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DNA barcoding of *Mugilogobius mertoni* and *M. rambaiae* from Siberut and Enggano Islands, the small outermost islands of Sumatra, Indonesia

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Abstract. *Roesma DI, Tjong DH, Syaifullah, Aidil DR. 2025. DNA barcoding of* Mugilogobius mertoni *and* M. rambaiae *from Siberut and Enggano Islands, the small outermost islands of Sumatra, Indonesia. Biodiversitas 26: 386-395.* Siberut and Enggano Islands are the small and outermost islands of Sumatra, Indonesia. These two islands' unique geological history allows evolutionary processes to produce high levels of endemicity. One of the interesting fish genera from the Siberut and Enggano Islands is *Mugilogobius*. Based on morphological identification, it was estimated there are two *Mugilogobius* species in the Siberut and Enggano Islands. Molecular identification of *Mugilogobius* using the Cytochrome Oxidase I gene (known as DNA barcoding) needs to be done to prove it. The liver tissue was used for molecular analysis. The BLAST analysis showed that *Mugilogobius* from Siberut and Enggano Islands have a similarity range of 97.49%-96.06% with *Mugilogobius* GenBank. Based on the 558 bp sequence analyzed, *Mugilogobius mertoni* and *Mugilogobius rambaiae* from Siberut and Enggano Islands have a low sequence of divergences at 0.0%-0.4%, respectively. *M. rambaiae* from Siberut and Enggano Islands share the same haplotype. The ability of the species to maintain their genetics and the similarity of conditions between the two islands share their high genetic similarities. *M. mertoni* and *M. rambaiae* from Siberut and Enggano Islands have a high sequence of divergences at 3.0-4.6% with *M. mertoni* and *M. rambaiae* GenBank, respectively. The long-distance location, the presence of the ocean as a barrier, and differences in habitat conditions contribute to the high variations between *Mugilogobius* from two islands and other populations. *Mugilogobius* from Siberut and Enggano Islands has a sequence of divergence at 12.4%-17.3% compared to other *Mugilogobius* species, supporting their differences at the species level in the same genera. This study contributed to presenting the first molecular data of *Mugiologius* that can be used as a sequence reference for identification and the sequences became the genetic richness data of fish in the small and outermost islands of Sumatra, Indonesia (Siberut and Enggano Islands).

Keywords: Cytochrome Oxidase I, genetic similarities, *Mugilogobius*, sequence divergences

INTRODUCTION

Indonesia is located in a tropical area with a complex geological history, making it one of the countries with the highest biodiversity in the world (Tumonggor et al. 2013; von Rintelen et al. 2017). Indonesia is an archipelago, most of which are small and outermost islands that form boundaries with other countries (Kodoatie 2012). Siberut and Enggano are two of the 111 small and outermost islands in Indonesia. Enggano Island is an oceanic island located in the western part of Sumatra Island and is part of the Mentawai Fault (Barber et al. 2005). Enggano Island is unique because, in its geologic history, it never joined the mainland of Sumatra Island (Voris 2000; Barber et al. 2005). The non-existence of small tree mammals such as squirrels and freshwater fish commonly found in Sumatra proves this (Senoaji 2006). Siberut is one of the islands of the Mentawai Archipelago, larger than the other island parts (Quinten et al. 2014; Aninta et al. 2022). Unlike Enggano Island, which never joined the mainland of Sumatra, Siberut Island was once joined with the mainland of Sumatra (Quinten et al. 2014; Aninta et al. 2022). However, it separated in the middle of the Pleistocene period about 500,000 years ago (Voris 2000).

The unique geological history of these two islands allows the evolutionary processes to produce flora and fauna with high levels of endemicity. In the mid-20th century, almost ten botanical explorations were conducted on Siberut Island (van Steenis 1950). Lutjeharms conducted the first biological exploration on Enggano Island in 1936 (Maryanto et al. 2017). Later, the United States Agency for International Development (USAID) team studied the aspects of biodiversity in 2004, and the Bengkulu University team in 2006 (Senoaji 2006). The initial data collection of flora and fauna potential on Enggano Island was conducted by Regen (2011), who reported that the forest on Enggano Island is one of the seven forests with the best conditions managed by Ministry of Environment and Forestry in Bengkulu. In 2015, researchers from the Biology Research Center, Indonesian Institute of Sciences (LIPI) (known as BRIN- National Research and Innovation Agency) carried out a new expedition to explore the biological resources on Enggano Island (Maryanto et al. 2017).

Expeditions to Enggano Island and Siberut Island have provided basic information on the flora and fauna. However, information on freshwater ichthyofauna groups on Siberut and Enggano Islands is limited, especially before the LIPI expedition in 2015. Previously, only two freshwater fish

species were reported on Enggano Island by Perugia (1893), such as *Acentrogobius janthinopterus* Bleeker 1853 and *Butis amboinensis* Bleeker 1853. LIPI Expedition in 2015 showed that there are 51 species of fish, many of which are unidentified at the species level, which need to be proven by further studies using molecular data (Hadiaty and Sauri 2017; Maryanto et al. 2017). Information on freshwater fish on Siberut Island was also limited (Goistepan 2016) before the freshwater fish study by Roesma et al. (2022, 2024). Biodiversity studies using morphological and molecular data found as many as 34 freshwater fish species in South Siberut and North Siberut. However, it does not rule out the possibility of other fish species that have not been found due to limited exploration that can be done due to flooding on Siberut Island.

One of the fish genera found in Enggano Island and Siberut Island, reported previously by the Maryanto et al. (2017) and Roesma et al. (2024), is *Mugilogobius*. *Mugilogobius* is one of the genera in the Oxudercidae, consisting of 25 valid species (Nelson et al. 2016; van der Lann 2018) and limited molecular data information (Larson et al. 2014; Huang et al. 2016a, 2016b), especially in Indonesia. Based on the results of morphological identification, Roesma et al. (2024) reported two types of *Mugilogobius* fish in North Siberut, namely *Mugilogobius mertoni* Weber 1911 and *Mugilogobius rambaiae* Smith 1945. A freshwater fish study conducted by the author on Enggano Island in 2024 has found fish morphologically similar to *M. mertoni*. Molecular identification of *Mugilogobius* Siberut and Enggano Islands fish using the Cytochrome Oxidase I (COI) gene known as the DNA Barcoding method needs to be done. The molecular data obtained in this study can be used as a reference for the identification of *Mugilogobius* species and provide information on the genetic richness data of fish on small and outermost islands of Indonesia, in this case, Siberut and Enggano Islands.

MATERIALS AND METHODS

Study area

The fish samples were collected from two rivers in North Siberut, Siberut Island, West Sumatra Province, Indonesia (locally known as Srilanggai River, Malancan Village and Purodottan River, Muara Sikabaluan Village) (August 2023) and one river in Enggano Island, Bengkulu Province, Indonesia (locally known as Kahyapu River, Kahyapu Village) (August 2024). The sampling location is shown in Figure 1. Srilanggai River is a large river with a width of 2-4 meters and a depth of 0.5-1.2 meters with a muddy ground substrate so that the water is turbid with a brownish-yellow color. Meanwhile, the Purodottan River is a small river with a width of 1-2 meters and a depth of 0.2-0.5 meters with rocks and soil substrate and turbid water with a brownish-yellow color. The sample collection in the two rivers can only be reached by sea using boats. Kahyapu River is a small river with a width of 2-3 meters and a depth of 0.2-0.5 meters. It has limestone and sandy substrates and clear water. The sample collection in Kahyapu River was reached by land access. Sample collection was only conducted at three rivers because *Mugilogobius* fish were found only in these three rivers among several rivers explored on the Siberut and Enggano Islands.

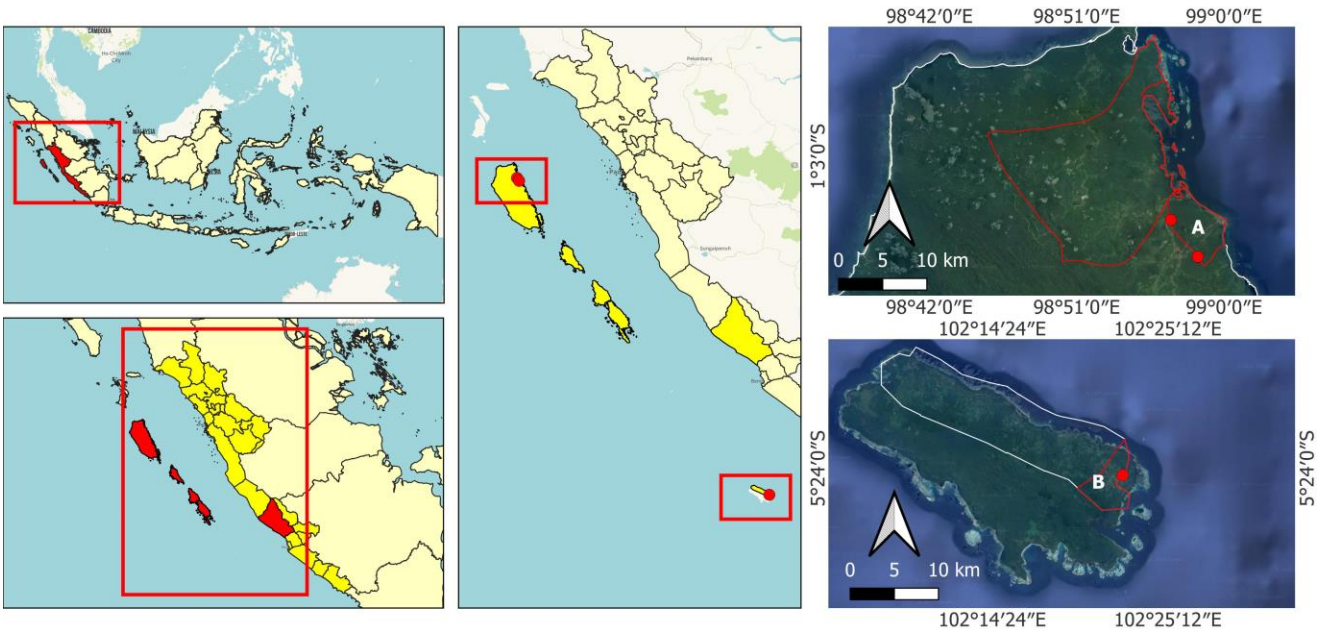

Figure 1. Location map of A. Siberut Island, Mentawai, West Sumatra and; B. Enggano Island, North Bengkulu, Bengkulu, Indonesia

Procedures

Samples collection

The sample collection was conducted using the observation method by direct sampling in the field (Ajayi 2023). Fish samples were collected using electrofishing (Thomas et al. 2019) and mentawai's special fishing nets known as *subba* (Roesma et al. 2022). A total of one *M. mertoni* individual from Siberut Island, one *M. mertoni* individual from Enggano Island, and two *M. rambaiae* individuals from Siberut Island were successfully collected. Representative samples of each fish species were photographed and labeled. Individual tissue samples were taken and stored in ethanol PA to prevent DNA degradation. Individual samples were preserved in 10% formalin. The samples were preserved for about 3-4 weeks. The samples were washed in running water and transferred to 70% alcohol for long-term storage at the Genetics and Biomolecular Laboratory, Department of Biology, Universitas Andalas, Padang, West Sumatra, Indonesia. Morphological identification was conducted by referring to identification books freshwater fish by Kottelat et al. (1993), Nelson et al. (2016), and van der Laan (2018), and based on Eschmeyer's Catalog of Fishes (2024).

Isolation, amplification, and sequencing of DNA

DNA isolation was performed on the tissue samples using the Zymo Research DNA Isolation KIT based on company protocol. Amplification of the COI gene was performed using the universal COI primers forward FISH FI (5'TCAACCAACCACAAAGACATTGGCAC3') and reverse FISH RI (5'TAGACTTCTGGGTGGCCAAAGAATCA3') (Ward et al. 2005). The composition of the PCR solution and the thermal cycling program was conducted following Roesma et al. (2022; 2024). The PCR product was checked by electrophoresis using 2% agarose gel and tested on a UV light plate. The good-quality PCR products were purified and sent to the first base in Malaysia for sequencing.

Data analysis

The sequencing results in forward and reverse sequences were assembled using the DNASTAR (DNASTAR, Inc., Madison, WI, USA) (Burland 2000). The DNA sequences were compared with sequences in GenBank, the National Center for Biotechnology Information (NCBI), using the Basic Local Analysis Search Tool (BLAST) to determine the similarity of sequences (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The other COI gene sequences were extracted from GenBank, NCBI, as comparison sequences. All sequences were aligned using Aliview and checked using the Bioedit software (Hall 2011; Reguant et al. 2020). The amino acids of aligned sequences were checked using the DNA translate to protein (http://insilico.ehu.es/translate/). If there are no stop codons in the amino acid translation, the DNA sequences can be submitted into the Barcode of Life Data System (BOLD System) to generate DNA Barcodes and the Barcode Index Number (BIN). Sequences that have 100% similarity with the registered sequence in the BOLD System will have the same BIN code (Kartavtsev 2018). The polymorphism sequence data were analyzed using the DNA SP 6.0 software (Rozas et al. 2017). The phylogenetic tree was reconstructed using the maximum likelihood (ML) method through IQ Tree using the 1000 bootstrap replications (Nguyen et al. 2015). The nucleotide composition and pairwise genetic distance (Kimura Two Parameter) were analyzed using the Molecular Evolutionary Genetics Analysis (MEGA) 11 software (Tamura et al. 2021). The range of genetic distance in mtDNA genes for fish species following Kartavtsev et al. (2016); Kartavtsev (2018).

RESULTS AND DISCUSSION

Nucleotide base variations

The universal primer has successfully amplified the partial COI gene of one individual of *M. mertoni* from Enggano and Siberut Islands, respectively, and two individuals of *M. rambaiae* from Siberut Island. All the sequences produced a final trimmed alignment of 558 bp, and no stop codons, deletions, or insertions were observed, which suggests there were no pseudogenes. This high level of accuracy in our sequencing process instills confidence in the reliability of our results. All sequences were aligned to GenBank, NCBI sequences databases using the BLAST tool. The BLAST analysis showed that *M. mertoni* from Siberut and Enggano Islands have a similarity of 97.49%-96.77% with *M. mertoni* GenBank, and *M. rambaiae* from Siberut and Enggano Islands have a similarity of 96.34%-96.06% with *M. rambaiae* GenBank (figure not shown). Meanwhile, the two species (*M. mertoni* and *M. rambaiae*) have a similarity of 89-90%. The similarity percentage between *M. mertoni* and *M. rambaiae* supported them to be classified into different species. The results showed that the gene COI can identify organisms up to the species level. The study using DNA barcoding is considered successful in identifying fish species and in evaluating the genetic variability within and interspecies (Rajkumar et al. 2015; Panprommin et al. 2019; Ghouri et al. 2020; Yulianto et al. 2020; Alshehri et al. 2022; Utama et al. 2022; Lamadi et al. 2023; Ndobe et al. 2023).

Four *Mugilogobius* sequences from the Enggano and Siberut Islands and 39 comparison sequences with a length of 558 bp have been analyzed. The alignment sequences (558 bp) consist of 331 bp (59.31%) conserved sites and 227 bp (40.69%) variable sites, of which 214 bp (38.35%) are parsimony informative sites. The average base composition was 30.6% (T), 27.0% (C), 24.3% (A), and 18.1% (G). The nucleotide content of all sequences showed that the frequency of A+T was higher than G+C. Nucleotide sequences have an AT ratio higher than the GC ratio, which is characteristic of the genome of mtDNA in vertebrates (Hubert et al. 2008). Other studies also reported that the content of A+T was higher than G+C (Roesma et al. 2022; Hasim et al. 2023). The COI gene analysis represented 413 mutations with an estimated transition/ transversion ratio bias of R: 1.445. Overall, 29 substitutions consist of 21 transitions and eight transversions for *M. mertoni*, and 26 comprise 22 transitions and four transversions for *M. rambaiae*. The mutations only cause the changes in the two amino acids for *M. mertoni*. *M. mertoni*

PP089071.1 from Singapore is a reference sequence for other *M. mertoni*, and *M. rambaiae* MW591103.1 from Malaysia is a reference sequence for other *M. rambaiae*. All *M. mertoni* sequences have nucleotide base variations, and no identical genetic sequences exist. *M. mertoni* Siberut and Enggano Islands only have two nucleotide base variations. Interestingly, for *M. rambaiae* sequences, there is an identical genetic sequence for *M. rambaiae* Siberut and Enggano Island. Based on the variations in nucleotide base, there are similarities in patterns of nucleotide base based on geographic region. *M. mertoni* and *M. rambaiae* from the same populations or nearby regions almost have the same pattern of nucleotide base variations.

A total of 40 haplotypes were identified among all sequences, consisting of three haplotypes for *M. mertoni* and *M. rambaiae* in this study and 37 haplotypes for GenBank sequences; a total of 22 haplotypes to *Mugilogobius*, six haplotypes to Stiphodon, three haplotypes to Redigobius, seven haplotypes to *Glossogobius*, and two haplotypes to outgroup species (*Danio rerio* Hamilton 1822) and *Danio choprae* Hora 1928) (Table 1). The 43 sequences have haplotype diversity (Hd) of 0.997 and nucleotide diversity (Pi) of 0.17098. Among 88 nucleotide bases mutated in *M. mertoni* and *M. rambaiae*, resulting in seven haplotypes for *M. mertoni* and six haplotypes for *M. rambaiae*. The central haplotype for *M. mertoni* is *M. mertoni* PP089071.1 from Singapore and *M. rambaiae* MW591103.1 from Malaysia for *M. rambaiae*. Interestingly, *M. rambaiae* from Enggano and Siberut Islands share the same haplotype. A high genetic similarity was found in *M. mertoni* from Enggano and Siberut Islands, with two base variations that created the two haplotypes. All mutations cause changes in two amino acids in *M. mertoni*. The high similarity of goby fishes in Siberut Island with other islands was also reported in the previous studies (Roesma et al. 2022).

Table 1. Haplotype list of sequences

Haplotype	Sequences
Haplotype 1	*Mugilogobius rambaiae* Smith 1945 Siberut Island, Indonesia 1*****, *Mugilogobius rambaiae* Siberut Island, Indonesia 2*****
Haplotype 2	MW591105.1 *Mugilogobius rambaiae* Smith 1945 Malaysia 2
Haplotype 3	MW591103.1 *Mugilogobius rambaiae* Smith 1945 Malaysia 4
Haplotype 4	MW591106.1 *Mugilogobius rambaiae* Smith 1945 Malaysia 1
Haplotype 5	MW591102.1 *Mugilogobius rambaiae* Smith 1945 Malaysia 5
Haplotype 6	MW591104.1 *Mugilogobius rambaiae* Smith 1945 Malaysia 3
Haplotype 7	*Mugilogobius mertoni* Weber 1911 Siberut Island, Indonesia*****
Haplotype 8	*Mugilogobius mertoni* Weber 1911 Enggano Island, Indonesia*
Haplotype 9	KM887180.1 *Mugilogobius mertoni* Weber 1911 Indonesia
Haplotype 10	KM887185.1 *Mugilogobius mertoni* Weber 1911 Indonesia
Haplotype 11	KX056135.1 *Mugilogobius mertoni* Weber 1911 Taiwan
Haplotype 12	PP089071.1 *Mugilogobius mertoni* Weber 1911 Singapore
Haplotype 13	PP089072.1 *Mugilogobius mertoni* Weber 1911 Singapore
Haplotype 14	MW591101.1 *Mugilogobius chulae* Smith 1932 Malaysia
Haplotype 15	KX223921.1 *Mugilogobius chulae* Smith 1932 Malaysia
Haplotype 16	KX056136.1 *Mugilogobius myxodermus* Herre 1935 Taiwan
Haplotype 17	NC 036070.1 *Mugilogobius myxodermus* Herre 1935
Haplotype 18	KX056131.1 *Mugilogobius abei* Jordan and Synder 1901 Taiwan
Haplotype 19	KJ669538.1 *Mugilogobius platynotus* Günther 1861 Australia
Haplotype 20	MW591109.1 *Mugilogobius tigrinus* Larson 2001 Malaysia
Haplotype 21	KX056138.1 *Mugilogobius tigrinus* Larson 2001 Malaysia
Haplotype 22	MW591107.1 *Mugilogobius tigrinus* Larson 2001 Malaysia
Haplotype 23	MK714087.1 *Glossogobius giuris* Hamilton 1822 India
Haplotype 24	JX260876.1 *Glossogobius giuris* Hamilton 1822 India
Haplotype 25	MW574837.1 *Glossogobius aureus* Akihito and Meguro 1975 Australia, MW574834.1 *Glossogobius aureus* Australia
Haplotype 26	MW574828.1 *Glossogobius circumspectus* Macleay 1883 Australia
Haplotype 27	MW574808.1 *Glossogobius circumspectus* Macleay 1883 Australia
Haplotype 28	MW574702.1 *Glossogobius olivaceus* Temminck and Schlegel 1845 Japan
Haplotype 29	MW574709.1 *Glossogobius olivaceus* Temminck and Schlegel 1845 Japan
Haplotype 30	PP132756.1 *Stiphodon semoni* Weber 1895 Java, Indonesia
Haplotype 31	PP132754.1 *Stiphodon semoni* Weber 1895 Java, Indonesia
Haplotype 32	PP132753.1 *Stiphodon annieae* Keith and Hadiaty 2014 Maluku, Indonesia
Haplotype 33	PP132749.1 *Stiphodon annieae* Keith and Hadiaty 2014 Maluku, Indonesia
Haplotype 34	PP132745.1 *Stiphodon maculidorsalis* Maeda and Tan 2013 West Sumatra, Indonesia
Haplotype 35	PP132727.1 *Stiphodon maculidorsalis* Maeda and Tan 2013 West Sumatra, Indonesia
Haplotype 36	KJ669608.1 *Redigobius macrostoma* Günther 1861 Australia, KJ669607.1 *Redigobius macrostoma* Australia
Haplotype 37	KU692832.1 *Redigobius bikolanus* Herre 1927 Bali, Indonesia
Haplotype 38	KU692830.1 *Redigobius bikolanus* Herre 1927 Bali, Indonesia
Haplotype 39	MK714084.1 *Danio rerio* Hamilton 1822 India
Haplotype 40	MN342410.1 *Danio choprae* Hora 1928

Notes: The asterisk (*) showed the *Mugilogobius* species from Siberut and Enggano Islands, Indonesia

Fish database, morphological, and taxonomic notes

Mugilogobius mertoni and *M. rambaiae* species were categorized as Least Concern (LC) based on the International Union for Conservation of Nature (IUCN) Red List categories (Huckstorf and Larson 2021) (Figure 2 and Figure 3). Information on both species is limited regarding distribution data, ecological studies, morphology, and genetics. Based on geographic range data at IUCN, the distribution of *M. mertoni* in Indonesia has only been reported on the Aru Islands and Raja Ampat, and *M. rambaiae* in Indonesia has only been reported on Bintan Island and Kalimantan.

The discovery of *Mugilogobius* fish on Enggano and Siberut Islands is a new report on the distribution of *Mugilogobius* fish in Indonesia. Morphologically, *M. mertoni* from Enggano and Siberut Islands have the main characteristics of (i) body sides with clear oblique or transverse black lines; (ii) predorsal scales less than 17; (iii) longitudinal scales less than 43; (iv) cheeks and operculum with a checkered pattern; (v) eyes small and positioned high on the head; (vi) brownish body with 7-11 darker narrow diagonal lines; (vii) chevrons or X-shaped markings along the sides; (viii) spaces between chevrons or pale lines; (ix) caudal peduncle without horizontal lines; and (x) two to three dark spots or short diagonal lines on the caudal peduncle. The morphological characteristics differ between *M. mertoni* Siberut and Enggano and other *M. mertoni* in the absence of a first dorsal fin that is shorter than the second dorsal fin and the pattern on the cheeks with a checkered pattern vs. three black stripes (Sreeraj et al. 2024). *M. mertoni* is widely distributed around the Indo-West Pacific region, and several different morphological types of *M. mertoni* from different regions were observed and considered synonyms of *M. mertoni* (Huang et al. 2016a). Based on the morphological and molecular study, Huang et al. (2016a) suggested that the *M. mertoni* could be regarded as a species complex, and several cryptic species might be included in the *M. mertoni* complex.

Mugilogobius rambaiae is different from *M. mertoni* in some characteristics, consisting of a greyish body, scales with short vertical or curved dark lines, and a brown oblique bar in the shoulder. The anal, pelvic, and pectoral fins do not display any specific color patterns. One to several scales, sometimes larger than others, scales on the body mostly ctenoid. Caudal fin rounded to rectangular. Based on morphology, both species have the main characteristics that classify them into different species. The molecular data also supported separating them into distinct species with 9.9-11% sequence divergences (based on Kartavtsev et al. 2016; Kartavtsev 2018). This study provided the first molecular data for *M. mertoni* from Enggano and Siberut Islands and *M. rambaiae* from Siberut Island. The author submitted the DNA barcodes of *M. mertoni* and *M. rambaiae* to the BOLD System, a global platform for DNA barcoding and biodiversity research. The sequence data become the genetic richness of fish on the Enggano and Siberut Islands.

Mean genetic divergence (K2P) analysis

The Kimura Two Parameter (K2P) model was used to calculate the genetic distance with MEGA 11.0 software. The genetic distance within the same species ranged from 0.0% to 4.6%. There is a low to high intra-species variation for the COI gene. *M. mertoni* from Enggano and Siberut Islands has low sequence divergences (0.4%); likewise for *M. rambaiae* from Siberut Island, which has low sequence divergences (0.0%). According to Kartavtsev et al. (2016) and Kartavtsev (2018), the values (0.0-0.4%) showed a difference at the population level within the same species. The low intraspecific distance in fishes was also reported in other studies (Renxie et al. 2018; Ceruso et al. 2020; Roesma et al. 2022; Haslawati et al. 2023; Abdulmalik-Labe and Quilang 2024; Chan et al. 2024; Eriegha et al. 2024; Modeel et al. 2024). This study revealed the most significant K2P distance increased from 0.0% between the investigated sequences (Enggano and Siberut Islands) to 4.6% with comparison sequences from GenBank from the same species in other countries. Limbu et al. (2024) reported that the intraspecies K2P genetic distances ranged from 0% to 5% and the genetic divergence of more than 2% in intraspecific, indicating that there might be putative species. According to Kartavtsev et al. (2016) and Kartavtsev (2018), the intraspecific genetic distance in this study differs at the subspecies/sibling species level within the same species.

Figure 2. *Mugilogobius mertoni*

Figure 3. *Mugilogobius rambaiae*

The mean pairwise distances (K2P) among the species, genera, and families of Goby fish (Gobiiformes) are shown in Table 2. The mean K2P genetic divergence increased at different taxonomic levels, from within species (1.4%) to within genus (13.5%), to within families (20.4%), to within orders (20.7%). The increased value at higher taxa levels shows that distinct species were observed. The mean sequence divergences were almost similar to the previous studies (Khedkar et al. 2014; Pandey et al. 2020; Roesma et al. 2022; Limbu et al. 2024). The mean genetic distances increased hierarchically from

Interspecific divergence between the species in the same genera ranged from 9.9% to 17.3%, and within species to the family ranged from 16.9% to 25.8%. Within *Mugilogobius*, the highest sequence divergences (17.3%) were observed between *M. rambaiae* and *M. chulae* (Khedkar et al. 2014; Pandey et al. 2020; Roesma et al. 2022; Limbu et al. 2024). Apart from *Mugilogobius*, other genera in the Oxudercidae (*Redigobius* and *Stiphodon*) were represented by two sequences for each species as comparison sequences. The highest sequence divergences between the species in the different genera (within the family) were observed between *Redigobius macrostoma* and *Mugilogobius platynotus* (25.8%) (Table 2).

Phylogenetic analysis

The relationships between goby fishes were reconstructed using the IQ tree represented by the maximum likelihood method with 1000 bootstrap replicates (Figure 4). The best analysis model used is TPM2u+F+I+G4 based on the Bayesian information criterion (BIC). The phylogenetic tree represented the clear separation between different genera, which was supported by high sequence divergences among them. The phylogenetic tree showed that each branch is monophyletic for its respective genera (*Mugilogobius*, *Stiphodon*, *Redigobius*, and *Glossogobius*) relative to the outgroup. The two main clusters were obtained with three subclusters in the first cluster and two subclusters in the second cluster. *Mugilogobius* is present in the first cluster, and other genera are in the second cluster. *Mugilogobius* descended from a single ancestor, similarly reported by Larson et al. (2014) and Abbas et al. (2017), who determined the *Mugilogobius* species as a monophyletic group. *M. mertoni* and *M. rambaiae* were clustered within themselves in the first subcluster. *M. rambaiae* became a sister taxon of *M. mertoni* with sequence divergences of 9.9-11.6% among them. According to Kartavtsev et al. (2016) and Kartavtsev (2018), the values showed the different species at the same genus level.

M. mertoni from Enggano and Siberut Islands are on the same node with high similarity and separated in different nodes than *M. mertoni* GenBank with sequence divergences of 3.0-3.7%. *M. mertoni* KM887180.1 and KM887185.1 are samples from the eastern part of Indonesia, and *M. mertoni* KX056135.1, PP089071.1, and PP089072.1 are samples from other countries (Taiwan and Singapore). *M. rambaiae* from Enggano and Siberut Islands are also present on the same node with high similarity and separated in a different node than *M. rambaiae* GenBank with sequence divergences of 4.2-4.6%. All *M. rambaiae* GenBank sequences are samples originating from Malaysia. The long distance location, the presence of the ocean as a barrier, and differences in habitat conditions are thought to contribute to the high variations between *Mugilogobius* from Enggano and Siberut Islands and *Mugilogobius* from other populations (GenBank sequences). According to Kartavtsev et al. (2016) and Kartavtsev (2018), the values showed a difference at the sub-species/sibling species level within the same species. Various factors, such as genetic drift and ecological factors, can lead to population-level diversification, resulting in genotypic and phenotypic variation features of species across geographic regions (Lostrom et al. 2015). The high sequence divergence of intraspecies suggests the potential presence of sibling species due to adaptation and the high evolutionary potential of the species (Garcia-Cisneros et al. 2018). Limbu et al. (2024) reported high intraspecies genetic distances (4-5%), indicating the presence of putative/subspecies. Meanwhile, for the Enggano and Siberut Islands, which are separated and never connected, the ability of the species to maintain their genetics and the similarity of conditions between the islands is thought to make them have high genetic similarities. The low genetic distance may indicate that species from some populations may have similar origins (Tapilatu et al. 2021; Dwifajri et al. 2022). The high genetic similarity of the samples from different locations suggests that these species resist different habitat conditions and may have been distributed from one region to another (Baylan 2023).

Other *Mugilogobius* are in the second and third subclusters with sequence divergences of 12.4%-17.3% with the first subcluster. According to Kartavtsev et al. (2016) and Kartavtsev (2018), the values showed the difference at the species level within the same genera. Therefore, the study result showed that the COI gene is able to differentiate species in the same genera. Other studies also showed the COI gene is able to identify species and represent the clear separation between different species (Roesma et al. 2018; Roesma et al. 2019; Roesma et al. 2020; Kainama et al. 2023; Abdel-Gaber et al. 2023; Afifi et al. 2023; Afrand and Sourinejad 2023; Marnis et al. 2024; Ziyadi 2024). *M. abei*, *M. chulae*, and *M. myxodermus* grouped in the second subcluster with highly supported bootstrap values. Huang et al. (2016a) also reported that *M. chulae*, *M. myxodermus*, and *M. abei* were in the same group.

Table 2. The mean pairwise distances (K2P) among the species, genera, and families of Goby fish (Gobiiformes)

Taxonomic level	Min (%)	Mean (%)	Max (%)
Within species	0.0	1.4	4.6
Within genus	9.9	13.5	17.3
Within family	16.9	20.4	25.8
Within order	17.2	20.7	28.1

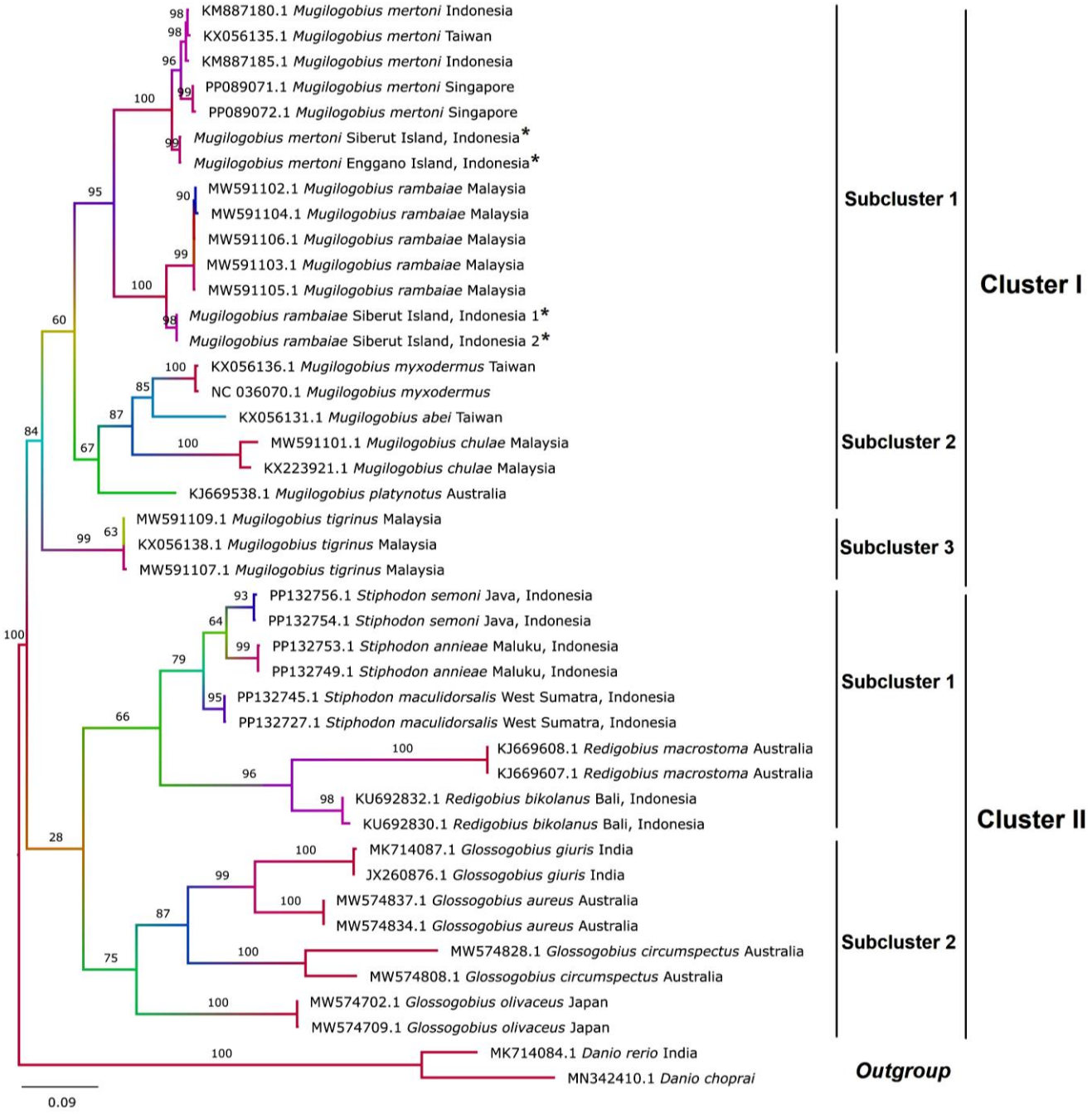

Figure 4. The phylogenetic tree (IQ Tree) of *Mugilogobius* using the Maximum Likelihood method with 1000 bootstrap replicates. The asterisk (*) showed the *Mugilogobius* species from Siberut and Enggano Islands, Indonesia

In the third subcluster, there is only a single species (*M. triginus*). Huang et al. (2016a) also reported *M. triginus*, which is present in different groups from other *Mugilogobius*. The second cluster comprises three other genera in Gobiiformes (*Stiphodon*, *Redigobius*, and *Glossogobius*). *Stiphodon* and *Redigobius* are present in the same subcluster (first subcluster), with sequence divergences of 17.8%-24.4% among them and 16.9%-25.8% among the members in the first cluster. Based on Kartavtsev et al. (2016) and Kartavtsev (2018), the value supports them in the different genera in the same family (Oxudercidae). *Glossogobius* presence in the second subcluster has sequence divergences of 17.2-28.1% and 18.3-25.4% with the second subcluster and first cluster, respectively. Based on Kartavtsev et al. (2016) and Kartavtsev (2018), the value supports them in the different families in the same order (Gobiiformes).

This study showed the ability of the COI gene to identify species and generate the DNA barcodes of *M. mertoni* and *M. rambaiae*. Our study contributed to presenting the first molecular data of the fish fauna in Enggano Island, and the sequences generated have been submitted to the BOLD System. The study revealed that the variation of genotype

and phenotype was suggested due to various factors such as genetic drift, ecological factors, and geographical isolation.

In conclusion, a total of four DNA barcodes of *Mugilogobius* species in Siberut and Enggano Islands were successfully generated and submitted to the BOLD System. *M. mertoni* and *M. rambaiae* from Siberut and Enggano Islands have low sequence divergences of 0.0-0.4%, respectively. The ability of the species to maintain their genetics and the similarity of conditions between the two islands make them have high genetic similarities. *M. mertoni* and *M. rambaiae* from Siberut and Enggano Islands have high sequence divergences of 3.0-4.6% with *M. mertoni* and *M. rambaiae* in GenBank, respectively. The long-distance location, the presence of the ocean as a barrier, and differences in habitat conditions are thought to contribute to the high variations between *Mugilogobius* from two islands with other populations. *Mugilogobius* from Siberut and Enggano Islands has a sequence divergence of 12.4-17.3% compared to other *Mugilogobius* species, which supported their differences at the species level in the same genera. This study contributed to presenting the first molecular data of *Mugiologius* that can be used as a sequence reference for identification and the sequences became the genetic richness data of fish in the small and outermost islands of Sumatra, Indonesia (Siberut and Enggano Islands).

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