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

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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 amplication 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 Lake 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 populations of fish. Estimed of Nei's (1972) unbiased genetic distance (D) values ranged from 0.0534 to 0.0610. Unweighted pair group method with arithmetic mean (UPGMA) dendogram contructed 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 [1], but still little has been conserved insitu and exsitu [2,3,4,5]. In terms of fish resources has contributed to the foreign exchange of 2,600.000.000 IDR/ year [6]. Therefore, it is very important to prioritize the development of new commodity by study of morphometric, genetic molecule, domestication and culture [7, 8, 9, 10, 11, 12, 13].

Asang fish (*Osteochilus vittatus* Cyprinidae) is a native fish in Indonesian inland waters[14], have a strategic value that are (a) as a source of food non cholesterol for rural community and urban[15], (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 [16,17], Singkarak Lake [18,19], Koto Panjang Reservoir and Kampar Kanan River [20,21], (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[22].Considering this, the present study on selected morpho 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 [16.15], changes in trophic lake's status from oligotrophic to heavy eutrophic [23,17], 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[18,19,4], in Koto Panjang Reservoir due to changes in water flow becomes stagnant in the Kampar Kanan River for hydropower Koto Panjang [20,24].

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.

2. Materials and Methods

2.1 Sample collection

The *O. vittatus* (Figure 1) samples were collected from Singkarak Lake, Solok Regency (00º 31′ 46" - 00º 42′ 20" S Latitude and 100º 26′15"–101º 31′46"E Longitude), Antokan River which is the oulet of Maninjau Lake Agam Regency (100º.24′.20"–100º.25′.20"S Latitude and 00º.16′.60"- 00º.27′.21"E Longitude) and Koto Panjang Reservoir Lima Puluh Kota Regency (101º23'64"–101º.24′.13"S Latitude and 00º.11′.13" – 00º.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-18 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 25 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 ºC for 5 min followed by 29 cycles at 94 ºC for 1 min. 40 ºC for 1 min and 72 ºC for 2 min with a final extension at 72 ºC for 10 min.

2.4 Agarose gel electrophoresis and visualization of bands

After amplification 8 µl of PCR products were electrophoresed ini 1.5% agarose gel containing ethidium bromide and 1X TBE buffer to visualize the band patterns generated by each primer. The moleculer weight of each band was estimated using a standard moleculer 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. District and well separated bands were selected for the 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 identitity (I) and genetic distance (D) values between *O.vittatus* populations were calculated using the data generated from RAPD profiles using TFPGA 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 four decamers 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 lenght between 400-1300 bp in lenght were assigned to 22 loci with an average of 5-12 per

primer. The perfomance 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 percentace is shown in Tabel 1.

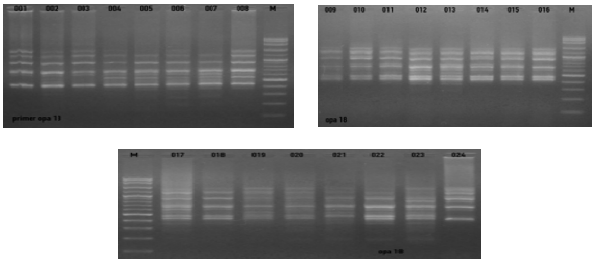

Fig 3: RAPD bands amplified by primer OPA-18 in three *Osteochilus vittatus* populations collected from various West Sumatra locations. (Lane M- standart moleculer weight marker; lanes 1 to 8- Singkarak lake population; lanes 9 to 16 - Antokan river population; lanes 17 to 24 - Koto Panjang reservoir

Table 1: Genetic varations 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
Percentace 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			*****

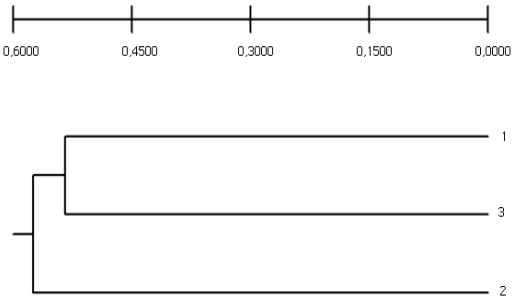

Figure 4. UPGMA dendrogram of three *O.vittatus* populations based on RAPD profiles.

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 percentace 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%[10], *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[37]. 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[3], the *Anabas testudineus* Anabantidae from South Kalimantan Province average gene diversity values ranged from 0.1347 to 0.3758 [38] and *Osphronemus gouramy* Osphronemidae from Bogor in West Java Province gene diversity values ranged from 0.2360 to 0.3050 [33].

Generally, genetic variation in freshwater fish was low migration due to natural limitations so that chances very small of mating with other populations [39, 10]. This phenomenon is possible because of *O.vittatus* populations lived in Antokan River (21 km-length) with characterized by water temperature 26ºC, 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 27ºC, 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 28ºC, 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 [40]. Furthermore [25] 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[10].

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 [29,3,41] 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* [32]**.** 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 [18]. This is caused by pressure of arrests carried out by fishermen every day. According [29] 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 [42].

Dendrogram contructed 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

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7. References

1. Ministry of Maritime Affairs and Fisheries of the Republic of Indonesia. Rare fish in Indonesia. Directorate-General of Marine, Coastal and Small Islands 2012, 210.
2. Gustiano R, Haryanti Sulaeman. Research future biotechnology of aquaculture breeding. Media Akuakultur 2007; 2(1):164-168.
3. Azrita, Syandri H, Nugroho E, Dahelmi, Syaifullah. Genetic variation of blue spotted snakehead (*Channa lucius* Channidae) from in Singkarak Lake West Sumatra, Tanjung Jabung floodplain Jambi, Kampar floodplain Riau Province using RAPD method. Biologi News 2011; 10(5):675-680.
4. Syandri H, Junaidi, Azrita. Management of resources of the bilih (*Mystacoleucus padangensis*) based on local wisdom in Singkarak Lake. Indonesia Journal of Fisheries Policy 2011; 3(2):11-18.
5. Aryani N, Nuraini, Suharman I. Morphological characterization of the green catfish (*Hemibagrus nemurus*) aquatic habitat on the different method based truss morfometrics. Journal of Fisheries and Aquaculture 2013; 4(3):139-142.
6. Kartamihardja ES, Purnomo K, Umar C. Inland resources in Indonesian waters ignored. Indonesia Journal of Fisheries Policy 2009; 1(1):1-15.
7. Haryono. Aspect biology of the tambra (*Tor tambroides*) rare as the basis for domestication. Biodiversitas 2006; 7(2):195-198.
8. Wibowo A, Sunarno MSD, Makmur S, Subagja. Stock structure identification of the belida (*Chitala* spp) and implication for natural population managemens. Indonesia Journal of Fisheries Research 2008; 1(14):31-44.
9. Krejszeff, Targońska K, Żarski D, Kucharczyk D. Domestication affects spawning of the ide (*Leuciscus idus*) preliminary study. Aquaculture 2009; 295:145-147.
10. Kusmini I I, Rudy G, Mulyasari. Genetic characterization of the kelabau (*Osteochilus kelabau*) from several locations in West Kalimantan using RAPD Method. Biology News 2011; 10(4): 449-454.
11. Akombo PM, Atile JI, Adikwu IA, Araoye PA. Morphometric measurements and growth patterns of four species of the genus *Synodontis* (Cuvier, 1816) from Lower Benue River, Makurdi, Nigeria. International Journal of Fisheries and Aquaculture 2011; 3(15):263-270.
12. Azrita, Syandri H, Dahelmi, Syaifullah,Nurgroho E. Morphological characterization of blue spotted snakehead (*Channa lucius* Channidae) in Singkarak Lake West Sumatra, Tanjung Jabung floodplain Jambi, Kampar floodplain Riau. Indonesia Journal of Natur 2013; 15(1):1-8.
13. Xiaolei Z, Risheng L, Yanfeng C, Guiju H, Dahui Y, Jixing Z *at. al.* Genetic characterization of northern snakehead (*Channa argus*) populations in China using microsatellite markers. Biochemical Systematics and

Ecology 2012; 43:25-31.
14. Kottelat M, Whitten AJ, Kartikasari SN, S. Wirjoatmodjo S. Freshwater Fishes of Western Indonesia and Sulawesi. Periplus Edition (HK), Jakarta.1993.
15. Azwir, Syandri H. The study of fish species diversity in Maninjau Lake West Sumatra . Journal Sigmatek 2010; 4(6):11-17.
16. Syandri H.The use of the asang (*Osteochilus vittatus*) and tawes (*Puntius javanicus*) as an agent of biological in Maninjau Lake. Indonesia Journal of Natur 2004; 6(2):87-91.
17. Sulastri, Hartoto DI, Yuniarti I. Environmental condition, fish resources and management of Maninjau Lake of West Sumatera**.** Indonesia Journal of Fisheries Research 2012; 1(18):1-12.
18. Uslichah U and Syandri H. Reproduction aspects of the sasau (*Hampala* sp.) and lelan (*Osteochilus vittatus*) in Singkarak lake. Indonesia Journal of Ichtiology 2003; 1 (3):41-48.
19. Syandri H and Azrita. Directory economically important of fish in Singkarak Lake. Bung Hatta University Press 2010; 110 p.
20. Warsa A, Nastiti AS, Krismono, Nurfiarini A. Fisheries Resources in Koto Panjang Reservoir**.** Bawal 2009; 2 (3): 93-97.
21. Fithra, RY and Siregar YI. Inventarisation fish species from Kampar Kanan River. Journal of Environmental Science 2010; 2 (4):139-147.
22. Syandri H, Azrita, Junaidi. Morphological characterization of the asang (*Osteochilus vittatus*, Cyprinidae) in Singkarak Lake, Antokan River and Koto Panjang Reservoir West Sumatra Province, Indonesia. Journal of Fisheries and Aquaculture 2014; 1(5):158-162.
23. Henny C. Dynamics of biogeochemistry of sulfur in Maninjau Lake. Limnotek 2009; 16(2):75-87.
24. Krismono ASN, Nurfiarini A, Kartamihardja ES, Sunarno MSD. Valuation of conformity candidate sites reserves of fishery in Koto Panjang Reservoir. Bawal 2009; 2(5):193-202.
25. Jeong JH, Byeung CL, Ki OY, Su KJ. Influence of small-scale habitat patchiness on the genetic diversity of the Korean endemic species *Saussurea chabyoungsanica* (Asteraceae). Biochemical Systematics and Ecology 2012; 43:14-24.
26. Harrison S, Hastings A. Genetic and evolutionary consequences of metapopulation structure. Trends Ecology Evolution 1996; 11:180-183.
27. Vrijenhoek RC. Conservation genetics of freshwater fish. Journal of Fish Biology 1998, 53:394-412.
28. Maskur. Conservation program of fish germplasm in inland waters. Indonesia Journal of Aquaculture 2002; 1(3):139-144.
29. Ruzafa AP, Wangüemert MG, Lenfant P, Marcos C, Charton JAG. Effects of fishing protection on the genetic structure of fish populations. Biological Conservation 2006; 129:244-255.
30. Kahl U, Stephan HI, Robert JR, Jurgen B. The impact of water level fluctuations on the year class strength of Roach: Implications for fish stock management. Limnologica 2008; 38:258-268.
31. Syandri H, Azrita, Aryani N. Size distribution, reproduction and spawning habitat of the bilih (*Mystacoleucus padangensis*) in Singkarak Lake. Bawal 2013; 5(1):1-8.
32. Nivet MD, Vandeputte M, Haffray P, Chevassus B. Effect of different mating designs on inbreeding, genetic variance and response to selection when applying individual selection in fish breeding programs. Aquaculture 2006; 252:161-170.
33. Nugroho E. 2011. Evaluation of genetic variation of Giant Gouramy strains revealed DNA marker. Journal Fisheries Science 2011; 13(2):86-90.
34. Saini A, Anish D, Vindhya M, Lakra WS. Moleculer discrimination of six species of Bagrid catfishes from Indus river system using randomly amplified polymorphic DNA markers. Mol. Bio. Rep 2010; 38(5):2961-2965.
35. Nei M. Genetic distance between populations. Am. Nat 1972; 106:283-292.
36. Miller MP. Tools for population genetic analysis (TFPGA) version 1.3. Departement of Biological Science. Northem Arizona University, Arizona USA 1997.
37. Asih S, Nugroho E, Kristanto AH, Mulyasari. Determination of genetic variation of the tambra (*Tor soro)* from North Sumatra and West Java with using RAPD method. Journal of Aquaculture Research 2008; 3 (1):91-97.
38. Slamat, Marsoedi, Athailah M, Diana A. Genetic conservation of the betok (*Anabas testudineus* Bloch 1972) in East Kalimantan floodplain. Indonesia Journal of Fisheries Research 2012; 1(18):9-15.
39. Rodrîguez CG, Morris MR, Dubois NS, de Queiroz K. Genetic variation and phylogeography of the swordtail fish *Xiphophorus cortezi* (Cyprinodontiformes, Poeciliidae). Molecular Phylogenetics and Evolution 2007; 43:111-123.
40. Liang J, Liu Y, Zhang X, Zhang X, Yue B, Song Z. An observation of the loss of genetic variability in prenant's schizothoracin, *Schizothorax prenanti*, inhabiting a Plateau Lake. Biochemical Systematics and Ecology 2011; 39:361-370.
41. Piou C, Prévost E. A demo-genetic individual-based model for Atlantic salmon populations: Model structure, parameterization and sensitivity. Ecological Modelling 2012; 231:37-52.
42. Nugroho E, Hadie W, Subagja J, Kurniasih T. 2005. Genetic and morphometric variation of the green catfish (*Mystus nemurus)* from Jambi, Wonogiri and Jatiluhur. Indonesia Journal of Fisheries Research 2005; 11(7):1-6.