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Genetic Variation of "Sacred Fish" Population in Tourism Site of Sungai Janiah West Sumatra

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Abstract: Sacred fish is one of the forbidden fish in West Sumatra which found in Sungai Janiah Tourism site and had been isolated for hundreds. That condition allows the low genetic variation due to the high incidence of inbreeding. The purpose of the study was to analyze the genetic variation among Sacred Fish in Sungai Janiah pond, West Sumatra. The study used the direct survey and sample collection. The analysis of genetic variation using Random Amplified Polymorphism DNA (RAPD). The result showed that there were 28 polymorphic loci (N) with polymorphic locus percentage (PLP) 45.16%. The heterozygosity value (H) is 0.1897. The Shannon diversity index (I) is 0.2827. That value concludes that the genetic variation of the Sacred Fish in Sungai Janiah is low.
Keyword: Genetic Variation, Sacred Fish, RAPD.

INTRODUCTION

Mostly, the waters area in Sumatra are well managed and widely utilized by the peoples. However, several waterbodies are not allowed to be exploited, such as a forbidden pond. The forbidden pond is a very well conserved area by the local peoples, and no intervention is allowed and consuming the fish is strictly prohibited. One of the tourist attractions of forbidden pond in West Sumatra is Sungai Janiah.

Fish in the forbidden pond is estimated to be highly isolated for hundreds of years. Therefore the inbreeding rate can be estimated to be high. Long-term inbreeding will decrease genetic variation, and increase the homozygosity. Until now, there is no genetic variation information of sacred fish from Sungai Janiah, West Sumatra. Based on the previous study (unpublished) the scientific name for that sacred fish is *Tor douronensis.*

Genetic variation is needed by the organism to maintain the viability and respond to changes in the environment [1]. Genetic variation in a population affects individual survival ability [2]. The higher genetic variation in a population, the better individual survival [1]. Genetic variation has an essential role in conservation since the survival ability and evolution process correlated to the genetic variation [3]. Genetic variation determination can be done using a genetic marker. One of the methods is Random Amplified Polymorphism DNA (RAPD). RAPD analyses DNA bands that formed through PCR amplification [4]. RAPD method does not require DNA sequence genome information [5], using universal primer, and neither probe [1].

MATERIALS AND METHODS

The study was conducted from February to May 2018. The fish scale was used as a DNA sample. The sacred fish scales were obtained from forbidden pond Sungai Janiah, West Sumatra. *Tor douronensis* from two different location was used as a sample comparison which was obtained from Batang Agam and Batu Putiah. The sample was analyzed in Genetic and Bio Molecular Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, Andalas University, Padang.

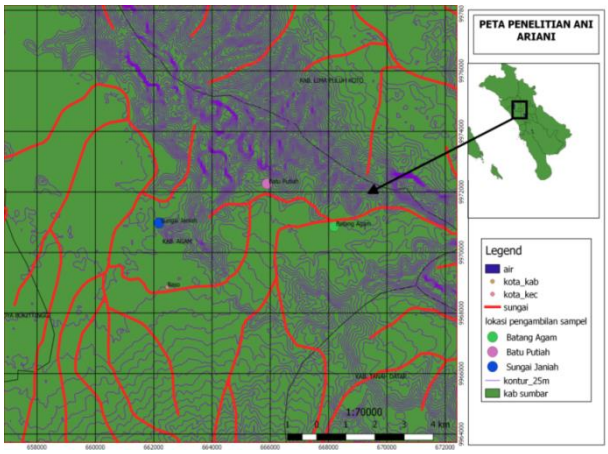

Fig-1: Map locations

Direct observation and collection were used as a method based on the previous study [6]. Genetic variation analysis was done using Random Amplified Polymorphism DNA (RAPD). DNA genome was isolated from scale using Invitrogen DNA isolation KIT. DNA isolation visualization was done using electrophoresis.

The screened primers referred to another study [7] in *T. douronensis* fish using PCR amplification method. Amplification was done using PCR with materials consisted of GoTaq green Master Mix (Bioline) 12.5 µl, Nuclease-free water 6.5 µl, Primer 2µl, isolated DNA product 4µl, so the total volume was 25 µl

PCR cycle consisted of three steps, denaturation of double helix DNA, annealing, and elongation DNA band. Pre-denaturation was done with a temperature of 94 ºC for two minutes to ensure complete denaturation; denaturation was done with a temperature of 36 ºC for one minute and elongation in 72 ºC for two minutes 30 seconds. The last cycle was done with 72 ºC for 10 minutes to ensure complete denaturation. There were 45 cycles of PCR in total. The primer selection was based on the resulted polymorphism band.

The analyzed parameter included polymorphic locus presentation (PLP), genetic diversity (H), Shannon phenotype diversity index (I), heterozygosity in subpopulation (Hs) and total heterozygosity in total population (Ht), genetic differentiation coefficient (GST) and gene flow (Nm) were analyzed using POPGENE 1.32 analysis program [8].

RESULTS AND DISCUSSION

DNA Isolation

Figure 2 showed the result of DNA electrophoresis

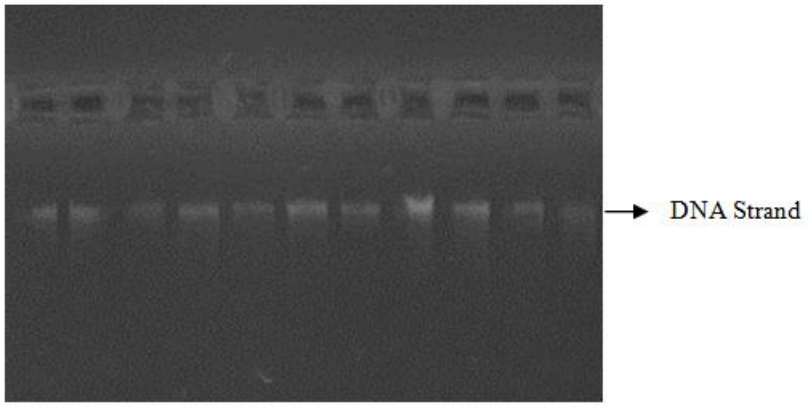

Fig-2: Electrophoresis of *T. douronensis* DNA isolated using agarose gel 1.2%

DNA was successfully isolated and run using agarose gel 1.2% (Figure 2). It shows that the isolated DNA has good quality and not contaminated with other substance which may cause smear.

Primer Selection using PCR

Primer selection shows that five primers produce the apparent and polymorphic bands. The polymorphic bands indicated that the primer had a nucleotide base sequence and attachment site to the DNA genome of *T. douronensis*. According to some authors [9], one of the contributing factor affecting PCR amplification is the

structure of the primer nucleotide base that has an attachment site by the base structure in the DNA genome. All the five primers were used as DNA fragment amplification (Table 1).

Table-1: Primers for DNA amplification

Primer	Primer sequence (5'→ 3')
OPA-01	5' CAG GCC CTT C 3'
OPA-08	5' GTG ACG TAG G 3'
OPA-16	5' AGC CAG CGA A 3'
OPB-08	5' GTC CAC ACG G 3'
OPB-10	5' CTG CTG GGA C 3'

Some authors used a universal primer to analyze genetic variation of *Tor* (subfamily: Cyprinidae).and showed various primer selection result. For examples, they analyzed 37 primers on *T. malabaricus* and showed fifteen polymorphic primers [10]. The others [11] tested 12 primers on *T. tambroides*, and each primer produced amplicon and polymorphic. While [12] tested 69 primers, 30 of them showed bands that can be counted. However, only eleven primers that were able to amplify the DNA genome in five species of *Tor*. Another study [13] showed sixteen polymorphic primers among twenty primers tested for *T. putitora* species. While other [7] analyzed fifteen primers in *T. dourunensis*, and 10 of the primaries were amplicon and polymorphic.

DNA Stand Profile from Amplification

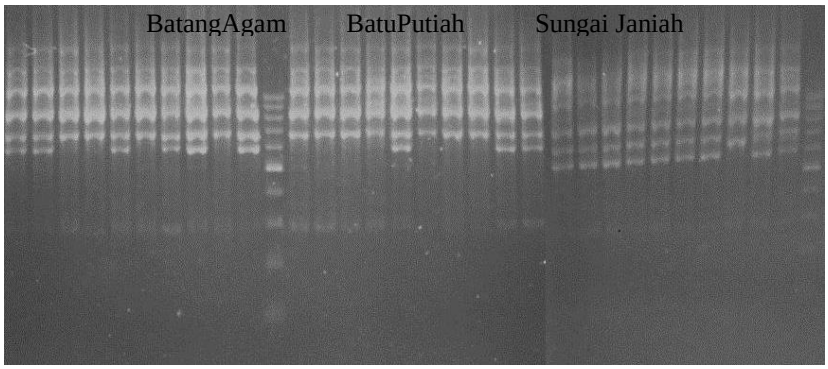

Fig-3: One of the DNA bands profile from amplification using OPB-10 in three Populations

The size of the DNA obtained ranged from 150 base pairs (bp) to 1090 bp and majority of the resulted fragment size was smaller than 1000 bp. OPA-01 was the primer that produced amplification with shorter fragment size compared with another primer, which was 700 bp to 1056 bp. Meanwhile, OPB-08 is one of the primers that produced more extended fragment size compared to another primer, 333 bp to 1090 bp.

Table-2: The performance of the primers and DNA Specific Bands Profile from 30 samples *T. douronensis*

No.	Primer	Total Bands	Polymorphic Bands	Polymorphic Bands Percentage	Description of the specific band (bp)		
					Batang Agam	Batu Putiah	Sungai Janiah
1	OPA-01	7	6	85.7%	-	-	(1056)
2	OPA-08	11	9	81.8%	(700)	-	(200)
3	OPA-16	14	14	100%	(450)	-	-
4	OPB-08	14	12	85.7%	-	(1021)	(333,400,450,500, 562,700,735)
5	OPB-10	7	2	28.5%	-	-	-
	Total	53	43	381.7%	2	1	9
	Mean	10.6	8.6	76.3%			

The result of the study on *Barbus* (Cyprinidae) [14] reported that eight species showed a 300 bp to 2000 bp bands. From a study on Mahseers fish [13] reported that eleven primers could amplify DNA genome from 200 bp to 2000 bp. Also reported [15] using seven primers for RAPD among eight species of a Cyprinidae fish family with 65 bp to 1444 bp band. While [8] reported there was 188bp - 2000 band in *T. duoronensis* species using ten primers.

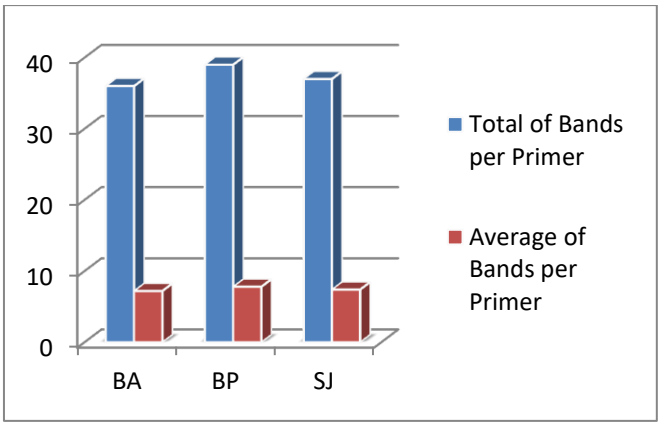

Fig-4: Total Bands in Populations
Note: BA (BatangAgam); BP (BatuPutiah); SJ (Sungai Janiah)

Each of primer in our study showed the different size of the fragment. Every primer had its attachment site. Therefore the amplified DNA with a particular primer produced a different amount of fragments (Table not shown). According to [16], PCR amplification is affected several factors such as annealing temperature, deoxyribonucleotide triphosphate (dNTP), primer oligonucleotide, DNA template, and buffer composition, amount of cycle, enzymes, and contamination. It argued [15] that three factors were affecting DNA fragment amplification such as a primer, a DNA template, and the reaction condition.

Based on the DNA amplification, there were 53 bands with a mean of 10.6 bands per primer and 76.3% polymorphic band obtained (Table 2). According to the authors [4], a locus is defined as polymorphic if there was more than one allele with the percentage of ≤95%. Another author [17] stated that the polymorphic band is a band when only found in a group of population and absence in another. The values from our result indicated that the primer in this study could analyze genetic variation in *T. douronensis.* Several similar studies reported that there were 60 bands amplified with 50% polymorphic locusand28.8% monomorphic in the *Schistura* [18]. A total of 102 bands with 37.25% polymorphic locus and 62.74% monomorphic locus found in *T. tor* [19]. There were 197 polymorphic bands reported with an average of 19.7 bands per primer and four monomorphic bands with an average of 0.4 bands per primer in *T. douronensis* [8]. The low percentage of polymorphic loci was due to the small population size and high inbreeding rate [10].

The result of our study also shows that the number of the band found in each population was quite similar. The highest number of the band found in the Batu Putiah population (39 bands with an average of 7.8 bands per primer). The lowest number of the band found in the Batang Agam population (36 bands with 7.2 average bands per primer). According to the authors [20], the genetic variations may reflect by the number, and the average of bands found. The more bands found in a population, the higher the genetic variation. Based on the results of this study, it can be stated that the genetic variation in each population was almost the same.

All populations in this study had specific bands (Table 2). According to the authors [21], specific bands could be used as genetic markers to see the variations in DNA levels. The population of Sungai Janiah has the most specific band (9 bands) indicating that the population has high genetic variation compared to other populations. While the Batu Putiah population has only 1 specific band, this illustrated that the population had lower genetic variation than other populations. The more specific bands present in a population, the higher the genetic variation in the population. According to [22] the specific bands in a population represent the level of genetic variation among populations, allowing for the occurrence of speciation processes.

The level of genetic variation will affect the ability of species respond to the environmental changes. Explained by the authors [23] that genetic variation was essential for the long-term viability of a species and can also guarantee the fitness of a species or population by providing such species or populations the ability to adapt to environmental change.

Table-3: Overall estimated genetic variation parameters in *T. douronensis* population

No	Populations	N	H	I	PLP
1	BatangAgam	17	0.1211	0.1774	27.42%
2	BatuPutiah	17	0.1025	0.1547	27.42%
3	Sungai Janiah	28	0.1897	0.2827	45.16%

Note: N = number of polymorphic locus, H = Heterozygosity value, I = Shannon Diversity Index, PLP = Percentage of Polymorphic Locus

The highest number of the polymorphic locus in Sungai Janiah population was 28 with polymorphic locus percentage (PLP) of 45.16%, heterozygosity value (H) 0.1897, and value of Shannon Index (I) 0.2827 which is also higher than Batang Agam and the Batu Putiah populations (Table 3). The values showed that the genetic variation in the Sungai Janiah population was relatively higher than in other populations. Appointed that the (H) value and (I) value could be used to observe the degree of genetic variation [4]. The higher the proportion of polymorphic loci, the heterozygosity value and the Shannon diversity index, the higher the genetic variation in the population [24]. Concluded that the high value of heterozygosity allowed that individuals in the population to adapt to environmental changes [25] and [23].

The lowest number of polymorphic loci (17 loci) was found in Batang Agam and Batu Putiah with 27.42% polymorphic locus percentage. The lowest heterozygosity was 0.1025, and the lowest Shannon diversity index value of 0.1547 was found in the Batu Putiah population. The range of heterozygosity values was0 <h <1 [4], Base on that value, the genetic variation of the *Tor* sp. in each population was low. The low heterozygosity and Shannon diversity index were due to the size of the population which allow inbreeding. Inbreeding was one of the factors that cause low genetic variation in a population [3].

The Genetic variation among Population and Genetic Structure of *T. douronensis*

Table-4: The Genetic VariationValue of *T. douronensis* Among Population

No	Number of Individuals	Ht	Hs	Dst	Nm	Gst
1	30 individu	0.2786	0.1377	0.1409	0.4891	0.5055

Ht = Total Heterozygosity, Hs = Heterozygosity in population, Dst = Intergroup heterozygosity value, Nm = Gene flow, Gst = Genetic Differentiation between populations

The heterozygosity value in the population (Hs) was calculated based on the mean heterozygosity value of each population. In this study, heterozygosity value in population and among the population (Dst) was low. That value indicated that the genetic variation of the whole *T. douronensis* was low. According to the authors [26], the value of heterozygosity between populations significantly affects the value of genetic differentiation between populations, the higher the value of heterozygosity between populations, the higher the genetic differentiation between populations.

In this study, Nm value was 0.4891 which meant that the flow of genes between populations was low. The value refers to the opinion of the researcher [27] who suggested that the gene flow was low if the value (Nm) $\leq$ 0.5. The value of genetic differentiation (Gst) was 0.505 which considered as high. The value of Gst ranges from 0-1 [28]. Low Nm and high Gst values might be due to population isolation and no migration, so the chances of inbreeding were very high. Those values showed genetic differences between populations. The inhibition of gene flow may increase the genetic variation between populations and reflected by specific alleles in each population.

Table-5: The Genetic distance among three populations of *T. douronensis*

Populations	BatangAgam	BatuPutiah	Sungai Janiah
BatangAgam	***		
BatuPutiah	0.1327	***	
Sungai Janiah	0.4020	0.3339	***

The higher genetic distance was between Batang Agam, and the Sungai Janiah population (0.4020), which indicated that there was a genetic difference between the two populations. The genetic difference might be due to geographically the locations. The Batang Agam and Sungai Janiah were far apart and different environmental conditions. Batang Agam is a river while Sungai Janiah is a pond. The smallest genetic distance was between the Batang Agam and the Batu Putiah population (0.1327), which showed both populations have a genetic similarity. The small genetic distance between the two populations might be the

cause of the Batu Putiah river flows to the Batang Agam River which allows the genetic mixing and reduces the genetic distance.

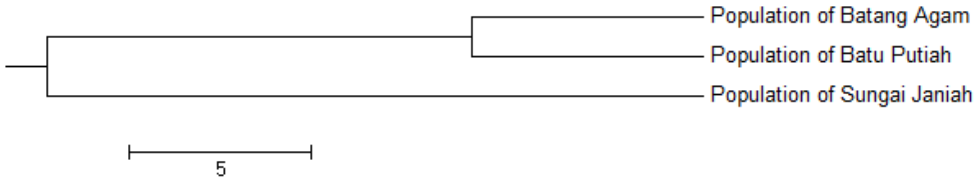

Fig-5: The Dendrogram of three populations of *T. douronensis*

Figure 5 shows the results of the UPGMA cluster analysis that classified the *T. douronensis* into two groups. The first group consisted of the population of Batang Agam and Batu Putiah population. In line with the value of their genetic distance, which caused by the possibility of genetic mixing between the two populations. The population of the Sungai Janiah separated from two other populations. Geographically population of the Sungai Janiah was isolated and located far from the Batang Agam and Batu Putiah, and therefore the gene flow is low.

In this study, a high inbreeding rate was evidenced by a lower Hs value compared to the value of Dst. High Dst values were in line with high genetic differentiation values. If inbreeding occurs in a long time and repeatedly, the chances of a homozygous individual will be higher.

Different environmental conditions in each population as well as adaptation of individuals in the population was also estimated induced the high of genetic differentiation. Information on the results of this study can help the cultivation and conservation efforts of the fish in Sungai Janiah pond. The RAPD technique had succeeded in detection the genetic variation of *T. douronensis.* The overall value of *T. douronensis* heterozygosity in each population studied was low. The Sungai Janiah population had higher heterozygosity value than other two populations. The genetic variation between populations was higher than the genetic variation in the population

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