1882)

Material examined: ♂♀, 22.05.2015, constructing web near tree bark, Poring Hot Spring Nature Reserve; ♀, 26.05.2015, resting at center of web, Crocker Range National Park.

Notes: the orb-web is usually found close to the ground. It is found in gardens, heath forests and montane forests at higher elevations and in cooler areas.

Leucauge celebesiana (Walckenaer, 1841)

Material examined: ♀, 19.05.2015, eating, Mesilau Resort Nature Reserve; ♂♀, 25.05.2015, spider constructing web in long grasses, Crocker Range National Park.

Notes: Normally found constructing orb-webs in groups in gardens and montane forests at higher elevations and in cooler areas. The species was found eating prey from Diptera (*Nematoceran* sp.) and Formicidae (*Oecophylla* sp.).

Leucauge decorata (Blackwall, 1864)

Material examined: ♂♀, 25.05.2015, spiders constructing webs in long grasses in groups. Crocker Range National Park.

Notes: This spider constructs orb-webs in grassy areas. It is normally found at higher elevations and in cooler areas in montane forests.

Leucauge liui Zhu, Song & Zhang, 2003

Material examined: ♀, 18.05.2015, resting at center of web, Mesilau Resort Nature Reserve; ♀, 25.05.2015, constructing web in lower shrubs, Crocker Range National Park.

Notes: This spider constructs orb-webs between 25 to180 centimeters from the ground.

Leucauge sabahan Dzulhelmi, sp. nov.

Type material: Female holotype (PHS033) from Poring Hot Spring Nature Reserve, Sabah (6°2'35"N, 116°42'7"E) was collected by hand picking at 2100 hours on 22st May 2015. Holotype specimen is stored in the Museum of Zoology, University of Malaya.

Etymology: The specific name is a noun, referring to the location where the holotype was collected.

Diagnosis: The *L. sabahan* resembles *L. tesellata* and *L. taiwanica* with the presence of dense hairs on tibia IV. The *L. sabahan* can be differentiated based on the following: **Abdomen** (1) The *L. sabahan* has an oval-shaped abdomen while *L. tessellata* has an elongate-shape abdomen. (2) The *L. sabahan* abdomen does not overhang the carapace, and does not extend posteriorly above the spinnerets as in *L. taiwanica*. **Coloration** (3) The abdomen of *L. sabahan* has a leaf-like shape pattern, with no pairs of anterior and posterior black spots, that differs significantly from the abdomen patterns and coloration of *L. taiwanica*.

Description: **Male.** Unknown. **Female.** Total length 7.06; **Carapace:** 2.83 long, 2.13 wide, carapace orange-brown in colour, carapace is longer than it is wide (Figure 2A-B), cephalic area markedly narrower in the thoracic area, sternum heart-shaped slightly wider than it is long with a similar colour to the carapace (Figure 2E); **Eyes:** diameters AME 0.15, ALE 0.10, PME 0.13, PLE 0.10; inter-distances AME-PME 0.15; AME-AME 0.11, AME-ALE 0.28, PME-PME 0.13, PME-PLE 0.28, PLE-PLE 0.05; clypeus 0.15 high; lateral eyes loosely contiguous or almost so, eight eyes in two slightly recurved rows, distance between PME-PME greater than between AME-AME, PME slightly smaller than AME, AME size one times the distance between them, PME size about one times the distance between them, distance between PME and PLE are about twice the PME eye size, clypeus height one times the AME size (Figure 2D); **Chelicerae**: promargin with 3 teeth, retromargin with 4 teeth; **Abdomen**: 4.19 long, 3.35 wide; oval-shaped light-brown abdomen, abdomen does not overhang carapace, the dorsal abdomen is covered with leaf patterns with silver pigments, two silvery line markings on the ventral abdomen (Figure 2C); **Spinnerets**: spinnerets pointing downwards and exceed abdomen end (Figure 2F); **Legs**: leg measurements (femur/ patella/ tibia/ metatarsus/ tarsus/ total): leg I (6.17/1.15/5.87/6.77/1.64/21.60), leg II (4.65/1.01/4.18/4.53/1.14/15.51), leg III (2.69/0.77/1.69/1.76/0.97/7.88); leg IV (4.75/1.02/3.39/3.58/1.24/13.98); legs are darker-brown in color with black annulations, leg formula (I-II-IV-III), Short spines on legs, Leg I: femur with 8-10 spines, tibia I with 1-3 spines, Leg II: femur II with 6-8 spines, tibia II with 6-8 spines, Leg III: femur III with 6-8 spines, tibia III with 6-8 spines, Leg IV: femur IV with 6-8 spines, tibia IV with 6-8 spines, long dense brush of hairs covering more than one-third of the tibia, metatarsus IV has dense brush of hairs, two rows of long trichobothria covering more than one-third of the prolateral femur IV. **Epigyne**: simple and weakly sclerotized, spermathecae round in shape with short copulatory duct (Figure 2G-H).

Distribution: It is known from the type locality in the dipterocarp forest in Poring Hot Spring Nature Reserve in Sabah. This species had been recorded in Kubah National Park and Gunung Gading National Sarawak, Malaysia (Dzulhelmi et al. 2016). It may be found in the Borneo rainforest (Figure 3.A).

Natural history: Nocturnal. Specimen is found resting at the center of the web during the night. The web was constructed at 70° angle between two shrubs in an open space above 100 cm from the ground which was covered with soil.

Taxonomic: There are currently more than 170 recognized *Leucauge* species (World Spider Catalog 2015), and some of the *Leucauge* species have dense brush of hairs on tibia IV, which resembles the *Opadometa* species. Yoshida (2009) classified *L. taiwanica* (Yoshida 2009), *L. tessellata* (Thorell, 1887), and *Opadometa fastigata* (Simon, 1877) into one genus group. Recently, two new *Opadometa* species, *O. sarawakensis* and *O. kuchingensis* were described (Dzulhelmi et al. 2015). Taxonomic revision of the tetragnathid species which have dense hairs on tibia IV should be done in the future.

Mesida gemmea (Hasselt, 1882)

Material examined: ♀, 22.05.2015, constructing web in lower shrubs, Poring Hot Spring Nature Reserve.

Notes: This spider constructs orb-web at 0° to 60° web orientation. It is found at dipterocarp forests.

Tylorida striata (Thorell, 1877)

Material examined: ♂♀, 25.05.2015, constructing web on grass on hill slope, Crocker Range National Park.

Notes: By 1000 hours, this spider takes about two minutes to dismantle its web by rolling it into a ball and consume it, while leaving the main frame. It is a common spider that is found on the forest fringe and in gardens.

Tylorida tianlin Zhu, Song & Zhang, 2003

Material examined: ♂♀, 18.05.2015, wrapping preys, Mesilau Resort Nature Reserve; ♀, 23.05.2015, construct web at lower shrubs, Poring Hot Spring Nature Reserve.

Notes: Prey wrapped by the spiders was from Coleoptera, Diptera (*Nematoceran* sp.).

THERIDIIDAE

Chikunia nigra (Pickard-Cambridge, 1880)

Material examined: ♀, 22.05.2015, spider was found under shrubs, Poring Hot Spring Nature Reserve.

Notes: This spider is often found hiding under leaves in dipterocarp forest.

Janula bubalis Yoshida & Koh, 2011

Material examined: ♂♀, 17.05.2015, both spiders on the same web, Mesilau Resort Nature Reserve.

Notes: This spider was found hiding under a leaf.

Parasteatoda mundula (Koch, 1872)

Material examined: ♀, 02.2015, resting on web, Pantai Bak Bak; ♀, 19.05.2015, resting under branch, Kinabalu National Park.

Notes: This common spider is found inside rolled leaves in gardens and forests.

Parasteatoda tapidariorum (Koch, 1841)

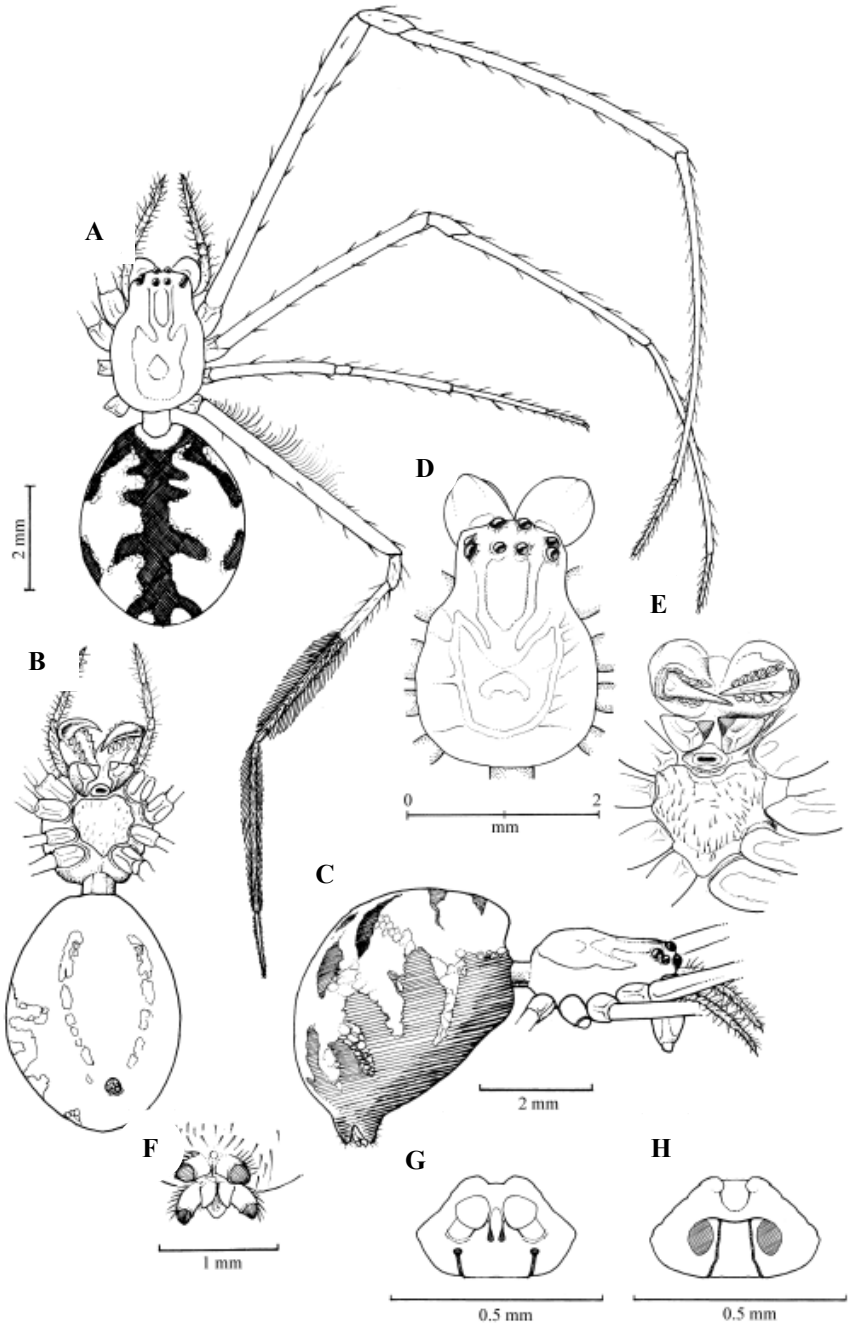
Material examined: ♀, 20.05.2015, build webs under railings, Kinabalu National Park.

Notes: This spider usually build tangled webs near human settlements.

Phoroncidia lygeana (Walckenaer, 1841)

Material examined: ♀, 23.05.2015, on leaves, Poring Hot Spring Nature Reserve (Figure 3.D).

Notes: This spider would rest using a fine silk from its spinnerets and attaching it to the tip of the leaves. It is commonly found in heath forests.



Figures 2. Female *Leucauge sabahan* new species. Body: (A) dorsal view, (B) ventral view, (C) lateral view; eye pattern: (D) dorsal view; (E) sternum; (F) spinnerets; epigyne: (G) dorsal view (internal), (H) ventral view (outer).

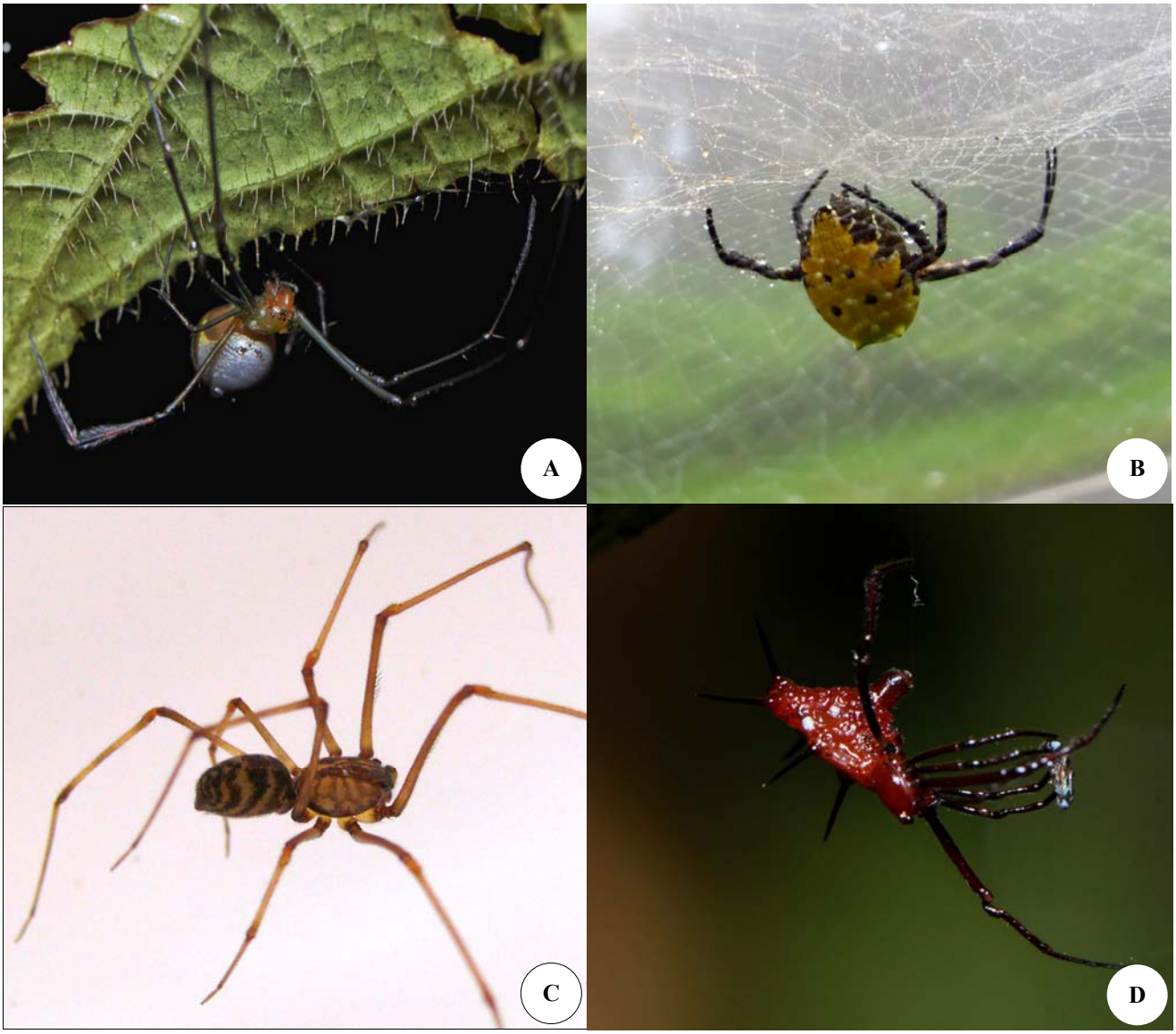


Figure 3. A. Tetragnathidae, *Leucauge sabahan* sp. nov,. B. Araneidae, *Cyrtophora cylindroides*, C. Sytodidae, *Scytodes fusca*, D. Theridiidae, *Phoroncidia lygeana*

THOMISIDAE

Amyciaea forticeps (Cambridge, 1873)

Material examined: ♀, 13.04.2015, on railing, Signal Hill.

Notes: This spider have the ability to mimic *Oecophylla smaragdina* ants before preying on them.

Lycopus rubropictus Workman, 1896

Material examined: ♀, 13.04.2015, on leaves, Signal Hill.

Notes: Female is sometimes found guarding their eggs inside their nest.

Mastira bipunctata Thorell, 1891

Material examined: ♀, 25.05.2015, spider was moulting, Crocker Range National Park.

Notes: This spider is normally found in flowers in gardens. During molting, it takes about five minutes for the exoskeleton to harden after the spider emerges and starts to move.

Platythomisus octomaculatus (Koch, 1845)

Material examined: ♀, 22.05.2015, on leaves, Poring Hot Spring Nature Reserve.

Notes: This cryptic species usually rest in-between leaves of the lower shrubs.

Stephanopis altifrons Cambridge, 1869

Material examined: ♀, 12.04.2015, on tree bark, Sri Kinabalu Resort.

Notes: This spider camouflages on lichen-covered wood during the night.

Thomisus guangxicus Song & Zhu, 1995

Material examined: ♀, 14.05.2015, on leaves, Sabah Tea Garden.

Notes: Normally found in agricultural areas.

Tmarus orientalis Schenkel, 1963

Material examined: ♀, 19.05.2015, resting on twigs, Kinabalu National Park.

Notes: Normally it blends itself with twigs by stretching both leg I and II frontward. This spider can be found in gardens and dipterocarp forests.

ULOBORIIDAE

Uloborus plumipes Lucas, 1846

Material examined: ♀, 24.05.2015, spider was found under shrubs, Poring Hot Spring Nature Reserve.

Notes: This spider constructs a horizontal orb-web which is placed under leaves. It is normally found in gardens and dipterocarp forests.

ZODARIIDAE

Mallinella annulipes (Thorell, 1892)

Material examined: ♂, 11.01.2015, on rock, Kampung Bangkau

Notes: Usually hunt on the base of the tree trunk, possibly feed on ants.

The state of Sabah, as an integral part of the hugely unexplored island of Borneo, is already internationally well-known to contain a truly huge assortment of fascinating and diversified habitat types. These habitats are able to support and sustain the existence of a myriad of fascinating creatures in Borneo's vast tropical jungles, including countless variations of Malaysia's very own indigenous spider species. It is highly probable that these habitats are still hiding many more mysterious new spider species which at present still remains unknown. The current official record certainly does not do justice to, nor reflect the true biodiversity present throughout the enormous range of habitat types present in Sabah.

A proper and accurate documentation of the spider species present is very important. More than 90% of the newly recorded species are common spider species which can be found in many parts of South East Asian countries (e.g. Barrion and Litsinger 1995; Murphy and Murphy 2000; Song et al. 2002; Jager et al. 2012). From the newly recorded list of spider species, about 28% had only just recently been recorded in Sarawak (i.e. Dzulhelmi et al. 2016), while 64% of the newly recorded species have been recorded in the neighboring country, Brunei (i.e. Koh and Ming 2013). Among the newly recorded species, *Leucauge liui* (Tetragnathidae) and *Stephanopis altifrons* (Thomisidae) represent newly discovered spider species in this country. In addition, the new *Leucauge* species found shows a very strong indication of the existence of many other unrecorded spider species in the state. However, there were also many specimens collected in this study which could not be identified, which had affected the total number of newly recorded spider species for this paper.

Therefore, there is a definite need for a continuous and sustained research effort in order to more accurately determine the number of spider species present in Sabah. This would undoubtedly contribute greatly to increasing the number of spider species recorded in the current inventory. Since the diversity and distribution of the spider species in the state of Sabah and Malaysia is so poorly known, each study is an important step forward and a scientific contribution to increasing the knowledge of the spider fauna for this country.

ACKNOWLEDGEMENTS

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The length-weight correlation and population dynamics of razor clams (*Solen regularis*) in Surabaya east coast, Indonesia

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Abstract. *Trisyani N, Herawati EY, Widodo MS, Setyohadi D. 2016. The length-weight correlation and population dynamics of razor clams (*Solen regularis*) in Surabaya east coast, Indonesia. Biodiversitas 17: 808-813. Solen regularis* is a pelecypod species living in intertidal areas at sandy substrate. In Surabaya east coast, *Solen regularis* is exploited by the locals for consumption, both as fresh seafood or processed one. This research aims to analyze the length-weight correlation of the clams and the population dynamics, conducted from August 2014 to July 2015 in Surabaya east coast. The results show that the correlation is explained in the equation $W = 0.038L^{2.798}$ with the correlation coefficient of 0.9. Also, the allometric growth pattern is proven negative as the increase in body length is faster than that in weight. Growth is measured with von Bertalanffy growth model $Lt = 8.0 (1\text{-}e (-0.7^{(t+0.003)})$ and the result from the parameter analysis using ELEFAN I method in FISAT II software show that $L_\infty = 8.0$ cm and $k = 0.7$/year. The estimated weight at t_0 in Pauly's empirical formula is -0.003 cm. Mortality rates are shown as follows: natural mortality (M) is 2.05/year, fishing mortality (F) is 2.01/year, and total mortality is 4.05/year. Exploitation rate (E) is 0.50/year indicating that the exploitation of *Solen regularis* is in optimal condition. The B/R and Y/R value is 1.4%/year. It can be suggested from the findings of the research that knowledge on population dynamics may be useful for the utilization and management of *Solen regularis* in order to preserve its environmental and ecological sustainability.

Keywords: Growth, mortality, razor clams, *Solen regularis*, Surabaya east coast

INTRODUCTION

Solen spp. or also known as r*azor clam* or *kerang bambu* (Indonesian) or *kerang lorjuk* (Maduranese) is a species of mollusk clams widely consumed and highly traded as a commodity in international markets (Baron et al. 2004). This species varies in several species, such as *Solen dactylus* living along the coasts with muddy sandy substrate in Oman Sea and Persian Gulf (Bruyne 2003), *Solen regularis* in the western part of Sarawak, Malaysia (Rinyod and Rahim 2011), and *Solen marginatus* in the coasts of Atlantic, Europe, northwestern part of African coasts, and Mediterranean Sea buried under the sand and/or tidal areas, both in intertidal and sub tidal parts at mud substrates and the sands (Hmida et al. 2012). In Indonesia, *Solen* spp. can be found in the Maduranese coast of Pamekasan District (Nurjanah et al. 2008), Eastern coast of Surabaya (Trisyani et al. 1999; Trisyani and Irawan 2008), and Kejawanan Beach, Cirebon (Subiyanto et al. 2013). The locals call this type of clam *lorjuk* or *kerang bambu*. The findings from investigation conducted by the Indonesian Institute of Sciences (LIPI) show that *Solen regularis* is widely found in the Surabaya east coast, and the DNA analysis using RAPD indicates that *Solen regularis* in the Surabaya east coast exhibits 13.1% of similarity with *Solen* sp. found in Pamekasan coast (Trisyani and Budiman 2015).

The higher demands for *Solen* spp. as comestibles have led to the more intensified fishing for this species which is feared as the possible cause of decreasing population of the clam. This problem, however, has not been officially identified because of the absence of data for fishing production and industry. A research carried out by Trisyani and Irawan (2008) shows that *Solen* sp. found along the eastern coast of Surabaya lives in the muddy sand substrate with the organic content of approximately 0.22-0.54 ppm, water temperature of about 28-31ºC, salinity around 26-31 ppt, and soil pH at 7.0-8.5. The clams are fished using dredging tools and sticks whose ends are immersed into a mix of chalk and soap. Substrate is dredged and sticks are pushed into the hole to pull the clams out. As the stick end touches the clams, they are immediately captured by hand.

Trisyani and Hadimarta (2013) have conducted a research and found that *Solen*'s reproduction cycle by observing their Gonad Maturity Level (GML). It is discovered that the GML of the clams in the Surabaya east coast reaches the mature level (GML III) in Mays and starts the insemination (GML IV) in Junes. Gonad growth declines until the resting phase (GML 0) in Septembers, and in Octobers, GML gradually refrains to the mature levels (GML II and GML III). As the GML rises, it is followed by an increase in the diameter of the oocytes with the equation of GML = 0.522 ± 0.528 of oocyte diameter with correlation of 79.2 %

Previous researches related to the razor clams growth have been conducted by Baron et al. (2004) on *Ensis*

macha, Saeedi et al. (2009) on *Solen dactylus*, and Otero (2014) on *Ensis arcuatus*. Growth is an important element as the information on age and shell size is beneficial for the precise management strategies in order to support the sustainable utilization of mollusk species (Peharda et al. 2007). Information on the growth level is required in order to recognize the age of which the individual becoming the part of biomass can be utilized and the time needed to achieve the commercial size (Haddon 2011). In addition, the growth data can be the reference to asses the status of fisheries and to determine the status of exploitation of a species (Hilbron et al. 1995). Considering the lack of regulations related to the fishing and industrial management of *Solen* spp. as a resource in Indonesia, it is entailed that studies on the length-weight correlation and population dynamics of *Solen regularis*, as we conducted in Surabaya east coast, to preserve the species for sustainable exploitation.

MATERIALS AND METHODS

This research carried out in the Surabaya east coast at the latitude of 07°08'33.5" and longitude of 113°35'27.1" starting from August 2014 to July 2015. Sampling on *Solen regularis* was made every two weeks. During the period of December to the beginning of March 2015, no sample was found in the location. There are 2929 specimens collected during the research and taken from intertidal areas at the sand substrate at the low tides. Samples were caught with a stick dipped in the chalk at its tip and penetrated into the substrate hole. As the clam peeps out, it is pulled out by hand and preserved in 5% of formaldehyde of sea water immediately after the fishing (Baron et al. 2004). The morphometric measurement consists of the length (L) and weight (W) of the clams. Length is measured using a calliper with the precision of 0.1 cm and weight is recognized by analytic scale with the accuracy of 0.01 gram.

Length-weight correlation

Analysis on the length and weight of *Solen regularis* was conducted using linear regression. The length-weight correlation is very important in the science of population dynamics, for example, in calculating the catch per recruit (yield per recruit, Y/R) and biomass (biomass per recruit, B/R). The weight of the clams can be considered as a function from their length and this correlation follows the cubic law stated with the formula $W = a L^b$. W is the weight (in gram) and L stands for length (in cm); a is the intercept (curve intersection of length-weight correlation with axis y and b is the probe of length-weight growth pattern. If b = 3 that shows isometric growth, it means that the length and the weight increase equally. Meanwhile, $b \neq 3$ shows allometric growth, $b < 3$ shows that length increases faster than weight, and b> shows weight increases faster than length (Park and Oh 2002).

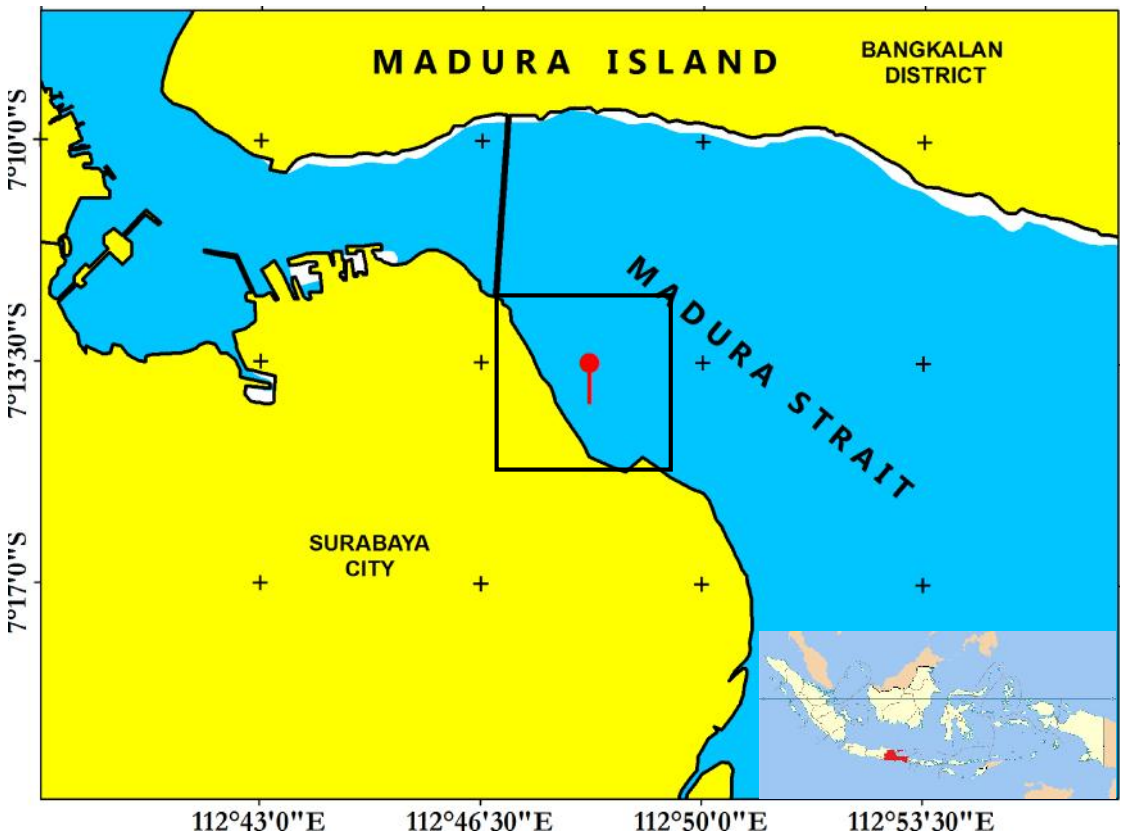

Figure 1. Study site at Surabaya East Coast, East Java, Indonesia

Von Bertalanffy's growth model

The growth of the clams measured with Von Bertalanffy's growth model (Sparre and Venema 1999) was $L_t = L_\infty (1\text{-}e^{-k(t-t0)})$. "t" is the number of growth checks marked by the clam since the beginning of shell formation, "Lt" is the valve's length (L) at growth check "t", L_∞ is the maximum length of *Solen regularis* theoretically (asymptotic length), k is the coefficient of growth rate (per time unit), and t_0 is the theoretical age of *Solen regularis* as the total length of the shell is equal to zero, Pauly's empirical equation (1984) is Log $(-t_0)$ = 0.3922-0.2752 (Log L_∞)-1.038 (Log k).

Mortality rate

Total mortality rate (Z) is the stock's declining rate. Mortality rate was determined by Gulland's formula (1971) with the equation $Z = \frac{k(L\infty - L)}{L - Lc}$. Normal mortality rate (M) was counted using Pauly's (1984), Log M =-0.0066-0.279 Log L_∞ + 0.6543 Log k + 0.4634 Log T. "T" is the average water temperature being measured every two weeks during the study. Fishing mortality rate (F) can be measured by subtracting M value to Z with the formula F = Z-M.

Exploitation rate (E)

To determine the rate of utilization or exploitation, Beverton and Holt (1957) formula was used by comparing the fishing mortality rate (F) and total mortality rate (Z). E>0.5 shows high exploitation level (over fishing); E<0.5 shows low exploitation level (under fishing), and E=0.5 shows optimal exploitation (Sparre and Venema 1999).

RESULTS AND DISCUSSION

Length-weight correlation

Length-weight correlation is generally used in researches in fisheries to explain changes in individual size, to show the growth pattern of the organism, to acquire the index of physical condition of the population, and to evaluate the quality of the habitat (Albuquerque et al. 2009).

From the analysis on the length-weight correlation of *Solen regularis*, it is acquired $W = 0.038\ L^{2.798}$ with the correlation coefficient of 0.95 (Figure 1). The b value shows negative allometric value (b < 3), which means that the length of the shell increases faster than the weight. This result is similar to that from a research by Saeedi et al. (2009) observing *Solen dactylus*, which is $W = 0.0001\ L^{2.5921}$ with correlation coefficient = 0.96 for all the analyzed specimens with $p < 0.001$. The b mean is 2.57 ± 0.1 for one year. The t student value determines that the length-weight correlation of this species is negative allometric. On *Ensis arcuatus*, the correlation coefficient from the length-weight correlation is 0.97 (Fahy et al. 2001) and on *Solen strictus* is 0.91 (Park and Oh 2002) with the average b value of 2.57 ± 0.1. The length-weight correlation of this species forms a negative allometric pattern. The difference on the growth patterns represented by the b value on length-weight correlation is affected by the growth phase, the size, food supply, sex, gonad growth, health, and breeding period (Miranda et al. 2006).

Attributes specifically found in fish and mollusks, such as the body shape, can be a reference for explanation on particular species' survival ability in waters and a guide to environmental factors that fish can adapt to (Allan and Castillo 2007). The length-weight development is influenced by several factors, such as food and environmental adaptation (Effendie 1997). Food supply in sandy substrate in the intertidal area relatively low, depends on the streams carrying planktons as clams are benthic organisms and suspension feeder. The ability to adapt with the body shape that is elongated in the intertidal areas dominated with sand enables *Solen* sp. to dig faster immediately as the passing waves translocate animals from substrate. Normally, *Solen* sp. has a tiny slender body with parts cleverly modified for the purpose of digging faster. Shortly after the organisms are pulled out of the substrate by the passing wave, they dig it back before the water sways them out. An example of organism with such ability is donax clam and razor clam (Nybakken and Bertness 2005). The research site is an exposed, therefore this adaptation process in the area requires a considerable amount of energy which causes declining weight development and single length growth that leads to negative allometric growth. Meanwhile, a fore-and-aft body type in some species such as razor clam allows them to dig deeper with less energy for protection from predators (Urban 1994). In addition, its lightweight shape prevents them from going too deep in the hole, and stability to go against the undercurrent is essential for survival (Stanley 1970). Such body type and shape as found in *Ensis macha* including *Solen regularis* has the proportion of length more superior than the weight that facilitates stability against the current, as well as adaptive strength to become an effective digging organism.

Von Bertalanffy's growth model

The result from parameter analysis on *Solen regularis* growth with ELEFAN I method in FISAT II is L∞ = 80 mm and k = 0.7/year. Estimation on t_0 is acquired from Pauly's empirical formula (1979) with the value of t_0 =-0.003. It can be deduced that the von Bertalanffy's growth equation is $Lt = 8.0\ (1\text{-}e\ (-0.7^{(t+0.003)}))$ (Figure 2)

Saeedi et al. (2009) found that *Solen dactylus* Persian Gulf, Iran might grow approximately 22-29 mm every year, and transects both near to and far from the coast have parameter estimation of L_∞: 101 and 108 mm respectively, k value of each transect is 0.27/year, and 0.28/year, and t_0 of each transect are-0.99 and-0.94. Baron et al. (2004), whose research was on *Ensis macha* living in Argentine and Chile, came up with L_∞ 154-153 mm, k = 0.27/year-0.28/year, and t_o =-0.08-0.72. Meanwhile, observation conducted by Fahy et al. (2001) on *Ensis arcuatus* showed in L_∞ 145-159 mm, k = 0.28-0.43/year, and t_0 =-0.26-0.3.

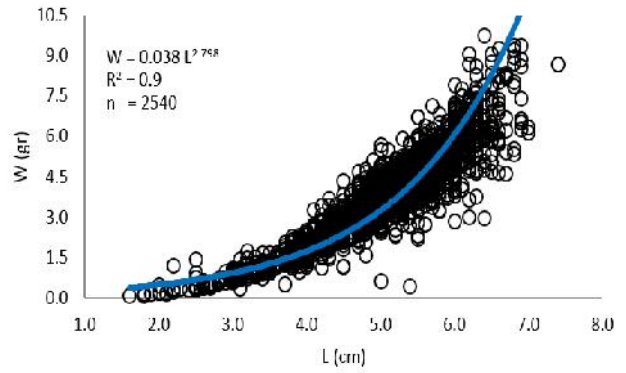

Figure 1. Graph of length-weight correlation in Solen regularis in Surabaya east coast

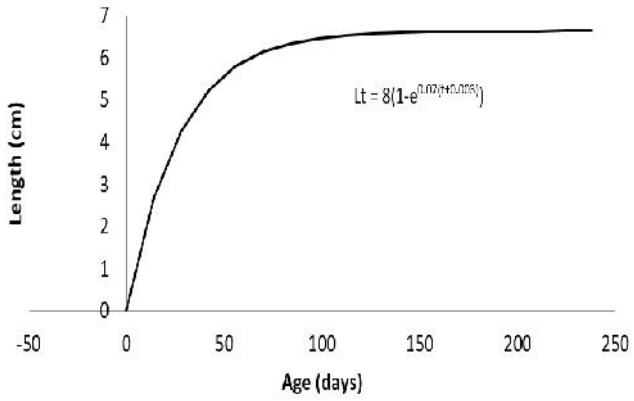

Figure 2. Solen regularis growth in Surabaya east coast

As researched by Otero et al. (2014) on *Ensis arcuatus*, the clams display a swift growth in the first three years of life. The rise on the first year is 50-80 mm; 33-37 mm between the first and second year, and14-18 mm between the second and third year. From there, the growth gradually declines between the forth to sixth year (with estimated rise only 5-10 mm), and finally will undergo asymptotic phase at 8-9 year (with growth rise less than 5 mm per year). The asymptotic size (L_∞) varies between 140 and 174 mm, while the growth constancy (k) is around 0.24 and 0.57 per year. The longest living expectancy of the species is approximately 13 years.

The *Solen regularis* growth in Surabaya east coast has relatively smaller L_∞ value compared to that in *Solen* sp. or *Ensis* sp. in other countries, but the growth coefficient is relatively faster, 0.7 per year. It is found that during the research, *Solen regularis* may grow as long as 15.2-75.4 mm. The difference growth pattern in the same species is caused by several components, such as the number of the samples, and other external factors, such as convenient environmental condition for the development of this species (Innal et al. 2015). *Solen regularis* living in tropical areas generally have smaller shell. In Malaysia, as observed by Rinyod and Rahim (2011), *Solen regularis* show similar size of shell as those observed in Surabaya, 60.72 ± 9.77 mm in Asajaya Laut, and 58.44 ± 5.65 mm in Kampong Buntal. The growth coefficient on *Solen regularis* is higher presumably due to the fact that tropical countries facilitate maximum metabolism as there is no seasonal problems. In subtropical areas such as the northern part of Persian Gulf, sea clams may grow and reproduce well due to seasonal winds, high nutritional concentration, and phytoplankton supply (Saaedi et al. 2009).

Mortality rates and exploitation

Normal mortality rate (M) during the research with average annual water temperature 28°C is 2.05/year. The average normal mortality is 2.05/year which indicates that deaths occur regularly in the research location as Pauly (1984) states that such number is higher than the maximum mortality rate, 1.5/year.

Fishing mortality rate (F) on *Solen regularis* during the observation is 2.01 per year. The number indicates intensive fishing activities. Pauly (1984) developed an optimum fishing rate concept that it may be achieved if the value is the same as the that of natural mortality rate (Foptimum = M). It also point to the fact that deaths of the clams in Surabaya east coast are mainly caused by fishing. Gulland (1971) states that if F > M, the status of fishing can be categorized as overexploited, while Amani et al. (2011) find that if fishing activities cause more mortality than the normal, it can be concluded that imbalance has occurred in the stock.

Analysis on the total mortality (Z) using the equation from Beverton and Holt (1957) in the FISAT II software shows that the total mortality number is 4.05 per year. Laudien (2002) find the total mortality (Z) in immature donax clams at 4: 26 per year. The high mortality caused by predation shore birds. The number is acquired from both normal mortality (M) and fishing mortality (F). A graph on mortality can be seen in Figure 3.

According to Sparre and Venema (1999), the considerable number of mortality from fishing is caused by the activity particularly those using fishing gears, the absence of operational area boundaries, lack of information from the local government or related institutions to the fishers on the importance of preservation, and the unavailability of regulations on the size of the fish allowed for fishing and trading. Those factors apply to fish and alike, such as clams. Regarding to this problem, Bahtiar (2005) states that when fishing activities in an area are massive or exactly equal to the number of parent population, the schools will lose their members gradually and at a certain level, the organisms are threatened to extinction.

The exploitation rate (E) of *Solen regularis* during the research is 0.50/year. This informs than 50% of the sample population are taken away by fishing activities. It is based on the concept of exploitation rate developed by Gulland (1971) and Pauly (1984) that the optimum exploitation rate value is 0.5/year (Eoptimum = 0.5/year). Also, referring to the concept, the exploitation rate of *Solen regularis* in the Surabaya east coast has achieved the exact optimum value of Eoptimum.

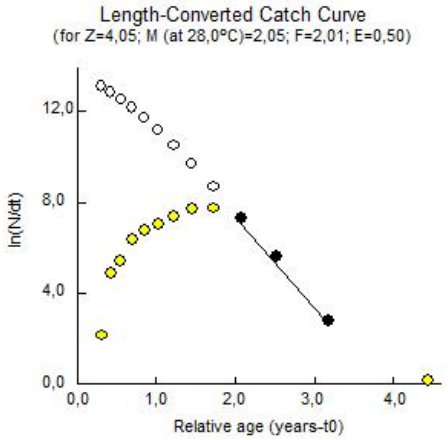

Figure 3. Mortality rates and exploitation of *Solen regularis* in Surabaya east coast

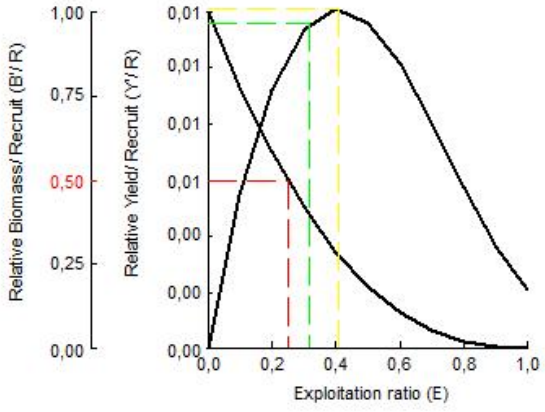

Figure 4. Y/R dan B/R value of *Solen regularis* in Surabaya east coast

Figure 4 shows the B/R value of 0.014/year that indicates the number of *Solen regularis* population in the water, 1.4%/year. Meanwhile, the Y/R value also displays the same number, meaning that 1.4%/year of *Solen regularis* is captured in the eastern coast. The same number of B/R and Y/R implies that the number of fishing is the same as that of biomass in the water.

The B/R value of 1.4%/year gives use information of then tiny number of population of *Solen regularis* in the Surabaya east coast, that it is highly encouraged that efforts on limiting fishing activities should be taken into consideration to restore the number of biomass in the water. The parameter that may be controlled is the fishing mortality rate (F) or time and number management of fishing outputs.

Solen regularis capture on Surabaya east coast must consider the existing stock, because the value is already optimal exploitation. Management is done with the cooperation between local governments and local communities for the clam sustainable use.

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Wati (*Piper methysticum*) medicinal plant: The ethnobiological and ethnomedicinal values of the Marind tribe in Merauke, Papua, Indonesia

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Abstract. *Suharno, Tanjung RHR, Sufaati S, Agustini V. 2016. Wati (*Piper methysticum *L.) medicinal plant: The ethnobiological and ethnomedicinal values of the Marind tribe in Merauke, Papua, Indonesia. Biodiversitas 17: 814-822.* Biological resources around neighborhood play important roles in the cultural development of the surrounding communities, including the use of plants. *Wati* (kava, *Piper methysticum*) is one of the species that has long been used as a traditional medicine and cultivated by Marind tribal community in the lowlands of Merauke, Papua. The aim of this study is to examine the use and domestication of *wati* plant by Marind tribe in Papua. Results of the study showed that *wati* plant has long been used by the Marind tribal community as a medicinal plant with high customary value. Each customary event includes *wati* plant as a complementary requirement for legitimate activities by the Marind tribe. It is the importance of customary values that led the domestication of *wati* plant done since 60 years ago on a small scale to eventually develop into plant called as "the Marind people's gold". Results of the observation showed that 93.8% of the Marind people have largely recognized *wati* plant, while 53.3% of the immigrant communities from outside the area recognized it, but only 33.3% knew about its utilization. Although not all indigenous elders cultivate it, they recognize, utilize, and understand the rules of using *wati* plant in traditional events and as traditional medicine. As traditional medicine, the parts used by Marind people use root (100.0%), stem (96.6%), and leaves (89.7%). For customary events, the most important parts are the whole plant (100.0%), stem (100.0%), leaves (98.3%) and roots (93.1%). Their children even recognize it and know its benefits, but most of them 31.25% only utilize it but are prohibited from participating in its preparation (0.0%). The domestication of this plant is quite unique because it is closed to the public and is still done by a conventional method.

Keywords: Ethnobiology**,** ethnomedicine, Marind tribe, Papua, *Piper methysticum*

INTRODUCTION

As an archipelagic country, Indonesia is abundant with natural resources, including the diversity of plants. More than 30,000 plant species are found there in where 9,600 species are medicinal plants (Suharno et al. 2011; Putri et al. 2016). Over than 1,800 plant species known exist and were planted in several forest formations, with 940 plant species used by local people for traditional herbal medicine and only 300 species by drug industries (Putri et al. 2016). In Papua, various medicinal plants species with cultural value are quite significant in number (Suharno et al. 2011).

Wati or *kava* plant (*Piper methysticum* L; Piperaceae) is one of the local plants in Papua. It grows well in lowlands in Merauke Regency (Nova 2009). The plants with woody shrubs stature growing in the wild, can reach a height of 5 m, and can be cultivated by slip and generative. In nature, most individuals are male species (Cassileth 2011; Tanjung et al. 2014). In internationally world, the plants are well-known as "kava" and largely mostly used by local people as traditional medicine (Lebot and Simeoni 2004; Nova 2009).

Wati plant is used to treat rheumatism, respiratory tract infections, tuberculosis, gonorrhea, and headache. Its leaves and stems contain several compounds that can be utilized as with anti-stress, analgesic, and psychoactive drugs (Backhauβ and Krieglstein 1992; Kavanagh 2009; Tanjung et al. 2014) as well as antibacterial (Amorim et al. 2007) and anticancer ones (Hashimoto et al. 2003; Tabudrayu and Jaspars 2005). The roots of *wati* plants have been utilized as a traditional beverage in many countries, including in the Pacific Islands (Balick and Lee 2002; Anke and Ramzan 2004). In the last few decades, *wati* plants have been known to have sedative and anxiolitic properties (Boon and Wang 2003; Garrett et al. 2003; Sarris et al. 2009; Shanti and Avinash 2013). The plants have potential as a source of compounds for pharmacy because they contain a variety of lactone compounds such as sedative, soporiphyic, analgesic, and actinoconvulsive ingredients as well as local anesthetics, muscle relaxants, and diuretics (Davis and Brown 1999; Briskin et al. 2001; Laporte et al. 2011). In adversely, other some studies indicated that *wati* plant might have implications on liver damage for users (Anon. 2002; Anke and Ramzan 2004; Ulbricht et al. 2005; Amorim et al. 2007; Martin et al. 2014; Hussein 2015).

As one of the regencies in Papua Province, Merauke has a population of 246,852 people (data per December 2013) and became one region in the eastern of Indonesia

directly that borders with Papua New Guinea (PNG). Before the splitting of region, Merauke regency had approximately 29% of the territory of Papua Province. However, after several regions were splitted, the remaining is 46,791.63 km^2, which covers 20 regencies, 8 urban villages, and 160 villages (www.merauke.go.id). Indigenous peoples that inhabit Merauke territory are from Marind tribe, consisting of several large groups at swamps, beaches, and inland.

In their life, the Marind tribe has very high traditional values and depends on environment and forest products. The community uses forest products in a variety of both official and unofficial events and activities. The position of various plant species as symbol and identity of the ethnic group is still high. *Wati* plant is one of the customarily invaluable plant species, so it is called as "the Marind people's gold". For the Marind people, *wati* plant has a significant value for the benefit of local customary people, thereby increasing economic value to the family. Davis and Brown (1999) and Shanti and Avinash (2013) revealed that change in peoples' attitudes also affects the change in utilization of natural resources for the survival and socialization process among them. The purpose of this study is to investigate the traditional utilization of *wati* plants as medicine by the Marind people in Merauke, Papua, Indonesia.

MATERIALS AND METHODS

Study area

The study was performed through survey from May to June 2016 in Merauke District, Papua Province, Indonesia. The survey was held for three weeks in several locations where *wati* (*P. methysticum*) plants were naturally found or cultivated by local people. It was made randomly in villages representing several sub-districts, i.e. Okaba, Merauke, and Sota (Figure 1).

Wati plants are evenly grown in the lowland areas in the southern part of Papua. On a large scale, the cultivation of *wati* plants is concentrated in Merauke Sub-district and Sota Sub-district . Mean while, the plant are considered as "sacred" for the Marind people, so that not all locations can be accessed for detailed information. However, some major

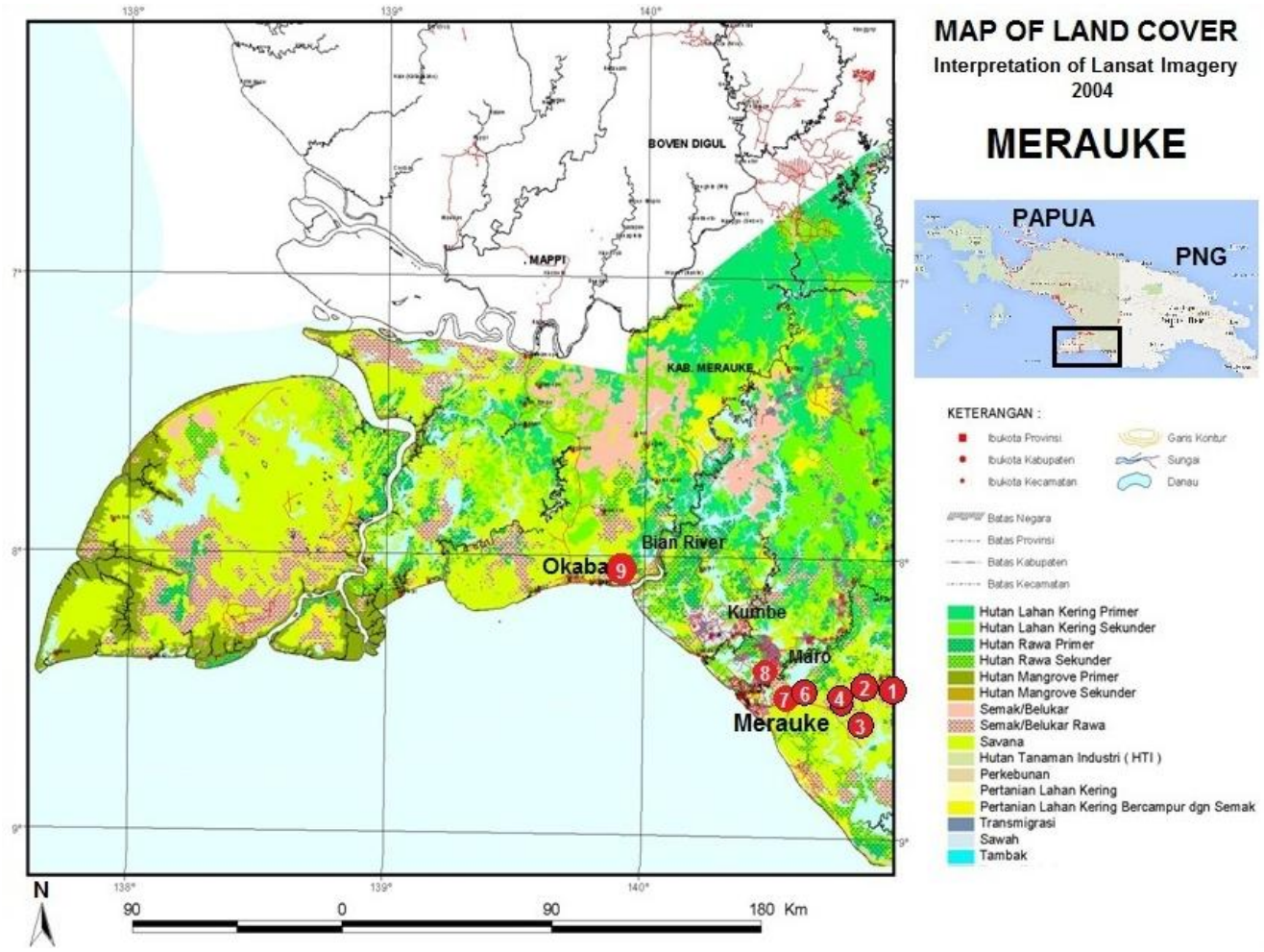

Figure 1. Location of the study in several Sub-districts in Merauke District, Papua, Indonesia: 1. Sota village, 2. Pinje, 3. Yanggandur, 4. Wasur I, 5. Wasur II, 6. Mangga Dua-Johar, 7. Mangga Dua, 8. Kuprik, 9. Sanggase

Table 1. Location of sampling in some major villages in Merauke, Papua

No.	Location		Coordinate position	Alt (m asl.)	Temperature (^oC)
	Village	Sub-District (Kecamatan)			
1.	Sota, Sota	Sota	S: 08°25'42.5"; E: 141°01'01.8"	22	31.0-33.0
2.	Pinje Km 67, Sota	Sota	S: 08°26'54.6"; E: 140°53'31.1"	20	31.5-34.0
3.	Yanggandur	Sota	S: 08°28'31.6"; E: 140°50'24.4"	26	31.0-32.5
4.	Wasur I, Rimba Jaya	Merauke	S: 08°31'26.3"; E: 140°31'10.2"	21	29.5-31.5
5.	Wasur II, Rimba Jaya	Merauke	S: 08°31'24.2"; E: 140°31'13.1"	21	30.0-31.5
6.	Mangga Dua-Johar, Kelapa Lima	Merauke	S: 08°28'35.1"; E: 140°24'21.5"	15	30.0-32.0
7.	Mangga Dua, Kelapa lima	Merauke	S: 08°28'45.4"; E: 140°24'20.1"	20	29.0-30.0
8.	Kuprik	Semangga	S: 08°28'01.7"; E: 140°26'12.2"	25	25.0-33.5
9.	Sanggase, Okaba	Okaba	S: 08°03'52.5"; E: 140°01'03.2"	19	26.0-29.0

locations such as Merauke Sub-district and Sota Sub-district are considered to represent as samples of other various locations where *wati* plants are cultivated (Table 1).

The utilization of *wati* plant by the Marind People

Direct observation was done to examine the utilization and cultivation of *wati* plants. Questionnaires and interviews were used to gain additional information. The study involved 74 respondents from 9 observation sites included Merauke Sub-district, Sota Sub-district, Semangga Sub-district, and Okaba Sub-district. Informants were selected with purposive sampling technique. The respondents can be categorized as key informants, main informants, and additional informants, who were from social/customary figures, farmers or users of *wati* plants. Of the 74 respondents, 58 were from the Marind tribe and 15 were migrant communities outside of the tribe. As for the migrants, they were specially selected based on the period (>5 years) of living in Merauke and were considered as adult. The detail of respondents were as follow; from social or customary figures; 10.3%, male or female adults 62.1%, and children 27.6%. Data were collected from key informants, then substituted by other informants such as customary figures and laypersons who use *wati* plants in customary events and as traditional medicine. Interviews were conducted using semi-structural and open-ended technique with all informants in the survey locations and other informants deemed relevant (Walujo 2004; Zebua and Walujo 2016).

The cultivation of wati plant by the Marind People

The techniques of cultivating *wati* plants were known through interviews with *wati* plant farmers. The latter was interviewed for more accurate and reliable information. The interviews were carried out in five active farmer groups when the direct observation was done in the field. The data obtained were presented in the form of descriptive information. Samples of soil taken for soil fertility levels were analyzed at the Laboratory of Soil, SEAMEO-Biotrop, Bogor.

Data analysis

Results of the observation were analyzed by a descriptive technique. The data process involved organizing, sorting, categorizing, evaluating, comparing, and synthesizing data, and drawing conclusions.

RESULTS AND DISCUSSION

The utilization of *wati* plant by the Marind People

Results of the study showed that *wati* plants have long been utilized by the Marind people with customarily high value. It unknown the use and domestication of wati plants within Marind tribe, but they have long been used since their ancestors for generations. In the other hand, intensive cultivation is is known to have started at small scale from 60 years ago.

Wati plant has a high value because it is used in various customary events of the Marind tribe. In fact, in the resolution of specific cases related to conflict between social groups, *wati* plant should be provided as a means to unite them in solving the problem. By brewing water with or directly chewing *wati* leaves, the entertainment is served during the community meetings or into the conflicting tribes. They used water as sedative to facilitate the consultation for consensus to resolve the conflict among communities. According to Davis and Brown (1999), *wati* plants are associated with mystical and ceremonial events as indicated in the Marind tribe people in Merauke. Most residents keep this issue because according to traditional elders some people take advantage of *wati* plants for the negative activities that harm others.

The observation showed that 93.8% of the Marind tribe were able to recognized *wati* plants, while 53.3% of the immigrant from outside the area have recognized the plants, with only 33.3% knew about its utilization. Although not all traditional elders or leaders cultivate the plants, they have recognized, utilized, and understood the rules of the use of *wati* plants in the traditional events and as a traditional medicine. Their children were also recognized *wati* plants and knew the benefits, but only 31.2% of them have utilized them and they were prohibited from participating in the preparation process (0.0%) (Table 2).
The knowledge of *wati* utilization among the Marind tribe is almost the same between men and women, with survey indicated that more men serve as *wati* farmers (90.5), while women were only 53.3%. Most children as respondents revealed that some of them (12.5%) actively helped their parents for cultivating *wati* plants, but they did not understand cultivation techniques done by their parents. The knowledge of customary rules for children is also not informed by their parents as indicated that no respondent (0%) among children knew such rules. For migrant

communities, information about *wati* plant is less. At least only 53.3% of respondents knew *wati*, and only 33.3% knew the benefits of *wati* (Table 2). Moreover, they also do not understand about the function of *wati* plant as traditional medicine and its utilization in traditional events of the Marind people.

The utilization of *wati* plant in customary events

The use of *wati* plants in customary event is to the role of pig in a variety of formal and informal events. Some important traditional events where *wati* plant plays complementary requirement in legitimated activities such as girl-proposing, wedding ceremony, celebration of special occasion, ceremonial meal for died people, special guest banquet, even the meeting of traditional leaders in solving inter-ethnic and other problems. Either large or less materials of *wati* plant and pig provided in these events show the social level of the relevant organizing community. For a customary event, the most important parts of *wati* plant utilised were all parts (100.0%) and stem (100.0%) of *wati*, followed by leaves (98.3%) and roots (93.1%) (Figure 2).

In a girl-proposing event and a wedding ceremony, *wati* plant is used as *ube rampe*. In such event, the utilization of *wati* is integrated as a customary function and a medicinal function. Usually *wati* plant is used as medicine, it is served at the end of the event. Some customary figures revealed that other roles of *wati* plants were highly confidential as to maintain security within an ethnic group and among the groups outside their tribe.

The utilization of *wati* plant as traditional medicine

Table 2 shows that all customary elders and both male and female adults understand about *wati* plant as medicine. Not all women know, however, how to process *wati* plant used as traditional medicine. Some information suggested that this plant is often used as drug of cough, influenza, aches, muscular pain, tonic for the body (fatigue), sedative, maag, injury, malaria, even AIDS. The preparation procedure is very simple and traditional, just by making *wati* plant in drinks. In use as a medicine, the parts mostly used by were roots (100.0%), stem (96.6%), and leaves (89.7%). For both customary elders and adults, they understand how to use the plant, and only 75.0% of children knew that *wati* plant can be used as a medicine and a small portion of them (12.5%) know how to use.

The cultivation of wati plant

The Marind tribes of Merauke lowland still has a farming habit with a nomadic agricultural field system. It is evident that the small-scale gardens cultivated are still nomadic, although they are in areas that are not too far from the settlement occupied. The gardening technique applied is still very simple. Most activities in *wati* plant cultivation are dominantly carried out by men as heads of the family, although mothers and other women also played an important role.

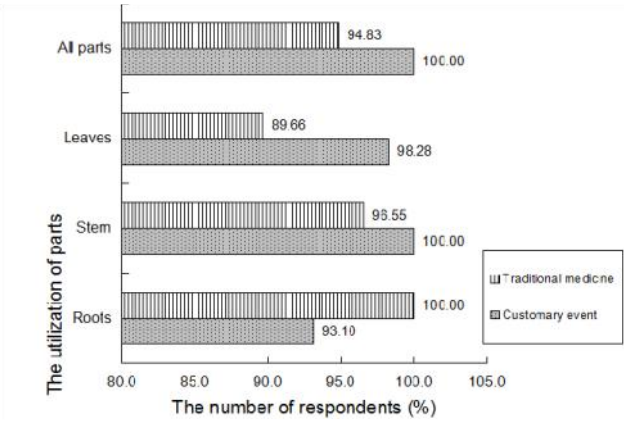

Figure 2. The utilization of parts of *wati* (*P. methysticum*) plant by the Marind tribe in traditional medicine and customary events in Merauke, Papua

Table 2. The Marind tribe people's knowledge about the utilization of *wati* plants in Merauke, Papua

Knowledge of communities on wati plant	The respondents of Marind tribe (percentage)					Migrant communities	
	Customary figures	Men	Women	Children	Total	Total	%
Know the plants wati	6 (100)	21 (100)	15 (100)	15 (93.75)	57 (98.28)	8	53.33
Know the benefits of wati plant	6 (100)	21 (100)	15 (100)	13 (81.25)	55 (94.83)	5	33.33
Never used	6 (100)	21 (100)	15 (100)	5 (31.25)	47 (81.03)	0	0.00
Never mix potions	6 (100)	21 (100)	4 (26.67)	0 (0.00)	31 (53.45)	0	0.00
Performers cultivation	5 (83.33)	19 (90.48)	8 (53.33)	2 (12.50)	34 (58.62)	0	0.00
Understand cultivation techniques	6 (100)	19 (90.48)	8 (53.33)	0 (0.00)	33 (56.90)	0	0.00
Know the rules of customary	6 (100)	21 (100)	8 (53.33)	0 (0.00)	35 (60.34)	0	0.00
Knowledge of wati as medicine	6 (100)	21 (100)	15 (100)	12 (75.00)	54 (93.10)	0	0.00
Know how to use as a traditional medicine	6 (100)	21 (100)	12 (80.00)	2 (12.50)	41 (70.69)	0	0.00
Knowledge of wati as customary	6 (100)	21 (100)	15 (100)	9 (56.25)	51 (87.93)	0	0.00
Know how to use a customary	6 (100)	20 (95.24)	14 (93.33)	0 (0.00)	40	0	0.00
Knowing the risks of misuse of customary	6 (100)	20 (95.24)	14 (93.33)	0 (0.00)	40	0	0.00

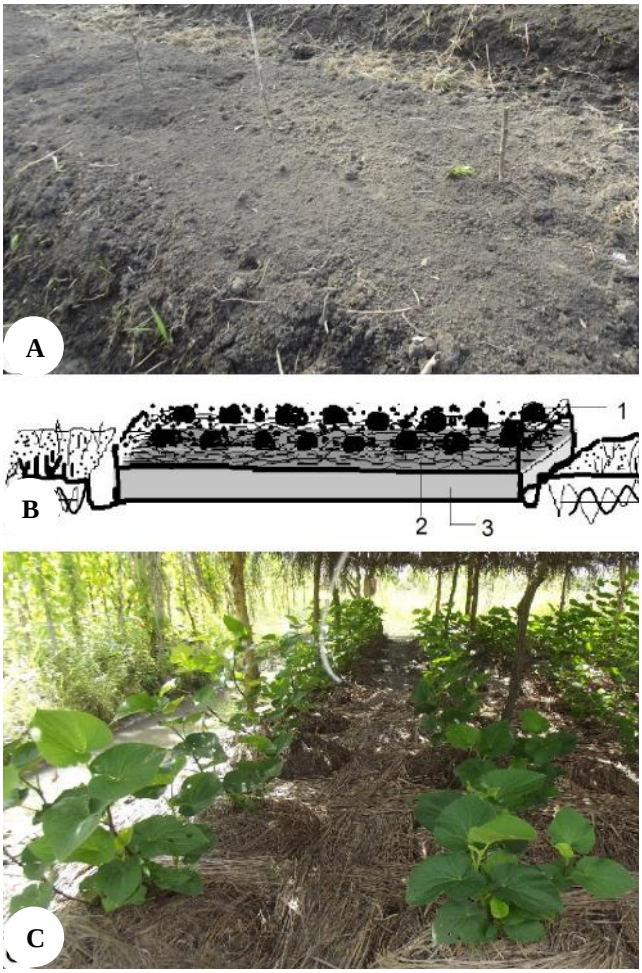

Figure 3. *Wati* plant preparation and cultivation. A. Making seedbed, B. Making the graphic line of land used in *wati* plant cultivation (1. mound (*kuming*), 2. litter, 3. soil seedbed). C. *Wati* cultivation land.

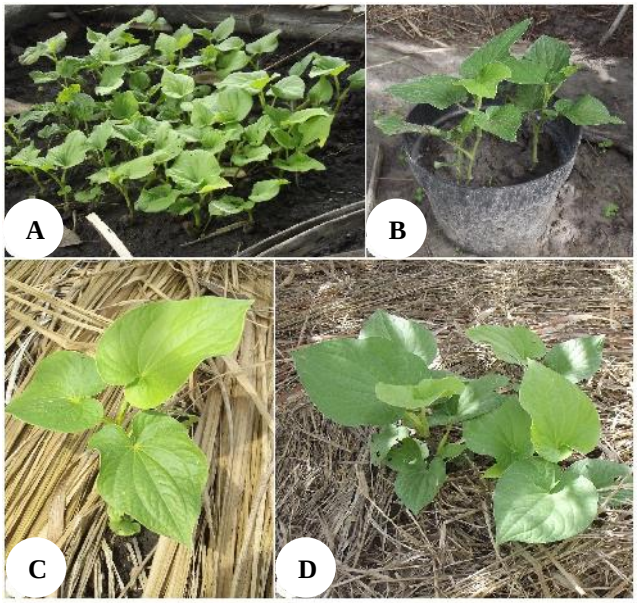

Figure 4. Overview of *wati* seeding and planting. A. Planting seeds that will be used by using a stem cutting technique on land, B. Preparation of seeds using stem cutting technique in pots, C. At one mound, there is one plant grown on land, and D. At one mound there are more than one plant

Table 3. Results of the analysis of soil physical and chemical properties of soil in cultivation land in the lowlands of Merauke, Papua

Parameters of physical and chemical properties		Location of village			
		Sota	Pinje, Km67	Wasur	Mangga Dua
pH (H_2O) (1:1)		5.3	5.6	5.7	5.1
pH ($CaCl_2$) (1:1)		4.6	4.5	4.5	4.4
C organic	(%)	1.75	4.54	0.58	5.35
N total	(%)	0.12	0.24	0.08	0.54
Ratio C/N		14.6	18.9	7.3	44.6
P (P_2O_5) available	(ppm)	14.0	7.3	8.2	3.4
K	cmol.kg^{-1}	0.51	0.61	0.23	0.78
Ca	cmol.kg^{-1}	3.66	1.96	1.98	2.06
Na	cmol.kg^{-1}	1.33	2.04	2.45	2.55
Mg	cmol.kg^{-1}	1.05	1.33	1.28	1.48
CEC	cmol.kg^{-1}	9.94	18.32	6.95	26.69
Base saturation	(%)	65.90	41.92	85.47	25.74
Al-H_{dd} KCl 1 N:					
Al^{3+}	(me/100g)	0.00	0.00	0.00	0.30
H^+	(me/100g)	0.10	0.10	0.10	1.63
Soil texture:					
sand	(%)	39.3	21.5	14.8	2.3
dust	(%)	18.6	42.0	45.6	67.0
clay	(%)	42.1	36.5	39.6	30.7

The land required for *wati* plant cultivation is generally very suitable for plant growth. The results of analysis of the soil in the Merauke lowlands showed that pH of soil ranged from 5.1 to 5.7 (H_2O) with an average of 4.5, content of soil organic C from 0.58 to 5.35%, total Nitrogen (N) from 0.08 to 0.54%, C/N ratio from 7.3 to 44.6, and phosphorus (P) content available from 3.4 to 14.0 ppm. Several cations can be exchanged such as calcium (Ca), ranging from 1.98 to 4.44 cmol.kg^{-1}, magnesium (Mg) from 1.05 to 1.56 cmol.kg^{-1}, potassium (K) from from 0, 23 to 0.78 cmol.kg^{-1}, natrium (Na) from 1.18 to 2.55 cmol.kg^{-1}, and CEC (cation exchange capacity) from 6.95 to 26.69 cmol.kg^{-1} (Table 2). Most areas have a clay loam soil texture to the dusty clay.

Discussion

The importance of *wati* plant is seen from a variety of customary activities that have utilized this plant as a part of the ritual. The customary elders/figures and the leaders of various clans in the Marind tribe consider that an event will not be "legitimate" without the presence of *wati* plant, meaning that *wati* plant must be absolutely available. The Marind community regards that *wati* plant is the first property, so that in any customary events *wati* plant is the main material that must be presented. *Wati* plant is also essential for certain traditional parties, for example, at grievance *wati* is used at the end of event, i.e. as a drink. The utilization of *wati* as a medicinal plant has also been done from generation to generation since the past. Someone who is exhausted usually consumes *wati* drink. However, for such case the consumption is only for adult, while children and unmarried people are still prohibited.

Scientifically, *wati* plant contains compound similar to anesthetic (Anon. 2004; Sarris et al. 2009; Laporte et al. 2011). People who drink it will feel "fly" and in a short

time (approximately 15-20 minutes) will make the person asleep. It is the time to sleep that will affect the rest of the body to work, because he/she will not remember anything else until waking up the next morning in a fresh condition. The duration of sleeping time varies, depending on the high or low content of *wati* drunk. This makes the Marind people knows that there are some *wati* plants with diverse types.

Wati plant with high-quality (easily intoxicating) is characterized by black or red color and short nodus. *Wati* black is often referred to as *palima*, while the short nodus *wati* is often referred to as *fangge yambad*. People are familiar with several types of *wati* plant leaves, some stated that there are three, five, and even seven types. This difference is due to different factors in certain regions. In view of the existing number of clan, each of the seven clans has generally its own characteristic. It is not clear whether or not the types of *wati* plant actually have specific difference to species level. However, if viewed morphologically, all "types" of diverse plants are still part of the plant species of *P. methysticum*. According to Davis and Brown (1999), there are many cultivars of *wati* plant species as indicated by the property of upright stems, stem color, internodes relative length (segments) its and hardness, lenticel distribution on stem, leaf color, and the aging process of leaves and hairs on the leaf surface. Lebot et al. (1999) revealed that there is the morphological variation of *wati* plant as a morphotype from various sources of different locations.

The product of *wati* plant is used as traditional drink. The way of making this drink is very simple, but it is only done by certain people such as customary elders, those who believed or elders who are usually agreed upon by community groups in the village they live (Table 2). The aim is to limit the making of *wati* drink in order that not all people can do it, so that the use and exploitation can be controlled. A product such as the filtered beverage liquid can be consumed. Some people still keep some information about how to make the traditional drink of *wati* as medicine. Ernst (2007) revealed that such anxiolytic herbal medicine has also been sold well in UK and other countries. In UK, *wati* plant is packaged in herbal medicine or dietary supplements. Many cases showed that *wati* is believed to be the cause of hepatotoxicity, even 100 cases reported in the world are associated with liver disease. According to Davis and Brown (1999), kava has been widely explored in South Pacific region, for example in Fiji, Tonga, Vanuatu, Samoa, and areas of Micronesia. This plant is important for traditional and ceremonial events in community and has been cultivated. According to Cassileth (2011), *wati* is also a native plant in the Hawaii islands where rhizome and its roots have widely been used as a non-fermented beverage with relaxant effect in order that people can be more relax. It can be used in social events and usually made as a ceremonial drink since hundred years ago in the Pacific islands.

In toxicological studies, some recent studies show that kavalactone and its extract have the effect of low toxicity level. *Wati* plant has also potential to cause drug interaction that inhibits cytochrome P450 enzymes. The constituents of kava, flavokavin, and pipermetistin showed cytotoxic in vitro, but in other studies there is no toxicity or hepatoprotective effect in treatment (Ernst 2007). Since 1999, the professional observers of health in some countries such as Germany, Switzerland, and the United States reported several cases possibly associated with the use of *wati*-related products (Anon. 2002), while in Canada the information is still minimal (Boon and Wong 2003).

All parts of *wati* plant can be utilized by community in a variety of events. However, people know and understand which parts can be utilized as needed. For wedding ceremony, particularly in proposing to a girl, the man's party is required to bring a few clumps (each clump is composed of some trees) as a requirement for "legitimate" proposing to the girl. Typically, these plants are taken as a whole from the roots to the stems and leaves, then strung together with other agricultural products such as bananas, batatas, lesser yam (*Dioscorea esculenta*), and fruits. All of these *ube rampe* are given to the family of the girl. Many interesting stories from results of this survey, but not all of the respondents are willing to share the story in depth as it relates to community tradition. Therefore, the research team also keeps some of the stories that are considered to be confidential in order that no misunderstanding occurred in the community, especially in Merauke.

Wati cultivation system among the Marind Tribal Community in Merauke

Cultivation system applied by indigenous people in Merauke (the Marind tribe) is a nomadic agricultural field system. According to Suharno (2001), most areas in Papua still use the nomadic agricultural field system, particularly local communities. This condition prominently appears in areas with locations far from town. Most lands in the lowlands of Merauke are under wet and marshy condition. Seasonal patterns affect the agricultural system. In the rainy season, most of the marshy areas will be inundated by water, while in the dry season most lands in the areas are dry. The type of muddy clay soil dominates the lowlands (Table 3).

Some fields are treated with a levee system or beds to avoid puddles when water is excessive due to high rainfall or other factors. Meanwhile, in suburbs and rural areas, people are free to choose land deemed suitable for gardening and free to determine their respective lands. In transmigration areas, people more use their land for planting vegetables and utilize the beds for growing rice paddy. Rice production in these areas is quite high, so Merauke is well-known as the granary of rice in the southern of Papua.

The prevailing customary system in Papua region affects the agricultural system in *wati* plant cultivation. Recently, *wati* plant is considered as a heritage with the highest cultural value as it is utilized as a part of the customary activities. In the cultivation system, the plant is still considered to be closed to the public, although there are some people who started to open up the information on how to develop and cultivate this sacred plant. The limitation of customary rule system is also important for safety and preventing from its misuse for the things that harm others.

***Wati* plant cultivation**

Information about *wati* plant cultivation for the common Marind people is very confidential, so that not all tribes/social groups can provide this information completely. The heads of family play a great role in cultivation of *wati*. Most farmers give credence to fathers/husbands/men to maintain *wati* garden. The reason is that the workload in taking care of the field is large. Mothers or women are not usually associated with this work due to such consideration. The role played by men to make *wati* garden can be seen from their involvement in farming from the process of clearing the garden, making beds, planting, and maintenance to harvesting. Some of them also involve their children. However, the research team found a farmer who involved a mother to maintain *wati* plant because he is less successful in farming, including in *wati* cultivation (Tanjung et al. 2014).

Selection of land. Location is selected based on the ease of access (such as yard and customary land), security (to minimize the misuse of *wati* plants), and the previous experience of quality crop, so that each tribe/community group has different methods.

Land clearing. Land clearing begins with clearing grass, shrubs, and trees at the location. This process is performed by a family together, especially the men, including their boys. The making of bed (*wambad*) is compulsory for *wati* garden. The beds are built with different sizes, averagely 3-4 m in wide and 10-25 m in long. Bed height greatly varies from one site to another, averagely ranges from 30 to 60 cm.

The process of making the cultivation land is done in steps (Tanjung et al. 2014), i.e.: 1) The making of beds (*wambad*); the height of *wambad* is adapted to the rising waters and to avoid the sinking of beds during the rainy season; 2) The addition of grass litter. The goal is to make the grass as organic fertilizer for the provision of nutrients for plant growth. Each village has different habits due to the experience of different communities. 3) A thin layer of black soil that is regarded as being able to strengthen plants to be more fertile. 4) The making of mound "*kuming,* local term". Mound is round-shaped of 8-15 cm in height with a diameter of 20 cm that serves to lay *wati* seeds. Mound is made with a planting distance of 60-100 cm between plants. 5) The grass cover. Mound is then covered with grass around it about 15-20 cm in tall, thinly covering mound.

Para-para of 160-180 cm in height must be made as shading protector for *wati* plant in the beds from direct sunlight. The condition will optimize the growth of *wati* plant. According to Davis and Brown (1999), several studies that used the treatment of shading and open land show that plant growth significantly increases in several parameters such as the number of stems per plant, stem length, leaf and dry weight of stem (kg), and sale value, while the parameter of leaf width did not affect it significantly.

Seeding and planting. The easiest and fastest way to provide seeds is to make stem cuttings as suggested also by Davis and Brown (1999). The observation also indicate that all social groups who have cultivated *wati* plants in Merauke have applied the same method. Nevertheless, some technologies such as tissue culture can also be developed in the effort to provide seeds, such as tissue cultures in root, crown, and trunk axial bud on *wati* plant stem (Li and Zheng 2012). The seeds are planted by putting on mound that was made before. In one mound, one or more seeds can be planted. This will affect the treatment when the plant grown (Figure 3).

The observation showed that the provision of seeds can be done by using pots, buckets, or directly in a specific land by adding "black" soil. The cut seeds are allowed to grow shoots and laid under shade. When shoots were grown about 10-20 cm, or 20-30 days old, these seeds can be transferred to the cultivated lands. There are two planting systems: "single" and "double" planting systems, i.e. in one mound a seed is planted and in other mound more than one (usually 3 or 4) seeds are planted.

In addition to *wati* plant, in bed other crops are also planted. Traditionally, there is a group of other crops used to support the cultivation of *wati* plant. First, ornamental plants are planted as "decoration" or well-known as "*anggin-anggin*". *Anggin-anggin* is placed in the corners or among *wati* plants where 2-3 trees are planted in one bed. The ornamental plants are various such as croton (*Codiaeum variegatum*) and each clan has a different kind of croton. *Anggin-anggin* serves as a clan identity. Every garden with a certain type of croton will become the identity of clan that cultivates *wati* plants. It also applies at buying or selling transactions. When buyer requires *wati* plants in large quantity, *anggin-anggin* will be included in a form of croton identity. Croton signifies the clan from which they bought *wati* plants. Second, red ginger (*halia*, local name; *Zingiber officinale*) plant. Third, galingale (*Kaempferia galanga*). Galingale "must" be planted in bed to ward off pests and plant diseases. The technique for use is still very simple, by chewing its rhizome and sprayed on the leaves or stems that are exposed to disease or pests. If the disease occurs extensively, galingales are mashed before and sprayed into the parts exposed to pest and disease.

Maintenance. Routine maintenance includes the land clearing of weeds, the addition of litter among plants (fertilization), watering if necessary, branch multiplication, and propagation of clumps. The process of fertilization is done by adding the litter of certain plant species. All *wati* farmers in Merauke never used chemical fertilizers as the materials for fertilizing plants. They said that using the chemical fertilizers make *wati* plant "unable to intoxicate"; in other words, it has low quality.

Maintenance is done to keep quality plants by preparation of seed plan and disease-free plants. The diseases that are often found in *wati* plants in Merauke have not been recognized yet. Some types of worm can be a pest on *wati* leaves. According to Davis and Brown (1999), one of the diseases often found in some countries are "dieback" caused by cucumber mosaic cucumovirus (CMV) with the characteristics of slow plant growth and then death. Diseases due to fungi include anthracnose, leaf spot of *Sphaerulina*, *Sclerotium rolfsii* and *Fusarium* spp., while bacteria of the genus *Erwinia* is such as *E. carotovora*. Several nematode groups include spiral,

reniform, and "root-knot" nematodes. To overcome this problem, Nelson (2005) revealed the importance of an integrated pest control management.

Harvesting. It is carried out in accordance with buyer's demand, provided *wati* plants met the qualification of growth. This is done when the plant has grown about one year or reached about 50 cm in tall. A good crop to be harvested usually has a height of 50-120 cm.

Post-harvest. *Wati* farmers are very careful with buyer because of fear that *wati* they bought will be misused. Buyers with demand in large quantities will be asked to confirm what they will do with *wati* they want to buy. It is customarily governed by the Marind tribal community in general.

In *wati* plant cultivation system, there are some customary restrictions, for example, prohibition in gardening, i.e. women that have menstruation or just gave birth are prohibited from entering into seedbed. Meanwhile, menopausal women are allowed to manage and maintain *wati* plant, as she is considered pure.

For large purchase of wati plant, should be known by local customary leaders or the Customary Community Institution (*Lembaga Masyarakat Adat*, LMA) in Merauke. LMA has imposed special rules on the cultivation and utilization of *wati* in the life of indigenous population. According to Tanjung et al. (2014), they also agreed to set prices of *wati* plant to keep them under control.

According to Tanjung et al. (2014); as well as Angelique et al. (2015), the prospect of *wati* plant cultivation is very promising. The relatively high price become potential source of family income. David and Brown (1999) mentioned some benefits of *wati* plant cultivation. In the Fiji Islands, the economic value of the plant is higher than that of other crops such as sugar cane, copra, mango, papaya, pineapple, and various other types of plants. Similarly in Tonga and Vanuatu, *wati* plant has high sale price. Loew and Franz (2003) studied the traditional and industrial quality of *wati* plant. This plant has long been cultivated traditionally and has no serious effect. However, recently it is known that various products have a significant effect on the health of users.

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Handicraft of butterflies and moths (Insecta: Lepidoptera) in Bantimurung Nature Recreation Park and its implications on conservation

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Abstract. *Putri IASLP. 2016 Handicraft of butterflies and moths (Insecta: Lepidoptera) in Bantimurung Nature Recreation Park and its implications on conservation. Biodiversitas 17: 823-831.* The abundance of butterflies in Bantimurung Nature Recreation Park of Bantimurung-Bulusaraung National Park, South Sulawesi, Indonesia provides economic benefits to the community through butterfly's handicrafts trading. This study aims to determine local species of commodified butterfly that are traded in various forms of craft and its implications for the conservation of butterflies. The study was conducted through the direct identification of butterfly species which are sold as crafts or deposited directly by the catchers to collectors. Data of commodified butterfly were collected using direct interviews. Data were analyzed by descriptive quantitative and qualitative. The results showed that there are 142 species of butterfly which are traded in the period of 2010-2015. The seller participants on butterfly handicrafts consist of the butterfly catchers, middlemen, craftsmen, stall employee, stall employers, and street vendors. The buyer participants consist of local tourists, tourists from outside district/province, traders from outside district/province, buyers from overseas and scientists or butterfly collectors. The butterfly price range was in between Rp. 500.00-Rp. 150,000.00/head at collectors' level. The butterfly selling prices increased up to Rp. 7,500.00-Rp. 1,000,000.00 when they were processed into various souvenirs forms. Considering that there were so many traded butterfly souvenirs in the market, it raised an impression that there were more butterflies trapped for souvenir than free-living butterfly escaped from the trap. Commodification of butterflies needs to be regulated by setting the butterflies harvesting quota based on population in nature, sex, season and age (especially for female butterfly), accompanied by socializing rules of law, increasing public awareness about the importance of conservation butterflies, and creating new jobs for the people who depend on the butterflies trading.

Keywords: Bantimurung Nature Recreation Park, butterfly and moth, handicrafts, tourism, trade

INTRODUCTION

Butterfly (Insecta: Lepidoptera) is the most popular insect (and New Sands 2013) and most beautiful (Wagner et al. 2008; Rau 2013) in the world. Butterfly is also the most familiar insect for human (Davis and Butler 2008), ranging from children to adults. Butterfly has long become an insect that gives economic benefits for society (Ramana 2010; Boppre and Vane-Wright 2012). Several species of butterfly, such as bird wings butterfly (Sands and New in 2013), are the most wanted butterfly by collectors and are traded at a high price because it has large wings with beautiful, colorful and interesting pattern.

In the forest having butterfly richness, such as Bantimurung Nature Recreation Park (Bantimurung NRP or *Taman Wisata Alam Bantimurung*) and its surrounding areas inside the area of the Bantimurung-Bulusaraung National Park (Babul NP), the butterfly is widely used as a source of income for the local community, particularly through butterfly trading. Various forms of handicrafts of butterfly can be found to be sold at souvenir stalls alongside the entrance to this tourism place. The role of butterfly in the tourism industry in Bantimurung was very interesting to be analyzed, so the study was conducted in order to determine the species of local butterfly traded in various forms of handicrafts and its implications for the conservation of butterflies in Bantimurung NRP of Babul NP, South Sulawesi, Indonesia.

MATERIALS AND METHODS

Study area

The study was conducted at the butterfly trade center in Bantimurung Nature Recreation Park of Bantimurung-Bulusaraung National Park, Maros, South Sulawesi, Indonesia (Figure 1). Observation on the local species of butterfly which are traded in 2010-2015 was done first. Gathering information on the price at the butterfly catchers and middlemen was based on recent data collected in August 2015.

Procedures

Data retrieval on butterfly species that are traded was done by direct identification of butterfly species which are sold in various forms of handicrafts at stalls in Bantimurung NRP. Identification is also done on butterflies deposited by the butterfly catcher to middlemen or craftsmen. Photograph is taken on handicrafts of butterfly which species is difficult to be identified, and it is used in observation and further identification using identification books namely Tsukada and Nishiyama (1982, 1981, 1985, 1991), Vane-Wright and de Jong (2003), Cassidy (1995), and Peggie and Amir (2006). In addition, the photos of

crafted butterfly were also identified by matching butterfly species on it with the result of identification that have been done before in the Research Center for Biology, Indonesian Institute of Sciences (LIPI), Cibinong-Bogor, West Java, Indonesia. Initial identification on butterfly handicraft market is done by a survey method. Once market participants are identified, data collection regarding the commodification of butterflies into the handicraft by traders used interviews method (Dawson 2010; Turner 2010), both semi-structured (Laforest et al. 2009), to the merchant of butterfly craft, or in-depth interviews (DiCicco-Bloom and Crabtree 2006; Guion et al. 2011), to middlemen and butterfly catchers. Respondent selection of butterfly catchers was conducted with accidental sampling method (Pereira et al. 2005), namely doing a direct interview to a butterfly catcher who coincidentally are catching butterflies in the forest. Respondents selection of butterflies middlemen is done by snowball sampling method (Pereira et al. 2005) that is based on information from key informants about the people who work as collectors of butterflies. Respondents selection of butterfly merchants is by picking up randomly the merchants who are selling their stuffs in stalls. Topics of interview were the selling price of butterflies, the newly caught butterflies and the crafted butterflies.

Data analysis

Analysis of data on traded species of butterflies is a descriptive quantitative, namely describing the number of traded species of butterfly, the number of species in each family, as well as forms of crafts made from any species of butterfly. Data taken from interview with butterfly catchers, butterfly middlemen, craft makers, and traders were analyzed descriptively and qualitatively (Creswell et al. 2007; Vaismoradi et al. 2013; Richard 2015).

RESULTS AND DISCUSSION

There are about 142 local species of butterflies and moths (Order Lepidoptera) from seven families (Hesperiidae, Lycaenidae, Nymphalidae, Papilionidae, Pieridae, Riodinidae, and Saturniidae) which were used as materials for butterfly handicrafts in the butterfly trade center of Bantimurung NRP-Babul National Parks. Species of butterflies which are most widely used as a craft comes from the family of Nymphalidae (86 species), Papilionidae (23 species), and Pieridae (23 species) (Table 1).

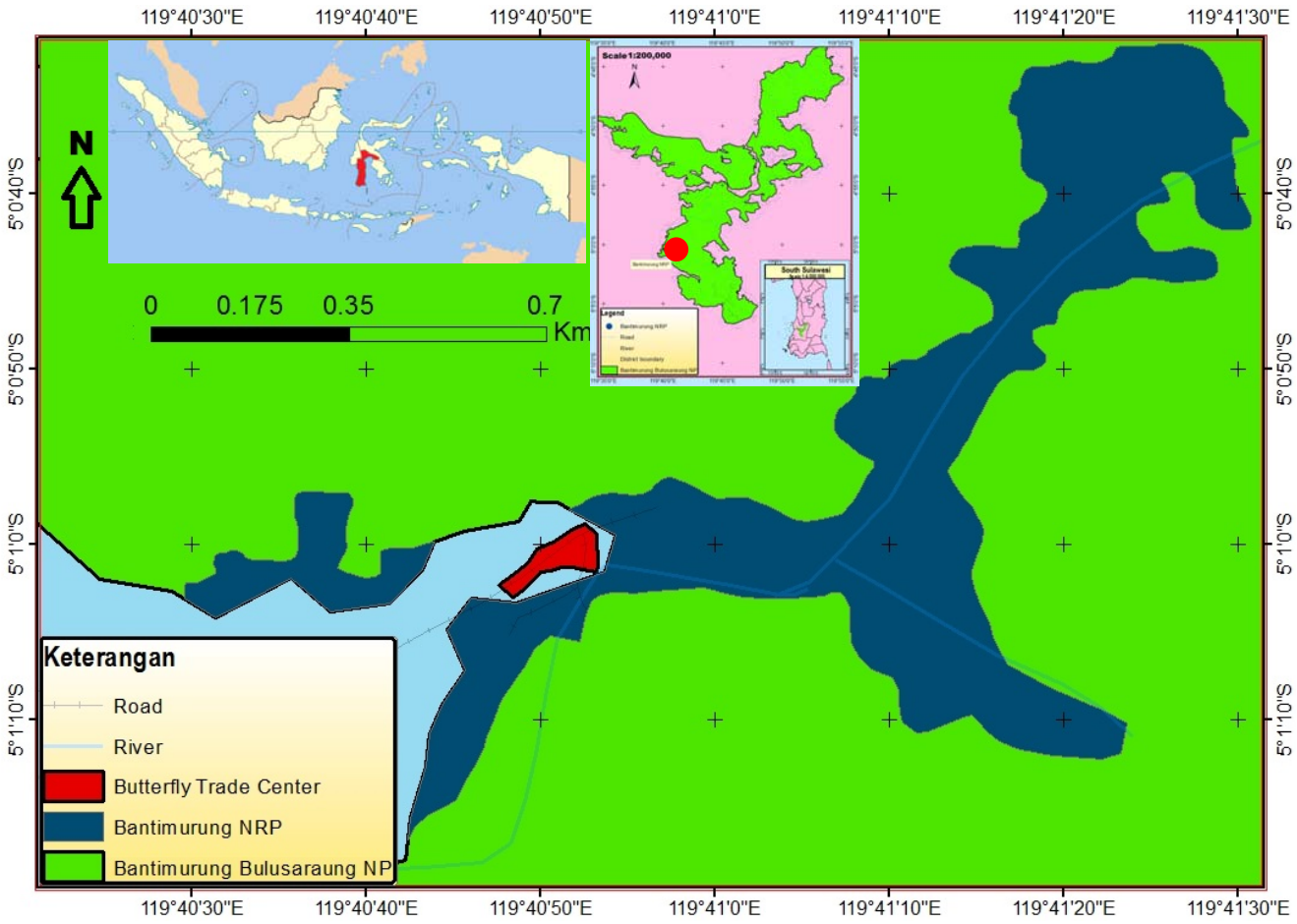

Figure 1. Location of the study at butterfly trade center of Bantimurung NRP of Babul NP, South Sulawesi, Indonesia

Table 1. Species of local butterflies traded at the butterfly trade center Bantimurung NRP of Babul NP, South Sulawesi, Indonesia

Scientific name	Family	Item price at the level of the middlemen (Rp.)	Form of craft
Tagiades sp.	Hesperiidae	500	Key chains
Arhopala irregularis	Lycaenidae	1,000	Key chains, frame
Arhopala argentea	Lycaenidae	1,000	Key chains, frame
Curetis sp.	Lycaenidae	2,000	Bracelet, necklace
Deudorix sp.	Lycaenidae	2,000	Bracelet, necklace
Jamides sp.	Lycaenidae	2,000	Bracelet, necklace
Rapala sp.	Lycaenidae	2,000	Bracelet, necklace
Tajuria sp.	Lycaenidae	2,000	Bracelet, necklace
Acraea moluccana	Nymphalidae	1,000	Key chains
Amathusia sp.	Nymphalidae	3,000	Key chains, frame
Amathuxidia plateni	Nymphalidae	3,000	Key chains, frame
Bassarona labotas	Nymphalidae	1,000	Key chains, frame
Bletogona mycalesis	Nymphalidae	1,000	Key chains, frame
Cethosia biblis	Nymphalidae	5,000	Triangular envelope, frame
*Cethosia myrina**	Nymphalidae	5,000	Triangular envelope, frame
Charaxes affinis	Nymphalidae	1,000	preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Charaxes nitebis	Nymphalidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Charaxes solon	Nymphalidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Chersonesia rahria	Nymphalidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Cirrochroa semiramis	Nymphalidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Cirrochroa thule	Nymphalidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Cupha maeonides	Nymphalidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Cyrestis strigata	Nymphalidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Cyrestis thyonneus	Nymphalidae	1,000	Key chains, frame
Danaus chrysippus	Nymphalidae	1,000	Preserved butterfly in triangular envelope, key chains, frame
Danaus genutia	Nymphalidae	1,000	Preserved butterfly in triangular envelope, key chains, frame
Danaus ismare	Nymphalidae	1,000	Preserved butterfly in triangular envelope, key chains, frame
Discophora bambusae	Nymphalidae	1,000	Key chains
Dophla evelina	Nymphalidae	1,000	Key chains
Elymnias cumaea	Nymphalidae	1,000	Key chains
Elymnias hewitsoni	Nymphalidae	1,000	Key chains
Elymnias hicetas	Nymphalidae	1,000	Key chains
Elymnias mimalon	Nymphalidae	1,000	Key chains
Euploea algea	Nymphalidae	1,000	Key chains, frame
Euploea configurata	Nymphalidae	1,000	Key chains, frame
Euploea eleusina	Nymphalidae	1,000	Key chains, frame
Euploea eupator	Nymphalidae	1,000	Key chains, frame
Euploea hewitsonii	Nymphalidae	1,000	Key chains, frame
Euploea latifasciata	Nymphalidae	1,000	Key chains, frame
Euploea phaenareta	Nymphalidae	1,000	Key chains, frame
Euploea redtenbacheri	Nymphalidae	1,000	Key chains, frame
Euploea westwoodii	Nymphalidae	1,000	Key chains, frame
Euripus robustus	Nymphalidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Euthalia amanda	Nymphalidae	♂5,000; ♀15,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Faunis menado	Nymphalidae	1,000	Key chains
Helcyra celebensis	Nymphalidae	2,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Hypolimnas anomala	Nymphalidae	1,000	Key chains, frame
Hypolimnas bolina	Nymphalidae	1,000	Key chains, frame
Hypolimnas diomea	Nymphalidae	1,000	Key chains, frame
Hypolimnas misippus	Nymphalidae	1,000	Key chains, frame
Idea blanchardi	Nymphalidae	3,000	Preserved butterfly in triangular envelope, frame

Ideopsis juventa	Nymphalidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Ideopsis vitrea	Nymphalidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Junonia almana	Nymphalidae	500	Bracelet, key chains
Junonia atlites	Nymphalidae	500	Bracelet, key chains
Junonia erigone	Nymphalidae	500	Bracelet, key chains
Junonia hedonia	Nymphalidae	1,000	Bracelet, key chains
Lamasia lyncides	Nymphalidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Lasippa neriphus	Nymphalidae	1,000	Key chains, frame
Lethe europa	Nymphalidae	1,000	Key chains
Lexias aeetes	Nymphalidae	1,000	Key chains, frame
Libythea geoffroy	Nymphalidae	1,000	Key chains, frame
Lohora decipiens	Nymphalidae	1,000	Key chains
Lohora dinon	Nymphalidae	1,000	Key chains
Lohora unipupillata	Nymphalidae	1,000	Key chains
Melanitis boisduvalia	Nymphalidae	2,000	Key chains, frame
Melanitis leda	Nymphalidae	2,000	Key chains, frame
Melanitis pyrrha	Nymphalidae	1,000	Key chains
Melanitis velutina	Nymphalidae	1,000	Key chains
Moduza libnites	Nymphalidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Moduza lycone	Nymphalidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Moduza lymire	Nymphalidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Mycalesis horsfieldii	Nymphalidae	1,000	Key chains
Neptis celebica	Nymphalidae	1,000	Key chains
Neptis ida	Nymphalidae	1,000	Key chains
Orsotriaena jopas	Nymphalidae	500	Key chains
Parantica cleona	Nymphalidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Parantica menadensis	Nymphalidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Parthenos sylvia	Nymphalidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Phaedyma daria	Nymphalidae	1,000	Key chains
Phalanta alcippe	Nymphalidae	500	Key chains
Polyura alphius	Nymphalidae	5,000	Key chains, frame
Polyura cognata	Nymphalidae	♂10,000; ♀50,000	Key chains, frame
Rhinopalpa polynice	Nymphalidae	1,000	Key chains, frame
Rohana macar	Nymphalidae	1,000	Key chains
Symbrenthia sp.	Nymphalidae	1,000	Key chains, frame
Tarattia lysanias	Nymphalidae	1,000	Key chains, frame
Terinos taxiles	Nymphalidae	1,000	Key chains, frame
Tirumala choaspes	Nymphalidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Vindula dejone	Nymphalidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Vindula erota	Nymphalidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Yoma sabina	Nymphalidae	1,000	Frame
Ypthima nynias	Nymphalidae	1,000	Key chains
Zethera incerta	Nymphalidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Graphium agamemnon	Papilionidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Graphium androcles	Papilionidae	5,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Graphium antiphates	Papilionidae	15,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Graphium codrus	Papilionidae	5,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Graphium deucalion	Papilionidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame

Graphium encelades	Papilionidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Graphium eurypylus	Papilionidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Graphium meyeri	Papilionidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Graphium milon	Papilionidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Graphium rhesus	Papilionidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Lamproptera meges	Papilionidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Pachliopta polyphontes	Papilionidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Papilio ascalaphus	Papilionidae	♂3,000; ♀5,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Papilio blumei	Papilionidae	10,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Papilio demoleus	Papilionidae	3,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Papilio fuscus	Papilionidae	3,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Papilio gigon	Papilionidae	3,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Papilio peranthus	Papilionidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Papilio polytes	Papilionidae	1,500	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Papilio sataspes	Papilionidae	1,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
*Troides haliphron***	Papilionidae	3,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
*Troides helena***	Papilionidae	♂5,000; ♀7,500	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
*Troides hypolitus***	Papilionidae	♂15,000; ♀25,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Aoa affinis	Pieridae	1,000	Key chains, frame
Appias albina	Pieridae	500	Key chains, frame
Appias hombroni	Pieridae	500	Key chains, frame
Appias lyncida	Pieridae	500	Key chains, frame
Appias paulina	Pieridae	500	Key chains, frame
Appias zarinda	Pieridae	500	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Catopsilia pomona	Pieridae	500	Key chains, frame
Catopsilia pyranthe	Pieridae	500	Key chains, frame
Catopsilia scylla	Pieridae	500	Key chains, frame
Cepora celebensis	Pieridae	500	Key chains, frame
Cepora timnatha	Pieridae	500	Key chains, frame
Delias rosenbergi	Pieridae	2,000	Preserved butterfly in triangular envelope or wrapped in a plastic, key chains, frame
Eurema alitha	Pieridae	500	Key chains, frame
Eurema blanda	Pieridae	500	Key chains, frame
Eurema celebensis	Pieridae	500	Bracelet, key chains
Eurema hecabe	Pieridae	500	Key chains, frame
Eurema tominia	Pieridae	1,000	Necklace, bracelet, key chains
Gandaca butyrosa	Pieridae	1,000	Key chains, frame
Hebomoia glaucippe	Pieridae	1,000	Key chains, frame
Leptosia lignea	Pieridae	1,000	Necklace, bracelet, key chains
Leptosia nina	Pieridae	1,000	Necklace, bracelet, key chains
Pareronia tritaea	Pieridae	500	Key chains, frame
Saletara panda	Pieridae	500	Key chains, frame
Abisara kausambi	Riodinidae	1,000	Key chains, frame
Attacus atlas	Saturniidae	10,000	Frame

Note: The protected status: *) Government Regulation No. 7 of 1999, **) Government Regulation No. 7 of 1999, CITES Appendix II, Annex B of European Union Wildlife Trade Regulation, and Forestry Ministerial Decree No. 57 of 2008

Commodification of butterflies as craft materials

Generally, in the international market, the majority of sales of butterflies are live butterflies (Nijman 2010; Boppre and Vane-Wright 2012), caterpillars (Ramos-Elorduy et al. 2011), pupa (Shambu and Heyden 2010; Heyden 2011; Boppre and Vane-Wright 2012), or specimens of dead butterfly (Leary 1991; Pyle 1995), whereas in Bantimurung NRP-TN Babul, most butterflies are sold in dead condition and has been processed into various forms of crafts. Commodification of butterflies as craft materials were from all kinds of butterflies and were caught from the wild regardless of species, size, condition, and quality. Craft making is done on butterflies with folded (vertical) wings or with stretched wings. Butterflies with folded (vertical) wings were crafted into small to medium sized key chains, preserved butterfly in a triangular envelope, pendant necklaces, and bracelets. Butterfly with stretched wings were used as preserved butterfly display in plastic containers, frames and large key chains.

Boppre and Vane-Wright (2012) states that trade on butterflies are generally conducted on species of large butterflies, such as butterfly from the family of Nymphalidae (*Danaus, Idea, Morpho, Caligo, Cethosia, Heliconius, Hypolimnas, Parthenos*), Papilionidae (*Papilio*), and Pieridae (*Hebomoia*). However, in Bantimurung NRP-Babul NP, commodification of butterflies is on various sizes. Small butterflies, like a butterfly coming from Family Lycaenidae (*Tagiades, Jamides*), are commonly used in the manufacture of bracelets and pendant necklaces. Medium-sized butterfly is generally used for a keychain or as a display in a frame. Sized butterflies are generally only on display in the frame although there is also used as a keychain-sized. Utilization of small-sized butterfly is harder to do than of bigger one. Smaller body size and wings causes fragile butterflies. Small size is more easily damaged than the larger size butterfly. This causes the making process of pendants, key chains, and bracelets using this species of butterfly are more difficult and requires more patience than using butterfly of medium to large size. However, the selling price of the craft using small-sized butterfly is quite cheap. This condition makes the quantity of crafts using small size butterfly is far less than the crafts using bigger size of butterflies.

Colorful and beautiful butterfly wings became the main interest of butterfly (Sandved and Cassie 2004), so the butterfly trade is generally conducted on the butterfly having attractive colors of wings (Bopprea and Vane-Wright 2012). But in Bantimurung NRP-Babul NP, utilization of butterflies were also conducted on the butterfly which color was less attractive, e.g. dark brown and black butterflies. Some species of butterflies with less attractive color actually have a slightly higher price at the collectors' level because it's harder to find in nature, e.g. in *Melanitis* sp. with brown wings.

Utilization of butterflies is also conducted on all genders. In some species of butterflies, individual male, female and transvestite have a different pattern, style, and color of the wings. Such differences lead to differences in price. At the collector's level, the females have a higher price than the male butterflies. This is mainly due to the number of catches of male butterflies in nature which is always more numerous than the female butterflies. Transvestite butterflies and butterfly with peculiar wings or body have a much higher price because it is very rare and have the distinction which will not be found in normal butterflies. Those butterfly price range were in between Rp 150,000.00 – Rp. 1,000,000.00/head at middlemen's level and becomes object of hunting by collectors, especially those collectors from abroad.

Collins and Morris (1995) states that the traded butterflies have a wide range of quality. Lower quality of butterflies is generally used for ornamentation or decoration materials. High quality butterflies sometimes are completed by additional data such as the date and location of capture, and are mostly purchased by the museum or collectors of butterflies. In Bantimurung NRP-Babul NP, commodification of butterflies is carried out on several quality or level of wings damage (wing quality). A1 Quality is a butterfly with good quality of wings and no flaw at all. A$^-$ quality butterfly is a butterfly that has a little torn on the wings. A2 quality butterfly is a butterfly having slightly faded wing colors or few defects. A3 quality butterfly is a butterfly having faded wings color and/or defective wings and/or torn wings. The butterfly collectors receive all butterfly caught in nature with varying levels of quality. With a little skill, a butterfly that was heavily damaged or lightly damaged can still be used as craft materials. Utilization of butterfly with severely damaged wings or body is by removing part of the damaged body then replaced by good body parts of other butterflies, taken from the same species or from different species of butterfly, as long as it looks congenial and beautiful. Then, this butterfly can be packed into butterfly with folded wings and put into triangular envelopes (papilot envelopes), in a plastic package, or in the form of a keychain, or a display in the frame. Torn wings of butterfly can be cut neatly, while butterflies with faded colors of wings can be used as craft materials by peeling its scales, so that the butterfly wings are transparent (Figure 2).

Commodification of butterflies as craft materials were also conducted on the following species, namely *Troides haliphron, T. helena, T. hypolitus,* and *Cethosia myrina*, which are protected species of butterflies, listed in Government Regulation No. 7 of 1999 as protected species, Appendix II of CITES, and Annex B of European Union Wildlife Trade Regulation, and are classified as species of high priority for conservation by Forestry Minister Regulation No. 57 Year 2008 (Government Regulation No. 7 of 1999). Though based on Government Regulation No. 8 of 1999 on the use of plants and wildlife, the protected species may not be traded but their second generation and third generation breeded in captivity are free to be traded.

Prices of butterfly crafts and market participants

Prices of butterflies traded are various. However, the selling price of a butterfly craft abroad is relatively much higher than the price at the local level. To trade on an international scale, etsy.com puts *Troides haliphron* at a price of $43.18 per pair (etsy.com 2015). Ebay.com puts up

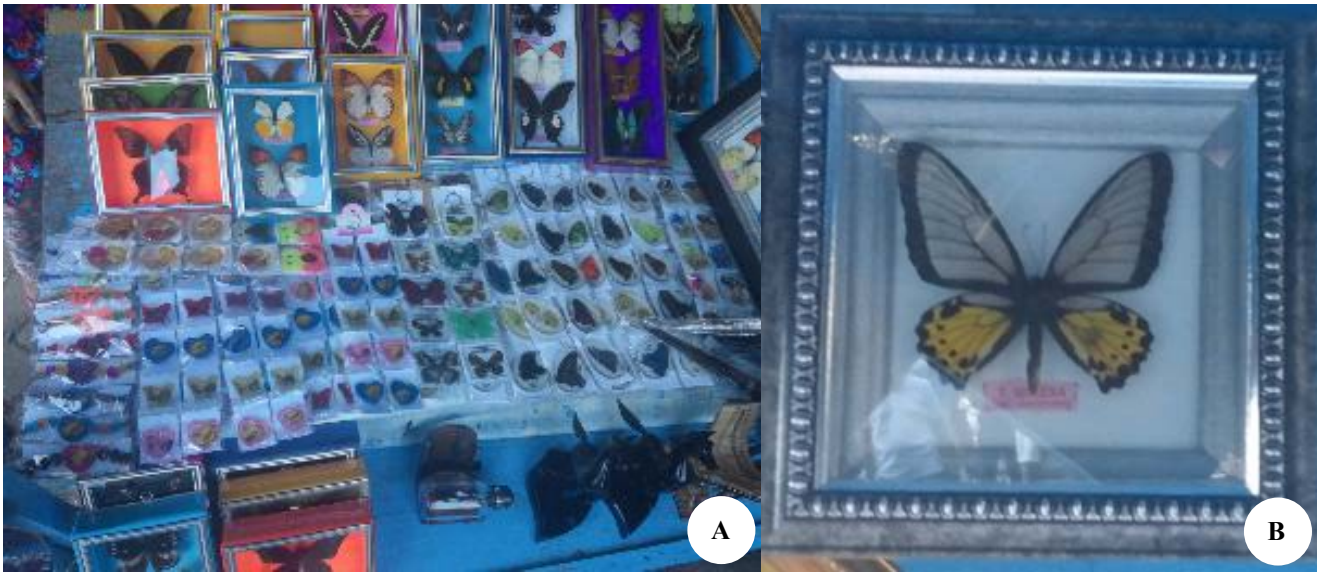

Figure 2. A. Various forms of butterfly handicrafts in Bantimurung NRP of Babul NP, South Sulawesi, Indonesia. B. Crafts on protected species of *Troides helena* in which the its scales on the wings has been peeled off

the price of $15 per head for *Troides helena*, $85 per pair for *Troides Hypolitus*, and $7.98 per head for *Cethosia myrina* (ebay.com 2015). In Bantimurung NRP-Babul NP, the selling price of butterflies depends on the level of trade, species of butterflies, quality, size, gender, season, inventories of butterflies and butterfly craft forms. For example, at the catcher level, the highest purchase price of the collectors is for *Polyura cognata*. The selling price of butterflies will increase as it is sold in the stalls. Butterflies that have been packed in the frame have a higher price than other craft forms.

There are a number of market participants (buyers and sellers) in butterfly crafts in Bantimurung NRP. Despite the fixed number of market players, there will be a change in number of individual market participants, depending on the season and market demand. First type of sellers is the butterfly catcher. Butterfly catchers are local people around Babul NP at various ages ranging from children to adults, but the butterfly catchers are mostly at the level of school age. The second type of sellers is the butterfly breeders (owner of butterflies breeding cage). Around Bantimurung NRP, the numbers of butterfly breeder left are only two persons. The advantage of butterfly breeders is that they can sell live butterflies. In general, the sale is made at the time the butterflies has reached cocoon phase. These cocoon will be sent anywhere, from Sulawesi island to overseas. Third type of sellers is butterfly middlemen who are also the local communities living around Babul NP. Some middlemen, in his spare time, are butterfly catchers. Some middlemen are also butterfly breeders. Fourth type of seller is the butterfly artisans. In general, these butterfly craftsmen are also collectors of butterflies. Butterfly middlemen and artisans whose business has gone well usually employ several craftsmen. The fifth type of seller is the owner of the kiosk. In Bantimurung NRP, there is a middlemen who also serves as a butterfly artisan as well as kiosk owner who hires employees as a kiosk assistant.

Other market participants are buyers. There are several species of butterfly buyers in Bantimurung NRP. The first kind of buyers is the local tourists who come to visit the Bantimurung NRP and, being attracted to insect's beauty of wings, they buy butterfly. Local buyers are generally not familiar with the species of butterflies that are marketed as well as having a low knowledge and understanding of the species of butterflies that exist. Local buyers also have little understanding of the condition of butterflies they buy, so they often buy the butterfly that has received specific treatment, for example, has a body of a different kind with wings, or an upper wing and a lower wing derived from different species of butterflies. The second type of buyers is traders of butterfly from outside the district/province but still in the territory of the Republic of Indonesia. These buyers generally come from the island of Java, Bali, Sumatra, and Borneo. They generally buy butterflies that will be sold again. The third type of buyer is a butterfly merchant from abroad. This kind of buyer has a good knowledge about butterflies and just buys a butterfly with good quality. Just like the second type of buyer, this buyer will also resell their purchase from Bantimurung NRP at much higher prices. The fourth type of buyer is a collector of butterflies. Butterfly collectors generally come from abroad and have a good understanding of the butterfly. Butterfly collectors from abroad often hunt for very rare butterflies, like an abnormal butterfly or a pansy butterfly and are willing to pay at a high price. Fifth type of buyer is researchers or scientists who buy butterflies for scientific purposes. In the 1970s and 1980s, the numbers of this type of buyers are still quite a lot and generally come from Japan. But this time, it can be said that there is almost no longer butterfly purchase for research purposes.

Conservation management

A large number of species of butterflies which are traded in the form of craft shows that the forest area around Bantimurung NRP of Babul NP is rich in species of butterfly. The richness of butterfly species in Bantimurung NRP even amazed Wallace while visiting Bantimurung in the past decade, so Wallace gave the nickname of The Kingdom of Butterfly on Bantimurung (Bantimurung-Bulusaraung National Park 2008; Koterman 2013). Unfortunately, the wealth of the butterfly can not be enjoyed to the fullest in the wild. When we are traveling in Bantimurung NRP, fluttering butterfly is very rarely to be found (Rahmanto 2012; Wijanarko 2012; Koterman, 2013; Gassing 2015). The disappointed visitors will only be informed that it was not in season of butterfly (Rahmanto 2012; Wijanarko 2012; Nofrianti 2015). In fact, a very contrastive situation can be seen at the entrance to the Bantimurung NRP, i.e. the numerous of butterflies are being traded continuously and abundantly (Wijanarko 2012; Nofrianti 2015) without season consideration. This gives the impression that more butterflies are displayed as a souvenir than flying freely in nature. In addition, these conditions may be indirect clues that the number of butterflies around the place was actually numerous, but most of them were captured and used as material for handicrafts and trade.

The impact of the excessive butterflies captures are the decreasing number of individual butterflies flying freely in Bantimurung NRP. In 2008, the authors conducted an interview with one of the collectors who stated that during the day, the collectors can collect up to 900-1000 butterflies from butterfly catchers. When the author interviewed him again in 2010, he claimed that butterfly catches has been reduced to only about 500-600 per day. And, at the last interview in 2014, he claimed that he could only collect 200-300 live butterflies per day (pers. comm. 2014). Based on interviews with former old butterfly catchers, in the late 1970s and 1980s, catching butterflies in large quantities can be done only around the yard. But now, to catch butterflies in large quantities, the catcher must go in a long distance into the woods (pers. comm. 2015).

Currently, the number of species of butterflies found in the area of Babul NP is still considered to be in great quantities, but, considering that many species of butterflies are traded in the form of the craft, the excessive captures will continue to be happened, and one day, the number of butterflies will not only be decreased, but certain species of butterfly will come to an extinction due to high levels of exploitation. To prevent further decline in butterfly populations, it is necessary to manage the butterfly wisely. The basic thing that is important to be done immediately is the enforceable regulation of use. This is in accordance with the opinion of Giles et al. (2006), Nijman (2006), Nekaris and Nijman (2007), Shepherd and Nijman (2007a, b), Eudey (2008) and Zhang et al. (2008) which states that in the Asian region, the laws governing wildlife trade classified as inadequate and needed initiative to create legal mechanisms in order to work more effectively. In trading butterflies in Bantimurung NRP of Babul NP, it is needed for legal enforceable regulations governing the number of individuals that can be captured based on availability in nature, gender, season, and age (especially for female butterflies). Rules of butterfly commodification should be set out in the binding and enforceable local rules and should be adhered by all participants involved in the exploitation of the butterfly. The rules need to be routinely monitored and enforcement of sanctions for offenders. Also socialization of the rules is needed, especially regarding endemic species which are rare and protected. Besides that, it would also require an increase in public awareness about the importance of conservation of butterflies, awareness improvement, and the community's role in the conservation of butterflies, for example, by no longer capturing protected butterfly in nature, by no longer catching young female butterflies that haven't laid eggs, by planting food plants around their neighborhood, as well as by increasing the number of breeding facility which is managed by the community. Another important step that can be done is to create new jobs that can provide promising income for people who depends his life on the butterfly trade.

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Botanical survey in thirteen montane forests of Bawean Island Nature Reserve, East Java Indonesia: Flora diversity, conservation status, and bioprospecting

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Abstract. Trimanto, Hapsari L. *2016. Botanical survey in thirteen montane forests of Bawean Island Nature Reserve, East Java Indonesia: Conservation status, bioprospecting and potential tourism. Biodiversitas 17: 832-846.* Bawean Island which located between Borneo and Java islands possessed unique and distinctive abiotic and biotic resources. Botanical survey has been conducted in Bawean Island Nature Reserve. This paper reported the results of inventory study of plant bioresources in 13 montane forests of Bawean Island, discussed their conservation status, bioprospecting on some wild plant species and potential development subjected to some conservation areas. Inventory results in montane forests showed that it was registered about 432 plant species under 286 genera and 103 families; comprised of 14 growth habits in which tree plants were the most dominant with about 237 species. Conservation status evaluation showed that there are at least 33 species of plants included in IUCN list comprised of 30 species categorized as least concern and 3 species considered at higher risk of extinction *i.e. Podocarpus rumphii* (Near Threatened); *Pterocarpus indicus* and *Memecylon myrtilloides* (Vulnerable). Bioprospecting results showed that 10 tuberous plants prospected as food sources; 19 woody plants prospected as timber sources, and 28 plants prospected as ornamental plants. There are at least 7 invasive alien plant species identified including *Ageratum conyzoides*, *Chromolaena odorata*, *Eupatorium inulifolium*, *Lantana camara*, *Imperata cylindrica*, *Stachytarpheta jamaicensis* and *Themeda arguens*. If well managed, the development of Bawean Island as nature-based and eco-tourism may contribute both to biodiversity conservation and alleviating prosperity of the local residents.

Keywords: Bawean Island, bioprospecting, bioresources, conservation, montane forest, plant

INTRODUCTION

Indonesia is the largest archipelago state in the world. It consists of more than 17,508 islands and about 70% of its territorial areas covered by oceans with more than 81,000 km of coastlines (Farhan and Lim 2010). Scientific studies revealed that impacts of climate change and the rising of sea-level on biodiversity in the island states are much greater than continental areas. Therefore, preservation of biodiversity in small islands which is under the pressure of climate change are more urgently needed than in the continent. Climate change and the rise of sea-level will cause unfavorable shifts in biotic composition and adversely affect competition among species (Nurse et al. 2001). One of the main targets of the global strategy for plant conservation is to understand and to document the diversity of plants, especially the endangered habitats in small islands (GSPC 2002). Limitations of the distribution area and the threat of the rise of sea level cause an area of small islands as vulnerable habitats and need to be prioritized in plant conservation efforts.

Bawean Island is one small island in Indonesia. Geographically, Bawean Island which is located among Borneo Island and Java Island brings about unique and distinctive of abiotic and biotic resources. Bawean island has lowland primary and secondary tropical rain forest type, with many water springs found across the island which support bioresources richness including flora, fauna, microorganisms, etc. (Trimanto 2014). It provides wide ranges of habitat in which animals and plants with different evolutionary lineage may evolve and undergo speciation in the island. The discovery of endemic species such as Bawean deer *Axis kuhlii* (Semiadi et al. 2015), Javan warty pig *Sus verrucosus* (Blouch 1995), butterfly *Atrophaneura coon* sub. sp. *sangkapurae* (Maurizio and Salla 1992), some birds of Falconiformes and Strigiformes (Nijman 2004), etc. indicated that Bawean Island, which is rich with bioresources, can be an interesting subject of study by many biologists.

Some inventory studies and assessments on flora biodiversity in Bawean Island have been conducted sporadically in few past years. Its montane forests were characterized by dense tree species and understory with predominance of ferns, bryophytes and orchids. The dominating tree species were different for each montane forest. Most important and common trees species found in some montane forests in Bawean Island, according to scholars and researchers' report from vegetation analysis study, include *Syzigium lepidocarpa*, *Irvingia malaya*, *Garcinia* spp., *Microcos tomentosa*, *Ficus variegata*, *Myristica guatteriaefolia*, *Tetrameles nudiflora*, *Canarium hirsutum*, *Litsea firma*, *Alstonia scholaris*, *Pittosporum* sp., etc. (Mansur et al. 2004; Trimanto 2014; Danarto and Rahadiantoro 2015).

Tourism has experienced rapid growth over the past 50 years and is expected to continue to develop, particularly in biodiversity 'hotspots'. Nature-based' tourism is the fastest growing element of tourism *i.e.* the segment in the tourism market in which people travel with the primary purpose of visiting a natural destination (Kuenzi and McNeely 2008). According to the document long-term plan management of Bawean Island Nature Reserves and Wildlife Reserves (2012-2021), it is stated that Bawean Island will be developed into a nature-based tourism destination (Achmad 2001). In order to support the plan, some infratructures i.e. road networks and airport has been built in Tambak Sub-District. However, several studies result that tourism activities which focus on the natural environment create some risks and pressures on the ecosystems, bioresources and their services. The significant alterations may include deforestation, drainage of wetlands, soil erosion, fragmentation and disruption of habitat, encroachment on protected areas, littering, air and water pollution, eutrophication, increased risk of fires, introduction of invasive alien species (weeds, pests and possibly animals), the changing behavior of wildlife and even the loss of biodiversity (Hay and Hunt 1995; Ning 1999; Kuenzi and McNeely 2008). Awareness about the impacts of tourism, the importance of biodiversity, and the need for conservation efforts have to be raised in order to prevent Bawean Island from the negative impacts of tourism

In 2014, Purwodadi Botanic Garden (Pasuruan, East Java) has conducted exploration study on the biodiversity of flora in Bawean Island and collected some plant materials to be *ex-situ* conserved. Botanical survey in small and remotes islands are necessary to reveal and study its plant diversity which may become valuable basic data of biodiversity for further development and also to evaluate the major intrinsic and extrinsic factors affecting long-term preservation (Singh et al. 2014). Furthermore, conservation and bioprospecting efforts are needed to conduct in order to keep the plant genetic resources in the island and also to utilize gene sustainability. This paper reports the results of inventory study of plant bioresources in Bawean Island, discusses their conservation status, carries out bio-prospecting of some wild plant species and takes some notes on its potential tourism development.

MATERIALS AND METHODS

Study site

Bawean Island is a small and remote island in Indonesia and is located in Java Sea, off the North Coast of Java, about 150 km from Surabaya, the capital city of East Java. It is administered by Gresik District of East Java Province, Indonesia. The island is about 190-200 km^2 with mostly hilly land topography and slopes between 5-75% with altitude up to 695 m above sea level. The island was originated from a volcano and was located near its center in which igneous rocks cover about 85% of its surface with occasional limestone, sandstone and dolomite. The climate is tropical monsoonal with slightly less humid (Blouch 1995; Mansur et al. 2004). Bawean Island has function as nature conservation area; in 1979 by Decree of Minister of Agriculture, two national nature preserves were created with areas of 3836.6 ha as wildlife sanctuary and 725 ha as Nature Reserve (Minister of Agriculture 1979; Achmad 2001). Botanical surveys were conducted in Bawean Island Nature Reserve and Wildlife Sanctuary covering 13 montane forests of the island i.e. Montane Forest of Langger, Pakem, Asakan, Pakotokan, Nangka, Payung-payung, Kastoba, Lumut, Gadung, Bangkuang, Pangambaan, Mandala, and Panjang (Figure 1).

Botanical survey method

Plant exploration was conducted using survey method to inventory and gather plant materials in forms of seedlings, seeds, cuttings, tubers, corms, etc. for ex-situ conservation purpose in Purwodadi Botanic Garden. Plants inventoried including Angiosperms, Gymnosperms and Pteridophytes (Bryophytes, Lichens and Algae were excluded). Direct identification were made for each plant in the field using main and specific morphological characters such as habit, stem or branch, petiole or rachis, stipules, leaf, inflorescence, flower, fruit and seed forms, also exudate, smell and glands. Supporting data was recorded and documented, i.e. coordinate and climatic factors (air temperature, relative humidity, soil pH and soil humidity). For those unidentified and particular suspect, more detailed identification were conducted by collecting voucher specimens (Damery et al. 2011) to be identified in Herbarium Bogoriense (BO) of the Research Center for Biology, Indonesian Institute of Sciences, Cibinong-Bogor, West Java, Indonesia.

Classification by plant habit

We classified diversity of plant based on its plant habit. Plant habit refers to the overall shape of a plant. It refers to the genetic tendency of a plant to grow in a certain shape and to attain a certain mature height and spread. The classification of plant habit includes trees, shrubs, vines, climber, woody climber, herbaceous (annual and perennial), epiphityc (fern and orchid), terrestrial (fern and orchid), tuber and rhizome (Jud et al. 2007).

Conservation status: evaluation, bioprospecting and plant invasiveness determination

The conservation status of plant species were evaluated using application of International Union for Conservation of Nature: Conservation Categories and Criteria (IUCN 2011) on its official website, i.e. http://www.iucnredlist.org/search. Whilst, interviews to local residents were conducted to gather bioprospecting information of the plants focusing on its uses as food sources, timber and ornaments. Prior informed consent (PIC) was obtained verbally before commencing each interview (Ellena et al. 2012). The plant invasiveness were determined by looking out from literature list of invasive plant species in Indonesia (Tjitrosoedirdjo 2005), from invasive alien plant species database of SEAMEO Biotrop and the State Ministry of the Environment (http://www.biotrop.org/database.php?act=dbias) and also from some world's invasive alien plant species databases.

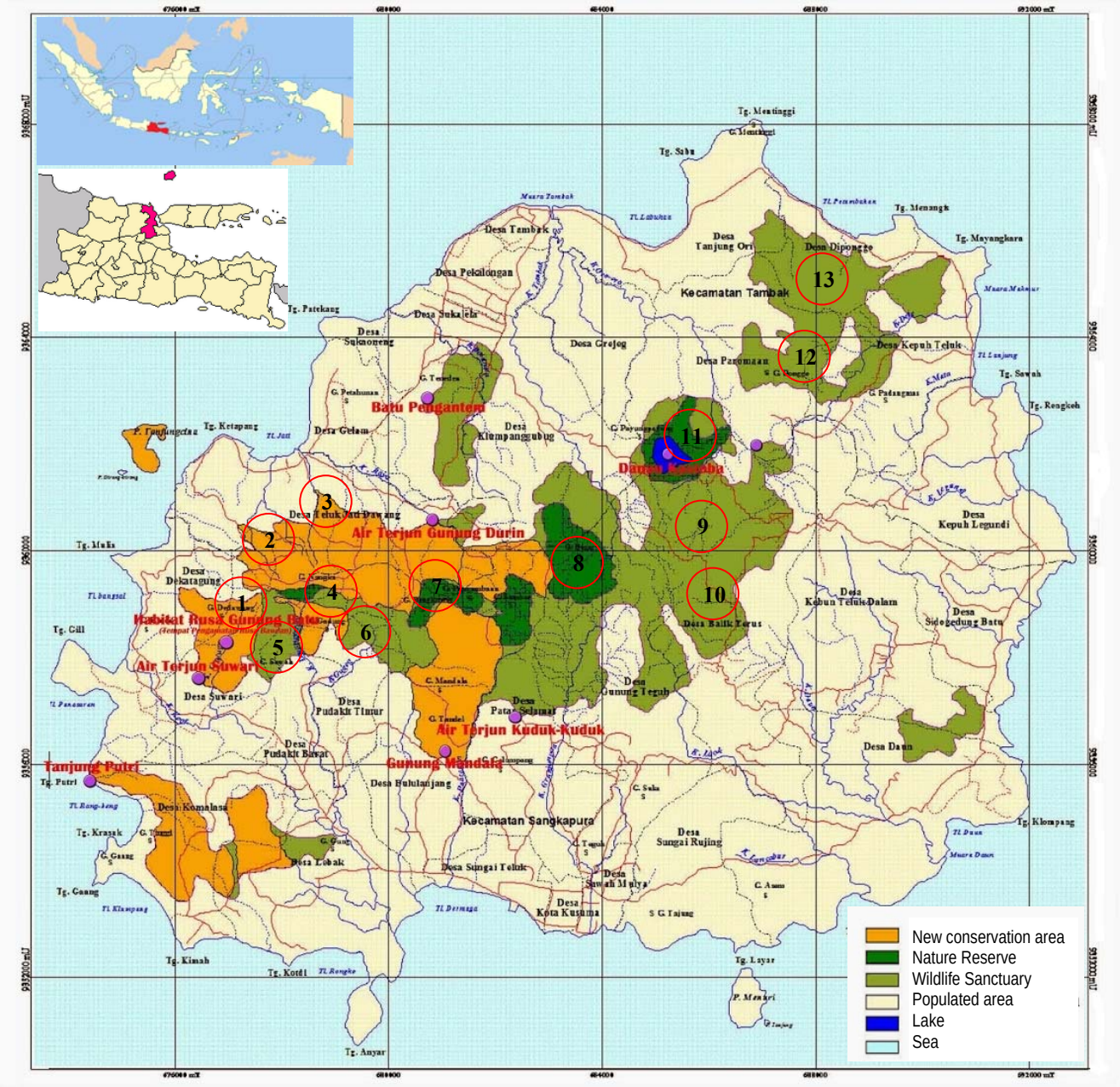

Figure 1. Maps of Bawean Island: botanical surveys covering 13 montane forests: 1. Langger, 2. Pakem, 3. Pakotokan, 4. Nangka, 5. Panjang, 6. Mandala, 7. Bangkuang, 8. Lumut, 9. Asakan, 10. Gadung, 11. Kastoba, 12. Pangambaan, 13. Payung-payung

RESULTS AND DISCUSSION

Plant inventory in 13 montane forests of Bawean Island

Plant inventory results showed that it registers about 432 plant species under 287 genera and 103 families in 13 montane forests of Bawean Island within altitudes 8 to 572 m above sea level. Plant species under Euphorbiaceae Family were the most commonly found; followed by Orchidaceae, Poaceae, Moraceae, Rubiaceae, and so on (Figure 2.A). The complete list of plant species is showed in Table S1. The abundance of Euphorbiaceae indicated that montane forests in Bawean Island has carried out succession process. Member of Euphorbiaceae i.e. *Homalanthus populneus* and *Macaranga tanarius* were categorized as pioneer plant species in early stages of secondary succession in montane forests (van Valkenburg and Ketner 1994). In addition there were about 13 species of *Ficus* spp. (Moraceae). Ficus is known as key species in tropical rain forests in which some of them are pioneer in dry habitats (Lee et al. 2013); like in limestone soil of Bawean Island.

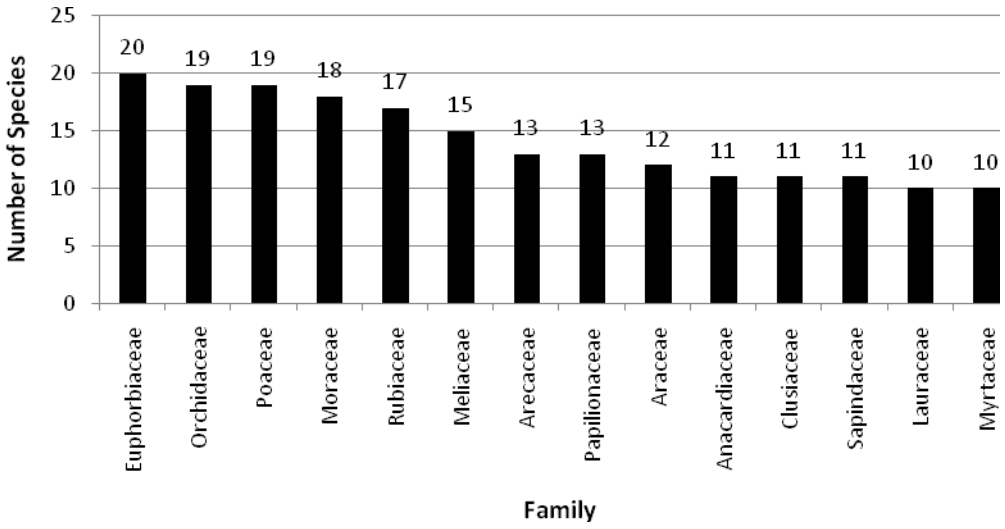

Figure 2. Inventory results of plant families with 10 and above species member in 13 montane forests of Bawean Island Nature Reserve, Indonesia

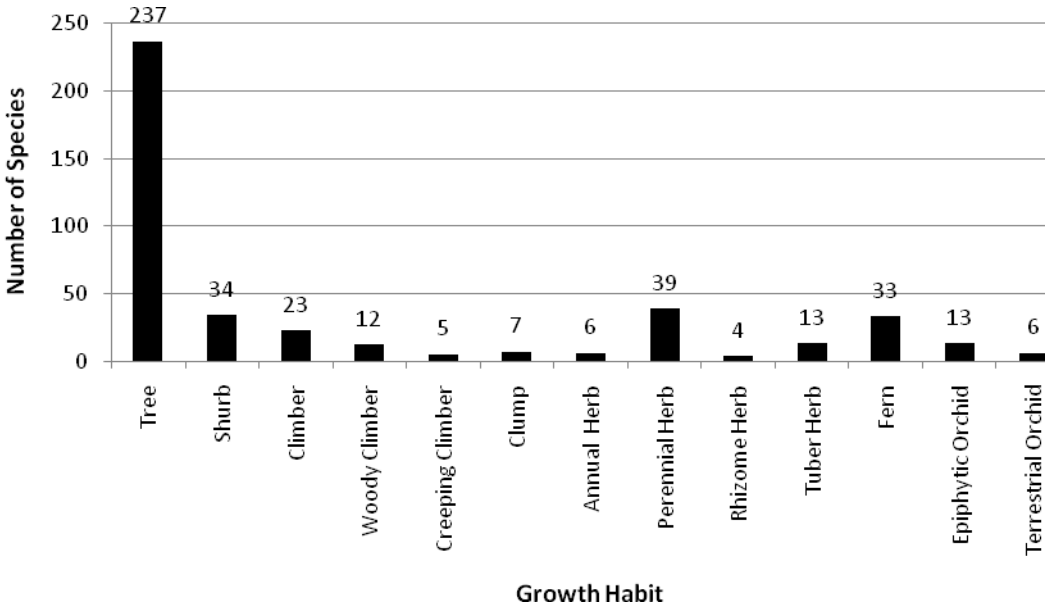

Figure 3. Growth habit diversity of plants inventoried in 13 montane forests of Bawean Island Nature Reserve, Indonesia

Based on its growth habit, it was grouped into 14 habits in which tree plants were found dominant with about 237 species and then followed by ferns, shrubs, perennial herbs and climbers which were found in moderate amount, and so on (Figure 3). Plant habit provides important information about its ecology (Jud et al. 2007). The high diversity of plant habits in 13 montane forests in Bawean Island indicates the diversity of macro and micro climates of the forests. This study showed that the soil temperature ranged 26,4 ^{o}C to 39,1 ^{o}C, relative humidity was 77 % to 95 %, pH was 5,1 to 6,8 and light intensity was 1760 to 93500 lux.

Trees

Tree plants are found dominant in 13 Montane Forests of Bawean Island. It was recorded about 237 species (Figure 3) under 144 genera and 51 families. High diversity and density of tree plants were supported by high relative humidity of the forest (79% to 99%). Tree plants provide shades as habitat for annual/perennial herbaceous plants and ferns. Tree plants also has important roles as host tree (phorophytes) and habitat of epiphytic plants and climber plants.

Irvingia malayana (Simaroubaceae) (Figure 4.A) and *Ficus variegata* (Moraceae) (Figure 4.C) were known as the dominant trees in Bawean Island. It was distributed in primary or secondary forests of the island. Furthermore, the high important value of tree plants in 7 montane forests of Bawean Island were *Myristica guatteriaefolia* (Myristicaceae), *Canarium asperum* (Burseraceae), *Syzygium garciniifolium* (Myrtaceae), *Pittosporum moluccanum* (Pittosporaceae),

Calophyllum soulattri (Clusiaceae), *Dysoxylum densiflorum* (Meliaceae), *Garcinia dioica* (Clusiaceae), and *Garcinia celebica* (Clusiaceae) (Trimanto 2014).

Tree plant species in Bawean Island were more similar to Java Island than to Borneo Island. *Podocarpus brateatus* (Podocarpaceae), *Aglaia lawii* (Meliaceae), *Canarium asperum* (Burseraceae), *Pongamia pinnata* (Papilionaceae), *Lepisanthes rubiginensis* (Sapindaceae), *Poliscias nodosa* (Araliaceae), *Caryota mitis* (Arecaceae) and *Suregeda glomerulata* (Euphorbiaceae) were commonly distributed at disjunctive locations in Sumatra, Sulawesi, Java, Flores Island and Papua. However, those species were not recorded to be available in Borneo (Lemmens et al. 1995; World Conservation Monitoring Centreb 1998a; Farjon 2013).

Shrubs

It was recorded about 34 shrub species (Figure 3) under 27 genera and 16 families. They were mostly from Rubiaceae Family comprised of 8 species, followed by Verbenaceae, Acanthaceae, Annonaceae, Euphorbiaceae, Malvaceae, *etc*. Some other shrub species found included *Barleria lupulina* (Acanthaceae), *Orophea enneandra* (Annonaceae), *Capparis micracantha* (Capparaceae), *Jatropha curcas* (Euphorbiaceae), *Scaevola taccada* (Goodeniaceae), *Hibiscus macrophyllus* (Malvaceae), *Ficus montana* (Moraceae), *Ardisia crispa* (Myrsinaceae), *Cephaelis ipecacuanha* (Rubiaceae), *Ixora javanica* (Rubiaceae), *Allophylus cobbe* (Sapindaceae), *Clerodendrum buchananii* (Verbenaceae), etc.

Climbers (general, woody and creeping)

Climber plant was classified into general climber, woody climber and creeping climber. In general climber plants, it was recorded about 23 species (Figure 3) under 18 genera and 10 families. Some climber plants were identified as new records in Bawean Island, i.e. *Hoya verticillata* (Asclepiadaceae), *Freycinetia excelsa* (Pandanaceae), *Freycinetia scandens* (Pandanaceae) and *Smilax zeylanica. Hoya verticillata* which are relatively widespread, occurring in India, Myanmar, Thailand, Indo-China, Malay Peninsula, and Sumatra to North Borneo (Kidyue et al. 2007). Those two Pandanaceae species were distributed in northern Australia to New Guinea (Hyland et al. 2010). *Smilax zeylanica* were found abundantly in Borneo Island (Purwodadi Botanic Garden and Indo Tambangraya Megah 2016) but no record in Java Island (Priyadi et al. 2010).

Woody climber plants were recorded about 11 species (Figure 3) under 10 genera and 9 families. Woody climber plants commonly found were *Gnetum gnemoides* (Gnetaceae) and *Tinospora crispa* (Menispermaceae). The plants grew very well and produced many fruits; it became fodder for wild animals on the island as evidenced by the bitten marks on the fruit peels. *Tinospora crispa* are widely cultivated in Java, it is used as herbal medicine (Backer 1963). The other woody climber species were *Uvaria* sp. (Annonaceae) (Figure 4.D), *Zizyphus oenoplia* (Rhamnaceae), *Poikilospermum suaveolens* (Cecropiaceae), *Hiptage benghalensis* (Malpighiaceae), *Schefflera elliptica* (Araliaceae), *Anamirta cocculus* (Menispermaceae), *Olax scanden* (Nephrolepidaceae), and *Harrisonia perforata* (Simaroubaceae). Whilst, creeping climbers were recorded about 5 species (Figure 3) under 1 genus and 1 family i.e. *Piper cubeba*, *Piper retrofractum* and *Piper* spp. of the Piperaceae Family.

Clumps

Bamboo plants (Poaceae) have growth habit in form of clumps. It was recorded about 7 species (Figure 3) under 4 genera. It was found mostly around the river banks and water sources. Ecological functions of the bamboos are very important on soil erosion control, water conservation, land rehabilitation, and carbon overcoming (Zhou et al. 2005). In addition, some bamboo species are also found distributed in the forest and forest border. Bamboo species found included Bambu Duri (*Bambusa blumeana*), Bambu Ampel (*Bambusa vulgaris*), Bambu Ater/Betung Jawa (*Dendrocalamus asper*), Bambu Apus (*Gigantochloa apus)*, Bambu Betung (*Gigantochloa ater*), Bambu Kuning (*Schizostachyum brachycladum*) (Figure 4.G), and Bambu Buluh (*Schizostachyum iraten*). Bamboos are utilized as popular material for building of traditional houses for local residents.

Herbaceous/herbs (annual and perennial)

Perennial herbs showed higher diversity than annual herbs. Annual herbs were recorded to have about 6 species (Figure 3) under 5 genera and 5 families *i.e. Cyathula prostrata* (Amaranthaceae), *Ageratum conyzoides* (Asteraceae), *Begonia* spp. (Begoniaceae), *Hyptis brevipes* (Lamiaceae) and *Hibiscus tiliaceus* (Malvaceae). Whilst, perennial herbs were recorded to have about 39 species (Figure 3) under 32 genera and 11 families including grasses (Poaceae), bananas (Musaceae), aroids (Araceae), etc (Table S1).

There are 19 species of grasses identified (Table S1). *Homalomena pendula* (Araceae) were mostly distributed in the wet land (Figure 4.H). *Crinum asiaticum* and *Pancratium zeylanicum* (Amaryllidaceae) were found only in coastal area. *Calathea lietzei* (Marantaceae) and *Curculigo orchioides* (Hypoxidaceae) were distributed in large populations but on specific location with high humidity. Wild banana species of *Musa acuminata* ssp. (Figure 4.H) and *Musa balbisiana* ssp. were found only in Kastoba Montane Forest. Wild *Musa acuminata* and *Musa balbisiana* were widespread in tropical and subtropical regions in Asia; however for *Musa balbisiana* in Bawean Island was suspected to be introduced by the local residents since it was endemic in northern India, China, most of Indochina to the Philippines (De Langhe et al. 2009).

Rhizome herbs

Rhizome herbs were recorded to have about 4 species (Figure 3) under 4 genera and 2 families *i.e.* Costaceae (*Costus speciosus*) and Zingiberaceae (*Alpinia galanga*, *Etlingera elatior*, *Gastrochilus panduratus*). Both families are mostly found in the lowland sites with high humidity. *Alpinia galanga* (local name= laos or lengkuas) is widely cultivated by local residents in their home garden for food spices and medicine. *Etlingera elatior* is consumed by local residents as side dishes.

Figure 4. Some of plant species found in 13 montane forests of Bawean Island Nature Reserve, Indonesia: A. *Irvingia malayana*, B. *Ficus variegata*, C. *Podocarpus rumphii*, D. *Uvaria* sp, E. *Hoya diversifolia*, F. *Freycinetia scandens*, G. *Schizolatium bracicladum*, H. *Homalomena pendula*, I. *Musa acuminata wild Musa)*, J. *Amorphophallus mulerii*, K. *Tacca leontopetaloides*, L. *Tacca palmata*, M. *Dioscorea hispida*, N. *Selaginella plana*,and O. *Phalaenopsis amabilis*

Tuberous herbs

Tuberous herbs were recorded to have about 15 species (Figure 3) under 5 genera and 3 families comprised of Araceae (*Amorphophalus* spp., *Colocasia esculenta*, *Xanthosoma sagittifolium*), Dioscoreaceae (*Dioscorea* spp.) and Taccaceae (*Tacca* spp.) (Figure 4.J-M). They were mostly found in the lower plains and grew well at dry and sandy soil. They were prospected as alternative food sources for consumption.

Tacca leontopetaloides is geophyte tuber which commonly grows in grasslands, forests, river banks, under shades or full sun (Contu 2013). It was distributed from Western Africa through Southeast Asia to Northern Australia. The finding of *Tacca leontopetaloides* (Figure 4.K) in Bawean Island was a new record. It was found in small population at Payung-payung Montane Forest.

Ferns (terrestrial and epiphytic)

Ferns were recorded to have about 33 species under 21 genera and 18 families. Terrestrial ferns were more diverse and abundant (26 species) than epiphytic ferns (7 species) (Figure 3). The terrestrial ferns were dominated by *Selaginella plana* (Sellaginellaceae) (Figure 4.N). In surrounding of water springs, there were several ferns grow well such as *Angiopteris evecta* (Angiopteridaceae), *Adiantum caudatum* (Adiantaceae), *Lygodium cyrcinatum* (Schizaeaceae), *Pteris ensiformis* (Pteridaceae). Tree fern such as *Cyathea contaminans* (Cyatheaceae) was only found in high elevation. According to Posthumus (1927), it is noted that ferns in Bawean Island are also found on Java Island, and some found in Borneo. There were about 49 species of ferns during 1924-1928 (Posthumus 1927). From this study, it can be revealed that there was a decrease of fern species in Bawean Island with about 32, 65% in 2014 (33 species).

Orchids (terrestrial and epiphytic)

Orchids were recorded to have about 19 species under 17 genera (Table S1). It comprised of 6 terrestrial orchids and 13 epiphytic orchids (Figure 3). The terrestrial orchids found were *Nervilia aragoana*, *Nervilia plicata*, *Habenaria digitata*, *Malaxis* sp., *Calanthe* sp., and *Geodorum* sp. with *Nervilia aragoana* as the dominant species. Whilst, ephipytic orchids found in Bawean Island included *Phalaenopsis amabilis* (Figure 4.O), *Aeredes odorata*, *Eria javanica*, *Cymbidium aloifolium*, *Dendrobium anosmum*, *Liparis condylobulbon*, *Rhynchostylis retusa*, *Pholidota imbricata*, *Taeniophyllum bicuspidatum*, *Cymbidium* sp., *Dendrobium* sp., *Aerides* sp. and *Eria* sp.

Phalaenopsis amabilis (Figure 4.O) was the most abundance epiphytic orchid found in several Montane Forests. The host trees (phorophytes) recorded included *Euonimus javanicus* (Celastraceae), *Leea angulata* (Leeaceae), *Schleicera oleosa* (Sapindaceae), *Antidesma petandrum* (Euphorbiaceae), *Ficus variegata* (Moraceae) (Figure 4.B) and *Tectona grandis* (Verbenaceae). Most of ephipytic orchids live on zone 3 (the basal; one third of large branches), 4 (the middle third) and 5 (the upper third) of the trunk (Marsusi et al. 2001).

Conservation status of plant bioresources in Bawean Island

Conservation status evaluation showed that there are at least 33 species of plants which were included in the IUCN list whereas the others were unknown. About 30 plant species were categorized as least concern which means at lower risk of extinction. It was found widespread and abundant in Bawean Island (Table 1). Three plant species considered in higher risk of extinction in the wild including *Podocarpus rumphii* (Near threatened/NT); *Pterocarpus indicus* and *Memecylon myrtilloides* (Vulnerable/VU). *Podocarpus rumphii* and *Pterocarpus indicus* are tree plant species which are good sources of timber, therefore special attentions are needed in advocating the conservation of those plant species.

Podocarpus rumphii is widely distributed in South East ASia to New Guinea (Lemmens et al. 1995). It is a constituent of lowland to lower montane tropical rainforests, where it can be locally common. Despite its vast range and occurrence in many locations where the forest remains undisturbed, there is evidence of decline due to logging, especially in the Philippines. The extent of the decline may be approaching 30% over the past 75 years (Farjon 2013).

Pterocarpus indicus has wide geography distribution ranges from southern Myanmar to the Philippines and throughout the Malay Archipelago to New Guinea and the Solomon Islands with considerable morphological and ecological variation when viewed throughout its range. Subpopulations of *Pterocarpus indicus* have declined because of overexploitation, sometimes illegal exploitation for its timber, as well as the increasing general habitat loss (World Conservation Monitoring Centre 1998b; Orwa 2009).

Memecylon myrtilloides is shrub or small tree reaching 3 m, mostly found in upland rainforest. *Memecylon* species is reported having potential pharmacological activities (Sivu et al. 2013). However, there is a continuing decline in the extent and the quality of habitat of this species so that it is concluded as vulnerable (IUCN SSC East African Plants Red List Authority 2013).

Bio-prospecting of local plant genetic resources

Food sources

Plants which are prospected to become alternative food sources in Bawean Island are mostly from tuberous plants belong to the families of Dioscoreaceae, Taccaceae and Araceae (Table 2). Root and tuber crops are plants that produced starchy organs in forms of roots, rhizomes, corms, stems and tubers. It contained of approximately 70-80% water, 16-24% starch and trace quantities (<4%) of proteins and lipids (Hoover 2000). Each of tubers has their own nutrition and anti-nutrition properties which are also potential characters (Table 2.A). Some antinutritional substances including total free phenolics, tannins, hydrogen cyanide, total oxalate, amylase and trypsin inhibitor however it can be inactivated and eliminated by moist heat treatment and soaking followed by cooking before consumption (Shajeela et al. 2011).

Table 1. Plant species in 13 montane forests of Bawean Island Nature Reserve, Indonesia which included in IUCN list

Species name	Local name (Indonesian)	Family	IUCN status
Aglaia lawii	Sampar Kidang	Meliaceae	Least Concern
Aglaia odoratissima	-	Meliaceae	Least Concern
Alstonia scholaris	Pulai	Apocynaceae	Least Concern
Arytera littoralis	-	Sapindaceae	Least Concern
Avicennia alba	Api-api	Avicenniaceae	Least Concern
Calophyllum inophyllum	Nyamplung	Clusiaceae	Least Concern
Calophyllum soulattri	Bintangur	Clusiaceae	Least Concern
Canarium asperum	Kenari	Burseraceae	Least Concern
Centella asiatica	Pegagan	Apiaceae	Least Concern
Colocasia esculenta	Bentul	Araceae	Least Concern
Dolichandrone spathacea	Kijaran	Bignonaceae	Least Concern
Eleusine indica	Rumput Belulang	Poaceae	Least Concern
Erythrina variegata	Dadap	Fabaceae	Least Concern
Euonymus javanicus	-	Celastraceae	Least Concern
Excoecaria agallocha	Kibuta	Euphorbiaceae	Least Concern
Gnetum gnemon	Melinjo	Gnetaceae	Least Concern
Gnetum gnemonoides	Melinjo	Gnetaceae	Least Concern
Horsfieldia irya	Kayu Rah	Myristicaceae	Least Concern
Irvingia malayana	Bongin	Simaroubaceae	Least Concern
Mangifera foetida	Mangga	Anacardiaceae	Least Concern
*Memecylon myrtilloides**	Tulangan	Melastomataceae	Vulnerable
Michelia champaca	Cempaka	Magnoliaceae	Least Concern
Myristica guatteriifolia	Pala Hutan	Myristicaceae	Least Concern
Paspalum conjugatum	Rumput Kerbau	Poaceae	Least Concern
Podocarpus bracteatus	Jamuju	Podocarpaceae	Least Concern
*Podocarpus rumphii**	Jamuju	Podocarpaceae	Near Threatened
Pogonatherum paniceum	Rumput pring-pringan	Poaceae	Least Concern
Pongamia pinnata	Kacang Kayu Laut	Papilionaceae	Least Concern
Prunus arborea	Kayu Tinggi	Rosaceae	Least Concern
Prunus javanica	Kayu Tinggi	Rosaceae	Least Concern
*Pterocarpus indicus**	Sena, Angsana	Fabaceae	Vulnerable
Tacca leontopetaloides	-	Taccaceae	Least Concern
Tetrameles nudiflora	Winong	Datiscaceae	Least Concern

Table 2. Plants bioprospected as food sources in Bawean Island Nature Reserve, Indonesia

Species name	Local name	Family	Notes
Dioscorea alata	Uwi	Dioscoreaceae	High carbohydrates, fiber, mineral and vitamin (Wanasundera and Ravindan 1994); high protein, carbohydrate and vitamin C (Udensi et al. 2008)
Dioscorea bulbifera	Uwi gantung	Dioscoreaceae	High carbohydrates and easy to produces tubers; rich of flavonoid, phenolics, reducing sugars, starch, diosgenin, ascorbic acid, and citric acid (Chopade et al. 2012)
Dioscorea hispida	Gadung	Dioscoreaceae	High demands, can be processed into variety of foods, hypoglycemic index which is good for diabetes diet (Estiasih et al. 2012),
Tacca leontopetaloides	Iles-iles	Taccaceae	High carbohydrates; contains of chemical compounds for medication, flavonoid, saponin and antioxidant (Contu 2003; Ubwa et al. 2011)
Tacca palmata	Iles-iles	Taccaceae	High carbohydrates; contains of chemical compounds for medication, potentially useful lead compounds for anti-cancer properties (Contu 2003; Hemscheidt 2004)
Amorphophallus campanulatus	Suweg	Araceae	High carbohydrates, glucomannan (for foods and pharmaceutical) and fiber. High demands in international market, have potentials as antibacterial, antifungal and cytotoxic activities (Sumarwoto 2005; Khan et al. 2007)
Amorphophallus variabilis	Suweg	Araceae	High carbohydrates, glucomannan (for foods and pharmaceutical) and fiber. High demand in international market, lowered blood cholesterol levels (Sumarwoto 2005; Harijati et al. 2011)
Amorphophallus muelleri	Iles-iles, Porang	Araceae	High glucomannan and fiber. As water purifier and floating colloid in beer, sugar, and oil industry (Indriyani et al. 2011)
Colocasia esculenta	Talas	Araceae	Easy to be cultivated and harvested; high carbohydrates, protein and fiber also low fat (Sefa-Dedeh and Agyir-Sackey 2004).
Xanthosoma sagittifolium	Kimpul	Araceae	Easy to be grown and harvested, produce big size of tubers. High carbohydrates, protein and fiber, low fat (Sefa-Dedeh and Agyir-Sackey 2004).

Table 3. Plants bioprospected as timber in Bawean Island Nature Reserve, Indonesia

Species name	Local name	Family	Wood density (gr/cm^3)
Diospyros blancoi	Bisbul	Ebenaceae	0.88
Irvingia malayana	Kayu buluh	Irvingiaceae	0.84
Garcinia celebica	Manggis hutan	Clusiaceae	0.76
Protium javanicum	Trenggulun	Burseraceae	0.75
Adenanthera pavonina	Saga	Mimosaceae	0.70
Leea angulata	Birang	Leeaceae	0.68
Aglaia lawii	Sampar kidang	Meliaceae	0.61
Calophyllum inophyllum	Nyamplung	Clusiaceae	0.60
Dysoxylum densiflorum	Keduya	Melastomataceae	0.57
Diospyros maritima	Kayu hitam	Ebenaceae	0.56
Pterocarpus indicus	Angsana	Papilionaceae	0.54
Sterculia foetida	Kepuh	Sterculiaceae	0.51
Canarium hirsutum	Kenari	Burseraceae	0.50
Canarium asperum	Kenari	Burseraceae	0.48
Podocarpus bracteatus	Jamuju	Podocarpaceae	0.46
Podocarpus rumphii	Jamuju	Podocarpaceae	0.46
Macaranga tanarius	Karahan	Euphorbiaceae	0.43
Calophyllum soulattri	Bintangur	Clusiaceae	0.43
Planchonella nitida	-	Sapotaceae	-

Wood density reference: Chave et al. 2009; Zanne et al. 2009)

Table 4. Plants bioprospected as ornamental in Bawean Island Nature Reserve, Indonesia

Species name	Local name	Family	Notes
Adiantum caudatum	Suplir	Adiantaceae	Beautiful foliages of fern
Adiantum hispidatum	Suplir	Adiantaceae	Beautiful foliages of fern
Tectaria polymorpha	-	Aspidiaceae	Liana fern, can be used to ornaments pergola
Asplenium nidus	Paku Sarang Burung	Aspleniaceae	Epiphytic fern, bird nest-like
Blechnum orientale	Paku Lencir, Paku Lubang	Blechnaceae	Epiphytic fern, unique foliages
Pteris biaurita	Paku Pedang	Pteridaceae	Terrestrial fern, beautiful foliages
Cyathea contaminans	Paku tiang	Cyatheaceae	Beautiful tree fern
Angiopteris evecta	Paku Gajah	Angipteridaceae	Beautiful tree fern
Crinum asiaticum	Bakung	Amaryllidaceae	White beautiful flower
Dischidia imbricata	Benikan	Asclepiadaceae	Liana, beautiful foliages and flowers
Hoya diversifolia	Hoya	Asclepiadaceae	Liana, beautiful foliages and flowers
Hoya verticillata	Hoya	Asclepiadaceae	Liana, beautiful foliages and flowers
Homalomena pendula	Nampu	Araceae	Herbaceous, beautiful foliages and fragrant, suitable for indoor plant
Curculigo orchioides	Bedur, Kokrok	Hypoxidaceae	Herbaceous, beautiful foliages and flowers
Phalaenopsis amabilis	Anggrek Bulan	Orchidaceae	Epiphytic orchid, beautiful flower
Aerides odorata	Anggrek Kuku Macan	Orchidaceae	Epiphytic orchid, beautiful flower
Cymbidium aloifolium	Anggrek Cymbidium Daun Gaharu	Orchidaceae	Epiphytic orchid, beautiful flower
Eria javanica	Anggrek Eria Kancil	Orchidaceae	Epiphytic orchid, beautiful flower
Rhynchostylis retusa	Angrek Ekor Tupai	Orchidaceae	Epiphytic orchid, beautiful flower
Liparis condylobulbon	-	Orchidaceae	Epiphytic orchid, beautiful flower
Dendrobium bracteosum	Anggrek Karang	Orchidaceae	Epiphytic orchid, beautiful flower
Dendrobium anosmum	Anggrek Mata Sapi	Orchidaceae	Epiphytic orchid, beautiful flower
Freycinetia excelsa	Pandan rambat	Pandanaceae	Creeping pandan , suitable for outdoor plants
Cissus javana	-	Vitaceae	Vine, color-full foliages, and climber
Costus speciosus	Pacing	Costaceae	Herbaceous, beautiful foliages and flowers
Areca catechu	Pinang	Arecaceae	Beautiful foliages of palm, suitable for outdoor plants
Areca montana	Jambe rende	Arecaceae	Beautiful foliages of palm, suitable for outdoor plants
Caryota mitis	Gendaru/ Palem Ekor Ikan	Arecaceae	Beautiful foliages of palm, suitable for outdoor plants

The tuber production of *Amorphophallus* spp. in agroforestry system may reach up to 8-9 tonnes/ha (Arisoesilaningsih et al. 2009). Farmers in Nganjuk Regency, East Java mostly planted *Dioscorea* spp. in agroforestry system intercropped with tree plants or in land between rice fields without any special agronomy practices and may harvested about 15-20 tubers per plant (Trimanto and Hapsari 2015). Agro-climatic condition of Bawean Island which is low land area with low intensity of rain and loose soil are optimum for the growth of tuberous plants.

Domestication of those tuberous plants followed by adoption of agricultural techniques may enhance its yield productivity.

Timber

The uses of woods to build local traditional houses (local name: rumah panggung) must take into account the conservation aspect of woody plants in Bawean Island. Local residents of Bawean Island cultivated some woody plants in their private lands to harvests its timber such as *Tectona grandis* (Verbenaceae), *Gmelina arborea* (Verbenaceae), and *Swietenia macrophylla* (Meliaceae) but yet illegal logging to forests in nature reserve are also still happening according to informations from the rangers of Bawean Island Nature Reserve.

There are at least 19 species of woody plants from the montane forests that have the potential to be developed as timber plants (Table 3). The wood of *Irvingia malayana* is good to build heavy construction, wall paneling, cabinet work, furniture, pulp for making paper, railway sleepers and supporting goods, etc. (Sosef et al. 1998). The local residents use its woods mostly for pole structures and roofs of the local house. Its wood density is quite high, only slightly lower than *Diospyros blanchoi* (Family of Ebony wood) (Table 3). Woods of the Genus *Diospyros* has good quality timber and it is easy to propagate in the low lands by seeds (Lemmens et al. 1995). In addition to its wood, *Diospyros blanchoi* also produced edible fruits for dessert. In some countries *Disopyros blanchoi* fruit are processed into various foods (Regucivilla 2013). *Podocarpus bracteatus* is an important timber species in Java. The wood is used for house construction and for making oars; spars and masts ship so that conservation is important for this species (Sosef et al. 1998).

Based on data obtained from the Central Conservation of Bawean Nature Reserve (unpublished), it is shown that illegal logging is a problem as it often happens in the conservation area. Illegal logging occurs in some montane forests *i.e.* Besar, Taneden, Payung-payung, and Alas Timur. Any cultivation efforts are needed instead of extracting from the forests in order to stop illegal logging practices and also to improve the economic level of surrounding local community. Cultivation efforts especially to those three higher risks IUCN list i.e. *Podocarpus rumphii, Pterocarpus indicus* and *Memecylon myrtilloides* may support and lower its conservation status.

Ornamentals

Most of the local houses in Bawean Island were decorated with ornamental plants gathered from the forests. There are at least 28 local plant species in the forest which are prospected as ornamental plant (Table 4). It consists of orchids, ferns, herbaceous plants, epiphytes, vines, palm, *etc.* Orchid species commonly found in the home garden are *Phalaenopsis amabilis* and *Dendrobium anosmum*. Plants of *Genus Hoya* also have potential as ornamental plants including *Hoya verticillata* which was considered as a new record in Bawean Island and *Hoya diversifolia* (Figure 4.E). Hoya plant is easy to propagate by its vegetative parts. They have beautiful flowers and suitable as indoor ornamental plants. The cultivation and development of ornamental plants in Bawean Island is very potential to be carried out.

Bawean Island as conservation area or tourism destination?

Small islands, whether located in the tropics or higher latitudes, have characteristics which make them especially vulnerable to the effects of climate change, sea-level rise, and extreme events of disasters. It is worsened by anthropogenic activities such as destruction and fragmentation of natural habitats, conversion of forests into residential areas and well-being for food, timber production/logging, tourism, *etc.* It becomes threats for plant bioresources degradation and extinction in small island (Mimura et al. 2007). Interview to forest police officers in Bawean Island Nature Reserve has given information that illegal logging and forest fires are common problem in the forest, and also tourism which are not well managed.

The increasing population, along with the development progress in Bawean Island, advocates the importance to note aspects of conservation of the island. However, the economic need enforces people to open the conservation area for tourism destination *i.e.* Kastoba Lake which is located in the hilltop of Kastoba Montane Forest with 725 ha width; it offers calm and crystal clear water with natural hilly landscape of forests (Figure 5.A) and the sanctuary of endemic Bawean deer in Tampo Sangkapura Village (Figure 5.B). The sanctuary is objected as conservatory for critically endangered Bawean deer due to deterioration of its natural habitat. Mandala and Lumut Montane Forests are also potential as tourism destinations. In addition to tourism in mountains environment, coastal environments in Bawean island are also attractive and potential as popular tourism destinations.

The development of some places in Bawean Island into tourism destination must consider its initial functions as conservation areas. It supposed to be eco-tourism destinations. Ecotourism focuses on socially responsible travel, personal growth, and environmental sustainability; is intended as a low-impact and often at small scale, as an alternative to standard commercial (mass) tourism (Honey 2008). Eco-tourists should be very caring towards environmental sustainability. In term of the opening access of natural protected areas in Bawean Island as eco-tourism destination, in addition to improved economic needs of local government and residents, it also give negative impacts to the environment which may occur in form of unexpected changes including pollution or degradation to landscapes, vegetation community structure, wildlife, invasive alien species, piled of garbage, *etc.* Therefore, some regulations must be created by local and central government to support the development of eco-tourism in Bawean Island.

It is widely recognized that invasive alien species are the second most severe threat to biodiversity after habitat destruction and that the impacts of invasive species are particularly severe on small island ecosystems (Clubbe and Hamilton 2010). Such movement, like tourism, may

Figure 5. Some tourism destinations in Bawean Island Nature Reserve, Indonesia: A. Kastoba Lake, B. Bawean deer sanctuary

become one of ways to introduce and spread the species wider. From this study, it is showed that at least 7 invasive alien plant species were identified to be occured in Bawean Island including *Ageratum conyzoides*, *Chromolaena odorata*, *Eupatorium inulifolium*, *Lantana camara*, *Imperata cylindrica*, *Stachytarpheta jamaicensis* and *Themeda arguens* (Lowe et al. 2000; Tjitrosoedirdjo 2005).

The invasive plant species were mostly in form of shrubs, small trees and herbaceous habitats. They grow abundantly, forms dense stands and mostly have allelopathic effecs that prevent the establishment of other plant species. It becomes serious problem as it spreads rapidly via seeds and root suckers (Hapsari et al. 2014). The environmental impact of an alien plant species whether it becomes invasive at its destination depends on its biological key point, what ecological role the species may play, and on additional factors such as its tolerance of the gross features of the environment in the new range (GISP-CITES 2000). It was reported that the invasion of exotic plant species *Chromolaena* and regrowth of *Tectona grandis* caused deterioration quality the natural habitat of Bawean deer, in which resulted population decrease of Bawean deer (Semiadi et al. 2015). Thus, active management of habitat through control of invasive plant species is needed.

Active conservation on the botanical heritage is needed to face rapidly acceleration impact of human development as well as global climate change. This study provides important information about the richness, conservation status, valuation of the economic prospects of plant genetic resources and some notes on potential tourism in Bawean Island. This information is essential for decision making process concerned with biodiversity conservation and sustainable natural resources management in the island.

In addition to in-situ conservation on the natural protected areas, botanic gardens have opportunity and responsibility for significant involvement in conservation of local plant genetic resources. Botanic gardens form effective network for biodiversity conservation and sustainable utilization besides their role in maintaining ecological balance preventing environmental degradation. Some conservation efforts may include ex-situ conservation in botanic gardens, develop seed banks, raise nursery and cultivation of economic prospected plants in the island to prevent from illegal logging and over extraction, eco-tourism development, etc. (Singh et al. 2014). From this botanical survey in Bawean Island, it was collected about 197 access numbers of plants to be ex-situ conserved in Purwodadi Botanic Garden, Pasuruan, East Java. It consists of 692 specimens in form of seedlings, cuttings, seeds, suckers, corms, tubers and bulbils.

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Table S1. List of inventory plant species in Bawean Island

Acanthaceae
Barleria lupulina
Clinacanthus nutans
Gendarussa vulgaris
Hypoestes polythyrsa
Pararuellia napifera
Ruellia tuberosa
Adiantaceae
Adiantum caudatum
Adiantum hispidulum
Amaranthaceae
Achyranthes aspera
Cyathula prostrata
Amaryllidaceae
Crinum asiaticum.
Pancratium zeylanicum
Anacardiaceae
Anacardium occidentale
Buchanania arborescens
Dracontomelon mangiferum
Dracontomelondao
Gluta renghas
Lannea coromandelica
Mangifera foetida
Mangifera indica
Spondias pinnata
Spondias malayana
Spondias sp.
Angiopteridaceae
Angiopteris evecta
Annonaceae
Orophea enneandra
Polyalthia lateriflora
Saccopetalum sp1
Saccopetalum sp2
Trivalvaria macrophylla
Trivalvaria sp.
Uvaria sp1
Uvaria sp2
Apiaceae
Centella asiatica
Apocynaceae
Alstonia scholaris
Cerbera manghas
Cerbera sp.
Wrightia tomentosa
Araceae
Alocasia macrorrhiza
Alocasia sp.
Amorphophallus campanulatus
Amorphophallus variabilis
Amorphophallus blumei
Amorphophallus muelleri
Colocasia esculenta
Colocasia sp1
Colocasia sp2
Homalomena pendula
Typhonium trilobatum
Xanthosoma sagittifolium
Araliaceae
Antrophyum sp.
Polyscias nodosa
Schefflera elliptica
Arecaceae
Areca catechu
Areca montana
Arenga pinnata
Caryota mitis
Caryota sp1
Caryota sp2
Cocos nucifera
Daemonorops sp.
Licuala sp.
Livistona chinensis
Metroxylon sagu
Pinanga caesia
Pinanga coronata
Plectocomia elongata
Asclepiadaceae
Dischidia imbicata
Hoya diversifolia
Hoya verticillata
Aspidiaceae
Tectaria polymorpha
Tectaria sp.
Aspleniaceae
Asplenium nidus
Asplenium sp.
Asteliaceae
Cordyline fruticosa
Asteraceae
Ageratum conyzoides
Chromolaena odorata
Eupatorium inulifolium
Eupatorium riparium
Synedrella nodiflora
Wedelia trilobata
Athyriaceae
Athyrium esculentum
Averrhoaceae
Averrhoa bilimbi
Avicenniaceae
Avicennia alba
Begoniaceae
Begonia sp1
Begonia sp2
Bignonaceae
Crescentia cujute
Dolichandrone spathacea
Oroxylum indicum
Radermachera gigantea
Radermachera sp.
Blechnaceae
Blechnum orientale
Stenochlaena palustris
Bombacaceae
Bombax ceiba
Ceiba pentandra
Durio zibethinus
Boraginaceae
Cordia bantamensis
Burseraceae
Canarium asperum
Canarium hirsutum
Canarium oleosum
Canarium sp.
Garuga floribunda
Protium javanicum
Caesalpiniaceae
Peltophorum pterocarpum
Senna alata
Senna multijuga
Senna siamea
Tamarindus indica
Capparaceae
Capparis micracantha
Cecropiaceae
Poikilospermum suaveolens
Celastraceae
Euonymus javanicus
Clusiaceae
Calophyllum inophyllum
Calophyllum soulattri
Calophyllum sp.
Calophyllum sp.
Garcinia celebica
Garcinia dioica
Garcinia dulcis
Garcinia glaucifolia
Garcinia parviflora
Garcinia sp.
Garcinia sp.
Combretaceae
Terminalia catappa
Terminalia microcarpa
Terminalia sp.
Convolvulaceae
Ipomoea pes-caprae
Merremia peltata
Costaceae
Costus speciosus
Cyatheaceae
Cyathea contaminans
Cyathea sp.
Cyperaceae
Cyperus kyllingia
Datiscaceae
Tetrameles nudiflora
Davalliaceae
Davallia trichomanoides
Dennstaedtiaceae
Microlepia speluncae
Dilleniaceae
Tetracera scandens
Dioscoreaceae
Dioscorea bulbifera
Dioscorea hispida
Dioscorea pentaphylla
Dioscorea sp1
Dioscorea sp2
Ebenaceae
Diospyros javanica
Diospyros maritima
Diospyros sp1
Diospyros sp2
Euphorbiaceae
Antidesma bunius
Antidesma montanum
Antidesma pentandrum
Antidesma sp.
Baccaurea sp.
Blumeodendron tokbrai
Codiaeum sp.
Codiaeum sp.
Croton argyratus
Croton caudatus
Drypetes neglecta
Drypetes sp.
Excoecaria agallocha
Glochidion molle
Glochidion sp.
Homalanthus populneus
Jatropha curcas
Macaranga tanarius
Phyllanthus buxifolius
Suregada glomerulata
Flacourtaceae
Flacourtia sp.
Scolopia spinosa
Flagellariaceae
Flagellaria indica
Gleicheniaceae
Gleichenia linearis
Gnetaceae
Gnetum gnemon
Gnetum gnemonoides
Gnetum latifolius
Goodeniaceae
Scaevola taccada
Hypoxidaceae
Curculigo orchioides
Curculigo sp.
Lamiaceae
Gmelina arborea
Gmelina asiatica
Hyptis brevipes
Salvia riparia
Vitex pinnata
Vitex trifolia
Vitex sp.
Lauraceae
Actinodaphne glomerata
Cinnamomum sp.
Cinnamomum verum
Dehaasia caesia
Litsea firma
Litsea glutinosa
Litsea sp1
Litsea sp2
Neolitsea cassia
Persea rimosa
Leeaceae
Leea angulata
Leea rubra
Loganiaceae
Fagraea fragrans
Lythraceae
Lagerstroemia speciosa
Magnoliaceae
Michelia champaca
Malpighiaceae
Hiptage benghalensis
Malvaceae
Abelmoschus manihot
Hibiscus macrophyllus
Hibiscus rosa-sinensis
Hibiscus tiliaceus
Urena lobata
Marantaceae
Calathea lietzei
Melastomataceae
Melastoma malabathricum
Memecylon floribundum
Memecylon myrsinoides
Memecylon myrtilloides
Meliaceae
Aglaia eximia
Aglaia lawii

Table S1. List of inventory plant species in Bawean Island (continued)

Meliaceae
Aglaia sp1
Aglaia sp2
Aglaia sp3
Aphanamixis grandifolia
Melia azedarach
Sandoricum koetjape
Aphanamixis sp.
Chisocheton sp.
Dysoxylum densiflorum
Dysoxylum gaudichaudianum
Dysoxylum sp.
Swietenia mahagoni
Toona sureni
Menispermaceae
Anamirta cocculus
Tinospora crispa
Mimosaceae
Acacia auriculiformis
Adenanthera pavonina
Albizia falcataria
Albizia lebbekoides
Albizia procera
Moraceae
Artocarpus elasticus
Artocarpus heterophyllus
Artocarpus integer
Artocarpus sericicarpus
Ficus ampelas
Ficus benjamina
Ficus callophylla
Ficus callosa
Ficus copiosa
Ficus fistulosa
Ficus hispida
Ficus montana
Ficus padana
Ficus retusa
Ficus septica
Ficus variegata
Ficus virens
Streblus asper
Musaceae
Musa acuminata
Musa balbisiana
Musa sp.
Myristicaceae
Ardisia crispa
Ardisia humilis
Ardisia sp1
Ardisia sp2
Embelia javanica
Knema laurina
Myristica fatua
Myristica guatteriifolia
Myristica sp1
Myristica sp2
Myristica sp3
Myrsine sp.
Rapanea hasseltii
Myrtaceae
Acmena acuminatissima
Acmena sp.
Syzygium cumini
Syzygium garciniifolium
Syzygium littorale
Syzygium polyanthum
Syzygium sp1
Syzygium sp2
Syzygium sp3
Syzygium sp4
Nephrolepidaceae
Nephrolepis cordifolia
Nephrolepis sp.
Jasminum multiflorum
Olax scandens
Ophioglossaceae
Helminthostachys zeylanica
Helminthostachys sp.
Orchidaceae
Aerides odorata
Aerides sp.
Calanthe sp.
Cymbidium aloifolium
Cymbidium sp.
Dendrobium bracteosum
Dendrobium sp.
Eria javanica
Orchidaceae
Eria sp.
Geodorum sp.
Habenaria sp.
Liparis condylobulbon
Malaxis sp.
Nervilia aragoana
Nervilia sp.
Phalaenopsis amabilis
Pholidota imbricata
Rhynchostylis retusa
Taeniophyllum bicuspidatum
Pandanaceae
Freycinetia exelca
Freycinetia scandens
Pandanus tectorius
Papilionaceae
Abrus precatorius
Centrosema pubescens
Derris elliptica
Derris sp.
Desmodium gangeticum
Erythrina orientalis
Erythrina crista-gali
Moghania strobilifera
Mucuna pruriens
Mucuna sp1
Mucuna sp2
Pongamia pinnata
Pterocarpus indicus
Piperaceae
Piper cubeba
Piper retrofractum
Piper sp1
Piper sp2
Piper sp3
Pittosporaceae
Pittosporum moluccanum
Poaceae
Axonopus compressus
Bambusa blumeana
Bambusa vulgaris
Centotheca lappacea
Chrysopogon aciculatus
Cynodon dactylon
Dendrocalamus asper
Eleusine indica
Eulalia amaura
Gigantochloa apus
Gigantochloa atter
Imperata cylindrica
Oplismenus burmannii
Oplismenus compositus
Paspalum conjugatum
Pogonatherum paniceum
Schizostachyum brachycladum
Schizostachyum iraten
Themeda arguens
Podocarpaceae
Podocarpus bracteatus
Podocarpus rumphii
Polypodiaceae
Drynaria quercifolia
Drynaria rigidula
Pyrrosia nummulariifolia
Proteaceae
Helicia serrata
Pteris biaurita
Pteris ensiformis
Pteris tripartita
Pteris sp1
Pteris sp2
Rhamnaceae
Zizyphus oenoplia
Rhizophoraceae
Rhizophora sp.
Carallia sp.
Rosaceae
Rubus rosaefolius
Prunus javanica
Prunus sp.
Rubiaceae
Anthocephalus cadamba
Cephaelis ipecacuanha
Ixora javanica
Ixora miquelii
Ixora paludosa
Nauclea coadunata
Nauclea lanceolata
Nauclea sp.
Paederia scandens
Pavetta indica
Psychotria adenophylla
Randia oppositifolia
Tarenna fragrans
Canthium glabrum
Neonauclea sp.
Ixora sp1
Ixora sp2
Rutaceae
Acronychia trifoliolata
Clausena excavata
Zanthoxylum rhetsa
Melicope sp1
Melicope sp2
Zanthoxylum sp.
Sapindaceae
Allophylus cobbe
Arytera littoralis
Chrysophyllum roxburghii
Harpullia arborea
Harpullia sp1
Harpullia sp2
Lepisanthes rubiginosa
Manilkara kauki
Mischocarpus pentapetalus
Mischocarpus sp.
Planchonella nitida
Sapotaceae
Schleichera oleosa
Tristiropsis sp1
Tristiropsis sp2
Simaroubaceae
Brucea javanica
Harrisonia perforata
Irvingia malayana
Picrasma sp.
Smilacaceae
Smilax zeylanica
Sterculiaceae
Helicteres hirsuta
Kleinhovia hospita
Pterospermum javanicum
Sterculia foetida
Sterculia sp.
Eurya nitida
Thelypteridaceae
Christella arida
Christella dentata
Sphaerostephanos polycarpus
Thymelaeaceae
Phaleria octandra
Tiliaceae
Microcos tomentosa
Schoutenia ovata
Ulmaceae
Celtis sp.
Urticaceae
Laportea stimulans
Pipturus argenteus
Verbenaceae
Clerodendrum buchananii
Sterculia sp2
Taccaceae
Tacca leontopetaloides
Tacca palmata
Theaceae
Planchonella nitida
Palaquium sp.
Palaquium sp.
Planchonella sp.
Schizaeaceae
Lygodium circinatum
Selaginellaceae
Clerodendrum serratum
Lantana camara
Selaginella plana
Stachytarpheta jamaicensis
Vitaceae
Cayratia trifolia
Cissus javana
Tetrastigma lanceolarium
Vittariaceae
Vittaria sp.
Zingiberaceae
Alpinia galanga
Etlingera elatior
Gastrochilus panduratus

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Temporal diversity of *Taraxacum kok-saghyz* plants reveals high rubber yield phenotypes

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Abstract. *Cornish K, Kopicky SL, McNulty SK, Amstutz N, Chanon AM, Walker S, Kleinhenz MD, Miller AR, Streeter JG. 2016. Temporal diversity of* Taraxacum kok-saghyz *plants reveals high rubber yield phenotypes. Biodiversitas 17: 847-856. Taraxacum kok-saghyz* is a diploid, out-crossing, rubber-producing species under development as an alternative natural rubber crop. About 17,650 seed were obtained from progeny of 20 wild collected plants. New populations of plants were developed in Ohio from a random subsample of these seed, which were then open pollinated. In November 2011, these seed were direct seeded in outdoor shallow raised beds and in high tunnel deep raised beds. Plants were harvested from the outdoor beds from July 2012 to July 2013 to provide temporal phenotypic data as plants developed and overwintered. The high tunnel beds were harvested July 2013, and provided data on 11 individual accessions, and their progeny, and on the effect of winter bed heating. Plants were highly variable. Rubber concentration in root tissue was not directly correlated with root, shoot or plant size. Across all growing conditions and developmental stage, the highest rubber yields per plant were found in plants with large roots combined with a large rosette, and an above average rubber concentration. These parameters appeared to segregate independently, and rubber concentration was heritable. Interbreeding plants selected for large root, large rosette and then high rubber concentration, should rapidly move *Taraxacum kok-saghyz* towards domestication and commercialization.

Keywords: Diversity, Kazak dandelion, natural rubber, phenotype, rubber dandelion, Russian dandelion, *Taraxacum kok-saghyz*, temporal

INTRODUCTION

Taraxacum kok-saghyz (TK, also known as rubber dandelion, Russian or Kazak dandelion) was identified as an alternative source of natural rubber in the early 1930's (Lipshitz 1934), and is suited to domestic rubber cultivation in temperate regions (Whaley and Bowen 1947; Mooibroek and Cornish 2000; van Beilen and Poirier 2007; Buranov et al. 2010). Producing *cis*-1, 4-polyisoprene rubber very similar, in terms of macromolecular structure and composition, to that from *Hevea brasiliensis* (Cornish et al. 2015), TK is an annual crop which may be managed using modern agricultural techniques. Although TK has long been known to produce rubber (Lipshitz 1934), the crop has not been domesticated in the modern era, and requires extensive agronomic optimization and genetic improvement before it can become a reliable, commercial, rubber crop.

Early cultivation of TK highlighted key issues associated with production of this emerging crop. High genetic variation was present in TK populations with root weights ranging from 2 to 150 g (Whaley and Bowen 1947). Rubber content also varied, with some TK plants producing only trace amounts of rubber, while others produced upwards of 30 percent of the total plant dry weight (Whaley and Bowen 1947). Intraspecific variation also was evident in root and leaf morphological characteristics, which seemed to be heritable, but no phenotype was identified that directly correlated with high rubber yield. By the 1940's, genetically distinct TK lines had been developed which exhibited greater uniformity in both root weight and rubber content (Krotkov,1945; Whaley and Bowen 1947). Also, yield improvements were obtained using tetraploids induced with colchicine (Warmke 1945). However, with the restored supply of low cost *Hevea* natural rubber following WWII, TK-based rubber production became economically uncompetitive, and cultivation of TK and all other alternative rubber sources ceased (Finlay 2009; Venkatachalam et al. 2013). Further, all improved TK lines, which had been maintained into the post-war era, were ultimately lost with the dissolution of the Soviet Union. A re-introduction of TK in the early to mid-2000s, which was widely distributed, was later found to be *Taraxacum brevicorniculatum*, a common seed contaminant (Kirschner et al. 2012). Because of this, our current efforts to develop TK as an alternative rubber source are based on the later 2008 USDA collection of seed from wild TK diploid outbreeding plants from Kazakhstan and Uzbekistan (Hellier 2011). This collection was guided by Dr. Alexander Sennikov, Curator of Vascular Plants, Herbarium, Russian Academy of Sciences, V. L. Komarov Botanical Institute, Russia, to ensure accurate TK identification.

In this study, we characterized phenotype diversity in a population generated by open pollination of several thousand plants grown from a subsample of 17,450 seed from 20 accessions. Diversity in growth and rubber concentration in TK plants was tracked from 7 to 20 months in outdoor shallow raised beds, and among 9-month old plants from different accessions grown in high tunnel raised beds with and without winter bed heating.

MATERIALS AND METHODS

Plant material

TK seed were obtained from the 2008 USDA collection of seed from wild TK plants in Kazakstan and Uzbekistan (Hellier 2011). The plants grown from these seed were compared with detailed descriptions of TK and *T. brevicorniculatum* (Kirschner et al. 2012). Although considerable variation existed among plants, the TK plants had thick blue-green leaves without small teeth on the leaf margins (Figure 1.A) whereas *T. brevicorniculatum* leaves were thinner and light in color (*cf.* Figure 1.A and D, and B and E). Also, the TK leaves were waxy (Figure 1.A), while the *T. brevicorniculatum* leaves were more papery (Figure 1.D). The flowers were relatively small, and had long "hornlike" bracts (Krotkov 1945; Kirschner et al. 2012) distinct from the shorter bracts on authentic *T. brevicorniculatum.* The latter species often exhibits a red/purple color on leaves and flower stalks (Figure 1.D) which almost never occurs in healthy TK (Figure 1.A-C). In addition, the genome sizes of over 1,000 TK plants were analyzed by flow cytometry (n=8, data not shown) and without exception were proved to be diploids, in contrast to the clearly triploid *T. brevicorniculatum* and *T. officinale* (common dandelion), which also were confirmed with flow cytometry (data not shown).

Cuttings from 91 different interbred TK lines developed in Ohio were transplanted into high tunnel beds in fall 2010 and overwintered. In spring 2011, bulk seed was collected by vacuum and hand harvest from 11 of the beds, forming 11 new seed accessions, and cleaned. The mean rubber content of three of the maternal parents of each accession was analyzed by accelerated solvent extraction (see method below).

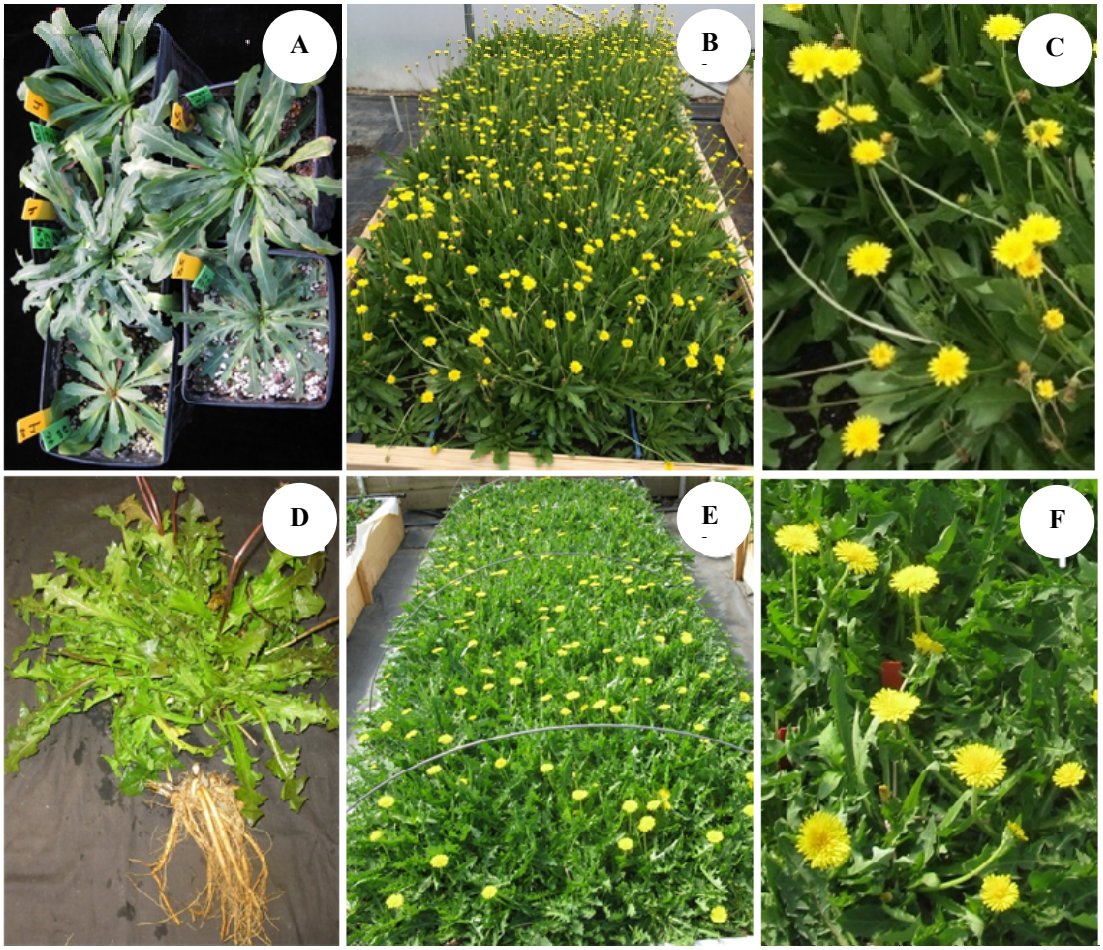

Figure 1. Photographs of *Taraxacum kok-saghyz* and *Taraxacum brevicorniculatum* plants in our germplasm collection. A. Some *T. kok-saghyz* leaf phenotypes; B. A bed of flowering *T. kok-saghyz*; C. *T. kok-saghyz* flowers; D. A typical *T. brevicorniculatum* plant; E. A bed of flowering *T. brevicorniculatum*; F. *T. brevicorniculatum* flowers

Growth and rubber content of TK in outdoor shallow beds

A subset of seed was pooled from 11 generated accessions and planted in autumn of 2011, in outdoor shallow raised beds at The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, OH. The shallow raised beds had a soil composition of 20% parboiled rice hulls, 30% peat moss, 25% compost from OARDC campus facilities, and 25% Wooster silt loam field soil. TK seed was mixed with a turface/sand mix, which acted as inert filler and reduced seed density, and was dispersed by hand into premade furrows in the beds. This method of seed distribution simulated direct seeding conditions. The shallow raised beds were 1.2 m wide, 9.1 m long, had a soil depth of 0.3 m and were drip irrigated. Low tunnels fashioned from clear plastic sheeting with a thickness of 0.15 mm (Klerks Hyplast, Chester, SC) were applied from November to March to provide insulation and protection from the elements. The outdoor beds were left exposed to the environment from April to October. A Hobo Pro v2 data logger (Onset Computer Corporation, Bourne, MA) was installed in the middle of a representative outdoor bed to measure both air temperature and soil temperature every 15 minutes for the duration of the experiment. The data logger was fitted with an aluminum radiation shield to deflect direct sunlight from the air temperature sensor and the soil temperature sensor probe was inserted 10-15 cm into the soil. Daily soil temperatures averaged 13°C for the duration of the experiment, with -7°C recorded as the minimum soil temperature and 44°C as the maximum soil temperature. Daily air temperatures also averaged 13°C for the duration of the experiment, with -13°C recorded as the minimum air temperature and 44°C as the maximum air temperature. The campus operated solar powered data logger and modem (Campbell Scientific, Logan, UT) inserted at 10 cm into the ground recorded an average soil temperature of 12°C during the experiments with extremes of 0°C and 29°C. This weather station also recorded air temperatures as low as -18°C in January 2012 and -17°C in January 2013.

Five randomly selected plants were harvested by hand on approximately a biweekly basis from July 2012 to July 2013. A total of 124 TK dandelions were harvested over the course of the experiment. Immediately following each harvest, plants were wrapped in moist paper towels and transported to the lab in clear plastic bags, where data were gathered on plant size. TK roots were separated from the crown to obtain fresh root weights, then placed in paper bags, and oven dried at 53°C for two days, and weighed.

Growth and rubber content of TK in high tunnel deep planting beds

Seed of 11 accessions were planted in autumn of 2011, in high tunnel deep raised beds at The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, OH. The high tunnel raised beds were protected by a roof made of plastic sheeting with a thickness of 0.15 mm (Klerks Hyplast, Chester, SC) throughout the year, with retractable siding allowing airflow and temperature regulation from April to October. During the cold season, November to March, the sidewalls were closed creating a protected, unheated environment. Data loggers were installed in representative beds as described above. To further protect the plants from low temperatures and freeze/thaw, tents made from Agribon™ row cover fabric (Polymer Group, Inc., Charlotte, NC) were fitted on to the high tunnel beds from November to March. High tunnel beds had dimensions of 3.65 m by 1.2 m with a soil depth of 0.61 m. Soil in the raised beds was a mixture of 33% Pro-mix, 16.5% vermiculite, 16.5% compost from OARDC campus facilities, 16.5% Wooster silt loam soil, and 16.5% perlite. Six high tunnel beds were sectioned crosswise into 1.8-m halves forming 12 plots, and each plot was divided into 10 rows. An accession was randomly selected to be planted in each row, with no accession being repeated in a plot. Mixtures of seed and turface were dispersed into furrows in each of the 10 rows, functioning as a simulated direct seeding method. Half of the high tunnel beds were outfitted with subterranean heating cables operational from December 2011 to March 2012, maintaining an average soil temperature of 21°C, but soil temperature dropped to as low as 2°C on the coldest days of the year. Soil temperatures in the unheated beds averaged 16°C and also dropped as low as 2°C. The high tunnel *Taraxacum kok-saghyz* plants were grown until the following July (2012), then harvested. Two dandelions of each accession were harvested on each harvesting day. Plants were labelled, wrapped in moist paper towels, placed in clear plastic bags, and transported to the lab and weighed. TK roots were cut from the crown, weighed, placed in paper bags, oven dried at 53°C for two days, and reweighed before rubber analysis. A total of 240 TK dandelion samples were harvested, with 120 samples from heated beds and 120 from unheated beds.

Rubber quantification

Rubber was quantified using accelerated solvent extraction (ASE). Dry root samples were finely ground using an analytical mill (A 11 basic, IKA, Wilmington, NC) and weighed to 0.2500g ± 0.0005g. Ground material was mixed with approximately 5 ml of inert Ottawa sand (20-30 mesh), to evenly distribute the sample throughout 11 cm^3 stainless steel cells (from the Dionex ASE 200) and to improve accessibility of the solvent to sample particles during extraction. The resulting mixture was poured into the stainless steel cells fitted with cellulose filters inserted on both ends, preventing plugging during the purge cycle and increasing the flow of the dissolved analyte (Thermo Scientific, Waltham, MA). ASE cells were placed into the Dionex ASE 200 (Thermo Scientific, Waltham, MA). A sand filled blank served as both a visual and numerical control for extraction protocols. During the extraction process, hexane was injected into each cell, which were sequentially pressurized to 10.34 MPa (1,500 psi) and heated to 120°C to extract high molecular weight rubber. After a 50-minute process, including two 20-minute static cycles and two repeated 60-second purges, hexane-dissolved rubber from each cell was collected in separate glass vials. Vials containing hexane and dissolved rubber were vortexed and poured into pre-weighed aluminum

pans. Additional hexane was used to rinse analyte remnants from the vial walls using repeated vortexing. Hexane was evaporated from aluminum pans during a 24 h drying period at room temperature in a fume hood, leaving behind the rubber. Aluminum pans were then reweighed and the amounts of dried rubber determined. The following equations were used to determine rubber content in plant biomass, rounded to 0.1 mg/g dry weight.

$$Final\ Pan\ Weight\ (g) - Empty\ Pan\ Weight\ (g) = Analyte\ Weight\ (g)$$

$$\left(\left(\frac{Analyte\ Weight\ (g)}{Sample\ Weight\ (g)}\right) * 1000\right) - correction\ factor = Analyte\ Content\ \left(\frac{mg}{g}\right)$$

A correction factor was used to account for the fraction of acetone-soluble non-rubber analytes that were co-extracted with the rubber by the hexane (Pearson et al. 2013). This factor is the mean of 15.22 $\pm$ 1.73 mg/g from 78 root samples (data not shown) from sequential accelerated solvent extraction in which acetone was used as the extractant before the hexane (data not shown). Rubber extraction efficiency of this ASE method is 91% (data not shown).

RESULTS AND DISCUSSION

Outdoor shallow beds

Plant size was highly variable (Figure 2.A) and this included variation in both root (Figure 2.B) and rosette (Figure 2.C) size. Exceptionally large plants (> 3x the median) were rare in this population. Also, larger plants may be especially susceptible to plant death during the winter (Figure 2.A-C) because no very large plants were present the following spring and summer. The variation among the plants, even discounting the exceptionally large plants, was also reduced in the following spring and summer. Roots across this time course were highly variable in rubber concentration (51.4 $\pm$ 16.5 mg/g dry root, mean of 129 plants $\pm$ sd), and plants harvested on a single day varied by as much as 68 mg/g dry root (Figure 2.D).

As the plants overwintered, there was a general overall decline in rosette weight (Figure 2.C), as many leaves senesced, and a decline in root mass also was observed (Figure 2.B). The plants in July of 2013 did not regrow to the plant, root and rosette biomass achieved a year earlier at the same time of year (Figure 2.A-C). Yet even with this variation, an overall increase in rubber concentration was apparent during the winter season, beginning in November 2012 and levelling off by February 2013 (Figure 2.D). Variability in rubber concentration among plants harvested on any single day was also greater in the spring than during previous autumn. However, when the total amount of rubber was calculated (Figure 2.E), the increase in rubber concentration (Figure 2.D) was countered by the reduction in root mass, leading to little change in rubber per plant over the year in most TK plants. It is also clear that the winter die-back affected rosettes more than roots (Figure 2.F) since the root:rosette ratio increased during the winter. However, not all plants suffered rosette senescence during the winter (Figure 2.F).

Two plants had higher rubber amounts than the general range (Figure 2.E). One of these plants (indicated by the dot and dash arrows) had a high rubber concentration in the roots (Figure 2.D) but, although above average in root biomass (Figure 2.B), was not an unusually large plant (Figure 2.A) and had a relatively small rosette (Figure 2.C). Thus, high root rubber concentration was the cause of its overall high rubber yield (Figure 2.E). The highest yielding plant in the study (indicated by the dashed arrow) had a moderate root rubber concentration (Figure 2.D), but was the largest of all the plants harvested (Figure 2.A), had the second largest rosette (Figure 2.C), and most notably, a remarkably large root mass (Figure 2.B). One other very large plant (indicated by the solid arrow, Figure 2.A) had a large rosette (Figure 2.C) but a quite small root system (Figure 2.B). Even though this plant also had an above average root rubber concentration (Figure 2.D), its small root biomass resulted in only a slightly above average plant rubber yield (Figure 2.E).

Overall there was a correlation of root fresh weight and rosette fresh weight but the high degree of between plant variation in root:rosette ratio caused a quite low correlation coefficient (Figure 3.A, $r^2 = 0.446$). No relationship of root fresh weight to the root:rosette ratio was found (Figure 3.B, $r^2 = 0.018$). However, rosette fresh weight was correlated to the root/rosette ratio in a nonlinear manner (Figure 3.C, $r^2 = 0.308$).

Rubber concentration and rubber yield per plant were then plotted against a variety of plant phenotypic parameters (Figure 4). The highest amount of rubber per plant (dashed arrow) occurred in an exceptionally large plant (Figure 4.B) with a very large root (Figure 4.D), a large rosette (Figure 4.F), and a root:rosette ratio of 0.65. In contrast, the highest concentration of rubber (dot and dash arrow) was observed in a plant with large root and, average rosette and plant biomass. However, this plant has a relatively high root:rosette ratio of 0.88.

Rubber concentration was not well correlated with plant phenotype (Figure 4.A, C, E, G) and this was confirmed by plotting concentration against root and shoot fresh weight (Figure 5). No clear trend was apparent from the weighted averages.

However, when total rubber per plant values were plotted (Figure 6.A) a clearer relationship was revealed. Small plants were invariably low rubber yielding. Plants with large rosettes and small roots had also low rubber yield. Plants with large roots and small rosettes were medium in rubber yield, although they had considerably more rubber than the first two classes described, and larger roots progressively resulted in higher total rubber. However, in this germplasm pool, plants with large root *and* large rosettes had the highest rubber yields. The weighted average plot (Figure 5.B) confirmed these trends and indicated an optimum relationship of maximum total rubber in plants with both large root and large rosette.

High tunnel deep beds

The 240 plants harvested in July 2012 from high tunnel deep beds had a wide range of root rubber concentrations (0.4 to 103 mg/g dry root). This is similar to what was

described in plants grown in outdoor shallow beds. Root rubber concentrations in individual plants were skewed towards lower rubber concentrations, but some plants had concentrations as high as twice the population mean of 51.4 mg/g dry root (Figure 7).

Plants from the 11 accessions exhibited considerable within variation for rubber concentration (Figure 8.A). However, an analysis of variance showed no significant difference among the 11 accessions (Table 1). In addition, the accessions did not significantly differ in root biomass (Figure 8.B, Table 2). Rubber concentration of mother plants did positively correlate with rubber concentration in their progeny (Figure 9, $r^2 = 0.530$).

Heating the deep beds in the winter had no effect on root rubber concentration (Figure 10.A, Table 1). However, heating had a significant effect on root biomass (Figure 10.B, Table 2), with root weight means of 6.6 and 4.7 g for the heated and unheated treatments, respectively. Maintaining the soil temperature at 21°C from December to March resulted in approximately 40% larger roots, compared to TK in unheated beds, when harvested the following July.

The relationship of plant phenotype to rubber concentration and total rubber per plant was investigated, combining data from all 11 accessions, because significant differences had not been detected among them for rubber or biomass parameters. As was found for plants grown in the outdoor shallow beds, phenotypes in the high tunnels were highly variable, and a wide range of rubber concentration and total rubber per plant was observed (Figure 11.A-F). As expected, since the outdoor shallow beds were planted with the same pool of seed, some outliers were again observed. Some plants were much larger than most (Figure 11.A and B), and had very large roots (Figure 11.C and D) or rosettes (Figure 11.E and F). In general, rubber yields increased with plant and root biomass, but rubber concentration was not correlated to biomass parameters, and the 3D plot (not shown) was similar to that in Figure 5. The overall trends in the relationship of root and rosette biomass to total rubber per plant (Figure 12) were very similar to those in the temporal study (Figure 6.B). Thus, small TK plants grown in deep high tunnel beds contained very little rubber, and the highest rubber yields were found in plants with both large roots and rosettes.

Discussion

Wide phenotypic variation was apparent in TK grown in outdoor shallow beds and in high tunnel deep beds, as expected since 91 distinct Ohio developed TK lines made up the parent accessions, and TK is a sexual, self-incompatible diploid (Hodgson-Kratky and Wolyn 2015). This high level of variation allows for determination of some phenotypic characteristics that are, and others not, related to rubber yield. It is clear that large root biomass, not high rubber concentration alone, is currently the principle determinant of rubber yield, particularly when coupled with a large rosette (Figures 6 and 12). The lack of correlation, in both temporal and high tunnel deep bed studies, between root, rosette, plant biomass and rubber concentration, means that large plants with above average rubber concentrations are present (Figures 4 and 11) albeit in relatively small numbers. Also, it suggests that these parameters are segregating independently of each other. Thus, while rubber concentration of the population was skewed towards lower concentrations, existence of high rubber concentrations in roots of some plants indicates that selection for higher rubber concentration should be possible in large plants. These results suggest that earlier studies on season and daylength (Borthwick et al. 1943) may not be pertinent to improve growth or rubber parameters of our population.

Yield per plant is a combination of root biomass and rubber concentration parameters. There was no negative correlation apparent between root, rosette, or plant biomass and root rubber concentration, or total rubber per plant. The highest yield per plant, in our study of unimproved plants, was 0.9 g rubber in a plant established from seed in autumn 2011, by direct seeding, and then harvested in early spring 2013, a longer growing season than could be readily added to a standard crop rotation. Nonetheless, 1 million plants per acre of this phenotype would yield 2160 kg/ha, grown under these conditions in northeast Ohio. This amount is already within the rubber yield/ha/year range of tropical rubber tree plantations (800-3000 kg/ha/year and very similar to the yield from the semi-arid lands rubber crop, guayule.

Table 1. A two-way analysis of variance of accession and heat treatment on *Taraxacum kok-saghyz* rubber content.

Response: Rubber Content	Degrees of Freedom	Sum of Squares	Mean Squares	F Value	Significance (P)
	10	445	444.7	1.32	0.25
Seed lot					
Heat treatment	1	143	142.9	0.43	0.52
Interaction	1	235	235	0.70	0.40
Residuals	235	79080	336.5		

Table 2. Two-way analysis of variance of accession and heating treatment on *Taraxacum kok-saghyz* root biomass.

Response: Root Growth	Degrees of Freedom	Sum of Squares	Mean Squares	F Value	Significance (P)
	10	24.7	24.7	1.96	0.16
Seed lot					
Heat treatment	1	188.1	188.12	14.89	0.00015
Interaction	1	0.3	0.33	0.026	0.87
Residuals	235	2968.7	12.63		

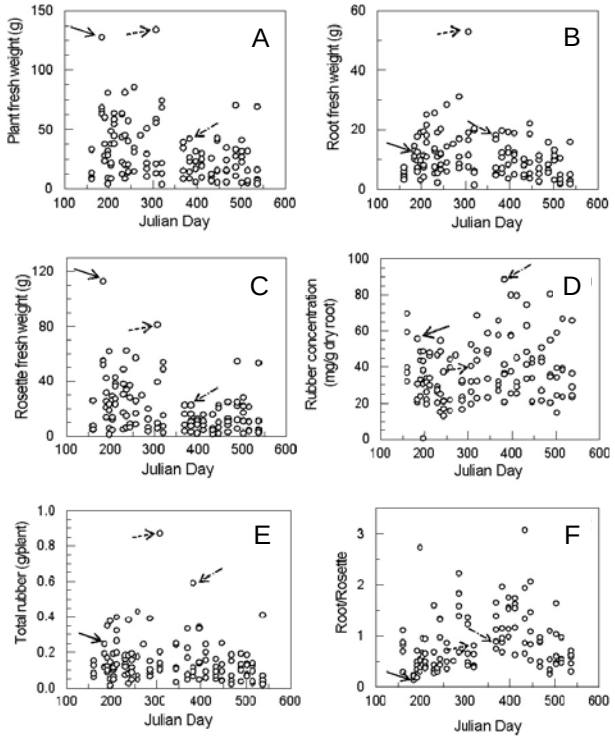

Figure 2. Phenotypic characteristics of *Taraxacum kok-saghyz* plants harvested from July 2012 to July 2013 from outdoor shallow beds. A. Plant fresh weight; B. Root fresh weight; C. Rosette fresh weight; D. Root rubber concentration on a dry weight basis; E. Total rubber per plant on a dry weight basis; F. Root:rosette ratio. Julian day #1 is 1 January, 2012. Julian day #366 is 1 January, 2013. The equinoxes during the time period shown, were September 22 (Julian day 264) December 21 (Julian day 356), March 20 (Julian day 446) and June 21 (Julian day 537). Three exceptional plants are indicated by plant-specific arrows on the different plots

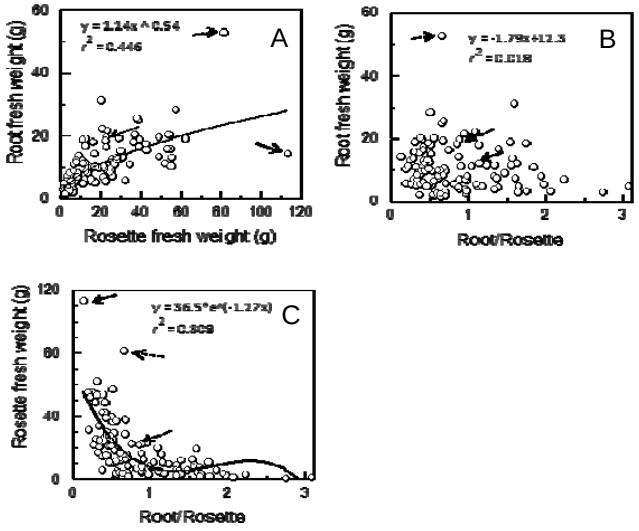

Figure 3. A. Relationship of root fresh weight and rosette fresh weight; B. Root fresh weight and the ratio of root and rosette fresh weight; C. Rosette fresh weight and the ratio of root and rosette fresh weight. *Taraxacum kok-saghyz* plants were harvested from July 2012 to July 2013, from outdoor shallow beds. The three outlier plants in Fig. 1 are indicated here by the same plant-specific arrows on the different plots.

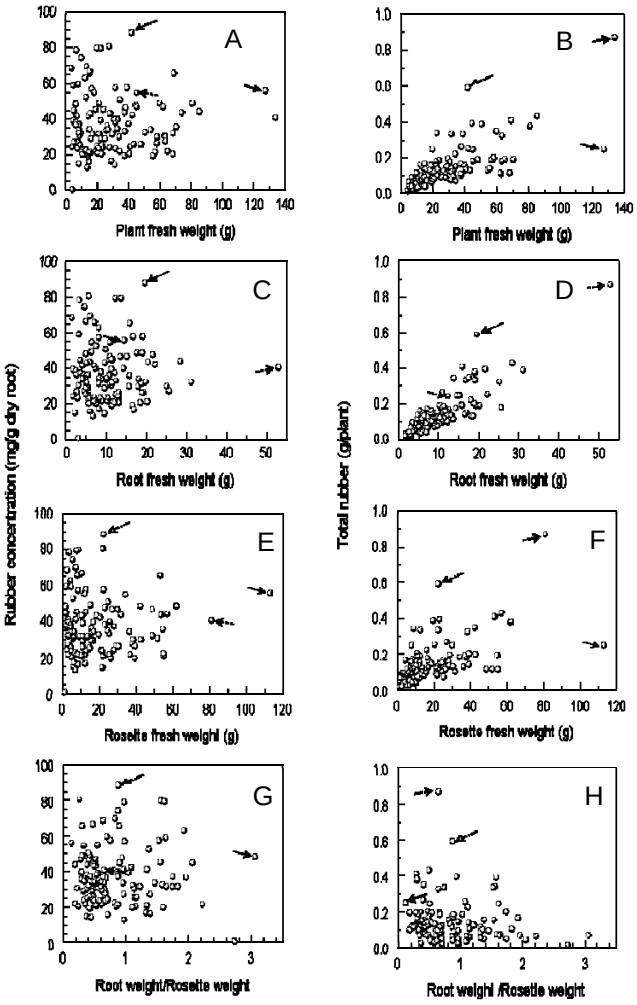

Figure 4. Relationship of root rubber concentration (A, C, E, G) and total rubber per plant (B, D, F, H) with plant fresh weight (A,B), root fresh weight (C, D), rosette fresh weight (E, F) and the ratio of root to rosette fresh weight (G, H) in *Taraxacum kok-saghyz* plants harvested between July 2012 to July 2013 from outdoor shallow beds. The three outlier plants in Fig. 2 are indicated here by the same plant-specific arrows on the different plots.

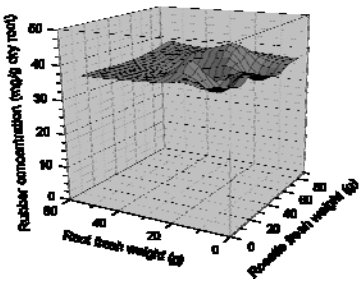

Figure 5. Surface 3D plot of root rubber concentration as a function of root and rosette fresh weights in *Taraxacum kok-saghyz*. The data were plotted as weighted averages

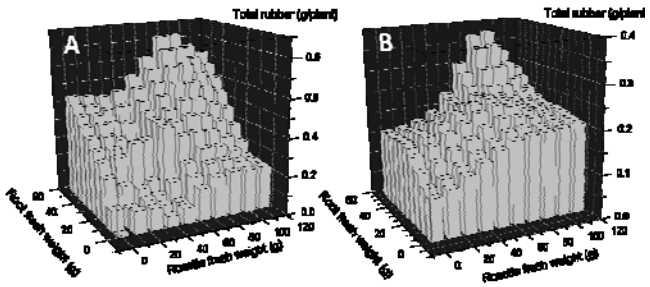

Figure 6. 3D plots of the relationship of total rubber in *Taraxacum kok-saghyz* as a function of root and rosette fresh weights by (A) linear interpolation (B) weighted average methods

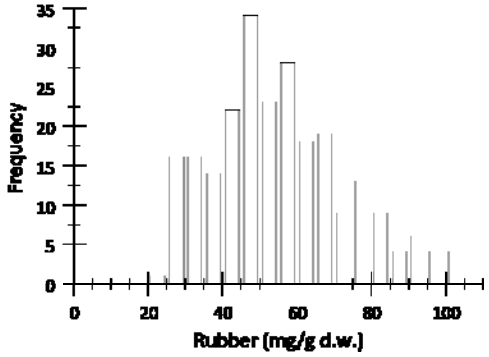

Figure 7. Distribution of rubber concentration across 10 mg intervals in 240 individual *Taraxacum kok-saghyz* root systems harvested from high tunnel raised beds during July, 2012. The plants were seeded in November, 2011

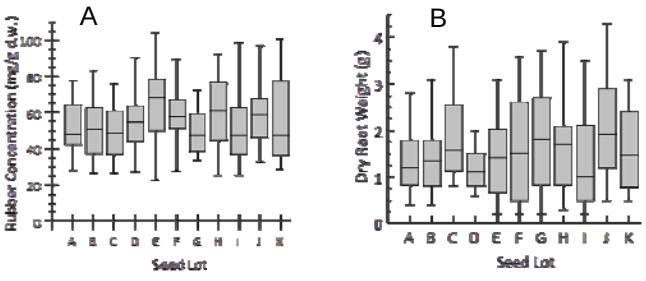

Figure 8. Box and whisker plots of (A) rubber concentration, and (B) root dry weight distribution, for the 11 *Taraxacum kok-saghyz* accessions planted from seed in November 2011 and harvested from high tunnel raised beds in July 2012. The solid boxes display the distribution of middle quartiles with the midline representing median concentrations. The extended lines display the full range representing the upper and lower quartile limits. Numbers of plants in each lot are: A-24, B-24, C-16, D-25, E-24, F-24, G-24, H-24, I-24, J-23, K-8.

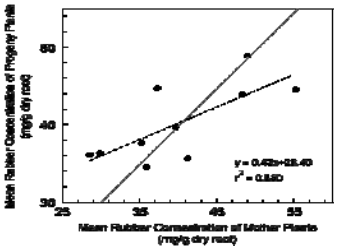

Figure 9. Correlation of rubber concentration in the progeny of 10 intrabred *Taraxacum kok-saghyz* accessions, with rubber concentration in the mother plants of the same 10 accessions. Plants were grown in high tunnel deep beds. Each progeny value is the mean of 24 plants. The dashed line is the linear regression plot. The solid line is the 1:1 ratio of progeny and maternal rubber which would occur if progeny had the same rubber concentration as their mothers

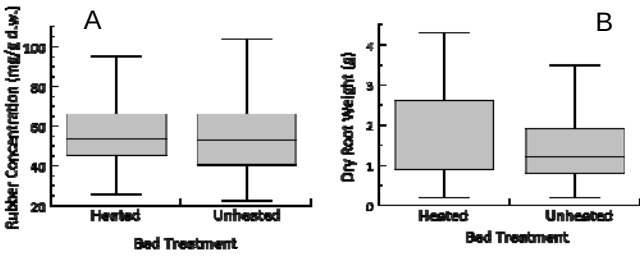

Figure 10. Box and whisker plots comparing (A) the distribution of root rubber concentrations and (B) root biomass, in 240 *Taraxacum kok-saghyz* plants of beds heated with subterranean cables from December to March, to those left untreated. The solid boxes display the distribution of middle quartiles with the midline representing median concentrations. The extended lines display the full range representing the upper and lower quartile limits

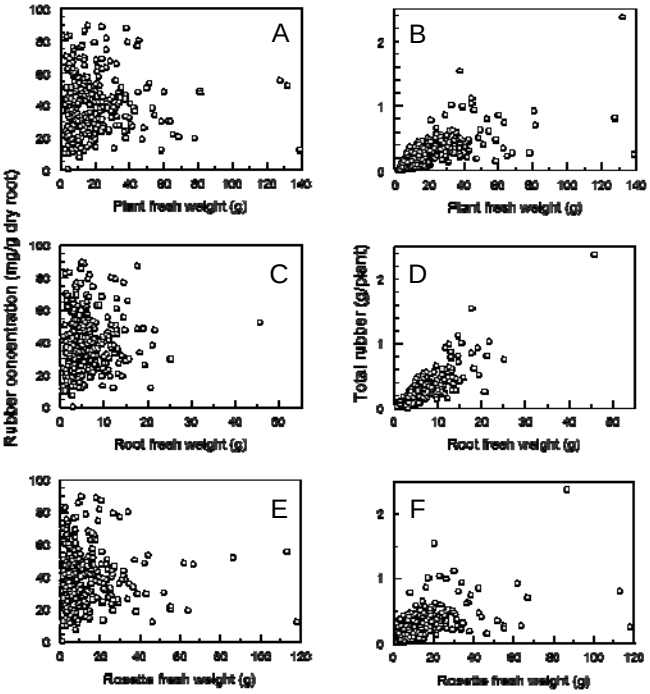

Figure 11. Relationships of root rubber concentration (A,C,E) and total rubber per plant (B,D,F) to plant fresh weight (A,B), root fresh weight (C,D) and rosette fresh weight (E,F) in 270 *Taraxacum kok-saghyz* plants grown in high tunnels and harvested in July 2012.

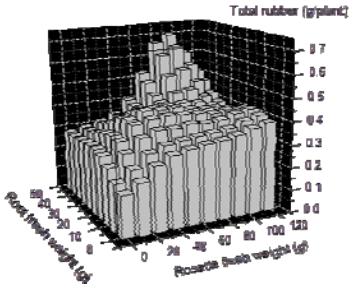

Figure 12. Relationship of rubber concentration to root and rosette fresh weights in 270 *Taraxacum kok-saghyz* plants grown in high tunnels in deep beds. Data are plotted as weighted averages

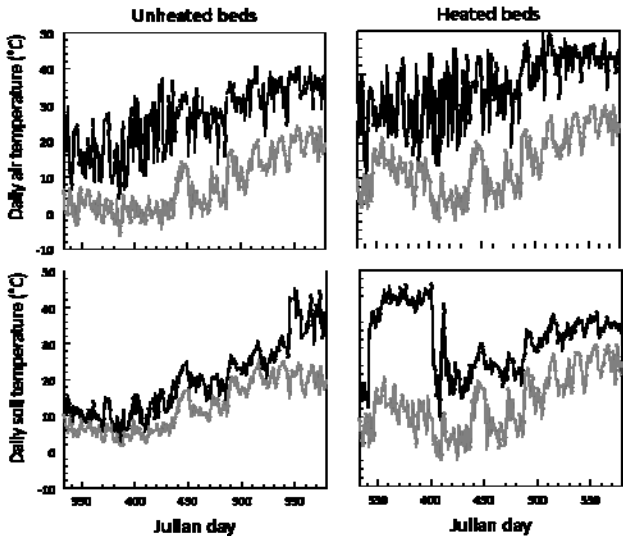

Figure 13. Maximum (black lines) and minimum (grey lines) daily air and soil temperatures in unheated and winter month-heated deep beds in high tunnels 31, from 29 November, 2011 to 31 July, 2012

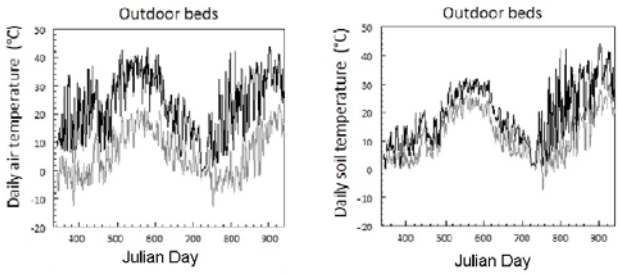

Figure 14. Maximum (black lines) and minimum (grey lines) daily air and soil temperatures in shallow outdoor beds from 29 November, 2011 to 31 July, 2013

The correlation between rubber concentration in parental accession and progeny (Figure 9), indicates some degree of heritability of rubber concentration. So interbred large high rubber plants may generate progeny with improved rubber yield. Our results indicate that the root system should be at least 30 g fresh weight and that these plants need to have a rosette fresh weight of at least 60 g to support high rubber production in roots (Figures 9 and 12). As plants are selected and bred for high biomass and high rubber concentration, it is possible that higher yielding plants may have different rosette and root biomass requirements to those described here. It is also possible that, because rubber is a metabolic end product, increasing rubber concentrations eventually may begin to inflict an unsustainable drain on plant resources and that these yields will plateau, as for other phenotypic traits in domesticated crops (Meredith 2000). Interestingly, rubber quantities above 300 mg rubber/g plant have been previously reported (Whaley and Bowen 1947). In our current study, highest rubber concentrations in these essentially wild plants were below 100 mg/g dry root, which would be less than 30 mg per average sized plant and less than 10% of the highest value reported. Nonetheless, since this study was performed, we have observed rubber concentrations in roots of up to 220 mg/g dry root in large field plants grown in Ohio.

Historically, TK has been tested as an annual crop and as an overwintered crop, and both of these can be considered with either a fall planting or a spring planting, and with direct seed or transplants as propagules. A high density direct seeded crop will be preferable for commercial viability due to the high cost of transplants. It has previously been shown that TK roots will more than double in biomass between spring and autumn (Cornish et al. 2013) in their first year of growth, but after the major flowering and seed set the next spring, field-grown plants were highly prone to summer dormancy, die back of rosettes, and root senescence after flowering, as was also described previously (Scarth et al. 1947).

There are many advantages to establishing TK as an annual production crop using an autumn planting scheme with a harvest the following autumn, especially with respect to maximizing growing days. Unfortunately, autumn-planted field plants, established in Wooster, Ohio with transplants or with direct seed, have not yet been carried through *en masse* into the spring because of the very high level of young plant death caused by frost-heaving. It is possible that a nurse crop will facilitate overwintering of young plants, but preliminary trials have not been encouraging in our region. Until overwintering of young plants is validated, a spring planting seems the only viable option, and this should be initiated as early as possible to maximize the growing season and to generate the large plants needed to produce a high rubber yield. Spring crop establishment is also needed for TK seed crops. Such plants are sufficiently large at the onset of winter that they can survive frost heaving that would otherwise kill younger plants. Overwintering allows these plants to vernalize and ensures good seed set in the following spring (Hodgson-Kratky et al. 2015).

Cold induction of rubber biosynthesis in TK has been reported (Cornish et al. 2013), and was observed in some plants in the outdoor shallow beds (Figure 2.D), but the threshold temperature for induction has not been established. Similar rubber concentrations in plant roots produced in high tunnel deep beds to those grown in

outdoor shallow beds, clearly indicate that cold temperatures are not required (Figures 13 and 14). This conclusion is supported by the high tunnel study, in which roots in heated and unheated beds produced very similar rubber concentrations. The high tunnel plants in heated beds did not become dormant like the plants in unheated beds (Figure 10) and so were able to grow more rubber-producing root tissue than the other plants. Since they maintained the same concentration of rubber as the roots grew, this resulted in a higher rubber yield per plant. This suggests that rubber concentration is genotype specific (a genotype is a genetically-unique individual or set of identical clones), is not inhibited by warm soil temperatures, and that the growing region for TK may extend to milder climates than those found in Ohio. However, variability in rubber concentration increased among the different plants harvested during the winter (Figure 2.D). This indicates that some plants were more responsive to cold induction during the winter than others (Figure 2.D). These data suggest that only some genotypes are capable of responding to cold by increasing rubber biosynthesis. Selecting these responsive genotypes may be key to establishing effective postharvest cold storage practices, which can be used to increase rubber content in stored fresh roots (Cornish et al. 2013). The presence of ambient and cold-inducible components of rubber production is similar to another species, *Parthenium argentatum* (guayule) which also has both features (Downes and Tonnet 1985; Madhavan et al. 1989; Cornish and Backhaus 2003).

In the outdoor shallow beds, rubber concentration increased during the winter, but roots did not become larger on average (Figure 2.B). Root weight per plant decreased during the winter and the resultant rubber yield per plant in the spring was essentially the same as in the previous autumn (Figure 2.E) indicating no overall yield benefit from the additional months in the outdoor beds. The additional months also pose a risk of plant death and yield loss in severe winters. In the severe "polar vortex" winter of 2013/14, in which temperatures dropped to -26°C, half of the plants grown outdoors (field and outdoor raised beds) died (unpublished results). A previous report described roots that were harvested in the autumn and then stored at 4°C in the dark after clipping off their rosettes. Root inulin reserves were catabolized during the next month, and new rubber biosynthesis then occurred, approximately doubling the rubber content of roots above 10g fresh weight in 45-60 days (Cornish et al. 2013). This method may prove to be a viable production practice for cold-responsive genotypes, especially as no roots are lost during storage.

In conclusion, clear initial selection targets have been identified - plants with large roots, large rosettes and high root rubber concentration. Even though rubber concentration and root biomass are not correlated, the relative abundance of target plants in the TK population suggests that utilization of small plants with high rubber concentration as parents and then recovering large biomass through backcrossing is unnecessary. These targets hold true independent of season for plants between 6 and 18 months of age. Rubber concentration does appear to be heritable and interbreeding selected target plants has considerable promise in rapidly converting this species into a domesticated crop.

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Polycyclic aromatic hydrocarbon degrading bacteria from the Indonesian Marine Environment

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Abstract. *Yetti E, Thontowi A, Yopi. 2016. Polyaromatic hydrocarbon degrading bacteria from the Indonesian Marine Environment. Biodiversitas 17: 857-864.* Oil spills are one of the main causes of pollution in marine environments. Oil degrading bacteria play an important role for bioremediation of oil spill in environment. We collected 132 isolates of marine bacteria isolated from several Indonesia marine areas, i.e. Pari Island, Jakarta, Kamal Port, East Java and Cilacap Bay, Central Java. These isolates were screened for capability to degrade polyaromatic hydrocarbons (PAHs). Selection test were carried out qualitatively using sublimation method and growth assay of the isolates on several PAHs i.e**.** phenanthrene, dibenzothiophene, fluorene, naphtalene, phenotiazine, and pyrene. The fifty-eight isolates indicated in having capability to degrade PAHs, consisted of 25 isolates were positive on naphthalene (nap) and 20 isolates showed ability to grow in phenanthrene (phen) containing media. Further, 38 isolates were selected for dibenzothiophene (dbt) degradation and 25 isolates were positive on fluorene (flr). On the other hand, 23 isolates presented capability to degrade in phenothiazine (ptz) and 15 isolates could grow in media with pyrene (pyr). Based on homology analysis of partial 16S rDNA gene, we obtained six taxonomy classes of PAH degrading bacteria, namely α-*Proteobacteria* (31%), γ-*Proteobacteria* (43%), *Firmicutes Bacilli* (12%), *Actinobacteria; Micrococcales* (9%), *Actinobacteria; Propionibacteriales* (2%), and *Bacteroidetes; Flavobacteriia* (3%). In this research, we obtained diverse PAH degrading bacteria from marine areas.

Keywords: Bacteria, degradation, marine environment, polyaromatic hydrocarbon

INTRODUCTION

Polycyclic aromatic hydrocarbon compounds (PAHs) is one of the oil component that has 37 % contribution of all (Baek et al. 2004). PAHs are aromatic compounds containing from two to eight conjugated ring systems which have properties cytotoxic, mutagenic, and carcinogenic. They can have a range of substituents such as alkyl, nitro, and amino groups in their structure. Nitrogen, sulfur, and oxygen atoms can also be incorporated into their ring system. PAHs are a concern environmental problem due to their persistency. Moreover, these compounds can stay in the environment for long periods of time. One of the most common ways of PAHs to enter the body is through breathing contaminated air (Crone and Tolstoy 2010).

PAHs in marine environment were distributed due to oil spill contamination noticed by some reports. Baumard et al. (1998) and Witt (1995) reported that PAHs are widespread in marine coastal sediments. Other researchers also found them in surface sediments of the Arctic Ocean with variable concentrations from the shelf to basin (Yunker and Macdonald 1995; Yunker et al. 2011; Zaborska et al. 2011).

Microorganisms play an essential role in the transformation of polycyclic aromatic hydrocarbons (PAHs) and their biological degradation is the main process of natural decontamination in ecosystems. It is well known that bacterial degradation plays an important role in PAH removal from marine environments. Many researchers reported about PAH degrading bacteria isolated both of marine and terrestrial areas. More recent advances, some researchers have revealed PAH degrading bacteria from marine such as *Cycloclasticus* (Dyksterhouse et al. 1995), *Marinobacter* (Hedlund and Staley 2001), *Pseudoalteromonas*, *Marinomonas* (Melcher et al. 2002), *Halomonas* (Melcher et al. 2002), *Sphingomonas* (Demaneche et al. 2004) and *Vibrio* (Hedlund and Staley 2001) found in coastal sediment. Further, biodiversity of PAH degrading bacteria from Indonesia marine environment have been reported by Thontowi and Yopi (2013) that focused in Pari Island. They concluded that *α-Proteobacteria* was the majority class of PAH degrading bacteria existed in Pari Island, Seribu Islands, Jakarta.

The purposes of this study were to screen marine bacteria as our collection in Laboratory of Biocatalyst and Fermentation (LBF), Research Center for Biotechnology, Indonesian Institutes of Sciences (LIPI) on PAHs, identify molecularly, and determine their biodiversity.

MATERIALS AND METHODS

Microorganisms, chemicals, and media

Isolates used in this study were our collection in Laboratory of Biocatalyst and Fermentation, Research Center for Biotechnology, Indonesian Institute of Sciences (LIPI), Cibinong-Bogor, West Java, Indonesia. We isolated these bacteria from several marine areas in Indonesia, i.e.

Pari Island, Jakarta, Kamal Port, East Java and Cilacap Bay, Central Java. The PAHs were purchased from Nacalai Tesque (phenothiazine, napthalene), TCL Tokyo Kasei (dibenzothiophene, fluorene), and Wako (phenanthrene, pyrene). Each PAH stock solution was prepared in dimethyl sulfoxide (DMSO) with a concentration of 3000 ppm. The growth medium, Marine Agar (MA), and Marine Broth (MB) were supplid by BD Difco, while Artificial Sea Water (ASW) that was used as screening media was prepared by PE, PET Japan.

Screening of marine bacteria

The screening test to select the best and the most potential strains were conducted using ASW agar by sublimation method as described by Alley and Brown (2000). The isolates were also growth in ASW broth medium contained 50 ppm of PAHs as carbon source (Juhasz et al. 1997. The isolates were incubated for 7-14 days to select the positive candidates. The candidates with ability to degrade PAHs from sublimation test was shown by one of followed indicator i.e. color change of media; clear zone appearance around the isolates; and both of them.

PCR amplification of the 16S rDNA genes and sequencing

The partial 16S rDNA genes of isolates were amplified from genomic DNA using the universal primer set 9f (5′GAGTTTGATYMTGGCTCAG-3′) and 1541r (5′-AAGGAGGTGWTCCARCC-3′). The thermal cycling parameters were a min, hot start at 95°C, 2 min (1cycle), followed by 95^{o}C, 2 min (1 cycle); 95^{o}C, 30 sec; 65^{o}C, 1min; 72^{o}C, 2 min (10 cycles); 95^{0}C, 30 sec; 55^{o}C, 1 min; 72^{o}C, 2 min (30 cycles); 72^{o}C, 2 min (1 cycle). The sequences were determined directly using conserved bacterial 16S rDNA sequencing primers by First Base.

Phylogenic tree analysis

The 16S rDNA sequences were aligned with published sequences from the GenBank database using the NCBI BLASTN comparison software. The program was run via internet through the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/blast/). Multiple alignment and phylogenetic trees were constructed by the neighbour-joining method using Mega 3.1 ABI *sequencer software* (Kumar et al. 2004). Nearly full-length 16S rDNA sequences of the most phylogenetically related strains were selected from the Gen Bank database as reference strains.

RESULTS AND DISCUSSION

Screening test

One hundred thirty two isolates were tested on six PAHs i.e. naphthalene, dibenzothiophene, fluorene, phenanthrene, phenothiazine, and pyrene. Due to their insolubility characteristic, the selection of PAH degrading bacteria on solid media was conducted by sublimation method as described by Alley and Brown (2000). With this test, qualitative indicators for candidates that have capability on PAHs degradation were clear zone and/or color change. Fifty eight isolates were selected as potential isolates for PAHs biodegradation.

Among candidates of PAHs degrading bacteria, 25 isolates were selected for naphthalene (naph) degradation and 20 isolates were able to degrade phenanthrene (phen). Further, 38 isolates could grow in dibenzothiophene (dbt) containing media, while 25 isolates were positive for fluorene (flr). Moreover, 23 isolates showed capability to degrade phenothiazine (ptz,) and 15 isolates were potential for pyrene (pyr) degradation. Results of sublimation performance of some isolates were shown in Figure 1, while growth test were performed in Figure 2.

Due to their difference performance, there were interesting phenomena showed by positive candidates of isolates possessing capability to degrade PAHs. According to some research, growth on PAHs in solid media was considered positive by the formation of a clear zone around the growing colonies or appearance of pigments (Johnsen et al. 2002; Kumar et al. 2006). In this research, the potential isolates for four PAHs degradation i.e. naphthalene, fluorene, phenanthrene, and pyrene, presented clear zone and/or color change of media from clear to yellow. On the other hand, dibenzothiophene (DBT) degrading bacteria showed on the plate as well as in liquid media the bacterium produced orange or reddish brown water-soluble product or metabolite (Kumar et al. 2006; Andreolli 2011). While, isolates candidates for phenothiazine degradation had clear zone and/or color change media to blue (Figures 1 and 2). The existence of color complex and clear zone on the media proved that the bacteria could utilize PAHs compound as a carbon source for their growth (Marino et al. 1998). The phenomenon shows that there has been metabolism of PAHs by these isolates. An example from observed the formation of a diffusible yellow color during microbial degradation of biphenyl and have shown that this color is caused by a meta-cleavage product (Ahmad et al. 1991).

Bacterial identification and phylogenis tree

Analysis of bacterial rRNA gene sequences revealed that the characterized isolates belong to 21 genera and six taxonomy classes within four phyla (*Proteobacteria*, *Firmicutes*, *Bacilli*, and *Actinobacteria*). Molecular identification of 58 isolates by homology analysis with BLAST search was provided completely in Table 1. Several isolates showed identity less than 96% such as LBF-1-0103, LBF-1-0108, LBF-1-0130, LBF-1-0136, and LBF-1-0137. The first tree isolates were identified as *Brachybacterium saurashtrense* strain JG 06 (94%), *Shewanella algae* strain KJ-W37 (95%), and *Janibacter limosus* strain DSM 11140 (95%). Whereas, the last two isolates were identified as *Halomonas cupida* strain NBRC 102219, respectively. The identity value less than 96% of isolate can be assumed as candidate of new species (Fox, et al. 1992), but definitely, these results need further assay such as morphological and biochemical test.

The relationship among 58 isolates as candidates of PAHs degrading bacteria was described by phylogenic tree as shown in figure 3. The phylogenetic tree was

constructed based on partial 16S rDNA gene fragments and MEGA 3.1 software using the maximum composite likelihood model with gamma-distributed rates and pairwise deletion.

Biodiversity of PAHs degrading bacteria

According to analysis of 16SrDNA gene, fifty eight isolates that selected as PAH degrading bacteria classified in six taxonomy classes designated as α-*Proteobacteria* (31%), γ-*Proteobacteria* (43%), *Firmicutes Bacilli* (12%), *Actinobacteria; Micrococcales* (9%), *Actinobacteria; Propionibacteriales* (2%), and *Bacteroidetes; Flavobacteriia* (3%) (Figure 4). According to the data, the majority of the isolates were members of *Proteobacteria* phylum (76%) followed by *Firmicutes* (12%), *Actinobacteria (9%),* and *Bacteroidetes* (3%), respectively.

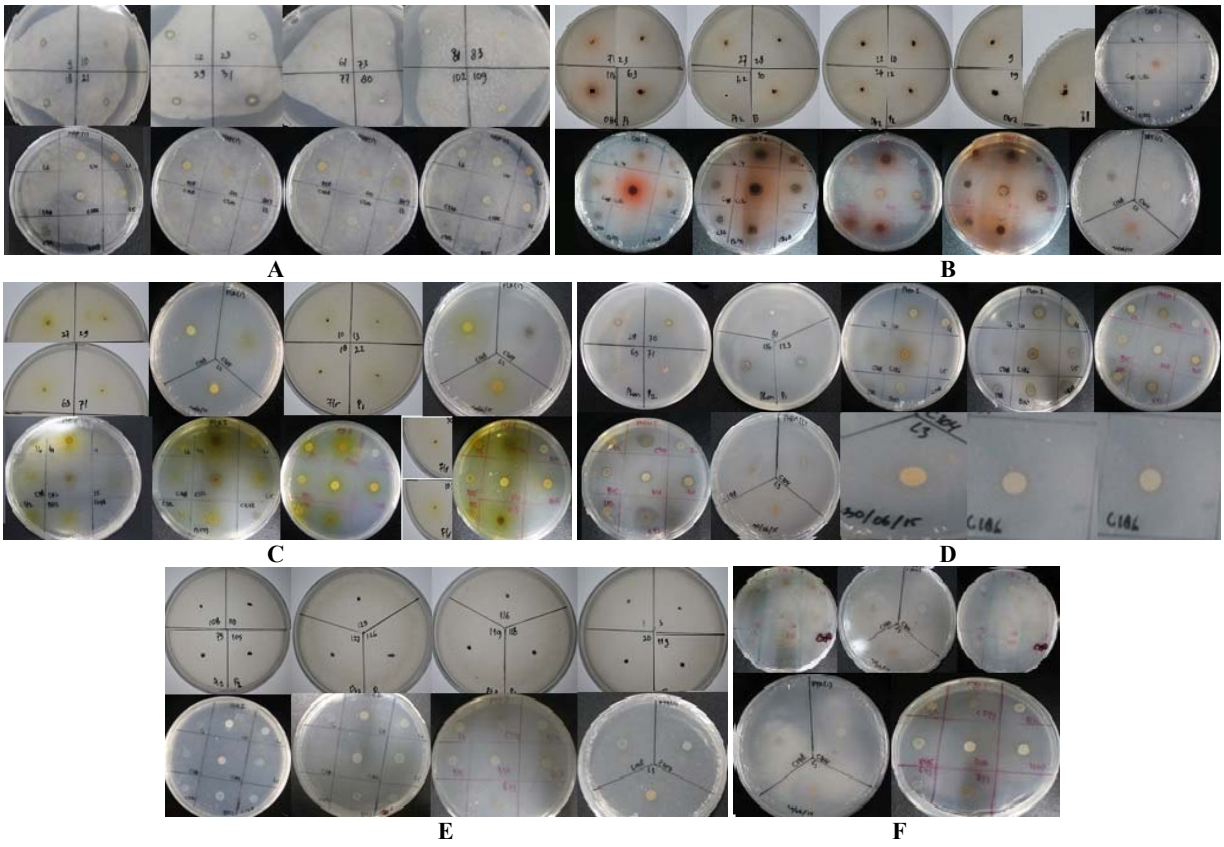


Figure 1. Screening result of marine bacteria on six PAHs by sublimation methods, A. Naphthalene, B. Dibenzothiophene, C. Fluorene, D. Phenanthrene, E. Phenothiazine, and F. Pyrene

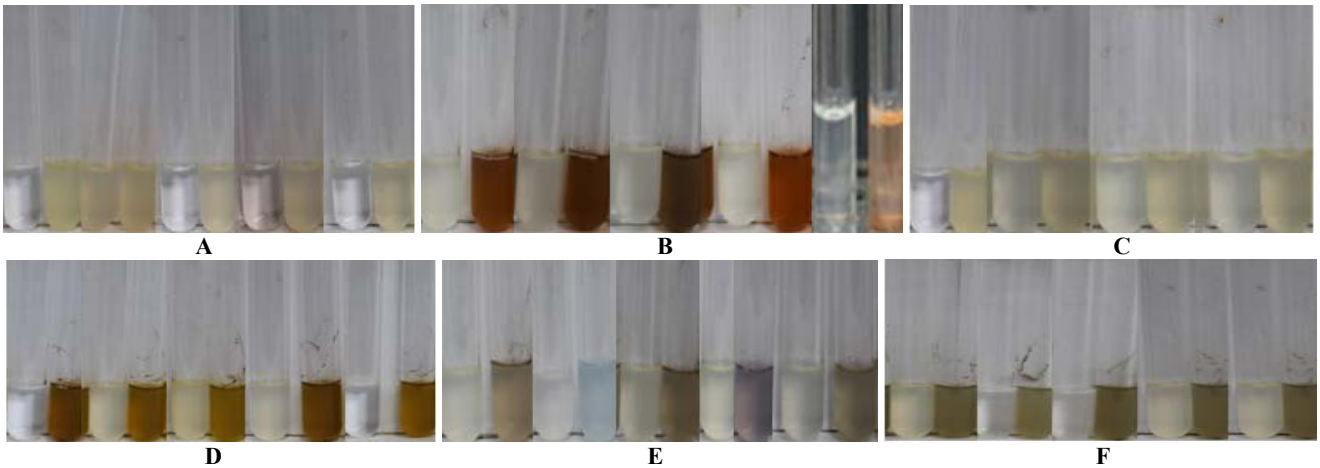


Figure 2. Screening results of marine bacteria on six PAHs by growth test in Broth medium; A. Naphthalene, B. Dibenzothiophene, C. Fluorene, D. Phenanthrene, E. Phenothiazine, and F. Pyrene

Table 1. Homology analysis of isolates with BLAST search

Isolate code	Closest strain	Class	Accession No	Similarity (%)
LBF-1-0001	*Labrenzia aggregata* IAM 12614	Proteobacteria; Alphaproteobacteria	NR-115659	99
LBF-1-0003	*Thalassospira permensis* strain SMB34	Proteobacteria; Alphaproteobacteria	NR_042909	99
LBF-1-0009	*Muricauda aquimarina* strain SW-63	Bacteroidetes; Flavobacteriia	NR_042909	100
LBF-1-0010	*Pseudoalteromonas shioyasakiensis* strain SE3	Proteobacteria; Gammaproteobacteria	NR_125458	99
LBF-1-0011	*Pseudoalteromonas shioyasakiensis* strain SE3	Proteobacteria; Gammaproteobacteria	NR_125458	99
LBF-1-0012	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR_116368	99
LBF-1-0013	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR_116368	99
LBF-1-0018	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR_116368	99
LBF-1-0019	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR_116368	99
LBF-1-0020	*Pseudoalteromonas shioyasakiensis* strain SE3	Proteobacteria; Gammaproteobacteria	NR_125458	99
LBF-1-0021	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR_116368	99
LBF-1-0022	*Alcanivorax xenomutans* strain JC109	Proteobacteria; Gammaproteobacteria	NR_133958	99
LBF-1-0023	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR-116368	99
LBF-1-0024	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR_116368	99
LBF-1-0026	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR_116368	99
LBF-1-0027	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR-116368	99
LBF-1-0028	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR_116368	99
LBF-1-0029	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR_116368	99
LBF-1-0030	*Bacillus subtilis subsp. subtilis* strain OS-44.a	Firmicutes; Bacilli	NR_114997	99
LBF-1-0031	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR_116368	99
LBF-1-0040	*Bacillus stratosphericus* strain 41KF2a	Firmicutes; Bacilli	NR_042336	98
LBF-1-0046	*Vibrio alginolyticus* strain NBRC 15630	Proteobacteria; Gammaproteobacteria	NR_113781	99
LBF-1-0050	*Brachybacterium conglomeratum* strain J 1015	Actinobacteria; Micrococcales	NR_104689	98
LBF-1-0054	*Microbacterium amylolyticum* strain X5	Actinobacteria; Micrococcales	KJ151779	99
LBF-1-0056	*Pseudomonas aeruginosa* strain KUN2	Proteobacteria; Gammaproteobacteria	KT966462	99
LBF-1-0057	*Pseudomonas aeruginosa* strain SNP0614	Proteobacteria; Gammaproteobacteria	NR_118644	99
LBF-1-0060	*Muricauda olearia* strain CL-SS4	Bacteroidetes; Flavobacteriia	NR_044579	99
LBF-1-0061	*Novosphingobium pentaromativorans* strain US6-1	Proteobacteria; Alphaproteobacteria	NR_025248	99
LBF-1-0062	*Pseudomonas balearica* strain SP1402	Proteobacteria; Gammaproteobacteria	NR_025972	99
LBF-1-0070	*Pseudomonas stutzeri* strain ISA12	Proteobacteria; Gammaproteobacteria	HQ189755	99
LBF-1-0072	*Nocardioides zeae* strain JM-1068	Actinobacteria; Propionibacteriales	NR_134102	98
LBF-1-0074	*Idiomarina zobellii* strain SBU4	Proteobacteria; Gammaproteobacteria	KF052992	99
LBF-1-0076	*Shewanella indica* strain 0102	Proteobacteria; Gammaproteobacteria	KP236237	99
LBF-1-0079	*Lysobacter* sp. 3070	Proteobacteria; Gammaproteobacteria	AM111012	99
LBF-1-0080	*Lysobacter concretionis* strain Ko07	Proteobacteria; Gammaproteobacteria	NR_041003	97
LBF-1-0082	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR_116368	99
LBF-1-0101	*Sphingomonas* sp. 2MPII	Proteobacteria; Alphaproteobacteria	U90216	96
LBF-1-0102	*Brachybacterium* sp. XJ133-127-6NF2	Actinobacteria; Micrococcales	JX975428	97
LBF-1-0103	*Brachybacterium saurashtrense* strain JG 06	Actinobacteria; Micrococcales	NR_116516	94
LBF-1-0107	*Shewanella indica* strain KJW27	Proteobacteria; Gammaproteobacteria	NR_108899	98
LBF-1-0108	*Shewanella algae* strain KJ-W37	Proteobacteria; Gammaproteobacteria	JQ799131	95
LBF-1-0111	*Hydrothermal vent* strain TB66	Proteobacteria; Alphaproteobacteria	AF254109	97
LBF-1-0114	*Stenotrophomonas maltophilia* strain G7	Proteobacteria; Gammaproteobacteria	KC136824	98
LBF-1-0118	*Bacillus subtilis* strain A2	Firmicutes; Bacilli	KC433738	97
LBF-1-0122	*Bacillus stratosphericus* strain 41KF2a	Firmicutes; Bacilli	NR_042336	97
LBF-1-0124	*Bacillus aerius* strain 24K	Firmicutes; Bacilli	NR_118439	96
LBF-1-0125	*Bacillus altitudinis* strain LZLJ004	Firmicutes; Bacilli	KR018737	96
LBF-1-0128	*Alcanivorax dieselolei* strain B5	Proteobacteria; Gammaproteobacteria	NR_043106	97
LBF-1-0130	*Janibacter limosus* strain DSM 11140	Actinobacteria; Micrococcales	NR_026362	95
LBF-1-0131	*Pseudomonas stutzeri* strain ATCC 17588	Proteobacteria; Gammaproteobacteria	NR_041715	98
LBF-1-0133	*Pseudomonas stutzeri* strain W31	Proteobacteria; Gammaproteobacteria	KT380576	97
LBF-1-0134	*Pseudomonas aeruginosa* PAO1	Proteobacteria; Gammaproteobacteria	NR_074828	97
LBF-1-0135	*Bacillus zhanjiangensis* strain JSM 099021	Firmicutes; Bacilli	NR_117854	96
LBF-1-0136	*Halomonas cupida* strain NBRC 102219	Proteobacteria; Gammaproteobacteria	NR_114046	95
LBF-1-0137	*Halomonas cupida* strain NBRC 102219	Proteobacteria; Gammaproteobacteria	NR_114046	95
LBF-1-0141	*Alcanivorax xenomutans* strain JC109	Proteobacteria; Gammaproteobacteria	NR_133958	96
LBF-1-0142	*Marinobacter koreensis* strain DD-M3	Proteobacteria; Gammaproteobacteria	NR_043718	96
LBF-1-0143	*Marinobacter koreensis* NBRC 106396	Proteobacteria; Gammaproteobacteria	AB682412.1	96

Table 2. Classification of PAHs degrading bacteria based on their capability to degrade PAHs from sublimation test

Group	Isolates Code	Capability of PAHs degradation					
		LMW PAHs					HMW PAHs
		NAPH	DBT	FLU	PHEN	PTZ	PYR
I	LBF-1-0001	-	-	-	-	CC	-
	LBF-1-0003	-	-	-	-	CC	-
	LBF-1-0011	-	CC	-	-	-	-
	LBF-1-0012	-	CC	-	-	-	-
	LBF-1-0019	-	CC	-	-	-	-
	LBF-1-0020	-	-	-	-	CC	-
	LBF-1-0024	-	CC	-	-	-	-
	LBF-1-0026	-	CC	-	-	-	-
	LBF-1-0028	-	CC	-	-	-	-
	LBF-1-0040	-	-	-	-	CC	-
	LBF-1-0046	-	-	-	-	CC	-
	LBF-1-0050	-	-	-	-	CC	
	LBF-1-0054	-	CC	-	-	-	-
	LBF-1-0056	-	-	-	-	CC	-
	LBF-1-0057	-	-	-	-	CC	-
	LBF-1-0061	-	CZ, CC	-	-	-	-
	LBF-1-0072	CZ	-	-	-	-	-
	LBF-1-0074	-	-	-	-	CC	-
	LBF-1-0076	CZ	-	-	-	-	-
	LBF-1-0079	CZ	-	-	-	-	-
	LBF-1-0082	CZ, CC	-	-	-	-	-
	LBF-1-0107	-	-	-	-	-	-
	LBF-1-0111	-	-	-	-	CC	-
	LBF-1-0118	-	-	-	-	CC	-
	LBF-1-0124	-	-	-	-	CC	-
	LBF-1-0125	-	-	-	-	CC	-
II	LBF-1-0009	CZ	CC	-	-	-	-
	LBF-1-0010	CZ	CC	CC	-	-	-
	LBF-1-0013	-	CC	CC	-	-	-
	LBF-1-0018	CZ	CC	CC	-	-	-
	LBF-1-0021	CZ	CC	CC	-	-	-
	LBF-1-0022	CZ	CC	CC	-	CZ, CC	-
	LBF-1-0023	CZ	CC	-	-	-	-
	LBF-1-0027	-	CC	CC	-	-	-
	LBF-1-0029	CZ	CC	CZ, CC	CZ	-	-
	LBF-1-0030	-	CC	CC	CZ	-	-
	LBF-1-0031	CZ	CC	CC	-	-	-
	LBF-1-0060	CZ	-	CC	-	-	-
	LBF-1-0103	-	CC	CC	-	CC	-
	LBF-1-0070	-	CC	CC	CZ	-	-
	LBF-1-0131	CZ	CZ	CC	CZ, CC	-	-
	LBF-1-0141	CZ	CC	-	-	-	-
	LBF-1-0142	-	CZ, CC	CZ, CC	CZ, CC	-	-
III	LBF-1-0062	-	CC	CC	CZ	CC	CZ
	LBF-1-0080	CZ	-	-	CZ	-	CZ
	LBF-1-0101	CZ, CC	CC	-	CZ	CZ	CZ
	LBF-1-0102	CZ	CZ, CC	CC	CZ	CZ	CZ
	LBF-1-0114	CZ	CC	CC	CZ	CZ	CZ
	LBF-1-0122	-	CZ	-	CZ	-	CZ
	LBF-1-0128	CZ	CZ, CC	CC	CZ	-	CZ
	LBF-1-0129	CZ	CZ, CC	CC	CZ	CZ	CZ
	LBF-1-0130		CZ, CC	CC	CZ	CZ	CZ
	LBF-1-0133	CZ	CC	CZ, CC	CZ, CC	CZ	CZ
	LBF-1-0134	-	CZ, CC	CC	CZ, CC	-	CZ
	LBF-1-0135	-	CZ, CC	CC	CZ, CC	CZ	CZ
	LBF-1-0136	CZ	CC	CC	CZ, CC	-	CZ
	LBF-1-0137	-	CZ, CC	CZ, CC	CZ, CC	-	CC
	LBF-1-0143	CZ	CZ, CC	CZ, CC	CZ	CZ	CZ

Note: Tree indicator for positive candidates of PAH degrading bacteria namely Clear Zone (CZ), Colour Change (CC), and Both of them (CZ, CC)

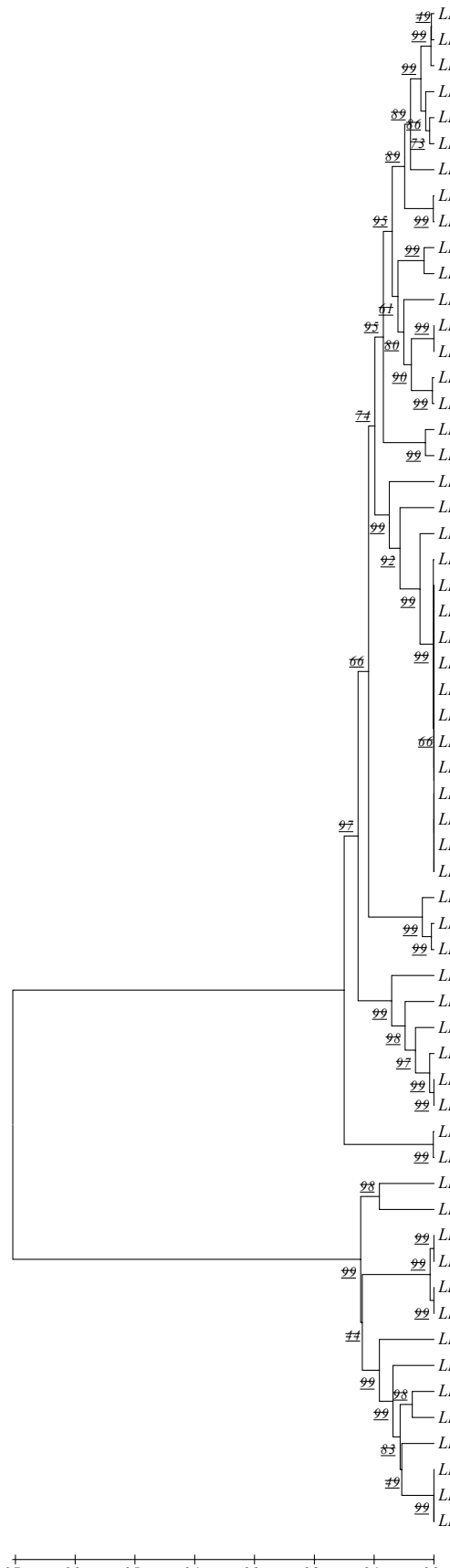
The information from this research confirm that *Proteobacteria* is a common phyla of PAH degrading bacteria from marine areas (Yuan et al. 2015; Isaac et al. 2013; Dong et al. 2015). The data of this research also supported several researches that informed *Proteobacteria* as dominant phyla of PAH degrading bacteria from Indonesia marine area (Yopi et. al. 2006; Harwati et al. 2007; Thontowi and Yopi 2013).

More specifically, *Stakelama, Pseudomonas,* and *Bacillus* were the predominant genera of PAHs degrading bacteria from tree marine areas i.e. Pari Island, Kamal Port, and Cilacap Bay with composition 22%, 12%, and 12%, respectively (Figure 5). Overall, *Stakelama* is the most dominant genera of PAH degrading bacteria from tree marine areas (Pari Islands, Kamal Port, and Cilacap). Actually, the bacteria from this genus is rarely reported as PAH degrader previously. Dong et. al. (2015) released that *Pseudomonas*, *Cycloclasticus*, and *Alcanivorax* are predominant genus of PAH degrading bacteria from deep-sea sediments of Arctic Ocean; while *Alcanivorax* is the most dominant in deep sea water of Southwest India (Yuan et al. 2015) .

The remaining other genera were *Alcanivorax, Pseudo-alteromonas, Shewanella, Halomonas, Brachybacterium, Marinobacter, Muricauda, Lysobacter, Novosphingobium, Labrenzia, Thallaspira, Hydrothermal, Vibrio, Nocardioides, Stenotrophomonas, Janibacter, Microbacterium,* and *Sphingomonas.* Previously, most of these genus have been reported as PAH degrader such as *Alcanivorax, Pseudo-alteromonas, Shewanella, Halomonas, Brachybacterium, Marinobacter, Muricauda, Lysobacter, Novosphingobium, Vibrio* (Hedlund and Staley 2001; Hedlund and Staley, 2006; Melcher et al. 2002; Demaneche et al. 2004; Dong et al. 2015). Particularly, *Alcanivorax* is also noteworthy because it has been recognized as one of of obligate marine hydrocarbon degraders (Yakimov et al. 2007). In addition, from this research, we also obtained several genus that are rarely reported as PAH degrader namely *Labrenzia, Thallaspira, Hydrothermal, Nocardioides, Steno-trophomonas, Janibacter,* and *Microbacterium*. Therefore, the result of this research revealed that PAH degrading bacteria from studied marine areas were very diverse.

Degradation characterization of PAH degrading bacteria

Interesting phenomena were shown in screening result using sublimation test. There were different indicators showed among positive isolates for PAHs degradation i.e clear zone, color change or both of them. Further, we also obtained information about diversity of PAH degrading bacteria corresponding with their capability in PAHs degradation (Figure 3). The phylogenic tree gave information of how far relationship among isolates. On the other hand, the tree also described how diverse the characteristics of each isolate in PAHs degradation. Although some isolates have close relationship even originated from same species, they have different characteristic in PAHs degradation. This information could be seen from some species such as *Pseudomonas aeruginosa*, *Stakelama pacifica, Bacillus stratosphericus*, and others.



Capability of PAHs Degradation					
LMW					**HMW**
NAPH	**DBZ**	**FLU**	**PHN**	**PTZ**	**PYR**
-	-	-	-	CC	-
-	-	-	-	CC	-
CZ	CC	CZ, CC	CZ, CC	CZ	CZ
-	CZ, CC	CC	CZ	CZ	CZ
-	CC	CC	CZ	CC	CZ
-	CC	CC	CZ	-	-
-	CZ, CC	CZ, CC	CZ, CC	-	-
CZ	CC	CC	-	CC, CZ	-
CZ	CC	-	-	-	-
-	-	-	-	CC	-
-	-	-	-	CC	-
-	-	-	-	CC	-
CZ	CC	CC	-	-	-
-	CC	-	-	-	-
CZ	-	-	-	-	-
-	-	-	-	CC	-
CZ	-	-	-	-	-
CZ	-	-	CZ	-	CZ
-	-	-	-	CC	-
-	-	-	-	CC	-
-	CZ, CC	-	-	-	-
CZ	CC	CC	-	-	-
CZ	CC	-	-	-	-
-	CC	-	-	-	-
CZ	CC	CC	-	-	-
-	CC	CC	-	-	-
CZ	CC	CZ, CC	CZ	-	-
-	CC	-	-	-	-
-	CC	-	-	-	-
CZ, CC	-	-	-	-	-
-	CC	-	-	-	-
-	CC	CC	-	-	-
CZ	CC	-	-	-	-
-	CC	-	-	-	-
-	CZ, CC	CC	CZ, CC	-	CZ
-	-	-	-	CC	-
-	CC	-	CZ	-	CZ
CZ	-	-	-	-	-
-	CC	-	-	-	-
CZ	CZ, CC	CC	CZ	CZ	CZ
-	-	-	-	CC	-
CZ	CZ, CC	CC	CZ	CZ	CZ
-	CC	CC	-	CC	-
CZ	CC	-	-	-	-
CZ	-	CC	-	-	-
CZ, CC	CC	-	CZ	CZ	CZ
-	-	-	-	CC	-
-	CC	CC	CZ		-
-	-	-	-	CC	-
-	-	-	-	CC	-
-	-	-	-	CC	-
CZ	CC	CC	CZ	CZ	CZ
CZ, CC	-	-	-	-	-
-	CZ	CC	CZ, CC	-	-
CZ	CZ, CC	CZ, CC	CZ	CZ	CZ
CZ	CZ, CC	CC	CZ	-	CZ
-	CZ, CC	CC	CZ, CC	CZ	CZ
CZ	CC	CC	CZ, CC	-	CZ
-	CZ, CC	CZ, CC	CZ, CC	-	CC

Figure 3. Phylogenetic tree derived from 16S rDNA gene sequence of 58 isolates and their capabilities for PAHs degradation. The NJ-tree was constructed using neighbour joining algorithm with Kimura 2 parameter distances in MEGA 3.1 software. Bar, 1% estimated sequence divergence. LMW: low molecular weight, HMW: high molecular weight, NAPH: naphthalene, DBT: dibenzothiophene, FLR: fluorene, PHEN: phenanthrene; PTZ: phenothiazine; PYR: pyrene; CC: colour change; CZ: clear zone

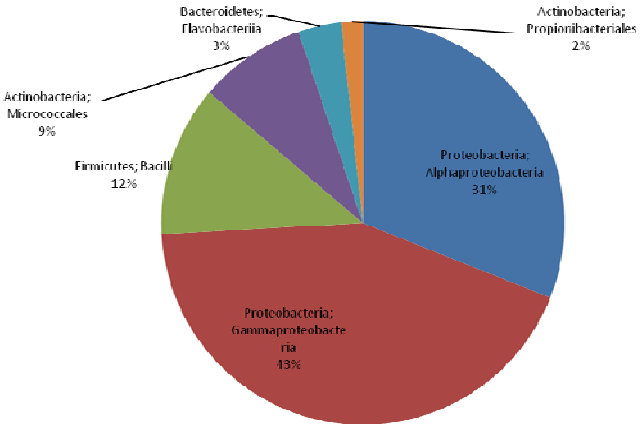


Figure 4. Biodiversity of PAH Degrading Bacteria from Marine Area in Indonesia. The 58 Selected Bacteria were divided into six Classes

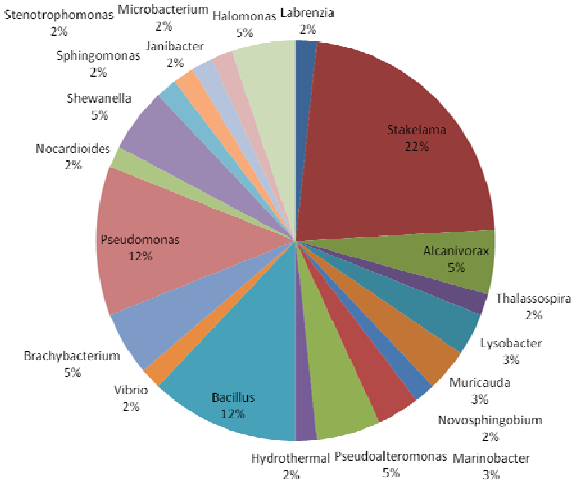


Figure 5. Biodiversity of PAH Degrading Bacteria from Marine Area in Indonesia. Analysis of bacterial rRNA gene sequences revealed that the characterized isolates belong to 21 genera

Furthermore, some data overview could be analyzed related to classification of PAHs used in this study. Naphthalene, dibenzothiophene, fluorene, phenanthrene, phenothiazine are categorized as Low Molecular Weight (LMW) PAHs, consisted of 2 ring benzene rings. Whereas, pyrene is High Molecular Weight (HMW), contained 3 (three) benzene rings (Gong et al. 2007). Otherwise, from the isolates, we could see that some isolates have capability to degrade 1 (one) or more PAHs. Therefore, from these data and information, we classified the isolates into 3 (three) group (Table 2). First, isolates that could degrade only 1 LMW PAHs (26 isolates); second, isolates that could degrade more than 1 LMW PAHs (17 isolates); and third, isolates that could degrade LMW and HMW PAHs (15 isolates).

Based on this study, we obtained six taxonomy classes and 21 genera of PAH degrading bacteria from marine areas in Indonesia within four phyla i.e. *Proteobacteria*, *Firmicutes*, *Bacilli*, and *Actinobacteria*. Therefore, we concluded that PAH degrading bacteria from studied areas were very diverse. These potential isolates have different characteristics in PAHs degradation. Furthermore, we classified these isolates into three groups designated as isolates that could degrade only 1 LMW PAHs (group I); isolates that could degrade more than 1 LMW PAHs (group II); and isolate that could degrade LMW and HMW PAHs (group III).

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Crown shape dynamics of dense mangrove *Kandelia obovata* stands in Manko Wetland, Okinawa Island, Japan

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Abstract. *Analuddin K, Septiana A, Sharma S, Hagihara A. 2016. Crown shape dynamics of dense mangrove Kandelia obovata stands in Manko Wetland, Okinawa Island, Japan. Biodiversitas 17: 865-872.* The objectives of this study were to elucidate the crown structure dynamics for dense mangrove stands, and to know the crown shape maintenances and its important role for ensuring the stability and vitality the crowded mangrove forest. The growth parameters of *K. obovata* Shue, Liu & Yong stands, such as tree height H (m), height at the lowest living leaves H_L (m), crown length C_L (m) and crown width C_W (m), were measured in the summer from 2004 to 2008. The crown shape dynamics were analyzed. The results showed that the H_L was significantly increased with increasing H, which suggests that the crown changed to be dumpy as the stands grew. However, the C_L of young stands increased and then decreased continuously as the stands grew, while the C_L of mature stands decreased from 2004 to 2007 and then increased in 2008. Meanwhile, the C_L/C_W ratio of young stands decreased as the stands grew, while the C_L/C_W ratio of mature stands decreased and then increased, which imply that dense *Kandelia obovata* trees might transform their crown shape for reducing of competition for light among trees. Therefore, these results suggested that the crown shape of dense mangrove treesare dynamics as developing stands.

Keywords: Crown length, crown width, crown shape, crown volume, *Kandelia obovata*, stand dynamics

INTRODUCTION

Mangrove forests are known to play many important roles in the subtropical and tropical coastal areas of the world including nursery grounds and breeding sites for various animals, an essential resource of wood; sites for accumulation of sediment and nutrients in coastal areas (Twilley 1995; Alongi 2002; Manson et al. 2005); maintain the ecological equilibrium in coastal waters and preserve species diversity (Saenger 2002). The exchange of carbon between mangroves and coastal ocean and its fate in the ocean is, therefore, increasingly recognized as potentially important components in the ocean carbon budget. Mangrove ecosystems play important role in the global carbon cycle. The crown structure of mangroves plays an important role for mangrove forest productivity, and the analysis on crown dynamics in mangrove stands is very important to understand the maintenance their productivity and stability.

A tree's crown plays important roles for plant functioning and forest productivity through its effect on light penetration (Kellomaki 1995). The crown of trees influences their competition and survival abilities in the community (Kuulivainen 1992). Crown structure, leaf morphology and photosynthesis are closely related, which influence whole-crown carbon gain (Ellsworth and Reich 1993; Bond et al. 1999; Koike et al. 2001; Pons and Anten 2004). The crown of trees is as a dynamics system, because the leaves composing the crown are dynamic in term of their development and senescence. The leaves arrangement within the crown influences many aspects of whole-plant function including photosynthesis, transpiration and energy balance (Campbell and Norman 1989; Pearcy and Yang 1996).

As the trees grow larger, they produce more precise crown to be sound in their life. In the dense stands, the trees have to maintenance their crown shape to ensure their functioning. This maintenance may be accompanied with the changes in the crown and structure, which are essential for ensuring the life and growth of trees. Therefore, studies on tree crown shape dynamics provide critical information to assess the ecological functioning of mangrove forests.

Several theoretical studies on crown dynamics have been done by models (Mori and Hagihara 1991; Grote 2003; Shaw et al. 2003; Mottus et al. 2006; Weiskittel et al. 2007), but few field data are available (Jimenez-Perez et al. 2006), even there was few studies mentioned crown shape dynamics for mangroves till now. Measurement of a tree crown is often used to study of individual (tree) growth (Kozlowski et al. 1991). Taking into consideration the tree crown as a parameter of the vegetation development, several scientific studies relating to the crown and growth of trees have been undertaken to determine tree growth through models for tree crown profiles (Biging and Gill 1997; Gadow 1999; Gill et al.2000). On the contrary, little information is known about crown shape dynamic on mangrove forests, which are among the most productive ecosystems and play an important role throughout tropical

and subtropical coastal areas of the world (Ewel et al. 1998). Therefore, it would be worthwhile to explore the dynamics of crown shape for dense mangrove stands, knowledge of which can improve the understanding of the structural maintenance of mangrove forests.

Kandelia abovata is one of among the most dominant mangroves in Okinawa Island, Japan. Although intensive studies have been done in this mangrove *K. obovata* forest including allometric model and mangrove productivity (Khan et al. 2004, 2005, 2007), mangrove photosynthesis capacity (Suwa et al. 2006, 2008; Suwa and Hagihara 2008) and mangrove self-thinning (Analuddin et al. 2009b) and foliage dynamics (Analuddin et al. 2009a), butthe information about how the spatial and temporal maintenances of tree crown shape in dense mangrove forest are scarce. In this study, we monitored the crown shape dynamics of the dense *K. obovata* forest over five years. The dynamics of crown length, width and volume were investigated. The objectives of this study were: (i) to elucidate the crown structure dynamics for dense mangrove stands, and (ii) to know the crown shape maintenance of the dense mangrove forest.

MATERIALS AND METHODS

Study site

The present study was carried out at Manko Wetland (Figure 1), which is located along the Kokuba river beside Tomigusuku city of Okinawa Island, Japan (26º11′ N and 127º40′ E). The wetland has been recognized as an important conservation area and has been registered under the Ramsar Convention. The mangrove *Kandelia obovata* (S., L.) Yong was formerly recognized as *K. candel* (L.) Druce. Recently, it was split into two species, one of which is *K. candel* and the other is *K. obovata* distributed in China and Japan (Sheue et al. 200). Tree density of *Kandelia obovata* stands decreased year by year, which ranges from 2.68 to 4.88 (ind./m^2) in 2004, and decreased from 1.58 to 3.28 (ind./m^2) in 2008 (Analuddin et al. 2009b). *Kandelia obovata* is the dominant species in the study site. Besides, some small groups of *Rhizophora stylosa* Griff., *Bruguiera gymnorrhiza* (L.) Lamk. and *Excoecaria agallocha* L. are also observed, but they grew separately with *K. obovata* stands. The mean annual temperature in the years from 2004 to 2007 was 23.4± 0.1 (s.e.) ºC, while the mean precipitation at the same periods was 2190 ±211 (s.e.) mm yr^{-1}.

Tree inventory

A belt-transect 125 m long and 5 m wide was established in the *K. obovata* forest, whose canopy has been completely closed, perpendicularly to the current river and divided into 25 subplots (5 × 5 m^2). All trees in the subplots were recorded. The stem age of trees was determined by ring analysis from sample trees that taken from each subplot. The individual sample tree was taken on the basis of mean stem diameter in each plot. The stem of tree was cut and the annual ring was counted. Stem visualization revealed that tree age continuously increased from 6 yrs near the riverside to 10 yrs near the land (as of 2005), so that trees within a subplot could be assumed as uniformed age. The young and mature trees have been distinguished from their stem age. Trees are having age of

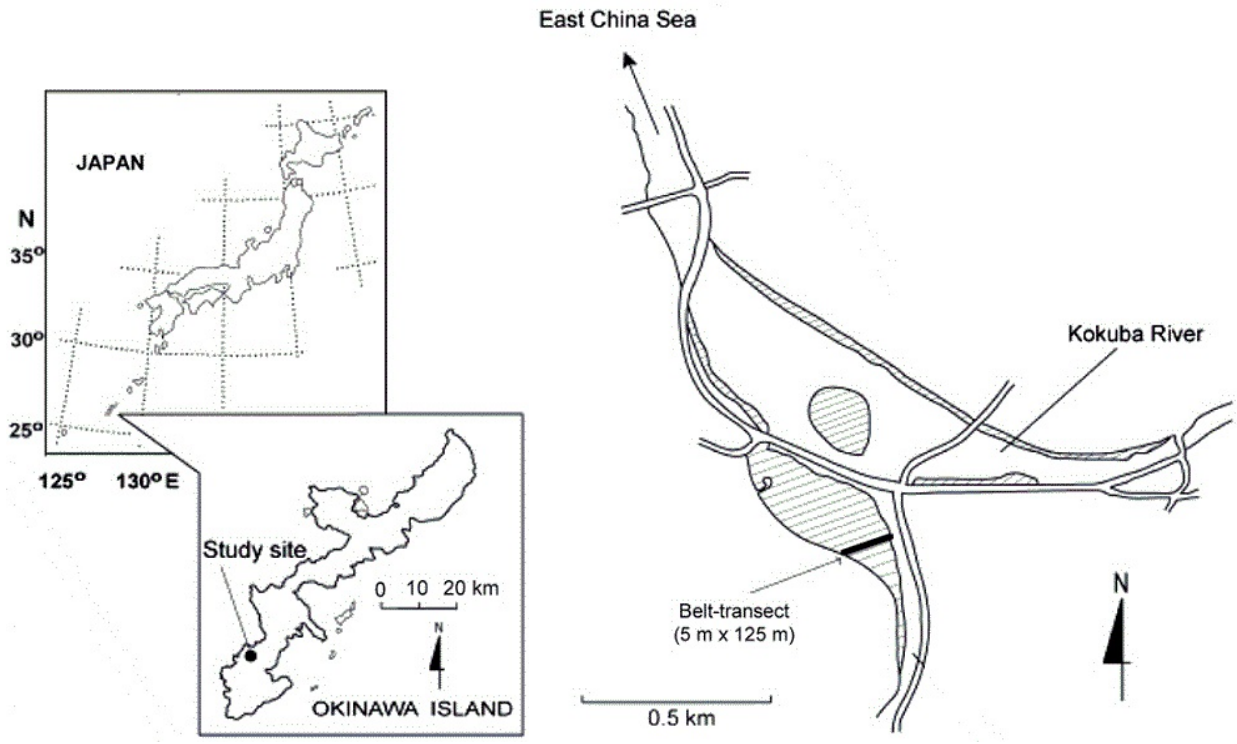

Figure 1. Study site at Manko Wetland, Tomigusuku city of Okinawa Island, Japan

less than 7 years assumed as young stands, while the trees are having age of 10 years assumed as mature stands. The young stands are located near the river side and middle area about 25 m and 60 m from the river edge, while mature stands are located near the inland area about 100 m from the river.

Growth parameters of mangrove trees, such as tree height H (m), stem diameter at 10% of $HD_{0.1H}$, height at the lowest living leaves H_L (m), crown length C_L (m) and crown width C_W (m), were measured in the summer of 2004, 2005, 2006, 2007 and 2008. Crown length C_L was defined as the difference between tree height (H) and height of the lowest living leaves H_L. Crown width C_W of a tree was estimated by the arithmetic mean of two perpendicular directions of the crown, including the widest projection, while crown shape estimated from C_L/C_W ratio. Furthermore, the tree crown volume was estimated according to Frank (2010), i.e. C_L/C_W ratio = 1 assumed as a cylinder, C_L/C_W ratio=0.817 assumed as a sphere, and C_L/C_W ratio = 0.577 assumed as a cone. The growth rate of H and H_L between successively two years was also estimated in each subplot. In addition, scaling between crown structure to stand density, and tree age was performed to verify their effect on the crown shape dynamics.

The relative photon flux density (RPFD)under canopy of *Kandelia obovata* trees was measured in the beginning and end of experimental periods in every subplot by using a data logger (LI-1400, LI-COR, USA) and a pair of horizontally placed quantum sensors (LI-206.8 and LI-151.91, LI-COR, USA). One sensor was mounted at a height of 1.5 m under canopy and the other sensor was placed at above canopy. In this case, a hundred replications were taken in every subplot. The relative PFD (RPFD) from the bottom to the above canopy was calculated on the basis of the stationary sensor that placed above the canopy.

Statistics

The mean crown length C_L, crown width C_W, crown shape or C_L/C_W ratio and crown volume C_V were evaluated for all subplots. The C_L/C_W ratio and the age of trees when C_W reaches the higher value were estimated. Scaling the relationships of crown structure to tree density and tree age, as well RPFD were established by Spearman rank correlation, while Kaleida Graph ver. 4.0, Synergy Software was used for best fitting curves (higher R^2 value) for non-linear equations.

RESULTS AND DISCUSSION

Tree height and height at the lowest living leaves dynamics

Figure 2 shows the dynamics of tree height H and height at the lowest living leaves H_L during the five years. The H_L significantly increased with increasing H (Figure 2a), but the increasing rate of H_L was much higher than that of H except the increasing rate of H in 2008 (Figure 2b). The relationship between H and H_L based on individuals trees showed higher correlation (Figure 2c, r = 0.90, p< 0.05).These results suggested the decreasing size of crown length of *K. obovata* with increasing tree height.

Crown length and crown width dynamics

Figure 3 describes the growth dynamics of crown length C_L over five years. The growth of crown length showed spatial and temporal variation (Figure 3a). Mostly trees near the riverside showed small decreasing in crown length as compared to the trees near the land site. The trees grown near the river site (young stands) seemed to increase in the second year then decrease in the third and fourth year, but then increase again in the 2008 (Figure 3b), while the trees grown from the middle to near the landsite tended to decrease from the second up to the fourth year, though they showed small increased again (Figure 3c).The C_L decreased year by year with tree density (r = 0.39, p< 0.05, Figure 3d). A decreasing trend of C_L seemed to correlate with tree age (r = -0.549, p< 0.05; Figure 3e). These trends indicated that the C_L of *K. obovata* trees in dense stands are dynamics as the stands grew and correlated with their density and age.

Figure 4 shows the crown width dynamics of dense mangrove *Kandelia obovata* stands over five years. The crown width C_W of trees grown in the middle toward the river side showed increasing size, while the C_W of trees grown near the inland site tended to decrease continuously (Figure 4a). However, the C_W of young stands increased continuously as the stands grew (Figure 4b), while the C_W of mature stands increased and then decreased (Figure 4c). The growth of C_W was not significant correlated with tree density (Figure 4d, r = 0.23, p> 0.05), and tree age, r = 0.10, p> 0.05, Figure 4e). Meanwhile, the age of trees when the C_W attain their maximum decreased and increased from the riverside landward (Figure 5), which imply that the trees in the middle area later attains their maximum C_W as compared with trees near the riverside.

Crown shape and crown volume dynamics

Figure 6 shows the C_L/C_W ratio or crown shape dynamics over five years. The C_L/C_W ratio of *K. obovata* trees is dynamic as stands grew, and showed clearly different trend (Figure 6a). It seemed that the C_L/C_W ratio of young stands decreased continuously as the stands grew (Figure 6b), while the C_L/C_W ratio of mature stands decreased and then increased (Figure 6c). The crown shape showed mostly cylinder in 2004, while other years showed as sphere. However, the dynamics of C_L/C_W ratio showed significantly correlated with tree density (r = 0.44, p< 0.05; Figure 6d), and tree age (r = -0.46, p< 0.05; Figure 6e). These results realized that the dense mangrove trees might transform their crown shape to make their life function.

Figure 7 shows the crown volume C_V dynamics of *K. obovata* stands during five years. The C_V increased and decreased in general, though the C_V of young stands located near the riverside still increased as the stands grew (Figure 7a). The dynamics of C_V was positively correlated with tree density (r = 0.41, p<0.05, Figure 7b), but it was negatively correlated with tree age (r = -0.554, p< 0.05; Figure 7c) indicating that *K. obovata* trees might have to maintain sound crown volume to sustain in their life.

Figure 8 shows trends of relative photon flux density RPFD under the canopy of *Kandelia obovata* stands. The RPFD under the canopy layer of *K. obovata* stands showed spatial and temporal variations (Figure 8a). The RPPFD seemed to be higher near the landside as compared near the riverside. Many stands showed RPFD were more 10% in 2004, even all stands showed RPFD were more than 5% in 2004, but most of RPFD in all stands showed decreasing trends for less than 5% in 2008,excepting RPFD in two plots of near the river, in the middle and landsite areas. However, the RPFD in 2008 showed negatively correlated with crown volume (r =-0.32, p<0.05, Figure 8b), while the RPFD in 2004 was not correlated with crown volume in 2004 (r =-0.017, p>0.05, Figure 8b).

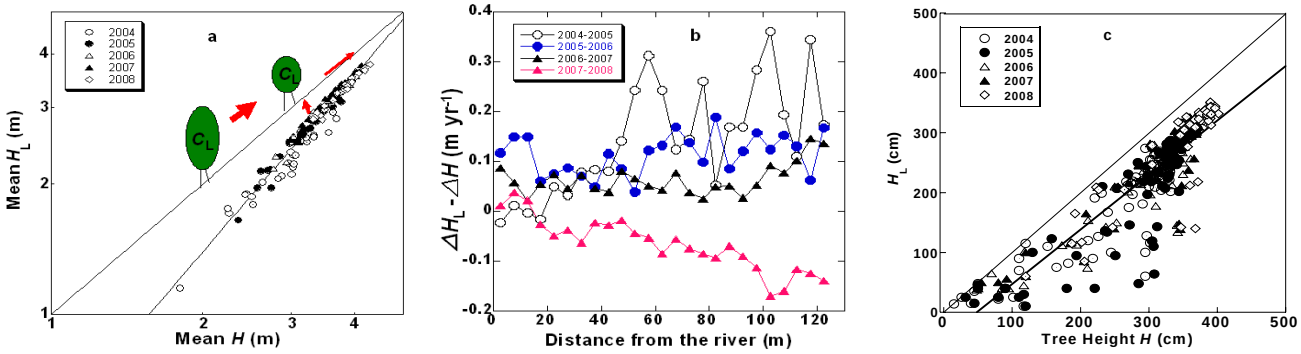

Figure 2. The dynamics of tree height H and height at the lowest living leaves H_L during the five years. A. Relationship between tree height H and height at the lowest living leaves H_L. Open circles 2004, filled circles 2005, open triangles 2006, filled triangles, 2007, open diamonds 2008. B. Trends of ΔH_L- ΔH increments from the riverside landward. Open circles 2004-2005, filled circles 2005-2006, filled triangles 2006-2007 (black), filled triangles 2007-2008 (red). C. Example the relationship between H and H_L of individual trees. Symbols are the same as Figure 2.A.

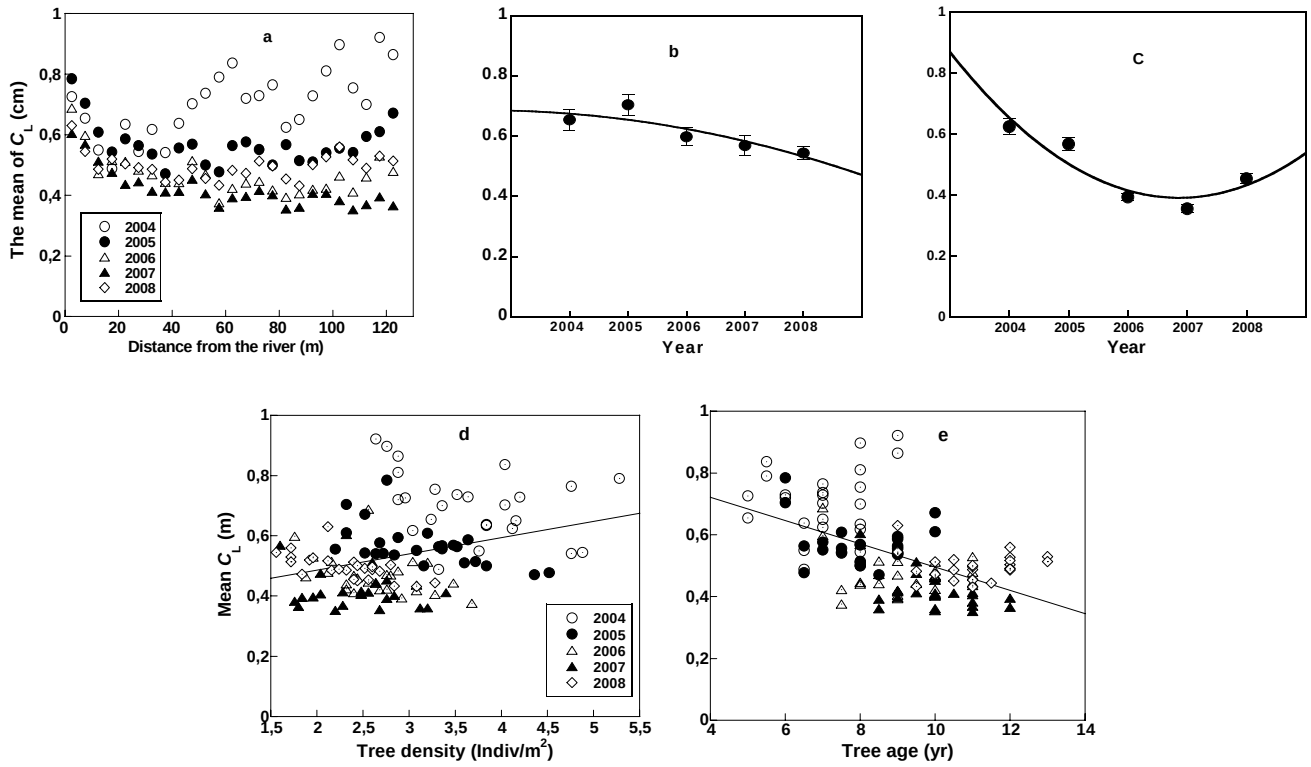

Figure 3. A. Spatial trends of crown length C_L dynamics during five years. B. Examples of C_L dynamics for young stand, and C. mature stand. D. Relationships of crown length to tree density, and E. tree age. Open circles 2004, filled circles 2005, open triangles 2006, filled triangles, 2007, open diamonds 2008

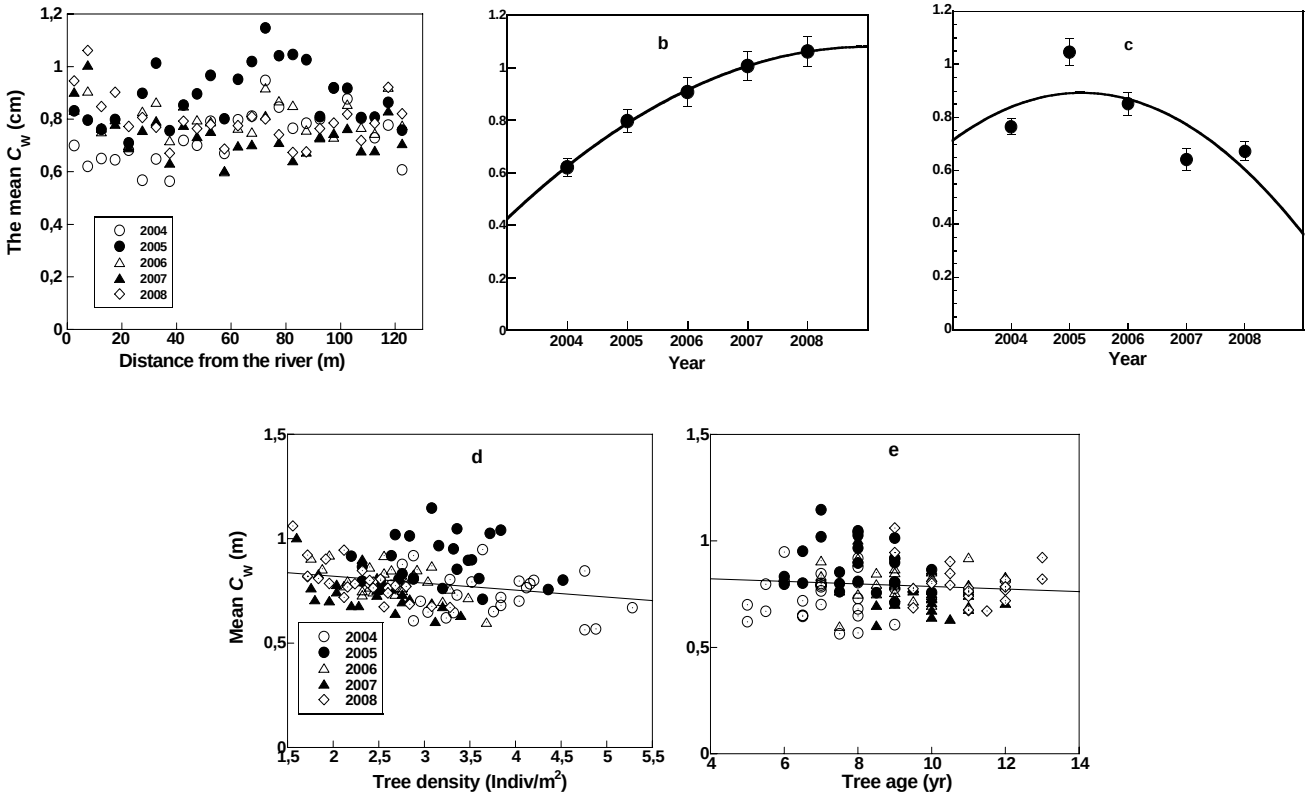

Figure 4. Spatial trends of crown width C_W over five years (a). Examples of C_W growth for young stand (b) and mature stand (c). Relationships of crown width to tree density (d) and tree age (e). Open circles 2004, filled circles 2005, open triangles 2006, filled triangles, 2007, open diamonds 2008

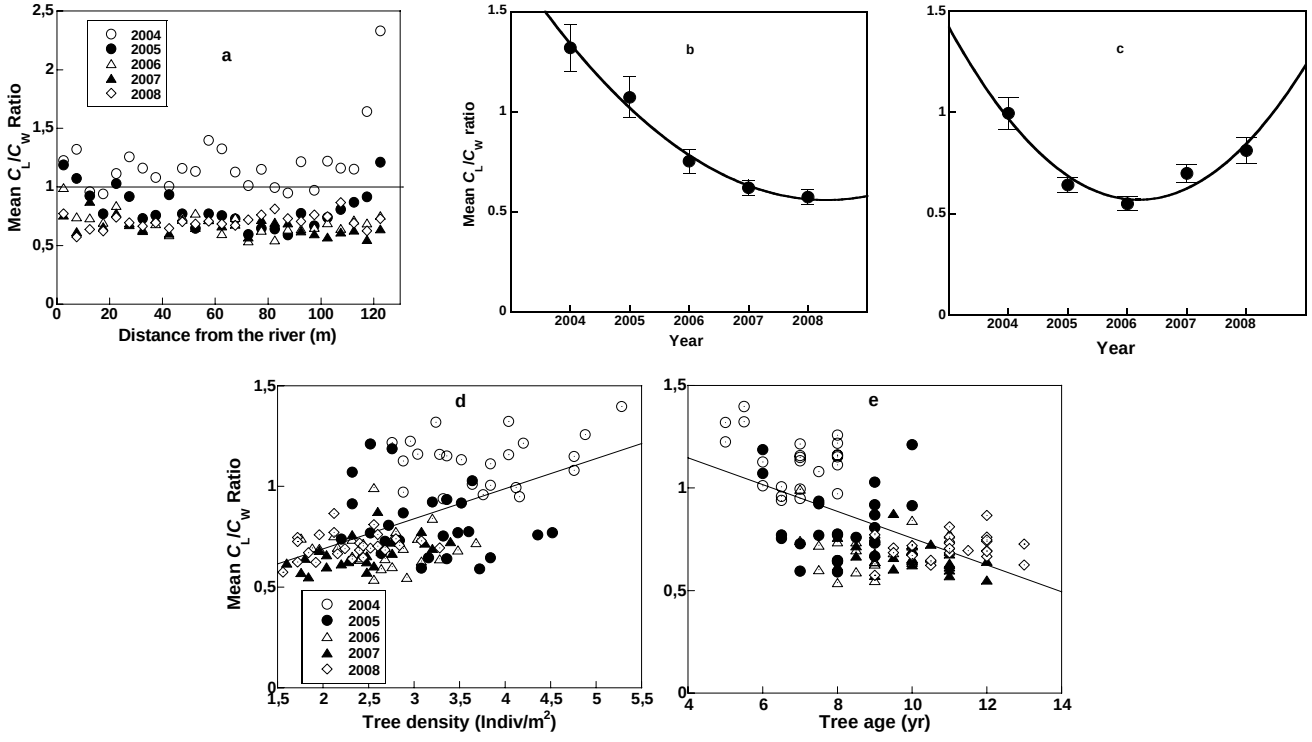

Figure 6. A. Spatial trends of C_L/C_W ratio or crown shape dynamics during five years. B. Examples of crown shape dynamics for young stand, and C. mature stand. D. Relationships of C_L/C_W ratio to tree density, and E. tree age. Open circles 2004, filled circles 2005, open triangles 2006, filled triangles 2007, open diamonds 2008

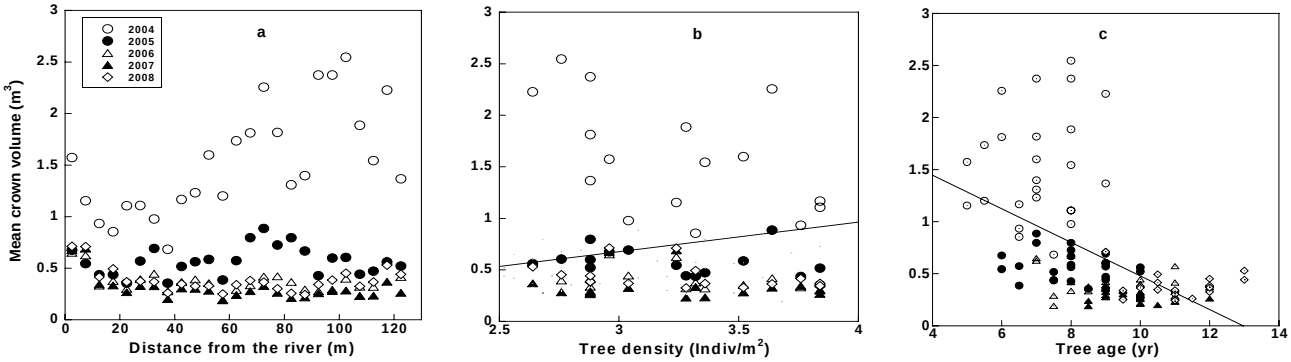

Figure 7. A. Spatial trends of crown volume dynamics during five years. B. Relationships of mean C_V to tree density, and C. tree age. Open circles 2004, filled circles 2005, open triangles 2006, filled triangles 2007, open diamonds 2008

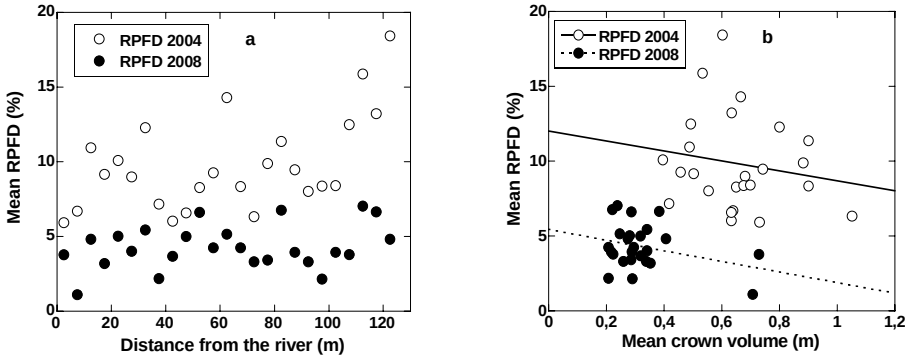

Figure 7. Spatial trends in relative photon flux density RPFD under the canopy of *Kandelia obovata* stands (a), and relationship of RPPFD to crown volume (b).Open circles2004 and filled circles 2008

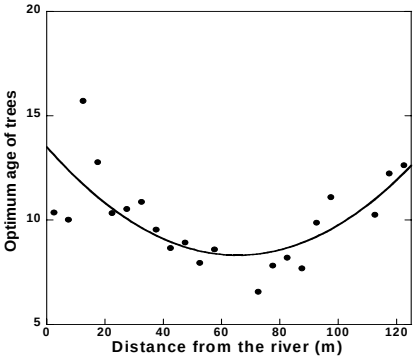

Figure 5. Spatial trend of optimum age when trees attain their maximum crown width from the riverside landward

Discussion

The obtained results confirmed that crown structure of dense *Kandelia. obovata* stands is dynamics. The C_L of the young stand increased and then decreased as the stands grew (Figure 3a), i.e. in the 2004, the C_L of young stand was a little thin then increase in the second year, but then decreased again in the third year and fourth year. This is because high irradiance was found in the first year, which was mostly over 5% for all plots, but it was decreased to less than 5% for most of plots in 2008 (Figure 8a). As the results, C_L tended to increased in the 2004, but then decreased due to reduced RPPDF under canopy of trees. The dynamic of C_L was correlated to tree density (Figure 3d), and tree age (Figure 3e). In contrast, as illustrated in Figure 4, the C_W increased and then decreased as the stands grew (Figure 4a), though the C_W of the young stand increased as the stand grew (Figure 4b), while it increased and then decreased for the mature stand (Figure 4c). The growth dynamic of C_W was not significantly correlated to tree density (Figure 4d), but it was negatively correlated to tree age (Figure 4e). However, the year when crown width attains its maximum decreased from the riverside landward (Figure 5), which indicates that trees near the riverside later attain the year to reach the maximum C_W as compared with trees near the landward. These trends indicate that the tree crown development is not only depending on competition but it also corresponding with their age. Meanwhile, crown shape of dense *Kandelia obovata* stands are dynamics as stands grew though they showed different trends for young and mature stands (Figure 6a). The C_L/C_W ratio decreased for the young stands (Figure 6b) as the stands grew, while it decreased and then increased for mature stands (Figure 6c). Hashimoto (1990, 1991) found that the ratio of crown length to crown width increased with age. Therefore, differences in the tree crown dynamics may reflect how the *K. obovata* trees produce sound crown to function in dense stands.

Although Kohyama et al. (1990) found that the tree crown length of a crowded stand increased as the stand grew, but our results showed that C_L of dense *K. obovata* stands decreased as the stands grew, which implies that crown changes to be dumpy as the stands grow. In fact, as revealed in Figure 6, C_L/C_W ratio decreased significantly as the stands grew, which suggests that the tree crown shape changes with developing stands. The crown volume of dense mangrove *K. obovata* stands are dynamics as stands grew (Figure 7a), and it was correlated with tree age (Figure 7c). This study results suggested that the tree crown shape of the dense mangrove stands is dynamic, and seems to correspond with the developmental stage of the stands. However, this dense mangrove stands were actively self-thinned for small trees (Analuddin et al. 2009b), while foliage in their crown are dynamic as the stands grew (Analuddin et al 2009a). Therefore, there may be existed internal factors (i.e. tree density, competition, and tree age) and external factor such as light intensity for maintenance the crown structures of dense *K. obovata* stands. As the crowded stands grow, the competition for light might be increased among individual trees. It shows that RPFD reduced from more than 5% up to 10% for most of plots in 2004 to less than 5% in 2008 (Figure 8a). The thick crown might result in low light intensity at the bottom canopy layer where leaves could not be doing better photosynthesis. As the results, carbon uptake by bottom leaves may become low. It has been reported by Khan et al (2007) that leaf carbon content of *K. obovata* trees in this forest decreased from the top to the bottom canopy layer. Uemura et al (2006) found on the leaves of winter-deciduous mature trees that leaves chlorophyll content those of trees decreased from the top to the bottom of the canopy. Less photosynthetic ability those of bottom leaves may be influenced their surviving ability, and the death of leaves or branches at the bottom canopy changed the crown shape of mangrove trees. In addition, nutrients from leaves at the bottom of the canopy might be re-translocated to support new leaves flushed in the top of the canopy. Khan et al. (2007) reported that N concentration in the leaves of *K. obovata* decreases from the top to the bottom of the canopy. This decrease might be induced for the rapidly dropped leaves of the bottom canopy or many bottom leaves might die as the stands grew. Therefore, the change in crown shape from a thick type to a thin type may imply that trees might transform their crown shape for reducing the stress of competition. Such change in the crown shape reflects that the dense mangrove stands might need to create sound crown shape to adapt growth and sustain in their life.

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The utilization of bioresources by local communities at Giam Siak Kecil-Bukit Batu Biosphere Reserve, Riau Province, Indonesia

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Abstract. *Titisari PW, Syamsudin TS, Sjarmidi A. 2016. The utilization of bioresources by local communities at Giam Siak Kecil-Bukit Batu Biosphere Reserve, Riau Province, Indonesia. Biodiversitas 17: 873-886.* This study aims to assess the use of bioresources of Giam Siak Kecil Bukit Batu Biosphere Reserve by the local community. The use of bioresources was characterized by the level of utilization of bioresources in the household economy. It classified into five groups; (i) subsistence group (SG), (ii) supplementary group (SpG), (iii) integrated group (IG), (iv) specialized extraction group (SEG), and (v) specialized cultivation group (SCG). The results showed that the local community used bioresources as main source of livelihood at the core zone and buffer zone. There are 36 species of fish, 17 species of non-timber, and 28 species of timber used by local community. Core area and buffer zone are dominated by SEG and SCG. In the core area, the relationship between SEG non-timber and timber is very significant. In buffer zone, the exploitation of fisheries, and timber were dominated by SEG. The correlation between the use of bioresources in the core area showed that the exploitation of non-timber and timber are very high by SEG community.

Keywords: Giam Siak Kecil Bukit Batu Biosphere Reserves, Specialized Extraction Group, Specialized Cultivation Group
Abbreviation: GSKBBBR = Giam Siak Kecil Bukit Batu Biosphere Reserves, SEG = Specialized Extraction Group, SCG = Specialized Cultivation Group

INTRODUCTION

Biosphere reserves are a conservation area terrestrial and coastal/marine or a combination of more than one type of ecosystem, which is internationally recognized within the framework of the MAB (Man and the Biosphere) program (MAB, 2008), formation is to balance and harmonize the human relationship with nature (UNESCO 2011). Biosphere reserves explicitly recognize the human and human interest in the conservation of the landscape while preserving the ecological values of protected areas (Coetzer et al. 2013). In other words, the biosphere reserve should be able to integrate aspects of cultural, social, economic and natural capital for the sustainability of local livelihoods and ecosystems on a broad scale (Bozak 2008; UNESCO 2011; Coetzer et al. 2013; Agnoletti et al. 2015).

Unfortunately, setting up community involvement in conservation actually cause conflicts between stakeholders with local communities living in the biosphere reserve (Hill et al. 2015; Jacobs et al. 2015; García-Llorente et al. 2016). The conflict arises because policies that build on the biosphere reserve restrict the activities of local communities (Hill et al. 2015, Jacobs et al. 2015). The conflict arises because policies that build on the biosphere reserve restrict the activities of local communities (Hill et al. 2015; Jacobs et al. 2015). The local community considers the closing of the core zone which has been the main source of livelihood will affect the economic prosperity that led to changes in their livelihood strategies (Jorda-Capdevila et al. 2015; Kolka et al. 2016). The problems that arise in the biosphere reserve caused by human activities such as illegal logging, poaching of wildlife, land use and sharing of economic benefits that do not fit (Habibah et al. 2010, 2011; Coetzel et al. 2012).

As well as in Giam Siak Kecil Bukit Batu Biosphere Reserve (GSKBBBR), the problems are also caused by human activities, such as land ownership conflicts, illegal logging, poaching of wildlife, land use, burning of land and conflicts with policy makers. Since the beginning of 2000 to 2014, approximately 47,200 hectares buffer zone of GSKBBBR disrupted due to illegal logging and forest fires, and followed by illegal palm oil plantations (Partomiharjo et al. 2007). There are around 126 plants (52 of rare plants and protected), 150 birds, 10 mammals and 8 reptiles are protected (Partomiharjo et al. 2007; LIPI 2008a, b).

Indeed human and biodiversity not be separated. Biodiversity is an asset for human beings to lives in the present and future, and the current biodiversity is threatened due to human activities. As shown in the Outlook Report (GEO-3) (UNESCO 2011), human activity is an important trigger factor for the rate of land use, climate change, pollution, and unsustainable the use of bioresources. Reduction of biodiversity caused by the human population growth is in line with the consumption patterns of unsustainable use of bioresources, land ownership conflicts, and injustice the distribution of economic and bioresources (Jacobs et al. 2015; Newton et al. 2015; Renaud et al. 2016). Reduction of biodiversity is closely related to sustainable development, so that the management of biosphere reserves require a more holistic

approach, integrated and comprehensive (Santhanam-Martin et al. 2015; Schmeller et al. 2016). To change unsustainable consumption and production patterns, it is important to calculate every sustainable contribution value and services including human wellbeing, biodiversity and cultural aspects (Szabo et al. 2016; Wyborn et al. 2016). The aim of this study to explore the use of the bioresources of the biosphere reserve by the local community in the GSKBBBR, Riau Province, Indonesia.

MATERIALS AND METHODS

Study area

This study was conducted in the Giam Siak Kecil-Bukit Batu Biosphere Reserves, located in Bengkalis Districts and Siak Districts in Riau Province. This area is one of seven biosphere reserves in Indonesia. Based on Proposal Management Plan of GSKBBBR (MAB Indonesia 2008). The area covered 705,271 ha. Administratively this area belongs to Bengkalis and Siak districts. The core zone is about 25% of biosphere reserve (178,722 ha), which is a combination of natural forest conservation and forest production. Forest production has been converted into oil palm plantations, industrial tree plantations and settlements. The buffer area is about 32% of biosphere reserve (222,425 ha). The transition zone is about 43% (304,23 ha).

Research methods

The method used were survey method, focus group discussions, key informant interviews, ethnographic study, and secondary data. Household income data is a combination of social and economic data, qualitative and quantitative data, participatory data, and extractive data.

Samples were taken randomly by a wide variety of backgrounds demography of population, economic families and access to information about GSKBBBR, based on this approach then determined the percentage of household sample of 60% of the total population of households in each village that represent the core zona and the buffer area.

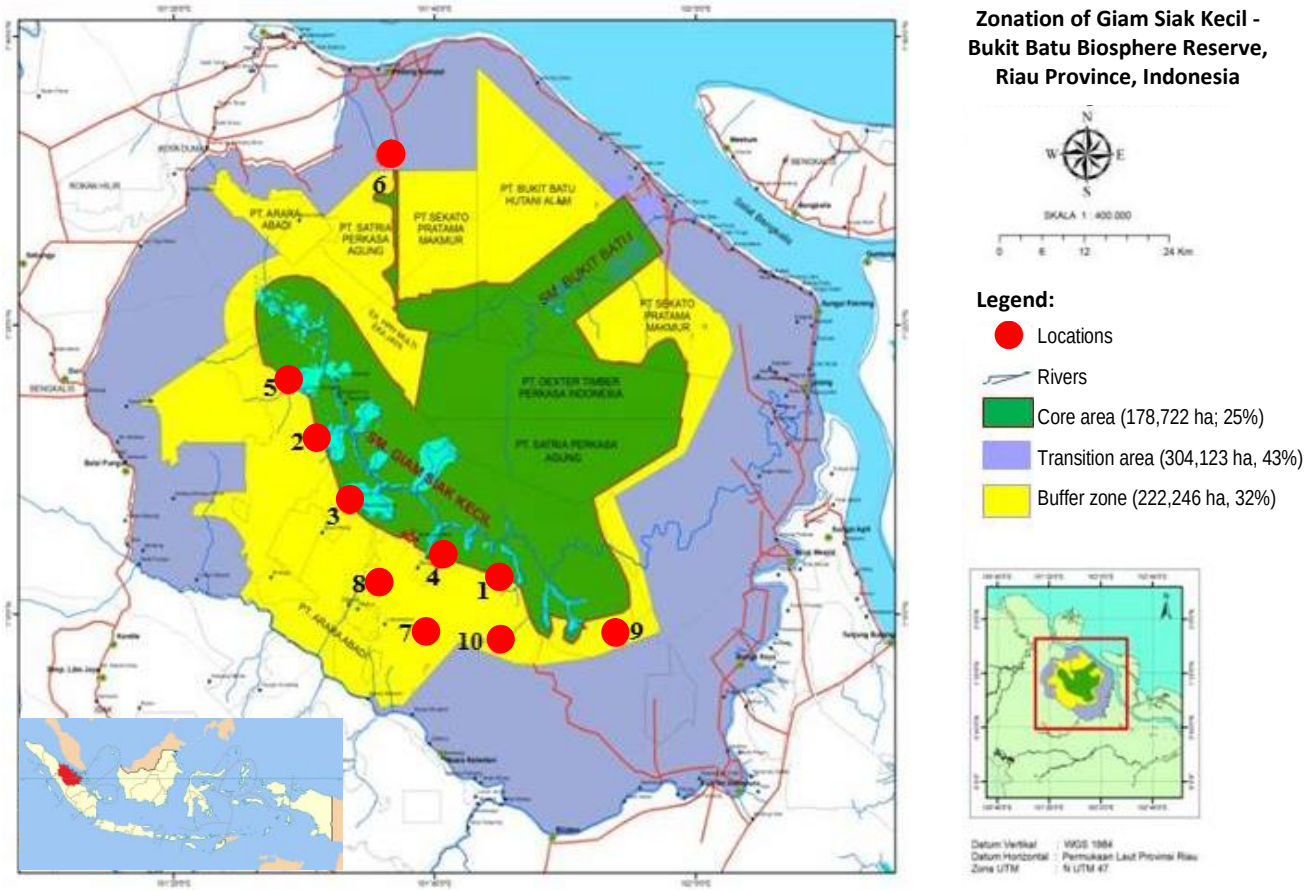

Figure 1. Giam Siak Kecil Bukit Batu Biosphere Reserve, Riau Province, Indonesia (LIPI 2008a). 1. Tasik Betung village, 2. Tasik Serai village, 3. Tasik Serai Timur village, 4. Tasik Tebing Serai village, 5. Tasik Serai Barat village, 6. Bukit Kerikil village, 7. Muara Kelantan village, 8. Sungai Selodang village, 9. Olak village, 10. Muara Bungkal village

Data collection

There were two types of data collected, i.e. primary and secondary data. The primary data were collected through key informant interviews, general observation, and focused group discussion (FGD). Key informant interviews were used to collect detail of information on local knowledge about forest use, land-use and bioresources. General observations carried out to illustrate the use of forest, land characteristics and utilization of bioresources. Observations were also conducted to test the cross-checking information collected from the community. FGD done basically to obtain general data from various people representing different groups in community. A community meeting initialized the methods of data collection. The secondary data consisted of demography, education level, public facilities, and land use systems. The related literature was collected from several sources.

Community meeting

A community meeting was attended by most of the community members, comprising young and senior inhabitant, men and women, and traditional leaders. During the meeting, the participants were asked to discuss how they described the forest land uses around them.

Interview with key informants

Key informants interviews conducted in 50 households by using a semi-structured questionnaire. The interview focused on local knowledge about land use and the utilization of forest and bioresources. In addition, interviews were also conducted with five key informants who understand the process and their involvement in land management and bioresources. The key informants were including the village head, customary leader, old villagers, and informal community leaders.

Focused group discussion (FGD)

We facilitate and initiate FGD on several groups participating in each village. Some of the topics covered include specific information about the importance of bioresources, the role of society and the rules of forest use.

Data analysis

There are two main effects that occur due to the use of bioresources in biosphere reserves. First, the impact on the species of the bioresources, such as changes in population, distribution and existence of the species. Second, the impact on the ecosystem in the form of ecosystem change, and no less important is the community's decision about the use of bioresources. Determination of biological indicators used in this study was to assess the sustainable extraction of bioresources, and the factors that influence decision-making in land use and utilization of bioresources. Specified indicators chosen based on three levels, namely the level of utilization of bioresources, land use at the ecosystem level, which will generally depict a mosaic of land use, including plantations and forests. The use of bioresources refers to the opinion Belcher et al. (2005), which is indicated by the level of utilization of bioresources as a source of economic income family, which consists of (i) Subsistence Group/SG, (ii) Supplementary Group/SpG, (iii) Integrated Group/IG, (iv) Specialized Extraction Group/SEG, and (v) Specialized Cultivation Group/SCG.

RESULTS AND DISCUSSION

Community and livelihood

The population of households and communities in this research is dominated by the ethnic Malay. The level of education is generally only up to primary school, do not complete primary school, and there was never gone to school. Traditional regulations still applied to life as guidelines and rules to define norms for the whole community. The main source of the people's livelihood comes from plantations and fisheries. Most community work as shifting cultivators, rubber tappers, loggers, weavers (especially for women) and plantation workers. Most community plant rice to meet their daily needs. They also plant rubber intercropped with vegetables and fruit trees. In addition, they are also hunting, fishing and gathering products of non-timber forest such as rattan, fruits, vegetables, honey, and *nira* (for coconut sugar).

The utilization of bioresources as a source of livelihood

Giam Siak Kecil Bukit Batu Biosphere Reserves have a diversity of plants is high. According to the geographical location, the dominant plants are relatively the same as the vegetation lowland tropical rain on the Sumatra, Borneo and Malay Peninsula. Some species of plants and found several species of unique and rare allegedly include: *Johannesteijsmannia altifrons* HE.Moore, *Areca catechu* L, *Iguanura wallichiana* (Mart.) Benth. & Hook f., *Dyera costulata* (Miq.) Hook.f, *Daemonorops draco* (Willd.) Blume), *Shorea peltata* Sym, *Aquilaria malacensis* Lamk, *Calamus ciilegalis* Blume, *Calamus exilis* Griffith, *Gonistylus bancanus* Kurz, *Styrax benzoin* Dryand, *Eurycoma longifolia* Jack, *Nenga* sp., *Archidendron bubalinum* (Jack) Kosterm, *Phanera kochiana* (Korth.), *Baccaurea racemosa* (Reinw. ex Blume) Müll, *Baccaurea stipulata* J.J.Sm, *Palaquium* sp., *Alstonia scholaris* (L.) R.Br, *Koompassia excelsa* (Becc.) Taub, *Shorea* sp., *Litsea* sp., *Dehaasia* sp., *Parashorea* sp., *Pterospermum javanicum* Jungh, *Eugenia* sp., and *Pometia pinnata* J.R. Forster & G.Forster.

Giam Siak Kecil Bukit Batu Biosphere Reserves has a high diversity of animals, some of which are endangered, as *Panthera tigris ssp. sumatrae* Pocock, *Elephas maximus sumatranus* Temminck, *Aonyx cinerea* Illiger, *Neofelis nebulosa* Griffith, *Catopuma temminckii* Vigors and Horsfield, *Balionycteris maculata* Thomas, and *Megaerops wetmorei* Taylor, but it also found six species of primates, *Presbytis melalophos* Rafflei, *Macaca fascicularis* Rafflei, *Macaca nemestrina* Linnaeus, *Hylobates agilis* Cuvier, *Symphalangus syndactylus* Rafflei, *and Presbytis femoralis* Martin. It was also found various species of birds, among which *Ciconia stormi* Blasius, *Leptoptilos javanicus* Horsfield, *Anhinga melanogaster* Pennant, *Cairina scutulata* Muller, *Melanoperdix nigra* Vigors, *Lophura erythrophthalma/L. pyronota* (del Hoyo and Collar),

Lophura ignita (del Hoyo and Collar), *Batrachostomus auritus* Gray, *Buceros vigil* Sibley and Monroe, dan *Pitta granatina* Sibley and Monroe. Some of the bird species are endemic species in Sumatra, *Pycnonotus melanicterus* Gmelin, *Trichastoma tickelli* Blyth and *Lonchura striata* Linn.

CBGSKBR bioresources utilized in the community as a source of livelihood consists of three groups: (i) fishery, (ii) timber, and (iii) non-timber. Utilization as well as the current condition of existence of the three bioresources is illustrated in the tables below.

Based on Table 1, Figure 2 and 3, illustrated condition the existence of various types of timber in the core zone and buffer area GSKBBBR. Since 2005 there has been a significant decline existence of different kinds of timber, including some types of protected categories, as *Gluta renghas* L, *Campnosperma auriculata* Blume, *Comnosperma macrophyla* Hook.f, *Fragraec fragrans* Roxb, *Anisoptera costata* Korth, *Palaquium leiocarpum* Boerl, *Koompassia malaccensis* Maing, and *Shorea parvifolia* Dyer. The condition of the existence of timber due to illegal extraction of timber excessive. These conditions are in addition to affecting the existence of a type as well as the conservation aspects in the core zone will also affect the livelihood resources that they extract the bioresources of timber as a source of livelihood, even though what they are doing is against the law and undermine the preservation of biodiversity in GSKBBBR. In Figure 2, seen the rate of decline of seven types of timber which since 2005 has been in dangerous conditions and getting very hard to find. Figure 2 data derived from illegal loggers, illegal timber collectors and traders of illegal timber, they stated that within a period of fifteen years there has been a significant decline, until this study is completed, illegal logging is ongoing, especially in the area of the buffer that have greater access to the timber stolen.

Table 1. The existence of bioresources timber used as a source of livelihood by local community at the core zone and buffer area at GSKBBBR, Riau Province, Indonesia

Biological resources		**Times (years)**					
Local vernacular name	**Scientific name**	**< 1990**	**1990 s/d 2000**	**2000 s/d 2005**	**2005 s/d 2007**	**2008 s/d 2010**	**2011 s/d 2012**	**2013 s/d 2015**
Balam/Jongkang	*Palaquium leiocarpum* Boerl.							
Bengku	*Santiria laevigata* Bl., Mus. Bot.							
Bintangur	*Calophyllum soulattri* Burm.f.							
Durian Burong	*Durio carinatus* Mast.							
Gaharu	*Aquilariella malaccensis* (Lamk.) van Tiegh							
Geronggang	*Cratoxylum arborescens* (Vahl) Blume, Mus. Bot. Lugd. Bat.							
Kelat	*Eugenia* spp.							
Kempas	*Koompassia malaccensis* Maing							
Keranji	*Dialium indum* L., Mant.							
Ketiau	*Ganua motleyana* (de Vriese) Pierre ex Dubard							
Mendarahan	*Myristica inners* Blume							
Mengeris	*Kompassia excelsa* Benth							
Meranti bakau	*Shorea uliginosa* Foxw.							
Meranti mersawa	*Anisoptera costata* Korth							
Meranti rawa	*Shorea parvifolia* Dyer.							
Meranti bungo	*Shorea teysmannia* Dyer.							
Meranti merah	*Sloanea* guianensis Aletón							
Nyamplung	*Calophyllum inophyllum* Linn.							
Nyatoh	*Payena leerii* (Teijsm. & Pinn.) Kurz.							
Para	*Aglaia ignea* Bark.							
Pulai	*Alstonia pneumatophora* Back.							
Punak	*Tetramerista glabra* Miq.							
Rasak	*Vatica rassak* (Korth.) Blume							
Rasak	*Vatica umbonata* Hook.f.							
Rengas	*Gluta renghas* L.							
Terentang	*Campnosperma auriculata* Blume							
Terantang	*Comnosperma macrophyla* Hook.f.							
Trembasah	*Fragraec fragrans* Roxb.							

Note:

= Still easily found in any collection/logging timber species is still widely available, and can be used as a source of income and the remainder can be used for its own needs and other needs

= Easy to found in any collection / logging timber species is still available but not many and distance timber extraction has been away to the middle of the forest, including the price of timber is expensive

= Difficult to found in any logging timber species is still available but not many and distance timber extraction has been away to the middle of the forest, including the price of timber is expensive, specialy *Koompassia malaccensis* Maing, *Vatica rassak* (Korth.) Blume, and *Palaquium leiocarpum* Boerl.

The condition of the existence of various types of non timber bioresources use by the communities in the core zone and buffer area CBGSKBR illustrated in Table 2. Bioresources are exploited non-timber comprising two groups of plants and animals. Both of these bioresources is the substitution of the bioresources of timber with adequate economic value, the level of dependence of some communities to bioresources for non-timber high enough, for enough help their household income, of 280 households in the sample, 13% rely on their economic returns from non-timber biological resources. Based on Table 2, since 2005 the availability of non-living resources of timber began to decline, and since 2011 some type of non timber biological resources, especially from animals getting hard to come by. The saddest thing is the exploitation is too much on some types of protected animals, such as *Chitra chitra* Nutphand, *Amyda cartilaginea* Boddaert, and *Manis javanica* Desmarest who mythologized as a traditional medicine. Based on information from the respondents, namely catcher and sellers of these animals in Bengkalis, since 2014 almost no longer sell these animals, because there is no supply from the field. Arrest activity and sales are illegal, because the traded animals are protected by government regulations, but based on field observation illegal activity is still ongoing. Illegal activities like this if allowed to continue, it will damage the biodiversity of protected animals in CBGSKBR.

The condition of the existence of various types of bioresources utilized by the fish in the core zone and buffer area GSKBBBR illustrated in Table 3. The dependence of society to the bioresources of fish is very high, because it helps their household income, from 280 households in the sample, 37% rely on their economic income of the bioresources of fish. Since 2005 has been the availability of biological resources, the fish began to decline, and since 2011 some kind of fish is getting hard to be obtained. The decline in fish catches, in addition affected by the excessive exploitation, also due to seasonal factors, in a year there are only four months (October-December) the effective time to catch fish, it is influenced by conditions of drought or dry months. In 2011 through 2013, most of his lakes and creeks in GSKBBBR experiencing a severe drought, which resulted in almost no retrieval or fishing. In Figure 5, since 2008 there are six kinds of fish presence is very difficult to find, *Scleropages formosus* Müller & Schlegel, *Hemibagrus nemurus* Kottelat & Whiten**,** *Bagrichthys macropterus* Bleeker**,** *Notopterus notopterus* Pallas, *Kryptopterus lais* Bleeker, *Lepidocephalichthys hasselti* Valenciennes, dan *Wallago attu* Bloch & Schneider.

Based on data from Table 4 and Table 5, all categories of Belcher et al. (2005) can be found at the study site, the group Subsistence group/SG, Supplementary group/SpG, Integrated group/IG, Specialized extraction group/SEG and Specialized cultivation group/SCG the details are as follows:

Table 4 to explains the level of dependency of people in the core zone GSKBBBR to the extraction of bioresources at 62% SG, SPG, IG, and SEG, this percentage is very large, especially in the SG and SPG, contributions extraction of bioresources become the main source of

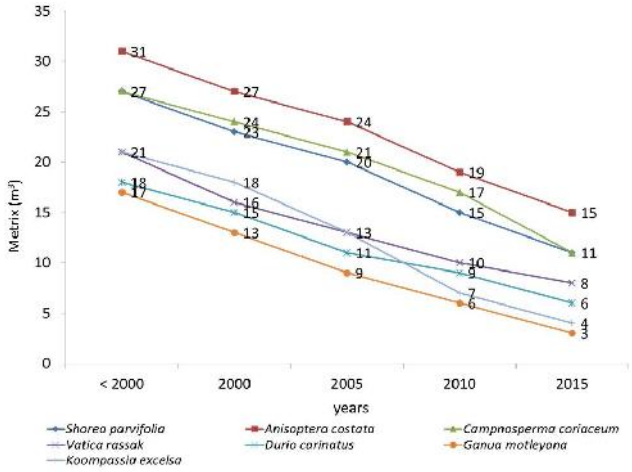

Figure 2. Reduction in the quantity of harvest some high value timber species taken through illegal logging in the core zone and buffer area at GSKBBBR, Riau Province, Indonesia

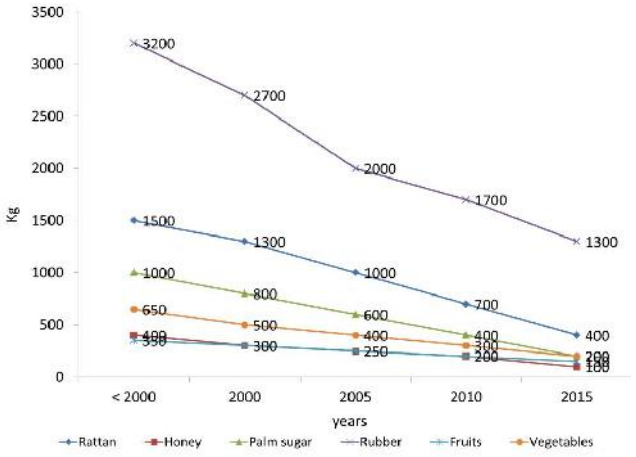

Figure 4. Reduction in the quantity of harvest some high value non timber species taken through illegal logging in the core zone and buffer area at GSKBBBR, Riau Province, Indonesia

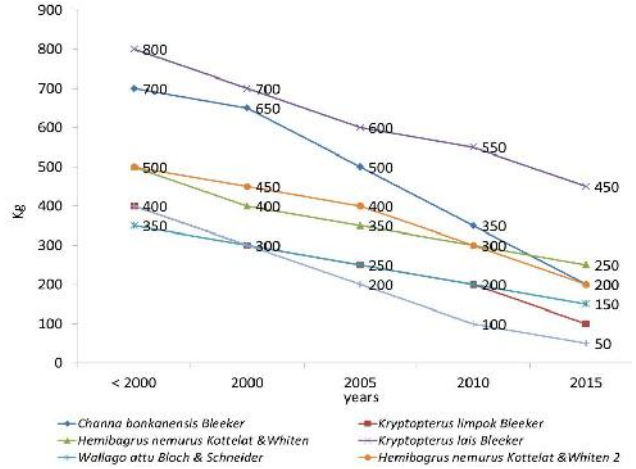

Figure 5. Reduction in the quantity of harvest some high-value fish species at GSKBBBR, Riau Province, Indonesia

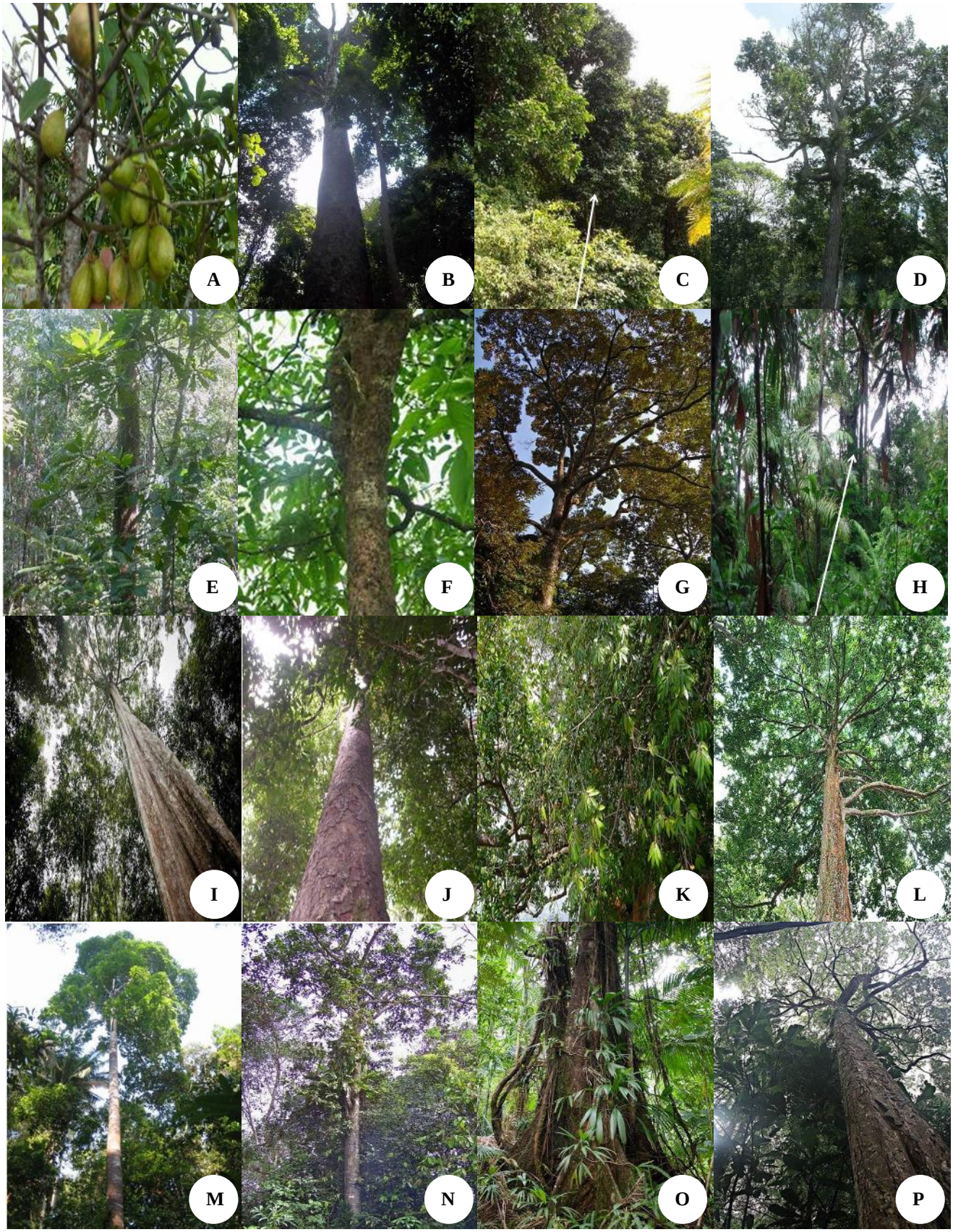

Figure 3. The diversity of high value timber in the core zone and buffer area which is utilized local community at GSKBBBR, Riau Province, Indonesia. A. *Anisoptera costata***,** B. *Aquilariella malaccensis*, C. *Calophyllum inophyllum*, D. *Calophyllum soulattri*, E. *Cratoxylum arborescens*, F. *Dialium indum*, G. *Durio carinatus*, H. *Ganua motleyana*, I. *Kompassia excelsa*, J. *Koompassia malaccensis*, K. *Myristica inners*, L. *Palaquium leiocarpum*, M. *Shorea parvifolia*, N. *Shorea teysmannia*, O. *Shorea uliginosa***,** P. *Vatica rassak*

Table 2. The existence of bioresources non timber used as a source of livelihood by local community at GSKBBBR, Riau Province, Indonesia

Biological resources	Times (years)					
	< 1990	1990 s/d 2000	2000 s/d 2005	2005 s/d 2007	2008 s/d 2010	2011 s/d 2012	2013 s/d 2015
Plant resources							
Rattan							
Honey							
Various kinds orchids flowers							
Various kinds vegetables							
Various kinds fruits							
Wild rubber							
Palm sugar							
Pandanus roofs							
Wild animal resources							
Muntiacus muntjak Zimm.							
Tragulus kanchil Milne-Edwards							
Helarctos malayanus Raff.							
Chitra chitra Nutphand							
Amyda cartilaginea Boddaert							
Manis javanica Desmarest							
Varanus salvator Laurenti							
Paradoxurus hermaphroditus Pallas							
Sus scrofa Linnaeus							

Note:

= Still easily found in any collection non timber is still widely available, and can be used as a source of income and the remainder can be used for its own needs/making home/cage and other needs, the price of non timber is cheap

= Still quite easily found in any collection non timber is still available but not many and distance non timber extraction has been away to the middle of the forest, including the price of non timber is expensive

= Difficult to find in any decision non timber is still available but not many and distance non timber extraction has been away to the middle of the forest, including the price of non timber is expensive, specialy *Chitra chitra* Nutphand, *Amyda cartilaginea* Boddaert, and *Manis javanica* Desmarest.

livelihood is above 50%, the number of respondents who entered this group is quite large, as 33%. Unlike in the buffer area, Table 5 illustrates the level of dependence of communities on biological resource extraction is smaller, at 56% SG, SPG, IG, and SEG. Besides extracting biological resources, groups SG and SPG seek additional livelihood through agriculture, plantation, fisheries and other businesses. Its growth is composed of rubber and oil palm plantations, with the limited area of land ownership gardens, plantation crops generally are not sufficient as the primary source of livelihood, especially the trend of the price of rubber and oil palm tends to decrease.

All the groups have made of fishery resources as a source of livelihood, based on Table 3 and Figure 5, there was a downward trend in the number of fish caught, in addition due to the large number of people involved, the season also be a determinant factor, especially in the dry season or drought. Since 2010, the fish caught is generally not worth the high economic, especially the fish sold fresh, to increase the sale value of the fish processed into smoked fish.

Almost all the group does not have a plantation area and considerable agricultural, most of the land belonging to the indigenous and certified, thus weakening their position as landowners. From the aspect of economics and law, land certificates can be used as capital that can help improve their access to financing sources. The group has its own land is the SEG and the SCG, but not certified, so it not yet be used as morally effort.

Difficult access roads in and out of the village, making the access to the market is also limited, they can only access the market of the village and market of the district which takes place weekly. This causes the processed products plantation, fishery, agriculture and household industry they produce does not have a high value. The level of dependence on middlemen and collectors very high, so they do not have the bargaining value of the price of the products they produce.

Figure 7 illustrates the relationship between the use of the bioresources of fish, timber and non-timber typology biological resource user community by Belcher et al. (2005), five groups, SEG has a strong relationship as extracting or utilizing bioresources fishery, timber and non-timber. This represents a large role SEG group as a group of contributors decline in the quality and sustainability of the diversity of bioresources in GSKBBBR, Based on personal interviews with SEG group, generally illegally clearing land for oil palm and rubber that occurs mainly in the core zone, forest fires, illegal logging and overfishing carried out by them.

Table 3. The existence of bioresources of fish are used as a source of livelihood by local community at the core zone and buffer area at GSKBBBR, Riau Province, Indonesia

Biological resources		**Times (years)**						
Local vernacular name	**Scientific name and family**	**< 1990**	**1990 s/d 2000**	**2000 s/d 2005**	**2005 s/d 2007**	**2008 s/d 2010**	**2011 s/d 2012**	**2013 s/d 2015**
Arwana	*Scleropages formosus* Müller & Schlegel							
Baung kunyit	*Hemibagrus nemurus* Kottelat &Whiten							
Baung Munti	*Bagrichthys macropterus* Bleeker							
Belida	*Notopterus notopterus* Pallas							
Betok	*Anabas testudineus* Bloch							
Gabus/haruan	*Channa bankanensis* Bleeker							
Julang	*Hemirhampodon phaisoma* Collette							
Kelabau	*Osteochilus spilurus* Bleeker							
Kemuringan	*Puntius eugrammus* Silas							
Kemuringan	*Puntius lineatus* Duncker							
Kepar selinca	*Belontia hasselti* Cuvier							
Lais Kuning	*Kryptopterus limpok* Bleeker							
Lais Putih	*Kryptopterus macrocephalus* Bleeker							
Limbek bolang	*Clarias teijsmanni* Bleeker							
Limbek patil	*Clarias nieuhoffi* Valenciennes							
Pantau codiak	*Rasbora einthovenii* Bleeker							
Pantau kuning	*Rasbora tornieri* Ahl							
Pantau merah	*Rasbora kalochroma* Bleeker							
Pantau perak	*Rasbora sumatrana* Bleeker							
Riu	*Pseudeutropius brachypopterus* Bleeker							
Sasau	*Rasbora cephalotaenia* Bleeker							
Selais	*Kryptopterus lais* Bleeker							
Selais hujan	*Kryptopterus macrocephalus* Bleeker							
Seluang/Mengkarik	*Rasbora argyrotaenia* Blkr							
Sepat Rawa	*Trichogaster trichopterus* Pallas							
Sepimping	*Parachela oxygastroides* Bleeker							
Silok	*Lepidocephalichthys hasselti* Valenciennes							
Sopek Hitam	*Parambassis macrolepis* Bleeker							
Sopek Hitam	*Pristolepis fasciata* Bleeker							
Sopek Batik	*Sphaerichthys osphromenoides* Canestrini							
Sopek Mujair	*Helostoma temminckii* Cuvier							
Tabak	*Luciocephalus pulcher* Gray							
Tapah	*Wallago attu* Bloch & Schneider							
Tempalo	*Betta anabatoides* Bleeker							
Toman	*Channa micropeltes* Cuvier							

Note:

= They are easy to find (a) in any arrests, this fish are still many caught, and can be used as a source of income and the remainder can be used for its own needs, (b) general price of fish is not too expensive, and more often processed into smoked fish, in order to fish selling price remains high.

= Still pretty easy to find (a) in any arrests, this fish they caught, but not so much, and no longer be used for its own needs, but rather used as a source of income, (b) the price of this fish is expensive, and more often processed into fish smoked, so that the fish selling prices remain high.

= Hard to find (a) in any form of arrest, the type of fish caught is extremely difficult, if caught because the price is expensive, it is more often processed into smoked fish, that fish selling price remains high. Except for *Scleropages formosus* Müller & Schlegel are generally sold alive.

Discussion

Local community perceptions towards CBGSKBR

The results showed low levels of participation and awareness among local communities in the conservation program CBGSKBR. Approximately 7% of the 280 respondents who know and realize the importance of the conservation program and be willing to be involved in conservation programs in CBGSKBR (eg meeting / focus group discussions, workshops, and training).

Based on the results of the FGD respondents perceptions of the conservation program, 39% better assess the conservation activities, 28% rate it very good, and 33% said it was not well and did not care about the conservation program in CBGSKBR. Respondents welcomed the conservation program only if they see no benefit to them for the long term as well as the involvement of local communities. It requires good management of the conservation program to provide pro-people programs so


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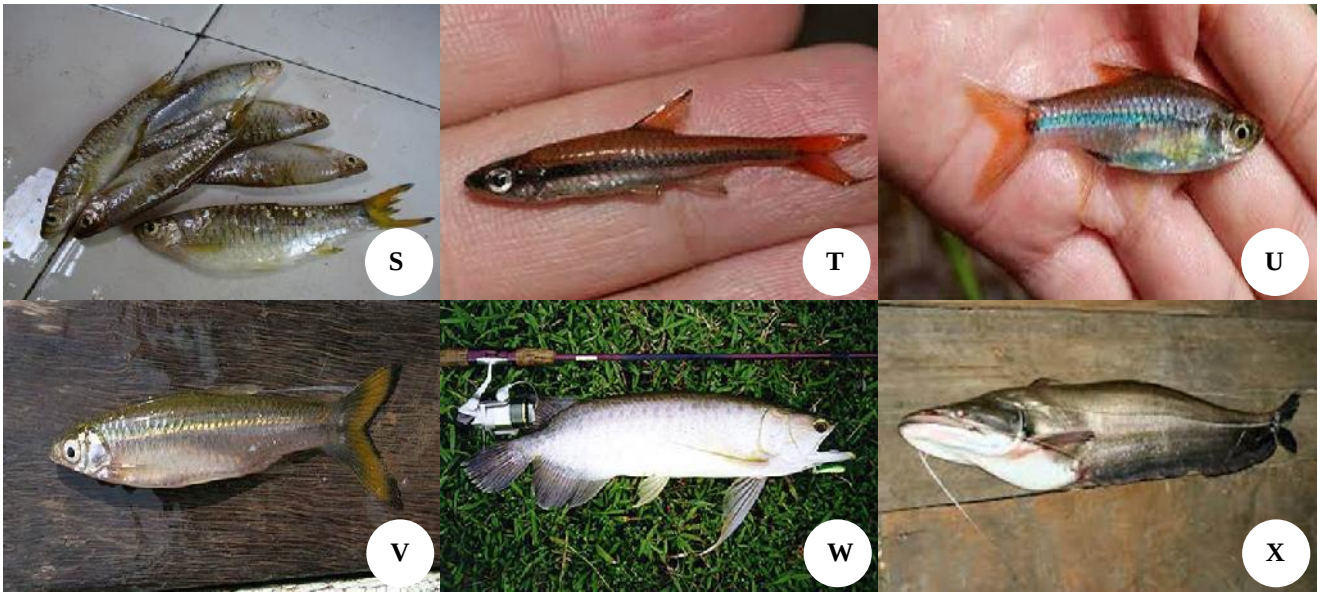


Figure 6. Diversity valuable fish are utilized by people in the core zone and buffer area at GSKBBBR, Riau Province, Indonesia. A. *Anabas testudineus*, B. *Anabas testudineus*, C. *Bagrichthys macropterus*, D. *Belontia hasselti*, E. *Belontia hasselti*, F. *Betta anabatoides*, G. *Channa bankanensis*, H. *Channa micropeltes*, I. *Clarias nieuhoffi*, J. *Clarias teijsmanni*, K. *Hemibagrus nemurus*, L. *Kryptopterus lais*, M. *Kryptopterus limpok*, N. *Kryptopterus macrocephalus*, O. *Luciocephalus pulcher*, P. *Notopterus notopterus*, Q. *Parachela oxygastroides*, R. *Pseudeutropius brachypopterus*, S. *Rasbora* spp. T. *Rasbora kalochroma*, U. *Rasbora sumatrana*, V. *Rasbora tornieri*, W. *Scleropages formosus*, X. *Wallago attu*

Table 4. Typology of local community on the utilization of bioresources in the core zone in the GSKBBBR, Riau Province, Indonesia

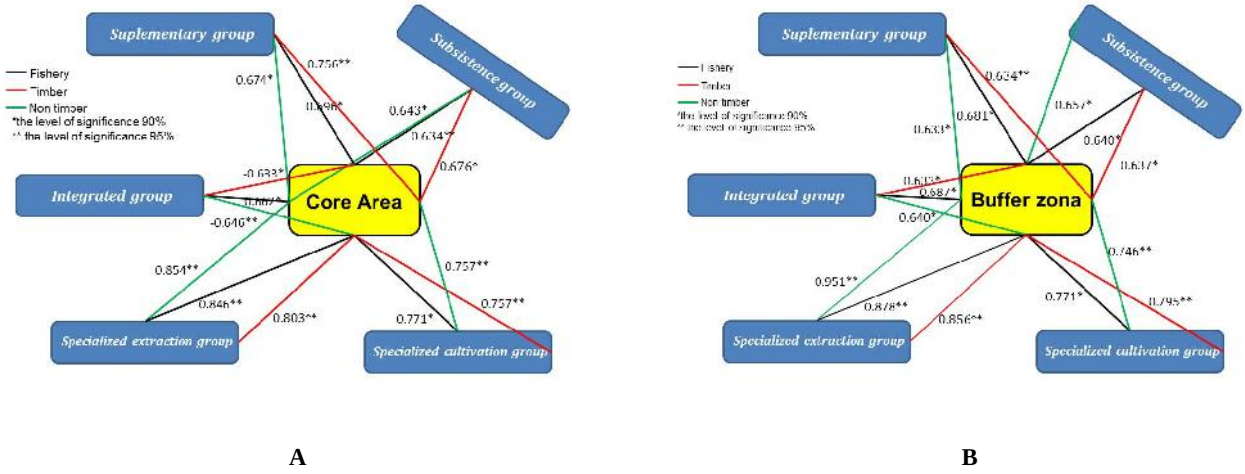
Household strategy	Subsistence group	Suplementary group	Integrated group	Specialized extraction group	Specialized cultivation group
Household (N = 140)	6%	18%	16%	28%	32%
Bioresources contribution in household (income)*	>50%	>50%	<50%	<50%	<50%
Distribution integrated or cashflow in household **	<50%	>50%	>50%	>50%	>50%
Agri culture	yes	yes	no	No	no
Plantation cultivation	yes	yes	yes	Yes	yes
Fishery culture	yes	yes	yes	Yes	yes
Land ownership	communal	communal	communal	Private	private
Product value	low	medium	medium	High	high
Markets	Local	Local	Regional	Regional	Regional

Note: * = Contributions utilization of bioresources economic value to the fulfillment of the minimum requirements of households; ** = Percentage of total income received in the form of money

Table 5. Typology of community based on the utilization of bioresources in the buffer area in the GSKBBBR, Riau Province, Indonesia

Household strategy	Subsistence group	Suplementary group	Integrated group	Specialized extraction group	Specialized cultivation group
Household (N = 140)	3%	6%	18%	29%	44%
Bioresources contribution in household (income)*	>50%	>50%	<50%	<50%	<50%
Distribution integrated or cashflow in household **	<50%	>50%	>50%	>50%	>50%
Agri culture	yes	yes	no	No	no
Plantation cultivation	yes	yes	yes	Yes	yes
Fishery culture	yes	yes	yes	Yes	yes
Land ownership	communal	communal	private	Private	private
Product value	low	medium	high	High	high
Markets	Local	Local	Regional	Regional	Regional

Note: * = Contributions utilization of bioresources economic value to the fulfillment of the minimum requirements of households; ** = Percentage of total income received in the form of money



A **B**

Figure 7. The relationship between the utilization of bioresources by local community based on Belcher et al. (2005) in the GSKBBBR, Riau Province, Indonesia

that people want to lend a hand to contribute to the conservation programs and change their perceptions of the functions and existence CBGSKBR. CIFOR (2012) and Santhanam-Martin et al. (2015) and Szabo et al. (2016) supports the view that decentralized participatory conservation programs can help and minimizing the barriers between conservation and sustainable development program, if implemented carefully.

In developing countries dependence on bioresources is very high. This is due to the economic value of high bioresources as well as a major source of livelihood (García-Llorente et al. 2016; Hill et al. 2015; Jacobs et al. 2015). Attitudes and perceptions of performed the visible benefits of the added value of bioresources (Asara et al. 2015). In this study, respondent participation is low, but most of them have a positive outlook on conservation programs and activities in CBGSKBR. Positive attitudes and perceptions are a good indicator if the initiatives taken conservation program, for example, community-based conservation approach, the greater likelihood of further enhancing the participation of local communities in conservation activities. However, the approach to conservation programs in CBGSKBR currently has limited public participation in decision making and planning.

Extraction of bioresources

During FGD, a majority of the participants raised concerns about the degradation of the GSKBBBR ecosystem from excessive resource extraction. Habitat destruction, as a result of illegal logging, and illegal hunting has reduced habitat for bigger animals. Large mammals such as *Panthera tigris* ssp. *sumatrae* Pocock, *Elephas maximus sumatranus* Temminck, and *Helarctos malayanus* Raff., has not been seen since 2012. Local and migratory bird species are also in a declining state, and this information has been verified by an analysis of resource sustainability trends. The current population pressure and increased settlements, fragmentation and thinning of the forest, and expansion of plantations and agroforestry are the major factors contributing to habitat degradation.

Bioresources and the income of the household economy

Our survey indicates that nearly 52% of households have adopted plantation and agriculture, followed by oil palm plantation workers (12%), as fisherman, small businesses, and other types of jobs. Percentage of respondent households rely on bioresources CBGSKBR both for their own consumption or for sale by 64%. Fahmi et al. (2015) found that 77% of the population living in the wetlands of South Sumatra depend on bioresources to maintain household food security and livelihoods. Najiyati et al. (2005) found that 73% of the local communities residing around the wetlands in Riau and Jambi rely on bioresources to supplement their income.Overall, we found that each local household extracted wetland resources with an annual economic value of Rp. 4.7 million ($361 USD; range: Rp 13,000).

Statistical analysis showed that the bioresources make a significant contribution to the economy of the household. When compared between groups with the extraction of bioresources do not extract, obtained significant economic benefit of Rp. 4.7 million, or $ 361 USD in the group of extractors and Rp 1.8 million, or $ 138 USD in the group not extract. This shows the economic contribution of GSKBBBR resources to the utilizing bioresources users.

Bioresource sustainability trends

In the FGD activity, respondents were asked to express their perceptions of the general trend in the utilization of bioresources CBGSKBR over a period of 15 years from 2000 to 2015 (Table 4).Three options for the indication of trends were given to the respondents: increasing, constant, and decreasing, and respondents were free to choose an option on the basis of their own understanding. The conditions of the GSKBBBR resources, including timber, non timber and fish, declined sharply during the last fifteen ten years. The majority of the utilizing bioresources and

non utilizing bioresources households were convinced of an increase in some types of fish available at the complex during the same period. In contrast, both groups stated that mammals, reptiles, amphibians, and resident and migratory birds had decreased, which corroborates the trend reported by (Partomihardjo 2007). Direct human impacts such as landslides and erosion were found to have increased, resulting in the degradation of vegetation stock and condition, confirming the information obtained during FGD. These results are consistent with the findings of Wyborn et al. (2016), who reports intense socioeconomic activities and poor management practices as the main causes of degraded wetland ecological status in the Lake Tana region of Ethiopia. Rahmawaty et al. (2014) and Bosma et al. (2012) provide a similar observation regarding the East Kalimantan, where 80% of the population perceived that the degraded condition of the wetland ecosystem was due to overexploitation. Overall, both the utilizing bioresources and non utilizing bioresources households communities had similar perceptions of each of the goods and services provided by the GSKBBBR.

Socio-economic factors affecting the extraction of bioresources

Socio-economic variables are factors that most influence the extraction of bioresources in CBGSKBR. Revenue earned from the extraction or sale of bioresources is considered as the main indicator of bioresource extraction. Revenues derived from CBGSKBR significantly affected by the number of members in the household, the length of time have made use of bioresources, age, level of education and access to other sources of livelihood. Number of family members and significant positive effect on the extraction of bioresources. More family members who could be involved to bioresources extracting it will increase the amount of revenue. The long duration of biological affect their mindset, because they are used to extract, they are not trying to find alternative sources of income.

The level of education also affects the extraction of bio-resources, the higher the educational level, the less dependent on the extraction of bioresources. This is because they have other alternatives to earn their livelihoods. Ownership plantations (rubber or palm plantations) positive and significant effect on the income derived from CBGSKBR. Own planter with plantation fairly broad ownership tends no longer extract the bioresources compared with smaller land holdings or none at all. In some cases though the respondents had a fairly extensive plantations, but they are still extracting bioresources CBGSKBR.

Local community views on the use of bioresources

Some focus group meetings and activities have been conducted to gather the views of local communities on the use of bioresources in CBGSKBR. This view is required as a long term solution that may be understood by the local community about the issues and consequences of the excessive use of bioresources in CBGSKBR.

More than 11 ideas collected from the FGD participants, from 11 to the idea, can be grouped into three main ideas. The first idea is the adoption of a model of community forestry in CBGSKBR. Along with the application of the idea of community forests, the participants believe that aspect of the legality of the law submitted to the government as the initiator and primary responsibility CBGSKBR management. If transferred into a model of community forestry, forest user groups will be formed strong, taking into account the poor and disadvantaged who have been totally dependent on the extraction of bioresources CBGSKBR. Forest user groups will be entitled to develop, preserve, use, and manage bioresources CBGSKBR. Thus, the model community forest will suppress the illegal use of bio-resources, facilitate sustainable utilization of biological resources, the application of strong management and participatory.

The second idea is to look for alternative forms of utilization of bioresources by utilizing a variety of technologies of cultivation without affecting the function of the core zone as a conservation area. FDG participants realized that the population is increasing, so is the dependence on natural resources. The lack of livelihood options for alternative means of overexploitation of biological resources. Lakes and rivers as well as the buffer and transition zones could be the basis of the provision of sustainable bio-resources to provide raw materials. The development of forest and lake resource-based non timber forest products community is possible.

The third idea is the introduction of ecotourism in the complex to diversify the economic opportunities of the local people and reduce their direct dependency on the lake resources. The indigenous Malay culture, rich biodiversity, and strategic location of the lake complex between two wildlife reserve, Giam Siak Kecil and Bukit Batu, could facilitate the publicity and attraction for tourism development. Basic tourist infrastructure would be needed, such as lodges, cafés, and walking trails inside the complex to view wildlife and natural scenery. The requirement of sufficient tourist guides would provide direct employment opportunities to local youth.

Lessons learned and study limitations

This study shows that although the local people are very dependent on the utilization of bioresources CBGSKBR, they also wanted to reduce such dependence. They expect CBGSKBR developed as ecotourism potential. However, to develop tourism, the existence of biodiversity CBGSKBR need to be maintained and preserved well, local communities may not have the expertise to do so. It provides a lesson that although the participation of local communities is the key to conservation, they also need support from outside agencies, initially to support their livelihoods through alternative sources of income.

Such support can help to create awareness among the local people and enable a smooth transition from consumptive to non-consumptive use without compromising the sustainability of the wetland resources. However, outside support for conservation of the wetlands should not interfere in local decision making for resource

conservation. The GSKBBBR resources are contributing a significant amount to the gross income of the households. We also found that there is ethnic sentiment associated with GSKBBBR. This shows a clear linkage between social and ecological systems, as advocated by Olsson et al. (2004). Therefore, its conservation is necessary through improved social transformation that helps sustainable conservation and improved livelihood. Adoption of a community-based conservation approach, along with alternative livelihood strategies, justifies the sustainability of resource use without its degradation, which has been successful in Bangladesh (Thompson et al. 2007). These lessons are valid not only for Indonesia, but are equally applicable to all developing countries that are struggling to manage and conserve biosphere resources.

Finally, some limitations of this study should be noted. This study had limited sample sizes, with a focus mainly on households that live close to the core area and buffer area GSKBBBR; therefore, our findings should be interpreted with caution. We also investigate only a small number of bioresources and excluded the valuation of tangible resources such as culture, tourism and climate mitigation. Future studies should take this area into consideration so that a holistic picture of bioresources of biosphere reserves can be obtained to formulate conservation policies and sustainable livelihoods better.

This study leads us to recommendations, the management system of the GSKBBBR could be transformed into a form of community-based conservation with options for to develop ecotourism to reduce resource dependency. Finally, social and ecological systems are linked, so more awareness programs are needed, focusing at all levels of community members in the study area, as peoples participation in conservation activities is comparatively low.

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Spatial and temporal description of water pollution status of Gajah Mungkur Reservoir Wonogiri, Central Java, Indonesia

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Abstract. *Wiryanto. 2016. Spatial and temporal description of water pollution status of Gajah Mungkur Reservoir Wonogiri, Central Java, Indonesia. Biodiversitas 17: 888-893.* Gajah Mungkur Reservoir (GMR) Wonogiri, Central Java, Indonesia was established with the purpose of: to control downstream flood, as hydropower generation, as fish farming, as a tourism destination and as an irrigation system in the dry season. As tourism destination and aquaculture, based on PPRI No. 82 2001, this reservoir is included in the category of class 2. The main purpose of this study was to determine the status of water pollution and activities of pollutant contributor to Gajah Mungkur Reservoir (GMR) Wonogiri spatially and temporally, in accordance with its purpose as the tourist destination and fish farming. Survey method study, purposive sampling method. The sampling was conducted at 8 stations (estuaries of Wuryantoro Sub-Drainage Basin (SubDAS), Alang-Solo Hulu Sub-Drainage Basin, Temon Sub-Drainage Basin, Keduang Sub-Drainage Basin, Tourism area, Fish culture area and aquaculture free zone, as well as the spillway or outlet), 4 times (in March, June, September and December) in the year 2011. The result shows that some reservoir water pollution parameters such as TSS, BOD_5, COD, Cu, Mn and Escherichia coli have been above the boundary required as class 2 water category based on PPRI No. 82 of 2001. Based on Storet calculations model, GMR Wonogiri waters are spatially distributed as heavily polluted on estuaries of Temon Sub-Drainage Basin and Keduang Sub-Drainage Basin, as moderately polluted on other 6 stations and temporally, on samplings on March 2011, GMR is heavily polluted, and the others are moderately polluted. Based on the calculation of Pollution Index spatial distribution, station 4 (Keduang estuary) is moderately polluted, and the other stations are lightly polluted, but based on the temporal distribution, the pollution status of GMR is lightly polluted. Pollutants flowing into GMR are dominated by wastes produced by households and industry activities from outside the reservoir (on the rainy season) and from KJA and tourism activities.

Keywords: Gajah Mungkur, pollution status, pollutant contributor activities

INTRODUCTION

Gajah Mungkur Reservoir (GMR) Wonogiri, Central Java, Indonesia was started to be built in 1975 and was completed in 1981. The purposes of the construction of GMR are (i) to overcome the flooding problems in the Bengawan Solo River downstream on the rainy season, (ii) to be the irrigation system in dry season, (iii) to be hydropower electric generator, (iv) to be water tourism area, and (v) to be aquaculture. The reservoir was built with an economic life estimation of 100 years, based on the estimation of actual erosion rate of 1.2 mm/year. From results of the monitoring, the erosion rate on Gajah Mungkur's catchment area is high, causing high sedimentation in the inundation area. The erosion rate is estimated at 8.58 mm/year (1982) and increasing into 26.0 mm/year (1985). With this erosion rate, it is estimated that the economic life of reservoir is only 27 years (Faculty of Geography UGM and SBRLKT Solo (1996). This requires the integrated management continuously to extend the life and function of the reservoir with the consideration of the environment.

Gajah Mungkur Wonogiri, is one of the reservoirs in Central Java, with a catchment area (DTA) which is located at 7°32' LS-8015' S and 110004 'BT-110 018' BT. Administratively, the location is mostly located in Wonogiri, Central Java and the other is in the district of Pacitan, East Java which in total consists of 21 districts and 224 villages, with total wide of 135,000 ha, 121,014 ha of land, and the remainder (13,986 ha) is in the form of puddle (Sudibyakto et al. 2005). The area includes 7 Sub-Drainage Basin (SubDAS), namely; Wuryantoro Sub-Drainage Basin, Unggahan Sub-Drainage Basin, Alang Sub-Drainage Basin, Solo Hulu Sub-Drainage Basin, Temon Sub-Drainage Basin, Wiroko Sub-Drainage Basin, and Keduang Sub-Drainage Basin.

Gajah Mungkur is potentially tainted by the influx of waste activity as results of activities of inhabitants who stays on land above the reservoir (activities of households, industries, offices, farms, ranches, restaurants, markets, hotels, etc.), which supplies waste and community activities in the waters of the reservoir (cultivation fish, floating stalls and tourism). The great quantity of waste affects water pollution, so the quality of the waters is low. That situation needs to be controlled to maintain the water quality of the reservoir.

The problems that can be formulated in this study are: (i) how is water pollution status of Gajah Mungkur Reservoir Wonogiri, and (ii) what activities which contribute greatly to the pollution of Gajah Mungkur Reservoir Wonogiri.

MATERIALS AND METHODS

Survey research, purposive sampling method. The research was conducted during 2011, with 4 times water sampling (March, June, September and December 2011) in 8 stations, namely; (i) estuary of Wuryantoro Sub-Drainage Basin (SubDAS), (ii) estuary of Alang-Solo Hulu Sub-Drainage Basin, (iii) estuary of Temon Sub-Drainage Basin, (iv) estuary of Keduang Sub-Drainage Basin, (v) KJA surrounding, (vi) the tourism zone, (vii) free zone (without KJA and tourism), and (viii) the outlet zone (spillway) (Figure 1).

Analysis of water samples (Alaerts and Santika 1987; Bapedal 1994; APHA 1995; AOAC 2005) was conducted in SubLab Chemistry and Biology, Laboratory Center of Mathematics and Natural Sciences, Universitas Sebelas Maret, Surakarta, Jawa Tengah, Indonesia, then the results were associated with the pollution parameter (Appendix of PPRI No. 82 of 2001). To determine the status of pollution, it was calculated with the method of Storet and Pollution Index (KepMNLH No. 115 of 2003). To determine the sources of pollution, an analysis of activities and pollutants that exceed the Water Quality Standards (class 2 based on PPRI No. 82 of 2001) should be carried out then they should be associated with the activities that produce or cause high pollution parameter.

RESULTS AND DISCUSSION

Environmental parameters

The result of water samples analysis of Gajah Mungkur Reservoir Wonogiri is completely presented in Table 1.

Temp. (temperature); from the calculation results, water temperature ranges from 27.7 to 32.9 ^{o}C. This temperature range is good for fish farming activities, in accordance with the statement of Effendi (2003).

Total Suspended Solids (TSS); almost the entire estuary of the Sub-Drainage Basin exceeds Water Quality Standard (WQS/BMA) (except Keduang Sub-Drainage Basin). This is because Keduang River provides water supply throughout the year so that most of the solids which are carried by the flowing water along the portion have already been precipitated in the river. In the dry season, the only river that feeds water into the reservoir is Keduang, so there is riptide on reservoir water. When the water flows back into the reservoir, it brings along the surface soil layer which is being eroded by water. The impact of these circumstances makes the TSS becomes larger than the other stations.

Total Dissolved Solids (TDS); TDS parameter at all sampling stations and sampling time was under water quality standard (BMA), namely under 1,000 mg/L.

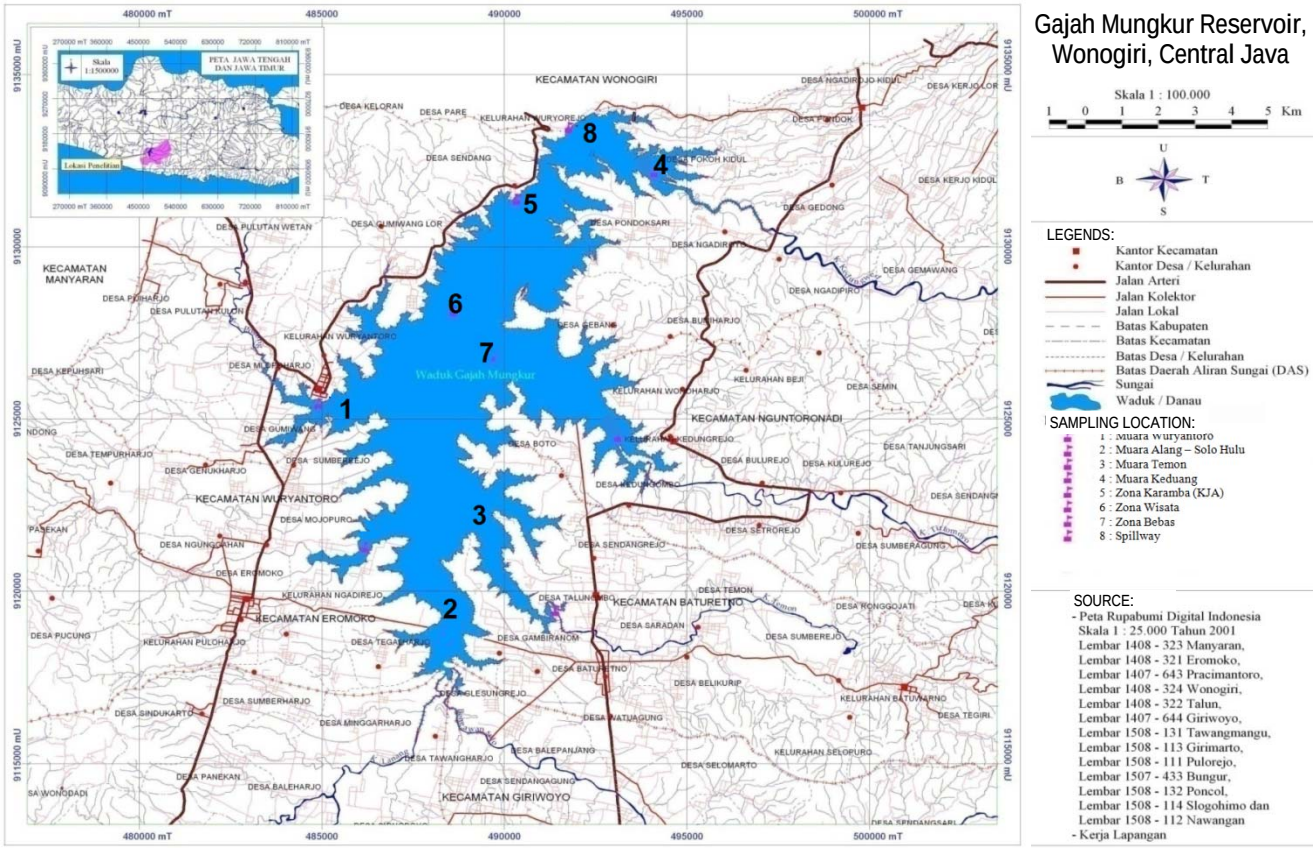

Figure 1. Location of water sampling of Gajah Mungkur Reservoir, Wonogiri, Central Java, Indonesia

Table 1. Measurement of water environment parameters of Gajah Mungkur Reservoir, Wonogiri, Central Java, Indonesia which is Associated with Water Quality Standard (BMA) class 2 PPRI No. 82 of 2001

Parameter	Unit	BMA	S1 (Wuryantoro SubDB estuary)				S2 (Alang – Solo Hulu SubDB estuary)				S3 (Temon SubDB estuary)				S4 (Keduang SubDB estuary)				S5 (Keramba)				S6 (Tourism Zone)				S7 (Free Zone)				S8 (Spillway)			
			1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Temp.	^{o}C	dev 3	32.9	31.4	30.8	30.0	32.6	31.8	27.7	31.7	32.8	30.2	29.9	31.8	32.1	30.4	27.9	30.8	33.1	29.2	28.3	30.7	32.9	30.0	28.7	32.5	32.2	33.4	27.7	30.7	31.2	28.3	31.3	31.1
TSS	mg/L	50	46.0	50.5	55.0	51.0	44	54.5	53.3	54.0	59.0	54.0	52.5	52.5	85	46.5	38.0	49.5	45	48.5	43.5	49.0	47	47.0	44.5	46.0	47	59.0	48.0	47.0	48	47.5	49.0	49.0
TDS	mg/L	1000	112	72	121	131	181	122	115	108	151.0	83.0	119.0	92.0	105	212	116	68	107	267	107	127	107	244	117	107	132	112	117	97	108	48	113	141
pH	-	6-9	8.7	8.4	9.0	8.6	8.1	7.7	9.0	8.5	8.2	8.5	8.4	9.0	8.7	8.4	8.7	8.3	8.4	7.8	8.5	7.5	8.5	8.5	8.2	8.5	8.7	8.6	8.8	8.7	8.3	8.4	8.4	8.0
BOD	mg/L	3	6.8	.9.6	6.3	8.1	6.3	10.1	8.9	7.2	6.5	10.5	9.6	13.7	5.8	10.1	6.6	11.1	7.5	7.4	6.6	10.9	7.8	9.8	6.4	9.2	7.4	11.6	5.5	9.6	9.1	9.1	6.1	8.5
COD	mg/L	25	13.1	26.6	16.8	23.9	15.2	25.2	20.8	19.5	17.9	28.8	25.1	39.1	14.8	38.5	18.5	32.7	18.3	19.9	17.7	31.6	20.6	26.7	17.0	26.2	18.7	32.2	15.6	27.5	23.3	24.9	16.5	24.1
DO	mg/L	4	7.9	5.9	7.2	7.4	7.6	5.3	7.2	7.5	6.9	6.9	7.0	7.3	7.3	6.5	7.1	7.3	7.2	4.3	7.2	7.3	7.3	6.9	7.1	7.4	7.8	7.7	7.2	7.4	7.1	6.3	7.2	7.4
Total-P	mg/L	0.2	Ttd	ttd	0.02	0.03	ttd	0.01	0.06	0.01	ttd	ttd	0.02	0.03	0.05	0.01	0.06	0.03	0.15	0.10	0.07	0.20	0.06	0.09	0.05	0.08	ttd	0.01	0.03	0.04	Ttd	0.01	0.03	0.07
NO3	mg/L	10	0.88	0.76	0.34	0.30	0.88	0.82	0.24	0.57	0.91	0.82	0.27	0.24	0.90	0.76	0.26	0.36	0.89	0.80	0.28	0.38	0.92	0.80	0.40	0.32	1.03	0.80	0.24	0.37	0.89	0.90	0.23	0.40
NO2	mg/L	0.06	Ttd	ttd	0.01	0.02	0.01	0.01	0.01	0.01	ttd	0.01	0.01	0.02	0.04	ttd	0.01	0.02	ttd	ttd	0.01	0.02	ttd	0.01	0.01	0.02	ttd	0.01	ttd	0.02	0.01	ttd	0.01	0.02
NH3	mg/L	(-)	0.11	0.33	0.24	ttd	0.06	0.14	0.03	ttd	0.19	0.33	0.03	ttd	0.48	0.01	0.01	ttd	0.21	0.16	0.09	ttd	0.18	ttd	0.08	ttd	0.20	0.01	0.01	ttd	0.14	0.20	0.01	ttd
Cd	mg/L	0.01	Ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	Ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	Ttd	ttd	ttd	ttd
Cr	mg/L	0.06	0.02	ttd	ttd	Ttd	0.02	ttd	ttd	ttd	0.02	ttd	ttd	ttd	0.03	ttd	ttd	ttd	0.02	ttd	Ttd	ttd	0.02	ttd	ttd	ttd	0.02	ttd	ttd	ttd	0.02	ttd	ttd	ttd
Cu	mg/L	0.02	0.25	ttd	ttd	ttd	ttd	ttd	ttd	ttd	0.03	ttd	ttd	ttd	0.025	ttd	ttd	ttd	0.025	ttd	Ttd	ttd	0.025	ttd	ttd	ttd	0.025	ttd	ttd	ttd	0.025	ttd	ttd	ttd
Fe	mg/L	(-)	0.12	0.05	0.17	0.18	0.33	0.23	0.62	0.33	0.42	0.19	0.76	0.20	3.59	0.66	0.61	0.81	0.09	0.28	0.23	021	0.14	0.11	0.23	0.35	0.10	0.55	0.24	0.13	0.15	0.05	0.26	0.43
Pb	mg/L	0.03	Ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	Ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	ttd	Ttd	ttd	ttd	ttd
Mn	mg/L	(-)	0.03	0.04	ttd	ttd	0.03	0.84	0.14	0.04	0.03	0.15	0.19	0.03	0.06	0.72	0.13	0.14	0.03	0.03	0.02	0.02	0.03	0.02	0.01	0.10	0.03	0.32	0.03	0.01	0.03	0.01	0.01	0.24
Zn	mg/L	0.05	0.07	0.01	0.01	ttd	0.14	0.01	0.01	ttd	0.49	0.01	0.02	0.01	1.88	0.11	0.02	0.02	0.06	ttd	0.001	Ttd	0.06	0.01	0.01	0.01	0.06	0.02	0.02	ttd	0.11	0.01	0.01	0.04
Fecal coliform	Ind/100 mL	1000	240	<3	75	9	15	11	460	9	1100	7	7	4	1100	3	3	9	11	75	15	75	15	3	4	<3	<3	<3	4	<3	<3	43	460	<3
Total coliform	Ind/100 mL	5000	1100	3	43	<3	≥2400		460	15	1100	9	4	4	150	3	3	9	1100	75	15	43	15	3	4	<3	1100	<3	<3	<3	≥2400	43	460	<3

Note: Sub DB: sub-drainage basin (sub DAS), ttd.: not detected

pH; the acidity (pH) of water in reservoirs, in general, is between 6-9 (tolerance limit of BMA). This pH range can support the life of aquatic biota (Boyd 1982). In Wuryantoro Sub-Drainage Basin, Alang-Solo Hulu Sub-Drainage Basin and Temon Sub-Drainage Basin, are at the highest value (upper limit BMA of 9) in the dry season, it is possible due to the evaporation effect that lasts for a long time, leading to an increased concentrations of water and finally, pH is increased too. The situation is still within the limits of tolerance for the activities of living aquatic organisms.

Biological Oxygen Demand (BOD); in all stations and all the sampling time, the value of BOD exceeds BMA water class 2 (> 3 mg/L). This indicates that the metabolic activity of aquatic organisms that occur in the reservoir water is high. This situation is possible because of the many organic compounds flowing into the waters of the reservoir, either from community activities in the area above the reservoir (especially in the rainy season) or from community activities in the reservoir itself (KJA, floating stalls, and Tourism), thus, it increases the metabolism of aquatic organisms.

Chemical Oxygen Demand (COD); at some stations, it is found that COD exceeds Water Quality Standard (WQS/BMA) class 2. It means that there are organic compounds which are hard to be decomposed biologically at that station, so the decomposing process should be done through a chemical one. This shows that there are organic compounds which are easy as well as hard to be decomposed biologically in the reservoir as a result of the accumulation of various human activities. At the station of the spillway, COD content is still under Water Quality Standard (WQS/BMA). This may be due to the flowing water which is always released through the spillway (outlet).

Dissolved Oxygen (DO); in all stations and at all the sampling time, DO value is above Water Quality Standard (WQS/BMA) namely 3 mg/L. This demonstrates the high photosynthetic activity of aquatic flora and aeration of the atmosphere above. With a high content of O_2, the availability of O_2 in the waters is not perilous. Although BOD and COD are high, it has no effect on the content of O_2 in the waters of GMR, so it does not disrupt the continuity of the normal life of the reservoir water biota. DO which is <2 mg/L can lead to fish mortality (UNESCO/WHO/UNEP in Effendi 2003).

Cuprum (Cu); Cuprum (Cu) were found exceeding the Water Quality Standard (WQS/BMA) class 2 at all stations on first sampling in March 2011 (except at estuary of Alang-Solo Hulu Sub-Drainage Basin), but it cannot be found at the next sampling. It is possible due to the activity in the catchment area (CA/DTA) producing Cu which is directly disposed into the river, and then is carried by the flow of the river and headed into reservoir water. The trace of the source of Cu in the catchment area (CA/DTA) is needed to be done and the efforts to prevent the entry of such materials into the river, which then flows into the reservoir water, are needed to be carried out.

Zinc (Zn), as well as Cu, is found exceeding the Water Quality Standard (WQS/BMA) class 2 on the first sampling in March 2011. Therefore, it needs necessary attention for such activities that generate waste Zn and Cu simultaneously.

Fecal coliform (*Escherichia coli*) which is derived from human excrement and exceeding Water Quality Standard (WQS/BMA) class 2 is found at station 3 (Temon estuary) and station 4 (Keduang estuary) on the first sampling (March 2011) during the rainy season. It is possible since the habits of farmers who cultivate their paddy as well as dispense their excrement in the river flow. Despite these circumstances are incidentals, sufficient attention is needed to change their behavior that can cause environmental pollution.

Pollution status

The calculation results of the water pollution status of GMR which is based on KepMNLH No. 115 of 2003 are presented in Table 2 and Table 3 and also in Figure 2.

Table 2. Status of water pollution on Gajah Mungkur Reservoir, Wonogiri, Central Java, Indonesia at various stations (with Storet model calculations and Pollution Index of MNLH Ordinance No 115 of 2003)

Station	Storet Method		Pollution Index Method	
	Score	Pollution Level	Pollution Index	Pollution Level
Wuryantoro	-20	Moderate	2.8280	Light
Solo Hulu-Alang	-20	Moderate	2.2970	Light
Temon	-36	Heavy	3.3286	Light
Keduang	-35	Heavy	5.3319	Moderate
KJA	-16	Moderate	2.2785	Light
Tourism zone	-16	Moderate	2.3333	Light
Free zone	-26	Moderate	3.4669	Light
Spillway zone	-20	Moderate	2.3181	Light
Average	-24	Moderate	3.0228	Light

Table 4. The level of water pollution of Gajah Mungkur Reservoir, Wonogiri, Central Java, Indonesia at various sampling times

Sampling time	Storet Method		Pollution Index Method	
	Score	Pollution level	Pollution Index	Pollution level
March 2011	-35	Heavy	3.0169	Light
June 2011	-24	Moderate	2.2289	Light
September 2011	-15	Moderate	2.1331	Light
December 2011	-24	Moderate	2.6007	Light
Average	-25.5	Moderate	2.4949	Light

Note:

Pollution level	Storet Method	Pollution Index Method
Meet quality standard	0	PI≤ 1.0
Light polluted	– 1s/d-10	1.0 < PI ≤ 5.0
Moderate polluted	-11 s/d-30	5.0 < PI ≤ 10
Heavy polluted	≥-31	< PI

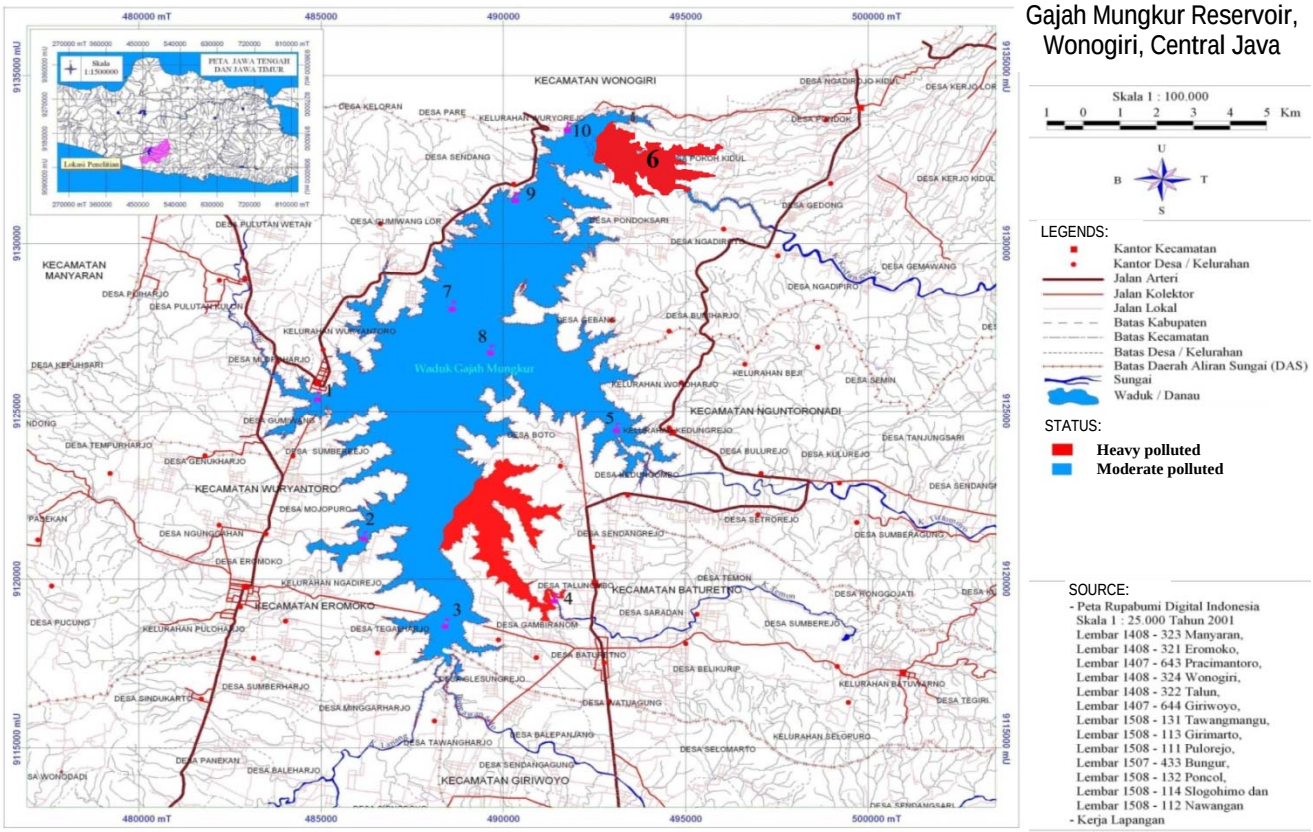

Figure 2. Status of water pollution in Gajah Mungkur Reservoir, Wonogiri, Central Java, Indonesia

Water pollution status of GMR is in the range of moderate to heavy polluted (based on the calculation of Storet model). Two observed stations have the status of heavy polluted (estuaries of Keduang River and Temon River). Major contribution to pollution is the presence of organic materials (either easy to be decomposed or hard to be decomposed), causing the requirement of O_2 to decompose them (BOD and COD become high), and it becomes worse due to the availability of metal pollutants namely Cu and Zn that come from the workshop activities in the catchment area (DTA), that finally flows into the water of GMR. Based on the calculation model of Pollution Index (IP), the pollution is in the range of light polluted to moderate polluted. This indicates that the various activities carried out by the community, both in the catchment area (DTA) and inside the reservoir, has not caused significant pollution (can be tolerated). Nevertheless, caution must be taken in order not to increase activity that can increase the formation of waste which in turn will cause an increase in the pollution status of GMR.

Based on the calculation of parameters exceeding the Water Quality Standard (WQS/BMA) in GMR on sampling of March, June, September and December 2011, there is a conclusion that the result, as shown in Table 4, shows that only sampling in March GMR was on the status of heavy polluted, while on other sampling times, it was only moderate polluted. This happened due to the entry of waste from many activities in the Catchment Area (DTA), so the pollution status WG Reservoir was increased. But with the IP model calculation, it gave a better result, namely from the calculation result of 4 sampling times, all was in light polluted status.

Differences in the calculation results for the pollution status of the same data by different methods (methods Storet and Pollution Index contained in the Decree of the Ministry of Environtment, KepMNLH No. 115 of 2003) can raise doubts against the effectivity of pollution analysis of a body of water. This situation will affect the businesses or activities that throw their waste directly into the water body by desire or choose the status analysis of water pollution by using one of the analysis methods which is more profitable for them.

Differences in status of water pollution will affect the biodiversity in it, both the physical and genetic characteristics. For organisms that can withstand environmental changes, they will be able to continue to grow and continue to do their life activity, while those who are not able to withstand can do a migration or become extinct. Although this polluting activity has happened for a very long time, but from now on, the preservation of biota in these areas needs to be done. Moreover, by introducing a new species into the water of GMR, this will have an impact on the elimination of endemic species, which in turn can lead to the loss of this population.

Activities contributing pollutant waste

Total Suspended Solids, almost all stations of Sub-Drainage Basin estuaries exceed Water Quality Standard (WQS/BMA), which means that the tidal waves on reservoir bring the solids which are eroded when the water flow back into the reservoir. BOD is high at all stations and at all time of the study, indicating a large number of organic waste that goes into the waters of the reservoir so that the metabolic activity of aquatic organisms is increased, which in turn the content of BOD will be increased too. The high number of COD at some stations is due to the large number of organic materials which are hard to be decomposed by aquatic organisms, so the decomposing process is done through a chemical reaction. The emergence of Cu and Zn in most research stations is happened at the first sampling (March 2011). This is probably due to industrial activities on land at the upper course which produces Cu and Zn waste and disposes them directly into the river, which in turn they will flow into the water of GMR.

The presence of Escherichia coli exceeding Water Quality Standard (WQS/BMA) class 2 (PPRI No. 82 of 2001) was possibly due to the activities of farmers that throwing their excrement to the water of river at the time they worked on their lands. But this situation is no longer happened now, as the dry season comes, agricultural activities are decreased causing land processing activities are also reduced; often the land is left fallow.

Tourism activities and aquaculture (KJA) also gave significant contribution especially the high number of BOD. Based on the results of the calculation of DO value that is high, the amount of BOD and COD does not preclude the availability of oxygen dissolved in the water, so it will not interfere the life of aquatic biota.

From the description above, the conclusion is as follows: Pollution of the waters of GMR is the pollution with the status of light-moderate-heavy. Activities that potentially give great contribution in water pollution of GMR are households and activities outside GMR. To manage this reservoir as an ecosystem cannot be done partially, the reservoir has to be considered as part of the Drainage Basin (DAS), Drainage Basin (DAS) approach in an integrated manner in the management of the reservoir covers aspects of Catchment Area (DTA) conservation and control of dilapidating force of water in the reservoir. It still needs deeper study of the quality and quantity of water GMR, to determine a management model which is appropriate, sustainable and environmentally friendly.

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Morphological diversity and the cultivation practice of *Abelmoschus manihot* in West Papua, Indonesia

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Abstract. *Prabawardani S, Djuuna IAF, Asyerem F, Yaku A, Lyons G. 2016. Morphological diversity and the cultivation practice of* Abelmoschus manihot *in West Papua, Indonesia. Biodiversitas 17: 894-999.* Papua is considered to be the second diversity centre of this plant; however, its diversity is declining, due to habitat destruction for regional development or land fragmentation, and hence Aibika preservation is a priority. This study aimed to assess the status of Aibika (*Abelmoschus manihot* L. Medik) diversity by collecting, preserving, conducting Aibika morphological characterization and preliminary assessment of its cultivation technique. Diverse germplasm can then be used to improve Aibika. The study was conducted between April and June 2015 in Mandopi, Warmare, Prafi of Manokwari Regency and Minyambouw of Arfak Mountain Regency. Descriptive method was used in this study, and the relationships among cultivars were analyzed according to Cluster Analysis using Excel Stat. Phenotypes, comprising 29 morphological characters, were recorded for cluster analysis. There were 39 Aibika cultivars collected from 4 locations of West Papua. Based on the UPGMA dendrogram, it was revealed that two primary clusters (A and B) separating separate the cultivars. Cluster A clearly separated from Cluster B at a dissimilarity value of about 0.57 (57%). Around 3.3 (33%) of variance separated the cultivars into four groups, consisting of cluster A, B1, B2, B3. Cluster A (MAD-01, Man-9, Man-11, Minyam-03, Imbenti-02, SP-02) is the most diverse area which consisted of cultivars of three different clusters. In Papua, Aibika is cultivated in a traditional mixed-cropping system, without appropriate planting distance, fertilizer, and pesticide application. This has resulted in suboptimal growth and high susceptibility to pests.

Keywords: *Abelmoschus manihot*, aibika, diversity, gedi, leafy vegetable, morphology

INTRODUCTION

Papua is known to have enormous crop diversity; however, there has been a lack of scientific study in this area. Among the biodiversity that has received little attention is Aibika or Gedi in the Indonesian language. Aibika is a tropical perennial shrub and belongs to the family Malvaceae. This plant originated in China, then spread through India, Papua, South Pacific Islands, and northern Australia (Zeven and Zhudwosky 1980). The greatest diversity is found in Papua New Guinea, the Solomon Islands and Vanuatu (Kambuou et al. 2003). The plants show great variability in leaf shape and size, petiole and stem colour, branching, and flowering characteristics. Therefore, the island of Papua is estimated to have a wide variety of this plant. Most Papuan food gardens are planted with some Aibika cultivars, which can be distinguished by leaf shape and petiole colour differences.

Aibika is among the popular leafy vegetables consumed by Papuan people and communities in other eastern parts of Indonesia. The edible part of Aibika is the young shoot tip or succulent young leaves. It is cultivated extensively and throughout the year in some Melanesian countries for its highly nutritious leaves and shoots tips (Kambuou et al. 2003). Aibika is reported to contain high nutrients; especially protein and micronutrients (Westwood and Kesavan 1982; Yalambing et al. 2015). Nutritionist (Susan Parkinson) in her personal communication with Westwood and Kesavan (1982) suggested that every household in the South Pacific region cultivate this plant. This plant is also used as a traditional medicine by indigenous people throughout the island of Papua and the Pacific Islands region, for relieving kidney pain, reducing high cholesterol, treating pregnant women to ease childbirth, stimulating lactation, treating diarrhea, and protecting against osteoporosis. Because of the secondary metabolites present in the plant, particularly antioxidants, it is efficacious to contribute contributing to the prevention of some diseases (Hodgson et al. 2006; Puel et al. 2005; Goebel et al. 2010).

In some parts of India, Aibika is used as a source of traditional medicine for kidney pain, heartburn, high cholesterol, osteoporosis, as well as to induce labour in pregnant women (Todarwal, et al. 2011). The flowers of *A. manihot* have been used as a traditional Chinese medicine for the treatment of chronic renal disease and diabetic nephropathy (An et al. 2011). Aibika has drawn much attention recently due to its potential beneficial health effects. Studies on Aibika have led to the isolation of two main kinds of plant secondary metabolites, flavonoids, and alkaloids. Aibika contains quercetin-3-O-robinoside, hyperin, Isoquercetin, gossipetin-8-O-glucuronide, and myricetin (Liu et al. 2006). The flowers contain quercetin-3-robinoside, quercetin-3'-glycosides, hyperin, myrecetin, anthocyanins, and hyperoside. Leaves of Aibika when

tested, have been shown to prevent ovariectomy-induced femoral ostopenia (condition of bone mineral density lower than normal range in the joints limbs as a result of surgical removal of the uterus/ ovaries) (Lin-lin et al. 2007; Jain et al. 2009). Aibika can also improve the function of glomerular filtration, reduced proteinuria, hyperplasia messangium which can reduce the damage of kidney tissue (Shao-Yuetal., 2006). Flavonoids in Aibika have many important functions for health, including reducing the risk of cardiovascular disease, hypertension, atherosclerosis, and as an antioxidant (Hodgson et al., 2006), and it is an important cash crop in local markets in Melanesia (Preston 1998).

According to Westwood and Kesavan (1982), Aibika is a highly productive plant vegetable which is highly adapted to the lowlands up to an altitude of 800 m height above sea level. It is also cultivated in higher altitude areas above 2000 m with annual rainfall of more than 2000 mm (Paofa and Kambuou, 2006). Aibika grows throughout the year and provides a continuous supply of highly nutritious leaves and shoot tips. Karafir and Vokames (2003) reported that there are 7 cultivars of Aibika in Nimboran district and 6 cultivars in Kemtuk district of Papua. This diversity can be seen from the shape and size of the leaf, petiole and stem colour, branching and flower characters. However, the diversity of Aibika is reported to be decline in recent years, probably due to rapid development, land fragmentation and global climate change (Kayadu 2013). Comprehensive studies on Aibika need to be done, as research on this plant is still limited.

The objective of the study was to observe the morphological diversity of Aibika through exploration, identification and collection. This study is expected to enrich the understanding of the diversity of Aibika and prevent the loss of the genetic base of this plant. The research also aimed to assess the cultivation techniques applied on Aibika by the local farmers.

MATERIALS AND METHODS

Exploration of Aibika was carried out from May to June 2015 in the lowland areas of Mandopi, Warmare, Prafi and the highland areas of Arfak (Minyambouw), West Papua, Indonesia (Figure 1) as supply of Aibika in the markets is from these areas. Exploration in each of these areas of known distribution was done to collect all cultivars of Aibika.

The descriptive method with a direct observation technique was applied in this research. Additionally, interviews with residents and local tribe leaders were conducted to determine the cultivars of Aibika, local names and cultivation of Aibika. Traditional Aibika cultivation methods (including land preparation, planting, and maintenance) were recorded.

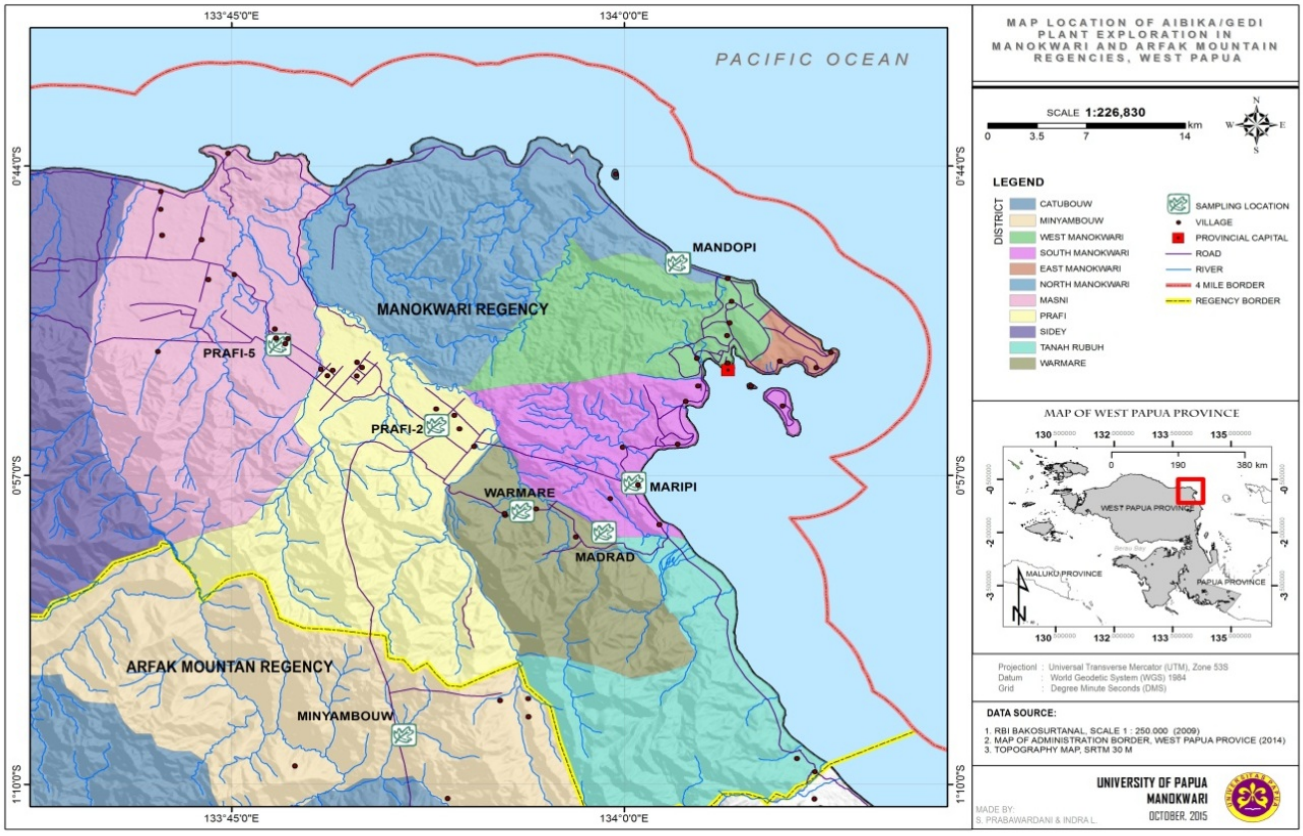

Figure 1. Map of exploration sites of Aibika in Mandopi, Warmare, Prafi (lowland areas of Manokwari) and Minyambouw (Highland area of Arfak), West Papua, Indonesia

Characterization was performed in the field at each location. The identification of Aibika was based on morphological characters, using descriptor list by Kambuou et al. (2003). The morphological characters of Aibika consisted of stem (pith, hairiness, internode length, diameter, primary stem colour, secondary stem colour, stem pigmentation, branch number); leaf (leaf shape, leaf segment shape, margin, leaf tip, leaf base, leaf shape variability, leaf lustre, leaf vein colour, petiole colour, petiole length. However, no flowers were observed, due to frequent pruning. Morphological characters were taken from Aibika plant samples which aged from 6 to 9 months (plants were still in productive age).

The collected cultivars were taken from farmer's gardens for the purpose of *ex-situ* collection or preservation in the experimental field of the Agriculture Faculty, the University of Papua, Manokwari. Data on the morphological diversity were analyzed using Unweighted Pair Group Method with Arithmetic UPGMA method using Excel stat program.

RESULTS AND DISCUSSION

Morphological diversity of Aibika

During the exploration, 36 Aibika cultivars were collected from 4 locations. The location I was Mandopi where 16 cultivars were found, denoted Man-01 - 16. Location II was Warmare, where 9 Aibika cultivars were collected, namely MARI-01 - 05, MAD-01 - 04. Location III was the transmigration area of Prafi, where five cultivars were found, namely SP1-01 - 03, SP5-01 - 02. Location IV was the highland area of Arfak (Minyambouw), where six cultivars were collected; Imbenti-01, Imbenti-02, Minyambouw-01, Minyambouw-02, Minyambouw-03, Ungga-01.

Based on the identification at 4 locations, the diversity of the morphological characters of Aibika was not only revealed to the individual among different locations (among population), but it was also observed in individuals at the same locations (within the population).

The magnitude of morphological characters within the population and between Aibika populations was perceived based on similarity or dissimilarity levels of morphological characters using cluster analysis with Unweighted Pair Group Method with Arithmetic (UPGMA). Based on this method, individuals, or population that have similar morphological characters were collectively or closely clustered. The cluster pattern of individuals or population is based on the similarity matrix, which is described by dendrogram with the character's dissimilarity distance lay between 0.00 (0%) and 1.00 (100%).

The UPGMA dendrogram resulting from the fusion matrix based on dissimilarity model revealed two primary clusters (A and B) separating the cultivars (Figure 1). It is evident that Cluster A (Mad-01, Man-09, Man-11—Minyam-03, Imbenti-02, SP1-020 clearly separated from Cluster B at a dissimilarity value of about 0.57 (57%). Around 3.3 (33%) of variance separated the cultivars into four groups, consisting of cluster A, B1, B2, B3. However, if 0.2 (20%) dissimilarity was used to distinguish the cultivars, eight groups were recognized. The groups were A1, A2, A3, B1a, B1b, B2, B3a and B3b. The members of Cluster A were the most widely distributed cultivars which can be found in all locations including I, II, III, and IV. The cultivars of cluster B3 were spread out in three locations (II, III, IV). Meanwhile, the cultivars of cluster B1 only grow in location I. It also can be noted that location IV is the most diverse area which consisted of cultivars of three different clusters which were Imbenti-02, Minyambouw-03 (cluster A), Minyambouw-01, Minyambou-02 and Ungga-01 (cluster B2), and Imbenti-01 (cluster B3). The diversity of Aibika occured as a consequence of differences in morphological characters of plant organs.

Based on the identification, the most prominent diversity of morphological characters among locations or population within a location were leaf shape characters and length, plant height, stem colour and diameter, internode length, petiole length, and colour. Each cluster generated sub-clusters of population based on the same growth location, except cluster A. It shows that individuals from the same location or close location were clustered in proximity or a close distance. This clustering pattern suggests a closer genetic relation among the population of similar areas compared to different locations. It means that each location has unique plant characters, and therefore three populations of Aibika are indigenous in each original location. This phenomenon supports the theory that the closer the geographical areas between two individuals or population, the shorter the genetic distance between those individuals and population. However, cluster A consisted of cultivars from diverse areas, probably was due to the migration of people who brought the genetic material of Aibika from one location to another, resulting in diverse cultivars in cluster A.

The different characters of Aibika among locations were due most likely to the ecological and geographical isolation (ecogeographic). Ecogeographic isolation is induced by the external factors such us climate, water, soil and topography. These factors function as a catalyst in inducing a barrier for gene exchange among populations, and hence each population in a particular ecosystem provides unique characters in each region.

Individuals or plant populations that are separated because of ecological isolation have specific habitats and specific environments. From this point of view, succeeding populations will not be adaptive if grown in a different habitat from those of the parents. They will only grow in a similar parent habitat or in between habitats of both parent populations (Grant 1971).

Different characters among individuals in one species, apart from environmental factors or geographical isolation, are also induced by migration, mutation, and hybridization. The migration of individuals or plant populations from one continent to another, or from one location to another, and followed by geographical isolation and hybridization, may

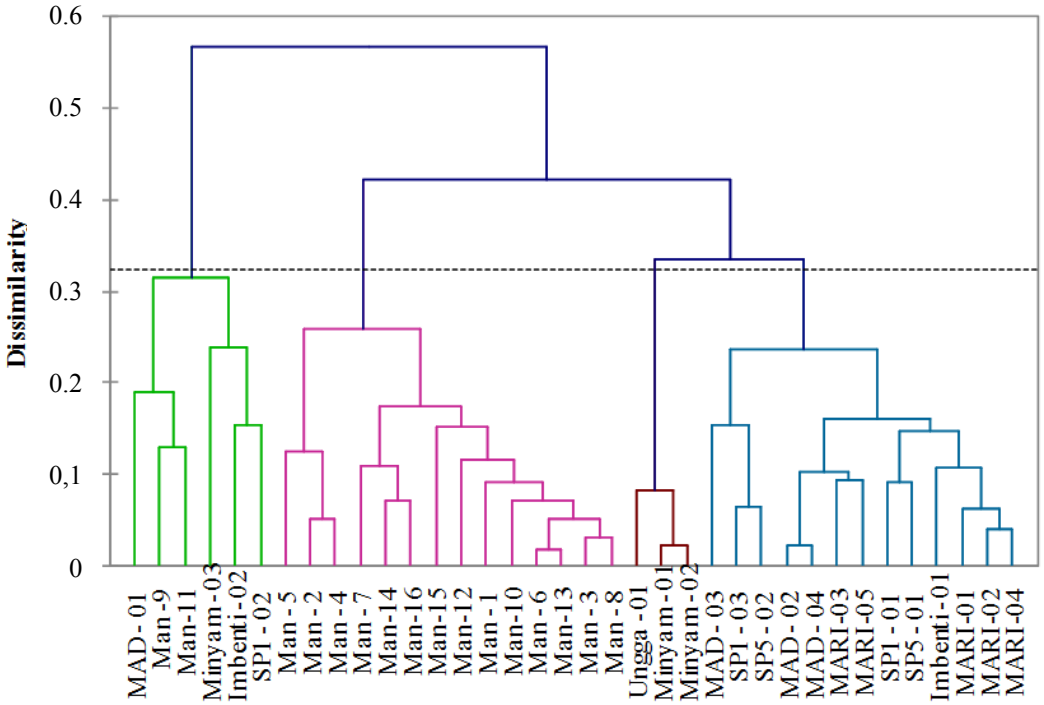

Figure 2. Dendrogram showing the dissimilarity relationship of Aibika cultivars collected from 4 different locations in Mandopi, Warmare, Prafi (lowland areas of Manokwari) and Minyambouw (the highland area of Arfak), West Papua, Indonesia

result in gene flow. Gene flow among plant populations increases the consequences of evolution and also may increase the character diversity. These create new gene combinations, raising the adaptability in location from one to other population (Nagi 1997).

Traditional cultivation of Aibika

In general, Aibika is traditionally cultivated and has not been grown in accordance with common agronomical practice. The cultivation system observed during the study consist of land clearing, preparation of planting material, planting method, maintenance, and harvesting.

Land clearing is usually carried out when farmers make a new garden, as they practise shifting cultivation. This activity is done by cooperation of the family members. The gardens which are located 2-3 km from their home generally belong to the family members. The field crop area is divided among members of the nuclear family or blood relatives. For field crop areas, farmers first clear grass or shrubs, then large trees are burned, which takes about 5 days for large gardens or 2-3 days for smaller gardens. The dry season is a good time to dry the remaining plants, as they will dry and decompose more quickly. Farmers believe that the ash remaining after burning increases the soil nutrient availability. When a new garden is opened, the previous land is rested (followed) after being used 3 to 4 times. Minimum tillage is practiced by Arfak people in their field crop area, where after completing burning and cleaning trees, farmers directly plant Aibika by using wooden drills. Equipment such as axes and machetes are used to cut wood and make fences. A crowbar is used to move large rocks and remnants of the roots of trees that remain in the field.

Preparation of planting material

Aibika is generally propagated using stem cuttings. Some farmers also cut old or unproductive Aibika and leave the primary stem in the field to regrow a new shoot. According to farmers, all parts of the trunk or branches can be used as planting materials, but it is best to use a stem or branch that is not too young and not too old. If the cuttings are taken from the old trunk or branch, the plant will grow slowly. The length of cuttings used by farmers is 30-40 cm (with 4-6 nodes). Cuttings are usually taken from the top and middle portions of healthy, mature stems. Stems are planted directly in the field. Between 2 and 3 nodes are buried, depending on the length of the cutting. The number of cutting per hole is around 3-4 cuttings. Irrigation depends on rainfall.

Planting

Aibika is cultivated in subsistence and semi-subsistence gardens. When planting time coincides with the beginning of the rainy season, Aibika growth will be faster. However, waterlogged soils will slow Aibika growth. Cultivation system practised by the local farmers in all studied locations is mixed-cropping. Aibika is commonly intercropped with some other food crops such as root crops,

Figure 3. A. Aibika cultivars with deep lobe leaf was marketed as a leafy vegetable; B. Less marketed cultivar due to highly sap content

banana, and various vegetable crops. With a cropping pattern like this, plant spacing is not applied on a regular basis, but a few farmers use spacing of 100 × 100 cm for Aibika. The tool used to make holes for planting is made from a wood stick with a length of about one metre. No fertilizer is used for improving the growth of Aibika.

Maintenance

Aibika plants are not intensively maintained after cuttings are planted. Plant maintenance is generally done earlier when the stem is newly planted. Cuttings will begin to produce shoots about two weeks after planting. Once the plants grow, farmers rarely maintain them, except for clearing grass at uncertain time intervals. Pruning is done to produce more young leaves and with an intention that Aibika plants do not grow too high to facilitate harvesting. Pruned leaves are taken for home consumption. Farmers usually only clean weeds around Aibika plants. Aibika pests that attack crops are mostly grasshoppers, caterpillars, and aphids. Grasshoppers damage the surface of the leaves while the caterpillars cause shoots to curl. The level of damage caused by pests is very high under shaded conditions and reduce the quality of Aibika leaves. Most farmers do not regularly clean up the Aibika garden to prevent pest infestation.

Harvest

Aibika plants have the advantage of being able to be grown throughout the year, and hence, it is classified as an annual plant. As stated by Goebel et al (2010), this plant can be grown all year in most tropical locations, but growth often slows with cooler, shorter days and drier conditions. Based on information from respondents, Aibika is usually first harvested at three months after planting in the lowland areas, and about six months after planting in the Arfak highland. Aibika grew in the highland generally have shorter stems, narrower leaves, shorter internodes and petiole lengths than those found in lowland locations. Aibika is generally harvested by picking young shoots and directly processing (cooked or sold in the local market). Storage for too long will cause Aibika leaves to wither and suffer damage. The productivity of Aibika plants usually declines after two years.

In conclusion, of the 39 cultivars collected from 4 areas, 16 cultivars were present in the northern part of Manokwari, West Papua. Aibika is also grown in the highland areas of Arfak as shown by the six cultivars collected from this area. Aibika traditionally cultivated in most gardens of local Papuan farmers. There is no agronomic inputs and maintenance in cultivating this plant, resulting in suboptimal growth and high susceptibility to pests. Further research is needed to develop this plant, particularly in cultivation techniques and nutritional aspects.

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The ethnobotany of medicinal plants in supporting the family health in Turgo, Yogyakarta, Indonesia

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Abstract. *Nahdi MS, Martiwi INA, Arsyah DC. 2016. The ethnobotany of medicinal plants in supporting the family health in Turgo, Yogyakarta, Indonesia. Biodiversitas 17: 900-906.* The knowledge of healing using medicinal plants among the people of Turgo Hamlet, Purwobinangun, Sleman, Yogyakarta, Indonesia has been inherited from generation to generation. This knowledge must be studied and preserved. This study was conducted from January to June 2014 with an objective of studying the ethnobotany of medicinal plants in Turgo Hamlet community, including the local knowledge of medicinal plants to support the family health, the parts of plants used as medicines and the processing of medicinal plants. Qualitative and quantitative methods were used to collect data, using in-depth interview with 40 respondents selected purposively. The results showed that the people of Turgo Hamlet used 69 plant species from 36 families as medicinal plants. The most used part of plant was leaf (51%), followed by fruit (15%), rhizome (11%), stem (5%), root (4%), sap (3%), flower (3%), all parts (3%), tubers (3%), and endosperm (2%). The medicinal plants were processed or directly used as medicines. Most of the medicinal plants were boiled (62%); others were smeared on skin (15%), directly consumed (12%), cooked (4%), used for bathing (3%), burned (3%), and crushed using a kitchen blender (1%). The medicinal plants were used for external (33%) and internal (67%) diseases.

Keywords: Generation, heritage, in-depth interview, plant organs, purposive sampling

INTRODUCTION

It is important to study ethnobotany because it is related to sustainable rural development in a region. Ethnobotany can also be used to know the dynamics of traditional ecological knowledge as an effort for biodiversity conservation in the future (Pieroni et al. 2014). Conservation of specific mountain ecosystem requires a multidisciplinary approach, so that the utilization of the ecosystem can be done on a sustainable basis in order to preserve the ecosystem service as a life supporting system (Idolo et al. 2010; Kandari et al. 2012; Khan et al. 2013).

Indonesia has abundant natural resources, including many species of plants, more than 2,039 of which have medicinal effect (Zuhud 2009). Each community has their own knowledge of the use of plants, not only for economic and cultural purposes but also for medicinal use (Kandari et al. 2012; Matthew et al. 2013). The current medicines can be divided into two categories, namely modern and traditional medicines (Muhammad 2000). Modern medicines are produced by pharmaceutical industries using sterile and reactive chemicals. On the other hand, traditional medicines are usually processed using a simple technology, based on recipes inherited from generation to generation, following local traditions and belief. Some are based on magical power, while others are based on traditional knowledge. Although they have slow reaction, traditional medicines have some benefits: they are cheaper, easy to get, easy to digest and do not have side effects (Bodeker 2000; Martin 2004). The use of natural substances as medicines and other products has been increasing. The substances have been used by lower-and middle-class families especially for prevention and curation of diseases, and rehabilitation and promotion of health. The researches on medicinal plants are increasing along with the increasing awareness of people of healthy life, and the demand on food has shifted. People not only care about the taste but also the effect of food on health (Setyowati 2010).

Turgo Hamlet is located in the slope of Mount Merapi with a total area of 200 hectares. It has an ecosystem influenced by the people's wide knowledge of medicinal plants. So, many species of medicinal plants are found in the hamlet and the people use the organs of the plants or the whole plants to maintain the family health. Their livelihood as farmers is in line with their hobby to preserve medicinal plants and to consume traditional medicinal plants for curation of diseases and maintenance of family health. Currently, there is a shift of their opinion regarding medicines. They know modern medicines and some of them abandon traditional medicines and prefer to use modern medicines which can react quickly. However, some of them, especially the native residents and farmers, still care about traditional medicines and preserve traditional healing.

Based on the above background, it is necessary to develop the local wisdom of Turgo Hamlet community in using belief and knowledge of medicinal plants as traditional healing heritage to maintain family health. There are other reasons why the medicinal plants in Turgo Hamlet needs to be documented: the potential of medicinal

plants is high; some residents still have knowledge of medicinal plants; there is land ecologically suitable for cultivation of medicinal plants which will help biodiversity conservation. Based on the above reasons, it is important to do research on the ethnobotany of medicinal plants in Turgo Purbowinangun Village, Yogyakarta, Indonesia, in order to know the local knowledge of medicinal plants to support family health, the parts of plants used as medicines and the processing of medicinal plants.

MATERIALS AND METHODS

This study was conducted in Turgo Hamlet, Purwobinangun, Pakem, Sleman, Yogyakarta, Indonesia from January to June 2014. This is the highest hamlet found in this rural area, located only 7 km from Mount Merapi. Geographically, it is located in 07°35.668 S and 110°25.118 E, with an altitude of 900-1000 m above sea level (Figure 1). The tools used in this study were: recorder, digital camera, note books, pens, scissors, plastic bags and questionnaire. The materials used were all medicinal plants found in the study site. The plants were identified using Steenis (1972) and Backer (1973).

A combination of qualitative and quantitative methods were used to collect information of ethnobotany of medicinal plants to support family health in order to know local knowledge of medicinal plants, the parts of plants used as medicines and the processing of medicinal plants. Ethnobotanical data were gathered through in-depth interview with respondents using open questionnaire. Respondents were selected using purposive sampling, based on certain criteria.

The number of respondents was 40. They were residents of Turgo Hamlet, consisting of native residents who were still concerned with medicinal plants, represented by 2 traditional midwife (5%), 7 old people (17.5%) and local community represented by 10 members of farmer group (25%), native residents represented by 16 medicinal plant farmers (40%). Based on their education, 40% of respondents graduated from elementary school, 30% from junior high school, and 30% from senior high school. Based on gender, 65% of respondents were female and 35% were male. Most respondents were farmers (85%), and the rest were civil servants and traders (15%) (Figure 2).

Information of medicinal plants were obtained from the community. Then the plants were collected from home gardens and forest around the hamlet. Every plant was identified using local name and scientific name. Unidentified plants were photographed and made into herbarium for further identification by botanist in the Laboratory of Botany and Ecology, Faculty of Science and Technology, University Islam Negeri Sunan Kalijaga Yogyakarta, Indonesia. The results of plant inventory were analyzed descriptively and quantitatively using tables.

RESULTS AND DISCUSSION

The results showed that the people of Turgo Hamlet have used 69 plant species, from 36 families as medicines, consisting of 14 species of trees (20%), 22 of shrubs (31%), 4 of lianas (5%), 26 of herbs (37%), and 3 of grasses (4%) (Figure 3). Family Zingiberaceae (10.14%) was the most used plants to cure various diseases and to maintain health and skin beauty (Tables 1 and 2).

Figure 1. Location of study on the south slope of Mount Merapi, i.e.: Turgo Hamlet, Purwobinangun Village, District of Sleman, Yogyakarta, Indonesia

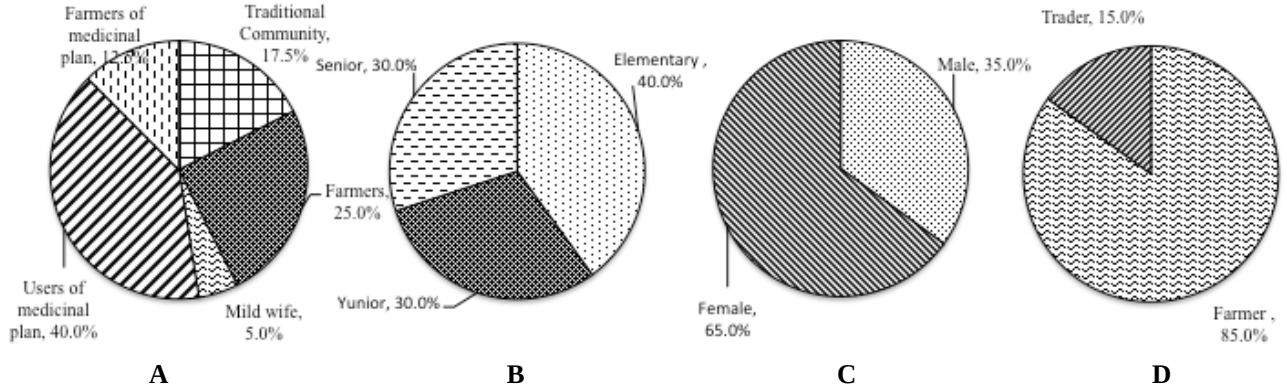

Figure 2. Qualification of respondents based on: A. Representation of community, consisting of native residents, traditional community and local community, B. Education level, C. Gender, and D. Occupation

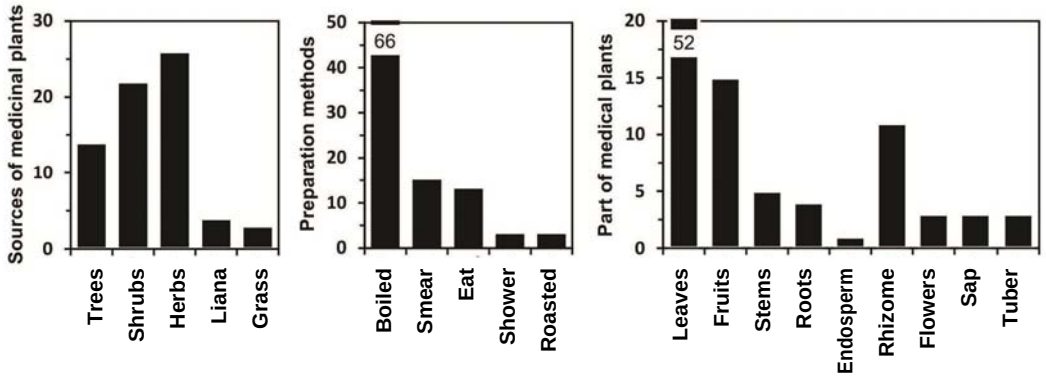

Figure 3. Medicinal plants in Turgo Hamlet based on: A. growth form, B. Processing, and C. parts of plants used

Species from Zingiberaceae, such as Dringo (*Acorus calamus* L.) was used to maintain body's immunity, tumeric kunir or kunyit (*Curcuma domestica* Val.) to cure liver disease, rheumatic, thypoid and diarrhea. Laos or lengkuas (*Alpinia galanga* (L.) Sw) to cure skin disease, temu giring (*Curcuma heynena* Vahl.) to lighten the skin for bride, Temuireng (*Curcuma aeruginosa* Roxb.) to increase appetite and to be used as vermicide. In addition, there were other species used for a variety of purposes, such as purple ginger or bengle (*Zingiber purpureum* Roxb.), jahe or ginger (*Zingiber officinale* Rosc.), and temulawak (*Curcuma xanthorrhiza* Roxb) (Table 1).

Family Asteraceae (8.7%) was the second most used for medicines for various diseases (Table 2), consisting of *bawukan* (*Ageratum conyzoides* L.), marsh fleabane or *beluntas* (*Pluchea indica* L.), *dewa* (*Gynura pseudochina* L.), *ireng-ireng* (*Eupatorium riparium* Reg.), *legetan* (*Synedrella nodiflora* L.), and *tempuyung* (*Sonchus arvensis* L.) (Table 1). The plants of this family were commonly found because they have made adaptation to tropical environment, and they have wide distribution and healing effect, so they are needed as solution to deal with the high price of modern medicines and the negative impacts of chemicals in modern medicines (Tjitrosoepomo 2010). Euphorbiaceae ranked third as medicinal plants (7.2%), consisting of *katuk* (*Sauropus androgynus* L.), *patikan cina* (*Euphorbia prostrata* Aiton), *patikan kerbau* (*Euphorbia hirta* L.), cassava or *singkong* (*Manihot utilissima* Crantz), and *yodium* (*Jatropha multifida* L.) (Tables 1 and 2). The plants of this family are used as medicines because they are commonly found in cultivation and in the wild.

Leaf is the most used part of plant for medicines, taken from 37 species (51.39%) of plants (Figure 3), because it is the easiest part to get, easy too process, having healing effect, and its removal is not destructive to plants (Setyowati 2010). Leaf has high moisture content (70-80%), a place of photosynthesis, containing organic elements having medicinal effects and anti oxidants. Most green plants, such as avocado or alpukat (*Persea americana* Mill.), Indian pluchea or beluntas (*Pluchea indica* L.), mignonette vine or binahong (*Anredera cordifolia* (Ten.) Steenis), common comfrey or komprei (Symphytum officinale L.), dadap serep (*Erythrina lithosperma* (Hassk.) Merr), and daun dewa (*Gynura pseudochina* L) have leaves rich in carbohydrate, fiber, vitamin, and mineral (Table 1). Leaf was also the most used part of plant in traditional community of Gunung Simpang Nature Reserve, West Java, which was 31% of 74 species of medicinal plants, and also in the communities of Bulgaria and Gemeda, North Ethiopia, which was 50% of all total plants (Mesfin et al. 2013; Nedelcheva et al. 2013; Handayani 2015).

Table 1. Medicinal plants in Turgo Hamlet: local name, scientific name, family, processing and health benefit/ health problems solved by each species

Family	Scientific name	Local name	Parts of plant	Processing	Health benefit or health problems solved
Annonaceae	*Annona muricata* L.	Sirsat	Leaf	Boiled	Uric acid, high blood pressure
	Stelechocarpus burahol Blume	Kepel	Leaf	Boiled	Uric acid
	Centella asiatica (L.) Urban	Pegagan	Leaf	Boiled	Blood circulation
Apiaceae	*Eryngium foetidum* L.	Musi Arab	Leaf	Smear	Vermicide
	Foeniculum vulgare Mill.	Adas	Leaf	Boiled	Body's immune system
Araliaceae	*Panax ginseng* L.	Gingseng Jowo	Rhizome	Boiled	Body's immune system
	Acorus calamus L.	Dringo	Rod	Boiled	Body's immune system
Arecaceae	*Cocos nucifera* L.	Kelopo	Root	Boiled	Nerve system
			Endosperm	Directly consumed	Itchy skin
	Monstera pertusa (L.) de Vrise	Jalu Mampang	Leaf	Boiled	Appetite
	Ageratum conyzoides L.	Wedusan	Leaf	Smeared	Skin cut
	Eupatorium riparium (Regel)	Ireng-Ireng	Leaf	Boiled	Malaria
Asteraceae	*Gynura pseudochina* Cass.	Dewa	Leaf	Boiled	Tumor
	*Pluchea indica (*L.)Less.	Beluntas	Leaf	Boiled	Increasing breast milk
	Sonchus arvensis L.	Tempuyung	Leaf	Boiled	Kidney stone
	Synedrella nodiflora (L.)Gaerth	Legetan	Leaf	Boiled	Skin cut, toothache
Araliaceae	*Polyscias scutellaria* Burm.F.	Mangko'an	Leaf	Boiled	Skin cut, diuretic
Bassellaceae	*Anredera cordifolia* (Ten.) Steenis	Binahong	Leaf	Smeared	Skin cut
Boraginaceae	*Symphytum officinale* L.	Comprei	Leaf	Mashed	Breathing problem, diabetes
Bromeliaceae	*Ananas comosus* Mill.	Nanas	Fruit	Boiled	Fever
Cactaceae	*Epiphyllum sp.* Haw	Sambung Otot	Leaf	Boiled	Nerve system
Caricaceae	*Carica papaya* L.	Kates	Leaf	Boiled	Diarrhea
Cucurbitaceae	*Cucumis sativus* L.	Ketimun	Fruit	Eat	High blood pressure
	Sechium edule (Jacq) Sw.	Jipang	Leaf	Cook	High blod pressure
	Jatropha multifida L.	Yodium	Leaf	Smear	Skin cut
	Sauropus androgynus (L.)Merr.	Katuk	Leaf	Boiled	Increasing breast milk
Euphorbiaceae	*Euphorbia prostate* Aiton	Patikan Cina	All Species	Boiled	Diuretic, antipyretic
	Euphorbia hirta L.	Patikan Kerbau	All Species	Boiled	Asthma, diarrhea, kidney infection
	Manihot utilissima Crantz	Singkong	Leaf	Smear	Cold
Equisetaceae	*Equisetum debile* Roxb	Sangkal Putung	Leaf	Boiled	Cholesterol, skin cut, Broken bones
Fabaceae	*Gliricidia sepium* (Jacq.) Kunth ex Walp.	Kleresede	Leaf	Shower	Itchy Skin
Liliaceae	*Aloe vera* L.	Lidah Buaya	Leaf	Smear	Skin burn
	Allium sativum L.	Bawang Putih	Tuber	Directly consumed	Cholesterol
Lamiaceae	*Orthosiphon stamineus* Benth.	Kumis Kucing	Leaf	Boiled	Kidney
Lauraceae	*Persea americana* Mill	Alpokat	Leaf	Boiled	Uric acid, high blood pressure
Leguminosae	*Erythrina lithosperma* (Hassk.) Merr	Dadap Serep	Leaf	Boiled	Fever
	Leucaena leucocephala (Lam.)De Wit	Lamtoro gung	Leaf	Boiled	Skin cut, cancer, vermicide
Loranthaceae	*Loranthus* sp. Jacq.	Kemladean	Parasit	Boiled	Cancer
Malvaceae	*Hibiscus similis* Bl.	Waru Gombong	Getah	Smear	Eye problem
Marantaceae	*Maranta arundinacea* L.	Garut	Tuber	Roasted	Dyspepsia (gastritis)
Melastomataceae	*Medinella speciosa* Reinw. Ex Blume	Parijoto	Flower	Directly consumed	Strengthening embryo
Meliaceae	*Swietenia macrophylla* King.	Mahoni	Fruit	Directly consumed	Hemorrhoid
Menispermaceae	*Tinospora tuberculata* (Thunb.)	Brotowali	Rod	Boiled	Appetite, diabetes
Moraceae	*Artocarpus heterophyllus* Lam.	Nongko	Fruit	Directly consumed	Diarrhea
Musaceae	*Musa paradisiaca* L.	Pisang Kepok	Fruit	Roasted	Kidney stone, gastritis, female fertility
	Musa textilis Nee	Pisang Raja	Sap	Smeared	Skin burn
	Syzygium aromaticum L.	Cengkeh	Fruit	Boiled	Warming the body
Myrtaceae	*Eugenia polyantha* Wight.	Salam	Leaf	Boiled	Uric acid, cholesterol
	Psidium guajava L.	Jambu klutuk	Leaf	Boiled	Diarrhea
	Piper betle Linn	Suruh	Leaf	Boiled	Fluor albus, cough, body's odor
Piperaceae	*Piper betle* var. nigra	Suruh ireng	Leaf	Boiled	Fluor albus, cough

	Piper crocatum Ruitz & Pav.	Suruh abang	Leaf	Boiled	High blood pressure
	Saccharum officinarum L.	Tebu	Rod	Directly consumed	Kidney, eye inflammation
Poaceae	*Cynodon dactylon* L.	Suket grinting	Leaf	Boiled	Uric acid
			Root	Boiled	Nerve system
	Imperata cylindrica L.	Alang alang	Leaf	Boiled	Skin cut
			Rod	Boiled	Fever, uric acid
Rubiaceae	*Morinda citrifolia* L.	Pace	Leaf	Boiled	High blood pressure, uric acid
	Paederia scandens L.	Sembukan	Leaf	Cooked	Increasing flatulence
Rutaceae	*Citrus aurantifolia* (Christm.) Swingle	Jeruk pecel	Fruit	Directly consumed	Cough
Solanaceae	*Capsicum annum* L.	Lombok	Fruit	Cooked	Influenza, appetite, aphthous stomatis
	Solanum lycopersicum L.	Tomat	Fruit	Directly consumed	Reducing risk of Cancer
Theaceae	*Camellia sinensis* L.	Teh	Leaf	Boiled	Cancer, Asam Urat, Rematik
Verbenaceae	*Clerodendrum japonicum* L.	Pagoda	Flower	Boiled	Diuretic, antiseptic, Haemostatic
	Vitex trifolia L.	Legundi	Leaf	Boiled	Uric acid
Zingiberaceae	*Zingiber purpureum* Roxb.	Bengle	Rhizome	Boiled	Body's immune system
	Zingiber officinale Roscoe	Jahe	Rhizome	Boiled	cold, stiff muscles
	Alpinia galangal (L.) Willd	Laos	Rhizome	Smeared	Skin fungus
	Curcuma heyneana L.	Temugiring	Rhizome	Shower	Skin smoothing cream
	Curcuma aeruginosa Roxb.	Temuireng	Rhizome	Boiled	Vermicide, appetite
	Curcuma xanthorrhiza Roxb	Temulawak	Rhizome	Boiled	Liver, appetite

Fruit was also frequently used as medicines. The medicinal fruits were taken from 11 species (15%) (Table 2), such as fennel or *adas* (*Foeniculum vulgare* Mill.), *cabai* or chili pepper (*Capsicum annum* L.), *mahoni* or magahony (*Swietenia macrophylla* King.), *nangka* or jackfruit (*Artocarpus heterophyllus* Lam.), and cheese fruit or *pace or mengkudu* (*Morinda citrifolia* L.). Fruits are used as medicines because they contain nutrient needed by human body such as potassium, pectin, beta-carotene and vitamin C. In addition, fruits also contain elements capable of cleansing food waste, has ready-to.-use energy (Gunawan 2007). Rhizomes of 8 species (11%) were also used as medicines, such as, *empon empon* (Zingiberaceae): purple ginger of *bangle* (*Zingiber purpureum* Roxb.), tumeric or *kunyit* (*Curcuma domestica* Val.), Javanese ginger ot *temulawak* (*Curcuma xanthorrhiza* Roxb.), and *jahe* or ginger (*Zingiber officinale* Rosc.). Rhizomes are are used as medicines because they contain substances benefecial for health, such as zingeberene found in ginger (*Z. officinale*) which can be used to cure impotence and as beverage to warm the body (Figure 3).

Stem of four 4 species (5%), namely *brotowali* (*Tinospora crixspa* L.), sweet flag or *dringo* (*Acorus calamus* L.), blady grass or *rumput ilalang* (*Imperata cylindrica* L.), and sugar cane or *tebu* (*Saccharum officinarum* L.) were also used as medicines. Roots of 3 species (4%) namely Bermuda grass or *rumput grinting* (*Cynodon dactylon*), common comfrey or *comprei* (*Symphytum officinale*) and coconut or *kelapa* (*Cocos nucifera* L.) were used as medicines. Other organs, namely flower, sap, tuber, and the whole plant were rarely used. Medicinal flowers were taken from showy Asian grape or *parijoto* (*Medinilla speciosa* Reinw.) and bleeding heart or *pagoda* (*Clerodendrum japonicum* L.), while medicinal sap was taken from "king" banana or *pisang raja* (*Musa paradisiaca* L) and *waru gombong* (*Hibiscus similis* L). Medicinal tubers were taken from garlic or *bawang putih* (*Allium sativum*) and *garut* (*Maranta arundinacea*), while the the medicines from whole plants were found in prostate sandmat or *patikan cina* (*Euphorbia prostate* Aiton.) and asthma plant or *patikan kerbau* (*Euphorbia hirta* L). Only few people used parasitic plants and endosperm as medicines. Only 2 species (1%) of parisitic plants, namely parasitic plant in tea or *benalu teh* (*Loranthus* sp.), and coconut or *kelapa* (*Cocos nucifera*) were sed as medicines (Table 1, Figure 3).

The people of Turgo Hamlet have used various processes of medicinal plant materials. Boiling (62%), in order to dissolve the active substance into the water, was conducted for leaves of 28 species, rhizomes 5 of species, fruits of 4 species, stems of 3 species, roots of 1 species, and 1 species of parasitic plant. Smearing on skin (15%) was done for leaves of 7 species, roots of 2 species, rhizomes of 1 species, and sap of 1 species, namely "king" banana *pisang raja* (*Musa paradisiaca*). Some parts of medicinal plants were consumed directly without processing (12%), such as showy Asian grape or *parijoto* (*Medinilla speciosa*), coconut's endosperm (*Cocos nucifera*), stem of sugar cane (*Saccharum officinarum*), tuber of garlic (*Allium sativum*). Cooking of plant materials were rarely done (4%). It was done for chilli pepper (*Capsicum* sp.), chayote or *jipang* (*Sechium edule*), and stinkvine or *sembukan* (*Paederia scandens*). Burning of plant materials was also rarely done (3%). It was done for tuber of arrowroot or *garut* (*Maranta arundinacea*) and banana or *pisang kepok* (*Musa paradisiaca*) (Table 1). In addition, some plant materials were used for bathing (3%), such as quickstick or *klereside* (*Gliricidia sepium*) and *temugiring* (*Curcuma heyneana*).

Table 2. Percentage of families used as medicinal plants in Turgo Hamlet

Family	Number of genera	Number of species	Proportion of species (%)
Annonaceae	2	2	2.90
Apiaceae	3	3	4.35
Araliceae	1	1	1.45
Arecaceae	3	3	4.35
Asteraceae	6	6	8.70
Bassellaceae	1	1	1.45
Boraginaceae	1	1	1.45
Bromeliaceae	1	1	1.45
Cactaceae	1	1	1.45
Caricaceae	1	1	1.45
Cucurbitaceae	2	2	2.90
Equisetaceae	1	1	1.45
Euphorbiaceae	4	5	7.25
Fabaceae	1	1	1.45
Lamiaceae	1	1	1.45
Lauraceae	1	1	1.45
Leguminosae	2	2	2.90
Portulacaceae	1	1	1.45
Liliaceae	2	2	2.90
Loranthaceae	1	1	1.45
Malvaceae	1	1	1.45
Marantaceae	1	1	1.45
Melastomataceae	1	1	1.45
Meliaceae	1	1	1.45
Menispermaceae	1	1	1.45
Moraceae	1	1	1.45
Musaceae	1	2	2.90
Myrtaceae	3	3	4.35
Piperaceae	1	3	4.35
Poaceae	3	3	4.35
Rubiaceae	2	2	2.90
Rutaceae	1	1	1.45
Solanaceae	2	2	2.90
Verbenaceae	2	2	2.90
Zingiberaceae	3	7	10.14
Total	**61**	**69**	**100**

Table 3. Types of diseases cured with medicinal plants

Types of diseases	Name of diseases and health benefit
Mild diseases	Skin cut, skin burn, aphthous stomatis, stomach gas, fever, stiff muscles, rheumatic, toothache, gastritis, hemorrhoid, diuretic, *fluor albus*, influenza, skin infection, diarrhea, cough, itchy skin, worm, skin ulcer, skin fungus, eye inflammation, warming the body, binding skin cut, maintaining body's immune system, maintaining brain health, increasing fertility, anti inflammation, reducing body odor and mouth odor
Serious diseases	Cholesterol, cancer, broken bones, diabetes, kidney, diuretic, liver, uric acid, asthma, kidney stone, nerve, high blood pressure, diuretic, neutralizing intestines

The people of Turgo Hamlet had the capability of classifying diseases into two categories: mild and serious. A disease is considered mild if it occurs to many people, and a disease is considered serious if it takes long time to heal it and it may cause death (Table 3). An example of mild disease is cold due to cold weather. The people cured this disease by consuming ginger (*Z. officinale)* which contains curcumin, capable of proliferating T cells, so it has good prospect to increase immunity system (Varalakshmi et al. 2008). A serious disease, diabetes was generally cured with *brotowali* (*Tinospora crispa)* which contains alkaloid and flavonoid, capable of reducing sugar concentration in the blood.

In addition, the community of Turgo Hamlet had knowledge of the use of medicine for external application, namely smearing, and internal application, namely consuming (Table 3) An example of external application was the smearing of coral bush or *daun yodium* (*Jatropha multifida)* on the skin cut, because the leaves contain alkaloid compound beneficial for blood coagulation and thus useful for new cut. The leaves of common guava (*Psidium guajava*) was used to cure diarrhea, because the leaves contain astringent (a substance which can line mollusc intestine wall with a layer, protecting the wall from the stimulation of the intestine content), which is alkaline in nature, and capable of killing bacteria, *Escherichia coli* and *Staphylococcus aureus*.

The belief of Turgo Hamlet people on traditional healing is inherited from generation to generation, developed and governed together by the community. In Turgo Hamlet medicinal plants were usually found in homegardens, plantations, shrubs and in cultivated land. This fact is in line with the results of research by Hariyadi (2011) that medicinal plants are not taken from natural forest, but from human-dominated ecosystems, especially shrubs and cultivated land. The local wisdom of Turgo Hamlet community has long influenced the paradigm of the people on health, and indirectly has encouraged them to conserve biodiversity of tropical forest, which consist of various ecosystem types and serve as storage of biodiversity (Hidayat et al. 2010). More than 2,039 species of medicinal plants are found in tropical forest, useful to maintain health and to cure various diseases of human and cattle (Zuhud 2009). Therefore, the belief and knowledge of community of the use of medicinal plants must be developed and protected as heritage of traditional healing to maintain family health.

The people of Turgo Hamlet have used 69 plants species, from 36 families, growing in home gardens and in forest nearby, as medicinal plants. Their knowledge of medicinal plants has been inherited from generation to generation. The most used part of plant was leaf, followed by fruit, rhizome, stem, root, sap, flower, all parts or the whole plant, tuber, and endosperm. The medicinal plants consisted of several growth forms, namely tree, shrub, herb, liana and grass. The processes of medicinal plants were: boiled, directly smeared on skin, directly consumed, cooked, used for bathing, and burned.

It is obvious that the people of Turgo Hamlet have achieved health sovereignty for themselves and their families, based on their knowledge inherited from their ancestors, supported by the potential of natural resources in

the village enabling them to do self healing for themselves and their families. In addition, they also have conducted ecosystem conservation through plant utilization for medicines. The community's knowledge will be improved if it is complemented with scientific research on the active substances of the medicinal plants, conducted by universities. So, sustainable research is needed in order to develop the knowledge qualitatively and quantitatively. It is also important to protect this local wisdom, so it will stay as the property of Indonesian nation.

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Bacterial spatial distribution in the sediments of Gajah Mungkur Reservoir, Central Java, Indonesia

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Abstract. Pujiastuti P, Masykuri M, Gunawan T, Sutarno. *2016. Bacterial spatial distribution in the sediments of Gajah Mungkur Reservoir, Central Java, Indonesia. Biodiversitas 17: 907-914.* The study aims to obtain the spatial dynamic pattern of bacterial sediment in the Gajah Mungkur Reservoir, Wonogiri, Central Java, Indonesia. This study supplied with colony morphology characterization, gram staining and biochemical test on the sediment samples that were obtained from eight contaminated zones. Furthermore, the spatial dynamic map based on the distance function using contour interpolation technique is processed using ArcView GIS 10 software. The research result shows that the distribution pattern of bacterial diversity is dynamic enough, identified by gram-positive bacteria: *Bacillus* sp., *Bacillus cereus,* and *Staphylococcus* sp.; and gram-negative bacteria: *Klebsiella, Escherichia coli, Aeromonas hydrophila, Aeromonas sobria, Aeromonas caviae, Aeromonas schubertii, Plesiomonas shigelloides, Acinetobacter* sp., and *Pseudomonas (Comamonas) acidovorans.* Gram-positive anaerobic bacteria show similar distribution pattern in all samples, including *Clostridium sphenoides,* and *Clostridium paraputrificums*.

Keywords: Bacteria, distribution pattern, reservoir, sediment

INTRODUCTION

The waters of Gajah Mungkur Reservoir (GMR), Wonogiri, Central Java, Indonesia have degraded from year to year, identified by some findings, including: the occurrence of sedimentation (JICA 2007), which poses the life of GMR to a threat and the activities in the river basins Wiroko and Keduang, which have eutrophication in the GMR waters (Wiryanto et al. 2016). The fish feed residues stacking for years have decreased water acidity level and the availability of dissolved oxygen and increased N-NO_2 and N-NH_3 contents. According to (Casali et al. 2010), farming activities produce sediment runoff, nitrate (N-NO_3), and phosphate (P-PO_4) which enter the stream, and therefore, this causes pollution in the water. The outlet of river basin continuously carries sediment runoff and dissolved nutrients (N-NO_3, N-NH_3, H_2PO_4 and K) of 32% from the river basin, 18% from the forest, and 17% from the farming land (Duran Zuazo et al. 2012). 17.07-36.7% of lands in the catchment area of GMR are utilized for farming activities (Bapeda 2012). The use of fertilizer causes a problem to the influx of a large number of Nitrogen to environment and farming activities that accelerate the Nitrogen transformation to the body of water (Xia et al. 2011). Fish-farming activities using floating fish cage and agricultural activities in catchment area have enriched nitrogen and phosphor in GMR waters (Pujiastuti et al. 2013). The high nutrient content makes water and sediment rich nutrients so it is a good habitat for microorganisms. There are a number of microorganisms populations in the sediment with high diversity (Bissett et al. 2007). In the 25-meter-depth sediment, bacteria population is found with the quantity of 4-90 x 10^6 cells/mL (Nuchsin 2007). In the waters and sediment in Cirata, Saguling and Jatiluhur reservoirs of West Java, Indonesia, some pathogenic bacteria are found, including *Bacillus badius, Bacillus brevis, Bacillus pumilus,* and *Pseudomonas* (Jumiarni 2008). Some bacteria can cause the disease to the fish in Batam waters, such as *Pseudomonas fluorescens, Pseudomonas alcaligenes, Enterobacter aerogenes, Citrobacter freundii, Aeromonas hydrophila, Aeromonas caviae, Aeromonas sobria*, etc are found (Syarief 2013). From the samples of tropical seafood in India, including squid, shrimp, and fish, five species of *Aeromonas* are found, comprising *Aeromonas hydrophila, Aeromonas enteropelogenes, Aeromonas caviae, Aeromonas punctata,* and *Aeromonas aquariorum* (Joseph et al. 2013).

The Storet test based on water quality class 2, some point in the waters of GMR has experienced pollution at the moderate to severe category. At every turn of the dry season to the rainy, death of fish en massive at GMR. In floating net area, an amount of dead fish, average 70 tons per day. Bacteria *Streptococcus* sp. caused the death of *Oreochromis niloticus* in GMR The existence of pathogenic bacteria in water reservoirs, can cause a decrease in dissolved oxygen and causing infections in fish. GMR *Oreochromis niloticus* contain *Streptococcus agalactiae* and *Streptococcus pneumonia,* with pathogenecity of up to 100%. Pathogenic bacteria in the waters do not only cause disease to some living creatures in the Lake, but also have

an important role in the natural purification process, for instance *Bacillus* sp. (Azlina and Norazila 2013). *Comamonas kerstersii* KSM7 (Swamy et al. 2014), *Staphylococcus aureus* (Nurhayati et al. 2012), *Escherichia coli*, and *Pseudomonas* sp. (Badjoeri and Widianto 2008); they can produce protease enzyme which makes a contribution in hydrolyzing or degrading organic pollutants containing protein to be a simpler substance. These research aims at obtaining spatial dynamic pattern of sediment bacteria in GMR, as an environmental information system, functioning to identify the local biodiversity which has an important role in natural purification of the pollutant in GMR waters. Several studies have been conducted to determine the status of water quality in the zone of floating net and outlet GMR, on the parameters of physics, chemistry, and biology. The Biological parameters that have been investigated are *Escherichia coli* and total coliform. This study reinforce some previous research, through the study of the distribution of pathogenic bacteria in the sediment at 8 points GMR polluted zone.

MATERIALS AND METHODS

Study area and sample collection

This research material is the sediment of Gajah Mungkur Reservoir (GMR), Wonogiri, Central Java, Indonesia in the contaminated zone taken at the peak of the dry season in 2014 at 5-7 meters depth. The sampling point was taken in Station 1 in traditional floating fish cage area (S 07°52'01.1'', E 110°54'13.6''), Station 2 in modern floating fish cage (S 07°52'12.1'', E 110°54'17.9''), Station 3 in tourism area (S 07°51'30.50'', E 110°54'47.06''), Station 4 in reservoir center (S 07°54'1.19'', E 110°53'40.73''), Station 5 in free area (S 07°54'09.0'' , E 110°53'36.9''), Station 6 in Wuryantoro estuary (S 07°54'39.3'', E 110°52'38.9''), Station 7 in Alang estuary (S 07°54'36.0'', E 110°53'44.4''), Station 8 in Wiroko estuary (S 07°53'48.6'', E 110°54'24.0'') (Figure 1).

The sampling tools employed were Ekman grab sampler and GPS. The bacteria were grown on Nutrient Agar with Cappuccino and Sherman method (2005), and then colony morphology characterization test, gram staining, and biochemical test were conducted. The obtained data were compared with standard description provided in Helt et al. (1994). The spatial dynamic mapping of the diversity of sediment bacteria was carried out using ArcView GIS 10 software (ESRI, Redlands, CA, USA).

Instrument, chemical and microbiological media

Media to growth all bacteria in sediments, used universal media Agar Nutrient. Special instrument and chemically anaerobic, used (i) Anaerobic jar oxoid, E Merck, BBL. (ii) Gas generating kit and anaerobic indicator. (iii) Catalisator, (iv) Anaerocult A and Anaerotes. (v) Metronidazole disc 5 mcg. Media to growth anaerobic used Thioglycolate broth. Media culture bacteria aerobic used Blood agar plate and MacConkey agar plate.

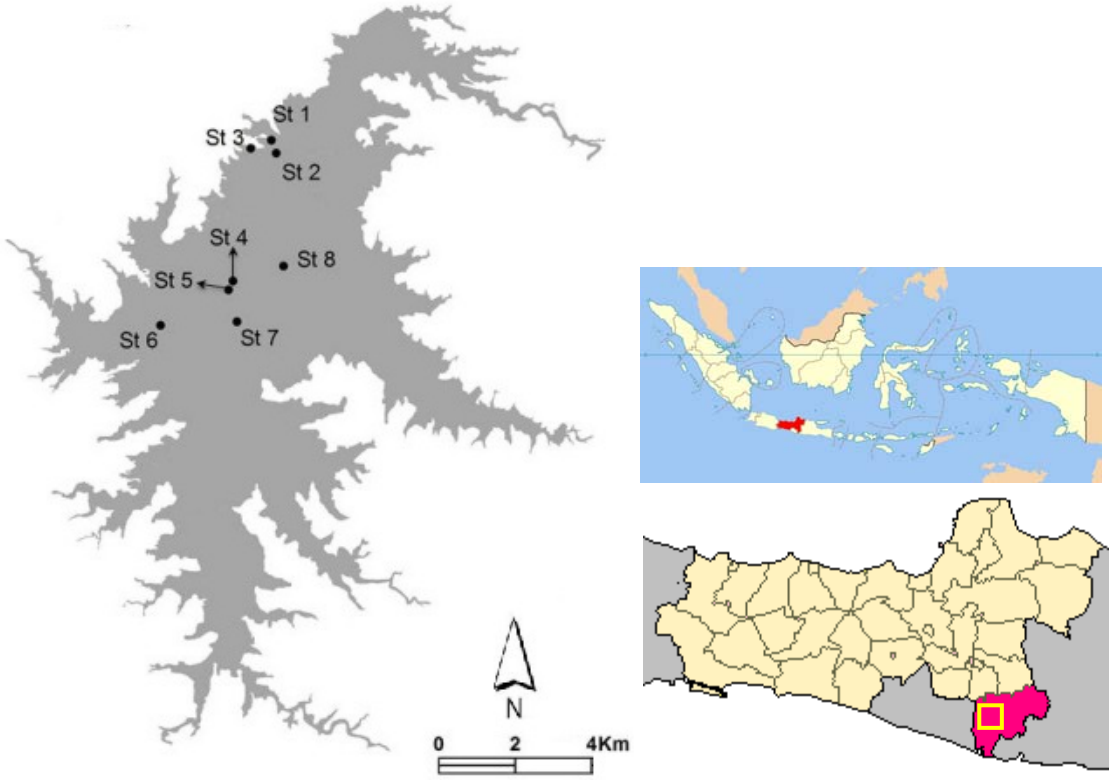


Figure 1. The map of sediments station sampling in Gajah Mungkur Reservoir, Wonogiri, Central Java, Indonesia

Isolation of reservoir bacteria sediment

Bacterial culture isolation: (i) Aseptically inoculate samples onto Nutrient Agar plates labeled with pertinent case history information, (ii) Incubate aerobically for 24-48 hours at 20-24°C. If no growth occurs at 24 and 48 hours. If no growth occurs after 96 hours, samples are discarded. (iii) When growth does occur on field collection tubes or plates, use a sterile loop or needle to select a single colony to subculture onto fresh Nutrient Agar. If colonies are not well isolated, the plate will have to be re-inoculated on Nutrient Agar and thoroughly struck over the entire plate surface to achieve isolation of bacteria. (iii) Incubate at 20-24°C for 24 hours to allow bacterial growth; all test should be performed on 24-48 hour cultures. (iv) Inoculate biochemical tubes. (v) Treat all bacterial cultures as potential human pathogens.

Identification of bacterial strain

Gram staining, it is a differential staining technique used to characterize bacteria as Gram-positive and Gram-negative. Steps of Gram staining of bacteria: (i) Fixation of sediment smear, (ii) Flood the fixed smear sediment with crystal violet solution, and allow to remain for 1 minute, and then rinse of the crystal violet with distilled water, (iii) Flood the slide with iodine solution, allow to remain for 1 minute, and then rinse off the iodine solution with distilled water, (iv) Flood the slide with decolorizer for 30 second, after that rinse off the decolorizer with distilled water, (v) Flood the slide with safranin, allow to remain for 30 second, after that rinse off the safranin with distilled water. (vi) Dry the slide, and see the slide for bacterial organism on microscope binocular under 100x objective. Observe several fields on slide for bacteria organisms. Describe the gram reaction of any organisms seen. Gram-positive bacteria stain deep violet, and gram-negative bacteria stain pink to red.

Biochemical test: Indole, catalase, oxydase test, coagulase test, H_2S test, citrat test, etc. Biochemical activities were determined according to the recommended scheme of Helt et al. (1994).

RESULTS AND DISCUSSION

Identification of aerobic and anaerobic bacteria in samples of GMR sediment

Indole test is performed to help differentiate species of the family Enterobacteriaceae. Bacteria that possess the enzyme tryptophanase are capable of hydrolyzing and deaminating tryptophan with the production of indole, pyruvic acid and ammonia. Interpretation Indole test is development of bring red color at the interface of the reagent and the broth within seconds after adding the reagent is indicative of presence of Indole and positive test. Indole positive: *E. coli* and *Proteus vulgaris*. Indole negative are *Salmonella* sp., *Klebsiella* sp., *Enterobacter aerogenes*. The result biochemical tests are shown in Table 1. The results of study of colony characteristics and Gram stain are presented in Tables 2 and 3.

Discussion

On the basis of the results of laboratory analysis on water samples of estuaries of the sub-river basin, floating fish cage area, and the central area of the reservoir, and later the test of water quality class 2 using Storet method, some sampling points reveal moderately to highly polluted water. Reservoir water system has the natural capability to carry out self-purification process. However, in case that the presence of organic compounds exceeds the capability of self-purification, accumulation of organic compounds and formation of toxic materials in water are uncontrollable, and hence, result in the decrease in water quality (Badjoeri and Widianto 2008). Aquatic pathogenic bacteria contribute to aquatic self-purification. As the purification is ongoing, biotransformation in which enzymes produced by micro-organisms modify toxic pollutants by changing their chemical structure occurs. This biotransformation leads to bio-gradation in which the toxic pollutants are degraded, their structures become in complex and finally transform to harmless and non-toxic metabolites (BPPT 2014).

Table 1. The results of biochemical tests in the sediment of Gajah Mungkur Reservoir, Wonogiri, Central Java, Indonesia

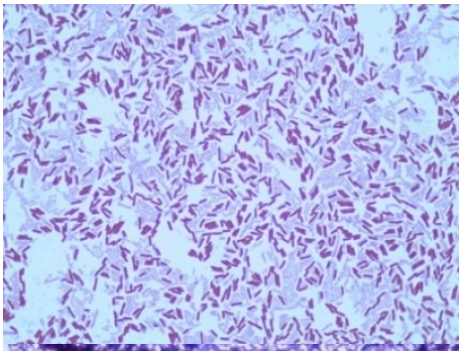
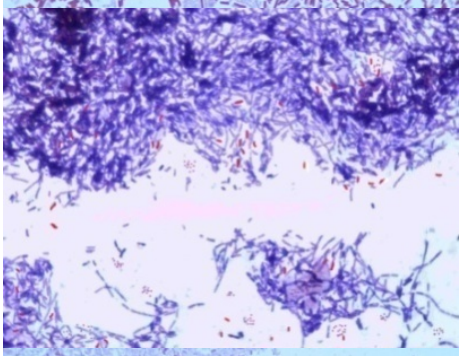
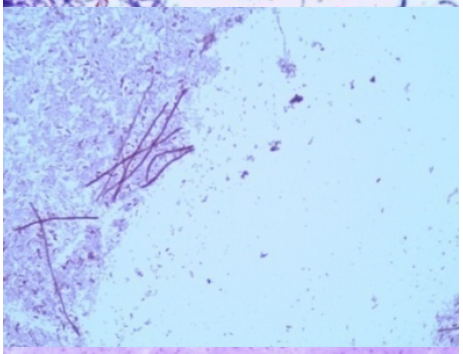
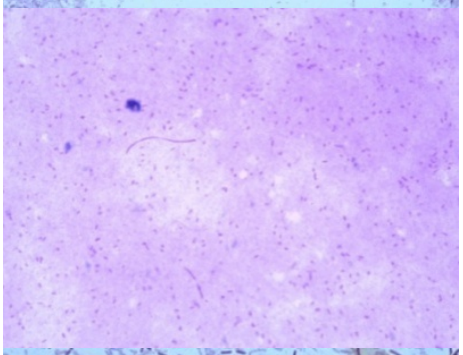
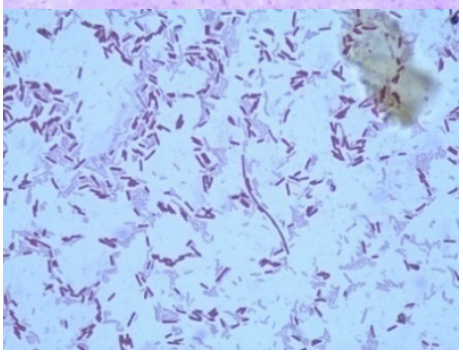
Bacteria	Biochemical Test					Morphology	
	Ind	Cat	Oxy	Ure	Glu	Gram	Stain
Bacillus sp.	+	+	-	-	+	+	Rods
Bacillus cereus	+	+	-	-	+	+	Rods
Staphylococcus sp.	-	+	-	-	-	+	Cocci
Klebsiella sp.	-	+	-	+	+	-	Rods
Escherichia coli	+	+	-	-	+	-	Rods
Aeromonas sobria	+	+	+	-	+	-	Rods
Aeromonas caviae	+	+	+	-	-	-	Rods
Aeromonas hydrophila	+	+	+	-	+	-	Rods
Aeromonas schubertii	-	+	+	-	-	-	Rods
Plesiomonas shigelloides	+	+	+	-	-	-	Rods
Acinetobacter sp.		+	-	+	-	+	Rods
Pseudomonas (Comamonas) acidovorans	-	+	+	-	-	-	Rods
Clostridium paraputrificums	-	-	-	-	+	+	Rods
Clostridium sphenoides	-	-	-	-	+	+	Rods

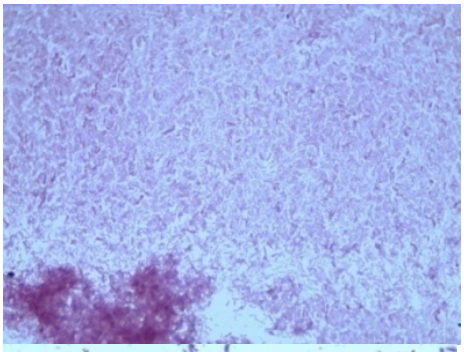
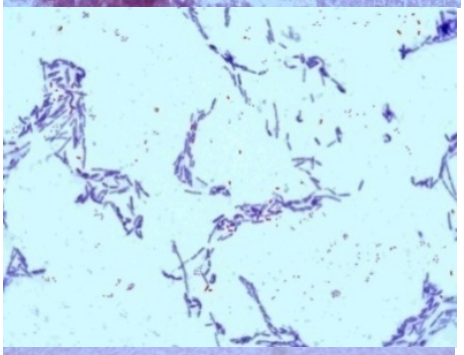
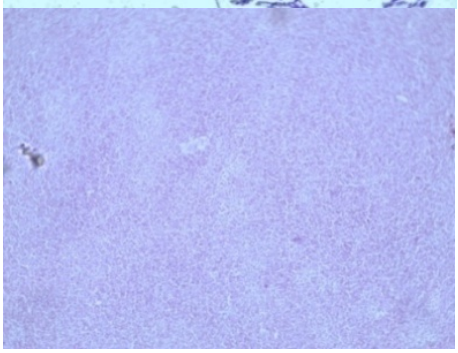
Note: Test Ind: indole, Cat: catalase, Oxy: oxydase, Ure: urease, Coa: coagulase, Glu: glucose

Table 3. Anaerobic bacteria in the sediment samples of Gajah Mungkur Reservoir, Wonogiri, Central Java, Indonesia

Sampling station	Anaerobic bacteria	
	Gram +	Gram-
St. 1	*Clostridium paraputrificums*	Not found
St. 2	*Clostridium sphenoides*	Not found
St. 3	*Clostridium sphenoides* *Clostridium paraputrificums*	Not found
St. 4	*Clostridium sphenoides* *Clostridium paraputrificums*	Not found
St. 5	*Clostridium sphenoides* *Clostridium paraputrificums*	Not found
St. 6	*Clostridium sphenoides*	Not found
St. 7	*Clostridium sphenoides* *Clostridium paraputrificums*	Not found
St. 8	*Clostridium sphenoides*	Not found

Table 2. Aerobic bacteria in the sediment samples of Gajah Mungkur Reservoir, Wonogiri, Central Java, Indonesia

Sampling station	Figures of bacteria	ALT cfu/mL)	Aerobic	
			GRAM +	GRAM-
St. 1		5.01 x 10^5	*Bacillus* sp.	*Klebsiella* sp. *E. coli* *Aeromonas sobria* *Aeromonas caviae*
St. 2		3.50 x 10^5	*Bacillus* sp. *Bacillus cereus* *Staphylococcus* sp.	*Klebsiella* sp. *E. coli* *Aeromonas hydrophila* *Plesiomonas shigelloides* *Acinetobacter* sp.
St. 3		1.16 x 10^4	*Bacillus* sp.	*E. coli* *Aeromonas schubertii* *Pseudomonas (Comamonas) acidovorans*
St. 4		4.06 x 10^5	*Bacillus* sp.	*Klebsiella* sp. *E. coli* *Aeromonas sobria*
St. 5		4.,18 x 10^5	*Bacillus* sp. *Bacillus cereus*	*E. coli* *Aeromonas caviae*

St. 6		4.67×10^5	*Bacillus* sp.	*Klebsiella* sp. *E. coli* *Aeromonas sobria*
St. 7		1.12×10^5	*Bacillus* sp. *Staphylococcus* sp.	*Klebsiella* sp. *E. coli* *Aeromonas sobria*
St. 8		6.35×10^5	*Bacillus* sp. *Bacillus cereus*	*E. coli* *Plesiomonas shigelloides*

The distribution of bacteria in the sediment of GMR

Sediment microorganism plays a crucial role in a variety of biogeochemical processes in freshwater ecosystems (Liu et al. 2014). Bacteria commonly reproduce well in reservoir sediment since it provides nutrition for microorganisms. *Escherichia coli, Citrobacter, Klebsiella,* and *Enterobacter* are defined as all types of aerobic, facultative anaerobic and rod-shaped bacteria which are able to ferment lactose and produce gasses in 48 hours with the temperature of 35°C (Marganof 2007). Distribution of both aerobic and anaerobic bacteria found in GMR can be seen in the following figure 2. Observation result of sediment taken from polluted zone shows that both aerobic and anaerobic bacteria have been identified. This is shown in Table 2 and Table 3.

Bacillus

Bacillus species, gram-positive and rod-shaped bacteria, can grow in aerobic environment. They are found separately in whole sampling areas of Alang estuary, Wuryantoro estuary, floating fish cage and the central of GMR. They play a role in the process of nitrification and denitrification, and they function as nitrogen binder, Se oxidizer, and Mn reducer/oxidizer. Moreover, they are able to dissolve carbonate and phosphates, decrease substrate pH due to acidic properties which are produced, mineralize complex organic compounds such as polysaccharides, protein, and cellulose. Alang and Wuryantoro Estuaries have 0,01 mg/L of $N\text{-}NO_3$, 0,013 mg/L of $N\text{-}NH_3$ and 0,0015 mg/L of $N\text{-}NO_2$, fulfilling the standard of quality. The small amount of nitrogen exists as a result of the role of *Bacillus* sp.

Aeromonas

Aeromonas species are gram-negative bacteria, potential pathogenic to the environment, and they produce cytotoxin (Balaji et al. 2004). They can reproduce in highly-polluted fresh water. *Aeromonas hydrophila, Aeromonas sobria, Aeromonas caviae* and *Aeromonas schubertii* are *Aeromonas species* found in GMR sediment. Their distribution is figured 3 out below. *Aeromonas sobria* bacteria are found in the sediment of Alang estuary, that of floating fish cage and spread to that of a center of the reservoir. *Aeromonas hydrophila* bacteria are identified in the sediment of floating fish cage and are not found in other sampling points. Camus et al. (1998) state that

Aeromonas hydrophila, *Aeromonas sobria* and *Aeromonas caviae* are associated with fish. The latter appear in the sediments of floating fish cage and free zone, while *Aeromonas schubertii* bacteria are only found in the sediment of tourism water area. The former have more vicious characteristic than the middle do (Cipriano 2001). As pathogenic bacteria, *Aeromonas* may bring about MAS (Motile *Aeromonas septicemia*) which is also known as red sore disease, *hemorrhagic septicemia*, and *ulcer disease* (Samcookiyaei et al. 2012). Angka (2005) highlights that bacterial infection of *Aeromonas hydrophila* causes fish to suffer from this disease. It attacks all life cycle stages of fish specifically larval and fry stages (Camus et al. 1998). In addition, it can infect common carp (*Cyprinus carpio*) and walking catfish (*Clarias batrachus*), while *Aeromonas caviae* may attack goldfish (*Carassius auratus*) Minaka et al. (2012). *Aeromonas veronii* bv. *sobria* were highly pathogenic to Oreochromis niloticus (Eissa et al. 2015). *Aeromonas veronii* Av27, highly resistant to tributyltin (TBT 3mM) uses this compound as carbon source and degrades it to less toxic compounds (Cruz et al. 2007).

Escherichia coli

Escherichia coli bacteria are found in sediments of all sampling points of a polluted zone in GMR. Their presence serves as an indicator of water pollution due to fecal matter. Determination of fecal coliform is used as an indicator of pollution since its number of colonies must be positively correlated with the presence of pathogenic bacteria. It is possible that other enteric pathogens, along with *E. coli*, are also found in the zone. *E. coli* bacteria enter GMR through river flow of all sub-river basins and spread along waters and sediments of the reservoir. The number of *E. coli* in GMR reservoir, in Alang estuary, in Wuryantoro estuary, in floating fish cage and in the center of the reservoir ranges between 780.10^2/100 mL-33.10^1/100 mL, 12.10^0-23.10^0/100 mL, 94.10^1-140.10^1/100 mL, 23.10^0-540.10^0/100 mL, and <1,8.10^0-19.10^0/100 mL respectively. Pathogenic bacteria, found along with *E. coli*, appear mostly in fish cage owned by Aquafarm Company, in the sediment of Alang estuary and in the sediment of the free zone with a total number of 8 bacteria, 6 bacteria, and 4 bacteria respectively.

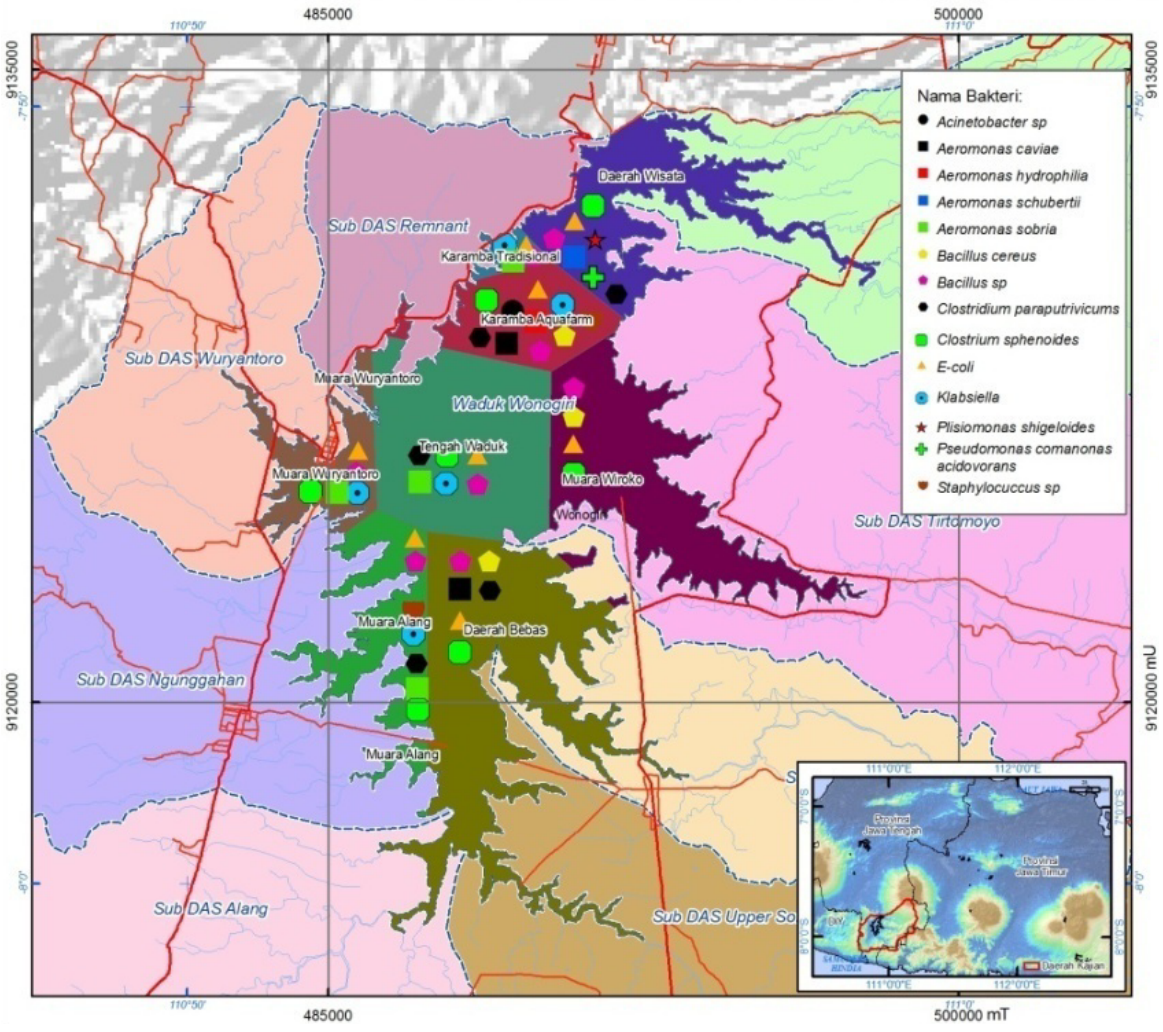


Figure 2. Distribution of aerobic and anaerobic bacteria in polluted zone of Gajah Mungkur Reservoir, Wonogiri, Central Java, Indonesia

Klebsiella

Klebsiella sp. is gram-negative, rod-shaped, and facultative anaerobic bacteria which are unable to produce spores. They can grow at temperature of 12-43°C. *Klebsiella* is pathogenic, which means that they are able to ferment carbohydrate to form acid and gas, and hydrolyze urea. They belong to the group of *coliform* bacteria. The existence of *Klebsiella* sp. is identified in the sediments of Alang estuary, Wuryantoro estuary, and floating fish cage areas. *Klebsiella sp* are also found spreading into the central area of GMR. The existence of the bacteria has a close relation with the organic pollutant content, like carbohydrate, which is measured with BOD_5 and COD parameters. In dry season, the water area of Alang estuary has the BOD_5 level of 1.2 mg/L below class 2 water quality standard of 3.0 mg/L, and COD level of 19.6 mg/L below class 2 water quality standard of 25 mg/L. Wuryantoro water area has the BOD_5 level of 2.3 mg/L and COD level of 19.6 mg/L. Floating fish cage area has the BOD_5 level of 2.6 mg/L and COD level of 15.3 mg/L. Meanwhile, the central area of the reservoir has the BOD_5 level of 2.5 mg/L and COD level of 25.5 mg/L. The low level of BOD_5 and COD scores are predicted to have a close relation with the existence of *Klebsiella sp* in the sediment, which has an ability to degrade organic pollutant in the form of carbohydrate into simpler molecule and gas.

Pseudomonas (Comamonas) acidovorans

Pseudomonas (Comamonas) acidovorans are only found in the sediment of tourism area of GMR. These bacteria are gram-negative. They play important roles in degrading complex organic pollutants and reducing chromium contents in contaminated water (Rudakiya and Parwar 2014). These species do not infect fish. There are three types of *Pseudomonas* which can infect fish in reservoir, including *Pseudomonas anguilliseptica*, *Pseudomonas chlororaphis,* and *Pseudomonas fluorescens.* The BOD_5 and COD levels in tourism water area are 2.8 mg/L and 21.6 mg/L below the water quality standard level two. The government regulation of the Republic of Indonesia 82/2001 regarding water quality management is estimated to have a relation with the existence of bacteria and their abilities, *Pseudomonas (Comamonas) acidovorans*, to degrade complex organic pollutants.

Acinetobacter sp.

Acinetobacter sp. are anaerobic and gram-negative bacteria. Growth on MacConkey agar, the biochemical characteristics are catalase positive, oxydase negative and glucose positive (Constantiniu el al. 2004). They need oxygen as the terminal electron in metabolism. They can grow in 20-30°C. They are able to use hydrocarbon chain as nutrient source, and therefore, they can remediate the oil content in water. In Gajah Mungkur Reservoir, *Acinetobacter* sp. can only be found in the sediment of floating fish cage. Oil, carbohydrate, and protein are organic pollutants, and therefore, they can be measured with BOD_5 and COD. The existence of these bacteria in floating fish cage sediment can help reduce the number of organic pollutants.

Clostridium sphenoides and Clostridium paraputrificums

Clostridium sphenoides and *C. paraputrificums* are gram positive rods, which may possess a single endospore. There are anaerobic bacteria. *Clostridium sphenoides* can be isolated in almost all of sediments of contaminated zones in GMR, except for traditional floating fish cage sediments. Meanwhile, *Clostridium paraputrificums* can be isolated in the sediments of Alang estuary, tourism area, and free and central area of GMR. Those types of bacteria are non-pathogenic to water biota. *Clostridium sphenoides* and *Clostridium paraputrificums* give variable indole test result usually negative (PHE 2015). This bacteria can't produce indole from the degradation of the amino acid tryptophan.

In conclusion, from sediment in polluted zone 8, the water area of GMR, which is taken as the sample in dry season, 14 bacteria are identified and isolated. The anaerobic bacteria found to consist of 12 genus/species, including gram-positive bacteria such as *Bacillus* sp., *Bacillus cereus,* and *Staphylococcus* sp., and gram-negative bacteria such as *Klebsiella, E. coli, Aeromonas hydrophila, Aeromonas sobria, Aeromonas caviae, Aeromonas schubertii, Plesiomonas shigelloides, Acinetobacter* sp., and *Pseudomonas (Comamonas) acidovorans.* Gram-positive anaerobic bacteria have the same distribution patterns at every sampling point. Those bacteria are *Clostridium sphenoides, Clostridium paraputrificums.* Meanwhile, there are no gram-negative anaerobic bacteria found. The distribution of bacteria in the sediment of GMR is quite dynamic. The most types of bacteria, totally 9 bacteria, are found in the sediment of floating fish cage. *E. coli*, *Bacillus* sp. and *Clostridium sphenoides* dominate in all sediments in contaminated zones used as samples.

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