

Morphogenetic and population structure of two species marine bivalve (Ostreidae: *Saccostrea cucullata* and *Crassostrea iredalei*) in Aceh, Indonesia

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Abstract. Ramadhaniaty M, Setyobudiandi I, Madduppa HH. 2018. Morphogenetic and population structure of two species marine bivalve (Ostreidae: *Saccostrea cucullata* and *Crassostrea iredalei*) in Aceh, Indonesia. *Biodiversitas* 19: 978-988. Oysters (Family Ostreidae) are mollusks, with high levels of phenotypic plasticity and wide geographic distribution. Oysters are a challenging group for morphological identification and genetic populations study. *Saccostrea cucullata* and *Crassostrea iredalei* are oysters from bivalve class that lives in the intertidal area and mangrove ecosystem. To clarify the morphology, genetic diversity and population structure of the two forms of *S. cucullata* and *C. iredalei*, we collected and studied oysters from three locations along the coastal region of Aceh by using morphometric method and 16 S mtDNA sequences analysis. We also added more oysters sequences from China, Japan, and Thailand to determine the connectivity between all populations. Morphometric characteristics of the oyster showed a negative allometric growth pattern, which means the rate of length gain is faster than that of the weight gain. The genetic distance from *S. cucullata* was 0.003-0.004 ($F_{st} = 0.708$) and *C. iredalei* was 0.000 ($F_{st} = 0.971$). The long genetic distance and high fixation index (F_{st}) in the oysters population are caused by the close geographical distance of the species in the three populations. The haplotype diversity value from *S. cucullata* and *C. iredalei* were 20 and 3, respectively. The haplotype showed the connectivity among the oyster populations which indicated by the gene flow pattern. The gene flow was affected by geographical distance and environmental complexity.

Keywords: 16S mtDNA, genetic, morphometric, oyster, tiram

INTRODUCTION

Morphometric characters and genetic analysis of oysters are the combinations of information about the effects of environmental (Abidin et al. 2014) and geographic conditions that can distinguish the size and genetic variation of oysters (Lam and Morton 2006). Growth is a three-dimensional process that changes over time. Oysters have an irregular shell shape and their growth is strongly influenced by biotic, abiotic factors (Gaspar et al. 2002), environmental, and geographical factors causing high variations in the formation of oyster shells (Gunter 1950; Gosling 2003). High-level phenotypic plasticity in oyster morphology is often of limited value for unambiguous identification of specimens and taxonomy as a whole (Boudry et al. 2003). Accordingly, identification of oyster species based on morphological characters alone is extremely difficult and insufficient (Klinbunga et al. 2005; Jiafeng et al. 2014). A factor that differentiates one oyster species from another is the shell. However, it is difficult to distinguish the shell shape due to its similarity (Boudry et al. 2003). Morphometric is an analysis based on measurements of shell's length and weight (Mass et al. 1999). The length-weight relationship may indicate the stock composition to estimate the availability of oyster sizes, mortality, growth, reproduction, and life cycle in the

aquatic environment, providing information about the range of oyster size that should be caught (Fafioyee 2005).

Various oysters live in Aceh coastal, such as *Saccostrea cucullata* and *Crassostrea iredalei*. Oysters are benthic marine species inhabiting intertidal areas and widely distributed throughout the tropical and subtropical regions (Hedgecock et al. 1995). Oyster has two weeks pelagic larval duration before it becomes a pediveliger larva that has feet to search for suitable substrate and grow into an adult oyster (Gosling 2003). Long larval dispersal distance combined with other factors has often caused uncertainty in gene flow between a wide geographic range of many oyster species (Buroker 1985; Boudry et al. 2003). The larvae dispersal pattern will lead to high levels of genetic variability within populations of intertidal species (Grassle 1972). Aceh coastal in the northern part of Sumatra is situated in a region with three different types of currents. The western part of Aceh water is associated with the Indian Ocean, the northern part with the Andaman Sea and the eastern part with the Malacca Strait (Nurhayati 2009). The geographic difference causes differences in the distribution and characteristics of marine biota among the three coastal sections. The physical oceanography factors such as currents and tides greatly affect the pattern of dissemination and recruitment of marine biota (Findly and White 2003).

Rapid species identification, proper management, and availability of stocks are important to avoid local extinction (Madduppa et al. 2017). In recent years, molecular techniques have become essential tools for identification of marine species (Boudry et al. 2003; Madduppa et al. 2014; Prehadi et al. 2015; Kusuma et al. 2016; Maulid et al. 2016). Molecular techniques have been proven to overcome the flaw of morphological identification approach (Boudry et al. 1998) and is widely used to answer questions that cannot be answered ecologically (Madduppa et al. 2016). The use of molecular DNA eases the systematic and identification of oyster species as well as the knowledge of their geographical range (Boudry et al. 2003). Mitochondrial DNA (mtDNA) is a single molecule that evolves more rapidly than the nuclear DNA. Therefore, it is suitable and recommended to be used for DNA barcoding (Hebert et al. 2003). DNA barcoding is an efficient method used to identify species level and plays a role in taxonomy as well as population structure (Hajibabaei et al. 2007). The 16S mtDNA used in this research was based on other previous studies on oysters. Genetic diversity determines population capacity to adapt to the environment (Taylor and Aarssen 1988). A population with high genetic diversity has a better chance of survival because every gene has a different response to

environmental conditions (Akbar et al. 2014). Various researches on oyster molecular systematics have been conducted in several countries in the world such as by Klinbunga et al. (2005) who combined the analysis of CO2, 16S and 18S rDNA of oysters in Thailand; Liu et al. (2011) identified oysters using mitochondrial DNA; Sekino et al. (2014) used mitochondrial DNA of *Crassostrea* in Japanese waters. The results of those studies answered the researchers' doubts about oyster species. By combining morphological and molecular data, a previous study even successfully found a new species of oyster namely *C. hongkongensis* (Lam and Morton 2003).

Oysters have great economic importance (Christo et al. 2010) and are consumed by people because of its good taste and high protein content (Ruesink et al. 2005). Oyster has been a source of livelihood for Aceh people for a long time. Oyster fishing activities are carried out daily, and as the numbers of fisherman increase, oysters become difficult to grow up normally. In Aceh, this activity has affected oyster populations and led to changes in oyster population structure (Octavina et al. 2014). In our present study, we analyzed morphological characters, genetic diversity, the population structure of oysters between and within populations to understand the wide-scale connectivity of the oyster population.

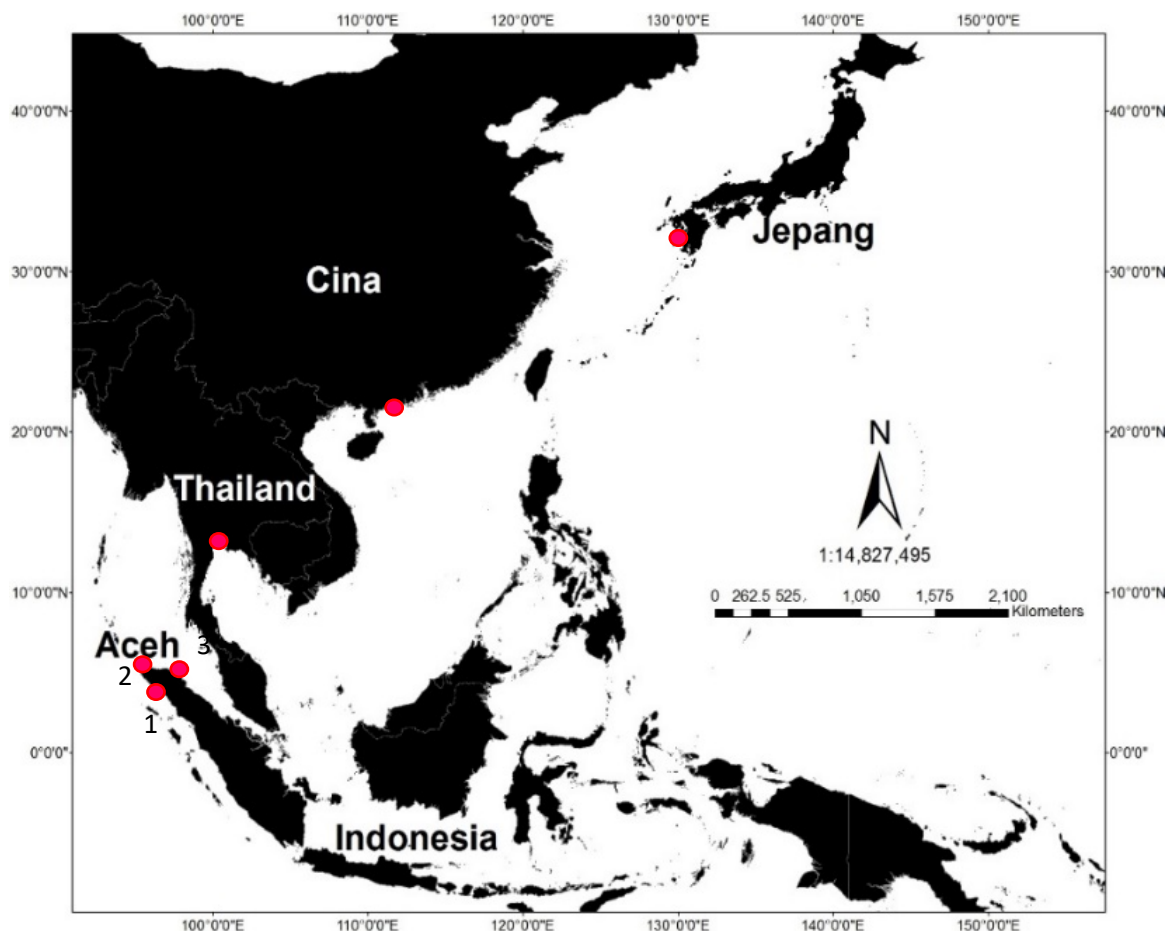


Figure 1. Research sites of *S. cucullata* and *C. iredalei* in Aceh Coastal area, Indonesia: 1. Labuhan Haji 6° 24' 46.8" S 97° 36' 43.2" E, 2. Kuala Gigieng 6° 33' 57.6" S 98° 6' 47.88" E), and 3. Loskala 3° 41' 22.2" S 97° 9' 3.6" E), and sequences from China, Japan dan Thailand obtained from Genbank

MATERIALS AND METHODS

Study area and sampling

Oysters were collected from three sites in Aceh namely Labuhan Haji (South Aceh), Kuala Gigieng (Aceh Besar) dan Loskala (Lhokseumawe) (Figure 1). Labuhan Haji is a region in South Aceh that faces the Indian Ocean, the sampling location at this site was around the Fishing Market. Kuala Gigieng is a site located in Aceh Besar that is adjacent to the Andaman Sea; this site is close to the settlement area and mangrove area. Loskala is an area in North Aceh precisely at Lhokseumawe, and the sampling site is nearby fish ponds.

The sampling was conducted from June 2016 to July 2016. A total of 77 samples were collected from three observation sites using Purposive Random Sampling (Sulistiyarto et al. 2007). Samples were collected when the lowest tides were in the intertidal area of the mangrove ecosystem. The samples were then identified morphologically by using identification book of Poitiers (1998) and Dharma (2005) as references. The length and weight of oysters were measured. Subsequently, oyster samples were removed from their shells, then placed into tubes containing 96% ethanol.

DNA extraction, amplification, and DNA sequencing

For DNA isolation, an adductor muscle of oyster was dissected from the sample and extracted using Extractions kit GeneJet Genomic DNA Purification Kit and gSYNC™ DNA Extraction Kit (Thermo Fisher, Waltham, MA, USA) (Liu et al. 2011). The molecular marker used in this study was 16S mtDNA. Amplification of a partial 16S segment by PCR was done using a primer pair F, 5'-CGC CTG TACA AAC AA AT-3'; and R 5'-GGT CTAAC TCA GAT CAG ATC ACG T-3' (Banks et al. 1993, Small and Chapman 1997). PCR reaction was set up in a total volume of 25 µL, containing 1-3 µL of DNA template, 1.25 µL of Kapa Master Mix (Kapa Biosystems, Wilmington, MA, USA), 1.25 µL (10 mM) of each primer, and 9 µL ddH₂O. The PCR condition is as follows: denaturation at 95°C for 2 min by 30 cycles, denaturation at 95 °C for 1 min, annealing at 57 °C for 1 min, extension at 72°C for 1 min and the final extension at 72 °C for 5 min (Bank et al. 1993). The PCR product was assessed by electrophoresis in 1.0% agarose containing Ethidium Bromide (EtBr). The electrophoresis was done at 100 V and 400 mA for 25 minutes and then visualized using a Gel Doc machine. Positively-amplified PCR products were then sent to a sequencing service company (First Base Malaysia) to be sequenced using the Sanger et al. (1977).

Data analysis

Morphometric length-weight relationship

The oyster shell morphometric was performed by analyzing the length and weight of oysters. The length-to-weight ratio was calculated to investigate the growth pattern of oyster using the following equations (Effendie 1997):

$$W = aL^b$$

Where:

W = Body weight (gram)

L = Body length (mm)

a and b = Constants

The equation is changed into a linear form, as follows:

$$\text{Log } y = a \log x + \log b$$

$$\text{Log } b = \frac{\sum \text{Log } y \cdot \sum (\text{Log } x) \cdot \sum \text{Log } X \sum (\log x \cdot \log y)}{\sum (\log x)^2 - (\sum \log x)^2}$$

Explanation:

$$\text{Log } b = \frac{\sum \log y - N \log b}{\sum \log x}$$

The determination of oyster growth criteria is performed by using the length-to-weight ratio based on the b value that is: when $b < 3$, the length is greater than the weight, a negative allometric. When $b > 3$, the weight is greater than the length, a positive allometric. When $b = 3$, the length increase, and the weight gain are balanced or called isometric (Effendie 1997).

Genetic diversity

Sequences from 77 individual oysters and additional 20 oysters 16S gene sequences from Genbank were aligned and edited in MEGA 6 software (Tamura et al. 2013). The sequences were aligned (Clustal W) to determine the homologous region of those sequences. A phylogenetic tree was constructed based on *Neighbour-Joining* method, Kimura 2 evolution model and 1000x bootstraps replication (Tamura et al. 2013). Additional oyster sequences from China, Japan, and Thailand were obtained from GenBank, National Center for Biotechnology Information (NCBI), as additional data to know the connectivity and genetic distance of the oysters on a wide scale in Asia. The analysis of genetic diversity (Nei 1987) and nucleotide diversity (Nei and Jin 1989) were calculated using Arlequin program (Schneider et al. 2000).

Genetic population structure and connectivity

The population structure of molecular variance was analyzed using AMOVA (Excoffier et al. 1992), pairwise using the difference of the subdivision of genetic distance in the population using Fixation index (F_{st}). Both statistical differences between populations and Chi-square probability test for population differentiation were tested using 1000 permutations from data estimated using DNASP (Hudson et al. 1992). Population connectivity was analyzed using PopART software with *Median-Joining* method (Bandelt et al. 1999).

Tabel 1. Additional sequence of oyster that was used in this research from China, Japan dan Thailand

Species	Lokasi	Acc. Genebank	References
<i>S. cucullata</i>	China	AF458901.1	Lam and Morton 2003
		AF458902.1	Lam and Morton 2003
		AF458903.1	Lam and Morton 2003
		AF458904.1	Lam and Morton 2003
		AF458905.1	Lam and Morton 2003
		AF458906.1	Lam and Morton 2003
	Jepang	AB748908.1	Sekino and Yamashita 2013
		AB748909.1	Sekino and Yamashita 2013
		AB748910.1	Sekino and Yamashita 2013
		AB748911.1	Sekino and Yamashita 2013
		AB748912.1	Sekino and Yamashita 2013
		AB748913.1	Sekino and Yamashita 2013
		AB748914.1	Sekino and Yamashita 2013
<i>C. iredalei</i>	Jepang	AB972008.1	Sekino and Yamashita 2013
		AB972007.1	Sekino and Yamashita 2013
	Thailand	EF122386.1	Bussarawit et al. 2006
		EF122385.1	Bussarawit et al. 2006
		EF122384.1	Bussarawit et al. 2006
		EF122387.1	Bussarawit et al. 2006

Note: *S. cucullata* = *Saccostrea cucullata*; *C. iredalei* = *Crassostrea iredalei*; Acc Genebank: Accept genebank

RESULTS AND DISCUSSION

Allometric growth of the oyster populations in Aceh

The morphological characteristics of the oyster in this study are described by the morphometric of length-to-weight ratio of oyster shell. The morphometric data of oyster samples from the three study sites are summarized in Table 2. The weight calculation of *S. cucullata* in Labuhan Haji, Kuala Gigieng and Loskala was $W = 0.1311L^{2.9203}$ $R^2 = 0.1831$, $W = 0.2508L^{2.7744}$ $R^2 = 0.2474$ and $W = 3.0144L^{7.895}$ $R^2 = 0.769$, respectively. The calculation result for *S. cucullata* in the whole sites was $W = 0.7111L^{2.037}$ with R^2 value = 0.158 (Table 2). Based on the graph of the length-weight ratio of *S. cucullata* in Labuhan Haji, Kuala Gigieng and Loskala, it was showed R^2 percentage of 18.3%, 24.7% and 76.9%, respectively. The calculation of the weight of *C. iredalei* in Labuhan Haji, Kuala Gigieng

and Loskala were $W = 0.765L^{4.923}$ $R^2 = 0.9623$, $W = 0.611L^{1.5909}$ $R^2 = 0.14$ and $W = 0.3569L^{1.3461}$ $R^2 = 0.0312$. The calculation of *C. iredalei* for the whole sites was $W = 0.787L^{2.197}$ $R^2 = 0.384$. The result shows that the percentage of R^2 was 96.2% (Labuhan Haji), 14% (Kuala Gigieng), 3.12% (Loskala) and the total was 38.4%. □

Genetic diversity of *Saccostrea cucullata* and *Crassostrea iredalei*

Genotypes analysis of the 77 samples revealed that there are two species of oysters. Sequence analysis of *S. cucullata* and *C. iredalei* using 16S mtDNA loci along Aceh waters and 19 additional oyster sequences from China, Japan, and Thailand obtained an indented result of 98-99%. As many as 96 DNA fragments of oyster were sorted, and there were 445-569 basepairs obtained. The phylogenetic tree shows two large clades namely *S. cucullata* and *C. iredalei* (Figure 2). The tree shows that the Chinese *S. cucullata* clade is separated from those of Japanese and Aceh waters with a genetic distance of 0.071, while the genetic distances among other populations were ranged from 0.006-0.002. The genetic distance among populations of *C. iredalei* is same, while the genetic distance within the population was 0.000. This value indicates a low genetic distance and close relationship among *C. iredalei* populations (Nei 1972). The knowledge of genetic distance and the phylogenetic tree can identify the closeness between the two oyster species (Jefri et al. 2015).

Table 3. Genetic diversity of *Saccostrea cucullata* and *Crassostrea iredalei* based on haplotype number (Hn), haplotype diversity (Hd), nucleotide diversity, and sample size (n)

Species	Population	N	Genetic diversity		
			Hn	Hd	π
<i>S. cucullata</i>	Labuhan Haji	27	16	0.931	0.005
	Kuala Gigieng	13	12	0.987	0.007
	Loskala	6	4	0.800	0.003
	Japan	7	2	0.285	0.001
	China	6	6	1.000	0.024
<i>C. iredalei</i>	Labuhan Haji	2	2	1.000	0.006
	Kuala Gigieng	18	4	0.634	0.003
	Loskala	11	3	0.618	0.003
	Japan	2	1	0.000	0.000
	Thailand	4	1	0.000	0.000

Table 2. Length-weight relationship of *S. cucullata* and *C. iredalei* with sample size (n), constants (a), growth index value (b), correlation value (r), and determinant (R^2)

Species	Site	n	a	b	r	r^2	Growth pattern
<i>S. cucullata</i>	Labuhan Haji	28	0.131	2.920	0.427	0.183	Negative allometric
	Kuala Gigieng	13	0.250	2.774	0.490	0.247	Negative allometric
	Loskala	6	3.014	2.895	0.840	0.769	Negative allometric
	Total	47	0.711	2.037	0.397	0.158	Negative allometric
<i>C. iredalei</i>	Labuhan Haji	3	0.765	4.923	0.980	0.962	Positive allometric
	Kuala Gigieng	18	0.611	1.590	0.370	0.140	Negative allometric
	Loskala	11	0.356	1.346	0.170	0.031	Negative allometric
	Total	32	0.787	2.197	0.619	0.384	Negative allometric

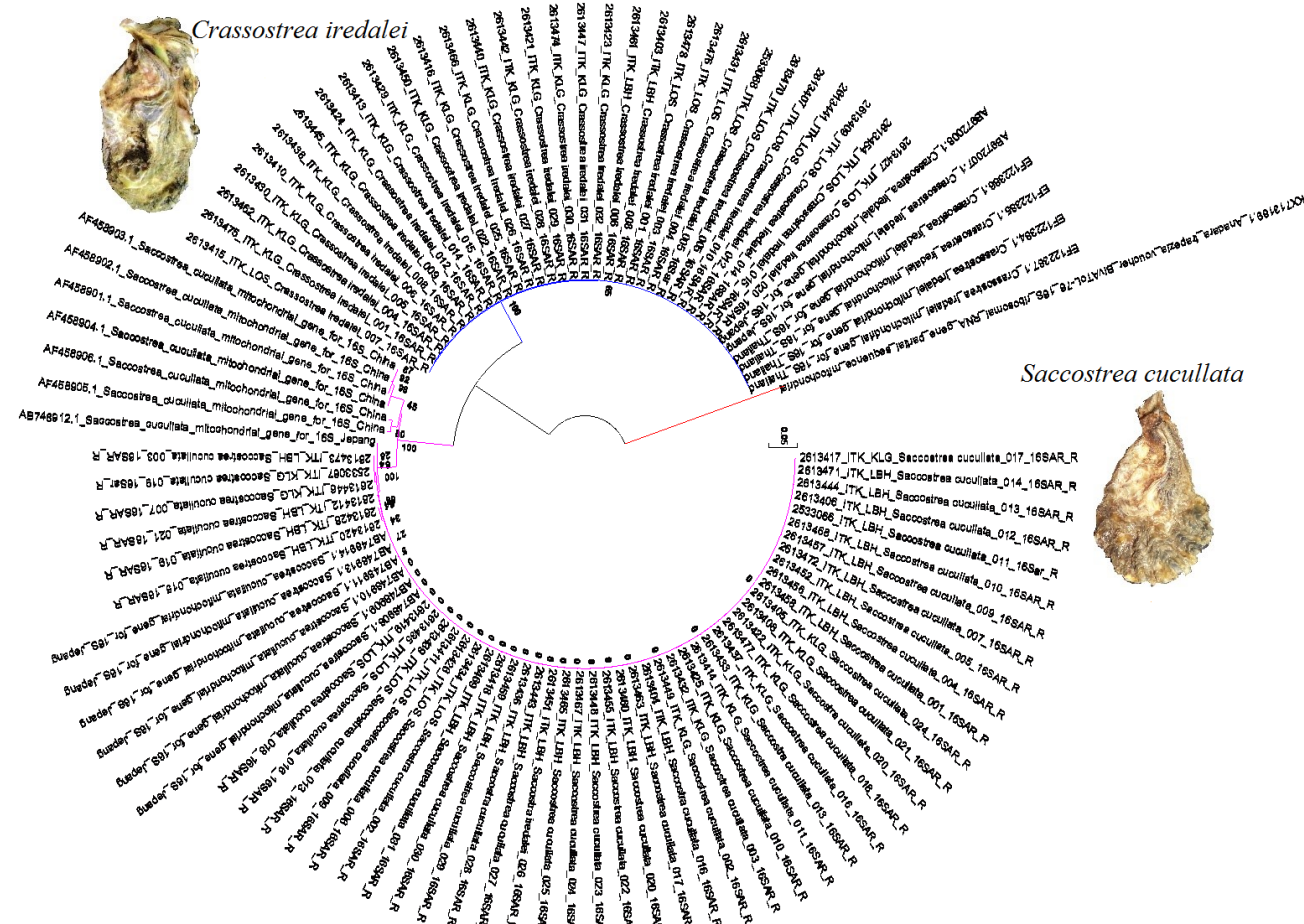


Figure 2. Reconstruction of the phylogenetic tree of *S. cucullata* dan *C. iredalei* using *Neighbour-Joining* method with Kimura 2 parameter model with a *bootstrap* value of 1000x

The genetic diversity of *S. cucullata* in each population was fairly high, with an index ranging from 0.800 to 0.987. The lowest nucleotide diversity was in the Loskala population ($\pi = 0.003$) and the highest in the Kuala Gigieng population ($\pi = 0.007$). The Genetic diversity index of the Chinese oyster population is very high, reaching 1.000 ($\pi = 0.024$). The Japan oyster population has the lowest genetic diversity compared to other regions, namely 0.285 ($\pi = 0.001$) (Table 3).

C. iredalei oyster population sampled from Kuala Gigieng site showed a low genetic and nucleotide diversity (0.634; $\pi = 0.003$). Labuhan Haji site showed a higher value of Hd and π of both species per population, (Nei 1987). The highest values of Hd and π in the oyster population of Labuhan Haji station were 1.00 and 0.006. The Hd and π values for Japan and Thailand population showed the lowest value of 0.000. The nucleotide chains undergoing polymorphisms in *S. cucullata* were 16, in which 10 nucleotides underwent transition and 6 nucleotides underwent transversion. There were 5 nucleotide substitutions of nucleotides in *C. iredalei* consisting of 3 nucleotides transition and 2 nucleotides transversion. A nucleotide transition is much more frequent than a nucleotide transversion (Kochzius and Nuryanto 2008).

Genetic population structure

The evolutionary relationship among 96 oyster samples is shown by the Haplotype network (Figure 4). The results showed that *S. cucullata* had more haplotypes than *C. iredalei*. However, viewed at each population, Kuala Gigieng site showed the highest number of *S. cucullata* haplotypes (9 haplotypes), followed by Japan (7 Haplotypes), Labuhan Haji (7 Haplotypes), Loskala (3 Haplotypes) and China (1 Haplotype). Meanwhile, *C. iredalei* showed 2 haplotypes for Japan, 2 haplotypes for Loskala and 1 haplotype for Labuhan Haji and Kuala Gigieng.

The results of the haplotype network analysis (Figure 4) showed that 7 haplotypes of *S. cucullata* were present only in Japanese water and 5 haplotypes were present only in Kuala Gigieng population. Other haplotypes could be found in all three populations which indicate that there is a combination of haplotypes among the three populations. However, in the Japanese oyster population, there is no combination with other populations as seen from the haplotype network in which there are many isolations occurred between the Japanese oyster population and other populations. The haplotype 1 was found in all the populations whereas the haplotype 2 was only found in the

Loskala oyster population. The same results were also shown in the haplotype network of the Japanese oyster population which exhibit more isolation than that of other populations.

The statistical analysis of population structure between and within populations was tested using molecular variance analysis AMOVA (Excoffier et al. 1992) and paired genetic distance test used F_{st} value. These molecular statistic calculations used Arlequin software version 3.5.2.2 based on the results obtained from Mega 6 software. The

genetic distance value of *S. cucullata* between populations in Aceh waters was 0.003-0.004 (Table 4), while the range of genetic distance within the population was 0.002- 0.005. The genetic distance between populations in *C. iredalei* was the same for all stations, with a value of 0.000. The F_{st} for *S. cucullata* was 0.708 (P-value = 0.000), whereas that of *C. iredalei* was 0.971 (P-value = 0.000) (Table 5). The F_{st} values of these two species belong to the high category of 0.6-1.00 (Excoffier et al. 1992).

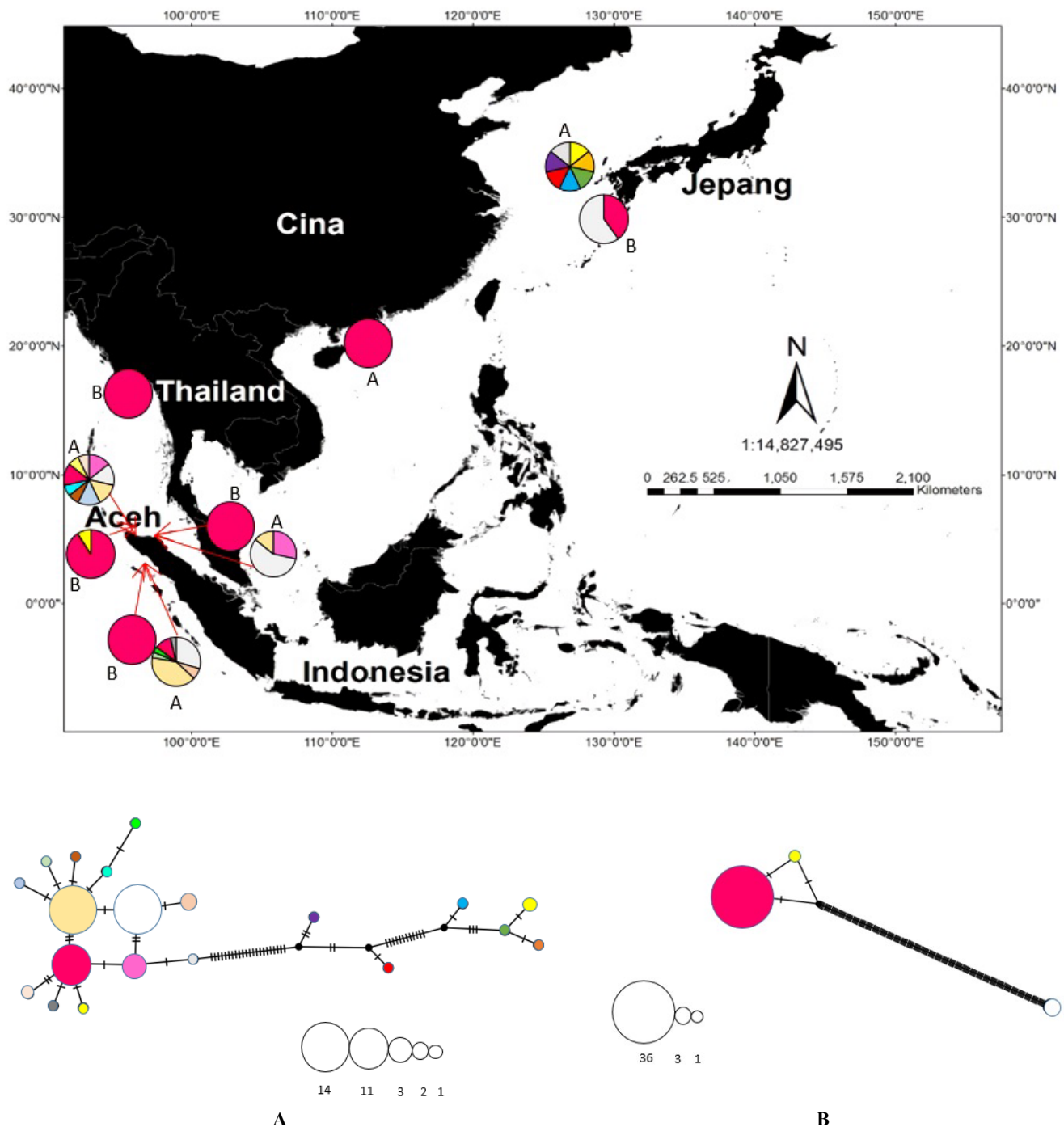


Figure 4. Haplotype network of *Saccostrea cucullata* (A), *Crassostrea iredalei* (B) in each population. The circle line demonstrates the presence of change in nucleotides and the circle size illustrates the frequency of change in haplotype composition

Table 4. Genetic distances analysis between and within populations of *Sacrosstrea cucullata* and *Crassostrea iredalei* for the whole populations

Species	Population	Labuhan Haji	Kuala Gigieng	Loskala	Japan	China	Thailand
<i>S. cucullata</i>	Labuhan Haji	0.003	-	-	-	-	-
	Kuala Gigieng	0.004	0.005	-	-	-	-
	Loskala	0.003	0.004	0.002	-	-	-
	Japan	0.005	0.004	0.006	0.001	-	-
	China	0.071	0.071	0.071	0.067	0.024	-
<i>C. iredalei</i>	Labuhan Haji	0	-	-	-	-	-
	Kuala Gigieng	0	0	-	-	-	-
	Loskala	0	0	0	-	-	-
	Japan	0	0	0	0	-	-
	Thailand	0	0	0	0	-	0

Table 5. Pairwise Fst values between and within populations of *Sacrosstrea cucullata* and *Crassostrea iredalei*

Species	Source of variation	d.f.	Variation (%)	Fst	P-value
<i>S. cucullata</i>	Within a population	4	70.84	0.708	0
	Between populations	54	29.16		
	Total	58			
<i>C. iredalei</i>	Within a population	4	97.10	0.971	0
	Between populations	32	2.90		
	Total	36			

Discussion

Allometric growth of the oyster populations in Aceh

Morphometric characters are measured to know the morphological variations within a species resulted from the difference in environmental conditions and the availability of nutrient in the environment (Chiu et al. 2002). Allometric growth is defined as the increase in size of different organs or parts of an organism at various rates (Gould 1966). Morphometric measurements can give information on the stock composition, age at maturity, life span, mortality, growth, and production (Beyer 1987; Bolger and Connolly 1989; King 1996; Diaz et al. 2000). The total morphometrics showed $b < 3$ for the three observation populations. This b value of *S. cucullata* indicates a faster increase in the length of the oyster shell compared to its weight gain, manifested in very thin body size. Effendie (1997) stated that $b < 3$ indicates an allometric growth in which the increase in body length is faster than the increase in body weight. Interestingly, oyster populations from Labuhan Haji showed $b > 3$ which means the increase in the total weight of the oyster shell is faster than that of the shell length. These results suggest that the number of samples may also affect the b value. The oyster length and total weight of *C. iredalei* showed $b < 3$ in all observation sites. Overall, our morphometric analysis showed that the growth of the two oyster species in all three stations is classified as negative allometric. Negative

allometric growth is the disproportionate growth of parts of an organism as the organism changes in size, in this case, the growth of the oyster shell length is faster than that of the body weight. (Gaspar et al. 2002). Octavina et al. 2014 found that oysters in Kuala Gigieng Station showed a negative allometric growth pattern.

We observed that oysters measured in this study were young oysters and still in the growth stage. This finding was likely due to rapid and continuous consumption of adult oysters in the area. Oysters are hermaphrodite animals (Satino 2003). According to Wang et al. (2004), oysters are born as males until they are able to release sperm, then they will turn into females if environmental condition is suitable (Galtsoff 1964). During this male phase, the oysters focus their growth on the elongation of the shell and sperm production, before lately turn into female oysters (Yang et al. 2014). When a male oyster turns into a female, the oyster will focus its growth on gaining the body weight. Therefore females tend to be heavier than males (Needler 1941). The change of sex in oyster is strongly influenced by the environment (Fulford et al. 2010). If the environmental condition is not conducive, for example, food crisis or population explosion, then the oyster will tend to stay male. This situation might result in a decrease in oyster population that would severely disrupt both their ecological and economic functions (Octavina et al. 2014). In the case of Labuhan Haji site, the growth of oysters was allometric positive because at this station the oysters are not consumed and sold. The difference in the growth rate in the three observation sites is due to the different growth rate of individuals composing each population. Every individual has their own capacity in utilizing energy and minimizing the effect of physiological and other factors (Dody 2010).

Genetic diversity of *Sacrosstrea cucullata* and *Crassostrea iredalei*

DNA analysis has been a successful approach to solving the problem of species identification. In addition, DNA analysis is also capable of answering questions that have not been able to be answered by the morphological analysis approach (Reeb and Avise 1990; Boom et al. 1994; Small and Chapman 1988; Boudry et al. 1998). In

this study, we reconstructed a phylogenetic tree from the three observation stations using the Neighbour-joining method and evaluate it with a bootstrap of 1000x. The distance calculation is based on Kimura 2 parameter method (Tamura et al. 2013), because this option considers the nucleotide transition and transversion rates (Jefri et al. 2015). Neighbor-joining trees obtained from molecular analysis not only solve some taxonomic issues but also identify patterns of biogeographic distribution (Lam and Morton 2005). The sequences of *S. cucullata* and *C. iredalei* from several countries obtained from GenBank are also used in this analysis to see the grouping of all sequences. Furthermore, the out-group included in this analysis come from the same class that is *Anadara Trapezia*. The constructed phylogenetic tree has two large clades namely *S. cucullata* and *C. iredalei*. Clade *S. cucullata* is separated into 2 sub-clusters namely oysters from Aceh waters and Japanese waters with a bootstrap value of 80 and oyster from Chinese waters with a bootstrap value of 45. Meanwhile, *C. iredalei* sequences from Japan and Thailand grouped into one large clade together with *C. iredalei* originated from Aceh waters with a bootstrap value of 65. The phylogenetic trees can be clarified by the genetic distance value of *Saccostrea cucullata* which is relatively high compared to *Crassostrea iredalei*. The genetic distance is determined by the amount of base undergone changes (Ubaidillah and Sutrisno 2009), the more the differences, the more mutations occur which lead to far genetic distance. Oysters are a type of sessile animal that can only mobile during their larval period with the help of water currents of their original habitat (Seliger et al. 1982). In general, overly large oysters will be distributed more widely compared with the less large oysters. For example, *Saccostrea cucullata* can be found in tropical indo-pacific whereas *Crassostrea iredalei* is only found in the South China Sea, Andaman Sea and Gulf of Thailand (Yoosukh and Duangdee 1999). The genetic distance of *C. iredalei* population is low probably because this oyster is not a native biota of Indonesia but originally from the Philippines (Poutiers 1998). From the Philippines, the larva disperses and reach Indonesia and the surrounding waters, causing a very limited gene flow. In contrast, *S. cucullata* has a very wide geographical range in which the larva is originated from the Indo-Pacific and disperse to South Africa (Poutiers 1998), the Malay Archipelago and North Australia (Buroker 1985); therefore, the gene has high heterozygosity level (Permana et al. 2006).

Nucleotide composition of *S. cucullata* species was 26.5% (Thymine), 18.1% (Cytosine), 28.9% (Adenine) and 26.5% (Guanine). The biggest nucleotide composition was the A+T pair with the average amount of 27.7% while the lowest was the pair G + C with the average amount of 22.3. The *C. iredalei* showed an average nucleotide composition of 29.6% (Thymine), 16.2% (Cytosine), 29.4% (Adenine) and 24.8% (Guanine). The most predominant nucleotide pair was the pair A + T with a frequency of 29.5%, and the lowest was the G + C pair with an average amount of 20.5%. If two different species have more A + T base composition, then the species will have many similarities because of the independent parallel substitution.

Consequently, they will be grouped by the similarity in their base composition (Lam and Morton 2006). Therefore, the similarity in nucleotide composition may explain morphological similarities observed on the two oyster species, and hence it is very difficult to distinguish them morphologically.

There are several mutation-driven nucleotide polymorphisms in the two oyster species (Ubaidillah and Sutrisno 2009). There are two types of nucleotide substitution mutation namely transition and transversion. The transition is the change between Adenine and Guanine (Purine) or between Citocyne and Thymine (pyrimidine), while transversion is the change between purine or pyrimidine (Ubaidillah and Sutrisno 2009). The polymorphisms occurred in the nucleotide chain of *S. cucullata* were 10 nucleotide transitions and 6 transversions. Nucleotide substitutions in *C. iredalei* occurred in 5 nucleotides of which 3 were transitions and 2 were transversions. Nucleotide transition is more predominant than transversion (Kochzius and Nuryanto 2008). Mollusks living in the sea have high levels of polymorphism due to the widespread of larvae, abundant populations and extensive dispersal (Ward et al. 1992; Bazin et al. 2006). Mutation or gene variation in population has proven to be very useful as an indicator for the population structure and is particularly sensitive to reproductive restrictions (Chapman 1989; Boom et al. 1994). Genetic variation and genetic diversity are important and has been linked to organismal complexity (Lynch and Conery 2003), long-term population survival in ecosystem recovery (Reusch et al. 2004), and species adaptability to environmental changes (Ellegren and Galtier 2016; O'brien 1994). Heterozygosity in many cases is considered the primary parameter to reflect the overall population genetic variability (Zhong et al. 2016).

Several comparative studies have demonstrated the diversity of *Tridacna crocea* haplotype ranging from 0.77 to 1.00 (Kochzius and Nuryanto 2008); the diversity of *S. cucullata* 0.6285 and *C. iredalei* 0.1857 (Klinbunga et al. 2005). In this study, the nucleotide diversity of *S. cucullata* ($\pi = 0.3501$) and *C. iredalei* ($\pi = 0.09$) are low compared to that of the previous report by Klinbunga et al. 2005. Based on the Hd and π values of both species per population, the Labuhan Haji population showed a higher average value. This is because oysters in Labuhan Haji are not exploited as heavily as those in the other observation stations wherein oysters become the livelihood of the local people. Exploitation is one of the key factors that determine the number of haplotypes and the nucleotide diversity in a population (Kochzius and Nuryanto 2008).

Genetic population structure and connectivity

The goal of population genetics is to understand the processes that influence the interconnectivity among populations of a species as a result of local adaptation, and eventually speciation based on the genetic structure (Fauvelot et al. 2003). The results of haplotype diversity analysis indicate a close relationship between oysters in all populations, haplotype diversity closely related to genetic diversity. Another factor that might influence close genetic

distance between populations is the absence of geographic complexity (Avice 1992). Oceanographic factors such as currents and tides have contribution to the pattern of dissemination and recruitment of marine biota (Findly and White 2003). The ocean current is different from place to place. This difference is probably caused by the irregular current movements within a year in the study sites. Ocean currents change every half year and correspond to the movement of the seasonal wind, resulting in even larval distribution to the north and south (Sverdrup et al. 1942). The larvae from these oysters can easily be carried away by the Indian Ocean currents along the Aceh coast which are only distinguished by the small current patterns between the Malacca Strait and the Andaman Sea. Those oceanographic factors might have maintained high and relatively similar levels of genetic diversity between populations. The tidal currents in the Indonesian waters are strong and affect the vertical water mass mixing. Potential factors that play a role in organism distribution are the current conditions and duration of larvae in the waters (Saleky et al. 2016).

The isolation observed in the Japanese oyster population is due to the genetic distance. The cycle of cold currents surrounding Japanese waters keeps the sessile biota that relies on their larval period to find a rich habitat in food supplies to remain in the vicinity of the Japanese waters. The Japanese water is flanked by two types of currents, namely Oyashio and Kuroshio currents (Nomura and Yamazaki 1977; Nomura 1991). The Oyashio is a cold current that moves from the southern Pacific Ocean, while the Kuroshio is a hot current that moves from the North Coast of Japan (Buesseler et al. 2011). Both currents are from the North Pacific Currents. The Kuroshio current is the continuation of the northern equatorial currents: after arriving in the Philippines, the direction of the current goes to the north and its movement is driven by the west wind.

Based on the results a very high-level F_{st} test and the genetic distance (Table 5) showing that the population was highly structured. The F_{st} shown in this study much higher than observed by Wang et al. (2004) reported that the F_{st} value of oysters in Chinese waters ranged from 0.0036-0.2705. This value tends to be high because there were large numbers of the oyster species found in this region, leading to high genetic diversity and in accordance to the population structure (F_{st}) (Wang et al. 2004). The genetic distance is determined by the amount of base that undergoes changes. The more the difference, the higher the mutation frequency, and the farther the distance (Ubaidillah and Sutrisno 2009). The greater the value of the genetic distance, the greater the gene flow into a population, so it can be explained that the current lion's share in the genetic distribution of each population. Gene flow is associated with the geographic isolation that is affected by the geographical distance and complexity of environmental diversity (Arnaud et al. 1999).

This study concludes that the growth pattern of *S. cucullata* and *C. iredalei* is negative allometry. This finding shows that the environmental impact might directly affect the oyster genetic structure. This genetic diversity in *S. cucullata* and *C. iredalei* indicates the presence of gene

flow in each population and their contribution in response to different environmental conditions. The population structure and phylogenetic reconstruction suggest that oysters from Aceh waters originated from the same population and had the same dispersal pattern because of the close geographic distance and the connection of a direct current pattern. *S. cucullata* and *C. iredalei* oysters from China, Thailand and Aceh waters have the same haplotype, demonstrating the presence of the respective gene (mtDNA 16S) in their populations.

Research on the population structure and genetic diversity of *S. cucullata* and *C. iredalei* may be helpful in developing conservation management because environment strongly affects oyster growth. This information may also increase the awareness of fishermen on oyster sustainability and educate them to be more selective in catching oysters and developing oyster cultivation systems.

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