Freshwater fish diversity from Siberut Island, a small island in the western part of Sumatra, Indonesia

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Abstract. *Roesma DI, Tjong DH, Aidil DR, Prawira FDL, Saputra A. 2024. Freshwater fish diversity from Siberut Island, a small island in the western part of Sumatra, Indonesia. Biodiversitas 25: 836-845.* Indonesia is an archipelagic country with thousands of islands, including small islands with a variety of biodiversity, such as freshwater fish. Data and information on the fish biodiversity of small islands in the western part of Sumatra are limited, including Siberut Island. A study on freshwater fish diversity in Siberut Island was carried out using morphological and molecular methods. The study was conducted on seven rivers in the North and South Siberut Districts, Siberut Island. The study obtained 676 individuals, including 34 species, 26 genera, 16 families, and 10 orders. Oxudecidae is the family with the largest species (8), and *Rasbora jacobsoni* has the highest number of individuals (341). Species richness ranged from 2 to 18 species per site and was highest in the Srilanggai River. Siberut Island has low-moderate freshwater fish diversity. As many as 90 DNA barcodes of individuals from 34 species have been submitted to the Barcode of Life Data System. Freshwater fish groups do not receive enough attention from local communities and the government because of the richness of marine fish on Siberut Island. Apart from functioning as evidence of species richness, the results of this study are also useful for local communities' education.

Keywords: Barcode of life data system, DNA barcode, North Siberut, Oxudercidae, South Siberut

INTRODUCTION

Indonesia has the largest archipelago in the world and the second-highest biodiversity (Tumonggor et al. 2013; von Rintelen et al. 2017). Indonesia is an archipelagic country with the highest freshwater fish richness, with around 1,300 species (Nguyen and de Silva 2006). Apart from large islands, Indonesia has small and outermost islands (Pulau-Pulau Kecil Terluar, abbreviated as PPKT). The study of freshwater fish biodiversity on large islands has been a focus and concern of researchers for a long time (Hadiaty and Kottelat 2009; Roesma 2011; Allen et al. 2014; Keith et al. 2014; Ng et al. 2015; Roesma et al. 2016; 2017; 2018; 2019a; 2019b; 2020). However, this differs from other small and outermost islands in Indonesia. Small island ecosystems tend to have high levels of endemic species but have environments with high risks, and the island's carrying capacity (availability of freshwater and food) is limited (Kementerian Koordinator Bidang Kemaritiman dan Investasi 2021).

Moreover, data and information on flora and fauna biodiversity in several small islands is limited. Although biodiversity data in Indonesia has been reported through websites and research studies, the existing data is mainly based on a morphological method (Schmidt et al. 2017). Morphological methods must be supported by molecular methods to provide accurate data because some species show the same morphology but are genetically different and vice versa (Roesma 2011). Limited road access and funding make obtaining data and information on

biodiversity on small and outermost islands difficult. The small islands region has become a national development priority, which can pave the way for researchers to provide biodiversity data and information on these islands (Kementerian Koordinator Bidang Kemaritiman dan Investasi 2021).

Several studies have been carried out by the Indonesian Institute of Sciences (LIPI) since 2015 on expeditions to many small islands in Indonesia (15 islands), the main focus being the central and eastern regions of Indonesia (Hadiaty and Kottelat 2009; Allen et al. 2014; Keith et al. 2014; Allen et al. 2015). Those studies revealed many new species and several species that could potentially be new species; further molecular studies were needed to prove those findings (Hadiaty and Kottelat 2009; Allen et al. 2014; Keith et al. 2014; Allen et al. 2015). Meanwhile, research on the small islands in the western part of Indonesia (Sumatra Islands), including Siberut Island, is rarely conducted. Siberut Island has a high density of endemic species with relatively different biodiversity than the surrounding islands because it has been isolated from Sundaland for the last 500,000 years (Voris 2000). Despite the flora and fauna in Siberut Island having unique biodiversity, the freshwater fish group receives less attention than other taxa.

Only two studies reported the freshwater fish in several rivers on Siberut Island. Goistepan (2016) found 16 freshwater fish species using morphological data in the Siluitung River, North Siberut. Roesma et al. (2022) reported 13 freshwater fish species from several rivers in

South Siberut using the DNA barcoding method, and some species can potentially become new species. The results of Roesma's study are the basis for further study regarding the possibility of other new species that have not yet been revealed that impact recording data of the richness of freshwater fish globally in Siberut Island. Further surveys must conducted using morphological and molecular methods. This study aims to determine the freshwater fish diversity from Siberut Island and its characteristics. The resulting biodiversity data will provide knowledge about the freshwater fish species on Siberut Island to the local community and help determine appropriate efforts to implement policies for managing freshwater fish biodiversity, especially on Siberut Island.

MATERIALS AND METHODS

Study area

Biodiversity surveys of freshwater fish on small islands in the western part of Sumatra (Siberut Island) were conducted using the direct samples collection method. Fish samples were collected in the South Siberut (October 2021) (Roesma et al. 2022) and North Siberut (August 2023) Districts, Siberut Island, Mentawai Islands. Road access from one district to another on Siberut Island can only be reached by sea using boats, and sampling can not be conducted in all districts because of some limited road access. The selection of river locations was based on rivers representing villages in North and South Siberut districts. Sample collection was carried out in three rivers in the South Siberut District: Siri Tengah River (Maileppet Village), Kulu Kubuk Waterfall (Madobag Village), and Majobulu/Majububiu River (Muntei Village) and four rivers in North Siberut District: Sirilogui River (Sirilogui Village), Srilanggai River (Malancan Village), Purodottan River (Muara Sikabaluan Village), and Sikabaluan River (Muara Sikabaluan Village). The sampling location is shown in Figure 1. Only two rivers can be reached by land access (the Siri Tengah River and the Majobulu/Majububiu River), and other rivers can only be reached by sea using boats.

Procedures

Sample collection

Sample collection was conducted using the direct sample collection method. Sample collection was carried out with the help of fishermen using fishing nets (small net and large net). Sampling also used mentawai's special fishing nets with rattan frames and a net of corn thread known as Subba (Roesma et al. 2022). Collecting samples at one location was carried out for 1-1.5 hours. The morphological characteristics of body color, fin color, body size, and others were recorded for additional data. Individual samples were photographed, labeled, and preserved in 10% formalin. Liver tissue samples from several individuals were taken for molecular analysis and stored in ethanol absolute. Ecological data from several rivers were recorded (pH, water and air temperature, width and depth of river, substrate, and surrounding habitat). Samples preserved in formalin were washed in running water and transferred into a 70% alcohol solution for long-term storage in the Genetic and Biomolecular Laboratory, Biology Department, Andalas University. Morphological identification refers to identification books (Kottelat 2013; Nelson et al. 2016) and molecular identification using DNA sequencing.
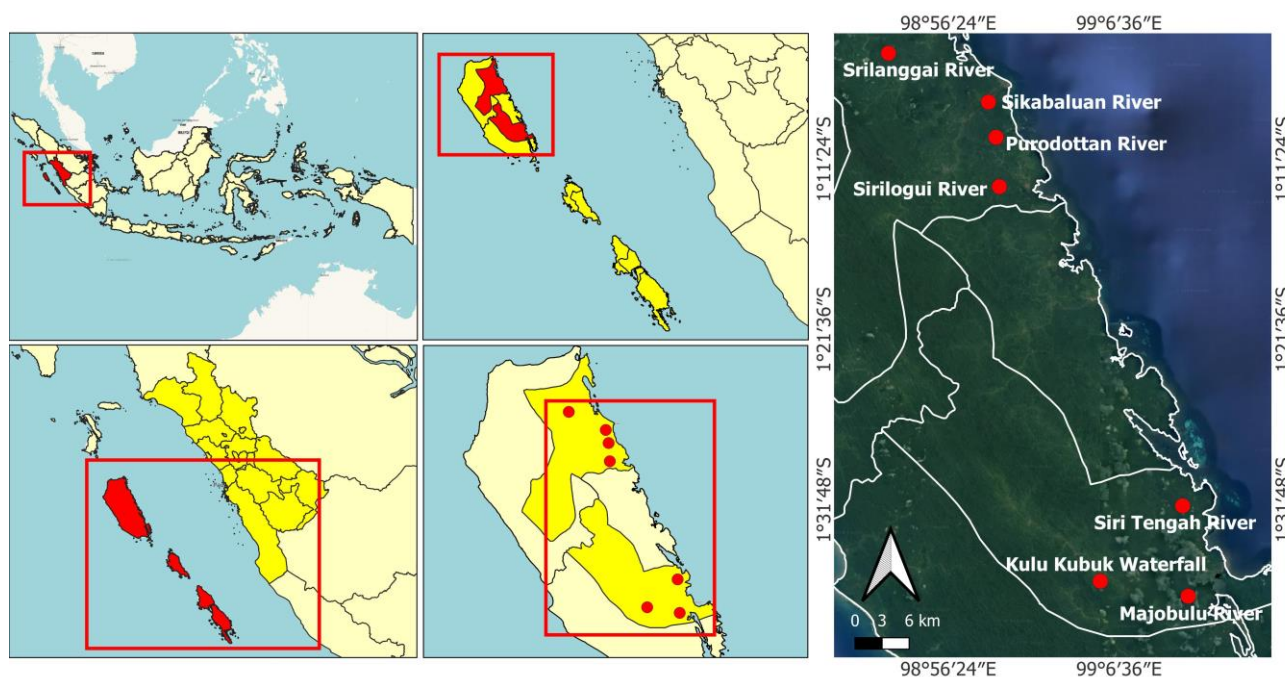

Figure 1. Map of sampling location of freshwater fish in Siberut Island, Mentawai Islands, Indonesia

DNA extraction, DNA amplification, and DNA sequencing

Tissue samples were isolated using the Extragene Gene All DNA Isolation mini KIT based on company protocol. The DNA samples were amplified using Cytochrome Oxidase I (COI) universal primer: FISH primers FI and R1. The solution composition of amplification following Roesma et al. (2022). The amplification procedure was conducted on one cycle of initial denaturation (95°C for 2 min) and continued for 35 cycles of denaturation (94°C for 0.5 min), annealing (54°C for 0.5 min), and extension (72°C for 1 min) and the one final cycle for final extension (72°C for 10 min) (Roesma et al. 2022). PCR products were stored at 4°C for long-term storage. The PCR products were purified and sent to First Base Malaysia for DNA sequencing.

Data analysis

Biodiversity indices analysis

Biodiversity indices analysis of fish species on Siberut Island was calculated using several index formulas, including Diversity Index (H') (Shannon and Wiener 1949), Species Richness Index (R) (Magurran 1988), Dominance Index (C) (Odum 1993), and Evenness Index (E) (Ludwig and Reynolds 1988).

Molecular analysis

The result of DNA sequencing was contig using DNASTAR software, and all sequences were aligned using Aliview (Reguant et al. 2020). Bioedit was used to check and remove ambiguous bases on aligned sequences (Hall 2011). Species identification from DNA sequences was conducted using the DNA barcoding method (COI gene). The similarity of sequences was checked with reference sequences in GenBank, NCBI database using the Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi). We used a 90-100% similarity threshold to assign samples to species level (Kartavtsev et al. 2014). All sequences were also translated into amino acids to check the reading frame. The fasta sequences were registered into the Barcode of Life Data (BOLD) System to generate DNA Barcode species. The BOLD System will match sequences with a database based on the Barcode Index Number (BIN) (Kartavtsev 2018). Sequences 100% similar to the reference sequences in the BOLD System will have the same BIN code. The registered DNA Barcode can be accessed on the BOLD System and Genbank and used as a reference to identify species (Purty and Chatterjee 2016).

RESULTS AND DISCUSSION

Fish composition

Freshwater fish biodiversity surveys have been successfully carried out in seven rivers from two districts (South Siberut and North Siberut) on Siberut Island. A total of 676 individual fish were obtained from 34 species, 26 genera, 16 families, and 10 orders. The list of freshwater fish species obtained is shown in Table 1. Based on the category of the IUCN Red List conservation status, the fish found were divided into three categories: Least Concern (LC) for 32 species (94.12%), Vulnerable (VU) for one species (2.94%%), and data deficient (DD) for one species

(2.94%). Regarding fish potential, 17 species (50%) have potential as consumption fish, 27 species (79.41%) have potential as ornamental fish, and 7 species (20,59%) have potential as consumption and ornamental fish (Table 1). More than 70% of the fish have the potential to be ornamental fish due to the many Goby fish that were obtained. Goby is the fish group with economic value, especially as a freshwater ornamental fish commodity (Gani et al. 2020). Based on their origin, 7 fish species are introduced (Table 1). Based on the morphological identification, three species are assigned to the species level as close form (cf. species), and 6 can only be assigned to the genus level. These 6 species obtained are small and very similar to closely related species; therefore, these species are only classified to the genus level to avoid misidentification.

Among the 34 species, 13 found in the South Siberut District have been reported by the author in the previous study (Roesma et al. 2022), and 27 species were found by this study in the North Siberut District. Thus, the total number of species obtained on Siberut Island was 34. Fish species found in North Siberut and South Siberut Districts are quite different. Only 8 of the same type species were found in the two districts (*T. trichopterus, Rasbora jacobsoni, Eleotris fusca, Giurus margaritacea, Redigobius bikolanus, Gobiopterus* cf. *brachypterus, Butis* sp., and *O. niloticus*). A previous study (Goistepan 2016) found 16 freshwater fish species in the Siluitung River, Sirilogui Village, North Siberut. No similar fish species were found between this and previous studies (Goistepan 2016). Differences in sampling locations, time, and fishing gear between studies are thought to influence the fish species found. Manangkalangi (2018) reported that sampling locations, variations of fishing gear, and collection times could affect variations in fish species composition. Collection samples using various fishing gear and extended fishing periods resulted in a higher fish diversity possibility.

The majority of species (18) were found in the Srilanggai River, Malancan Village, North Siberut District. Meanwhile, in other locations, only 10 species were found in the Purodottan and Siri Tengah Rivers, 9 species in the Sirilogui and Sikabaluan Rivers, 6 species in the Kulu Kubuk Waterfall, and the lowest species (two species) were found in the Mojobulu River. The highest number of individual fish was found in the Sikabaluan River (236 individuals), followed by the Srilanggai River (149 individuals) and the Kulu Kubuk Waterfall (126 individuals). Meanwhile, only 20 to 68 individuals were found in other locations. Many species were found in the Srilanggai River because they relate to the time and sampling locations. Sample collection in the Srilanggai River was conducted when flooding upstream occurred. Therefore, sampling can only happen in the river downstream. Usually, many fish species are found downstream of rivers or rivers around mangrove areas. Besides, the surrounding area in the Srilanggai River is not inhabited by the local community, so the fish population is well maintained. Freshwater fishes generally decline with increasing distance from the sea (McDowall and Taylor 2000; Muhtadi et al. 2022). Further downstream, the width and depth of the river increase, so the size and heterogeneity of the habitat for fish increase

compared to upstream. Hadiaty (2016) stated that the downstream part of the river has a higher species abundance because of more varied vegetation and warmer temperatures, thus providing broader habitat niches for the fish.

Table 1 shows that no species of the same type are present in all site locations. *R. jacobsoni, G. margaritacea,* and *E. fusca* were found in five locations, and *R. bikolanus* was found in four locations. Meanwhile, other species were only present in three, two, or one location. *R. jacobsoni* is the species with the highest number of individuals (341), followed by *T. tricopterus* (71), *G. margaritacea* (49), and *E. fusca* (48). *R. jacobsoni* is a native species in Indonesia that is only present (endemic) on Sumatra Island based on geographical range data in IUCN (Lumbantobing 2020); this fish is often found in rivers with rocks and sandy soil substrates, such as the Srilanggai and Sikabaluan Rivers. *T. trichopterus* is a fish introduced by migrant communities from outside Siberut Island, and it has become a consumed fish by communities around Kulu Kubuk Waterfall, Madobag Village. Hadiaty and Sauri (2017) reported, *T. trichopterus* was introduced to Enggano Island by transmigrant communities from Java. The fish species on Siberut Island are almost similar to species on another small island in the western part of Sumatra, namely Enggano Island; eight of the same species are found on Siberut Island and Enggano Island (Hadiaty and Sauri 2017) (*Anguilla bicolor*, *M. brachyurus*, *Anabas testudineus*, *T. trichopterus*, *Ophiocara porocephala*, *E. fusca*, *G. margaritacea*, and *R. bikolanus*).

Gobiiformes is the order with the highest number of species (18) among other orders. Most of the Gobiiformes members are small species that live in marine, brackish, and freshwater. Gobiiformes are an order of fish that includes gobies and their relatives, previously considered a suborder of Perciformes (Nelson 2006). However, the morphological and molecular studies (Agorreta et al. 2013; Thacker et al. 2015; Betancur et al. 2017) supported the Gobiiformes becoming a different order from Perciformes. Gobiiformes generally live in marine and brackish water, and only around 10% inhabit freshwater (Thacker et al. 2015). Nelson et al. (2016) rearranged family members within the Gobiformes compared to the previous classification in Nelson (2006). The most significant changes occurred in the Oxudercidae and Gobiidae, which are split into two families. Therefore, based on the revisions, Nelson et al. (2016) determined that Gobiiformes consists of 8 families. Meanwhile, van der Laan (2018) determined 7 families of Gobiiformes like Nelson et al. (2016) except for Thalasseleotrididae.

The Oxudercidae (Gobiiformes) is the family with the most species (8 species), followed by the Eleotridae (5 species) (Table 1). Oxudercidae is one of the families with the most species within Gobiiformes after Gobiidae (Nelson 2006; Nelson et al. 2016). Most of the Oxudercidae species in this study were previously members of Gobiidae (Nelson 2006), but taxonomic changes by Nelson et al. (2016) reclassified them into Oxudercidae. Currently, the Oxudercidae contain the species formerly classified as the Gobiidae subfamilies (Gobiinae, Amblyopinae, Gobionellinae, Oxudercinae, and Sicydiinae) (Nelson et al. 2016). Meanwhile, Gobiidae currently consists of species previously members of Kraemeriidae, Microdesmidae, Ptereleotridae, and Schindleriidae (Nelson et al. 2016). Hadiaty and Sauri

(2017) reported that the Gobiidae are the dominant family on Enggano Island. Goistepan (2016) reported Gobiidae as one of the dominant families in the Siluitung River, North Siberut. Based on the current taxonomic classification, the species belonging to the Gobiidae in the Enggano and Siberut Islands has been included in the Oxudercidae.

Generally, fish species in the western part of Indonesia are dominated by the Cyprinidae (Hadiaty and Sauri 2014; Nuryanto et al. 2016; Hadiaty and Sauri 2017; Pramanik et al. 2017; Maghfiriadi et al. 2019; Suryaningsih et al. 2020), and eastern part of Indonesia are dominated by Gobiidae (currently most of them belong to Oxudercidae) (Hadiaty and Kottelat 2009; Hadiaty 2018; Hadiaty et al. 2018; Rusdianto and Sauri 2021). However, this study revealed the dominant species belongs to the Oxudercidae. Oxudercidae became the dominant family, allegedly related to the geographical history of Siberut Island, which ever joined with Sundaland but was separated for a long time (more than 500,000 years ago) (Voris 2000). This separation resulted in the isolation of Siberut Island, located directly adjacent to the sea and ocean. Therefore, the freshwater fish found are mostly Gobiidae/Oxudercidae, which generally live in the sea and freshwater (Agorreta et al. 2013). Most goby species live in the sea, and the goby species that live in freshwater today result from long-term adaptation (Hadiaty and Sauri 2017). Although not the dominant family, 3 species of Cyprinidae (*B. banksi, R. jacobsoni,* and *R. vulcanus*) are also found on Siberut Island. These species are only distributed in some regions in Indonesia (Sumatra and Java). The presence of Cyprinidae species proves that Siberut Island previously ever joined with Sundaland, as Voris (2000) explained.

Sample collection in the North Siberut District area can only conducted in the middle to downstream part of the river because there is flooding in the upstream part, making it impossible to continue collecting samples using a boat. Among all the sampling locations, the Kulu Kubuk Waterfall and the Siri Tengah River have better environmental conditions than other rivers. The river around Kulu Kubuk Waterfall is a source of clean water for the people of Madobag Village. The Kulu Kubuk Waterfall is located upstream, and the Siri Tengah River, which is closer to the Maileppet estuary, has the same river type: rocky, gravel, and sand substrates, water pH ranges from 6-7, water temperature ranges from 22-24°C with moderate current speed, and have a width of 2-4 m and a depth of 0.3-1 m. Meanwhile, other rivers, such as Sirilogui, Srilanggai, and Sikabaluan Rivers, have the same river type: sandy soil substrates, murky brownish rivers, pH ranges from 5-6, and water temperature ranges from 25-28°C. The rivers are large, closer to the downstream, with a width of 4-8 m and a depth of 1-2 m. The rain during sample collection in the North Siberut District influenced the river condition, which became murky due to flooding upstream, which impacted the downstream. The Mojobulu and Purodottan rivers are smaller rivers with a width of 1-1.5 m and a depth of 0.3-0.7 m. The Purodottan River has a habitat type with rocky and sandy soil substrates, moderate current speed, and less clear water. Meanwhile, the Mojobulu River has a muddy soil substrate with reddish-brown water because the river is located around a swamp with many sago trees cut down around the river.

Table 1. Species list of freshwater fish in Siberut Island, West Sumatra, Indonesia

Order	Family	Scientific name	Indonesian name	Local name	IUCN Status	Fish potential			Location and individual numbers						
						Ornamental	Food	Introduction	ST	KK	MB	SR	PR	SL	SK
Anabantiformes	Anabantidae	*Anabas testudineus*	Ikan Puyu		LC	√	√		-	-	-	1	-	-	-
	Osphronemidae	*Trichopodus trichopterus*	Ikan Sepat	Sapek	LC		√	√	-	59	-	-	1	-	11
Anguilliformes	Anguillidae	*Anguilla bicolor*	Ikan Panjang	Lojo	NT		√		-	-	-	-	-	-	2
Beloniformes	Adrianichthyidae	*Oryzias javanicus*	Ikan Medaka		LC	√			4	-	-	-	-	-	-
		Oryzias sp.	Ikan Medaka		LC	√			-	-	-	-	3	-	-
Cichliformes	Cichlidae	*Oreochromis niloticus*	Ikan Nila	Togoigoi	LC		√	√	-	-	1	-	-	-	1
Cypriniformes	Cyprinidae	*Barbodes banksi*	Ikan Spotted barb	Mututek	LC	√	√		-	8	-	-	-	-	-
	Danionidae	*Rasbora jacobsoni*	Ikan Wader	Laitak	LC	√	√		-	23	50	-	5	63	200
		Rasbora vulcanus	Ikan Bada	Laitak Tenga	LC	√	√		8	19	-	-	-	-	-
Gobiiformes	Butidae	*Butis gymnopomus*	Ikan Belosoh	Poroiboi	LC	√	√		-	-	-	4	-	10	-
		Butis sp.	Ikan Belosoh	Nung-nung	LC	√	√		1	-	-	2	-	4	-
		Ophiocara porocephala	Ikan Belosoh	Poroi-poroi/Gai-gai	LC	√	√		-	-	-	-	1	-	-
	Eleotridae	*Eleotris fusca*	Ikan Belosoh	Gai-gai/pucek	LC	√	√		14	7	-	7	3	17	-
		Eleotris melanosoma	Ikan Belosoh	Gai-gai/pucek	LC	√	√		-	-	-	1	-	-	1
		Hypseleotris compressa	Ikan Gudgeon	Tai rok so	LC	√			1	-	-	-	-	-	-
		Hypseleotris sp.	Ikan Gudgeon	Jumbairat	LC	√			-	-	-	-	2	1	4
		Giurus margaritacea	Ikan Snackehead	Poroi-poroi/Gai-gai	LC	√	√		12	-	-	5	1	18	13
	Gobiidae	*Glossogobius tenuiformis*	Ikan Nyereh	Poroiboi	LC	√		√	-	-	-	-	-	5	-
		Glossogobius sp.	Ikan Nyereh	Poroiboi	LC	√			-	-	-	-	-	1	-
	Oxudercidae	*Mugilogobius mertoni*	Ikan Saffron	poncot	LC	√			-	-	-	-	2	4	-
		Mugilogobius rambaiae	Ikan Saffron	poncot	LC	√			-	-	-	-	-	2	-
		Oligolepis acutipennis	Ikan Sharptail goby	Poroiboi	LC	√			-	-	-	-	-	2	-
		Taenioides cirratus	Ikan Dragon goby	Lojo	LC	√			-	-	-	-	-	9	-
		Gobiopterus cf. *brachypterus*	Ikan Rinuak		DD		√		19	10	-	-	-	1	-
		Periophthalmus argentilineatus	Ikan Gelodok		LC	√	√		1	-	-	-	-	-	-
		Redigobius bikolanus	Ikan Goby		LC	√			2	-	-	3	1	1	-
		Stiphodon semoni	Ikan Goby		LC	√			6	-	-	-	-	-	-
Perciformes	Ambassidae	*Ambassis* sp.	Ikan Seriding	Sanggok	LC	√			-	-	-	1	-	4	-
	Scatophagidae	*Scatophagus argus*	Ikan Kiper		LC		√		-	-	-	-	-	1	-
Scorpaeniformes	Scorpaenidae	*Vespicula* cf. *trachinoides*	Ikan Mangrove	Nampot	LC				-	-	-	-	-	-	3
Siluriformes	Clariidae	*Clarias* cf. *fuscus*	Ikan Lele	Lele	LC		√	√	-	-	-	-	1	-	1
Syngnathiformes	Syngnathidae	*Hippichthys heptagonus*	Ikan Pipefish	Pipingok Laiboyak	LC	√		√	-	-	-	2	-	-	-
		Microphis brachyurus	Ikan Pipefish	Pipingok Laiboyak	LC	√		√	-	-	-	-	-	4	-
		Microphis sp.	Ikan Pipefish	Pipingok Laiboyak	LC	√		√	-	-	-	-	-	2	-
Total									68	126	51	26	20	149	236

Note: Location: ST: Siri Tengah River; KK: Kulu Kubuk Waterfall; MB: Mojobulu River; SR: Sirilogui River; PR: Purodottan River; SL: Srilanggai River; SK: Sikabaluan River

The species number and abundance of fish individuals are higher in the North Siberut rivers than in the South Siberut rivers. It is related to the distance of the river from the sea and the presence of the local community. The sampling locations in the South Siberut Rivers (Siri Tengah River and Mojobulu River) are rivers close to community areas and used for various purposes. It is thought to have affected the low species richness because community activities have polluted the river. Besides, the Mojobulu River's low water quality also caused fewer species. Hadiaty and Sauri (2017) reported that the species number and abundance of fish individuals are lower in rivers in the western region than in the central and eastern regions of Enggano Island because this area was inhabited by transmigrants, who used rivers for various needs, including for catching fish because of the location far from the coast. Meanwhile, rivers in the central and eastern regions are relatively close to the coast, and local communities prefer to fish in the sea for their needs. Thus, the fish population in the rivers is well maintained. Hasan et al. (2022) reported that rivers close to shrimp ponds and settlements have lower diversity because they are polluted by human activities, which have changed the natural function of the river.

Fish diversity indices

Analysis of the fish diversity indices on Siberut Island includes the Diversity Index (H'), Species Richness Index (R), Evenness Index (E), and Dominance Index (C) at each location is shown in Table 2. Diversity Index (H') values ranged from 0.1 to 2.13 for all locations. Based on the category, the diversity index value H' is low if it is less than 1 and high if it is more than 3 (Magurran and McGill 2011). The values obtained show that no locations have high species diversity. The Purodottan River is a location that has the highest diversity index among other locations (H' = 2.13) with a moderate diversity category. Meanwhile, the Mojobulu and Sikabaluan Rivers are the locations that have the lowest diversity index (H' = 0.10 and 0.68) with a low diversity category. The Purodottan River has a higher index than other rivers because it has the second-highest number of species and has an even distribution of individuals for each species. The diversity index increases if many species are found, and the abundance of individuals for each species is more evenly distributed and vice versa (Sihombing et al. 2017; Wahyuningsih et al. 2022).

The species richness index shows the number of species richness in a location. The richness index value ranges from 0.25 to 3.59 for all locations. Based on the category, the Richness Index (R) value is low if it is less than 2.5 and high if it is more than 4. The values obtained show that no

locations have high species richness. The Srilanggai is a river that has the highest richness index among other rivers (R = 3.40) with a moderate species richness category. Meanwhile, the Mojobulu and Siri Tengah rivers have low richness index values (R = 0.25 and 1.03), while others have moderate species richness index. The Srilanggai River has a higher species richness than the others, allegedly because of heterogeneous habitat conditions and sampling closer to the downstream of the river. Walrath et al. (2016) and Huang et al. (2019) stated that homogeneous habitats will cause a decrease in species richness and diversity compared to heterogeneous habitats.

The Evenness Index (E) is the evenness of the number of individuals of species for all species in a location. The evenness index value ranges from 0.14 to 0.92 for all locations. Based on the category, if the evenness index value is close to 1, the number of individual fish is evenly distributed in each species and vice versa. The Purodottan River has the highest evenness index among other locations (E = 0.92). It shows that the number of individual species is almost evenly distributed for all species at Purodottan River. Meanwhile, the Mojobulu and Sikabaluan rivers have the lowest evenness index (E = 0.14 and 0.31). The Dominance Index (C) shows the dominant species based on the number of individuals in a location. Dominance Index (C) values ranged from 0.14 to 0.96 for all locations. Based on the value category, if the Dominance Index (C) value is close to 1, it shows the presence of dominant species and vice versa. The Mojobulu River is the location that has the highest dominance index among other locations (C = 0.96). It shows the presence of dominant species in the Mojobulu River. Meanwhile, the Purodottan River is the location that has the lowest Dominance Index value (C = 0.14) and shows no species dominant.

The evenness index value is inversely proportional to the dominance index; if uniformity is high, the dominance will be low, and vice versa (Hossain et al. 2012). The Purodottan River has an almost uniform number of individuals in each species, so no dominant species exists. Meanwhile, the Mojobulu River has a low number of species, and the number of individuals is not uniform, so there is a dominant species. This occurred because a sample collection in the Mojobulu River was conducted briefly; therefore, only a few species and individuals were found. The Mojobulu River also has different water conditions from other rivers, with muddy soil substrates and reddish brown water due to the river being located around a swamp, and there is sago tree waste. Rizwan et al. (2017) and Dewi et al. (2019) stated that higher diversity and low dominance indicate stable ecosystem conditions and vice versa.

Table 2. Freshwater fish biodiversity indices on Siberut Island, West Sumatra, Indonesia

Index	Location						
	Siri Tengah River	Kulu Kubuk Waterfall	Mojobulu River	Sirilogui River	Purodottan River	Srilanggai River	Sikabaluan River
Diversity Index (H')	1.91	1.49	0.10	1.99	2.13	2.06	0.68
Dominance Index (C')	0.20	0.29	0.96	0.16	0.14	0.22	0.72
Species Evenness Index (E)	0.83	0.83	0.14	0.90	0.92	0.71	0.31
Species Richness Index (R)	2.13	1.03	0.25	2.46	3.00	3.40	1.46

Molecular analysis

Moreover, 90 individual freshwater fish on Siberut Island have been sequenced using universal primers for the COI gene. The total length of nucleotide bases obtained from sequencing is around 650-700 bp. The BLAST analysis results of DNA sequences have similarity values ranging from 84% to 100% with reference sequences in GenBank, NCBI. The similarity value shows the similarity of the species' DNA with reference DNA sequences for determining the species' taxonomy. Based on the results, a total of 34 species were identified. Among the 34 species, six are assigned to genus level (*Oryzias* sp., *Butis* sp., *Hypseleotris* sp., *Glossogobius* sp., *Ambassis* sp., and *Microphis* sp.) because they had a genetic distance of 11%-16% with reference sequences for each species in the same genus. According to Kartavtsev et al. (2014), the genetic distance values range from 11% to 16%, indicating sequence differences for different species in the same genus. Based on these values, the six fish species found on Siberut Island have high sequence divergences with the reference sequences in the same species. *Ambassis* sp., *Glossogobius* sp., and *Butis* sp. have 13%-15% genetic distances with some species for each genus. For example, *Butis* sp. has a genetic distance of 13% with *Butis butis*, 14% with *Butis amboiensis*, 15% with *Butis koilomatodon*, and 16% with *Butis humeralis* in Genbank, NCBI. Meanwhile, the genetic distance value for the same species ranges from 0% to 10% (Kartavtsev et al. 2014). Therefore, sequences are only assigned to the genus level to avoid taxonomic errors at the species level. The high nucleotide base variations showed the potential for new species.

Three species were assigned as cf. species, including *Vespicula* cf. *trachinoides*, *Clarias* cf. *fuscus,* and *Gobiopterus* cf. *brachypterus*. *Vespicula* cf. *trachinoides* is morphologically similar to *Vespicula trachinoides*, but the *Vespicula* sequence as references has not yet been reported in Genbank, NCBI and Bold System. *Vespicula* cf. *trachinoides* only has a similarity of 83% with the sequence in Genbank, NCBI. *Gobiopterus* cf. *brachypterus* is morphologically similar to *Gobiopterus brachypterus* but has a high genetic distance from *Gobiopterus* species. The previous studies (Roesma et al. 2020; 2022) reported that *Gobiopterus* cf. *brachypterus* has potential as a new species because of the high genetic distance from *G. brachypterus*. *Clarias* cf. *fuscus* has the closest genetic distance to *Clarias fuscus* (8.37%). Based on Kartavtsev et al. (2014), the value shows sequence differences at the population level within the same species. However, *C. fuscus* is a native species from Hong Kong based on the IUCN data (Chui et al. 2021), and there is no data on its presence in Sumatra Island, especially Siberut Island. The limited number of individuals and the difficulty distinguishing morphological characters from other *Clarias* make it assigned as *Clarias* cf. *fuscus*. Among all species, eight species do not yet have DNA sequences registered in the Genbank, NCBI and BOLD System, namely *Oryzias* sp., *Butis* sp., *Gobiopterus* cf. *brachypterus*, *Hypseleotris* sp., *Glossogobius* sp., *Taenioides cirratus*, *Ambassis* sp., and *Vespicula* cf. *trachinoides*. Therefore, all sequences are registered to the BOLD system and processed to obtain a BIN code.

The taxonomic classification species only assigned up to the genus level has also previously been reported in various studies (Deichmann et al. 2017; Iyiola et al. 2018; Nneji et al. 2019). Ali et al. (2020) reported four species have similarities only up to the genus level with reference sequence in Genbank, NCBI and are only assigned as "sp.". Hinchliff and Smith (2014) stated that the classification of a species is only assigned up to the genus level due to the lack or absence of reference data for target species sequences in the repository database. Many studies have reported that a lack of database species (DNA barcodes) can influence the accuracy of species identification (Deichmann et al. 2017). DNA barcoding has effectively differentiated organisms up to the species level (Lakra et al. 2016; van der Walt et al. 2017; Bingpeng et al. 2018; Ali et al. 2019).

The results of morphological and molecular identification have proven that the presence of species is morphologically similar but genetically different, such as in six species that were only assigned to the genus level. Roesma (2011) states that some species are morphologically identical but genetically different and vice versa. This study showed morphological and molecular approaches are needed to avoid misidentification. Thus, the availability of freshwater fish DNA barcodes registered in the BOLD System and Genbank, NCBI becomes critical to species identification (Roesma et al. 2021a; 2021b; 2023). The available data will contribute as basic data to future studies, especially for species with potential as new species. Freshwater fish groups do not receive enough attention from local communities and the government because of the richness of marine water fish on Siberut Island. Apart from functioning as evidence of species richness, the results of this study are also useful for local communities' education. Species richness and DNA barcoding data will help the government make policies that pay attention to the survival of freshwater fish on small islands, especially Siberut Island.

In conclusion, the study obtained 676 individuals from 34 species, 26 genera, 16 families, and 10 orders. Oxudercide is the family with the most species (8 species), and *Rasbora jacobsoni* has the highest number of individuals (341). Species richness ranged from 2 to 18 species per site and was highest in the Srilanggai River, Malancan Village. All river populations have low-moderate species diversity, with the Purodottan River having the highest diversity. Furthermore, 90 DNA barcodes of freshwater fish have been submitted to the BOLD System. Eight species are the new report for fish in Siberut Island that previously did not have DNA barcodes registered in the BOLD system.

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