ANALYSIS OF PYLOGENETIC RELATIONSHIP BETWEEN SOME RESIDENT FOODFISHES IN LAKE TOBA, INDONESIAN LARGEST LAKE

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Abstract

Fish diversity is relatively high in Indonesian waters supported by the presence of varied aquatic habitat conditions. But we lack robust data on our various economically important but threatened fish population in various lake systems. In this work, aspects of fish genetic variability in the Toba Lake in Sumatra, Indonesia was analyzed using sequence mtDNA as a tool. The pairwise distance was calculated and NJ dendogram based on Kimura Two parameter was constructed to take a look on fish phylogeny. PCR products of the *Cytochrome Oxidase Subunit I (COI)* mtDNA regions for the fish samples yielded different fragments which ranged in size from 573 to 671 bp. The genetic distance indicated segregation of *G.affinis, H. fasciatus, O. hasseltii, N.* stracheyi, *H. macrolepidota* and *P. reticulata* in separate cluster. The sequence profile showed high level between group genetic diversity and little within group genetic diversity suggesting improverishment of genetic variability in fish stock. This suggests the existence of a separate gene pool for these species.

Key words: fish diversity, genetic distance, genetic variability, mtDNA

Introduction

Indonesia is a country of about 17.000 islands streching 5.000 km along the equator and spanning two major biogeographical regions: Indo-Malayan and Australasian realms. The country itselft consists of seven biogeographic regions centered on the major islands and the surrounding seas: Sumatra, Kalimantan, Java and Bali, Sulawesi, Nusa Tenggara, Maluku and Papua. In any case, Indonesia is unquestionably one of the top two countries for biodiversity (Abdulgani, 2011).

However, there is paucity of pristine water bodies and lack of reliable trend data concerning aquatic fauna in Asia. We are uncertain about total species richness and precise rates of species loss, but the combination of high biodiversity and the magnitude of anthropogenic threats may make Asian inland water among the most endangered ecosystems on earth (Dudgeon, 2003). Loss of genetic resources has resulted in major concerns about future food and nutrition security. The vulnerability of aquatic systems towards pests, diseases and climate change makes the biodiversity urgent and has led to the development of a range of conservation strategies.

Biological information about different fish species though necessary, is not sufficient for undertaking conservation and genetic upgradation programmes (Kapoor & Sarkar, 2003). Conservation of a species should maintain the evolutionary potential of that species under natural environmental conditions. The evolutionary potential of a species may be defined in terms of its genetic variation (Frankel & Soule, 1981). Therefore, it is essential to quantify the amount and distribution of genetic variation within natural population if they are to be properly managed and conserved (Ryman, 1981).

Molecular markers derived from polymerase chain reaction (PCR) amplification of mitochondria DNA are an important part of the toolkit of evolutionary geneticists. Information on the genetic structure of fish species is useful for optimizing identification of stock, stock enhancement, breeding programs, management for sustainable yield and preservation of genetic diversity (Dinesh *et al*., 1993; Garcia & Benzie, 1995). In this paper we discuss aspects of genetic variability of some economically important fish species found in Toba Lake, a tectonic lake harbouring some of the economically important as well as threatened resident fish species. This

study was conducted through mtDNA COI gene sequence to discuss phylogenetic relationship among fishes and also to hightlight possible measures of conservation of such fish resources in a lake template.

Material and methods

Area study and sample collection

Sampling was conducted at 6 sites on Toba Lake, North Sumatra Province (Figure 1). Fish collections were undertaken from April 2013 and November 2013. Sampling and collecting representative samples of the adult fish community and fish juvenile were carried out using various types of fishing gear, Sulangat (a complex local fishing gear equipped with lamps), scoope net and gill nets.

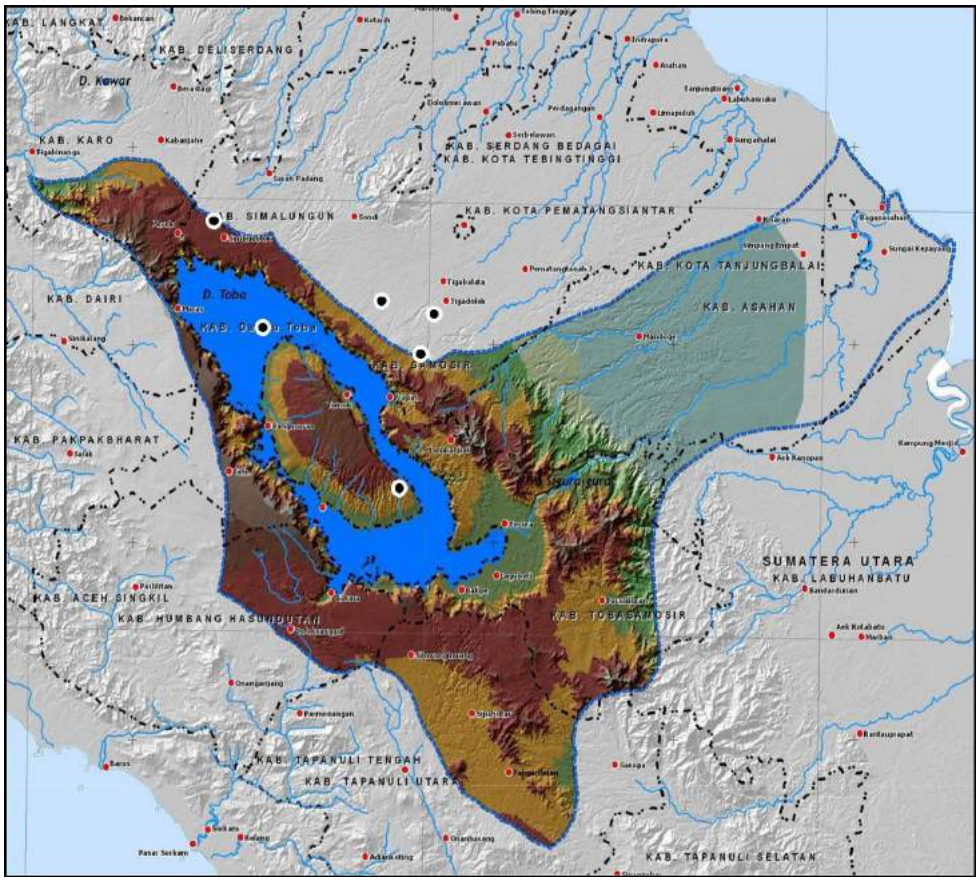

Figure 1. Sampling site in Toba Lake, North Sumatra Province (white dot).

Sample preservation and DNA analysis

Target species caught were identified to the species level following Kottelat *et al.* (1993). Among the fish species collected from Toba Lake, six species were selected for studying genetic diversity. These were *G. affinis, P. reticulate, H. fasciatus, O. hasseltii, N. stracheyi* and *H. macrolepidota.* Immediately after fish was caught, tissue was taken from every individual and stored in absolute alcohol of 1.5 mL. Sampled fish were preserved in 10% buffered formaldehyde for several days after which the specimens were transferred to 70% ethanol. A voucher collection is stored at the Research Institute of Inland Fisheries, Palembang, South Sumatra, Indonesia. Juvenile fish at early life stages (from preflexion to postflexion larvae) were collected using a set of 30 cm diameter modified bongo nets. The nets were maintained submerged for around 5 cm from surface. Larvae were kept in water and manually sorted after collection and were stored individually in absolute ethanol.

Total genomic DNA was extracted from muscle tissue of each specimen using the Extraction Kit procedure 'DNeasy Blood & Tissue' (Geneaid). The partial fragment of mitochondrial Cytochrome C Oxidase Subunit-1gene (CO1) was amplified using modified universal primers described by Ivanova *et al.* (2007):

Fish-COI-F (5'-ACT TCA AAC TTC CAY AAA GAY aty GG-3) and
COI-Fish-R (5'-TAG ACT TCT GGG TGG CCR AAR Aay CA-3 ').

Polymerase Chain Reaction (PCR) amplifications were made in a 50 µL of reaction volume consisted of 5 µL DNA samples, 16 µL double distillate water, 2 µL of each primer and 25 µL of PCR ready mixture solution (KAPPA). PCR cycling parameters included an initial denaturation phase at 95 °C for 10 min, followed by 35 cycles at 94 °C for 1 min, 48 °C for 1 min and 72 °C for 1.5 min and ended with a final extension at 72 °C for 7 min. Finally, all amplicons were automatically sequenced in both directions at First Base, Singapore (www.firstbase.com).

Chromatograms were controlled and checked manually edited with BioEdit (version 7.0.4.1) (Hall, 1999) and multiple alignments were done using Clustal W (Thompson *et al*., 1994). Following alignments, COI sequences were translated to amino acids to check for the presence of premature stop codons that indicate the presence of nuclear pseudo genes or sequencing errors. Sequence divergence was estimated using the Kimura two parameters (K2P) model of base substitution (Kimura, 1980). Phenetic reconstruction was done using a distance based method, Neighbor-Joining (NJ), carried out in MEGA5 software (Tamura *et al.,* 2007) with the K2P model of substitution. Support for nodes in NJ analyses was assessed using non-parametric bootstrapping with 100 full heuristic pseudo-replicates. For comparative purposes, we used the several sequences of the freshwater fish species in Genbank (Accession Number view in figure) to root the tree.

Results and Discussion

Knowledge of the effects of genetic variability in fish species is vitally important for understanding of how diversity is distributed within natural fish assemblage. In order to decipher genetic structure and genetic variability of some of the fishes, sequence of mtDNA COI gene is a useful tool. PCR products of the *Cytochrome Oxidase Subunit I (COI)* mtDNA regions for the fish samples yielded different fragments which ranged in size from 573 to 671 bp (Table 1).

Table 1. Sizes of amplified product of the COI region from the samples.

No.	Sample Code	Verified PCR sizes (bp)	Identification
1.	DT 12	675	*Neolissochilus stracheyi*
2.	DT 13	653	*Osteochilus hasseltii*
3.	DT 14	671	*Osteochilus hasseltii*
4.	DT 39	648	*Gambusia affinitis*
5.	DT 40	661	*Hemichromis fasciatus*
6.	DT 43	573	*Poecilia reticulata*
7.	DT 44	611	*Hampala macrolepidota*
8.	DT 45	601	*tidak teridentifikasi*
9.	DT 46	648	*Gambusia affinitis*
10.	DT 47	629	*Gambusia affinitis*
11.	DT 48	654	*Hampala macrolepidota*
12.	DT 49	654	*Hampala macrolepidota*
13.	DT 50	650	*tidak teridentifikasi*
14.	DT 51	658	*Gambusia affinitis*

The final aligned data of COI yielded 654 characters including 653 conserved and 1 variable sites, 611 characters with 608 conserved and 3 variable sites and 657 characters including 654 conserved and 3 variable sites for *Osteochilus hasseltii, Gambusia affinities* and *Hampala macrolepidota,* respectively. The COI sequences were very rich in A+T content ranging from 55,4% (*Osteochilus hasseltii)* to 53,2% (*Gambusia affinities)* with an average of 54,3% (Table 2).

Table 2. Sequence characteristics of COI region of mtDNA in some fishes of Toba Lake.

No.	Parameters	*Osteochilus hasseltii (n=2)*	*Gambusia affinitis (n=4)*	*Hampala macrolepidota (n=3)*
1.	Aligned length (bp)	654	611	657
	No. of conserved sites	653	608	654
	No. of variable sites	1	3	3
2.	G + C content (%)	45.5	46.8	44.6
	A + T content (%)	54.5	53.2	55.4
3.	Nucleotide frequencies of			
	Adenine	0.256	0.238	0.263
	Thymine	0.289	0.293	0.290
	Cytosine	0.266	0.293	0.269
	Guanine	0.188	0.173	0.176

The NJ dendogram based on pairwise genetic distance indicated segregation of *G. affinis* and *P. reticulata* in one cluster (Figure 2.) whereas *H. fasciatus, O. hasseltii, N. stracheyi* and *H. macrolepidota* in another cluster with node distance ranging from 0.174 to 0.291 (Table 3). The NJ analysis and bootstrap estimates in this study showed minimum genetic distance (0.174) between these two species (*N. stracheyi* and *H. macrolepidota*), emphasizing their evolutionary relationship as well as divergence in recent times.

When the sequences of individual fishes were compared belonging to the same species, they showed almost identical profile. Therefore, we can conclude that the population of each species to be nearly homogeneous (Meffe & Vrijenhoek, 1988). The average genetic distance between six fish species is found to be considerable higher that within species. This suggests the existence of a separate gene pool for these species.

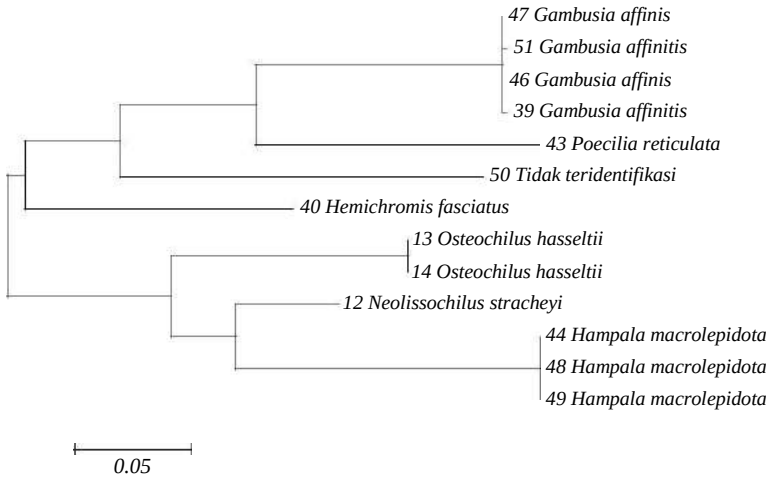

Figure 2. NJ dendogram constructed on the basis of genetic distance calculated from Kimura 2 Parameter among 7 fish species.

Table 3. Pairwise distance between fish populations studied.

		1	2	3	4	5	6	7	8	9	10
1	*12_Neolissochilus_stracheyi*										
2	*13_Osteochilus_hasseltii*	0,166									
3	*14_Osteochilus_hasseltii*	0,166	0,000								
4	*39_Gambusia_affinitis*	0,251	0,284	0,284							
5	*40_Hemichromis_fasciatus*	0,221	0,254	0,254	0,234						
6	*43_Poecilia_reticulata*	0,249	0,279	0,279	0,232	0,286					
7	*44_Hampala_macrolepidota*	0,174	0,228	0,228	0,296	0,264	0,293				
8	*46_Gambusia_affinis*	0,248	0,281	0,281	0,002	0,231	0,229	0,293			
9	*47_Gambusia_affinis*	0,248	0,281	0,281	0,002	0,231	0,229	0,293	0,000		
10	*48_Hampala_macrolepidota*	0,174	0,228	0,228	0,296	0,264	0,293	0,000	0,293	0,293	
11	*49_Hampala_macrolepidota*	0,174	0,228	0,228	0,296	0,264	0,293	0,000	0,293	0,293	0,000
12	*50_Tidak_teridentifikasi*	0,276	0,272	0,272	0,292	0,267	0,291	0,308	0,289	0,289	0,308
13	*51_Gambusia_affinitis*	0,251	0,284	0,284	0,004	0,234	0,232	0,296	0,002	0,002	0,296

However, low level of genetic diversity among species or impoverishment of genome variability could be a threat for the future survival of the species. Estimating the degree of genetic variation in a population is a crucial step in conservation genetic work, as it allows the channelling of conservation efforts to make better use of available resources. A natural fish assemblage with high genetic variability is usually considered to compose good fish culture stocks (Ramella *et al*., 2006). If a threatened species occupying a given area is shown to posses high degree of genetic variation, the conservation strategy should endeavour to preserve its diversity in that area, since there might be local adaptations that might lost in competition with other populations introduced in that area. On the other hand, for a population of a species which is homogeneous throughout its territory, efforts to protect those species can be concentrated on just one area and individuals from that area may be used to restock other area when necessary (Leuzi *et al*., 2004). All the fish species studied show high level of interspecific genetic variability and little intraspecific genetic variability.

As the genetic variation within group is found to be at the lower side, the conservation efforts should, therefore in agreement with Leuzi *et al*. (2004), be directed to protect the preferred habitat of Toba Lake. In order to protect various threatened fish species, a spatial conservation approach should be followed by protecting unique habitats like Toba Lake from degradation.

Conclussions

PCR products of the *Cytochrome Oxidase Subunit I (COI)* mtDNA regions for the fish samples yielded different fragments which ranged in size from 573 to 671 bp. The genetic distance indicated segregation of *G. affinis, H. fasciatus, O. hasseltii, N. stracheyi* and *H. macrolepidota* and *P. reticulata* in separate cluster. The sequence profile showed high level between group genetic diversity and little within group genetic diversity suggesting improverishment of genetic variability in fish stock. This suggests the existence of a separate gene pool for these species.

References

Abdulgani, R. 2011. Problems and issues affecting biodiversity in Indonesia. The workshop on training needs assessment for biodiversity conservation in Indonesia. Bogor. 33-35 pp.

Dinesh, K.R., T.M. Lim, K.L. Chua, W.K. Chan & V.P.E. Phang. 1993. RAPD analysis: An efficient method of DNA fingerprinting in fishes. Zoological science. 10: 849-854

Dudgeon, D. 2003. The contribution of scientific information to the conservation and management of freshwater biodiversity in tropical Asia. Hydrobiologia. 500: 295-314

Frankel, O.H & M.E. Soule. 1981. Conservation and Evolution. Cambridge University Press. Cambridge.

Garcia, D.K. & J.A.H. Benzie. 1995. RAPD markers of potential use in penaeid prawn (*Penaeus monodon)* breeding programs. Aquaculture. 130:137-144

Hall, T.A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser.41: 95-98

Ivanova N.V., T.S. Zemlak., R.H. Hanner & P.D.N. Hebert. 2007. Universal primer cocktails for fish DNA barcoding. Molecular Ecology Notes. 7: 544-548

Kapoor, D. & U.K. Sarkar. 2003. Expanding initiatives for conservation and sustainable exploitation of fish genetic biodiversity of India. Fishing Chimes. 23: 57-65

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution. 16: 111-120

Kottelat, M.A., J. Whitten, S.N. Kartikasari & S. Wirjoatmodjo. 1993. Freshwater fishes of Western Indonesia and Sulawesi. Barkeley Books. Pte Itd, Terrer Road. Singapore. 317 p.

Leuzi, M.S.P., F.S. de Almeida & M.L. Orsi. 2004. Analysis by RAPD of the genetic structure of *Astyanax altiparanae* (Pisces, Characiformes) in reservoirs on the Paranapanema river, Brazil. Genetics and Molecular Biology. 27: 355-362

Meffe, G.K. & R.C. Vrijenhoek. 1988. Conservation genetics in the management of desert fishes. Conservation Biology. 2: 157-169

Ramella, M.S., M.A. Kroth, S. Meurer, A.P.O. Nuner, E.Z. Filho & A.C.M. Arisi. 2006. Genetic variability in four fish species (*Pimelodus maculates, Prochilodus lineatus, Salminus brasiliensis, Steindachneridion scripta)* from Uruguay River Basin. Brazilian Archives of Biology and Technology. 49: 589-598

Ryman, N. 1981. Conservation of genetic resources: Experiences from brown trout (*Salmo trutta).* Ecological Bulletin. 34: 61-74

Tamura K, J. Dudley, M. Nei & S. Kumar. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution 10.1093/molbev/msm092.

Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin & D.J Higgins. 1997. The clustal X windows interface: Flexible strategies for multiple sequences alignment aided by quality analysis tool. Nucleic Acid Res. 25 (24): 4876-4882