

Genetic diversity of Yellow Finnedbarb *Mystacoleucus marginatus* (Valenciennes, 1842) (Teleostei, Cyprinidea) in Brantas basin Upstream, Indonesia

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ABSTRACT

Mystacoleucus marginatus, a native freshwater fish in the family Cyprinidae, is known from parts of Southeast Asia and Western Indonesia Archipelago (Borneo, Sumatra, and Java). This study aims to identify the genetic diversity of *M. marginatus* in the Brantas basin upstream, Java, Indonesia based on the Cytochrome C Oxidase Subunit I (COI) gene. The results showed that *M. marginatus* in the Brantas basin upstream had 2 Haplotypes with Haplotype diversity (Hd) = 0.222 and nucleotides diversity (Pi) = 0.00041.

Key words : Cyprinid, Freshwater fish, Java, COI, Genetic diversity, Phylogenetic

Introduction

Yellow finned barb *Mystacoleucus marginatus* Valenciennes, 1842 is one of the native species cyprinid in the Western Indonesia (Nelson, 2006; Weber and de Beaufort, 1916) with the specific characteristics that have horizontal spines in front of the dorsal fin and has a bright yellow fin color (Roberts, 1989; Rainboth, 1996). *M. marginatus* was also found in Indochina (Yang *et al.*, 2010; Zheng *et al.*, 2016), Malaysia (Ikhwanuddin *et al.*, 2017) and Thailand (Kottelat, 2013).

Especially in Java, *M. marginatus* was spread in the Brantas basin, East Java (Dahrudin *et al.*, 2017; Valen *et al.*, 2019). The genetic diversity of *M. marginatus* in Brantas upstream has not been recorded. Identification of genetic diversity in this study was using DNA Barcoding method (Hebert *et al.*, 2003) with the specific gene which is the cytochrome c oxidase subunit I (COI) gene, a coding protein region of the mitochondrial (mtDNA) genome (Liu *et al.*, 2014). The purpose of this study is to provide information about the genetic diversity in the Brantas basin upstream, East Java.

Materials and Methods

The fish sampling and description of the study sites

We conducted a random sampling survey of the fish diversity in the Karangates reservoir 8°10'05"S; 112°28'37"E; Wlingi Reservoir 8°08'28"S; 112°14'49"E; Leksoriver 8°09'02"S; 112°16'35"E. All sampling sites are located in East Java province, Indonesia. Specimens of *M. marginatus* were obtained from a local fisherman during a fieldwork carried out on 2 June-14 July 2019. We collected specimens using cast-nets. We also obtained specimens from local fishermen, who used traditional fish traps. The twenty-four live specimens of *M. marginatus* had a total length between 11 and 19 cm. Nine (9) of them were used as preserved specimens in 96% alcohol solution (Hasan and Tamam, 2019) for molecular identification. The remaining fifteen (15) were kept as livestock at the Fish Reproduction Laboratory, Brawijaya University, Malang Indonesia.

DNA extraction, isolation and amplification

Genomic DNA extraction from all samples was carried out using the KIT method: Genomic DNA Mini Kit Animal Tissue (GENE AID). Amplification (PCR) of mitochondrial cytochrome C oxidase subunit I (COI) locus gene was carried out using GO TAQ Green PCR Mix method with universal primer Fish_F1 and Fish_R1 (Ward *et al.*, 2005). The PCR process includes pre-denaturation at 94 °C for 3 minutes, followed by 35 cycles consisting of denaturation at 94 °C for 30 seconds, annealing at 50 °C for 30 seconds and an extension stage at 72 °C for 45 seconds. PCR results were then carried out by electrophoresis process to separate, identify and purify DNA fragments, using 1% agarose gel with 50 ml Tris Borate EDTA (TBE). PCR results that have been successfully amplified are then sent to First Base CO (Malaysia) using Big Dye© terminator chemistry (Perkin Elmer), to get the basic arrangement that forms DNA or nucleotide sequences.

Data analysis

Sequencing results are aligned by the Clustal W method in MEGA 6.06 software (Tamura *et al.*, 2013). Furthermore, sequence data is matched with data obtained from the NCBI (National Center for Biotechnology Information) Genbank online (www.ncbi.nlm.gov) via BLASTn (Basic Local

Alignment Search Tool-nucleotide) method. Nucleotide diversity analysis (π), haplotype (h) and polymorphic DNA using DNASP 5.1. Genetic distance was calculated using the pairwise distance method in MEGA 6.06 software (Tamura *et al.*, 2013). Phylogenetic reconstruction using the Maximum Likelihood Trees method of the Kimura-2 parameter model (Kimura, 1980) and 10000× bootstrap value by using MEGA 6.06 software (Tamura *et al.*, 2013). Haplotype network reconstruction using Network 5.0.

Results

Species identification

The length of the fragments resulting from the amplification of the *Mystacoleucus marginatus* COI gene obtained from the Brantas basin upstream using the primary universal Fish_F1 and Fish_R1 (Ward *et al.*, 2005) was 680 bp (base pairs). According to Hebert *et al.*, (2003), fragments along 658 bp using COI genes can be used as a basis for differentiating between animals. All samples from the Brantas basin upstream were identified in Genbank using the BLASTn (Basic Local Alignment Search Tool-nucleotide) method. The sample was identified as *M. marginatus* with a Query cover value 100%, Identity values 100% and E-value 0.0 (Table 1). Species with 99-100% similarity level, it can be said that species are identical (Hebert *et al.*, 2003).

Genetic characteristics

DNA monomers contain 3 different parts, namely pentose sugar, nitrogen bases (A, T, G, C) and phosphate groups. The average nucleotide composition found in the COI gene *Mystacoleucus marginatus* in the Brantas basin upstream was C (Cytosine) of 28.70%, T (Thymine) of 27.8%, A (Adenine) of 27.60% and G (Guanine) of 15.90%. The whole sample of G + C is 44.60% and has a lower amount than the number of A + T which amounts to 55.4%, the low content of G + C makes it easier for us in the amplification process.

Genetic diversity

Mystacoleucus marginatus in the Brantas basin upstream had a diversity of haplotypes (H_d) = 0.2222, nucleotide diversity (P_i) = 0.00041 and 2 haplotypes number (h). The results showed 1 nucleotide mutation, and 1 polymorphic site were identi-

fied. Hobbs *et al.*, (2013) suggested that there are 2 categories of diversity, namely $> 0 \text{ hd} < 0.5$, including in the low category and $> 0.5 \text{ hd} < 1$ including into the high category. In addition, according to Nei (1987), the diversity of haplotypes (Hd) 0.1-0.4 belongs to the low category, (Hd) 0.5-0.7 medium category and (Hd) 0.8-2.00 high category. Based on this category, the value of the diversity of the *M.marginatus* haplotype in the Brantas basin upstream has a low level of diversity.

Phylogenetic tree

A phylogenetic tree or evolutionary tree is a mathematical structure that models evolutionary history based on the DNA sequence of a group of organisms. Phylogeny and relatedness of *Mystacoleucus marginatus* in the Brantas basin upstream was inferred by the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980). The tree with the highest log likelihood (-1173.5121) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree for the heuristic search were

obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 17 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.*, 2013) (Fig. 1).

Genetic distance

Genetic distance of *Mystacoleucus marginatus* in the Brantas basin upstream has a P-distance = 0.000-0.002 (Table 2). Where 0.000 is the closest distance and this value indicates that of 1000 base pairs, none of them have different base pairs. This is because Brantas basin upstream is still in one water-eyed water on Mount Arjuno, the village of Brantas source, Kecamatan Bumi Aji, Kota Batu and then flows to Karangates reservoir; Wlingi reservoir;

Table 1. The identification of *Mystacoleucus marginatus* sequence through BLASTn analysis.

Sample code	Species outcome	BLAST			
		Access code of NCBI	Query Cover (%)	E-value	Identity (%)
Karang kates ¹	<i>M. marginatus</i>	KU692642.1	100	0,0	100
Karang kates ²	<i>M. marginatus</i>	KU692641.1	100	0,0	100
Karang kates ³	<i>M. marginatus</i>	KU692642.1	100	0,0	99
Wlingi ¹	<i>M. marginatus</i>	KU692641.1	100	0,0	100
Wlingi ²	<i>M. marginatus</i>	KU692642.1	100	0,0	100
Wlingi ³	<i>M. marginatus</i>	KU692642.1	100	0,0	100
Lekso river ¹	<i>M. marginatus</i>	KU692642.1	100	0,0	100
Lekso river ²	<i>M. marginatus</i>	KU692641.1	100	0,0	100
Lekso river ³	<i>M. marginatus</i>	KU692642.1	100	0,0	100

Table 2. Genetic distance of *Mystacoleucus marginatus* in the Brantas basin upstream.

	1	2	3	4	5	6	7	8
1 K ¹								
2 K ²	0.000							
3 K ²	0.002	0.002						
4 W ¹	0.000	0.000	0.002					
5 W ²	0.000	0.000	0.002	0.000				
6 W ³	0.000	0.000	0.002	0.000	0.000			
7 L ¹	0.000	0.000	0.002	0.000	0.000	0.000		
8 L ²	0.000	0.000	0.002	0.000	0.000	0.000	0.000	
9 L ³	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000

Remarks: K (Karangates reservoir); W (Wlingi reservoir); L (Lekso river)

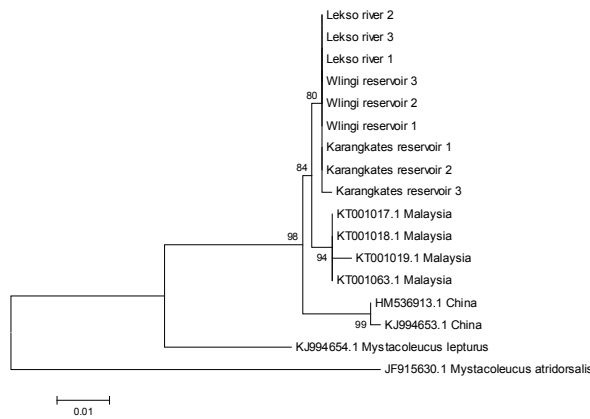


Fig. 1. Phylogenetic tree of *Mystacoleucus marginatus* in the Brantas basin upstream.

Lekso river. Current patterns result in high gene flow or exchange of genes between locations resulting in inhomogeneous genes.

Haplotype distribution

Haplotype distribution was calculated using DNASP 5.1 software (Table 2) and the network reconstruction of the haplotype has been described by Network 5.0 software (Fig. 2). *Mystacoleucus marginatus* in the Brantas basin upstream is closely related between locations, from 9 individuals of the Brantas basin upstream of them are in the same hap-

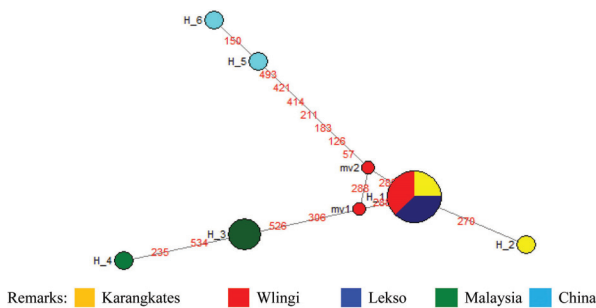


Fig. 2. The haplotype network of *Mystacoleucus marginatus*

lotype and one individual from Karangkates reservoir forms its own haplotype. The Malaysian group forms two haplotypes and China forms 2 haplotypes. Between Brantas basin upstream groups, Malaysia and China are not related to each other. This is because there is no mixing of genes between locations in a long period of time.

Conclusion

Mystacoleucus marginatus in the Brantas basin upstream with a sequence of 680 bp (base pairs) has a diversity of haplotypes ($H_d = 0.2222$) and nucleotides ($P_i = 0.00041$) shows a relationship with one another, this is supported by a 0.000-0.002 P-distance value, where 0.000 is the closest distance and this value indicates that of 1000 base pairs, none of them have different base pairs. The results of the phylogenetic analysis of *M. marginatus* produce the same topology in the Brantas basin upstream. Phylogenetic research on native species must be carried out continuously so that proper information is obtained regarding the presence of certain types of fish.

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References

Dahrudin, H., Hutama, A., Busson, F., Sauri, S., Hanner, R., Keith, P., Hadiaty, R. and Hubert, N.

Table 3. Haplotype distribution of *Mystacoleucus marginatus* in Brantas basin upstream.

Type	Haplotype Code (ind.)
Hap_1 (8 individu)	Karangkates reservoir ¹ ; Karangkates reservoir ² ; Wlingi reservoir ¹ ; Wlingi reservoir ² ; wlingi reservoir ³ ; Lekso river ¹ ;Lekso river ² ; Lekso river ³ .
Hap_2 (1 individu)	Karangkates reservoir ³
Hap_3 (3 individu)	KT001017.1 Malaysia ¹ ; KT001018.1 Malaysia ² ; KT001063.1 Malaysia ³
Hap_4 (1 individu)	KT001019.1 Malaysia ⁴
Hap_5 (1 individu)	HM536913.1 China ¹
Hap_6 (1 individu)	KJ994653.1 China ²

2017. Revisiting the ichthyodiversity of Java and Bali through DNA barcodes: taxonomic coverage, identification accuracy, cryptic diversity and identification of exotic species. *Mol Ecol Resour.* 17 (2) : 288-299.
- Hasan, V. and Tamam, M.B. 2019. First record of the invasive Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1758) (Perciformes, Cichlidae), on Bawean Island, Indonesia. *Check List.* 15 (1) : 225-227.
- Hebert, P.D.N., Cywinska, A. and Ball, S.L. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B : *Biological Sciences.* 270 (151) : 313-321.
- Hobbs, J.P.A., Lynne, V.H., Dean, R.J., Geoffrey, P.J. and Philip, L.M. 2013. High genetic diversity in geographically remote populations of endemic and widespread coral reef Angelfish (genus: *Centropyge*). *Diversity.* 5 : 39-50.
- Ikhwanuddin, M.E.M., Amal, M.N.A., Aziz, A., Sepet, J., Talib, A., Ismail, M.S. and Jamil, N.R. 2017. Inventory of fishes in the upper Pelus River (Perak river basin, Perak, Malaysia). *Check List.* 13 (4): 315-325.
- 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. 2013. The fishes of the inland waters of south-east Asia: a catalogue and core bibliography of the fishes known to occur in freshwaters, mangroves and estuaries. *Raffles Bulletin of Zoology Supplement.* 27 : 1-663.
- Liu, S., Yang, Z., Wang, D. and Liu, M. 2014. The mitochondrial genome of *Mystacoleucus marginatus* (Cypriniformes, Cyprinidae). *Mitochondrial DNA.* 26 (6) : 823-824.
- Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York, 512p.
- Nelson, J.S. 2006. *Fishes of the World*, Fourth edition. John Wiley & Sons, Inc. 601 p.
- Rainboth, W.J. 1996. Fishes of the Cambodian Mekong. FAO species identification field guide for fishery purposes. FAO, Rome, 265 p.
- Roberts, T.R. 1989. The freshwater fishes of Western Borneo (Kalimantan Barat, Indonesia). *Mem. Calif. Acad. Sci.* 14 : 210 p.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution.* 30 : 2725-2729.
- Valen, F.S., Widodo, M.S. and Kilawati, Y. 2019. Molecular Identification and Genetic Characteristics of Genus *Mystacoleucus* Based on Gene Cytochrome Oxidase C. Subunit I (COI) in Sengguruh Dam. *J. Exp. Life Sci.* 9 (1) : 40-46.
- Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R. and Hebert, P.D.N. 2005. DNA Barcoding Australia's Fish Species. *Phil. Trans. R. Soc. B.* 360 : 1847-1857.
- Weber, M.V.C. and de Beaufort, L.F. 1916. *The Fishes of the Indo-Australian Archipelago*. Vol. III. Brill, Leiden, 476 p.
- Yang, L., Mayden, R.L., Sado, T., He, S., Saitoh, K. and Miya, M. 2010. Molecular phylogeny of the fishes traditionally referred to Cypriniformes (Teleostei: Cypriniformes). *Zoologica Scripta.* 39 : 527-550.
- Zheng, L.P., Yang, J.X. and Chen, X.Y. 2016. Molecular phylogeny and systematics of the Barbinae (Teleostei: Cyprinidae) in China inferred from mitochondrial DNA sequences. *Biochemical Systematics and Ecology.* 68 : 250-259.
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