

# Genetic of asang

*by* Azrita Azrita

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**Submission date:** 19-Sep-2019 04:51PM (UTC+0700)

**Submission ID:** 1175732108

**File name:** dom\_amplified\_polymorphic\_DNA\_RAPD\_markers\_\_AZRITA\_INDONESIA.pdf (1.6M)

**Word count:** 3809

**Character count:** 21019



# International Journal of Fisheries and Aquatic Studies

ISSN 2347-5129

IJFAS 2014; 1(6): 168-172

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www.fisheriesjournal.com

Received: 16-06-2014

Accepted: 13-07-2014

## Azita

Department of Aquaculture, Faculty of  
Fisheries and Marine Science Bung  
Hatta University,  
West Sumatra, Indonesia.

## Hafrijal Syandri

Department of Aquaculture, Faculty of  
Fisheries and Marine Science Bung  
Hatta University,  
West Sumatra, Indonesia.

## Junaidi

Department of Aquaculture, Faculty of  
Fisheries and Marine Science Bung  
Hatta University,  
West Sumatra, Indonesia.

## Genetic variation among asang fish (*Osteochilus vittatus* Cyprinidae) populations using random amplified polymorphic DNA (RAPD) markers

Azrita, Hafrijal Syandri, Junaidi

### Abstract

Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) was applied to analyze the genetic variation among *Osteochilus vittatus* collected from three geographically distant location of West Sumatra Province. After initial PCR screening, four random oligodecamers viz OPA 07, OPA 09, OPA11 and OPA 18 which generated the RAPD profile for the three *O. vittatus* populations were selected. The best amplification using from four primers resulted are OPA 18 in fragments ranging in length 400 - 1300 bp assigned to 22 loci. The study results show that the genetic diversity of the populations Singkarak 30 ke is 0.1250, Antokan River is 0.0431 and Koto Panjang Reservoir is 0.1512. While pairwise Fst comparison test showed significant differences ( $p < 0.05$ ) between the three populati 34 of fish. Estimated of Nei's (1972) unbiased genetic distance (D) values ranged from 0.0534 to 0.0610. Unweighted pair group method with arithmetic mean (UPGMA) dendrogram constructed on the basis of genetic distance revealed very close genetic relationship among *O. vittatus* populations of Singkarak Lake and Koto Panjang Reservoir.

**Keywords:** Asang fish, *Osteochilus vittatus*, genetic diversity, populations, random amplified polymorphic DNA (RAPD)

### 1. Introduction

Inland waters of Indonesia have as many as 1,300 species of fish diversity<sup>[1]</sup>, but still little has been conserved insitu and exsitu<sup>[2,3,4,5]</sup>. In terms of fish resources has contributed to the foreign exchange of 2,600.000.000 IDR/ year<sup>[6]</sup>. Therefore, it is very important to prioritize the development of new commodity by study of morphometric, genetic molecule, domestication and culture<sup>[7, 8, 9, 10, 11, 12, 13]</sup>.

Asang fish (*Osteochilus vittatus* Cyprinidae) is a native fish in Indonesian inland waters<sup>[14]</sup>, have a strategic value that are (a) as a source of food non cholesterol for rural community and urban<sup>[15]</sup>, (b) as a origin of income for rural community close to the area Maninjau Lake because this species possesses a high cost in the local market ranged between 25,000 to 35,000 IDR/kg<sup>[16,17]</sup>, Singkarak Lake<sup>[18,19]</sup>, Koto Panjang Reservoir and Kampar Kanan River<sup>[20,21]</sup>, (c) it can be applied to restocking and introduction to lakes and reservoirs that is experience blooming phytoplankton fish farming activities due to floating net cages and (d) In conditions of socio-cultural, *O. vittatus* being mature gonad function as "indigenous fish" the wedding party in Minangkabau, specialy community in Agam Regency, Lima Puluh Kota Regency and Tanah Datar Regency, West Sumatra Province Indonesia<sup>[22]</sup>. Considering this, the present study on selected morpo physiographic features of tor Mahseer (*Tor tor*) from the Rana Pratap Sagar reservoir, Rajasthan (India) was conducted. The current issues of *O. vittatus* in Maninjau Lake and Antokan River are damage spawning habitat by hydropower dams upstream Antokan River and not selective capture<sup>[16,15]</sup>, changes in trophic lake's status from oligotrophic to heavy eutrophic<sup>[23,17]</sup>, and the introduction of new species inadvertently likes *Oreochromis niloticus*, *Oxyeleotris marmorata*, *Channa lucius*, so that elimination of native species. In Singkarak Lake rare of *O. vittatus* caused loss of habitat, spawning and food supply due to fluctuating water level in Singkarak Lake and not selective capture<sup>[18,19,4]</sup>, in Koto Panjang Reservoir due to changes in water flow becomes stagnant in the Kampar Kanan River for hydropower Koto Panjang<sup>[20,24]</sup>.

### Correspondence

#### Azita

Departement of Aquaculture,  
Faculty of Fisheries and Marine  
Science Bung Hatta University,  
West Sumatra, Indonesia

In vulnerable fish species often show the distribution pattern of small populations fragmented due to habitat destruction, climate change, and ecological succession [25]. In small and isolated populations, the risk of local extinction generally becomes greater due to the loss of genetic diversity on account of the genetic drift [26, 27]. Furthermore, habitat that is incompatible with the life of fish can cause a distressed population growth [28, 29, 30], decreased reproductive potential [31, 32] and loss of genetic diversity [25]. One effort that can be done in germplasm rescue increasingly rare of *O. vittatus* is doing the domestication program must be preceded by collecting genetic data of *O. vittatus* that live in various habitats, so it can be determined the genetic variation, because the genetic resources have a fundamental role in the context of the biodiversity crisis.

The present study aims to analyze genetic variation among *O. vittatus* populations in different habitats by *Random Amplified Polymorphism DNA* (RAPD) methods markers in order to obtain information about the genetic status of *O. vittatus*. The information obtained will be important in an effort to define the location of the broodstock to the domestication process and culture of *O. vittatus* in the future.

## 38 2. Materials and Methods

### 2.1 Sample collection

The *O. vittatus* (Figure 1) samples were collected from Singkarak Lake, Solok Regency (00° 31' 46" - 00° 42' 20" S Latitude and 100° 26' 15" - 101° 31' 46" E Longitude), Antokan River which is the outlet of Maninjau Lake Agam Regency (100° 24' 20" - 100° 25' 20" S Latitude and 00° 16' 60" - 00° 27' 21" E Longitude) and Koto Panjang Reservoir Lima Puluh Kota Regency (101° 23' 64" - 101° 24' 13" S Latitude and 00° 11' 13" - 00° 09' 32" E Longitude) (Figure 2).



Fig 1: Elongated body of *Osteochilus vittatus*



Fig 2: Map of West Sumatra Province and locations of *O. vittatus* sampling

### 2.2 DNA isolation

Approximately 100 mg of fin tissue from 8 individuals of each population was preserved in 95% ethanol. DNA was isolated from preserved samples following [33] with minor modifications.

### 2.3 Screening of primers and PCR amplification

A total of 20 arbitrary primers OPA series Operon Technologies Ltd. USA with random sequence were screened (Kusmini et al, 2011). Four primers OPA-7, OPA-9, OPA-11 and OPA-23, which gave reproducible results were selected. The PCR amplifications were carried out using Veriti 96 well Thermal Cycler Applied Biosystems in a reaction volume of 29 µl containing 50 ng genomic DNA, 10X PCR buffer (10 mM Tris-HCL pH 9.0, 50 mM KCL and 0.01% gelatin), 2.5 mM of each dNTP, 5 pmol of primer and 0.7 units of Taq DNA polymerase. The amplification conditions were 94 °C for 5 min followed by 29 cycles at 94 °C for 1 min, 40 °C for 1 min and 72 °C for 2 min with a final extension at 72 °C for 10 min.

### 2.4 Agarose gel electrophoresis and visualization of bands

After amplification 28 µl of PCR products were electrophoresed in 1.5% agarose gel containing ethidium bromide and 1X TBE buffer to visualize the band patterns generated by each primer. The molecular weight of each band was estimated using a standard molecular marker (Lambda DNA/Eco RI Hind III Double Digest) with Image master 1D Elite Ver.3.01 (GE Amsterdam Biociences USA) [34].

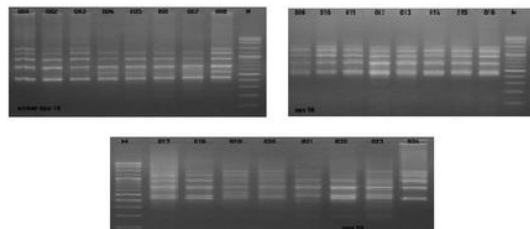
### 2.5 Statistical and dendrogram

Statistical analysis was carried out for the RAPD band pattern of all the three *O. vittatus* populations used for the present study. Using one selected arbitrary primers viz, OPA 18, the molecular characterization of *O. vittatus* populations and comparative analysis were made. The RAPD band pattern was visually analyzed and scored from photographs. Distinct and well separated bands were selected for comparative analysis. The genotypes were determined by recording the presence (1) or absence (0) of the bands and neglecting the weak and unresolved bands [35]. Unbiased genetic identity (I) and genetic distance (D) values between *O. vittatus* populations were calculated using the data generated from RAPD profiles using TFGPA program [36]. Genetic distance values were utilized to construct a dendrogram through clustering analysis (UPGMA) to determine the relationship between *O. vittatus* populations.

## 3. Results

Out of 20 decamer primers screened one primers viz OPA-18 showed reproducible results with good resolutions in banding patterns, whereas the other three primers produced highly inconsistent amplification products or did not amplify at all and hence they were excluded from further analysis. The RAPD band profile for three geographically *O. vittatus* populations for various selected oligodecamer are depicted in figure 3. The six oligodecamer primers that generated amplification fragments ranging in length between 400-1300 bp in length were assigned to 22 loci with an average of 5-12 per

primer. The performance of operon random markers among *O. vittatus* populations collected from Singkarak Lake, Antokan River and Koto Panjang Reservoir water systems which highlight the number of polymorphic bands, gene diversity and polymorphism percentage is shown in Tabel 1.



**Fig 3:** RAPD bands amplified by primer OPA-18 in three *Osteochilus vittatus* populations collected from various West Sumatra locations. (Lane M- standart molecular weight marker; lanes 1 to 8- Singkarak lake population; lanes 9 to 16 - Antokan river population; lanes 17 to 24 - Koto Panjang reservoir

**Table 1:** Genetic variations of *O. vittatus* based on the fragment of RAPD that was amplified with primers OPA-18

Parameter	Singkarak Lake	Antokan River	Koto Panjang Reservoir
Number of samples	8	8	8
Gene diversity	0.1250	0.0431	0.1512
No. of polymorphic loci	6	5	7
Percentage Polymorphism	27.2727	9.0909	40.9091

By using AMOVA (Analysis of Molecular Variance) there are significant differences ( $P < 0.05$ ) among populations genetic of *O. vittatus* of Singkarak Lake, Antokan River and Koto Panjang Reservoirs (Table-2). This indicates that is three populations geographically separate. This condition can be seen in the results of the analysis of genetic distance among locations (Table 3). TFPGA dendrogram constructed on the basis of genetic distances revealed that the genetic relationship was very close among *O. vittatus* populations of Singkarak Lake and Koto Panjang Reservoir. Whereas, *O. vittatus* populations of the Antokan River was found to be genetically distand from the rest of the populations (Figure 4).

**Table 2:** Result of Fst comparison pair test

Population	Singkarak lake	Antokan river	Koto Panjang reservoir
Singkarak lake	*****	0,0036**	0,0025**
Antokan river		*****	0,0018**
Koto Panjang reservoir			*****

\*\* :  $P < 0,05$

**Table 3:** Nei's 1972 genetic distance among three populations of *O. vittatus*

Population	Singkarak lake	Antokan river	Koto Panjang reservoir
Singkarak lake	*****	0.0539	0.0534
Antokan river		*****	0,0610
Koto Panjang reservoir			*****

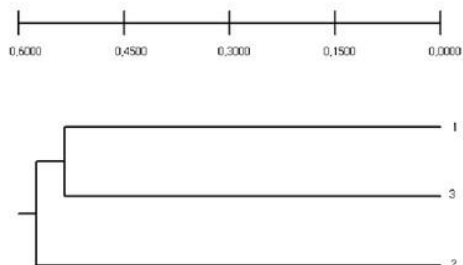


Figure 4. UPGMA dendrogram of three *O.vittatus* populations based on RAPD profiles.

#### 4. Discussion

In general of *O. vittatus* populations lived in three among locations has a relatively low level of diversity with gene diversity values ranged from 0.0431 to 0.1512 and the percentage of polymorphism was ranged from 9.09% to 40.91%. Close to the average values of gene diversity and percentage of polimorphism of *Osteochilus kelabau* Cyprinidae of three locations in West Kalimantan Province ranged from 0.0100 to 0.1651 and 5.0% to 58.8%<sup>[10]</sup>, *Tor Soro* Cyprinidae from Sumedang West Java Province with gene diversity values 0.0909 and *Tor Soro* populations from Tarutung North Sumatra Province with a gene diversity are 0.1407<sup>[87]</sup>. But in contrast to average gene diversity values of *Channa lucius* Channidae ranged from 0.2186 to 0.3668 from West Sumatra, Riau and Jambi Province<sup>[8]</sup>, the *Anabas testudineus* Anabantidae from South Kalimantan Province average gene diversity values ranged from 0.1347 to 0.3758<sup>[38]</sup> and *Osphronemus gouramy* Osphronemidae from Bogor in West Java Province gene diversity values ranged from 0.2360 to 0.3050<sup>[33]</sup>.

Generally, genetic variation in freshwater fish was low migration due to natural limitations so that chances very small of mating with other populations<sup>[9, 10]</sup>. This phenomenon is possible because of *O.vittatus* populations lived in Antokan River (21 km-length) with characterized by water temperature 26°C, pH 8.0, hardness 55.40 mg/l, alkalinity 50.44 mg/l, dissolved oxygen 8.10 mg/l. Almost same condition also occurred of *O. vittatus* populations lived in Singkarak Lake with a surface area of 11.200 ha with characterized by water temperature 27°C, pH 7.6, hardness 27.69 mg/l, alkalinity 24.93 mg/l, dissolved oxygen 4.29 mg/l. Whereas populations of *O. vittatus* lived in Koto Panjang Reservoir have a wider habitat and has several river basin are Mahat River, Kampar Kanan River, Mongan River, Kapur River and Malagiri River which can

be used by *O. vittatus* to migrated freely with characterized by water temperature 28°C, pH 6.0, hardness 24,18 mg/, alkalinity 83,14 mg/, dissolved oxygen 6.20 mg/l. The Schizothoracin (*Schizothorax prenanti*) populations of Wuxu Lake, China who experience barriers migratory due to hydropower dams have contributed to the low genetic diversity<sup>[40]</sup>. Furthermore<sup>[25]</sup> stated that small and isolated populations of *Saussurea chabyoungsanica* can be to extinction due to the smaller size of the populations and the more rapidly genetic drift due to habitat destruction, climate change and ecological succession. These conditions have occurred also of *Osteochilus kelabau* in Pontianak, Kapuas Hulu and Sintang waters in West Kalimantan Province<sup>[10]</sup>.

Gene diversity values of *O. vittatus* populations from Koto Panjang Reservoir better than of populations Singkarak Lake and populations Antokan River, so that of *O. vittatus* populations in Koto Panjang Reservoir have an total length average 169.49±24.37 mm, whereas the populations of Singkarak Lake average 139.67±31.6 mm and Antokan River average 117.0±15.67 mm. It can be stated that *O. vittatus* populations Koto Panjang reservoir is still be used as a potential candidate for domestication. According<sup>[29,3,41]</sup> that fish with high genetic diversity is able to adapt itself to environmental changes both natural and artificial.

The exchange genes with other populations are very small cause of *O. vittatus* Antokan River and Singkarak Lake population has rate limited and isolated migration. This condition in a long period of time will result in low genetic variation and will eventually appear higher homozygosity. Nivet *et al*<sup>[32]</sup>, stated that the effect of inbreeding are slow growth and decreased reproductive potential.

Furthermore *O. vittatus* populations in Singkarak Lake at the time of this study is rarely caught and size of fish caught ranged from 108,07 to 171.60 mm, smaller than the size of the fish in 2003 is 162.0 to 283.0 mm<sup>[18]</sup>. This is caused by pressure of arrests carried out by fishermen every day. According<sup>[29]</sup> decreased the density of fish due to fishing pressure populations can lead to loss of genetic diversity and fish grows increasingly smaller. Genetic diversity is largely determined by the density of the population, inbreeding, migration and genetic drift<sup>[42]</sup>.

Dendrogram constructed on the basis of genetic distance revealed very close genetic relationship among *O. vittatus* populations of Singkarak Lake and Koto Panjang Reservoir, compared the genetic distance among *O. vittatus* populations of Antokan River. The closest genetic distance among *O. vittatus* populations Singkarak Lake with Koto Panjang Reservoir indicated that it still derived from same common ancestor. Geographically the water stream of the Kampar Kanan River from Koto Panjang Reservoir and Ombilin River water flow from Singkarak Lake headed to Berhala Straits in east coast of the Sumatra island. Whereas geographically separated for *O. vittatus* populations from Antokan River because the water stream Antokan River headed to the Indian Ocean in west coast of Sumatra Island.

##### 5. Conflict of Interests

The author(s) have not declared any conflict of interests

##### 6. Acknowledgements

This research was funded by the Ministry of Research and Technology of the Republic of Indonesia in 2014, on the

Incentive Research Program of the National Innovation System with Basics Research Funding Scheme; and they wish to thank the reviewers at the IJFAS for their valuable criticism of the manuscript.

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