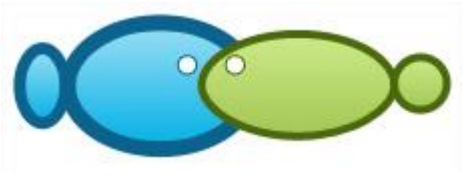

DNA barcoding of giant gourami (*Osphronemus goramy*) from West Sumatra, Indonesia

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Abstract. Giant gourami (*Osphronemus goramy*) is an economically important freshwater fish in some areas of Indonesia, such as West Sumatra. The *O. goramy* from this region has a distinctive character, i.e. red/albino color that can be used as geographical identification. The type of gourami of West Sumatra was identified using DNA barcoding. Sixteen DNA samples have been extracted from gourami muscle and amplified target genes using primers L14841 and H15149 then sequenced. DNA sequence was aligned with the sequences from genbank by BLAST program. Species identification was decided through the phylogenetic tree and similarity index with genbank sequences. The result showed that all of *O. goramy* samples from West Sumatra were identified as one group of *O. goramy* with similarity index of 99% compared to sequence data of genbank. A total of seven variable nucleotide sites and eleven specific haplotypes were detected on 475 bp of length. A common haplotype was found on two of four giant gourami strains (merah and krista). Values of genetic distance among *O. goramy* samples range from 8 x 10^{-6} to 7.5 x 10^{-4}.

Key Words: cytochrome B, genetic variation, haplotype, Kalui, character.

Introduction. Fish species belonging to the genus *Osphronemus* Lacepede 1801 are known as giant gourami. The four species of this genus are *Osphronemus goramy*, *O. laticlavius*, *O. septemfasciatus* and *O. exodon* (Roberts 1992, 1994; Kottelat et al 1993; Eschmeyer & Fong 2011; Eschmeyer & Fricke 2011). One of that species is commonly found in Indonesia as known as gourami (*O. goramy*), with the local name as gurami. This fish is an economically important freshwater commodity from the Sunda region, and spread widely to several regions such as West Sumatra, Jambi, Central Java, Jogjakarta, East Java, West Kalimantan, and South Kalimantan. There are four strains of giant gourami in West Sumatra, one of which has a distinctive character, namely red color (Azrita & Syandri 2015). Identification of this giant gourami needs to be done to ensure the type of fish and its relation to the nature of the character that is raised so that it can be more utilized in the management of fish resources.

Azrita & Syandri (2015) have grouped gourami strains into three groups based on the truss-morphometric technique i.e. the-red gourami fish (group 1), krista gourami fish (group 2) and palapah-tambago-jepun gourami fish (group 3). Further, the differences in morphology of gourami have not been detected genetically by using enzymatic methods (Nugroho & Kusmini 2007). Bickford et al (2006) argued that the existence of cryptic phenomena - and sibling - species causes an inappropriate identification or naming that is only morphologically based. Species identification has become more developed since the discovery of DNA chain propagation techniques using PCR (Polymerase Chain Reaction). The technique of analyzing MT DNA sequences has been widely used in the study of genetic structures (Zhou et al 2003), phylogenetic studies (Zhou et al 2004), and in establishing the origin of a fish population (Freufe et al 2002).

Taxonomic identification and determination of species can be carried out through barcoding of a single gene or DNA locus (Taylor & Harrist 2012; Sarma & Mankodi 2017). The use of barcoding can be performed with short gene sequences, namely with the

cytochrome oxydase (COI) gene (Hebert et al 2003; Ward et al 2005). Barcoding can identify various types of animals up to the species level (Waugh 2007) and can reconstruct phylogeny at the branch of species-level evolution (Palumbi 1996). Another gene that can also be used to identify species well is the Cytochrome b gene (Tobe et al 2009). This study aims to identify the types of gourami in West Sumatra and its correlationship structure.

Material and Method

Fish. The fish used in this study was the *O. goramy* collected from Lima Puluh Kota District, West Sumatra, Indonesia. A total of 16 fish samples consisting of 10 samples of red gourami (local namely sago strain) and 6 non-red color gourami samples (local namely krista, tambago, palapah strains) were considered.

DNA extraction. Samples of fish muscle (50-100 mg) were finely chopped, placed into a tube, and about 50 mL of digestion buffer solution (1% SDS; 0.5 M Tris-HCl, pH 9.0; 0.5 M EDTA, pH 8.0; 1 M NaCl) was added and this mixture was crushed. Then a 50 mL of digestion buffer solution and 20 mg/mL Proteinase K were added. This content was briefly shaken and incubated at 55ºC overnight (Duryadi 1993 in Elvyra et al 2009). Purification of total DNA followed the method of Sambrook et al (1989) using phenol and chloroform iso amyl alcohol (24:1). DNA was precipitated with absolute alcohol and rinsed with 70% alcohol.

Amplification and sequencing. Total DNA from purification results was amplified using PCR technique. The primers used to amplify the cytochrome b gene were L14841 (5'AAAGCTTCCATCCAACATCTCAGCATGATGAAA3') and H15149 (5'AAACTGCAGCCCCTCAG AATGATATTTGTCCTCA3') (Kocher et al 1989). PCR conditions are a cycle of pre PCR (94ºC for 5 minutes), and followed by 35 cycles of PCR which consisted of denaturation (at 94ºC for 30 seconds), annealing (at 55ºC for 45 seconds), extention (at 72ºC for 1 minute), and then followed by a cycle of post PCR (at 72ºC for 5 minutes) (Elvyra & Duryadi 2007).

Purification of PCR results and nucleotide sequencing of cytochrome b genes was carried out in Korea Data analysis. The homologous side of the nucleotide base sequence of the cytochrome b mitochondrial DNA obtained was then paralleled (multiple alignments). The comparative data used was the data of GenBank, which was the complete cytochrome b gene sequence K.Minor; partial cytochrome b gene from GenBank (Access number AY458895).

Results and Discussion

Genetic diversity. Throughout 475 base nucleotides from the cytochrome b region of mitochondrial DNA has been sequenced. The homologous side alignment results of the cytochrome b gene nucleotide sequence indicated that all gourami samples obtained from West Sumatra are *O. goramy* which are suitable for the sample isolates from GenBank (A414b) with a level of similarity of 99%. This shows that red gouramy (sago strain) and non-red (krista, tambago and palapah strains) are derived from *O. goramy*. Similar results were obtained from the study of Nugroho & Kusmini (2007) using enzymatic methods showing no differentiation in gourami of West Java. The differences that occur in morphology as reported by Azrita & Syandri (2015) using the truss morphometric method it is possible due to the contribution of environmental effects rather than the influence of genes.

Analysis of the composition of nucleotide bases for giant gourami from West Sumatra identified seven nucleotide sites that varied. All of them were informative parsimony that is a character containing 2 or more different states and 11 haplotypes on the site of the analyzed *O. goramy* samples. Samples of red and black *O. goramy* of Krista strain have the same number of haplotypes while the other *O. goramy* strains have their own haplotypes (Table 1). Nucleotide sequence variations are due to the influence of nucleotide substitution or reduction of nucleotides. According to Nei & Kumar (2000),

this nucleotide substitution can cause changes in amino acids if they are non-synonymous or do not cause changes in amino acids if they are synonymous. The possibility that occurs in *O. goramy* from West Sumatra is that there are changes in amino acids in parts that are not related to phenotypes.

The average composition of four nucleotide bases from 475 cytochrome b genes from the four gourami strains observed was the largest Cytosine (C) of 28.84% followed by Tymin bases (T = 28.44%), Adenine bases (A = 28.14%) and the smallest base is Guanine (G = 14.56%). The overall composition of Adenine + Tymin nucleotide bases overall in gourami was more (56.58%) than the average Guanine + Cytosine (43.29%). Low Guanine values in mitochondrial DNA are generally obtained in fish (Doadrio et al 2002; Peng et al 2004), including *Tor tambroides* from Sumatra (Wibowo 2012). Similarly, the overall value of Adenine + Tymin is higher than that of Guanine + Cytosine, also found in *T. tambroides* (Wibowo 2012) and Leuciscinae cyprinids (Ketmaier et al 2004). This variation in nucleic base composition can affect gene codon variation in the protein coding region of mitochondrial DNA. Variations in nucleotide bases that produce different codons can form or compose the same proteins or different proteins. This protein is then performed in the phenotype which can be observed visually or measured (non-visual). The possibility that occurs in *O. goramy* from West Sumatra is the variation of existing nucleotide bases to form the same protein or the occurrence of synonym substitution (Nei & Kumar 2000).

Table 1

Strain, sample number (n), number of haplotype (NH) and distribution of haplotype

Strain	*n*	*NH*	*H1*	*H2*	*H3*	*H4*	*H5*	*H6*	*H7*	*H8*	*H9*	*H10*	*H11*
Merah	10	7	1	1	2	2	2	1	1				
Krista	2	2		1			1						
Palapah	2	2								1	1		
Tambago	2	2										1	1

Genetic distance. The genetic distance among samples observed was ranging from 8 x 10^{-6} to 7.5 x 10^{-4}. The shortest genetic distance was found between the palapah and tambago strains. The genetic distance value of gourami was relatively smaller compared to those based on the RAPD method with an average value of 0.407 (Nugroho et al 2016a) and *Tor tor* with an average value of 0.370 (Nugroho et al 2006). The closest genetic distance between tambago and palapah strains are also strengthened the results obtained by Nugroho et al (2016a) and Azrita & Syandri (2015).

The DNA barcoding approach resolved some identification issues and explained the actual species composition in the region (Table 2).

Table 2

List of the species barcoded along with accession numbers

No.	*Order*	*Family*	*Species name*	*Accession no.*
1	Characiformes	Characidae	*Chalceus macrolepidotus*	AB054130.1
2	Perciformes	Helostomatidae	*Helostoma temminckii*	AB861523.1
3		Cichlidae	*Sarotherodon melanotheron*	JF894132.1
4		Ephippidae	*Platax teira*	KJ668153.1
5		Osphronemidae	*Osphronemus septemfasciatus*	AY763769.1
6		Osphronemidae	*Osphronemus exodon*	AY763767.1
7		Osphronemidae	*Osphronemus goramy*	AY763768.1
8		Osphronemidae	*Osphronemus goramy* isolate A414b	KR007731.1
9		Osphronemidae	*Osphronemus goramy* isolate A414a	KR007730.1
10	Anabantiformes	Channidae	*Channa asiatica*	KJ930190.1
11	Mugiliformes	Mugilidae	*Crenimugil crenilabis*	JF911707.1

The relationship of *O. goramy* and its relation based on the nucleotide base sequences are presented in Figure 1. This show that seven individual red gourami strains group together with 2 individual tambago strains, 2 individual strains of palapah and 1 individual strain of krista. These individuals are closed to the gene bank data which is an *O. goramy* strain. Whereas three individual red strains and one individual krista strains have their own groups that are outside from the first group. However, all of the samples belong to a large group. The group has a relationship with gourami *O. exodon* and *O. septemfasciatus* strain. While, Nugroho et al (2016b) categorized the red and krista gurami strains in one group, and tambago and palapah gurami strains in other groups. Azrita & Syandri (2015) separate red strain gurami from krista one, although there are still intersect areas in graph.

This phenomenon, especially the joining of three red strains and one krista strain gourami in one group further strengthens that there is no significant difference between the two strains of gourami, both morphologically and genetically. Furthermore, the joining of red, tambago, palapah and krista strains of *O. goramy* in other groups indicated that the fish might have had genetic mixing or genetic introgression, as along with the development of *O. goramy* culture. Generally, farmers use their own produced parents from West Sumatra so the chance of genetic mixing is quite high.

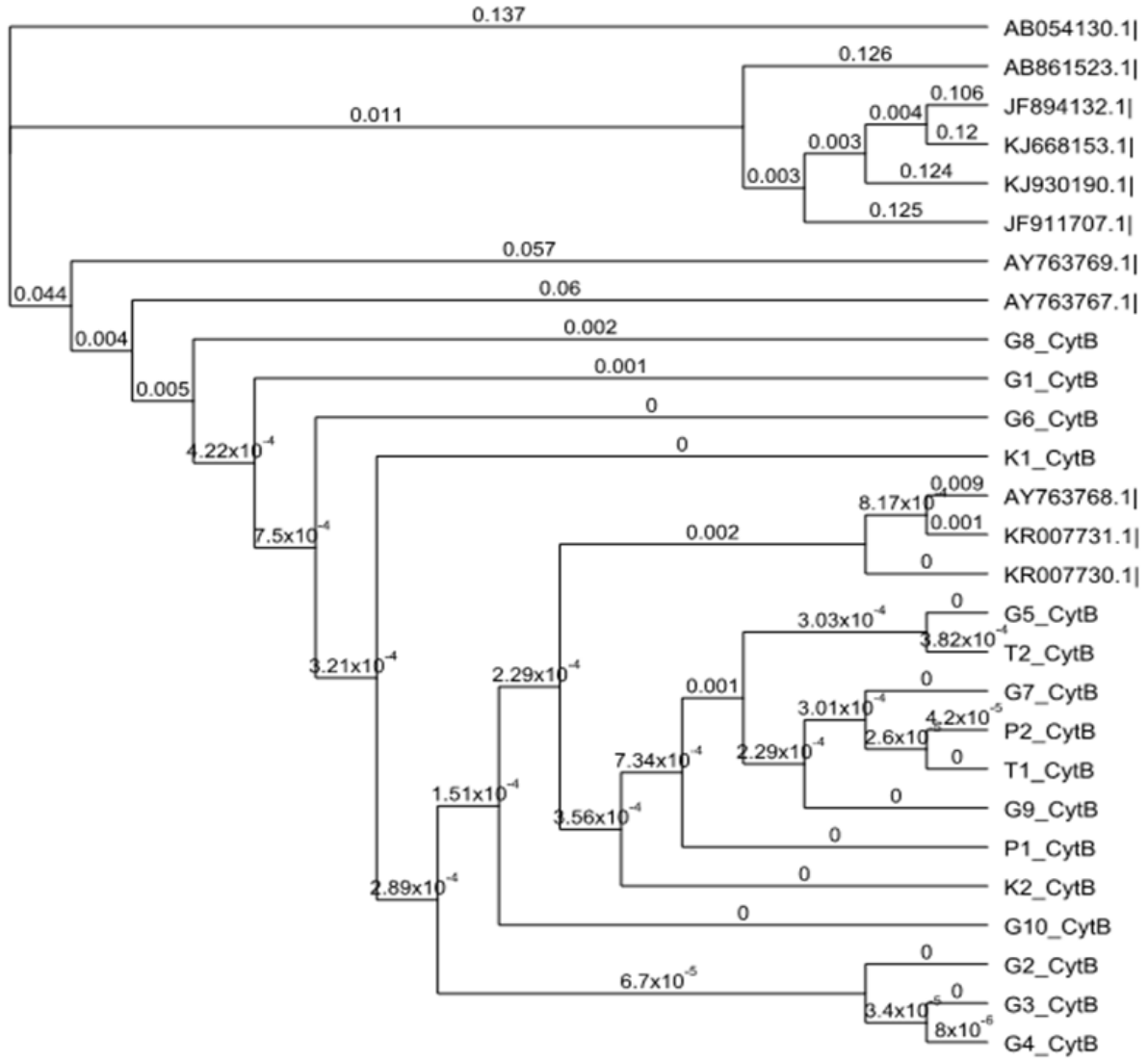

Figure 1. Neighbour-Joining phylogenetic tree of *Osphronemus goramy* at West Sumatra Province with comparison of cytochrome b DNA mitochondria from Gen Bank. (G=red, K=krista, P=palapah and T=tambago).

Conclusions. The type of *O. goramy* originated from West Sumatra had a similarity rate of 99% with the genbank data of *O. goramy*. There are 11 haplotypes from seven variable base sites on 475 sequencing nucleotide bases. The red and krista gourami strains have a common haplotype. The genetic distance between gourami samples ranges from 8 x 10^{-6} to 7.5 x 10^{-4}. The phylogram from Neighbor-Joining based mitochondrial sequences of cytochrome b DNA shows that the four types of gourami strains form one group.

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