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DNA barcoding of freshwater fish in Siberut Island, Mentawai Archipelago, Indonesia

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Abstract. *Roesma DI, Tjong DH, Janra MN, Aidil DR. 2022. DNA barcoding of freshwater fish in Siberut Island, Mentawai Archipelago, Indonesia. Biodiversitas 23: 1795-1806.* Information regarding molecular data of freshwater fish in Siberut Island, Mentawai Archipelago, Indonesia, is not available. This study was conducted to provide DNA barcodes of freshwater fish in Siberut island. The cytochrome oxidase subunit 1 (COI) gene was chosen to make DNA barcodes because it has been used as a tool capable of identifying organisms up to the species level. Specimens were collected directly captured in the three rivers from Siberut Island. A total of 40 individuals were selected from 200 individuals that have been identified morphologically and strengthened by the results of the molecular marker (DNA barcodes) consisting of 13 species, namely *Gobiopterus* cf. *brachypterus* (BOLD: ADF5945), *Eleotris fusca* (BOLD: AAF0108), *Butis* cf. *butis* (BOLD: AAL8960), *Stiphodon semoni* (BOLD: ACQ3312), *Giuris margaritacea* (BOLD: ACP9929), *Anabas testudineus* (BOLD: AAO2846), *Rasbora jacobsoni*, *Rasbora vulcanus*, *Barbodes banksi*, *Redigobius* cf. *bikolanus*, *Periophthalmus argentilineatus*, *Hypseleotris* cf. *compressa*, and *Oryzias* cf. *javanicus* (in a queue in the BOLD system). The average Kimura two-Parameter model (K2P) genetic distances within species, genera, families, and orders were 0.16%, 11.4%, 23.2%, and 26.0%, respectively. The interspecific K2P distance was higher than the intraspecific distance. The present study supports that COI Barcodes effectively identify fish up to the species level. Future research is needed, especially to species that may have potential as new species. In contrast, DNA barcodes as genetic information will contribute as the basic data to future studies in the monitoring, conservation, and management of freshwater fish in Siberut Island, Mentawai Islands. Therefore, the creation of DNA barcodes is immediately carried out.

Keywords: COI, DNA barcodes, genetic distances, mentawai archipelago, Siberut island

INTRODUCTION

Mentawai is an archipelago located approximately 150 kilometers off the western coast of Sumatra in Indonesia (Kkp.go.id 2017). Mentawai archipelago consists of 213 islands with four main island groups: Siberut, Sipora, North Pagai, and South Pagai (Kkp.go.id 2017). The Mentawai Islands have been separated from the main island of Sundaland since the mid-Pleistocene period or about 500,000 years ago (Voris 2000; Darmanto and Setyowati 2012). This condition may result in variations of flora and fauna, especially on the Siberut Island.

Siberut is the largest island from the Mentawai Archipelago, and most of its area (190.500 ha) has turned into Siberut National Park. There were approximately 900 vascular plant species, 20 reptile species with one endemic frog species, 134 bird species with one endemic species, and 31 mammal species where are 65% of them endemic species to Siberut Island (TFCA-Sumatera 2016; Kkp.go.id 2007). A total of four endemic Mentawai primate species are found only on Siberut Island, namely the Kloss's gibbon (*Hylobates klossii*), Mentawai macaque (*Macaca pagensis*), Siberut macaque (*Macaca siberu*), Mentawai langur (*Presbytis potenziani*), Siberut langur (*Presbytis siberu*), and pig-tailed langur (*Simias concolor*) with IUCN status as endangered species (Kkp.go.id 2007).

Based on the biodiversity data in Siberut Island, there are still other taxa which in-completed and unrevealed, such as the freshwater fish group. Meanwhile, freshwater fish are an important component of aquatic biodiversity and are critical riverine food webs. Sixteen freshwater fish species were reported in Siluitung River, North Siberut District, Siberut Island (Goistepan 2016). The Rapid Assessment by the Biology team of Andalas University (2020) reported four freshwater fish from Cyprinidae and Gobiidae in South Siberut District, Siberut Island. The species found in South Siberut were different from species in North Siberut. However, the studies have been conducted only from morphological and not confirmed by molecular studies. Morphological studies are considered insufficient to assess the species diversity because species may be genetically distinct yet morphologically similar, and vice versa, such as the study by (Roesma et al. 2018). Therefore, molecular data is required to provide biodiversity data of freshwater fish in Siberut Island.

The mitochondrial DNA cytochrome oxidase subunit 1 (COI) gene is often used as a DNA barcode to identify organisms in various taxa up to the species level (Hebert et al. 2004; Ward et al. 2005; Kartavtsev and Lee 2006; Dawnay et al. 2007). The COI gene has been used as an identification tool because it has a short nucleotide base sequence and a highly conservative region (Hebert et al.

2003). DNA barcoding has been widely used for various studies, including exploring the molecular diversity of Eleotridae (Guimaraes-Costa et al. 2016), new record of *Puntius* in West Sumatra (Roesma et al. 2018), DNA barcoding of freshwater fishes of Indo Myanmar biodiversity hotspot (Barman et al. 2018), fish biodiversity of Saint Peter and Saint Paul's Archipelago (Pinheiro et al. 2020), and revisiting the diversity of *Barbonymus* (Dahruddin et al. 2021). Unfortunately, DNA barcodes of freshwater fish in Siberut Island are not available. In contrast, DNA barcodes as genetic information will contribute as the basic data to future studies in the monitoring, conservation, and management of freshwater fish in the Siberut Island, Mentawai Islands. Therefore, the creation of DNA barcodes is immediately carried out.

MATERIALS AND METHODS

Sample collection

The samples from Siberut Island, Indonesia, were collected using the survey method, and the direct collection was referred to Cailliet et al. (1986). Samples were collected from several rivers in South Siberut (Siri Tengah River, Meileppet Village; Majobulu/Majububiu River, Muntei Village; and Kulu Kubuk Waterfall, Madobag Village) in December 2019 and October 2021 (Figure 1). Sample collection was carried out along the rivers with a depth of about 0.3-1 meters and a river width of 2-3 meters. Sample collection was carried out using fishing nets measuring 3-5 mm (small net) and 10-15 mm (large net). In addition, this activity also used the typical Mentawai fishing gear made of rattan which is rounded and given a net known as Subba. The Subba has varied in size, measuring at 2-15 mm from the bottom to the top. Individual samples of fish caught were taken and stored in plastic. Sample collection was carried out for two days. All individual fish caught were counted, photographed, and recorded morphological characters. The individual samples were stored in the collection box in 4% formalin. Liver tissue samples for DNA isolation were stored in microtubes containing 96% ethanol pro analyst (PA). The collected samples were held in the Laboratory of Genetics and Biomolecular, Biology, FMIPA, Andalas University. Individual samples in formalin were washed using running water for 4-6 hours and transferred to 70% alcohol for long-term storage. Individual samples were identified morphologically using identification books (Kottelat et al. 1993, 2013; Nelson et al. 2016).

DNA extraction, polymerase chain reaction (PCR), and DNA sequencing

DNA isolation was carried out following the KIT Extragene Gene All DNA Mini Kit protocol. The quality of isolated DNA was checked by electrophoresis using 1.2% agarose gel. DNA samples were amplified using Fish F1 (5' TCAACCAACCACAAAGACATTGGCAC 3') forward primer and Fish R1 (5' TAGACTTCTGGGTGGCCAAAG AATCA 3') reverse primer (universal primer) (Ward et al. 2005). PCR amplification for COI in a total volume of 25 µL containing; 11 µL PCR Supermix, three µL DNA isolate, ten µL DDH20, 0.5 µL forward and reverse primer, respectively.

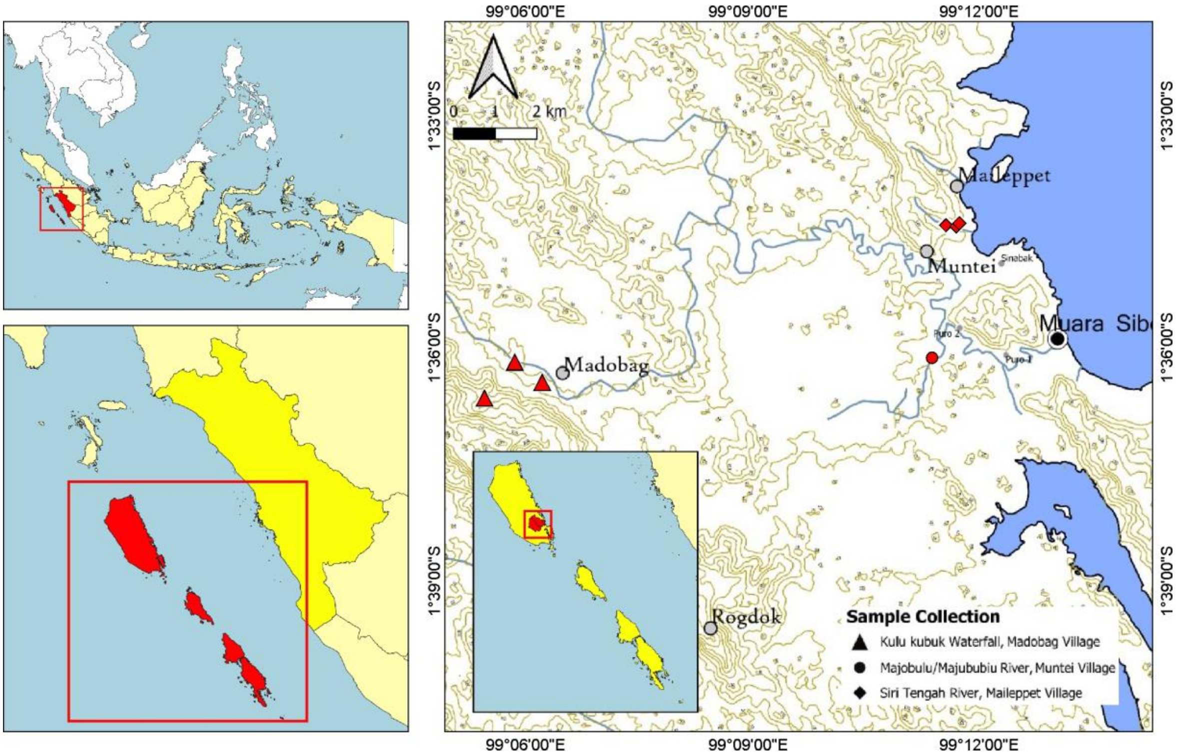

Figure 1. Map of sampling location of freshwater fish in South Siberut, Siberut Island, Mentawai Archipelago, Indonesia

Amplification cycles consisted of; initial denaturation (95°C for 2 min), 35 cycles of denaturation (94°C for 0.5 min), annealing (54°C for 0.5 min), extension (72°C for 1 min), one cycle final extension (72°C for 10 min), and at 4°C for the long-term storage (Ward et al. 2005). The amplified fragments were visualized under ultraviolet (UV) light using 2% agarose gels stained with ethidium bromide. PCR products were purified with Agarose Gel Extraction Kit (Promega) and sent to sequencing at First Base Malaysia.

Data analysis

Forward and reverse COI gene sequences from fish on Siberut Island were assembled and edited using DNASTAR software (Burland 2000). DNA sequences were compared with available data in GenBank for checked sequence similarity through the website http://blast.ncbi.nlm. nih.gov/Blast.cgi. All sequences were arranged with the Clustal X 2.0 (Thompson et al. 1997) and edited with the BIOEDIT Program (Hall 1999). The nucleotide bases were translated to amino acids using the online website http://insilico.ehu.es/translate. The molecular diversity consists of haplotype numbers (h), polymorphism sites (S), haplotype diversity (Hd), and nucleotide diversity (Pi) were calculated with the DNA Sequence Polymorphism (DNA SP) 5.10 program (Rozas et al. 2003). Genetic distances of sequences were computed with molecular evolutionary genetics analysis (MEGA) 6.0 using the Kimura two-parameter (K2P) model with 1000 bootstraps (Tamura et al. 2013). Phylogenetic trees were constructed independently based on Neighbors Joining (NJ) methods with 1000 bootstraps resampling using MEGA 6.0 (Tamura et al. 2013). The aligned sequences were submitted to the Barcode of Life Data (BOLD) System to acquire the DNA barcode and sequence accession code.

RESULTS AND DISCUSSION

Sequences analysis

A total of 200 fish individuals has successfully collected from the three rivers in South Siberut, Siberut Island, Mentawai, Indonesia. Morphological identification was conducted using the identification guide by Kottelat (2013) and Nelson et al. (2016). A total of 40 individuals were selected from 200 individuals that have been identified morphologically for making the DNA barcodes by sequencing of partial 5′ region of the COI mitochondrial gene because this number was sufficient to represent each species from each population. In addition, the results of the sequencing also show that each species found in the Mentawai has very small genetic differences or no differences so that they will have the same DNA barcode. The alignment of all COI sequences obtained 680 bp for analysis. Out of 680 bp were analysed, 262 bp (38.52%) as variable sites, whereas 254 bp (37.35%) of variable sites are parsimony sites, and eight bp (1.17%) are singleton sites. Overall, the composition of nucleotide bases and mean GC content at codon position 1-3 is served in Table 1. AT content and GC mean are 54.60% and 45.40%, respectively. No deletion, insertion, or stop codons, indicating that all fragments are functional mitochondrial COI sequences and not NUMTs (nuclear mitochondrial DNA sequences). The COI gene analysis represented 444 mutations with transitions to transversions (Ti/Tv) bias is R: 2.551. A total number of 24 haplotypes from 40 sequences were obtained. All the COI sequences showed the average haplotype diversity (Hd) was 0.956, and the average nucleotide diversity (Pi) was 0.17444. Hap03 is the dominant haplotype of six *Rasbora vulcanus* individuals from three rivers in South Siberut. *Eleotris fusca* has the highest number of haplotype variations (5).

Fish database and taxonomic notes

The present study preserved the first fish database revealed 13 species in adult stages in the South Siberut, Siberut Island. This study is the new record of freshwater fish in the South Siberut, Siberut Island, Mentawai Archipelago. A total of 40 sequences generated a library of data (DNA Barcodes), including four orders, six families, 12 genera, and 13 species (Table 2). Among 13 species, two species only consist of single samples. Gobiiformes was the order with the most species (8), followed by Cypriniformes (3). While out of six families were recorded, the Oxudercidae being the richest with 46% of the species (6). About 38.5% of the species (5 species) have a global distribution, 23% (3 species) occur in both Southeast Asia, and 38.5% (5 species) are restricted distribution (i.e., Indonesia and some regions in Southeast Asia). Among five species are restricted distribution, two species are endemic in Sumatra Island, one species occur in Indonesia and Malaysia, and two species occur in Australia, Indonesia, and Papua New Guinea. Based on IUCN Red List categories, 12 species were categorized as least Concern (LC), and one species was categorized as data deficient (DD) (Table 2). All COI sequences data as DNA barcodes were submitted to the BOLD System. All COI sequences which meet the quality (<1% N) and length (>500 bp) criteria for Barcode Index Number (BIN) assignment will get the BIN code.

Mean genetic divergence (K2P) analysis

The genetic divergence of each species was calculated based on the Kimura 2 Parameter (K2P) model using MEGA 6.0 (Table 3). The genetic distance represented the taxonomic proximity of sequences. The mean K2P genetic divergence increase in line with the increasing taxonomic levels from within a species (0.16%), to within genus (11.4%), to within families (23.2%), to within orders (26.0%) were reported (Table 3). The results showed low intra-specific K2P genetic divergence levels, with 80% of distances ranging between 0.0% and 2%. The highest mean intra-specific divergence (0.45%) was observed among *E. fusca* and *Gobiopterus* cf. *brachypterus* species, respectively. The mean inter-specific divergence (11.4%) was 70-fold higher than the mean intraspecific divergence (0.16%). The sequence divergences showed the presence of clearly distinct species among the reported species.

Table 1. Nucleotide bases composition, overall and order-wise GC content and GC at codon position 1, 2, and 3 of freshwater fish in South Siberut, Siberut Island, Mentawai Archipelago.

Nucleotide	**All species (%)**	**Order wise %**			
		Cypriniformes	**Gobiiformes**	**Beloniformes**	**Anabantiformes**
G	18.1	17.6	18.4	19.3	17.5
C	27.3	26.3	28.0	27.5	28.8
A	24.8	26.9	23.8	22.6	23.1
T	29.8	29.3	29.9	30.6	30.6
GC	45.4	43.9	46.4	46.8	46.3
GC1	42.9	43	55.9	52.9	42.7
GC2	37.5	32.6	42.6	42.7	40.5
GC3	55.9	56.2	40.7	44.7	55.5

Table 2. The list of freshwater fish was barcoded from South Siberut, Siberut Island, Mentawai Archipelago, Indonesia

Order	**Family**	**Genus**	**Species**	**IUCN status**	**N**	**Vername**	**BIN**
Cypriniformes	Cyprinidae	*Rasbora*	*Rasbora jacobsoni*	LC	6	Laitak	*in a queue in the BOLD system*
Cypriniformes	Cyprinidae	*Rasbora*	*Rasbora vulcanus*	LC	6	Laitak Tenga	*in a queue in the BOLD system*
Cypriniformes	Cyprinidae	*Barbodes*	*Barbodes banksi*	LC	4	Mututek	*in a queue in the BOLD system*
Gobiiformes	Oxudercidae	*Gobiopterus*	*Gobiopterus* cf. *brachypterus*	DD	5		BOLD:ADF5945
Gobiiformes	Oxudercidae	*Periophthalmus*	*Periophthalmus argentilineatus*	LC	1		*in a queue in the BOLD system*
Gobiiformes	Oxudercidae	*Stiphodon*	*Stiphodon semoni*	LC	3		BOLD:ACQ3312
Gobiiformes	Oxudercidae	*Redigobius*	*Redigobius* cf. *bikolanus*	LC	2		*in a queue in the BOLD system*
Gobiiformes	Butidae	*Butis*	*Butis* cf. *butis*	LC	1		BOLD:AAL8960
Gobiiformes	Eleotridae	*Eleotris*	*Eleotris fusca*	LC	5	Gai-gai/pucek	BOLD:AAF0108
Gobiiformes	Eleotridae	*Hypseleotris*	*Hypseleotris* cf. *compressa*	LC	1	Tai rok so	*in a queue in the BOLD system*
Gobiiformes	Eleotridae	*Giuris*	*Giuris margaritacea*	LC	2	Poroi-poroi	BOLD:ACP9929
Beloniformes	Adrianichthyidae	*Oryzias*	*Oryzias* cf. *javanicus*	LC	2		*in a queue in the BOLD system*
Anabantiformes	Anabantidae	*Anabas*	*Anabas testudineus*	LC	2		BOLD:AAO2846

Table 3. Mean genetic distances (Kimura-2 parameter) for the different taxonomic levels (excluding singletons) of the fish specimens collected from South Siberut, Siberut Island, Mentawai Arcchipelago.

Taxonomic level	**Min (%)**	**Mean (%)**	**Max (%)**
Within species	0.0	0.16	0.9
Within genus	11.4	11.4	11.6
Within family	18.9	23.2	28.2
Within order	21.3	26.0	29.9

The highest genetic distance among order appeared between Cypriniformes and Gobiiformes (20.6%-32.6%), and the lowest genetic distance among order seemed between Cypriniformes and Beloniformes (26.7%-28.3%). The highest genetic distances among species appeared between *R. jacobsoni and R. bikolanus* (32.6%). The lowest genetic distance among species seemed between *R. jacobsoni* and *R. vulcanus* (11.4%).

Phylogenetic tree

The phylogenetic tree (NJ) was constructed using the COI sequences, including sequences in the South Siberut, sequences from the Genbank database, and sequences from previous studies as comparison data (Roesma 2011; Roesma et al. 2018, 2020). The reconstruction of the phylogenetic tree showed species grouping based on the taxonomic proximity. Besides, the grouped species corresponds to the genetic distance, which indicates that the species could be effectively distinguished. The phylogenetic tree was constructed into four trees based on the orders group. The phylogenetic tree showed the same species clustered into the same branch, clearly separated from the other species into different clusters. The short branching was observed for some intraspecific individuals due to variable sites among different individuals within the species. All species which belong to the same genus were clustered together (*Rasbora*, *Barbodes*, *Periophthalmus*, *Stiphodon*, *Redigobius*, *Gobiopterus*, *Eleotris*, *Hypseleotris*, *Giuris*, *Butis*, *Anabas*, and *Oryzias*). The result of intra-specific shows the low genetic diversity (less than 1%).

Cypriniformes

The phylogenetic tree of Cypriniformes was constructed by sequences in South Siberut, sequences from previous studies, and the Genbank sequences (Figure 2). The members that occurs in South Siberut only from one family is Cyprinidae. Cyprinidae consist of three species with sequences divergence between them was 13.1%-24.4%. Among the Cyprinidae fishes obtained, *R. vulcanus* and *R. jacobsoni* are the most abundant species. Among the three species of Cyprinidae found (*R. jacobsoni*, *R. vulcanus*, and *B. banksi*), two species, namely *R. jacobsoni* (Weber and de Beaufort 1916) and *R. vulcanus*, are endemic fish on the island of Sumatra. *R. jacobsoni* has the type of locality from Sumatra (Kayu Tanam, Arau, Maninjau Lake, and Singkarak lake), and *R. vulcanus* has the type of locality from Batang Si Joontour, Painan, West Sumatra. *B. banksi* has the type of locality from Malaysia (Borneo and Sarawak) with restricted distribution in Malaysia, Singapore, and Kalimantan, Sumatra (Indonesia). The phylogenetic tree of Cypriniformes showed the monophyletic relationship. The sequence divergences within South Siberut populations of each species from cyrinidae are 0.0%. This value showed the low genetic diversity within species in South Siberut. On the other hand, species of Cyprinidae (*R. jacobsoni*, *R. vulcanus*, and *B. banksi*) from South Siberut populations have high genetic divergence with Sumatra and Genbank populations (4.0%-8.5%). The value shows sequence differences at the sub-species/sibling species level within the same species (Kartavtsev 2011; Kartavtsev 2013).

Rasbora vulcanus in South Siberut has sequence divergence of 4.0%-5.0% with *R. vulcanus* Genbank accession MN342816 and MN342817 (Collins et al. 2012) obtained from ornamental fish retailers in Inggris, Singapore, or New Zealand. However, there is no valid information associated with locality data. Specimens deposited in Raffles Museum of Biodiversity Research. In the same cases, specimens with Genbank accession EF452875 (Mayden et al. 2007), JF915673, and FJ753503 (Britz et al. 2009) no information about their sampling location. The value of high sequence divergences between *R. vulcanus* in South Siberut and Genbank sequences shows high genetic differentiation between them. *Rasbora jacobsoni* in South Siberut has sequence divergences of 4.0%-6.9% with other West Sumatra populations and Genbank sequences. Meanwhile, *R. jacobsoni* from other West Sumatra populations included *R. jacobsoni* Genbank accession MN869499 and MN869806 (Sholihah et al. 2020), and *R. jacobsoni* Batang katik have high genetic similarities with no sequence divergences (0.0%) between them. *Rasbora jacobsoni* in South Siberut has the highest genetic distance (6.9%) with *R. jacobsoni* Genbank accession HM224230 (Tang et al. 2010). *Rasbora jacobsoni* Genbank accession HM224230 was collected from the aquarium trade by Tang. There is no valid information about sampling location.

Rasbora is the most diverse freshwater fish genus of Sundaland and represents an excellent model to explore the evolutionary response of local freshwater biotas to a dynamic geological history (Hubert et al. 2019; Sholihah et al. 2020). However, most *Rasbora* species still suffer taxonomic confusion for decades due to the substantial cryptic diversity level (Hutama et al. 2017; Farhana et al. 2018). The previous study (Roesma 2011) reported that *Rasbora* species have a high morphological similarity; however, the phylogenetic analysis (Cytochrome b gene) showed that between species has significant genetic differences. Small-size species groups are more frequently confused and lumped together in the taxonomy determination (Kottelat et al. 2006). *Rasbora* included the small-size species more sensitive to fragmentation and experienced faster genetic mutations and cryptic diversity than the large-size species (April et al. 2013; Hubert et al. 2017). One of the reasons is the cryptic morphological diversity of Rasbora groups.

Barbodes banksi in South Siberut has sequence divergences of 2.6%-8.5% with other West Sumatra populations and Genbank sequences (MW168694 and MW168708 (Ng and Tan 2021); JF781237 (Song et al. 2013); MT483450 (Ren et al. 2020); KU692318 (Dahruddin et al. 2016); MG699597 (Thacker and Hardman 2005). *Barbodes* group in the phylogenetic tree separated into three subgroups. The first sub-group consists of *B. banksi* from other West Sumatra populations (Diatas Lake and Muaro Pingai River) and Genbank sequences (Malaysia and Java). The second sub-group consist of *B. banksi* in Batang Kuranji River from West Sumatra population. The third sub-group consists of *B. banksi* South Siberut and West Sumatra population (Maninjau Lake). *B. banksi* South Siberut has high genetic distances with the first and second subgroups were of 7.1%-8.5% and 7.5%, respectively. *Barbodes* cf. *banksi* with Genbank accession MW168694 and MW168708 (Song et al. 2013) from Gua Musang, Kelantan, Malay Peninsula have low sequence divergences with *B. banksi* Genbank accession JF781237 (Song et al. 2013) and MT483450 (Ren et al. 2020) from Malaysia (0.5%-0.7%). Ng and Tan (2021) reported *Barbodes* cf. *banksi* are likely *B. banksi* (Type locality: Kuching, Sarawak); however, the relationship between *Barbodes* cf. *banksi*, and *B. banksi* could not be further elucidated due to the lack of reliable data in GenBank at the time of writing.

Specimens with Genbank accession KU692318 (Dahruddin et al. 2016) and MG699597 (Hutama et al. 2017) reported from Java populations as *B. binotatus*, which also has synonym name as *B. banksi*. However, *B. banksi* South Siberut has a low genetic distance (2.6%) with the third subgroup from Maninjau lake. This result showed *B. banksi* in South Siberut has a high genetic similarity with *B. banksi* in Maninjau Lake. The divided to tree subgroups are *B. banksi* was reported in the previous study. Roesma et al. (2018) reported the previously species identified as *Puntius* cf. *binotatus* assigned as *B. banksi*, supported by high genetic similarity with *B. banksi* from Malaysia. The high genetic distances from three subgroups separated them into *B. banksi kuranji* and *B. banksi maninjau* as subspecies of *B. banksi.* The separation between *B. banksi kuranji*, *B. banksi maninjau*, and *B. banksi* in the tree is thought to be related to the presence of Bukit Barisan as a barrier between water flowing to the eastern and western of Bukit Barisan mountains range. The geographical distinction has triggered the emergence of genetic variation up to species diversity of fish in Sumatra.

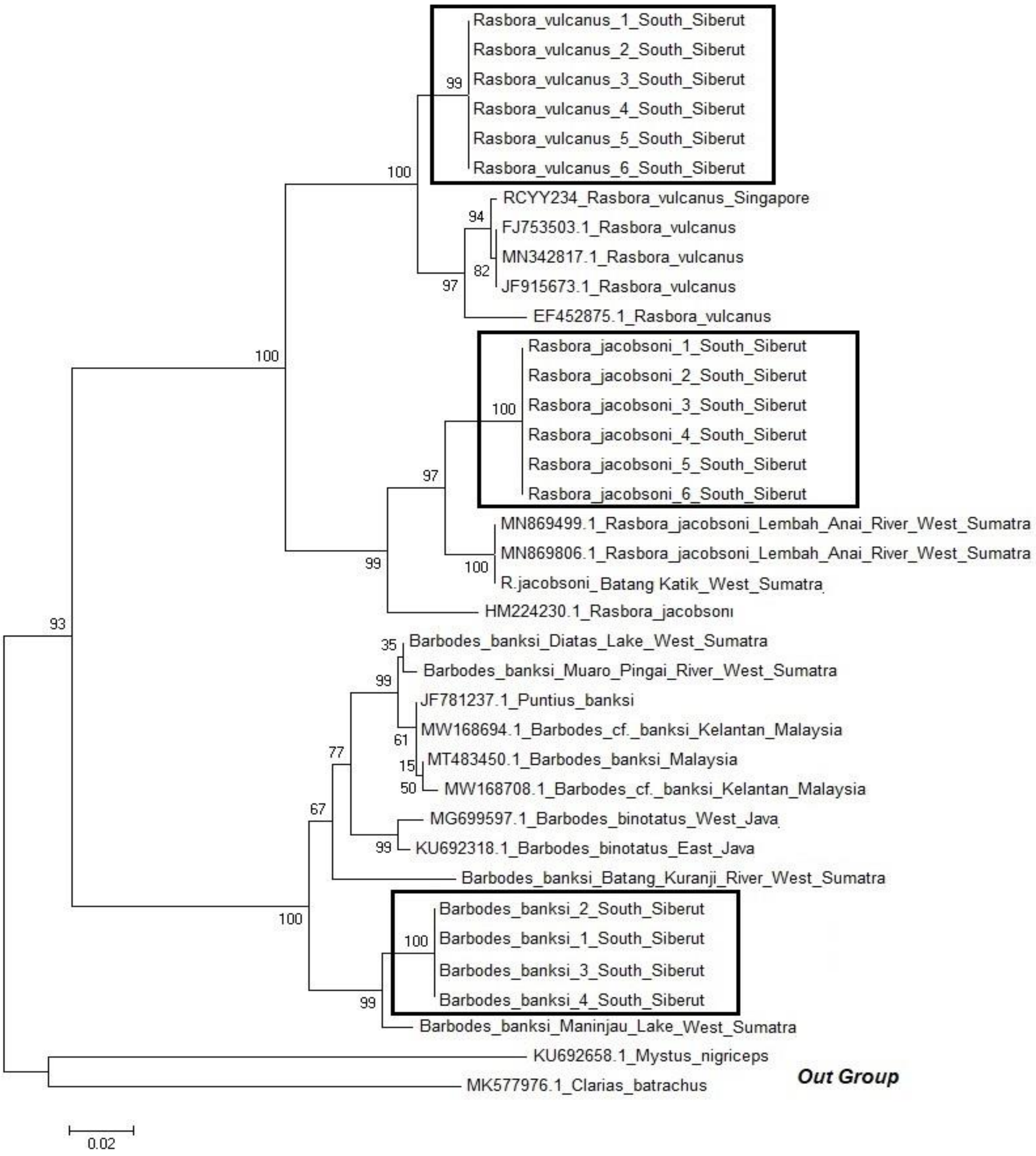

Figure 2. NJ tree analysis (based on K2P genetic distance of COI sequences) of Cypriniformes. The NJ tree was constructed from sequences found in South Siberut (samples marked with a black box), sequences from the Genbank database (samples with accession number), and sequences from the previous studies (samples without accession numbers from other Sumatran populations)

Previously, *B. banksi* was classified in the genus Puntius with the polyphyletic status. However, Kottelat (2013) published nomenclatural updates for Southeast Asian fishes, in which *B. banksi* as the former *Puntius* transferred into *Barbodes*. Members are distinguished from both related genera by ontogeny and color pattern aspects. Furthermore, Kottelat (2013) also assigned *B. banksi* with synonym names of *P. binotatus banksi* (*B. binotatus banksi*).

Gobiiformes

There are many recent studies and hypotheses related to the phylogeny and systematics of gobies. Kottelat (2013) used a classical classification for suborder Gobioidei with the seven families: Rhyacichthyidae, Odontobutidae, Eleotridae, Kraemeriidae, Gobiidae, Amblyopsidae, and Ptereleotrididae. Previously, Thacker and Hardman (2005), who recognized the gobies based on molecular characters, classified them into the six families; Rhyacichthyidae, Odontobutidae, Butidae, Eleotridae, Gobionellidae, and Gobiidae. Recently, we referred to the taxonomy classification from Fishes of the World book by Nelson et al. (2016) based on suggestions from Bold System. Nelson et al. (2016) adopted the classifications (Thacker and Hardman 2005; Thacker 2009; Chakrabarty et al. 2010; Gill and Mooi 2012; Agorreta et al. 2013; Mennesson et al. 2018), which recognized eight families within the

Gobioidei: Rhyacichthyidae, Milyeringidae, Odontobutidae, Eleotridae, Butidae, Thalasseleotrididae, Gobionellidae, and Gobiidae.

Three families represented the Gobiiformes group in Figure 3. Gobiiformes is an order with the most families (3) obtained in South Siberut. Gobiiformes members consist of Eleotridae (three species), Butidae (one species), and Oxudercidae (four species) with sequences divergence between them were of 20.6%-33.9%. Eleotridae consist of three species (*E. fusca*, *G. margaritacea*, and *H. compressa*) with sequences divergence between them was 14.2%-25.8%. The sequence divergences within South Siberut populations of each Eleotridae species of 0.0%-0.5%. Among Eleotridae species, *E. fusca* is the most abundant species. Besides, the *E. fusca* species has the highest number of haplotypes variations (5) among species in the South Siberut.

Eleotris fusca has the type of locality from Raitatea; Society Islands, French Polynesia, with global distribution from East Africa to French Polynesia. *Eleotris fusca* Siberut has low sequences divergences of 0.2%-0.7% with other Genbank sequences. *Eleotris fusca* specimens Genbank accession MH498407 (Page and Hughes 2010) known was collected from Vanuatu, Oceania. While for specimens Genbank accession AY722172 (Thacker and Hardman 2005) and KU727890 (Guimaraes-Costa 2016), no valid data about sampling location. The result showed *E. fusca* has widespread distributions but low genetic diversity.

Giuris margaritacea is Eliotridae species with type locality from Vanikoro Island, California, Santa Cruz Islands. Like *E. fusca*, *G. margaritacea* has low sequence divergences of 0.4%-1.6% with the *G. margaritacea* Genbank sequences. *Giuris margaritacea* specimen Genbank accession KU692503 (Dahruddin et al. 2016) was collected from Banten, West Java, but specimens with BOLD ACP9929 do not detail the valid data about sampling location. *Giuris margaritacea* also showed low genetic diversity with widespread distribution.

Hypseleotris is species with a single sample among Eliotridae species in South Siberut. *Hypseleotris* from South Siberut has high sequences divergences with other Genbank sequences (17.5%-18.1%). *Hypseleotris compressa* specimens Genbank accession HM006960 (Page and Hughes 2010) from Australia and KJ669476 were obtained from aquarium trade in Australia. *Hypseleotris compressa* (Empire Gudgeon) is endemic to Australia, Southern Central New Guinea, and Papua (Indonesia), with type locality from Clarence River and creeks near Port Denison, Australia. The high sequence divergences between the South Siberut population and other Genbank sequences led to assigned *Hypseleotris* in South Siberut as *Hypseleotris* cf. *compressa*. The presence of fish suspected as *Hypseleotris* cf. *compressa* is a new record of freshwater fish biodiversity in Siberut Island because it has restricted distribution. A future study with the high number of specimens in Siberut is needed to determine the taxonomy status.

In this study, the Butidae is represented by only one *Butis* species collected from the Siri Tengah river, Maileppet village. *Butis* in South Siberut has low sequence divergences (0.9%) with *Butis* sp. specimen with BOLD code AAL8960 and high sequence divergences (18.8%-19.5%) with *Butis butis* specimens Genbank accession KF714900 from Philippines and MH827972 from Bangladesh. The sequence similarity of *Butis* from South Siberut with the BOLD System showed 99% similarity with *Butis* sp. in BOLD System. However, the matching sequence with data in Genbank showed 85% similarity with *Butis butis* in Genbank. *Butis* sp. sequence registered in BOLD System is not yet submitted in the Genbank, NCBI. Data were written in the BOLD System only information about sampling locations; however, there is no data about the morphological characters of the *Butis* sp. specimen. *Butis butis* has distributions widely from east Africa to Fiji with the type of locality from India. The low sequence divergence between *Butis* from South Siberut with *Butis* sp. and high sequence divergences with *Butis butis* Genbank sequences led to assigned *Butis* in South Siberut as *Butis* cf. *butis*. A future study with the high number of specimens in Siberut is needed to determine the taxonomy status.

Oxudercidae is a family with the most species (4) obtained in South Siberut. Oxudercidae consists of four species (*P. argentilineatus*, *S. semoni*, *Redigobius* cf. *bikolanus*, and *Gobiopterus* cf. *brachypterus*) with sequences divergence between them was 18.9%-33.9%. Oxudercidae species has low sequence divergences within populations was of 0.0%-1.3%. *P. argentilineatus* from South Siberut sequence divergences were 0.2%-5.9% with Genbank sequences. *P. argentilineatus* Siberut has low genetic distances (0.2%) with *P. argentilineatus* Genbank accession MZ606683 from Yogyakarta. *P. argentilineatus* South Siberut has global distributions in Indo-pacific. *S. semoni* from South Siberut has low sequence divergence (0.0%-0.5%) with other GenBank sequences. *Stiphodon semoni semoni* Genbank accession KU693172 (Dahruddin et al. 2016) and MN812979 were collected from Sulawesi and Java, respectively. This result showed *P. argentilineatus* and *S. semoni* have low genetic diversity.

Redigobius from South Siberut has low sequence divergences (0.2%). The sequence similarity of *Redigobius* in South Siberut with sequences data in Genbank only showed 87% similarity with *R. bikolanus* Genbank accession KU692831 (Dahruddin et al. 2016) from Bali and KJ150224 (Huang et al. 2014). The sequence divergences between *Redigobius* from South Siberut with Genbank sequences was 17.1%-17.9%. *Redigobius bikolanus* Genbank accession KJ150224 is a specimen with complete genome DNA mitochondrial. This sequence was used as a sequences reference for identification. Based on the sequence divergence between *Redigobius* in South Siberut and *R. bikolanus* in Genbank can be asserted *Redigobius* in South Siberut as *Redigobius cf. bikolanus*. *Redigobius* species in South Siberut has potential as a new species, but sufficient samples and further study are needed.

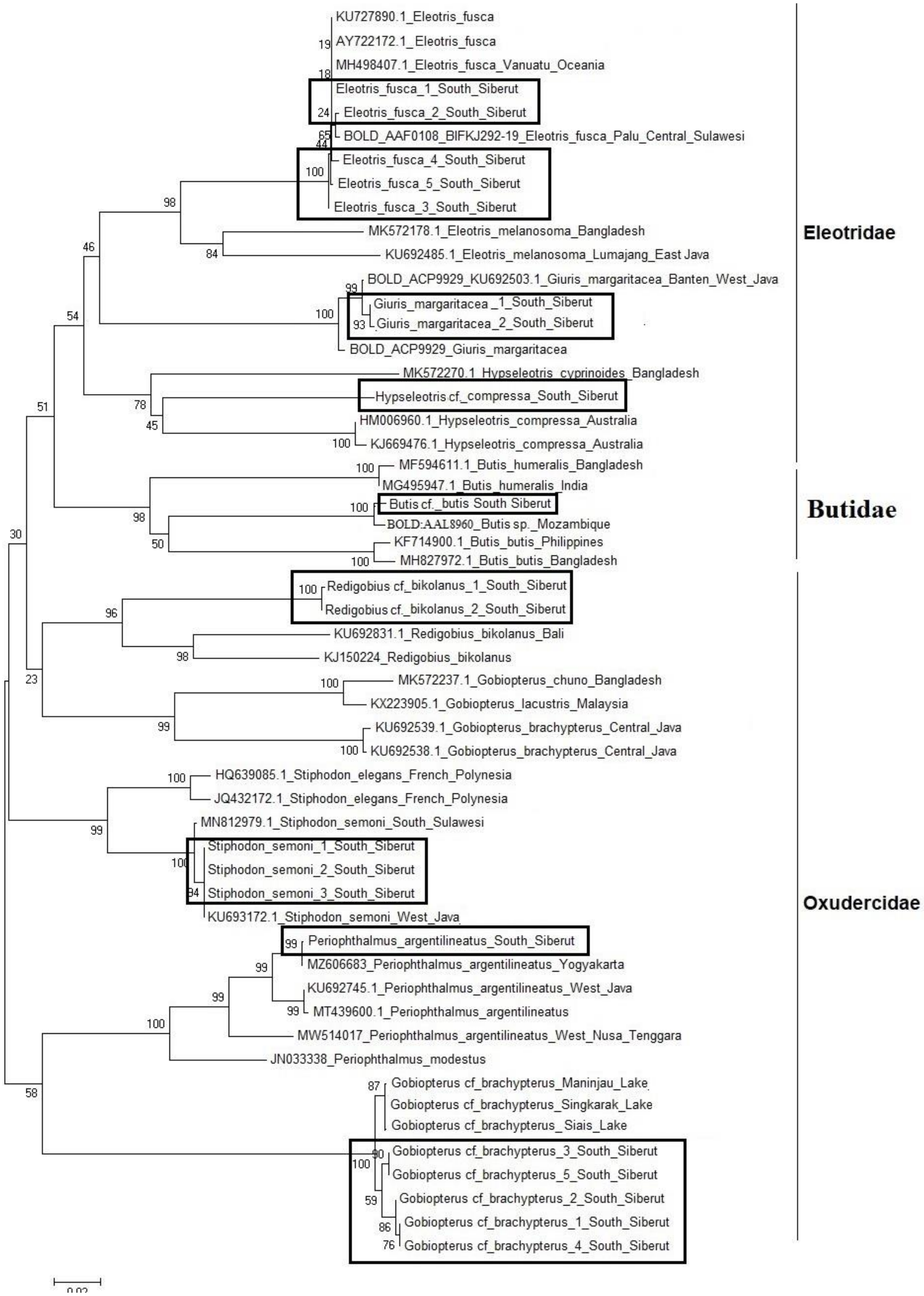

Figure 3. NJ tree analysis (based on K2P genetic distance of COI sequences) of Gobiiformes. The NJ tree was constructed from sequences found in South Siberut (samples marked with a black box), sequences from the Genbank database (samples with accession number), and sequences from the previous studies (samples without accession numbers from other Sumatran populations)

Gobiopterus is genus for transparent gobies in Gobiiformes group. *Gobiopterus* in South Siberut populations has low sequence divergences (0.0%-1.1%) with three haplotype variations. *Gobiopterus* from South Siberut has low sequences divergence with *Gobiopterus* cf. bracypterus from other West Sumatra sequences (1.1%-1.5%). However, *Gobiopterus* from South Siberut has high sequence divergences with *Gobiopterus* species in Genbank sequences (28.4%-29.2%). Besides, *Gobiopterus* species from Genbank separated with *Gobiopterus* from West Sumatra populations in the different branches. *Gobiopterus* Genbank sequence has the mean sequence divergences was 15.5%. According to Kartavtsev (2011) and Kartavtsev (2013), the values of 15.5% showed the difference in species level. However, the sequence divergences between *Gobiopterus* from South Siberut and *Gobiopterus* Genbank sequences showed the difference in genus/family level.

Referring to Kottelat (2013), the transparent gobies only consist of two genera (*Gobiopterus* and *Mistichthys*). *Mistichthys* only has one species, *Mistichthys luzonensis*. Thus, only one valid genus for the transparent gobies groups. The results align with the previous study (Roesma et al. 2020) in transparent gobies from three Lakes in Sumatra. Roesma et al. (2020) reported that despite having the morphological similarity, the high sequence divergences do not support the grouping of *Gobiopterus* cf. *brachypterus* from three lakes as the same species to *G. brachypterus* even to the genus *Gobiopterus*. However, there is no other genus for transparent gobies, assigned *Gobiopterus* from South Siberut as *Gobiopterus* cf. *brachypterus*. Therefore, *Gobiopterus* cf. *brachypterus* from Sumatra may have potential as a new species.

Anabantiformes

Anabantiformes group was represented by one family with single species (*Anabas testudineus*) (Figure 4). *A. testudineus,* including the low number of species, was found among the other species in South Siberut. *A. testudineus* has widely distributed in Asia (India to Wallace line). The genetic divergence among *A. testudineus* from South Siberut populations is 0.0%. The value shows the high genetic similarity of *A. testudineus* in South Siberut. *Anabas testudineus* on South Siberut population has low sequence divergences (0.0%-0.2%) with Philippines and West Nusa Tenggara populations and high sequence divergences (9.1%) with Bangladesh population. The inter-specific has over lower sequences divergences (8.2%-8.9%) than range value for different species in the same genus.

Beloniformes

Beloniformes group was represented by one family (Adrianichthyidae) with single species (*Oryzias*) (Figure 5). The sequence divergence among *Oryzias* from South Siberut populations is 0.0%. *O. javanicus* has the type locality from Java with distribution in Southern Asia (Indonesia, Singapore, Malaysia, and Thailand). *Oryzias* from South Siberut has high sequence divergences with *O. javanicus* Genbank sequences (14.2%-17.5%). Based on the high sequence divergence between *Oryzias* in South Siberut and *O. javanicus* Genbank, *Oryzias* in South Siberut is reported as *Oryzias* cf. *javanicus*. A future study with the high number of specimens in Siberut is needed to determine the taxonomy status.

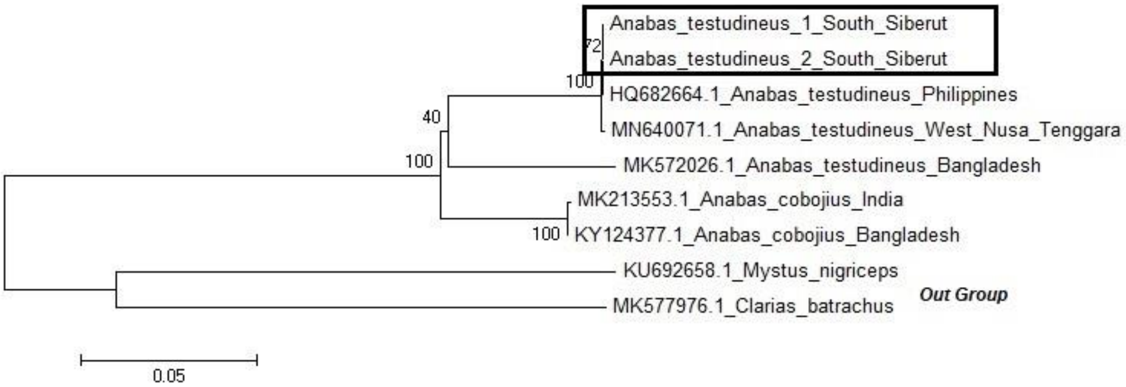

Figure 4. NJ tree analysis (based on K2P genetic distance of COI sequences) of Anabantiformes. The NJ tree was constructed from sequences found in South Siberut (samples marked with a black box) and sequences from the Genbank database (samples with accession number)

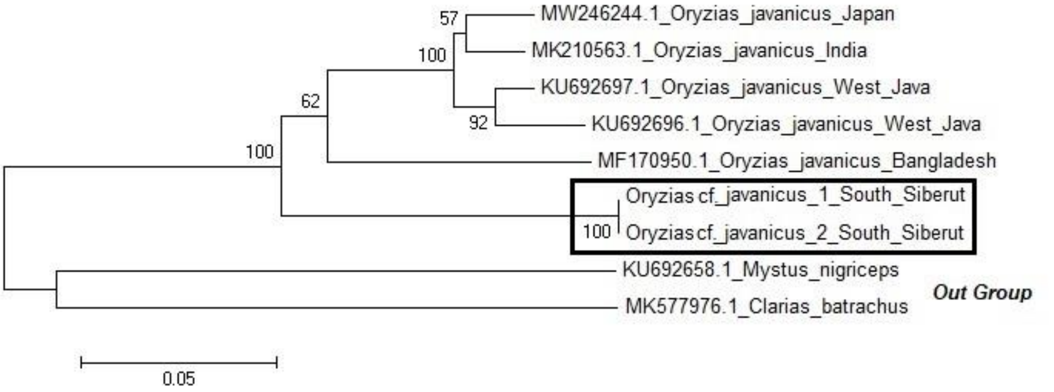

Figure 5. NJ tree analysis (based on K2P genetic distance of COI sequences) of Beloniformes. The NJ tree was constructed from sequences found in South Siberut (samples marked with a black box) and sequences from the Genbank database (samples with accession number)

This study successfully provided the DNA barcodes for 40 individuals (13 species) of freshwater fish in South Siberut, Siberut Island. The results are the new record of molecular data and freshwater fish biodiversity in Siberut Island. The low sequence divergences of each species intra-populations and inter-populations in South Siberut indicate that genetic variation within species is low. However, almost all species in South Siberut have high sequence divergences with other Sumatra populations and Genbank sequences. It's related to the evolutionary period of species in Siberut Island that has been separated from Sundaland since the mid-Pleistocene period or about 500,000 years ago. DNA barcode is important to determine the richness of freshwater from South Siberut, submitted to the BOLD System. The available data will contribute as the basic data to future studies, especially species with potential as new species. In addition, the data from DNA Barcode will help the governments take policies, paying attention to the fauna survival from islands.

In conclusion, a total of 40 DNA barcodes of freshwater fish in Siberut Island successfully consisted of 13 species, namely *Gobiopterus* cf. *brachypterus* (BOLD: ADF5945), *E. fusca* (BOLD: AAF0108), *Butis* cf. *butis* (BOLD: AAL8960), *S. semoni* (BOLD: ACQ3312), *G. margaritacea* (BOLD: ACP9929), *A. testudineus* (BOLD: AAO2846), *R. jacobsoni*, *R. vulcanu*s, *B. banksi*, *Redigobius* cf. *bikolanus*, *P. argentilineatus*, *Hypseleotris* cf. *compressa*, and *Oryzias* cf. *javanicus* (in a queue in the BOLD system). The average Kimura two-parameters model (K2P) genetic distances within species, genera, families, and orders were 0.16%, 11.4%, 23.2%, 26.0%, respectively. The interspecific K2P distance was higher than the intraspecific distance. Almost all species in South Siberut have high sequence divergences with other Sumatra populations and Genbank sequences. Future studies with a large number of samples and more comprehensive studies taking into account morphological, and molecular studies (mitochondrial DNA and nuclear DNA analysis), will help reveal species that have potential as new species.

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