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Research Article

Phylogenetic and genetic diversity of marble goby (*Oxyeleotris marmorata*) from the Brantas River, East Java, Indonesia

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Abstract

The marble goby (*Oxyeleotris marmorata*) is a commercially important freshwater fish in Southeast Asia with considerable potential for aquaculture development. However, information regarding its genetic diversity and phylogenetic relationships remains limited, particularly in Indonesia. This study aimed to assess the genetic diversity and phylogenetic relationships of *O. marmorata* populations from the Brantas River, East Java, using mitochondrial cytochrome c oxidase subunit I (COI) sequences. Specimens were collected from Malang and Kediri, and the resulting sequences were analysed together with reference sequences obtained from GenBank. Genetic diversity was evaluated using haplotype diversity (Hd), nucleotide diversity (π), haplotype network reconstruction, genetic distance analysis, and phylogenetic inference. A total of five haplotypes (H1-H5) were identified from 37 sequences. Haplotype H1 was the most common and widespread haplotype, occurring in all Indonesian populations as well as in Thailand and Cambodia. In contrast, H2 was unique to Vietnam, whereas H3-H5 were restricted to Thailand. All individuals from the Brantas River shared a single haplotype, resulting in zero haplotype diversity (Hd = 0) and nucleotide diversity (π = 0). Phylogenetic and haplotype network analyses consistently recovered two mitochondrial lineages, while genetic distances among haplotypes ranged from 0.16% to 1.69%, indicating shallow intraspecific divergence. The absence of mitochondrial variation in the Brantas River populations may reflect historical population connectivity, reduced mitochondrial variability, or past demographic events such as population bottlenecks. These findings provide baseline genetic information to support future conservation, broodstock management, and domestication programs for *O. marmorata*.

Keywords: aquaculture, coi, genbank, haplotype diversity, nucleotide diversity

1. INTRODUCTION

The genus *Oxyeleotris* comprises eighteen valid species, twelve of which are naturally distributed in Indonesia [1]. One of the most economically important species is the marble goby, *Oxyeleotris marmorata*, a freshwater fish widely distributed throughout Southeast Asia, including the Indonesian islands of Sumatra, Kalimantan, and Java [2,3]. This carnivorous species inhabits a variety of freshwater environments, including rivers, swamps, floodplain wetlands, and irrigation canals. Due to its distinctive taste and high market value, *O. marmorata* is highly favored by consumers and has considerable potential as an aquaculture and export commodity [4-6].

Despite its economic importance, the supply of *O. marmorata* still relies largely on capture fisheries because large-scale aquaculture production remains limited. Increasing fishing pressure, habitat degradation, and declining water quality may threaten the sustainability of natural populations [7]. Consequently, conservation and domestication programs are needed to support the sustainable utilization of this species. Successful implementation of these programs requires comprehensive biological information, including data on phylogenetic relationships and genetic diversity.

The Brantas River is the second-longest river in Java and one of the most important freshwater systems in East Java. The river supports diverse fish communities and serves as an important source of fisheries resources for local communities [8]. However, increasing anthropogenic activities, including urbanization, agriculture, and industrial development, have contributed to habitat alteration and declining water quality in several sections of the river [9]. Although *O. marmorata* is known to occur throughout the Brantas River, no previous studies have evaluated the genetic diversity or

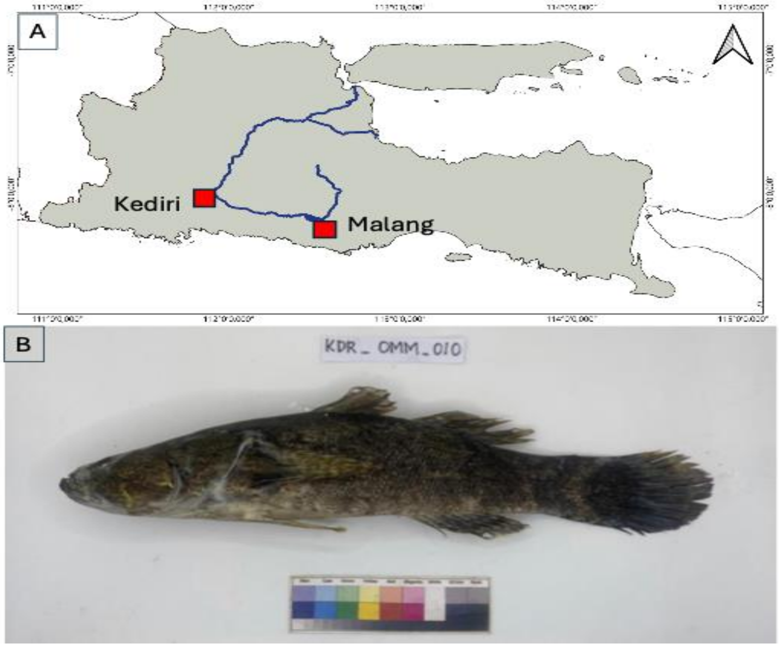

Figure 1. Map showing the sampling localities of *O. marmorata* in Malang and Kediri, East Java, Indonesia (A), and the left lateral view of a representative specimen collected from Kediri (specimen code: KDR_OMM_010; standard length = 239.0 mm) (B).

phylogenetic relationships of populations from this river basin. Existing genetic studies of *O. marmorata* have primarily focused on populations from other regions of Southeast Asia, while information from the Brantas River remains unavailable [e.g., 10]. Such information is essential for evaluating the genetic status of natural populations and supporting future conservation and domestication efforts.

Mitochondrial DNA (mtDNA) has been widely used in studies of fish phylogeny and population genetics because of its maternal inheritance, high copy number, and relatively rapid evolutionary rate [11,12]. Among mitochondrial markers, the cytochrome c oxidase subunit I (COI) gene has proven effective for species identification, phylogenetic reconstruction, and the assessment of genetic diversity in fishes [13,14]. Therefore, this study aimed to assess the phylogenetic relationships and genetic diversity of *O. marmorata* populations collected from Malang and Kediri in the Brantas River, East Java, using mitochondrial COI sequences. The findings of this study are expected to provide baseline genetic information to support future conservation, domestication, and aquaculture development programs for this economically valuable freshwater species.

2. MATERIALS AND METHODS

2.1. Sampling site and specimen collection

Specimen collection was conducted between February and March 2024 in the Brantas River, East Java, Indonesia. Fish were captured using environmentally friendly fishing gear, including fishing rods and fish traps. *Oxyeleotris marmorata* specimens were collected from two localities: Dempok Village, Kepanjen, Malang (8°11′16.5″ S, 112°31′42.1″ E), representing the upstream section of the Brantas River, and Tosaren Village, Pesantren, Kediri (7°50′10.9″ S, 112°01′27.6″ E), representing the midstream section (Figure 1). A total of 16 specimens were collected, comprising 10 individuals from Malang and 6 individuals from Kediri. Immediately after capture, specimens were identified based on external morphological characteristics and transported to the laboratory for molecular analyses. A small portion of the right pectoral fin or muscle tissue from the right side of the caudal peduncle was excised from each specimen and preserved in 96% ethanol prior to DNA extraction.

2.2. Molecular experiments

Genomic DNA was extracted from tissue samples using the Wizard® Genomic DNA Purification Kit (Promega, Madison, Wisconsin, USA) following the manufacturer's protocol. The COI gene was amplified using the polymerase chain reaction (PCR) method in a total reaction volume of 30 µL, consisting of 15 µL GoTaq® Green Master Mix (Promega, Madison, WI, USA), 11 µL nuclease-free water, 2 µL genomic DNA template, 1 µL forward primer FishF1 (5′-TCAACCAACCACAAAGACATTGGCAC-3′), and 1 µL reverse primer FishR1 (5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′) [15]. PCR amplification was performed using the following thermal cycling conditions: an initial denaturation at 94°C for 2 min; 40 cycles of denaturation at 94°C for 45 s, annealing at 58°C for 45 s, and extension at 72°C for 60 s; followed by a final extension at 72°C for 7 min. Amplification success was verified by electrophoresis on a 1.5% agarose gel prepared in 1× Tris-Borate-EDTA (TBE) buffer and stained with GelGreen® Nucleic Acid Stain (Biotium, Fremont, California, USA). Electrophoresis was conducted at 100 V for 30 min, and DNA bands were visualized under ultraviolet illumination. Successful PCR products were purified and sequenced bidirectionally using the Sanger

Table 1. List of sequence downloaded from GenBank

No	Species	Number of Individuals	Location	Accession Number	Reference
1	*O. marmorata*	10	Indonesia (Sukabumi)	KU692716.1 KU692717.1 KU692719.1 KU692720.1 KU692721.1 KU692722.1 KU692723.1 KU692724.1 KU692725.1 KU692726.1	[18]
2		1	Indonesia (Nunukan)	OK670610.1	[19]
3		1	Indonesia (Tana Tidung)	OK670609.1	[19]
4		1	Indonesia (Ambarawa)	KU692718.1	[18]
5		1	Indonesia (Bojonegoro)	PP085180.1	[20]
6		1	Vietnam	MH721190.1	[21]
7		1	Kamboja	EF609424.1	[22]
8		5	Thailand	MK448189.1 MK448069.1 MK448070.1 MK628379.1 MK049460.1	[23,24]
9	*O. selheimi*	2	Australia	AY722179.1 AY722166.1	[25]
10	*O. lineolata*	1	Australia	AY722165	[25]

Table 2. Geographic distribution of *O. marmorata* haplotypes

Locality		Haplotype				
		1	**2**	**3**	**4**	**5**
Indonesia	Malang	10				
	Kediri	6				
	Sukabumi	10				
	Ambarawa	1				
	Bojonegoro	1				
	Nunukan	1				
	Tana Tidung	1				
Cambodia		1				
Vietnam			1			
Thailand		1		2	2	1
Total individual		**31**	**1**	**2**	**2**	**1**

sequencing method by Apical Scientific Sdn. Bhd. (Selangor, Malaysia).

2.3. Sequence editing and dataset preparation

Forward and reverse sequence chromatograms were edited and examined for base-calling errors using Chromas v.2.6.6 (Technelysium Pty Ltd., South Brisbane, Australia). The edited sequences were subsequently assembled into consensus sequences using UGENE v.1.31.1 [16]. The dataset used for phylogenetic and haplotype network analyses consisted of newly generated COI sequences from this study combined with reference sequences obtained from GenBank. A total of 21 *O. marmorata* COI sequences were retrieved from GenBank, including 13 sequences from Indonesia and 8 sequences from Thailand, Vietnam, and Cambodia (Table 1). These sequences were selected to represent the known geographic distribution of the species and are listed in Table 1. All sequences were manually inspected for ambiguous nucleotide positions and aligned using Mesquite v.3.5.1 [17]. The final alignment (611 bp) was used for subsequent analyses of genetic diversity, phylogenetic relationships, and haplotype distribution.

2.4. Phylogenetic and haplotype network reconstructions

The phylogenetic relationships of the *O. marmorata* of our samples together with sequences downloaded from GenBank were evaluated using maximum likelihood method in GARLI v.2.0 software [26]. Bootstrap non-parametric method was used to evaluate each node of branching was conducted with 1000 times. The best substitution model was determined using jModelTest v.2.1.3 software [27]. Genealogical relationships among haplotypes from different populations were examined by reconstructing a haplotype network using the statistical parsimony algorithm implemented in TCS v.1.2.1 [28]. The resulting network was then visualized and refined using tcsBU [29].

2.5. Genetic distance and genetic diversity analyses

Pairwise genetic distances among haplotypes were estimated using both the p-distance and Kimura two-parameter (K2P) models implemented in MEGA v.11 [30]. Genetic diversity indices, including the number of haplotypes (H), haplotype diversity (Hd), and nucleotide diversity (π), were calculated using DnaSP v.6.0 [31]. These indices were used to evaluate the level of genetic variation within and among *O. marmorata* populations from the Brantas River.

3. RESULTS AND DISCUSSIONS

3.1. Haplotype data

Analysis of the mitochondrial COI dataset revealed 493 conserved sites, 118 variable sites,

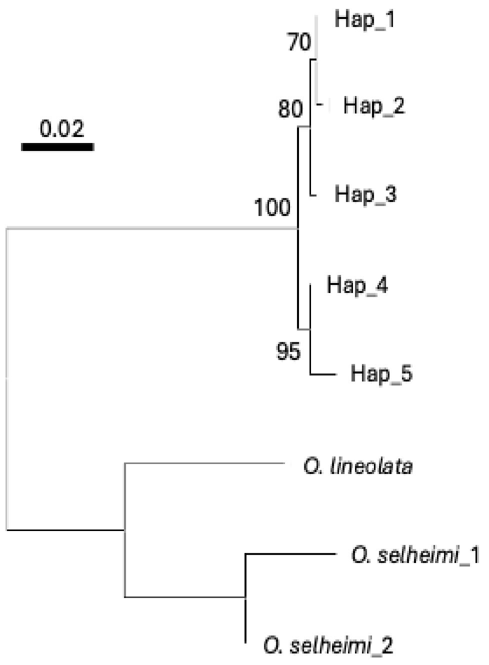

Figure 2. Maximum likelihood tree depicting the phylogenetic relationships among haplotypes of *O. marmorata*. Only bootstrap values greater than 70% are shown. See Table 2 for the composition of each haplotype.

78 parsimony-informative sites, and 40 singleton sites. A total of five haplotypes (H1-H5) were identified from 37 *O. marmorata* individuals, including newly generated sequences and reference sequences obtained from GenBank (Table 2). Haplotype H1 was the most common haplotype, comprising 31 individuals (83.8%) and occurring in all Indonesian populations (Malang, Kediri, Sukabumi, Ambarawa, Bojonegoro, Nunukan, and Tana Tidung) as well as in one individual each from Thailand and Cambodia. In contrast, H2 was unique to Vietnam, whereas H3, H4, and H5 were restricted to Thailand. Consequently, Thailand exhibited the highest haplotype richness, containing four of the five haplotypes identified in the dataset.

3.2. Phylogenetic relationships and haplotype network

Both phylogenetic reconstruction and haplotype network analyses revealed two major mitochondrial lineages within *O. marmorata* (Figures 2 and 3). The first lineage comprised H1, H2, and H3, whereas the second lineage consisted of H4 and H5, with the separation between lineages receiving strong bootstrap support (100%). The haplotype network further showed that H1 occupied a central position and represented the most widespread haplotype, whereas the remaining haplotypes exhibited restricted geographic distributions. Similar phylogeographic patterns have recently been reported in *O. marmorata* from Southeast Asia, where a widespread haplotype coexists with regionally restricted lineages [32,33]. The occurrence of H1 across Indonesia, Thailand, and Cambodia indicates that the distribution of mitochondrial lineages is not strictly associated with present-day geographic boundaries. One possible explanation for this pattern is historical dispersal facilitated by changes in drainage connectivity during the Pleistocene. Repeated sea-level fluctuations during glacial periods substantially altered river configurations throughout Southeast Asia, creating opportunities for freshwater fishes to disperse across areas that are currently isolated [34,35]. Such historical hydrological connections have been recognized as important drivers of phylogeographic patterns in freshwater fishes [36]. In contrast, the

Table 3. Genetic distances among *O. marmorata* haplotypes. Values below and above the diagonal represent p-distances and Kimura two-parameter (K2P) distances, respectively

	Hap_1	Hap_2	Hap_3	Hap_4	Hap_5
Hap_1	-	0.00164	0.00329	0.00832	0.01513
Hap_2	0.00164	-	0.00495	0.01002	0.01687
Hap_3	0.00327	0.00491	-	0.00830	0.01509
Hap_4	0.00818	0.00982	0.00818	-	0.00662
Hap_5	0.01473	0.01637	0.01473	0.00655	-

occurrence of geographically restricted haplotypes in Vietnam and Thailand may reflect subsequent regional isolation and local diversification following the re-establishment of contemporary drainage systems.

Despite the presence of two mitochondrial lineages at the regional scale, all Brantas River specimens belonged to H1, indicating limited mitochondrial variation within East Java populations. This pattern contrasts with previous COI-based studies on *Channa striata* and *C. gachua* from East Java, in which multiple haplotypes and higher levels of intraspecific variation were detected [37]. The low variation observed in *O. marmorata* may reflect reduced mitochondrial diversity or a relatively conserved evolutionary rate of the COI region in this species. However, because the present study was based on a single mitochondrial marker and limited geographic sampling, these biogeographical scenarios should be considered preliminary. Therefore, additional molecular markers, such as microsatellites, SNPs, or complete mitochondrial genomes, may be required to resolve fine-scale population structure and genetic connectivity more effectively.

3.3. Genetic distance among haplotypes

Genetic distance analysis revealed low levels of mitochondrial divergence among the five haplotypes of *O. marmorata* (Table 3). The smallest genetic distance was observed between H1 and H2 (0.16%), followed by H1-H3 (0.33%) and H2-H3 (0.49%), indicating a close evolutionary relationship among these haplotypes. In contrast, genetic distances between H1-H3 and H4-H5 ranged from 0.82% to 1.69%, supporting the separation of the two mitochondrial lineages recovered by both phylogenetic and haplotype network analyses. Nevertheless, the maximum divergence observed (1.69%) remained below the levels commonly reported for interspecific COI divergence in freshwater fishes [38,39].

3.4. Genetic diversity of O. marmorata from the Brantas River

No haplotype diversity (Hd = 0) or nucleotide diversity ($\pi = 0$) was detected in the Brantas River populations, indicating that all individuals from Malang and Kediri shared an identical mitochondrial COI haplotype. This result is consistent with the haplotype network and phylogenetic analyses, which assigned all Brantas River specimens to H1. The absence of detectable variation suggests a high degree of mitochondrial homogeneity within the study area and may be associated with historical connectivity within the Brantas River system, which could facilitate gene flow and promote the persistence of a single dominant haplotype [10,32,33]. In addition, long-term anthropogenic pressures, including habitat degradation, declining water quality, and fishing activities, may have contributed to reductions in effective population size and the loss of rare mitochondrial lineages [40-42]. Although the present dataset does not allow direct testing of demographic scenarios, historical population bottlenecks cannot be excluded as a contributing factor. Nevertheless, the presence of additional haplotypes and two mitochondrial lineages in populations from other regions of Southeast Asia demonstrates that genetic diversity exists within *O. marmorata* at a broader geographic scale.

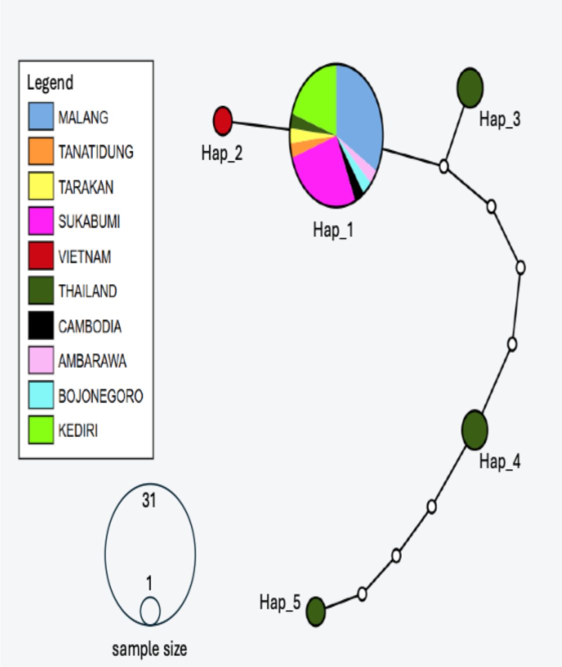

Figure 3. Statistical parsimony haplotype network showing the genealogical relationships among *O. marmorata* haplotypes. White circles represent inferred mutational steps between haplotypes.

Therefore, the observed lack of variation could be interpreted as population-level homogeneity in the Brantas River rather than an absence of genetic diversity in the species as a whole.

Low genetic diversity may have important implications for the long-term conservation of *O. marmorata* because genetic variation provides the evolutionary potential required to respond to environmental changes, emerging diseases, and other ecological disturbances [43,44]. Consequently, populations with limited genetic variation may exhibit reduced adaptive capacity and resilience to future environmental challenges. From a conservation perspective, efforts should prioritize habitat protection, improvement of water quality, and maintenance of connectivity among river sections to preserve existing genetic resources and support natural population processes. The observed genetic homogeneity may also have implications for future domestication and aquaculture programs. Although the present study does not provide direct evidence of inbreeding, the prolonged use of genetically similar broodstock may increase the likelihood of inbreeding and further reduce the adaptive potential of cultured populations over successive generations [41,44].

4. CONCLUSIONS

The present study revealed low mitochondrial genetic diversity in *O. marmorata* from the Brantas River, East Java, with all specimens sharing a single COI haplotype. Nevertheless, phylogenetic and haplotype network analyses identified two mitochondrial lineages across Southeast Asian populations, indicating broader genetic variation at the regional scale. These findings provide the first genetic baseline for *O. marmorata* in the Brantas River and emphasize the importance of conserving existing genetic resources through habitat protection and genetic monitoring. The results also provide a foundation for future broodstock selection, domestication, and aquaculture programs, which should be supported by broader genetic assessments using additional populations and higher-resolution molecular markers.

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Author Contributions

WEK, MD: designed the research, analysed the data and wrote the manuscript, research; BR, DF, LZ, NPAL, EA, IS: carried out the laboratory experiments, analysed the data, prepared figures and tables; MNH, KNAK: performed field sampling and morphological identification.

Conflict of Interest

The authors declare no conflict of interest.

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DECLARATION OF GENERATIVE AI

Not applicable.

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