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DNA barcoding of medaka fish *Oryzias marmoratus* in Lake Towuti, South Sulawesi, Indonesia

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Abstract. *Nur RA, Parenrengi A, Arisuryanti T, Widyastuti H, Andriani I. 2024. DNA barcoding of medaka fish* Oryzias marmoratus *in Lake Towuti, South Sulawesi, Indonesia. Biodiversitas 25: 3645-3652. Oryzias marmoratus* (Aurich, 1935), an endemic species of Lake Towuti, the second-largest lake in Indonesia, is facing a critical situation. Its population in nature is near threatened, as per the IUCN, necessitating immediate molecular studies such as DNA barcoding techniques. These studies are crucial to prevent a decline in the population of *O. marmoratus*. DNA barcoding is not just a tool for identification, monitoring, and protection but a call for genetic conservation. Therefore, the research was intended to identify the genetic variation of medaka fish. *O. marmoratus* was collected in Lake Towuti, and for DNA barcoding using primers, the cytochrome oxidase subunit I (COI) gene. Medaka fish samples (n=8) were collected from fishermen. DNA was extracted, and nucleotides were sequenced using sequencing provider 1st Base. The sequences of DNA were analyzed using Bioedit, BLAST-N, Mega11, DnaSP, and Network programs to obtain a consensus sequence, haplotype diversity, nucleotide diversity, genetic distance, phylogenetic, and haplotype network. The result showed that in the BLAST-N analysis, eight samples were closely related to *O. marmoratus* (LC154797.1) (AP005981.1) (94,42-99,84% in similarity). Genetic variation indicated a high value with a detailed 0.983 haplotype diversity, 0.026 a low nucleotide diversity, and six haplotypes; genetic distance with an average of 0.025 and 0.079. Phylogenetic analysis established five clades. Information on intraspecies genetic variation of *O. marmoratus* in terms of genetics, especially in Lake Towuti, is needed for genetic conservation and as an animal model of biology and molecular studies.

Keywords: COI, DNA barcoding, genetic variation, Lake Towuti, *Oryzias marmoratus*

INTRODUCTION

Medaka fish *Oryzias marmoratus* (Aurich, 1935) is one of the endemic species inhabiting Indonesian waters. There are 37 valid medaka fish species (Fricke et al. 2022), of which Sulawesi Island has 18 endemic species. *Oryzias marmoratus* is found in Lakes Towuti, Mahalona, and Lantoa (Kottelat et al. 1993; Dahruddin 2012). *Oryzias marmoratus* is not a migratory fish; it is benthopelagic. It is typically 4-5 centimeters in size and has a vibrant hue. *Oryzias marmoratus* adults have a brownish-yellow or olive-green body color, and the trunk of the body has dark brown patches that spread regularly, known as marmorated medaka. Because of the attractive body color of *O. marmoratus*, it is also used as an ornamental fish and can potentially be an experimental model (Said and Mayasari 2021). According to the International Union for Conservation of Nature (IUCN), *O. marmoratus* is near threatened (IUCN 2023). Lake Towuti is the second-largest lake in Indonesia and the largest in the Malili system of five tectonic lakes in Sulawesi (Wicaksono et al. 2015; Russel et al. 2020). According to research by Nursyahran et al. (2022) and Nursyahran et al. (2023), Lake Towuti has a unique biodiversity and endemic species, and several endemic fish species are vulnerable to extinction and population decline due to limitations. Distribution region, overfishing that is not ecologically friendly, and alien fish species competing with native fish for food.

Genetic variation in natural populations present in nature can be used to determine evolution; freshwater fish populations experience a decline in genetic diversity, affecting fish adaptation to environmental changes. Variation occurs due to mutation, migration, genetic drift, and hybridization (Xia et al. 2015; Mkare et al. 2021). Genetic variation can help fish species adapt to environmental changes necessary for survival. Information about morphology and genetic structure can help preserve fish species in nature. Fishery scientists explain the genetic diversity of fish species in nature using various biotechnology techniques (Carlson et al. 2015).

Morphological and molecular approaches are needed to prevent population decline in *O. marmoratus*. The morphological approach to fish is an initial method for identifying species, which includes body shape, color patterns, and the number of fins (Ikpeme et al. 2017). However, morphological identification has limitations in separating species within one genus. Therefore, a molecular approach to DNA barcoding is needed to

strengthen the results of a morphological (Bingpeng et al. 2018). These genetic identifications are typically supported with DNA barcoding analysis. The relationship between the minimum interspecific distance with a species' nearest neighbor and the maximum intraspecific distances within each species is known as the barcoding gap (Pandey et al. 2020).

DNA barcoding uses short gene sequences that can show genetic variations and kinship relationships intra and interspecies at the molecular level within a species. This method uses the Cytochrome Oxidase Subunit I gene's nucleotide base sequence (Kress et al. 2015; DeSalle and Goldstein 2019). Because it meets the requirements set forth by the Consortium for the Barcode of Life (CBOL), the COI gene is thought to be the most appropriate gene for animal DNA barcoding, namely that it is universal, has good sequence quality, and can differentiate species (Lou et al. 2011). With the use of DNA barcoding and the COI gene, some fish have been identified at the species level, including *Oryzias nigrimas* Kottelat, 1990 (Serdiati et al. 2020), grouper (Fadli et al. 2021), shark (French and Wainwright 2022), and *Caranx* spp. (Kainama et al. 2023). DNA barcoding is a taxonomic method that may precisely and accurately reveal the genetic composition of various *O. marmoratus* species, the DNA sequence of a species and its comparison with other species, as well as the phylogenetic structure of the species. Research regarding genetic variation, especially in *O. marmoratus* in Lake Towuti, still needs more information. Therefore, this research was carried out to molecularly identify and analyze the genetic variations of *O. marmoratus* based on DNA barcoding using the COI gene.

MATERIALS AND METHODS

Research area and sample collection

Eight samples of *O. marmoratus* were taken from Towuti Lake, East Luwu the District, Southern Sulawesi, Indonesia. There are two study stations on Lake Towuti, specifically Tanjung Timbala (2'42.5720'S 121'25.7850'E) and Tanjung Bakara (2'41.3470'S 121'25.5330'E) (Figure 1). Fishermen caught the samples using fishing nets. *O. marmoratus* has a body total length of 32.34-53.00 mm and a weight of 0.3-1.3 g. Eight samples (weight of 0.3-1.3 g) were collected, consisting of four samples from the first station (1T1, 1T2, 1T3, and 1T4) and four samples from the second station (2B1, 2B3, 2B4, and 2B5). The samples were preserved in 96% alcohol. Medaka fish were morphologically identified as *O. marmoratus* (Kottelat et al. 1993; Kobayashi et al. 2023). Next, samples were taken to re-identify their species using the molecular technique.

Procedures

DNA extraction, amplification, and sequencing

Using the DNeasy Blood & Tissue Kit technique (Qiagen, USA), muscle tissue from the caudal peduncle was used to extract DNA. Using COI primers, F1: 5'-TCAACCAACCACAAAGACATTGGCAC-3' and R1: 5'-TAGACTTCTGGGTGGCCAAAGAATCA-3' (Ward et al. 2005), the extracted DNA was used to amplify the target sequence. PCR amplification used was a 50 µL reaction consisting of 25 µL Ready Mix PCR (MyTaq™ HS Red Mix Bioline); 0,6 µM each primer; 1 mM, MgCl2; 11 µL sterile ddH2O; and 6 µL DNA. The PCR/Thermocycler cycle with three stages includes: (i) predenaturation for a minute at 95°C, (ii) 35 cycles for denaturation for 15 minutes at 95°C, annealing for 30 seconds at 50°C, expansion for 30 seconds at 72°C, and (iii) postextension for five seconds at 72°C (Arisuryanti et al. 2020). The PCR result was run across a 1% agarose gel, allowing the single DNA fragment to be seen to evaluate the success of DNA isolation. For sequencing, the amplification data were submitted to PT. Genetic Science Indonesia (Jakarta).

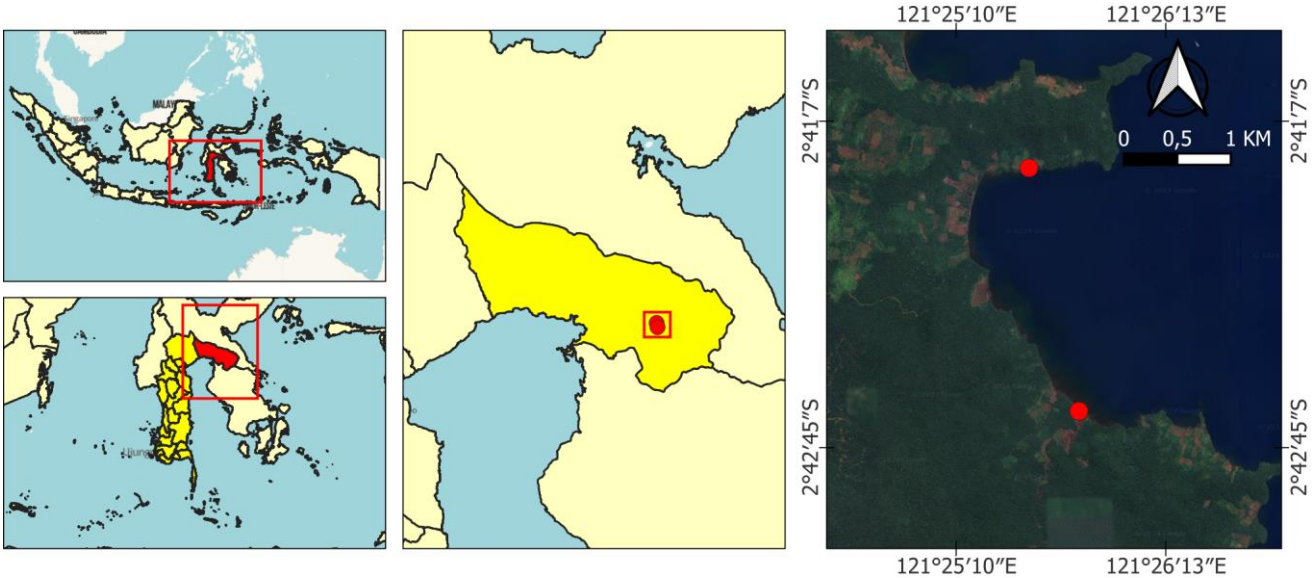

Figure 1. The samples of *Oryzias marmoratus*) are from Lake Towuti, East Luwu the District, Southern Sulawesi, Indonesia

Data analysis

The sequencing of the results was analyzed using the Bioedit program (Hall 1999) to obtain a consensus nucleotide sequence. The sequence consensus results were aligned by comparing those found on NCBI GenBank with Nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The online analysis results could predict medaka fish by looking at query cover and similarity percentage, which can show the sample's similarity to the information in GenBank. MEGA 11 software was used for the alignment process to construct phylogenetics with maximum likelihood and Neighbor-Joining techniques (Tamura et al. 2021).

The sample sequence data was then calculated to estimate genetic distance using Pairwise Distance with the Kimura-2-Parameter model and exported into MEGA".meg" format. Furthermore, the DnaSP 6.12.03 application examined genomic variation (Rozas et al. 2017). The genetic variation data discovered was the number of haplotypes, nucleotide diversity, and haplotype diversity. The relationship between haplotypes of the *O. marmoratus* species was analyzed using the Median Joining Network in the NETWORK ver.10.1 programs (https://www.fluxus-engineering.com).

RESULTS AND DISCUSSION

DNA amplification and sequence identification of *O. marmoratus* in Lake Towuti

The fragment length obtained from electrophoresis results was around 600 bp (Figure 2). The eight examined medaka fish samples gave consensus results for the COI gene sequence, showing a fragment length of roughly 593-629 bp and a translation result of 197-208 amino acids.

The BLAST outcomes compare the value of the sample base sequence with the base sequence in GenBank by analyzing the percentage of the query cover and similarity values. The NCBI uses this value as the identification standard derived from the BLAST method. Table 1 shows the results table for BLAST.

The BLAST results of eight samples from Lake Towuti based on the COI gene sequence (Table 1) identified *O. marmoratus* with a similarity between 94.42-99.84% and query cover between 98-100%. The outcomes show that the DNA sequencing from the sample shows the same length of sequence as the GenBank, 94.42-99.84%, so it can be said that the sample sequence shows a high degree of similarity.

Nucleotide composition

Table 2 shows the nucleotide composition of *O. marmoratus* in Lake Towuti according to the COI gene sequence. The alignment results of eight *O. marmoratus* samples from Lake Towuti produced a fragment length of 589 bp, and then nucleotide composition analysis was carried out using the MEGA11 program. The outcomes of the nucleotide composition study (Table 2) show that T, C, A, and G's nucleotide contents differ across eight *O. marmoratus* samples found in Lake Towuti, with an average of T being 28.89±0.22, C being 28.34±0.19, A being 24.36±0.24, and G being 18.39±0.22.

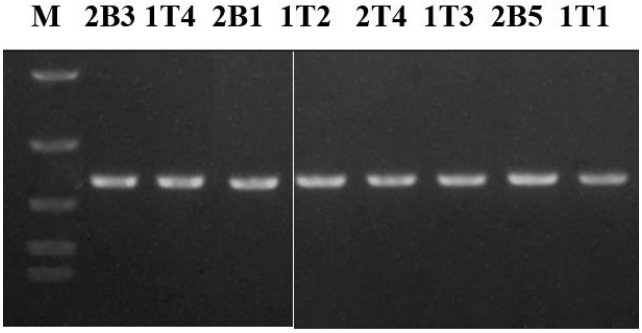

Figure 2. *Oryzias marmoratus* COI gene amplification result in Lake Towuti, Southern Sulawesi, Indonesia moved in 1% agarose electrophoresis. The example code is 2B3-1T1. A marker known as M can be seen from a ladder (GENEAID-1 kbp)

Table 2. *Oryzias marmoratus* nucleotide content (%) in Lake Towuti, Southern Sulawesi, Indonesia using the COI gene sequence

Sample	T(U)	C	A	G	A+T	G+C
2B4	29.13	28.27	24.19	18.39	53.32	46.67
2B3	29.20	28.18	24.10	18.50	53.31	46.68
2B1	27.42	29.64	26.06	16.86	53.49	46.50
2B2	29.03	28.01	24.10	18.84	53.14	46.85
1T3	29.20	28.18	24.10	18.50	53.31	46.68
1T1	29.37	28.01	23.93	18.67	53.31	46.68
1T4	28.91	28.23	24.14	18.70	53.06	46.93
1T2	28.86	28.18	24.27	18.67	53.14	46.85
Average	28.89	28.34	24.36	18.39	53.26	46.73
	±0.22	±0.19	±0.24	±0.22	±0.05	±0.05

Table 1. Outcomes of the COI gene sequence-based BLAST analysis of *O. marmoratus* in Lake Towuti, Southern Sulawesi, Indonesia

Code	BLAST				
	Query cover (%)	Similarity (%)	Species	Acc. No. GenBank	Location
1T1	100	96.78	*Oryzias marmoratus*	LC154797.1	Tanjung Timbala
1T2	100	99.84	*Oryzias marmoratus*	AP005981.1	Tanjung Timbala
1T3	100	97.41	*Oryzias marmoratus*	LC154797.1	Tanjung Timbala
1T4	99	99.68	*Oryzias marmoratus*	AP005981.1	Tanjung Timbala
2B1	98	94.42	*Oryzias marmoratus*	LC154797.1	Tanjung Bakara
2B3	98	99.68	*Oryzias marmoratus*	LC154797.1	Tanjung Bakara
2B4	99	97.28	*Oryzias marmoratus*	LC154797.1	Tanjung Bakara
2B5	99	96.47	*Oryzias marmoratus*	LC154797.1	Tanjung Bakara

Note: (-) = There are not any

Table 3. Based on COI gene sequences, *Oryzias marmoratus* genetic variation in Lake Towuti, Southern Sulawesi, Indonesia

Number of individual	Number of haplotype	Variable sites	Haplotype diversity	Nucleotide diversity
8	6	42	0.93	0.026

Table 4. Genetic distance of *Oryzias marmoratus* in Lake Towuti, Southern Sulawesi, Indonesia and outgroups from GenBank according to the COI gene sequence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1T1															
1T2	0.032														
1T3	0.003	0.028													
1T4	0.032	**0.000**	0.028												
2B1	0.045	0.061	0.041	0.061											
2B3	0.028	0.003	0.025	0.003	0.058										
2B4	0.003	0.028	**0.000**	0.028	0.041	0.025									
2B5	0.010	0.034	0.007	0.034	0.045	0.030	0.007								
O. marmoratus (LC154797.1)	0.030	0.005	0.026	0.005	0.059	0.002	0.026	0.032							
O. marmoratus (AP005981.1)	0.032	**0.000**	0.028	**0.000**	0.061	0.003	0.028	0.034	0.005						
O. celebensis (LC153105.1)	0.153	0.153	0.151	0.153	0.170	0.148	0.151	0.155	0.151	0.153					
O. matanensis (LC153099.1)	0.073	0.077	0.071	0.077	0.095	0.073	0.071	0.077	0.075	0.077	0.157				
O. nigrimas (LC153106.1)	0.105	0.112	0.103	0.112	0.123	0.108	0.103	0.107	0.110	0.112	0.131	0.131			
O. sarasinorum (LC154798.1)	0.105	0.113	0.103	0.113	0.127	0.111	0.103	0.105	0.113	0.113	0.127	0.113	0.109		
Oryzias sp. (JX311925.1)	0.143	0.142	0.140	0.142	0.155	0.138	0.140	0.141	0.138	0.142	0.149	0.151	0.127	0.136	
O. woworae (MK156277.1)	0.134	0.133	0.132	0.133	0.146	0.129	0.132	0.132	0.129	0.133	0.149	0.137	0.127	0.127	0.012

Genetic variation

Genetic variation originates from mutation and recombination events in the genome, which are mainly random phenomena. For this reason, genetic variation is an essential component of natural selection. Without mutations and other evolutionary factors, genetic variation will decrease and eventually disappear, especially in small populations, due to reduced allele frequencies (Ewens 2013; Jamniczky et al. 2010). Table 3 shows the outcomes of the genetic variation analysis of *O. marmoratus* in Lake Towuti samples. Eight samples of *O. marmoratus* from Lake Towuti with a length of 589 bp have six haplotypes of 42 variable sites. There is a 0.93 haplotype diversity value and a 0.026 nucleotide diversity value.

Genetic distance

Genetic divergence between species or populations within a species is measured by genetic distance (Nei 1987). The genetic distance of *O. marmoratus* fish from Lake Towuti was analyzed using the COI gene sequence utilizing the MEGA11 software and the Kumura-2-Parameter (K2P) model. Table 4 shows the outcomes of the genetic distance analysis. Table showing the genetic distance between eight samples (IT1-2B5) with outgroups from GenBank of 0.000, namely between samples 1T2 & 1T4; 1T3 & 2B4; *O. marmoratus* (AP005981.1) & 1T2; *O. marmoratus* (AP005981.1) & 1T4, genetic distance intraspecies has a low average value of around 0.025, and genetic distance interspecies has a high average value of around 0.079

Phylogenetics

Construction of a phylogenetic tree was performed using the alignment results of DNA sequences by the MEGA11 program. The phylogenetic structure has been evaluated using the Kimura-2-Parameter model, Maximum Likelihood (ML), and Neighbor-Joining (NJ) approaches. By examining transition and transversion substitution rates, the Kimura-2-Parameter model offers a more accurate computation model appropriate for short distances (Xiong 2006; Gogoi and Bhau 2018). The results of the phylogenetic tree reconstruction were then subjected to statistical tests using the bootstrap method to determine the confidence level (reliability) with the principle that random effects strongly influence data distribution (Hall 2018). Bootstrap testing was carried out 1000 times. Figure 3 shows the reconstruction of the phylogenetic tree that groups into five clades. The first clade comprises of eight *O. marmoratus* samples with *O. marmoratus* (LC154797.1) (AP005981.1) from GenBank. The second clade consists of *Oryzias matanensis* (LC153099.1) from GenBank. The third clade consists of *Oryzias sarasinorum* (LC154798.1) from GenBank. The fourth clade consists of *Oryzias celebensis* (LC153105.1), *Oryzias* sp. (JX311925.1), and *Oryzias woworae* (MK156277.1) from GenBank. The fifth clade consists of *O. nigrimas* (LC153106.1) from GenBank.

Haplotype network

Data from 8 samples and outgroups from GenBank were analyzed using DnaSP 6.12.03, producing 13 haplotypes. These findings were visually represented using the Median-Joining Network method in the image, as shown in Figure 4. The haplotype network functions as software that helps analyze genetic data to form an image representing genealogical relationships**.** The results of the analysis using the Median Joining Network from eight samples formed six haplotypes: H1, H2, H3, H4, H5, and H6; Haplotypes H2 and H3 comprised two samples.

Discussion

Using the COI gene sequences, we researched the identification and genetic variation of eight samples of *O. marmoratus* in Lake Towuti. It can be seen in Table 2 above that samples 2B3 and 1T3 have the same nucleotide composition. A+T has a higher average nucleotide content (53.26%) than G+C (46.73%). Variations in nucleotide composition indicate genetic variation in *O. marmoratus* from Lake Towuti based on the COI gene sequence. Kombong and Tuty (2018) argue that differences in nucleotide composition in the COI gene indicate genetic variation. According to Zhang and Hanner (2012), nucleotide variations can be a tool for distinguishing species.

The species is more primitive, as shown by the low proportion of GC (Niu et al. 2017). Yustinadewi et al. (2018) stated that the guanine and cytosine percentage (GC%) is the percentage of a sequence's total amount and should fall between 40 and 60%. By this opinion, the COI gene sequence of medaka fish with a GC composition of 46.73% is still classified as conserved. Meanwhile, according to Ismail et al. (2020), the AT composition is higher than the GC composition because of the significant diversity of nucleotide composition and increased nucleotide levels found in amplification genes.

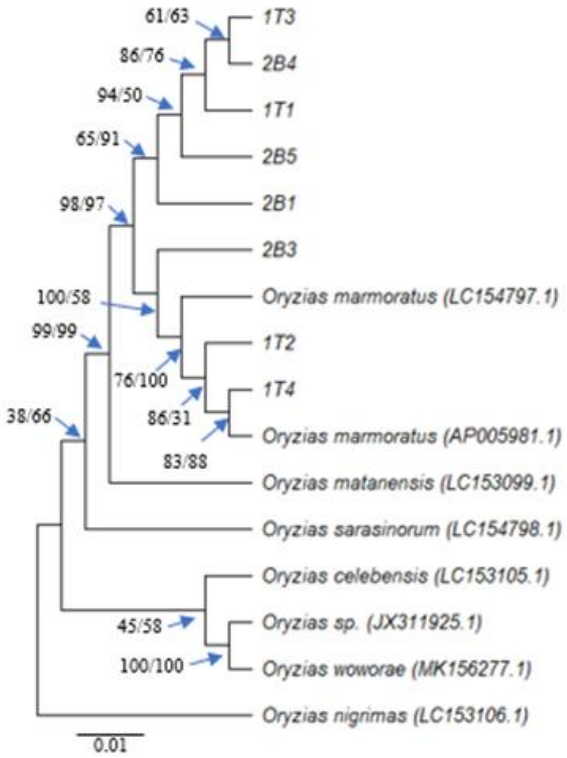

Figure 3. Reconstruction of 8 samples of *Oryzias marmoratus* in Lake Towuti and outgroups from GenBank using the NJ and ML methods based on COI gene sequences. Nodes indicate bootstrap values (NJ and ML, respectively). A scale bar value 0.01 indicates one nucleotide change for every 100 base pairs

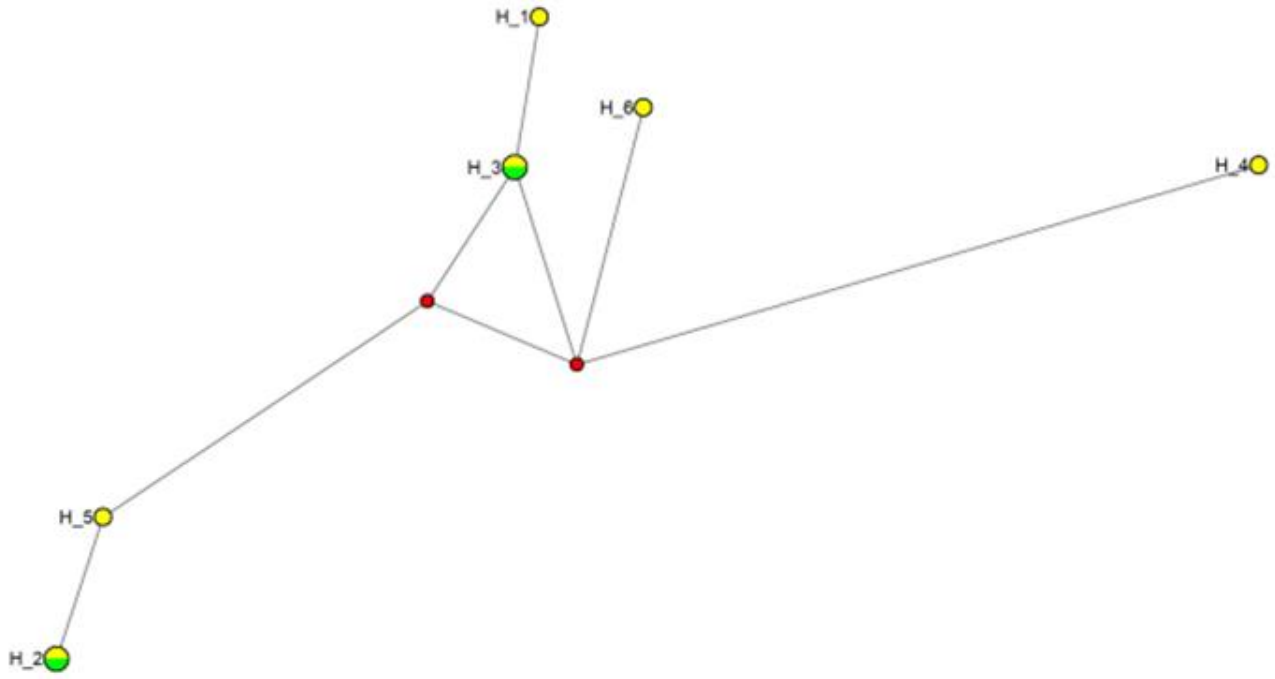

Figure 4. Haplotype network of *Oryzias marmoratus* in Lake Towuti, Southern Sulawesi, Indonesia based on COI gene sequences

These haplotype values indicate that *O. marmoratus* from Lake Towuti has high haplotype diversity, consistent with the varying nucleotide composition values (Table 2) and high genetic variations. Haplotype diversity indicates that the level of genetic variation can help a survival population in the natural environment. Numerous processes, including natural selection, recombination, and mutation, contribute to haplotype diversity (Stumpf 2004). The values obtained differ significantly from Nuryanto et al. (2019), with haplotype diversity (Hd) = 0.935±0.016 and nucleotide diversity (π) = 0.073±0.036. These two values indicate that *Hemibagrus nemurus* (Valenciennes, 1840) on Java Island has high genetic diversity. Previous and current research has shown that COI gene sequence is a highly variable marker in various animal species and is suitable for genetic studies.

Based on Table 3, eight samples have six haplotypes that show high haplotype values; this indicates genetic variation in the *O. marmoratus*. According to Leitwein et al. (2020), estimating selection and evolution over a range of timescales pertinent to conservation issues can be done using haplotype data. Based on research by Basith et al. (2021), results of haplotype diversity and nucleotide diversity in *Epinephelus* spp. on Madura Island with COI gene sequences of 0.978 and 0.12107 demonstrate that *Epinephelus* spp. has a significant level of genetic variation. Genetic diversity refers to the results of ecological, behavioral, and physical isolation and a limited number of individuals (Mignon-Grasteau et al. 2005). High genetic diversity populations may have a higher probability of surviving, indicating that genetic distance has a high similarity and low genetic distance, suggesting that intraspecies with genetic distance have high similarity and genetic distance is low. This is in line with the statement by Tallei and Kolondam (2015) that the closer the relationship between organisms is, the lower the genetic distance value.

According to Serdiati et al. (2020), an analysis of the genetic distance of the *O. nigrimas* COI gene sequence showed that the sample obtained had a low genetic distance (0.005); it was determined that this was the same species as *O. nigrimas* because there was no genetic separation and the genetic structure was well conserved (Brraich and Akhter 2015). Low genetic variety can contribute to poor survival and higher extinction, especially in populations facing environmental stress (Martinez et al. 2018). The genetic distance between *Caranx sexfasciatus* Quoy & Gaimard, 1825 and *Caranx tille* Cuvier, 1833, Papua, with the 16S gene sequence, is 0-0.02% and 0-0.2%. The highest genetic distance is 0.0023, and the lowest is 0.0188 (Kainama et al. 2023). If the genetic distance is low, the similarity is closer and may have the exact origin (Tapilatu et al. 2021; Dwifajri et al. 2022).

Analysis based on the phylogenetic tree of medaka fish shows that all members of the genus *Oryzias* are a monophyletic group (Figure 3). A monophyletic group is one in which all taxa are descended from the same ancestor (common ancestor), not from other lineages or taxa (Hall 2018). Five clades were identified by phylogenetic analysis, with a bootstrap value of 39-100%, and the bootstrap results showed that this clade grouping was strong. This indicates that eight samples are in the clade with *O. marmoratus* (LC154797.1) and *O. marmoratus* (AP005981.1), supported by good bootstrap values at the node (98%). A low bootstrap value means that the topology of the phylogenetic tree reconstruction at each sampling differs (Serdiati et al. 2020). *O. marmoratus* forms a clade with *O. nigrimas*, *Oryzias nebulosus* Parenti & Soeroto, 2004, *Oryzias sp.*, *Oryzias sarasinorum* (Popta, 1905), *Oryzias matanensis* (Aurich, 1935), *Oryzias woworae* Parenti & Hadiaty, 2010, and *Oryzias celebensis* (Weber, 1894), according to the grouping results published by Serdiati et al. (2020).

According to research by Zainal et al. (2022), the results of phylogenetic analysis of the genus *Oryzias* in Lake Lindu and the genus *Oryzias* recorded in GenBank COI gene sequences form 8 clades. The formation of 8 clades is supported by bootstrapping 80-100% using the NJ and ML methods. *Oryzias sarasinorum* (Popta, 1905) in Lake Lindu is the same clade as *O. sarasinorum* from the Japanese aquarium, and the bootstrap value is 100%.

Figure 4 shows that H3 has many connections or branches and is at the network's center, indicating that H3 is the ancestor. There is also a median vector with a red dot symbol, which connects bloodlines, one of which is H2 and H5 with the H3 kinship line. The haplotype network also shows that the highest mutation rate is 23, found between the median vector and H4, and the lowest mutation rate is found in several haplotypes, namely H3 with the median vector.

The high haplotype diversity and low genetic distance of sample *O. marmoratus* in Lake Towuti, which a limited distribution area may cause, are significant findings. These characteristics suggest a robust genetic makeup and a relatively homogenous population, which could be attributed to the species' non-migratory nature and exclusive freshwater habitat. In conclusion, the result showed that in the BLAST-N analysis, eight samples were closely related to *O. marmoratus* (LC154797.1) (AP005981.1). Genetic variation indicated a high value with detailed haplotype diversity, a low nucleotide diversity, and six haplotypes; genetic distance with an average of 0.025 and 0.079. Phylogenetic analysis established five clades. Information on intraspecies genetic variation of *O. marmoratus* in terms of genetics, especially in Lake Towuti, is needed for genetic conservation and as an animal model of biology and molecular studies.

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REFERENCES

Arisuryanti T, Firdaus NUN, Hakim L. 2020. Genetic characterization of striped snakehead (*Channa striata* Bloch, 1793) from Arut River, Central Kalimantan, inferred from COI mitochondrial gene. AIP Conf Proc 2260: 020001. DOI: 10.1063/5.0015905.

Basith A, Abinawanto, Kusrini E, Yasman. 2021. Genetic diversity analysis and phylogenetic reconstruction of groupers *Epinephelus* spp. from Madura Island, Indonesia based on partial sequence of CO1 gene. Biodiversitas 22 (10): 4282-4290. DOI: 10.13057/biodiv/d221020.

Bingpeng X, Heshan L, Zhilan Z, Chunguang W, Yanguo W, Jianjun W. 2018. DNA barcoding for identification of fish species in the Taiwan Strait. Plos One 13 (6): e0198109. DOI: 10.1371/journal.pone.0198109.

Brraich OS, Akhter S. 2015. Morphometric characters and meristic counts of a fish, *Crossocheilus latius latius* (Hamilton-Buchanan), from Ranjit Sagar Wetland, India. Intl J Fish Aquac Stud 2 (5): 260-265.

Carlson SM, Cunningham CJ, Westley PAH. 2015. Evolutionary rescue in a changing world. Trends Ecol Evol 29 (9): 521-530. DOI: 10.1016/j.tree.2014.06.005.

Dahruddin H. 2012. Rice fish (*Oryzias* sp.) from Sulawesi. Fauna Indonesia 11 (2): 28-32. ISSN 0216-9169. [Indonesian]

DeSalle R, Goldstein P. 2019. Review and interpretation of trends in DNA barcoding. Front Ecol Evol 7: 302. DOI: 10.3389/fevo.2019.00302.

Dwifajri S, Tapilatu RF, Pranata B, Kusuma AB. 2022. Molecular phylogeny of grouper of genus *Epinephelus* in Jayapura, Papua, Indonesia inferred from Cytochrome Oxidase I (COI) gene. Biodiversitas 23 (3): 1449-1456. DOI: 10.13057/biodiv/d230332.

Ewens WJ. 2013. Genetic variation. In Brenner's Encyclopedia of Genetics: Second Edition (Vol. 3). Elsevier Inc.

Fadli N, Muchlisin ZA, Siti-Azizah MN. 2021. DNA barcoding of commercially essential groupers (Epinephelidae) in Aceh, Indonesia. Fish Res 234: 105796. DOI: 10.1016/j.fishres.2020.105796.

French I, Wainwright BJ. 2022. DNA barcoding identifies endangered sharks in pet food sold in Singapore. Front Mar Sci 9: 836941. DOI: 10.3389/fmars.2022.836941.

Fricke R, Eschmeyer WN, Van Der Laan R. 2022. Eschmeyer's catalog of fishes: Genera, species, references, world wide web electronic publication (Eds). http:/ /researcharchive.calacademy.org [Accessed on 28 February 2022].

Gogoi B, Bhau BS. 2018. DNA barcoding of the genus *Nepenthes* (Pitcher Plant): A preliminary assessment towards its identification. BMC Plant Biol 18: 153. DOI: 10.1186/s12870-018-1375-5.

Hall TA. 1999. Bioedit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41: 95-98.

Hall BG. 2018. Phylogenetic Tree Made Easy: A How-to Manual. Oxford University Press, Oxford.

Ikpeme EV, Ekerette EE, Udensi OU, Ozoje MO. 2017. Assessment of morphological variation in wild and cultured populations of tilapia fish (*Oreochromis niloticus*). J Adv Biol Biotechnol 13 (2): 1-10. DOI: 10.9734/jabb/2017/33777.

Ismail M, Ahmad A, Nadeem M, Javed MA, Khan SH, Khawaish I, Sthanadar AA, Qari SH, Alghanem SM, Khan KA, Khan MF, Qamer S. 2020. Development of DNA barcodes for selected *Acacia* species using rbcL and matK DNA markers. Saudi J Biol Sci 27 (12): 3735-3742. DOI: 10.1016/j.sjbs.2020.08.020.

IUCN. 2023. The IUCN Red List of Threatened Species. Version 2022-2. ISSN 2307-8235. https://www.iucnredlist.org/ [Accessed: 10 September 2023].

Jamniczky HA, Boughner JC, Rolian C, Gonzalez PN, Powell CD, Schmidt EJ, Parsons TE, Bookstein FL, Hallgrímsson B. 2010. Rediscovering Waddington in the post-genomic age: Operationalising Waddington's epigenetics reveals new ways to investigate the generation and modulation of phenotypic variation. Bioessays 32 (7): 553-558. DOI: 10.1002/bies.200900189.

Kainama J, Louraine T, Wijayanti DP, Sabdono A, Dirgantara D. 2023. DNA barcoding and morphological characters of two trevally fish species (*Caranx* spp.) collected from Youtefa Bay, Papua, Indonesia. Indones J Mar Sci 28 (1): 97-104. DOI: 10.14710/ik.ijms.28.1.97-104.

Kobayashi H, Mokodongan DF, Horoiwa M, Fujimoto S, Tanaka R, Masengi KW, Yamahira K. 2023. A new lacustrine ricefish from Central Sulawesi, with a redescription of *Oryzias marmoratus* (Teleostei: Adrianichthyidae). Ichthyol Res 70: 490-514. DOI: 10.1007/s10228-023-00908-2.

Kombong CBS, Arisuryanti T. 2018. The 16S and COI mitochondrial DNA nucleotide composition of striped snakehead (*Channa striata* Bloch,1793) from Lake Sentani, Jayapura, Papua. Jurnal Perikanan Universitas Gadjah Mada 20 (1): 57-62. DOI: 10.22146/jfs.35551. [Indonesian]

Kottelat M, Whitten T, Kartikasari SN, Wirjoatmodjo S. 1993. Freshwater Fishes of Western Indonesia and Sulawesi. Periplus Editions, Hong Kong.

Kress WJ, García-Robledo C, Uriarte M, Erickson DL. 2015. DNA barcodes for ecology, evolution and conservation. Trends Ecol Evol 30 (1): 25-35. DOI: 10.1016/j.tree.2014.10.008.

Leitwein M, Duranton M, Rougemont Q, Gagnaire PA, Bernatchez L. 2020. Using haplotype information for conservation genomics. Trends Ecol Evol 35 (3): 245-258. DOI: 10.1016/j.tree.2019.10.012.

Luo A, Aibing Z, Simon Yw H, Weijun X, Yanzhou Z, Weifeng S, Stephen LC, Chaodong Z. 2011. Potential efficacy of mitochondrial genes for animal DNA barcoding: a case study using eutherian mammals. BMC Genomic 12 (84): 1-13. DOI: 10.1186/1471-2164-12-84.

Nei M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

Niu Z, Xue Q, Wang H, Xie X, Zhu S, Liu W, Ding X. 2017. Mutational biases and GC-biased gene conversion affect GC content in the plastomes of *Dendrobium* genus. Intl J Mol Sci 18 (11): 2307. DOI: 10.3390/ijms18112307.

Nursyahran N, Jayadi, Tamsil A, Harlina H. 2022. Growth patterns analysis of yellow–finned medaka (*Oryzias profundicola*) as endemic fish in Lake Towuti. IOP Conf Ser: Earth Environ Sci 1118: 012047. DOI: 10.1088/1755-1315/1118/1/012047.

Nursyahran N, Jayadi, Tamsil A, Harlina. 2023. Domestication of yellow-finned medaka fish (*Oryzias profundicola*). AACL Bioflux 16 (1): 524-533.

Nuryanto A, Komalawati N, Sugiharto. 2019. Genetic diversity assessment of *Hemibagrus nemurus* from Rivers in Java Island, Indonesia, using COI gene. Biodiversitas 20 (9): 2707-2717. DOI: 10.13057/biodiv/d200936.

Martinez AS, Willoughby JR, Christie MR. 2018. Genetic diversity in fishes is influenced by habitat type and life-history variation. Ecol Evol 8 (23): 12022-12031. DOI: 10.1002/ece3.4661.

Mignon-Grasteau S, Boissy A, Bouix J, Faure JM, Fisher AD, Hinch GN, Jensen P, Le Neindre P, Mormede P, Prunet P, Vandeputte M. 2005. Genetics of adaptation and domestication in livestock. Livest Prod Sci 93 (1): 3-14. DOI: 10.1016/j.livprodsci.2004.11.001.

Mkare TK, Jansen van Vuuren B, Teske PR. 2021. Conservation priorities in an endangered estuarine seahorse are informed by demographic history. Sci Rep 11: 4025. DOI: 10.1038/s41598-021-83754-4.

Pandey PK, Singh YS, Tripathy PS, Kumar R, Abujam SK, Parhi J. 2020. DNA barcoding and phylogenetics of freshwater fish fauna of Ranganadi River, Arunachal Pradesh. Gene 754: 144860. DOI: 10.1016/j.gene.2020.144860.

Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol Biol Evol 34 (12): 3299-3302. DOI: 10.1093/molbev/msx248.

Russell JM, Hendrik V, Satria B, Martin M, Alan D, Abdul H, Doug H, Ascelina KMH, Marina Morlock, Thomas von R, Rachel S, Bjorn S, Janella S. 2020. The late quaternary tectonic, biogeochemical, and environmental evolution of ferruginous Lake Towuti, Indonesia. Palaeogeography Palaeoclimatol Palaeoecol 556 (2020): 1-15. DOI: 10.1016/j.palaeo.2020.109905.

Said DS, Mayasari N. 2021. The development of adapted marmorated medaka *Oryzias marmoratus* (Aurich, 1935) at ex-situ habitat. IOP Conf Ser: Earth Environ Sci 744: 012069. DOI: 10.1088/1755-1315/744/1/012069.

Serdiati N, Arfiati D, Widodo MS, Lelono TJ, Ndobe S, Saranga R. 2020. Morphological variations and phylogenic analysis of *Oryzias nigrimas* Kottelat, 1990 (Rice Fish) from Lake Poso, Central Sulawesi, Indonesia. Biodiversitas 21 (3): 882-888. DOI: 10.13057/biodiv/d210305.

Stumpf MPH. 2004. Haplotype diversity and SNP frequency dependence in the description of genetic variation. Eur J Hum Genet 12 (6): 469-477. DOI: 10.1038/sj.ejhg.5201179.

Tamura K, Stecher G, Kumar S. 2021. MEGA11: Molecular evolutionary genetics analysis version 11. Mol Bio Evol 38 (7): 3022-3027. DOI: 10.1093/molbev/msab120.

Tallei TE, Kolondam BJ. 2015. DNA barcoding of sangihe nutmeg (*Myristica fragrans*) using math gene. HAYATI J Biosci 22 (1): 41-47. DOI: 10.4308/hjb.22.1.41.

Tapilatu R, Tururaja TS, Sipriyadi, Kusuma AB. 2021. Molecular phylogeny reconstruction of grouper (Serranidae: Epinephelinae) at Northern part of bird's head Seascape-Papua inferred from COI gene. Fish Aquat Sci 24 (5): 181-190. DOI: 10.47853/FAS.2021.e18.

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN. 2005. DNA barcoding Australia's fish species. Philos Trans R Soc Lond B Biol Sci 360 (1462): 1847-1857. DOI: 10.1098/rstb.2005.1716.

Wicaksono SA, Russell JM, Bijaksana S. 2015. Compound-specific carbon isotope records of vegetation and hydrologic change in Central Sulawesi, Indonesia, since 53,000 yr BP. Palaeogeography Palaeoclimatol Palaeoecol 430 (7): 47-56. DOI: 10.1016/j.palaeo.2015.04.016.

Xia JH, Bai Z, Meng Z, Zhang Y, Wang L, Liu F, Jing W, Wan ZY, Li J, Lin H, Yue GH. 2015. Signatures of selection in tilapia revealed by whole genome resequencing. Sci Rep 5: 14168. DOI: 10.1038/srep14168.

Xiong J. 2006. Essential Bioinformatics. Cambridge University Press, New York.

Yustinadewi PD, Yustiantara PS, Narayani I. 2018. Mdr-1 gene 1199 variant primer design techniques in pediatric patient buffy coat samples with lla. Metamorfosa: J Biol Sci 5 (1): 105-111. DOI: 10.24843/metamorfosa.2018.v05.i01.p16.

Zainal S, Tellu AT, Kasim A. 2022. Morphological variations and molecular phylogeny of *Oryzias sarasinorum* Popta, 1905 (Ricefish) from Lake Lindu, Central Sulawesi, Indonesia. Biodiversitas 23 (7): 3443-3451. DOI: 10.13057/biodiv/d230717.

Zhang J, Hanner R. 2012. Molecular approach to the identification of fish in the South China Sea. PLoS One 7 (2): e30621. DOI: 10.1371/journal.pone.0030621.