

ORIGINAL ARTICLE

Comparing genetic *Mystacoleucus marginatus* and *Mystacoleucus padangensis* (Cypriniformes: Cyprinidae) based on Cytochrome C Oxidase sub unit I (COI) gene

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Abstract

Mystacoleucus marginatus and *M. padangensis* are freshwater fish species that found in Indonesia. *Mystacoleucus marginatus* is native to Indonesia and *M. padangensis* is an endemic species to West Sumatra, Indonesia. This study aimed to investigate the relationship between *M. marginatus* from the Berantas River, East Java, and *M. padangensis* from Singkarak Lake, West Sumatra based on the Cytochrome Oxidase subunit I (COI) gene. Based on the results, *M. marginatus* and *M. padangensis* have a close genetic distance, with similarity of 99.81%, having difference of 3 nucleotide bases; then they can be identical or synonym. The nucleotide composition in *M. marginatus* and *M. padangensis* are almost similar where the percentage of nucleotide composition in *M. marginatus* is T (28.1%), C (29.2%), A (27.0%) and G (15.8%) and that of *M. padangensis* is T (28.3%), C (28.8%), A (26.8%) and G (16.2%). Based on phylogenetic tree, both species are closely related in the same cluster. Therefore, it is suggested they are a same species based on the COI gene, but suggesting further research using mitochondrial DNA complex and in-depth morphological analysis.

Keywords: COI, Cyprinidae, DNA Barcoding, *Mystacoleucus*.

INTRODUCTION

Indonesia is a country with high diversity having 1266 freshwater fish species (Fishbase 2022), which is seriously threatened due to habitat and environmental damages (Thushari & Senevirathna 2020). The presence of exotic fish is also a threat to the existence of native fish (Wood et al. 2017; Spikmans et al. 2020; Robin et al. 2023). *Mystacoleucus marginatus* is a native species to Indonesia with the IUCN Red List status of Least Concern (Vidthayanon 2012) and *M. Padangensis* is endemic to West Sumatra, Indonesia with the IUCN Red List status of Vulnerable (VU) (Lumbantobing 2020). Both species belong to Cyprinidae inhabiting tropical areas with a temperature range of 22-27°C as benthopelagic i.e. occupying the bottom depths of rivers and streams. They have different sizes where *M. padangensis* is 11cm in size and *M. marginatus* has a larger size of 20cm (Valen et al. 2020).

The genus *Mystacoleucus* has eight species, including *M. marginatus* (Valenciennes, 1842) in Southeast Asia to western Indonesia (Liu et al. 2015;

Ng et al. 2019; Valen et al. 2021), *M. padangensis* (Bleeker, 1852), in the Singkarak Lake, West Sumatra, Indonesia (Kottelat 2013), *M. atridorsalis* (Fowler 1937) in Mae Khlong basins (Kottelat 2015), *M. ectypus* (Baumgartner et al. 2020) in Laos, Thailand and Cambodia (Kottelat 2015), *M. chilopterus* (Fowler 1935) in Mae Khlong basins and Malay Peninsula (Kottelat 2013), *M. greenwayi* (Pellegrin & Fang 1940) in the Mae Khlong basins and Xe Bangfai (Kottelat 2015), *M. lepturus* (Huang, 1979) from Mae Khlong (Fu et al. 2021; Chen & Song 2022), *M. argenteus* (Day, 1888) in Myanmar and Thailand (Kottelat 2013) and *M. günther* in China (Kong et al. 2003). Of the eight species, 2 species are found in Indonesia, viz. *M. marginatus* is distributed on the island of Java and *M. padangensis* is endemic to Singkarak Lake, West Sumatera.

The genus *Mystacoleucus* has a horizontal spine in front of its dorsal fin (Kottelat et al. 1993). The Indonesian species have a fairly close resemblance, but there are several different characteristics e.g.

size differences (Valen et al. 2020). There is no molecular data of the two Indonesian species based on COI gene. Hence, this study aimed to investigate the relationship between *M. marginatus* from the Brantas River, East Java, and *M. padangensis* from Singkarak Lake, West Sumatra based on the Cytochrome Oxidase subunit I (COI) gene.

MATERIALS AND METHODS

Specimens of *M. marginatus* were collected from Brantas River, West Jawa Indonesia. All collected specimens were preserved in 95% ethanol (Valen et al., 2019b) for subsequent DNA analysis. The COI gene of *M. padangensis* were taken from the NCBI Genbank (MF062186, MF062185, and MF062184). We also obtained the COI gene of *M. atridorsalis* (JF915630) and *M. lepturus* (KJ994654) from NCBI Genbank. Extraction of DNA was done based on Robin et al. (2023). The amplification of COI gene following the Hotstart method with the FISH F1 and FISH R1 primers (Ward et al. 2005). The amplification proces was repeated for 38 cycles following the early denaturation at 94°C for 3 minutes, the next denaturing at 94°C for 30 seconds, annealing at 48°C for 30 seconds, and extension at 72°C for 45 seconds (Valen et al. 2022; Robin et al. 2023). The PCR results were then visualized in 1% agarose gel using electrophoresis by staining Nucleic Acid Gel Stain (GelRed®) (Hasan et al. 2021; Lutfiatunnisa et al. 2020). The samples are then sequenced at a sequencing service facility to obtain the nucleotide sequence, using the Sanger sequencing method (Sanger & Nicklen 1977).

Species identification was done using the BOLD SYSTEM (<https://www.boldsystems.org>) and the BLASTn (Basic Local Alignment Search Tool-nucleotide) NCBI GenBank (<https://blast.ncbi.nlm.nih.gov>). Nucleotide analysis was using the MEGA X (Kumar et al. 2018). The sequences were aligned using the Muscle algorithm (Edgar 2004). The phylogenetic tree was constructed using the Maximum Likelihood method (Tamura & Nei 1993). The analysis involved 8

nucleotide sequences. There were a total of 775 positions in the final dataset.

RESULTS AND DISCUSSION

In this study, species identification and their relationship analysis were done using the COI gene. Application of the COI gene has been proven informative for species identification on several types of animals and fish (Oba et al. 2015; Bingpeng et al. 2018; Panprommin et al. 2019). The length of the *M. marginatus* COI gene sequences were 665 bp (Table 1). The minimum of 658bp long fragment using the COI gene can be used as a basis for differentiation between animals and species identification (Hebert et al. 2003; Lin et al. 2015). Sequences of the specimens from the Brantas river, East Java were compared to the NCBI GenBank (National Center for Biotechnology Information) using the BLASTn (Basic Local Alignment Search Tool-nucleotide) method (<https://blast.ncbi.nlm.nih.gov>) for analyze homology of the sequences. We also ensure species validity through the BOLD SYSTEM (<https://www.boldsystems.org>) by specimen identification tools to check the species level barcode records (Table 2).

Based on the results, the specimens from the Brantas River have a 100% similarity with *M. marginatus* which means they are identical or same species. The same similarity also found between specimens from the Brantas River and *M. padangensis* with a similarity percentage of 99.81%. Sequences with a similarity of between 98-100% are identical or the same species, and the spesies with the differences of more than 3% can be different species (Hebert et al. 2003; Landi et al. 2014). Based on DNA BLAST, between sequences of the Brantas River and *M. padangensis* with up to 99.81% similarity was found showing that they are also identical or same species. In contrast, the similarity between the sequences of the Brantas river specimens, and *M. atridorsalis* and *M. lepturus*, were 90 and 94%, respectively meaning that the

Table 1. A Barcoding of *Mystacoleucus marginatus* from Brantas River, East Java, Indonesia.

<i>Myatacoleucus marginatus_1</i>			
CTTGTATTTGGTGCCTGAGCCGGAATAGTAGGAACCGCCTTAAGCCTTCTTATTCGAGCCGAACTGAGCCAACCCG GATCACTTCTAGGCGATGATCAAATCTACAACGTCATCGTTACTGCCACGCCTTCGTAATAATTTTCTTTATAGTA ATACCTATCCTTATTGGAGGGTTCGGAAACTGACTTGTACCACTAATGATTGGAGCCCCGACATAGCATTTCAC GGATAAATAACATAAGCTTCTGATTATTACCCCATCATTCTACTACTATTAGCCTCATCTGGTGTGAAGCCGGA GCCGGAACGGGGTGAACAGTATATCCCCCTCTTGCAGGGAACCTAGCCCACGCAGGAGCATCAGTAGACCTAACA ATTTTCTCACTCCACTTAGCAGGTGTATCATCAATTTTAGGTGCAATCAATTTTATTACTACAACCATTAACATGAA ACCCCAACCATCTCCAATATCAAACACCCCTTTTCGTTTGATCCGTAACCGCCGTAACCGCCGTAACCGCCGTA ACTACCCGTCTAGCCGCCGGGATCACAATACTCCTAACAGACCGAAATCTTAACACCACATTCTTCGACCCGGCA GGGGGAGGAGACCAATCTCTACCAACACCTATTTTGATTCTTTGGCCACCAAAAAAGTTCTAAA			
<i>Myatacoleucus marginatus_2</i>			
GCTGAGCCGGATAG2AGGAACCGCCTTAAGCCTTCTTATTCGAGCCGAACTGAGCCAACCCGGATCACTTCTAGGC GATGATCAAATCTACAACGTCATCGTTACTGCCACGCCTTCGTAATAATTTTCTTTATAGTAATACCTATCCTTATT GGAGGGTTCGGAAACTGACTTGTACCACTAATGATTGGAGCCCCGACATAGCATTTCACGGATAAATAACATAA GCTTCTGATTATTACCCCATCATTCTACTACTATTAGCCTCATCTGGTGTGAAGCCGGAGCCGGAACCCGGTGA ACAGTATATCCCCCTCTTGCAGGGAACCTAGCCCACGCAGGAGCATCAGTAGACCTAACAATTTTCTCACTCCACT TAGCAGGTGTATCATCAATTTTAGGTGCAATCAATTTTATTACTACAACCATTAACATGAAACCCCAACCATCTCC CAATATCAAACACCCCTTTTCGTTTGATCCGTAACCGCCGTAACCGCCGTAACCGCCGTAACCGCCGTAACCGCC GCCGGGATCACAATACTCCTAACAGACCGAAATCTTAACACCACATTCTTCGACCCGGCAGGGGGAGGAGACCA ATCTCTACCAACACCTATTTTGATTCTTCGGCAC			
<i>Myatacoleucus marginatus_3</i>			
TGANCCGGATAGTAGGACCGCCTTAAGCCTTCTTATTCGAGCCGAACTGAGCCAACCCGGATCACTTCTAGGCGAT GATCAAATCTACAACGTCATCGTTACTGCCACGCCTTCGTAATAATTTTCTTTATAGTAATACCTATCCTTATTGG AGGGTTCGGAAACTGACTTGTACCACTAATGATTGGAGCCCCGACATAGCATTTCACGGATAAATAACATAAGC TTCTGATTATTACCCCATCATTCTACTACTATTAGCCTCATCTGGTGTGAAGCCGGAGCCGGAACCCGGTGAAC AGTATATCCCCCTCTTGCAGGGAACCTAGCCCACGCAGGAGCATCAGTAGACCTAACAATTTTCTCACTCCACTTA GCAGGTGTATCATCAATTTTAGGTGCAATCAATTTTATTACTACAACCATTAACATGAAACCCCAACCATCTCCCA ATATCAAACACCCCTTTTCGTTTGATCCGTAACCGCCGTAACCGCCGTAACCGCCGTAACCGCCGTAACCGCCG CGGGATCACAATACTCCTAACAGACCGAAATCTTAACACCACATTCTTCGACCCGGCAGGGGGAGGAGACCAAT CCTCTACCAACACCTATTTTGATTCTTCGGCCANCCGG			

Table 2. Species identification and similarity.

Sspecimen	Similarity GenBank	Species Outcome	Accession Number (GenBank)
Sample sequence	100%	<i>Mystacoleucus marginatus</i>	KU692642.1
	99.81%	<i>Mystacoleucus padangensis</i>	<u>MF062186.1</u>
	90%	<i>Mystacoleucus atridorsalis</i>	JF915630.1
	94.45%	<i>Mystacoleucus lepturus</i>	<u>KJ994654.1</u>

difference between species i.e. exceeding 3%.

Based on geographical distribution (Fig. 1), *M. marginatus* is found in Brantas River, East Java and has been recorded in West java (Dahrudin et al. 2017; Valen et al. 2020; Valen et al. 2019a, b, 2021). *Mystacoleucus padangensis* (Bleeker 1852) is an endemic species to the Singkarak Lake, West Sumatra, Indonesia (Kottelat 2013). *Mystacoleucus aridorsalis* and *M. Lepturus* are found in Mae Khlong basins (Kottelat 2015; Fu et al. 2021), and both species have never been recorded in Indonesia.

Mystacoleucus marginatus and *M. padangensis* have close geographic distribution.

The basis for differentiation between animals and species identification is influenced by differences in nucleotides (Table 3). The variations in nucleotide composition can be used to support data information about genetic variation between species. The nucleotide composition in *M. marginatus* and *M. padangensis* are almost similar where the percentage of nucleotide composition in *M. marginatus* is T (28.1%), C (29.2%), A (27.0%)



Fig.1. Distribution of *Mystacoleucus marginatus* and *M. padangensis* in Indonesia; the Red Triangle is the distribution area of *M. padangensis* and The Blue triangle is the distribution area of *M. marginatus*.

Table 3. Nucleotide compound of *Mystacoleucus* genus.

Species	Nucleotide (%)			
	T(U)	C	A	G
<i>Mystacoleucus marginatus</i>	28.1	29.2	27.0	15.8
<i>Mystacoleucus padangensis</i>	28.3	28.8	26.8	16.2
<i>Mystacoleucus atridorsalis</i>	29.7	26.8	26.0	17.5
<i>Mystacoleucus lepturus</i>	29.4	27.5	26.6	16.5

and G (15.8%). while the nucleotide composition of *M. padangensis* is T (28.3%), C (28.8%), A (26.8%) and G (16.2%). In *M. Aridorsalis*, the percentage of nucleotide composition is T (29.7%), C (26.8%), A (26.8%) and G (17.2%) and *M. lepturus* T (29.4%), C (27.5%), A (26.6%) and G (16.5%). All species in the genus *Mystacoleucus* show the same pattern with high average amounts of adenine and thymine, so that the COI gene of this genus is categorized as an A-T rich group (A-T rich). The A-T hydrogen bond consists of 2 hydrogen bonds which are weaker than the G-C hydrogen bond which has 3 hydrogen bonds, so the possibility of species mutation is higher. In addition, the differences in the nucleotide bases of a species can be identified by conducting polymorphic site analysis. This analysis aims to determine the location of sites that experience variations within the same species (Table 4).

There are 3 nucleotide bases out of ±600 nucleotide bases between *M. marginatus* and

M. Padangensis. There is in 280th row *M. marginatus* has “C” and *M padangensis* has “G” nucleotide bases. The nucleotide differences between *M. marginatus* and *M. padangensis* also found in the 454 and 391th bases. These three DNA bases are also the difference between *M. marginatus* and *M. padangensis*. Differences in nucleotide bases between species result in genetic variation. The more differences between species, the more distance between species. While the difference in nucleotide bases between *M. marginatus* with *M atrodorsalis* and *M. lepturus* is quite large.

Genetic distance is the degree of difference in a gene which is calculated based on differences between species or populations (Nei 1972). The highest genetic distance between species in the genus of *Mystacoleucus* was found between *M. atridorsalis* to the *M. marginatus* as 0.330, followed by *M. lepturus* of 0.119 (Table 5). The closest distance between species occurs in

Table 4. Polimorphic Site of *Mystacoleucus* genus.

Species	Posisi Nukleutida														
	4	25	31	55	70	73	82	85	91	94	121	133	139	163	166
<i>M.marginatus</i>	C	C	G	C	C	C	T	T	C	C	A	T	G	A	G
<i>M.padangensis</i>
<i>M.atridorsalis</i>	T	T	T	A	T	T	C	.	T	A	G	C	A	.	A
<i>M.lepturus</i>	.	.	A	C	.	.	G	.	A	T	A
	169	181	184	193	196	205	218	221	226	238	242	256	259	268	271
<i>M.marginatus</i>	T	C	A	A	G	C	T	T	C	A	C	T	T	C	A
<i>M.padangensis</i>
<i>M.atridorsalis</i>	C	T	G	G	A	T	C	C	A	G	.	C	A	T	G
<i>M.lepturus</i>	T	.	.	.	G	T	.	.	T	G
	274	280	289	295	298	301	310	313	316	319	325	328	334	349	358
<i>M.marginatus</i>	C	C	A	T	C	T	G	C	A	C	A	A	A	T	C
<i>M.padangensis</i>	.	G
<i>M.atridorsalis</i>	.	A	G	.	G	C	A	T	G	T	.	.	T	C	.
<i>M.lepturus</i>	T	A	.	C	A	.	A	.	.	.	G	G	.	.	T
	361	362	364	367	383	385	391	394	400	406	409	418	427	431	433
<i>M.marginatus</i>	C	T	A	A	T	A	A	C	T	T	A	C	C	A	C
<i>M.padangensis</i>	G
<i>M.atridorsalis</i>	T	C	G	.	C	G	.	.	.	C	G	T	T	G	T
<i>M.lepturus</i>	.	.	.	G	.	.	.	T	C	.	.	T	.	G	.
	436	442	445	454	469	472	475	478	481	487	505	508	511	523	526
<i>M.marginatus</i>	C	A	T	C	C	A	T	A	C	A	A	C	C	G	C
<i>M.padangensis</i>	.	.	.	T
<i>M.atridorsalis</i>	T	G	C	A	G	.	C	T	T	G	T	T	T	C	T
<i>M. Lepturus</i>	.	.	.	A	.	G	T	.	.	T

Table 5. Genetic distances of *Mystacoleucus* genus.

Species	1	2	3
1 <i>Mystacoleucus marginatus</i>			
2 <i>Mystacoleucus padangensis</i>	0.0093		
3 <i>Mystacoleucus atridorsalis</i>	0.3309	0.2323	
4 <i>Mystacoleucus lepturus</i>	0.1394	0.0836	0.0502

M. marginatus and *M. Padangensis* as 0.009. According to Nei (1972), a genetic distance of 0.010-0.099 is included in the low category, 0.1-0.99 is included in the medium category and a genetic distance of 1.00-2.00 is included in the high category.

This genetic distance will determine the construction of the evolutionary tree in the species or a phylogenetic tree. Phylogenetic is often used to represent relationships among genes or individual organisms. Reconstruction phylogenetic relationship of *Mystacoleucus* genus were done base on mitochondrial COI gene (Zou et al. 2020; Kumari et al. 2020). The phylogenetic tree was used to

analyze the relationship between species of the genus *Mystacoleucus* involving samples of *M.marginatus* from the Brantas River and samples of *M. padangensis* sequences from NCBI Genbank (Fig. 2). Based on the results, the phylogenetic tree forms 3 clusters. One has a value of 100 which shows that with 1000x bootstrap (repetition) 100% will form the correct branch. According to Brinkman, (2001), a branch that is more than 70% has truth with a 95% confidence interval. *M. marginatus* and *M. padangensis* are come from a common ancestor and closely related. *Mystacoleucus marginatus* and *M. padangensis* are located on the same branch having low genetic

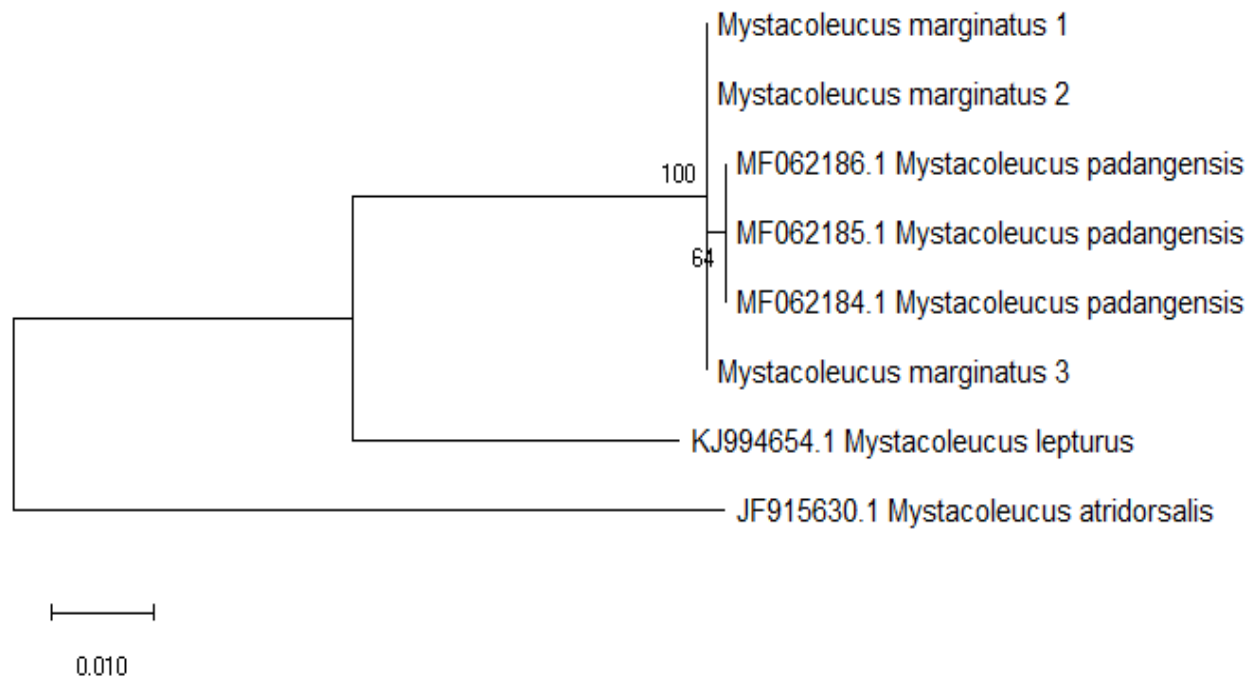


Fig.2. Phylogenetic of *Mystacoleucus* Based on Cytochrome Oxidase C Subunit I (COI) Gene.

distance of 0.009. The lower the genetic diversity, the closer the relationship between the two species, showing no genetic diversity, then they can be identical or synonym.

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مقاله کامل

مقایسه ژنتیکی *Mystacoleucus marginatus* و *Mystacoleucus padangensis* (کپور

ماهی شکلان: کپور ماهیان) بر اساس ژن سیتوکروم اکسیداز I (COI)

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چکیده: *Mystacoleucus padangensis* و *Mystacoleucus marginatus* از ماهیان آب شیرین هستند که در اندونزی یافت می‌شوند. *Mystacoleucus marginatus* بومی اندونزی و *M. padangensis* یک گونه بومی در غرب سوماترا، اندونزی است. این مطالعه با هدف بررسی رابطه بین *M. marginatus* از رودخانه برانتاس، جاوه شرقی و *M. padangensis* از دریاچه سینگاراک، غرب سوماترا بر اساس ژن سیتوکروم اکسیداز I (COI) انجام شد. بر اساس نتایج، *M. marginatus* و *M. padangensis* دارای فاصله ژنتیکی نزدیک با شباهت ۹۹/۸۱ درصد با اختلاف ۳ باز نوکلئوتیدی هستند. بنابراین آن‌ها می‌توانند مترادف هم باشند. ترکیب نوکلئوتیدی در *M. marginatus* و *M. padangensis* تقریباً مشابه است که در آن درصد ترکیب نوکلئوتیدی در *M. marginatus* (۲۸/۱٪) T، (۲۹/۲٪) A، C، (۰/۲۷،۰) G و (۱۵/۸)٪ است و برای *M. padangensis* (۲۸/۳٪) T، (۲۸/۸٪) A، C، (۲۶/۸٪) G و (۱۶/۲)٪ (۱۶/۲٪) است. بر اساس درخت فیلوژنی، هر دو گونه در یک خوشه نزدیک هستند. بنابراین، پیشنهاد می‌شود که بر اساس ژن COI آن‌ها یک گونه هستند، اما تحقیقات بیشتر با استفاده از DNA میتوکندری و تجزیه و تحلیل ریختی کامل را پیشنهاد می‌کند.

کلمات کلیدی: سیتوکروم اکسیداز I، کپور ماهیان، بارکد DNA، *Mystacoleucus*