EVALUATION OF GENETIC RELATIONSHIP AMONG SELECT SIX FISH SPECIES USING THE PARTIAL FRAGMENT OF MITOCHONDRIAL CYTOCHROME C OXIDASE SUBUNIT-1GENE (CO1)

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ABSTRACT

In modern conservation and sustainable management approaches of species, it is important to have knowledge about biodiversity, population structures and dynamics. In the present study, the genetic relationship among select fish species in Lubuk Lampam floodplain region was investigated, utilising partial fragment of mitochondrial Cytochrome C Oxidase Subunit-1gene (CO1). The aim of the study was (1) to reveal genetically relevant relationship of fish species in the Lubuk Lampam floodplain and (2) to find more pronounced substructures on the fine scale analysis. Tissue samples from 10 specimens of fish species were collected during several field trips in 2012 at sample sites across Lubuk Lampam floodplain Ogan Komering Ilir regency South Sumatra Province. The results showed different species that are deviated relatively new from a similar ancestor. The molecular marker, a 570 bp region of the mitchondrial cyctochrome c oxidase I gene (COI) has been successfully found to be species-specific, and was also more variable between species than within species. In summary, the present study represents an important step understanding evolution relationship for teleost fish living in tropical peat swamp in Sumatra. Producing of a COI sequences from the Lubuk Lampam system also assist to the global DNA barcoding library.

Keywords: Phylogeny, Lubuk Lampam and species assignment

INTRODUCTION

In modern conservation and sustainable management approaches of species, it is important to have knowledge about biodiversity, population structures and dynamics. Moreover, the detection of genetic variation at the species, population and within population level is of great importance for sustainable aquaculture practices. Especially in freshwater species, direct measurements about connectivity and differentiation are difficult to obtain, which is why indirect measures with the help of phylogeographic and population genetic approaches can be very helpful and necessary.

Genetic variation at species level helps to identify the taxonomic units and to determine the species distinctiveness. Variation at the population level can provide an idea about different genetic classes, the genetic diversity among them and their evolutionary relationship with wild relatives. The genetic variability within population is extremely useful to gather the information on individual identity, breeding pattern, degree of relatedness and disturbances of genetic variation among them (Schierwater et al. 1994). In order to evaluate biodiversity correctly it is important to clarify species boundaries, integrities, and phylogenetic relationships (Frankham et al., 2002).

The genetic tools available to date, vary from mitochondrial or plasmid DNA, to nucleus DNA, sequence based to length or single nucleotide polymorphism markers, and differ in the ways of possible analyses and information gain, all afflicted with different advantages and disadvantages (Timm et al., 2012). Mitochondrial sequence markers are widely used for phylogenetics of fish (e.g., Kochzius et al., 2003; Santini & Polacco 2006; Timm et al., 2008). As certain regions show high variability and the procedure for amplification and sequencing is comparably easy (Timm et al., 2012). Sequencing of the mitochondrial cytochrome-*c* oxidase subunit 1 (*COI*) gene fragment in animals has become one of the most widely used and effective tools for species identification and discovery. This approach, known as DNA barcoding, has been shown to provide unprecedented accuracy for the identification of various taxonomic groups of fish (Kochzius, 2009)

Not much is known about connectivity of freshwater populations in the Indonesia floodplain in general and especially for fish, despite the fact that such information is important to understand evolutionary and ecological processes in the centre of biodiversity. Data on connectivity are also urgently required to design effective conservation strategies for these living freshwater resources.

In the present study, the genetic relationship among select fish species in Lubuk Lampam floodplain region was investigated, utilising partial fragment of mitochondrial Cytochrome C Oxidase Subunit-1gene (CO1). This study aims (1) to reveal genetically relevant relationship of fish species in the Lubuk Lampam floodplain and (2) to find more pronounced substructures on the fine scale analysis.

METHODS

Sampling

Tissue samples from 10 specimens of fish species were collected during several field trips in 2012 at sample sites across Lubuk Lampam floodplain Ogan Komering Ilir regency South Sumatra Province. A small piece of tissue was cut off from each specimen. Tissue samples were preserved in 96% of ethanol and later stored at 4^0C.

DNA extraction, amplification and DNA sequencing

Genomic DNA was isolated from the muscle tissue using extraction kits from GENEAID, following the manufacturers' protocols. A fragment of around 550 bp from the mitochondrial Cytochrome C Oxidase Subunit-1gene (CO1) was amplified by polymerase chain reaction (PCR) using the primers 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 ') (Ivanova et al., 2007). Polymerase chain reaction (PCR) was carried out in a total volume of 50 µL. PCRs contained 5 µL DNA samples, 16 µL double distillate water, 2 µL of each primer and 25 µL of PCR ready mixture solution (KAPPA). The following temperature profile was used for the PCR, 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. The PCR products were purified using the The GenepHlow™ Gel/PCR Kit (GENEAID), following the manufacturer's protocol. Both strands of the purified DNA were automatically sequenced in both directions at First Base, Singapore (www.firstbase.com).

Genetic diversity DNA extraction, amplification and DNA sequencing

All sequences were edited with the program sequence navigator (version 7.0.1; Applied Biosystems) (Hall, 1999) and checked manually by eye. The sequences were translated to amino acids in order to exclude mistakes in sequencing and to verify if a functional mitochondrial DNA sequence was obtained and not a nuclear pseudogene. A multiple sequences alignment was obtained by using clustal w (Thompson *et al*. 1997) as implemented in the software bioedit (version 7.0.4.1; Hall 1999). 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 DISCUSSIONS

Information on the evolutionary relationships of genetic lineages can be obtained from DNA sequences through the reconstruction of phylogenies (Freeland, 2005). At the beginning, DNA was isolated from 20 specimens but amplification of the 501-bp target DNA fragment was successful only for 8 samples (voucher), despite multiple attempts to amplify DNA. The positioning of organisms on a tree is generally based on their genetic similarity to one another. This is illustrated in Figure 1, which shows a phylogeny tree showing the inferred evolutionary relationships among some fish species, genus and family. Organisms of different species of the same genus will be close to each other on the tree, different genera, such as *Barbichthys* and *Belodontichthys* (**Fig 1**), futher distance on the phylogeny tree.

The tree reflects how much genetic change has occurred and therefore roughly how much time has passed, since lineages split from one other, because branch lengths reflect the evolutionary distance between two points on a tree. Phylogenetic analyses have been invaluable in evolutionary biology, trees are appropriate for taxonomic groups at the species level and beyond, which have experienced a period of reproductive isolation long enough to allow for the fixation of different alleles (Freeland, 2005).

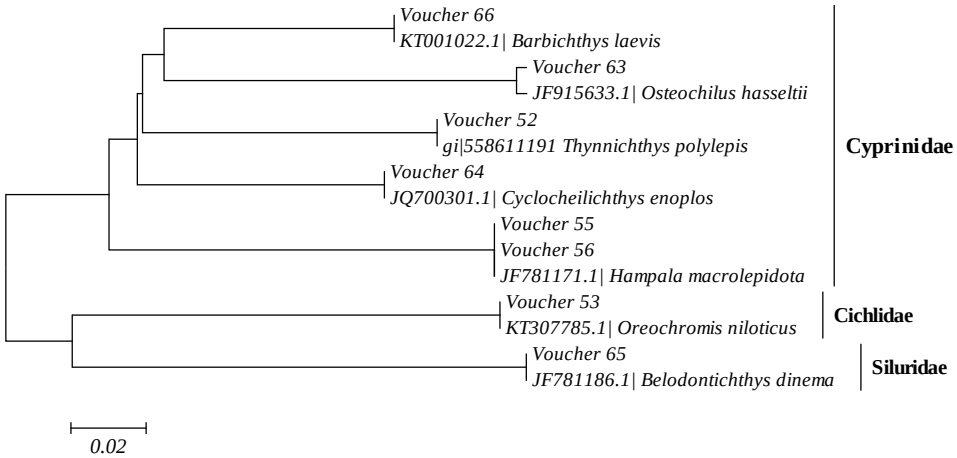

Fig. 1. A phylogeny of 15 fish species based on the partial fragment of mitochondrial Cytochrome C Oxidase Subunit-1gene DNA gene. First species names, then family names, are shown to the right of the tree.

One way in which we can measure the genetic similarly of two individuals is by estimating the genetic distance between them. There are many different ways in which this can be done, one of most common being Nei's (1972) genetic distance, D. The primer pair Fish-COI-F and COI-Fish-R used in this study was found to produce different number of base pairs with an average 570 bp.

The molecular marker, a 570 bp region of the mitchondrial cyctochrome c oxidase I gene (COI) has been successfully found to be species-specific, and was also an average of 18 times more variable between species (7.05-7.93 per cent) than within species (0.27-0.43 per cent) (**Table 1;** Herbert et al., 2004b). This suggests the existence of a separate gene pool for these species. Similar results were obtained when genetic distance calculated for other fish species (Lakra et al., 2007). However, low levels of genetic diversity among species or impoverishment of genome variability could be a threat for the future survival of the species (Sengupta & Homechaudhuri, 2012).

Table 1. Pairwise distance between fish species studied

		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Voucher_52														
2	gi|558611191_Thynnichthys_polylepis	0,000													
3	Voucher_64	0,146	0,146												
4	JQ700301.1|_Cyclocheilichthys_enoplos	0,146	0,146	0,000											
5	Voucher_66	0,145	0,145	0,134	0,134										
6	KT001022.1|_Barbichthys_laevis	0,145	0,145	0,134	0,134	0,000									
7	Voucher_63	0,178	0,178	0,171	0,171	0,159	0,159								
8	JF915633.1|_Osteochilus_hasseltii	0,183	0,183	0,167	0,167	0,156	0,156	0,005							
9	Voucher_55	0,188	0,188	0,171	0,171	0,177	0,177	0,221	0,221						
10	Voucher_56	0,188	0,188	0,171	0,171	0,177	0,177	0,221	0,221	0,000					
11	JF781171.1|_Hampala_macrolepidota	0,188	0,188	0,171	0,171	0,177	0,177	0,221	0,221	0,000	0,000				
12	Voucher_53	0,246	0,246	0,241	0,241	0,236	0,236	0,246	0,249	0,273	0,273	0,273			
13	KT307785.1|_Oreochromis_niloticus	0,246	0,246	0,241	0,241	0,236	0,236	0,246	0,249	0,273	0,273	0,273	0,000		
14	Voucher_65	0,255	0,255	0,239	0,239	0,244	0,244	0,283	0,286	0,256	0,256	0,256	0,234	0,234	
15	JF781186.1|_Belodontichthys_dinema	0,255	0,255	0,239	0,239	0,244	0,244	0,283	0,286	0,256	0,256	0,256	0,234	0,234	0,000

CONCLUSIONS

The molecular marker, a 570 bp region of the mitchondrial cyctochrome c oxidase I gene (COI) has been successfully found to be species-specific, and was also more variable between species than within species. In summary, the present study represents an important step understanding evolution relationship for teleost fish living in tropical peat swamp in Sumatra. Producing of a COI sequences from the Lubuk Lampam system also assist to the global DNA barcoding library.

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