BIODIVERSITAS
Volume 18, Number 3, July 2017
Pages: 1018-1025

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d180320

Genetic diversity of *Tor douronensis* (Pisces: Cyprinidae) in West Sumatra, Indonesia

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Manuscript received: 21 November 2016. Revision accepted: 7 June 2017.

Abstract. *Roesma DI, Tjong DH, Munir W, Agesi AV, Chornelia A. 2017. Genetic diversity of* Tor douronensis *(Pisces: Cyprinidae) in West Sumatra, Indonesia. Biodiversitas 18: 1018-1025.* A random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) analysis was applied to determine the genetic diversity within and among the populations of *Tor douronensis* in West Sumatra river system. The results provided information for proper breeding, maintenance, and conservation of *T. douronensis*. The highest and the lowest values of genetic diversity were the population of Batang Matur (H = 0.1571; I = 0.2411) and Batang Malalo (H = 0.0880; I = 0.1353), respectively. The value of genetic differentiation (G_{ST}) was 0.4266 while that of heterozygosity between populations (D_{ST}) and within a population (H_S) was, respectively, 0.088 and 0.1184. The value of gene flow between populations (Nm) was 0.6721. Grouping of individuals in a population based Principal Coordinates Analysis (PCO) and UPGMA was in line with the genetic distance values. Matur and Malalo were the most closely related populations (0.0174) while Batang Gumanti and Batang Sinuruik were the most distantly related populations (0.1918). The genetic variation between populations was lower than the genetic variation within the population of *T. douronensis* and population of Matur is recommended to be used as brood stock in the procurement of stock.

Keywords: Genetic variation, Cyprinidae, *Tor douronensis*

INTRODUCTION

Genetic diversity or variability is an essential characteristic of any population for the fitness of individuals as well as the survival of the whole population, permitting adaptation to the changing environmental conditions and stress (Mukhopadhyay and Bhattacharjee 2014). *Tor douronensis* is one of freshwater fish, belonging to the family of Cyprinidae, known as Mahseer, or Semah (named as Garing fish in local West Sumatra people). *T. douronensis* was a favorite freshwater fish in the fishing games and as a food source. This fish uses rivers to migrate from lowland to highland for food and breeding, thus they can reveal a wide range of genetic diversity as regards to adaptation to different ecological conditions. According to Roberts (1999), *Tor* species probably occur in large mountain streams throughout the Malay Peninsula and the larger Sundaic islands of Borneo, Sumatra, and Java. In general, the existence of the genus of *Tor* is under threat. Of these threats are including overfishing, pollution, habitat loss and change. Breeding experiment programs on *T. douronensis* has been carried out, but the program is not satisfactory because of the lack of supporting data both phenotypically and genetically. Information on genetic variation in fish species is important to support breeding and conservation programs. Turan et al. (2004) and Chandra et al. (2010) emphasized that the detail information on the population structure of the species is important for sound biodiversity management of fisheries resources. One of that information is genetic variation.

The evaluation of genetic variation has needed a rapid and precise method using the molecular marker. Random Amplified Polymorphic DNA (RAPD) marker has been widely applied in genetic variation of fish. According to Fuchs et al. (1998) and Bardakci (2001), RAPD-PCR can be used as an efficient molecular tool to differentiate allopatrically and/or sympatrically isolated populations and has been widely used to delineate the available gene pool of locally adapted populations of a species that may have arisen either by means of genetic selection under different environmental pressure or as a result of genetic drift. There is a serious decline of Mahseer population in different ecosystems and it will threaten its existence. The degradation of genetic diversity of a species that may be due to decreasing population size or damage of habitat will reduce its capability for adaptation and increases the risk of its extinction. Therefore, it is necessary to determine the genetic variation of *T. douronensis* between and within populations in West Sumatra to detect the populations which have a high genetic variation to be used as germplasm both for cultivation and conservation. Our results provide firsthand information that may be used in the proper breeding, maintenance, and conservation of this fish fauna as well as in other dwindled population.

MATERIALS AND METHODS

Samples collection

A total of 22 rivers have been surveyed to collect *T. douronensis* and the populations were found in 21 rivers. Six populations with the largest number of individuals were used for analysis. These population included *T. douronensis* from Batang Gumanti River, Batang Antokan River, Batang Malalo River, Batang Matur River, Batang Sinuruik River and Lubuk Mangkuih River (Figure 1). The DNA samples were taken from liver tissues.

DNA extraction

Genomic DNA of *T. douronensis* samples was isolated by using DNA isolation kit (High Pure PCR Template Preparation Kit, Roche, Germany) with a slight modification in water volume. The DNA quality and quantity were determined by using agarose gels electrophoresis.

Primer selection

Fifty arbitrary decamer primers of random sequences obtained from Operon Technologies Inc., Alameda, USA were tested for further analyses on the basis of the numbers, variability, and reproducibility of the bands obtained. The tested primers are listed in Table 1. All primers have 60-70% G-C content.

Table 1. Primers and primer sequences tested for detection of polymorphism in *T. douronensis* populations

Primer	Sequence (5'-3')	G + C Content (%)
OPA01	CAGGCCCTTC	70
OPA02	TGCCGAGCTG	70
OPA03	AGTCAGCCAC	60
OPA04	AATCGGGCTG	60
OPA05	AGGGGTCTTG	60
OPA06	GGTCCCTGAC	70
OPA07	GAAACGGGTG	60
OPA08	GTGACGTAGG	60
OPA09	GGGTAACGCC	70
OPA11	CAATCGCCGT	60
OPA12	TCGGCGATAG	60
OPA13	CAGCACCCAC	70
OPAA-01	AGACGGCTCC	70
OPAC-15	TGCCGTGAGA	60
OPB-10	CTGCTGGGAC	70

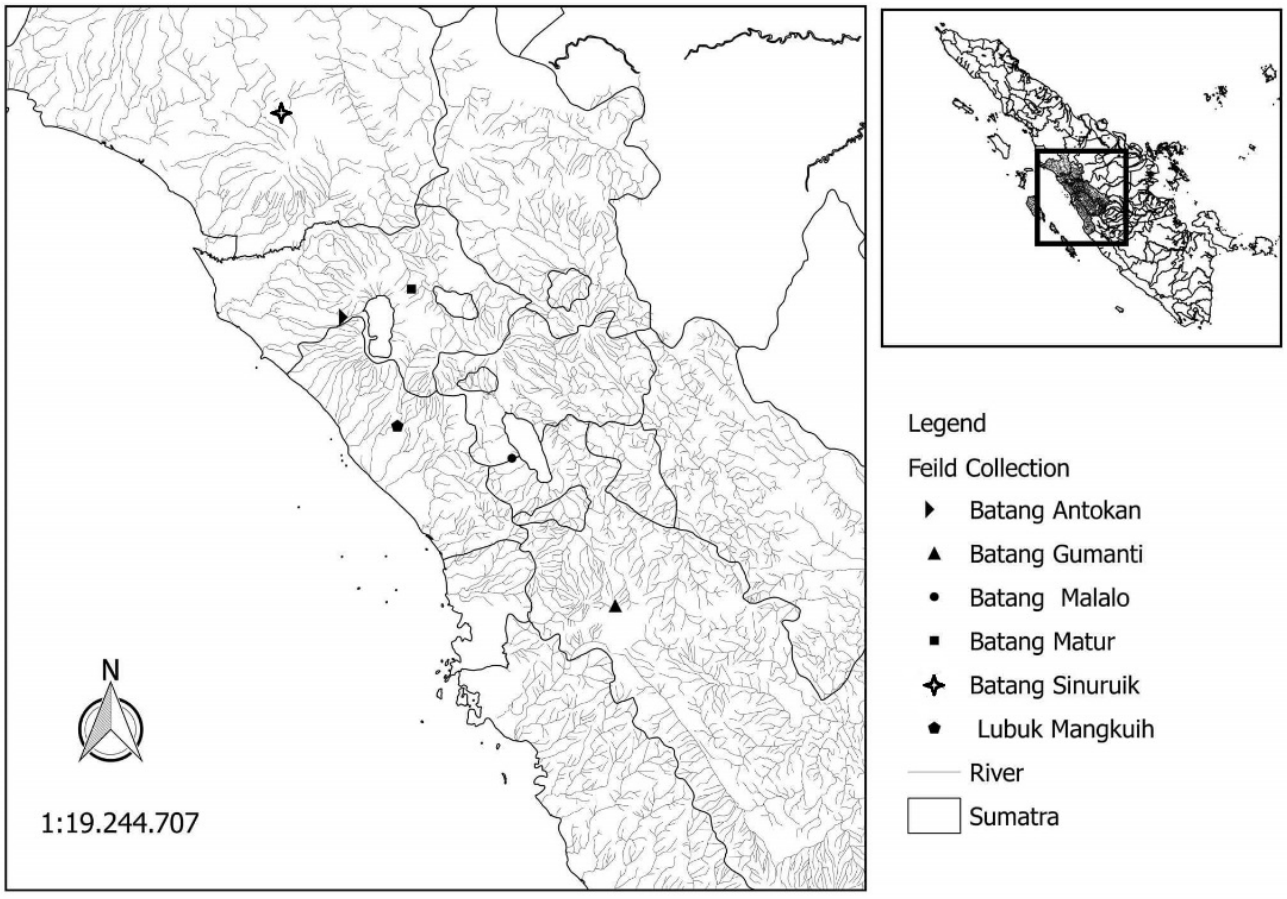

Figure 1. The locations map of sample collection in West Sumatra, Indonesia

RAPD-PCR assay and documentation of amplified products

PCR cycling programs were performed as follows: initial denaturation at 94^0C for 2min followed by 45 cycles of 94^0C, 1min for denaturation; 36^0C, 1min for annealing; 72^0C, 2.5 min for elongation; and finally an extension at 72^0C for 10 min (Yoon and Park 2001). The amplified products were size-fractionated in 2% (w/v) agarose gel with ethidium bromide 6.5 µl at a constant voltage of 100V and 100mA current in TAE buffer (40mM Tris-HCl, pH 8.0; 20mM acetic acid; and 1mM EDTA, pH 8.0) using electrophoretic apparatus. The molecular size of each band was estimated using a standard 100 base pair ladder. The gels were visualized on the UV-trans illuminator gel document system.

Statistical analysis

The RAPD is a dominant marker that cannot distinguish between homozygous and heterozygous dominant individuals. RAPD data was analyzed for assessing within and between populations genetic variability of *T. douronensis* in the six rivers. The RAPD marker profiles were determined by direct comparison of the amplified profiles and the obtained data were computed and analyzed in the form of binary variables (1 = band was present or 0 = band was absent). The RAPD data was analyzed using POPGENE 1:32 software (Yeh et al. 1997). The program analyzed both the Value of genetic distance (genetic distance) and the value of gene flow (*Nm*). Genetic variation within and between populations can be determined by calculating the average number of alleles per-locus (*Na*). The results of the analysis also showed the percentage of polymorphic loci (*Pp*), genetic diversity of Nei (*H*), phenotypic diversity index of Shannon (*I*), heterozygosity in a subpopulation (H_S), heterozygosity of total population (H_T), Value of heterozygosity between populations (D_{ST}), and genetic differentiation between populations (G_{ST}).

RESULTS AND DISCUSSION

Of the 15 selected primers, 10 primers showed amplification product and polymorphism. The primers were OPA 01, OPA 02, OPA 03, OPA 04, OPA 07, OPA 09, OPA 11, OPA 13, OPB 10 and, OPAA 01. Visualization of DNA electrophoresis resulted from amplification using the selected primers are shown in Figure 2.Different banding patterns produced by different primers. The results of 10 primers PCR amplification showed the varying size of bands which reflected the molecular weight of scorable bands generated by these primers. The band sizes ranged between 188 and 2000 bp (Table 2).The total number of fragments were 201. The number of band on each primer ranged from 13 bands (OPA 01) to 28 bands (OPA 11), with an average of 20.1 bands per primer (Table 3.)

From the present study, we obtained 197 polymorphic bands with an average of 19.7 bands per primer and 4 monomorphic bands with an average of 0.4 bands per primer. Polymorphic bands of each primer were also varied from 11 to 28 bands. A number of the polymorphic band also revealed the extent of genetic variation within a species. The value 11 to 28 bands indicated that genetic variation of *T. douronensis* in West Sumatra was high. Arora and Julka (2013) described in their research on genetic variation of *T. putitora* that the range of the number of the polymorphic band between two to six indicated that the species has low genetic variation.

Of the ten primers, seven primers produce DNA bands that were all polymorphic (100%). The primers were OPA 02, OPA 04, OPA 07, OPA 09, OPA 11, OPA 13 and OPAA 01. The other three primers (OPA 01, OPA 03 and OPB 10) showed polymorphism of 86.4%, 94.1% and 96.0%, respectively (Table 1). The high polymorphism bands obtained in this study indicated that these primers could be used to detect the genetic variation in populations of *T. douronensis* in West Sumatra.

From DNA band profiles, we observed that each of the population shows the existence of specific bands. Specific band is a band that is only found in one population and not found in other populations. The data in Table 4 shows that the populations with the high number of specific bands are populations from Batang Gumanti and Batang Sinuruik which consisted of nine and eight specific bands, respectively; meanwhile, no specific band was found in the population of Batang Matur. Specific band in a population can be used to differentiate the individuals in a population with that of other population. According to Mohindra et al. (2007), the number of specific bands indicates the occurrence of the genetic variation between populations. In addition, Dieckmann et al. (2004) concluded that the more specific band they have indicated the higher genetic differentiation between populations, and over the time it will give a possibility to the process of speciation.

The presence of a specific band in a population may be due to the geographic isolation. Population in perfectly isolated habitats enables the prevention of migration of individuals between populations. If there is a limited gene flow between populations, the frequency of genes in a population will be different from before, in which the genetic mixing between populations does still occur. Changes in gene frequencies will trigger the genetic differences between populations. The number of the specific band on Batang Gumanti and Batang Sinuruik populations indicated that these populations have been isolated so that the possibility of migration of individuals between populations is less likely to occur and no genetic mixing will take place. The absence of a specific band in Batang Matur population indicated that the population is in an equilibrium state and is able to maintain its genetic structure. In a large population, random mating is highly likely to take place so that genetic variation can be maintained and gene frequency in the population is stable.

Table 2. Type of the primers used and the size of the resulting band

Primer	Band Size (bp)
OPA 01	1.900, 1.780, 1.500, 1.125, 1.075, 1.013, 950, 733, 560, 425
OPA 02	1.730, 1.700, 1.600, 1.580, 1.550, 1.500, 1.375, 1.188, 1.063, 950, 850, 730, 670, 600, 550
OPA 03	1.600, 1.500, 1.375, 1.250, 1.125, 1.062, 1.000, 933, 800, 767, 733, 675, 625, 550, 440
OPA 04	1.409, 1.227, 1.136, 1.090, 1.000, 950, 925, 833, 750, 640, 525, 486, 443, 283
OPA 07	1.723, 1.580, 1.438, 1.250, 1.063, 700, 560, 475, 450, 425, 400, 320, 220
OPA 09	2.000, 1.500, 1.335, 1.285, 1.215, 1.167, 925, 800, 700, 650, 625, 575, 550, 440, 350
OPA 11	1.680, 1.500, 1.375, 1.312, 1.125, 950, 800, 725, 700, 633, 600, 550, 525, 500, 460, 400, 370, 340, 333, 320
OPA 13	1.700, 1.600, 1.428, 1.400, 1.357, 1.200, 1.188, 1.063, 950, 833, 775, 700, 675, 625, 600, 480, 383
OPB 10	1.870, 1.670, 1.600, 1.540, 1.437, 1.300, 1.250, 1.063, 950, 900, 850, 825, 800, 700, 600, 360, 300, 188
OPAA 01	1.725, 1.700, 1.600, 1.500, 1.313, 1.125, 1.000, 867, 817, 800, 725, 700, 540, 500, 450, 400, 385, 343, 300

Table 3. Types of the primers and RAPD band profiles generated from the 50 samples of *T. douronensis*

No.	Primer	Total number of band	Number of monomorphic bands	Number of polymorphic bands	Percentage of polymorphic bands
1.	OPA 01	13	2	11	84.6
2.	OPA 02	17	0	17	100
3.	OPA 03	17	1	16	94.1
4.	OPA 04	21	0	21	100
5.	OPA 07	21	0	21	100
6.	OPA 09	16	0	16	100
7.	OPA 11	28	0	28	100
8.	OPA 13	23	0	23	100
9.	OPB 10	25	1	24	96.0
10.	OPAA 01	20	0	20	100
Total		201	4	197	974.7
Average		20.1	0.4	19.7	97.47

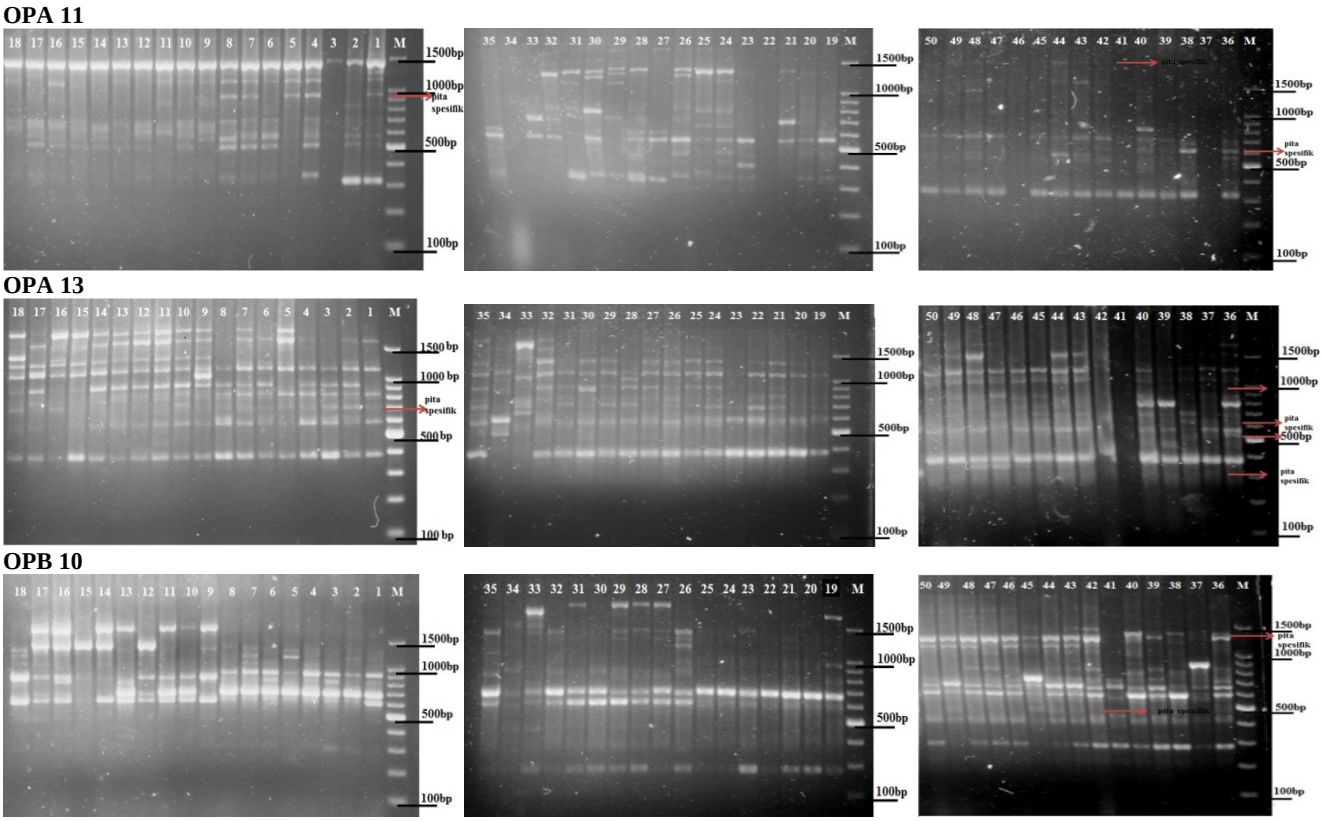

Figure 2. Representative 2% agarose gel showing RAPD *Tor douronensis* fragment patterns generated using OPA 11, OPA 13 and OPB 10 primers (three of ten primers) from Batang Gumanti samples (1-8); Batang Antokan samples (9-18); BatangMalalo samples (19-25); BatangMatur samples (26-35); Batang Sinuruik (36-40); Lubuk Mangkuih samples (41-50). M = Marker

Table 4. Profile of specific band on six populations of *T. douronensis*

Loci	Population					
	BG	BA	ML	MT	BS	LM
OPA 02-1.600	V					
OPA 02-1.730		V				
OPA 03-733	V					
OPA 03-1.660						V
OPA 04-329					V	
OPA 04-486	V					
OPA 04-500					V	
OPA 04-712					V	
OPA 09-575	V					
OPA 09-1.167		V				
OPA 09-1.285		V				
OPA 09-1.335	V					
OPA 09-2000	V					
OPA 11-612					V	
OPA 11-950	V					
OPA 11-1.900						V
OPA 13-360					V	
OPA 13-525					V	
OPA 13-612	V					
OPA 13-625					V	
OPA 13-1000					V	
OPB 10-460						V
OPB 10-1.445					V	
OPAA 01-300			V			V
OPAA 01-1.278						
Total	8	3	1	0	9	4

Note: BG = Batang Gumanti; BA = Batang Antokan; ML = Malalo; MT = Matur; BS = Batang Sinuruik; LM = Lubuk Mangkuih.

Table 5. Results of the analysis of genetic variation of *T. douronensis* in each population

Population	n	H	I	Pp (%)
Batang Gumanti	8	0.1033	0.1543	29.85
Batang Antokan	10	0.1074	0.1607	31.34
Batang Malalo	7	0.0880	0.1353	28.36
Batang Matur	10	0.1571	0.2411	52.74
Batang Sinuruik	5	0.1177	0.1708	28.86
Lubuk Mangkuih	10	0.1366	0.2001	36.32

Note: H: average of heterozygosity/Nei's gene diversity, I: Shannon information index, Pp%: Percentage of polymorphic loci, n: number of samples

Table 6. Results of the analysis of genetic variation in 35 individuals of *T. douronensis* and the value of gene flow.

Number of samples	H_T	H_S	D_{ST}	G_{ST}	N_M
50	0.2064	0.1184	0.088	0.4266	0.6721

Note: H_T: Total Heterozygosity in a population (HS+DST), H_S: Heterozygosity within population, D_{ST}: Heterozygosity inter-population, G_{ST}: Genetic differentiation inter-population, N_M: Gene flow value

Tor douronensis genetic diversity can be estimated by the percentage and the number of polymorphic loci. The highest percentage of polymorphic loci was found in *T. douronensis* population of Batang Matur (52.74%) with 106 polymorphic loci while the lowest percentage of polymorphic loci was found in the population of Batang Malalo (28.36%) with 75 polymorphic loci. The average of heterozygosity (*H*) and Shannon Index (*I*) were also used to determine the genetic diversity in a population (Nei and Kumar, 2000). Batang Matur population showed the highest mean values of heterozygosity and Shannon Index (*H*=0.1571 and *I* = 0.2411). Meanwhile, population of Malalo showed the lowest mean values heterozygosity and Shannon index (*H* = 0.0880 *I* = 0.1353) (Table 5.)

Our study revealed that the overall genetic diversity of *T. douronensis* in Batang Matur population was higher than other populations (Lubuk Mangkuih, Batang Antokan, Batang Gumanti, Batang Sinuruik and Batang Malalo) (Table 5). The high value of 1) the average heterozygosity, 2) the percentage of polymorphic loci and 3) Shannon index of Batang Matur population probably caused by the population size of *T. douronensis* in Batang Matur. According to Frankham et al. (2002), a large population can prevent the decline of genetic diversity due to inbreeding effects and fixation of some specific alleles in the population. Therefore, in a large population, the chance of random mating is higher, and the heterozygosity will increase. Avise (1994) and Dunham (2002) concluded that the high value of heterozygosity is important for a population to maintain its long-term survival and also to ensure fitness to enable the population to adapt to environmental changes. The lowest values of 1) the average value of heterozygosity, 2) the percentage of polymorphic loci and 3) the Shannon index was found in Batang Malalo population. These conditions may be due to the small population size of *T. douronensis* on this site. Silas et al. (2005) reported in his research on genetic diversity in Balamore river that percentage of polymorphic loci of *T. malabaricus* was low (21.79%), which presumably due to the small population size that triggered high levels of inbreeding.

The small population size can be caused by natural habitat damage of *T. douronensis* (Ng, 2004). Lack of suitable habitat depresses population growth and reduces the reproductive ability while reproduction is critical for the survival of gene diversity. Reproduction is a fundamental unit and its presence is important for the formation of new individuals and the diversity of genetic resources of a population. Additionally, an increase of exploitation of habitats will result in the increase of critical populations in their natural habitat. Kottelat et al. (1993) stated that most species of the genus *Tor* are threatened, especially by forest clearing and overfishing. In addition to population size, Nugroho et al. (2005) stated that the low genetic variation indicates that the population has low migration rate and is isolated, therefore, the exchange of genes between populations are less frequent. Restriction of gene exchange will result in inbreeding. This situation, in the long term, will result in low genetic variation and increased homozygosity. These conditions will get worse

with the damage of *T. douronensis* habitat because it will reduce the fitness of *Tor* population. Roesma et al. (2016) strengthened the importance of paying more attention to the water condition in Sumatran Rivers because it is important for Mahseer fish habitat. As this fish species is a migratory species heading to the headwaters for spawning, they need clear and fast flowing water.

Overall analysis of genetic variation shows that the D_{ST} value (0.088) is lower than H_S value (0.1184), meaning that genetic variation between populations is lower than the genetic variation within the population. This is supported by the calculated genetic differentiation value (G_{ST}) of *T. douronensis* in West Sumatra (0.4266) (Table 6.) G_{ST} value indicates that 42.66% of the total genetic variation was in between population and 57.34% was within the population. Beaumont and Hoare (2003) stated that low G_{ST} values had been affected by the *Ne* value (average number of effective alleles). If *Ne* is high, then the allele frequency differences between populations will be small, and G_{ST} will be low. G_{ST} value obtained in the present study was supported by the high value of gene flow (0.6721). Low G_{ST} and high gene flow (N_M) values showed that the population size in each population is large, and therefore, a random mating can take place so that the heterozygosity and genetic variation within a population can be maintained and increased. Based on those values it can be concluded that in West Sumatra there were no significant genetic differences among *T. douronensis* between populations.

The high N_M and low G_{ST} values obtained in the present study were in line with the view of PCO plot (Figure 3). The high gene flow value is reflected in the population of Batang Malalo with Batang Matur, Batang Gumanti with Batang Antokan and Lubuk Mangkuih with Batang Sinuruik, which are overlapping one to another. There was no relationship of groupings between populations with the geographical distance. Each group consisted of a pair of the population derived from rivers that do not share a geographical relationship. The separation or groupings that occur are estimated to be due to the number of specific bands possessed by the populations and the ownership of a shared allele.

The result of PCO analysis obtained is in line with the results of UPGMA cluster. The grouping patterns of *T. douronensis* between populations can be seen in the dendrogram (Figure 4). Dendrogram was generated using UPGMA (Unweighted Pair-Group Method with Arithmetic mean) (Nei 1991) using the software MEGA ver. 4 (Tamura et al. 2007).

Illustration in Figure 4 shows the genetic relationship among *T. douronensis* populations in West Sumatra. Grouping based on cluster analysis using UPGMA was supported by the value of genetic distance between each population (Table 7). Based on the calculated genetic distance of six *T. douronensis* populations, the smallest genetic distance value was found between Batang Malalo and Batang Matur populations (0.0174). Meanwhile, the largest value was found between Batang Gumanti and Batang Sinuruik populations (0.1918).

Figure 4. Dendrogram showing the genetic relationship among six *Tor douronensis* populations in West Sumatra

Table 7. Matrix of genetic distance in six populations of *T. douronensis*

No	Population	1	2	3	4	5	6
1	Batang Gumanti	0.0000					
2	Batang Antokan	0.1118	0.0000				
3	Batang Malalo	0.1194	0.1412	0.0000			
4	Batang Matur	0.1083	0.1140	0.0174	0.0000		
5	Batang Sinuruik	0.1918	0.1669	0.1191	0.1035	0.0000	
6	Lubuk Mangkuih	0.1845	0.1586	0.1172	0.0923	0.0414	0.0000

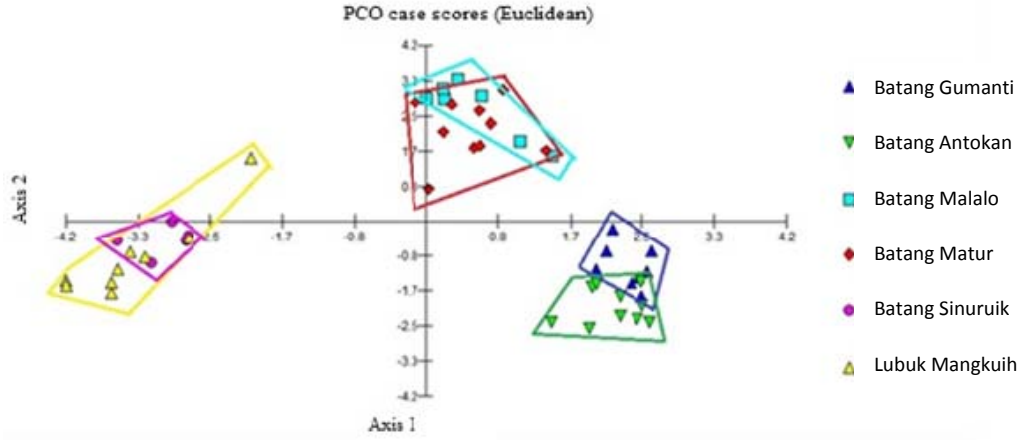

Figure 3. Plot of Ordinated Principal Coordinates Analysis (PCO) of all populations

The close relationship between BatangMalalo and Batang Matur populations indicated that fish from the two regions may have derived from the same lineage. The small value of the genetic distance between Batang Malalo and Batang Matur showed that the populations can maintain their allele which is indicated by the number of the common bands they have. The high value of genetic distance between Batang Gumanti and Batang Sinuruik population presumably because the two populations are not able to maintain their alleles as indicated by the number of specific bands they possessed. It is believed that the different environmental conditions between Batang Sinuruik and Batang Gumanti have given different influences in each population. Solue and Gilpin (1986) stated that environmental factors might influence the genotype as a process of adaptation or defense as a result of the change of environment. Geographically, the location between the two areas is quite far apart. These factors may also have affected the value of the genetic distance between these populations. Geographical distance caused the migration capability is limited so that the relationship between two populations become genetically apart. Iguchi et al. (1999) stated that the isolation due to differences in the distance is one of the factors that affect the rate of gene flow between far apart locations and eventually lead to genetic differences.

In the previous analysis, it was explained that based on PCO analysis (Figure 3) and UPGMA cluster (Figure 4) there was no relationship of groupings between populations with geographical distances (Batang Malalo-Batang Matur populations, Batang Gumanti-Batang Antokan populations, and Lubuk Mangkuih-Batang Sinuruik populations). Each of these groups also consisted of a pair of the population that came from rivers that have no geographical relationship. The grouping of individuals within each population was estimated because each population has an almost equal ability to maintain the inherited alleles of its ancestors. This can be observed from the number of specific bands possessed by the population and the specific allele they shared. Thus, for this pair of the allopatric population, the equality of shared allele has put this population within overlapping groups.

In conclusion, based on RAPD-PCR analysis, the genetic variation of *T. douronensis* inter populations is lower than that of intra-population. The population of Batang Matur is recommended to be used as brood stock in the procurement of stock. As Carvalho (1995) and Dinesh et al. (1996) state that generally individuals with greater genetic variation have higher growth rates, developmental stability, viability, fecundity and resistance to environmental stress and diseases.

ACKNOWLEDGEMENTS

This work was supported by Grants of Ministry of Research, Technology and Higher Education to Andalas University, Padang, Indonesia (No.31/H.16.UPT/LPPM/ 2015). We extend our thanks to the management for the funding support. Our thank also goes to Department of Biology, Andalas University for field and laboratory work permit. We also would like to thank our students who helped us in sample collection and laboratory works in the Laboratory of Genetic and Biology Cell, Faculty of Mathematic and Natural Sciences, Andalas University, Padang, Indonesia.

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