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by Mochamad Syaifudin

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Research Article

DNA Authentication of Indonesian Leaffish *Pristolepis grooti* from Kelekar River and Ogan River in South Sumatra Based on *Cytochrome C Oxidase Subunit I (COI)* Gene

Mochamad Syaifudin[1]*, Evitry Tamaria Gultom[1], Marini Wijayanti[1]
1)Aquaculture Study Program, Department of Fisheries, Faculty of Agriculture, Sriwijaya University. Jl. Raya Palembang-Prabumulih KM.32 Indralaya Ogan Ilir, South Sumatra, Indonesia. 30662.
* Corresponding author, email: msyaifudin@fp.unsri.ac.id

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ABSTRACT
Indonesian leaffish *Pristolepis grooti*, an endemic species, are distributed in the region of Sumatra, Riau, Bangka Belitung and Kalimantan. However, there has been a decline in the population recently. This research purposed to investigate the mitochondrial DNA *cytochrome c oxidase subunit I (COI)* gene, the genetic distance, the genetic tree of the leaffish and characterize the chemical physics of water of its habitat in the Kelekar River Muara Enim Regency and the Ogan River, Ogan Ilir Regency. The method used in species authentication was DNA isolation, amplification using PCR (Polymerase Chain Reaction) and sequencing of *COI* gene. The size of the *COI* mtDNA gene fragment was 704 bp (PM 1, PM 4, PP 2 and PP 4) and 723 bp (PM 2, PM 3, PP 1 and PP 3). A cryptic diversity of the species *P. grooti* is found based on the genetic distance value of 4.5-6%, both in the Kelakar and Ogan Rivers. The phylogenetic tree of the leaffish of this study formed 2 separate sub-clusters with a bootstrap value of 50%. The properties of water qualities in the two rivers included temperatures 28.3-31.8°C, pH 5.6-8.3, dissolved oxygen 4.82-10.89 mg L^{-1}, alkalinity 10-28 mg L^{-1} $CaCO_3$, water transparency 16-45 cm, ammonia 0.47-0.70 mg L^{-1}, water current 0.17-0.30 m s^{-1} and TDS 7-44 mg L^{-1}.

INTRODUCTION
The Indonesian leaffish *Pristolepis* sp., known locally as sepatung is one of the indigenous fishes in Indonesia. It is distributed in the Kelekar River South Sumatra (Muslim et al. 2019), the Ogan River, Ogan Ilir Regency, and the Musi River, Palembang (Emawati et al. 2017), also in the Kampar River (Riau), Borneo, Bangka and Belitung (Kottelat & Whitten 1993). The leaffish is a family group of *Pristolepidae* commonly found in flooded swamp waters and has high economic value. Currently, Fishbase reported 8 species recognized species in this genus, two of which are *Pristolepis grooti* known as Indonesian leaffish and *P. fasciata* known as Malayan leaffish (Froese & Pauly 2019) which also lives in the territory of Indonesia. The phenotypic diversity of the two species in nature makes it difficult to determine the phenotypic comparison of the two types of fish, furthermore, the two species possibly exist in the same water (Muslim et al. 2019). *P. grootii* has been reported as one of dominants species (C=0.05-

0.014) in Kelekar floodplain, Ogan Ilir Regency (Muslim & Syaifudin 2022). Identification and characterization of leaffish are essential, as they are related to the adaptability, cultivation techniques and conservation of the leaffish in its natural habitat, which is still very limited, so thus it is important to apply a relatively easy and more accurate method to identify the species using the molecular technique, relatively. One of which is through DNA barcoding by using mitochondrial DNA that matches the target for analysis on various species-level target genes (Masters et al. 2007). The samples of leaffish for DNA barcoding were taken from the Kelekar River, Muara Enim Regency and the Ogan River in Pemulutan, Ogan Ilir Regency.

Cytochrome C oxidase subunit 1 (COI) is one of the genes in the mitochondrial genome (mtDNA), which is precisely used as a barcode. This molecular technique can be used for determining genetic analysis systematically and at the taxonomic level of species, populations and also plays a role in the selection process of fish genetic diversity. The application of DNA barcoding has been conducted on several aquatic organisms i.e *Hemibagrus nemurus* (Syaifudin et al. 2017), *flatfish* from Vietnam (Truong et al. 2020), snake-skin gourami, blue gourami (Syaifudin et al. 2019), *Pristolepis fasciata* from Malaysia (Noikotr 2019) striped snakehead, catfish *Mystus singaringan* (Pramono et al. 2019), and ocellated snakehead (Syaifudin et al. 2020). In this study, the application of DNA barcodes has an important role in determining COI gene sequences as a taxonomic tool to reveal the genetics authentication of species among Indonesian leaffish from the Kelekar and the Ogan River in South Sumatra.

MATERIALS AND METHODS

Materials

Indonesian leaffish (Figure 1) and water samples were taken from two locations (Figure 2) i.e, Kelekar River in Segayam Village, Muara Enim Regency (given the PM code) and the leaffish from the Ogan River in Pemulutan District, Ogan Ilir Regency at sampling coordinates 3°03¢17¢¢S 104°46¢32¢¢E (given the PP code). Four individuals were taken from each river. All specimens were collected in the wild and determined morphologically in situ by visual observation based on manual identification (Saanin 1984). The length of leaffish from the Kelekar River ranged from 6.6-10.9 cm and weighed of 4.37-34.12 g while the leaffish from the Ogan River measures 11.5-13.5 cm in length and 28.66-50.53 grams of weight. Fin samples were put into a 1.5 ml tube with a 96% ethanol solution, then stored in a freezer at -20°C until DNA isolation was carried out.

Methods

DNA Isolation

The total genome of the leaffish DNA was isolated using the extraction kit of Genomic DNA (GeneAid) as described in the protocol. The DNA was extracted in six stages, i.e. the preparation of specimens, the lysis of cell, RNAse addition, DNA precipitation, cleaning and DNA dissolution. The extracted DNA of fin samples were stored in a freezer (-20 °C), until the electrophoresis process was carried out to check DNA integrity and PCR (Polymerase Chain Reaction).

DNA Amplification

DNA genome was amplified using Polymerase Chain Reaction (PCR) to target a 655 bp of *COI* gene with primer pairs of FishF2 (forward)-5' TCGACTAATCATAAAGATATCGGCAC 3' dan FishR2 (reverse)-5'

Figure 1. Indonesian leaffish (*Pristolepis grooti*).

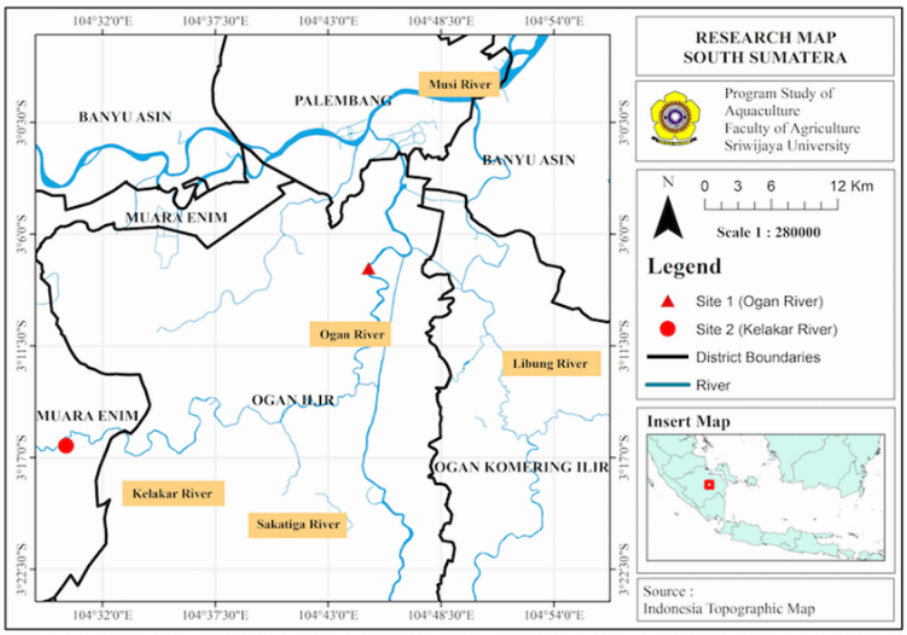


Figure 2. Map of research locations in the Kelekar and Ogan River.

ACTTCAGGGTGACCGAAGAATCAGAA 3' (Ward et al. 2005). PCR was performed in a final volume of 50 µL. Each reaction contained 17 µL ddH_2O, 25 µL My Taq Red Mix, 10 µL Fish R2 primer (10 pmol/µL), 10 µL Fish F2 primer (10 pmol/µL) and 6 µL DNA template. DNA amplification was carried out in stages: initiation cycle at 94 °C for 1 minute in 1 cycle, denaturation at 94 °C for 30 seconds, annealing at 52 °C for 30 seconds, extension or elongation at 72 °C for 15 seconds in 35 cycles and post extension 72 °C for 4 minutes in 1 cycle. Furthermore, the PCR product was visualized by electrophoresis of 1% agarose gel in 75 voltage for 35 minutes. The size of the DNA from the PCR was measured using a 1 kb DNA marker. The PCR products were sequenced bi-direction with both primers through the services of PT Genetics Science (Jakarta).

Water Quality

Water qualities were measured three times at each location during the

study. The parameters observed i.e. temperature (°C), water transparency (cm), dissolved oxygen (mg L^{-1}), pH, ammonia (mg L^{-1}), total alkalinity (mg L^{-1}), total dissolved solid/TDS (mg L^{-1}) and water current (m s^{-1}).

Data Analysis

The *COI* gene sequences in fasta format are then aligned using MEGA X software, then continue to BLAST (Basic Local Alignment Search Tool) for determining the homology of a DNA sequence with the data in NCBI (National Center for Biotechnology Information). Furthermore, all sequences were aligned to analyze the genetic distance and phylogenetic tree, including a sequence of *Oreochromis niloticus* (GenBank: KM438528) from Stirling collection as an outgroup. The phylogenetic tree was constructed using the Neighbor-Joining (NJ) method and the Maximum Composite Likelihood model on MEGA software X Version (Kumar et al. 2018; Stecher et al. 2020) and the genetic distance was analyzed using the Pairwise Distance method (Kimura 1980).

RESULTS AND DISCUSSION

DNA Authentication

The amplified DNA of the *COI* gene of leaffish was electrophoresed using 1% agarose gel, showing a size of 704 bp for PM 1, PM 4, PP 2 and PP 4 samples code and 723 bp for PM 2, PM 3, PP 1 and PP 3. All sequences of PCR product have been submitted in Barcode of Life Database Identification (BOLD-ID) under the accession number BOLD:ADO0531 and BOLD:ADN7493. The *COI* gene sequence of the leaffish is analyzed through nucleotide BLAST on the website of the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) for comparison with other species in the GenBank database. The percentages of nucleotide similarity of the leaffish were presented in Table 1.

Nine individuals of *P. grooti* were barcoded successfully using *COI* gene with universal primer followed (Ward et al. 2005) at annealing temperature optimization of 52°C for 30 seconds in 35 cycles of PCR. Annealing temperature in the PCR used to usually calculated from Tm-5°C to Tm+5°C (Muladno 2010). The table 1 indicated that the *COI* nucleotide sequences of the leaffish (*P. grooti*) had the highest identity of around 95.29%-96.13% with *P. fasciata* from Malaysia (accession code KT001055.1), then 94.70-95.85 to *P. fasciata* from Vietnam (accession code MH721176.1). The smallest percentage of similarity (87.48% - 89.22%) was *P. rubripinnis* from India (MG9234001, MG923399.1, MG923396.1).

There was none of the leaffish from the Kelekar River and the Ogan River 100% similar to other fish samples or the same species in GenBank (Table 1). *Pristolepis grooti* is very similar to *P. fasciata*, with slight differences in the presence of 3½ rows (fasciata: 4½ rows) of scales separating dorsal fin mid spines from lateral line scales; ventral fin that does not reach the anal canal, coloration, and profile of the top of the head towards the nape which is more convex (fasciata: tends to be straight) (Weber & Beaufort. 1936).

Based on data from meristic measurements of leaffish (*Pristolepis* sp.) from the Kelekar River and Ogan River, the leaffish had a total length between 12.7 cm - 13.5 cm. Table 2 Indicated the dorsal fins have 12-13 hard spines and 13-16 soft rays (D.XIII.15-16), 3-8 anal fins, 12-14 soft rays of pectoral fins (P. 12-14), 1 hard spine, 5 soft rays of ventral fins, 12-14 soft rays of caudal fins (C. 12-14). Research by (Muslim et al. 2019) on an Indonesian leaffish *Pristolepis grootii*, the dorsal fin has 13 hard spines and 15-16 soft rays (D.XIII.15-16), the anal fin consists of 3

Table 1. The similarity percentage of nucleotides of leaffish *P. grooti* from the Kelekar and Ogan rivers.

No.	Code	*Description*	Query cover (%)	Identity (%)	Accession	Origin
1.	PM 1	*Pristolepis fasciata*	91	95.62	KT001055.1	Malaysia
			94	95.28	MH721176.1	Vietnam
2.	PM 2	*P. fasciata*	91	95.29	KT001055.1	Malaysia
			95	94.70	MH721176.1	Vietnam
3.	PM 3	*P. fasciata*	91	95.62	KT001055.1	Malaysia
			93	95.39	MH721176.1	Vietnam
4.	PM 4	*P. fasciata*	92	95.29	KT001055.1	Malaysia
			96	94.96	MH721176.1	Vietnam
5.	PP 1	*P. fasciata*	87	96.13	KT001055.1	Malaysia
			92	95.85	MH721176.1	Vietnam
6.	PP 2	*P. fasciata*	91	95.96	KT001055.1	Malaysia
			94	95.72	MH721176.1	Vietnam
7.	PP 3	*P. fasciata*	91	95.96	KT001055.1	Malaysia
			94	95.72	MH721176.1	Vietnam
8.	PP 4	*P. fasciata*	91	95.96	KT001055.1	Malaysia
			95	95.32	MH721176.1	Vietnam
9.	All samples	*P. rubripinnis*	94–99	87.48-88.91	MG9234001 MG923399.1 MG923396.1	India

Table 2. Meristic parameter between *P. grooti* and *P. fasciata*.

Character	Sample		Species	
	Kelekar River	Ogan River	*P grooti*	*P. fasciata*
Dorsal fins	DXII-XIII.13-16	DXII-XIII.13-15	DXIII.15-16	D.XII-XIII.13-14
Ventral fins	V.I.5	V.I.5	V.I.5	V.1.5
Pectoral	P.12-14	P.12-14	P.13-14	P.12
Caudal	C.12-14	C.12-14	C.13-14	-
Anal	AIII.7-8	AIII3.7-8	AIII.7-8	A.III.7-8
Source			(Muslim et al. 2019)	(Sukmono & Margaretha 2017)

hard spines and 7-8 soft rays (A.III.7-8). As for the pectoral fins, there are no hard spines with 13-14 soft rays (P.13-14), the ventral fin has 1 hard spine and 5 soft rays (V.I.5) and the caudal fin which all consists of 13-14 soft rays (C.13-14). While the Malayan leaffish *Pristolepis fasciata* has dorsal meristic characteristics consisting of 12-13 hard spines and 13 -14 soft rays (D.XII-XIII.13-14), anal fin consists of 3 hard spines and 7-8 soft rays (A.III.7-8), pectoral fins consist of 12 soft rays (P.12), ventral fins consist of 1 hard spine and 5 soft rays (V.1.5) (Sukmono & Margaretha 2017). The meristic showed overlapping measurements between the two species, except the soft rays number in dorsal fins of *P. grooti* is slightly higher than *P. fasciata*. Molecularly, there was 94.70 – 96.13% identity based on nucleotide sequences of *COI* gene between *P. grooti* and *P. fasciata*. Determining the homology level of a sequence with other species sequences in GenBank data can be explained by the value of max score and total score, query coverage approaching 100%, E-value approaching 0 and percentage identity approaching 100% (Tindi et al. 2017).

Genetic Distance and Phylogenetics

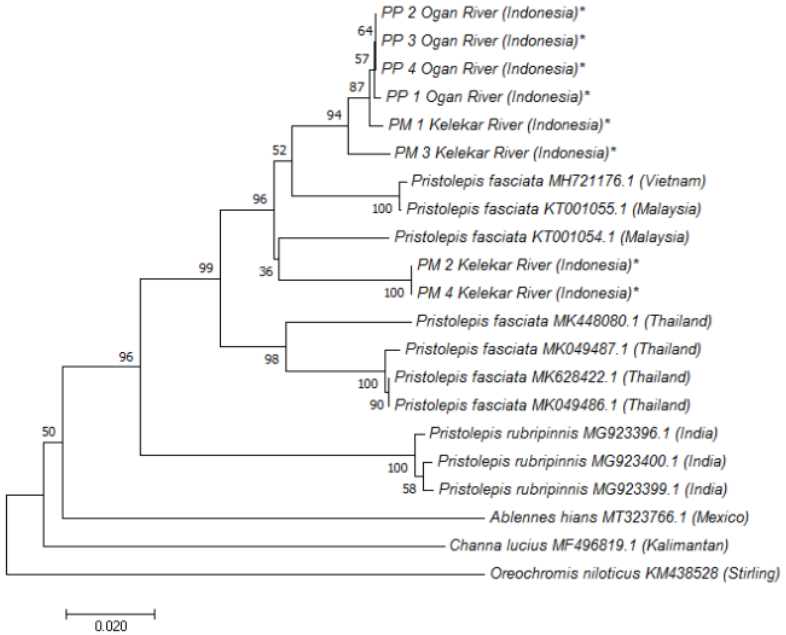
The genetic distance of the leaffish from the Kelekar and the Ogan River with other species in the GenBank data was presented in Table 3.

The genetic distance of the leaffish *P. grooti* from both rivers showed a range of 1.6-8.4% with *P. fasciata* from Genbank database. Sam-

ples PP 2, PP 3, PP 4, PM 1 have a genetic distance of 0.016 (1.6%) against PM3 and 0.045 (4.5%) to PM 4. The genetic distance of samples PM 1, PM 3, PP 2, PP 3 and PP 4 was 0.047 (4.7%) to *P. fasciata* from Malaysia (KT001055.1) and 0.049 (4, 9%) from Vietnam (MH721176.1).

The genetic distance within population from Kelekar or Ogan River is around 4.5-6%. This finding showed cryptic diversity of the fish species. Cryptic species are taxa which are distinguished by unique genetic differences, distinctive ecological preferences and the complete or practically complete absence of morphological discrepancies (Bączkiewicz et al. 2017). Therefore, they can be distinguished with the use of molecular methods, as it has been done for sea cucumbers (Muliani et al. 2020), triplophysa (Wang et al. 2020), and Asian bronze featherback (Lavoué et al. 2020).

The genetic construction of the leaffish from the Kelekar River and the Ogan River is presented in Figure 3. The genetic tree of the leaffish consisted of four clusters, namely first cluster of research samples from the Kelekar, Ogan River and *Pristolepis fasciata* from Vietnam (accession code: MH721176.1) and Malaysia (KT001055.1, KT001054.1) with bootstrap value of 91%. There were three sub clusters of the first cluster. Samples PP1, PP2, PP3, PP4, PM1 and PM3 were in separate sub cluster from PM2 and PM4 (bv= 91). The second cluster was a group of *P. fasciata* from Thailand (MK448080.1, MK049487.1, MK628422, and MK 049486.1) (bv=98), the third cluster was *P. rubripinnis* from India, while the fourth cluster was an outgroup of *O. niloticus* (KM438528). The phylogenetic of the leaffish of this study formed 2 separate sub-clusters consisting of the first sub-cluster, namely PP 2, PP 3, PP 4, PP 1, PM 1 and



PP 1, PP 2, PP3, and PP4 from Ogan River, PM1, PM2, PM4 from Kelekar River, *P. fasciata* KT001055 (Malaysia), *P. fasciata* MH721176 (Vietnam), *P. fasciata* KT001054 (Malaysia), *P. fasciata* MK628422 (Thailand), *P. fasciata* MK049486 (Thailand), *P. fasciata* MK049487 (Thailand), *P. fasciata* MK448080 (Thailand), *P. rubripinnis* MG923396 (India), *P. rubripinnis* MG923400 (India), *P. rubripinnis* MG923399 (India), *Channa lucius* MF496819 (Kalimantan), *Ablennes hians* MT323766 (Mexico), *Oreochromis niloticus* KM438528 (Stirling).

Figure 3. The genetic tree of the leaffish from the Kelekar and Ogan River.

Table 3. The genetic distance of the leaffish from the Kelekar and Ogan River.

	Species Name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	PM 3 Sungai Kelekar (Indonesia)*																				
2	PP 4 Sungai Ogan (Indonesia)*	0.016																			
3	PP 2 Sungai Ogan (Indonesia)*	0.016	0.000																		
4	PP 3 Sungai Ogan (Indonesia)*	0.016	0.000	0.000																	
5	PM 1 Sungai Kelekar (Indonesia)*	0.016	0.004	0.004	0.004																
6	PP 1 Sungai Ogan (Indonesia)*	0.018	0.002	0.002	0.002	0.006															
7	PM 2 Sungai Kelekar (Indonesia)*	0.045	0.057	0.057	0.057	0.060	0.057														
8	PM 4 Sungai Kelekar (Indonesia)*	0.045	0.057	0.057	0.057	0.060	0.057	0.000													
9	*P. fasciata* KT001055 (Malaysia)	0.047	0.043	0.043	0.043	0.047	0.041	0.055	0.06												
10	*P. fasciata* MH721176 (Vietnam)	0.049	0.045	0.045	0.045	0.049	0.043	0.057	0.06	0.002											
11	*P. fasciata* KT001054 (Malaysia)	0.055	0.047	0.047	0.047	0.051	0.045	0.055	0.06	0.066	0.064										
12	*P. fasciata* MK628422 (Thailand)	0.076	0.072	0.072	0.072	0.072	0.074	0.086	0.09	0.076	0.078	0.080									
13	*P. fasciata* MK049486 (Thailand)	0.076	0.072	0.072	0.072	0.072	0.074	0.086	0.09	0.076	0.078	0.080	0.000								
14	*P. fasciata* MK049487 (Thailand)	0.080	0.076	0.076	0.076	0.076	0.078	0.090	0.090	0.076	0.078	0.084	0.004	0.004							
15	*P. fasciata* MK448080 (Thailand)	0.084	0.080	0.080	0.080	0.080	0.082	0.084	0.084	0.086	0.088	0.068	0.051	0.051	0.055						
16	*P. rubripinnis* MG923396 (India)	0.129	0.117	0.117	0.117	0.117	0.115	0.136	0.14	0.117	0.115	0.117	0.123	0.123	0.119	0.127					
17	*P. rubripinnis* MG923400 (India)	0.129	0.121	0.121	0.121	0.121	0.119	0.136	0.136	0.117	0.115	0.121	0.123	0.123	0.119	0.127	0.004				
18	*P. rubripinnis* MG923399 (India)	0.129	0.121	0.121	0.121	0.121	0.119	0.136	0.14	0.117	0.115	0.121	0.123	0.123	0.119	0.127	0.008	0.004			
19	*Channa lucius* MF496819 (Kalimantan)	0.164	0.164	0.164	0.164	0.164	0.162	0.164	0.164	0.170	0.168	0.166	0.179	0.179	0.179	0.177	0.177	0.181	0.183		
20	*Ablennes hians* MT323766 (Meksiko)	0.173	0.160	0.160	0.160	0.160	0.162	0.173	0.173	0.179	0.177	0.177	0.170	0.170	0.168	0.172	0.177	0.181	0.181	0.189	
21	*Oreochromis niloticus* KM438528 (Stirling)	0.201	0.203	0.203	0.203	0.201	0.201	0.185	0.185	0.187	0.185	0.197	0.189	0.189	0.191	0.201	0.197	0.197	0.197	0.21	0.216

PM 3 with *P. fasciata* from Vietnam (MH721176) and Malaysia (KT001055) and the second sub-cluster, namely PM 2 and PM 4 with *P. fasciata* from Malaysia (KT001054) with a bootstrap value of 50%.

The genetic distance indicated that *P. grootii* differed genetically more than 3% than *P.fasciata*, genetically. The smaller the value of genetic distance denotes that the species is more related to other species and has a close relationship, while the greater the value of genetic distance indicates that the species is increasingly divergent. If the genetic distance is more than 3%, it indicates that in the group there are species that are different from other members, on the contrary, if the genetic distance value is equal to or less than 3%, it indicates that the group or cluster still comes from one species or the same species (Wong & Hanner 2008).

The construction of the phylogenetic relationship between species or related taxa was supported by the availability of a sequence database in many species (Hedrick 2005). However, when only one *COI* gene is used, a gene tree is produced, and it will not describe the broad evolutionary history of a group of species (Rubin et al. 2012). The genetic tree of all specimens of *P. grooti* in this study was separated from *P. fasciata* Thailand, but showed an indefinite cluster with the specimen from Malaysia and Vietnam. *P. fasciata* from Malaysia and Vietnam were located between two clusters of samples PP1, PP2, PP3, PP4, PM1, PM3 and PM2 and PM4 with low bootstrap value (bv=50). Bootstrapping is used to make inferences and evaluate the robustness of the tree (Holmes 2003). The data is relatively stable when the bootstrap value is greater than 70% (Lemey et al. 2009) relatively. Furthermore, *P. grooti* of this study also indicated different sub-cluster between Kelekar and Ogan River, where PM2 and PM4 from Kelekar River were separated from PM1 and PM3 (bv=91), where they belong to the same sub-cluster to PP1, PP2, PP3 and PP4 (originally from Ogan River). The proximity of the leaffish of the Kelekar and the Ogan River is interconnected because the Kelekar River flows into the Ogan River and empties into the Musi River (Wijaya 2001). The high similarity and genetic relationship of the leaffish are in line with the high level of diversity of these fish species that inhabit the Sunda shelf which consists of interconnected islands of Sumatra, Kalimantan, Java, Bangka

Table 4. Water quality measurements in the Kelekar and the Ogan River.

Water Quality	Kelekar River	Ogan River	Water quality standards
pH (Unit)	5.6-7.2	6.9-8.3	6-9 (Central Government 2001)
Temperature (°C)	28.3-31.8	30.8-31.5	25-32 (Central Government 2001)
Water current (m s^{-1})	0.17-0.30	0.19-0.21	0.25-0.5 (Supartiwi 2000)
Dissolved oxygen (DO) (mg L^{-1})	4.82-6.93	6.92-10.89	>4 (Central Government 2001)
Water transparency (cm)	16-20	41-45	30-60 (Cholik et al. 1991)
TDS (mg L^{-1})	7-11	24-44	<1000 (Central Government 2001)
Ammonia (mg L^{-1})	0.47-0.70	0.52-0.61	<0.2 (Central Government 2021)
Total alkalinity (mg L^{-1})	10-18	24-28	5-500 (Effendi 2003)

Belitung, which are dominated by fish that inhabit peatlands and freshwater, especially those that are endemic (Hubert et al. 2008), so that mating occurs in the distribution of the leaffish, geographically.

Water Quality

The water quality of the habitat of the leaffish in Kelekar and Ogan River was presented in Table 4. the pH value of Kelekar and Ogan River ranged 5.6-8.3, temperature values of 28.3-31.8°C, dissolved oxygen was between 4.82-10.89 mg L^{-1}, total dissolved solid (TDS) was about 7-44 mg L^{-1}. The water transparency value was approximately 16-40 cm, while the total alkalinity was about 10-28 mg L^{-1}. Mutagenic substances in the Reservoir 1 in the Canela National Forest have been altering the genetic integrity of the aquatic organisms that may be a threat for that aquatic ecosystem (Bühler et al. 2014). Furthermore, pollutants present in Madin Reservoir water were genotoxic and cytotoxic to *C. carpio* (Pérez-Coyotl et al. 2017).

Water pH media, dissolved oxygen and TDS in Kelekar and Ogan River were in accordance with the water quality standard class II for fishing activities, mainly for fish to grow and reproduce (Government Implementation of Environmental Protection and Management. 2021). The value of ammonia (NH_3) was 0.47-0.70 mg L^{-1}. This range was higher than required for fish production in freshwater based on the quality standard for grades 2 (< 0.2 mg/L (Central Government 2021). In this study, water current ranges from 0.17 to 0.30 m s^{-1}, indicated that the Kelekar River was classified as a slow current, while the Ogan River (0.19-0.21 m s^{-1}) was categorized as a slow current and water current of 0.25-0.5 m s^{-1} was at medium current (Supartiwi 2000). The water transparency value was approximately 16-40 cm, while the optimal value for water transparency required for the growth of freshwater fish is around 30-60 cm (Cholik et al. 1991). The total alkalinity was about 10-28 mg L^{-1} and still supports the survival of the leaffish. The total value of good alkalinity in waters is between 5-500 mg L^{-1} $CaCO_3$ (Effendi 2003).

CONCLUSIONS

This investigation utilizes the *COI* barcode for the molecular authentication of endemic fish *P. grooti* from Southern Sumatra. These results indicated the leaffish had the highest identity to *P. fasciata* from Malaysia (KT001055) with different percentages of each sample ranging from 95.29 to 96.13%. The genetic tree supported that *P. grooti* in this study was separated from *P. fasciata* in Thailand, but showed an indefinite cluster with the specimen from Malaysia and Vietnam.

AUTHOR CONTRIBUTION

The study was designed by MSF. ETG and MW were conducted the laboratory works. The data was analysed by MSF and ETG. All the authors wrote original draft, edited and approved the manuscript.

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CONFLICT OF INTEREST

The authors state that the research was created in the absence of any commercial or financial matters that could be a potential conflict of interest.

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