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Research Article

DNA Barcoding of Commercially Important Groupers (Epinephelidae) in Simeulue and Banyak Islands, Aceh, Indonesia

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ABSTRACT

The groupers are a group of demersal fish that live in tropical and subtropical areas and are mostly linked to coral reefs. The fish are highly valued in international markets and are subjected to overfishing in the wild. Accurate fish resource identification knowledge is essential for sustainable fisheries management. This research is aimed to generate a reference COI sequence library of grouper species caught in Simeulue and Banyak Islands, Aceh. The study was conducted from April-September 2021 at sixteen fish landing sites in Simeulue and Banyak Islands. In total, this study generated 70 COI sequences representing 20 grouper species. *Epinephelus* (54%) was the most prevalent grouper genus at the study site, followed by *Cephalopholis* (19%), *Plectropomus* (13%), *Variola* (12%), *Hyporthodus* (1%), and *Anyperodon* (1%). According to the IUCN classification, 17 species (85%) fall into the category of Least Concern, two (10%) fall into the category of Vulnerable, and one (5%) falls into the category of Data Deficient. The average genetic distance based on the Kimura-2-Parameter (K2P) between specimens was 0.51% at the species level and 8.34% at the genus level. Overall, this study has provided the COI sequence database of grouper for the Simeulue and Banyak Islands.

1. Introduction

Simeulue and Banyak Islands waters are located on the west coast of Sumatra, which is directly adjacent to the Indian Ocean (BPS 2014). According to Razi *et al.* (2022), both islands are significant hubs for grouper fisheries. The production of grouper was estimated at 30 tons/year in Simeulue and 4 tons/year in Banyak Islands in 2020 (BPS 2020). Moreover, Aceh, including these islands, contributed 8.26% of grouper fish to 16,414 tons of grouper production in Indonesia in 2020 (BPS 2020). Groupers are demersal fish that inhabit coral reef habitats in tropical and subtropical oceans (Heemstra & Randall 1993). Groupers are fast-growing and early-maturing fishes. Most groupers are protogynous hermaphrodites that are born female but change sex to male sometime in their lifespan (Heemstra dan Randall 1993; Alamsyah *et al.* 2013; Agustina *et al.* 2018; Astuti 2018). At least 163 species of grouper, belonging to 16 genera, have been recorded worldwide (Craig *et al.* 2011), and Indonesian seas have at least 77 species of groupers (FRCI 2021). As for many other economically important marine aquatic organisms, the high exploitation level of this group coupled with the destruction of its coral reef habitat has led to stock depletion with the risk of accelerating the extinction of these fishes in several regions in Indonesia (Burke *et al.* 2002; Yulianto *et al.* 2015). Sadovy de Mitcheson *et al.* (2013) reported that out of 163 grouper fish species globally, 12% (20 species) are classified as

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endangered, and 13% (22 species) of them are in the near-threatened category based on the IUCN category. Given the high importance of grouper fisheries in both islands, a precise species identification database must be developed to contribute to the conservation and sustainable management of fisheries resources. Therefore, understanding their genetic diversity and population structure is essential for implementing effective conservation strategies (Fadli *et al.* 2023). DNA barcoding is a relevant method for studying groupers in this region because it provides accurate species identification based on genetic information (Pavan-Kumar *et al.* 2016, 2018). This is particularly valuable in areas where traditional morphological identification may be challenging, such as in larval stages or for cryptic species (Rahayu *et al.* 2023). For example, DNA barcoding has been used to identify 27 commercial grouper species in the Philippines, 36 species in India, and 10 species in Malaysia, respectively (Alcantara & Yambot 2016; Aziz *et al.* 2016; Basheer *et al.* 2017). In another study, Qu *et al.* (2018) barcoded 57 grouper species in nine genera in the China Sea.

However, studies of grouper DNA barcodes in Indonesia, including in the western Sumatran region of Aceh, are still limited (Razi *et al.* 2021; Fadli *et al.* 2022; Razi *et al.* 2022). For example, Fadli *et al.* (2020) barcoded 72 commercially important reef fish on Weh Island, including 18 grouper species. The waters around Weh Island, which lies at the entrance of the Strait of Malacca, support an immensely high marine biodiversity (Baird *et al.* 2012; Rudi & Fadli 2012; Munandar *et al.* 2019; Octavina *et al.* 2021). In another study, Fadli *et al.* (2021) performed DNA barcoding for 26 grouper species along the coast of Aceh. In a recent study by Fadli *et al.* (2024), DNA barcoded six grouper species from Langsa, Aceh. However, many other areas in and around the biodiversity hotspot of Aceh have not been explored for their marine life, including the Simeulue and Banyak Islands.

Therefore, this study is aimed to conduct DNA barcoding accurately identify grouper species present, investigate the genetic diversity and population structure, assess the health of grouper populations and identify conservation priorities based on genetic data from commercially important grouper in Simeulue and Banyak Islands. This research is deemed vital for the enhancement of our understanding of groupers in the Simeulue and Banyak Islands and for contributing to their conservation. DNA barcoding approaches are a powerful tool to identify grouper species and assess their genetic diversity. The genetic information generated could address gaps in grouper fisheries to facilitate appropriate fisheries management and conservation planning to conserve fish resources, especially through conservation genetics, in the two islands. By elucidating the genetic makeup of grouper populations, decision-making processes aimed at preserving marine biodiversity and ensuring the sustainability of grouper fisheries in the region can be informed by this research.

2. Materials and Methods

2.1. Sample Collection

This research was carried out from April-September 2021. Grouper fish were collected from fishers and fish collectors or purchased from fish landing sites (TPI) in Simeulue and Banyak Islands (Permit Number: SI.07/K.20/TU/KSA.2.1/5/2021) (Figure 1). This study received full permission from Syiah Kuala University's committee on research ethics and animal care (Ethic Code No. 958/2015). The fish samples were morphologically identified up to the species level (Heemstra & Randall 1993; Craig *et al.* 2011; Froese & Pauly 2024). Fin clips of representative samples of each species were preserved in 96% ethanol and placed in a 2 ml tube. Tissue and whole-body samples of each fish species were documented according to the Fish-BOL collaborator's protocol (Steinke & Hanner 2011). Samples ranged from three to five individuals per species. The whole fish and tissue samples have been deposited at the Faculty of Marine and Fisheries, Universitas Syiah Kuala, Banda Aceh, Indonesia. All sequences have been deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/; Accession No. OK284518, OM273099-OM273167). Additional complementary data from the gathered fish samples are shown in Table 1.

2.2. DNA Extraction, Primer, and PCR Assay

DNA was extracted following the modified Cetyl trimethylammonium bromide (CTAB) protocol (Grewe *et al.* 1993). The final concentrations of the extracted DNA samples were quantified using the NP80 Implen Nanophotometer (https://www.implen.de/). PCR amplification of the COI gene was run applying primers F1 and R1 based on Ward *et al.* (2005) in a 25 µL master mix containing 12.5 µL MyTaq Red Mix, 2.0 µL DNA template, 1 µL of each primer, and 8.5 µL of ddH_2O water. The PCR was amplified in a Sensoquest gradient Thermal Cycler (https://www.sensoquest.de/). The thermal condition consisted of initial denaturation at 95°C (2 min) followed by denaturation at 94°C (30 cycles (45s)); annealing at 49.7-56°C (45s); elongation

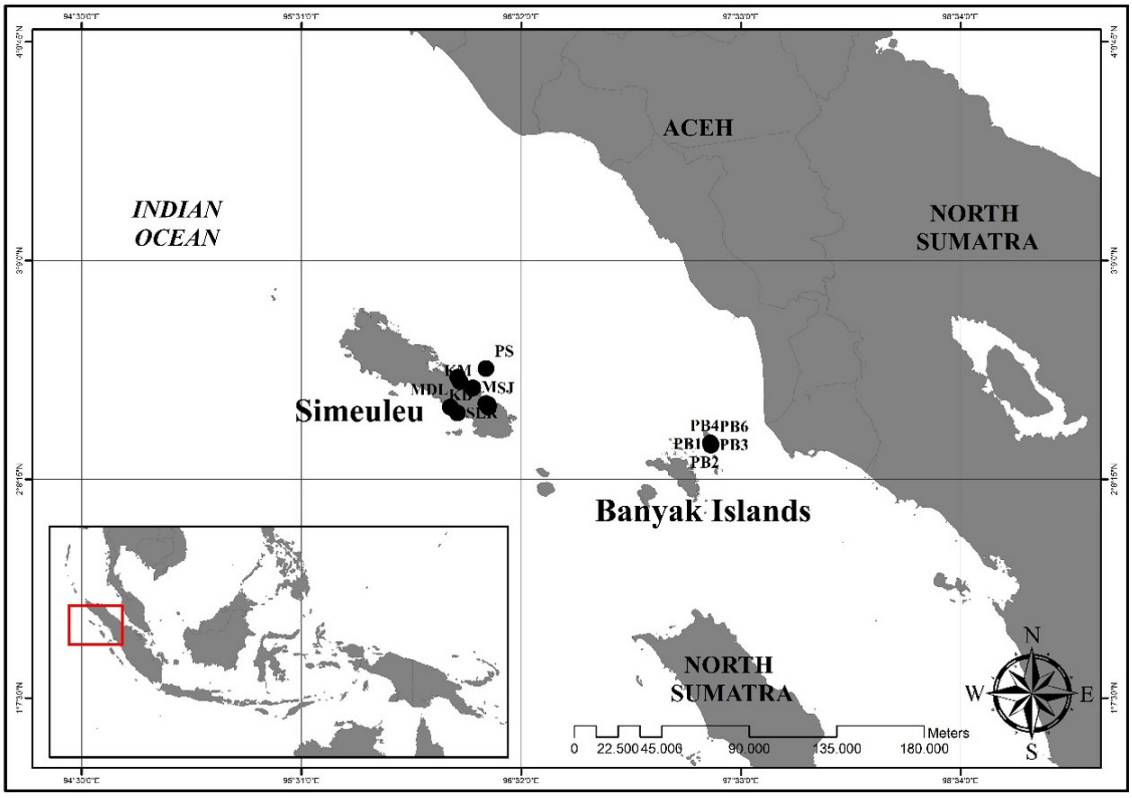

Figure 1. Research sites in Simeulue and Banyak Islands.

at 72°C (1 min), and a final extension of 72°C (10 min) before termination of the reaction at 4°C (Rahayu *et al.* 2024; Razi *et al.* 2024). Successfully PCR-amplified products were sent to the PT. Genetika Sciences, Jakarta, for bidirectional sequencing.

2.3. Data Analysis

Sequences were clipped and aligned using MEGA 6.06 software (Tamura *et al.* 2013). Later, the aligned sequences were translated into protein to ensure the correct alignment and recognition of stop codons. Species identification was conducted by comparing COI sequences in BLAST (Basic Local Alignment Search Tool; http://www.ncbi.nlm.nih.gov/BLAST) and the BOLD Identification System (IDS) (www.boldsystems.org). The Barcoding Gap Analysis tool examined the maximum intra-specific genetic distance at the species level with the minimum distance to the nearest neighbour (www.boldsystems.org). Furthermore, Automatic Barcode Gap Discovery (ABGD) (https://bioinfo.mnhn.fr/abi/public/abgd) was used for species boundary determination to determine the number of operational taxonomic units (OTUs) based on pairwise sequence distances between individuals in the dataset (Puillandre *et al.* 2012, 2021). The MEGA 6.06 software was also used to examine the base composition and number of variable sites and for calculation of pairwise genetic distances (conspecific, congeneric, and confamilial) based on the Kimura-2-parameter (K2P) model (Kimura 1980; Tamura *et al.* 2013; Rahayu *et al.* 2023). Haplotype distribution was summarised in DnaSP 5.10 (Rozas *et al.* 2003; Librado & Rozas 2009).

The phylogenetic tree was created using the Maximum Likelihood (ML) method with 1000x bootstrap with the Hasegawa-Kishino-Yano model (Kimura 1980; Hasegawa *et al.* 1985; Kumar *et al.* 2018). The outgroup used to root the tree was the *Lutjanus kasmira* sequence (Accession no. MK658130.1) (Delrieu-Trottin *et al.* 2019). In addition, the conservation status of the identified groupers was determined based on the International Union for Conservation of Nature (IUCN) website (http://www.iucnredlist.org) (Razi *et al.* 2023). Besides their conservation status, their trade status was determined using the Convention on International Trade in Endangered Species (CITES) online at http://www.cites.org/.

Table 1. The list of grouper species from Simeulue and the Banyak Islands

Genus	Species	Species																Total sample	Haplotype	Haplotype
		SLR	MDL	SNB	SJ	SM	AMT	PI	PS	KM	AP	KB	PB1	PB2	PB3	PB4	PB5			
Anyperodon	*Anyperodon leucogrammicus*									1								1	1	OK284518
Cephalopholis	*Cephalopholis boenak*														1			1	1	OM273099
	Cephalopholis leopardus							5										5	3	OM273100 - OM273104
	Cephalopholis miniata										1					2		3	2	OM273105 - OM273107
	Cephalopholis sonnerati							1						1		2		4	1	OM273108 - OM273111
Epinephelus	*Epinephelus areolatus*	1	1										1	1	1			5	3	OM273112 - OM273116
	Epinephelus coeruleopunctatus			2		2				1								5	1	OM273117 - OM273121
	Epinephelus fasciatus				2	1		1									1	2	2	OM273122 - OM273123
	Epinephelus flavocaeruleus			1		2		1										4	3	OM273124 - OM273127
	Epinephelus fuscoguttatus			2		1	1		1									4	4	OM273128 - OM273131
	Epinephelus longispinis			2	1			1										4	3	OM273132 - OM273135
	Epinephelus macrospilos			2														4	3	OM273136 - OM273139
	Epinephelus merra		2	1				1										4	4	OM273140 - OM273143
	Epinephelus morrhua							5										5	2	OM273144 - OM273148
	Epinephelus tauvina			1														1	1	OM273149
Hyporthodus	*Hyporthodus octofasciatus*					1												1	1	OM273150
Plectropomus	*Plectropomus areolatus*					2			1								1	4	4	OM273151 - OM273154
	Plectropomus leopardus					1			1	1			2					5	1	OM273155 - OM273159
Variola	*Variola albimarginata*								1		1	2					1	5	3	OM273160 - OM273164
	Variola louti				1		1					1						3	2	OM273165 - OM273167
	Total	1	3	9	4	10	2	15	4	3	2	3	3	2	2	4	3	70	45	

SLR: Salur; MDL: Maudil; SNB: Sinabang; SJ: Suka Jaya; SM: Suka Makmur; AMT: Amaiteng; PI: Pasar Ikan; PS: Pulau Siumat; KM: Kuala Makmur; AP: Air Pinang; KB: Kuala Baru; PB1: Kepulauan Banyak 1; PB2: Kepulauan Banyak 2; PB3: Kepulauan Banyak 3; PB4: Kepulauan Banyak 4; PB5: Kepulauan Banyak 5

3. Results

3.1. Species Composition

In total, 70 grouper sequences belonging to 20 species from six genera were acquired in this study (*Anyperodon, Cephalopholis, Epinephelus, Hyporthodus, Plectropomus*, and *Variola*). *Epinephelus* was the dominant genus found (54%), followed by *Cephalopholis* (19%), *Plectropomus* (13%), *Variola* (12%), *Anyperodon* (1%), and *Hyporthodus* (1%) (Figure 2). This is the first genetic record in Aceh of the following species: *Anyperodon leucogrammicus, Epinephelus fuscoguttatus, Epinephelus macrospilos, Epinephelus morrhua, Hyporthodus octofasciatus*, and *Plectropomus areolatus*.

3.2. Cytochrome Oxidase Subunit I Diversity Assessment

The 70 sequences produced represented 45 sequence haplotypes (Table 1). The length of the COI sequences was 639 bp after editing with a typical nucleotide composition of A = 24.37%, T = 29.60%, C = 27.82%, and G = 18.21%. The average GC level was 46.03%, while the AT level was 53.97% (Table 2). The GC content decreased from the first, second, and third position codons. No insertions, deletions, or stop codons were detected in the 70 generated sequences. The observed decrease in GC content from the first to the third codon positions is consistent with established principles of codon usage bias, evolutionary constraints, and genome composition. This finding can be interpreted in light of other analyses, such as codon usage bias, selective pressures, translational efficiency, or broader genomic trends, highlighting the interplay between molecular evolution, functional constraints, and genome organization.

The genetic distances based on the Kimura-2-Parameter increased from intraspecies to intragenera (between species within the genus) levels from 0% to 1.59% and 4.65 to 14%, respectively. The mean genetic distance was 0.51% within species (intra-species) and 8.34% between species within genus (intra-genera), which translates to a 16.4X higher K2P genetic distance between species than within species (Table 3). Pairwise comparisons of COI genes based on K2P distance (%) within species were highest in *Epinephelus flavocaeruleus*, 0.016, while the closest neighbor is between

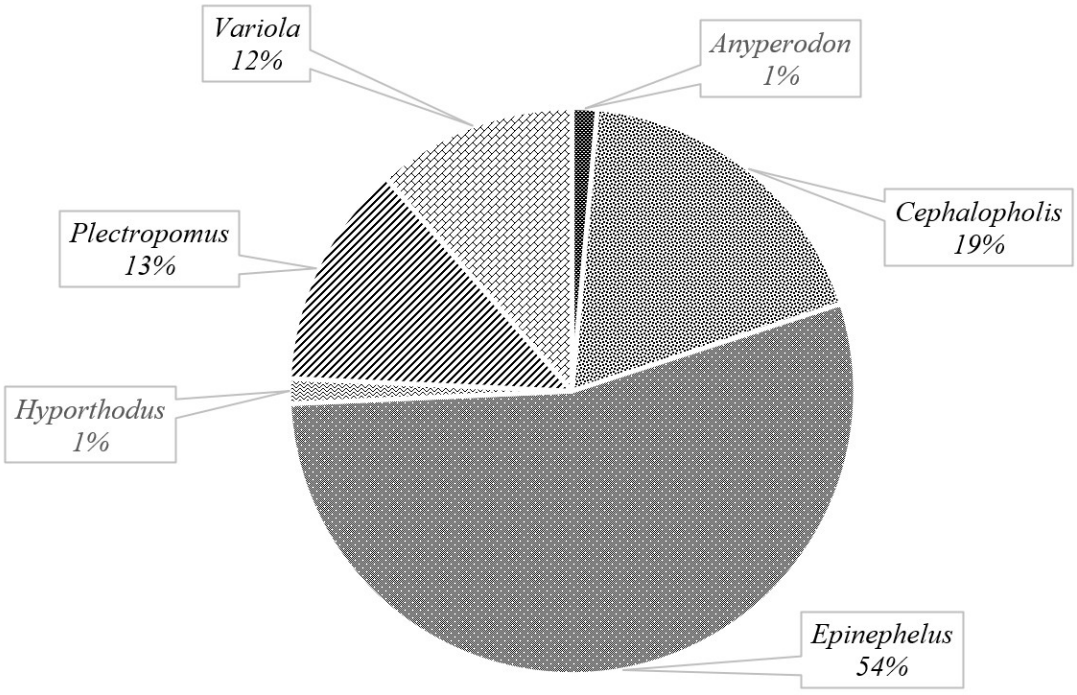

Figure 2. Composition of the grouper genus found in Simeulue and the Banyak Islands

Table 2. The summary statistics of the nucleotide frequency distribution of COI sequences of grouper samples collected in Simeulue and the Banyak Islands

	Mean	Min	Max
T %	29.60	26.45	32.24
C %	27.82	25.20	30.05
A %	24.37	23.32	25.51
G %	18.21	16.90	19.41
GC %	46.03	42.57	49.45
GC % Codon Pos 1	56.43	53.05	59.15
GC % Codon Pos 2	43.64	42.72	43.66
GC % Codon Pos 3	38.03	29.11	46.48

Table 3. The summary of genetic distance (K2P %) with taxonomic level

Taxonomy level	Taxa	Minimum %	Maximum %	Mean %
Species	20	0	1.59	0.51
Genus	6	4.65	14.00	8.34

Plectropomus leopardus and *Plectropomus areolatus*, with a distance of 8.04 (Tables 4 and 5).

3.3. Species Delimitation

The BLAST and BOLD databases produced similarity values for species validation that ranged from 98.12-100% (Table 6). This showed the accuracy of the COI gene used for grouper identification of the sampled specimens to the species level. No ambiguous sequence was observed. Moreover, Barcoding Gap Analysis revealed that all the observed species had intra-species distances <2% (mean 0.51%). Each species exhibited high distance values to their closest relatives, indicating an obvious "barcode gap" among the twenty species observed (Figure 3, Table 5). Similar to the Barcoding Gap Analysis, the ABGD analyses also yielded 20 operational taxonomic units (OTUs) with initial partitioning on the previous intra-specific divergence (P) (P = 0.001-0.060) (Figure 4). The resulting phylogeny tree showed all sequences clustered into their respective species with high support (Figure 5).

3.4. Conservation and Trade Status

According to the IUCN categorization, out of the twenty species, seventeen (85%) are classified as Least Concern (LC), two (10%) as Vulnerable (VU), and one (5%) as Data Deficient (DD). The species under the VU category were *P. areolatus* and *E. fuscoguttatus* (Figure 6). In addition, the populations of four species (*E. fuscoguttatus, P. areolatus, P. leopardus,* and *V. albimarginata*) showed a declining trend in the population (http://www.iucnredlist.org, accessed 20 June 2023). However, none of the sampled species have been reviewed by CITES (accessed 20 June 2023) (http://www.cites.org) (Table 7).

Table 4. Pairwise comparison of COI genes based on the mean K2P distance (%) within (bold) and between grouper species (Kimura 1980; Kumar *et al.* 2018)

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Anyperodon leucogrammicus	N/A																			
Cephalopolis boenak	21.07	N/A																		
Cephalopolis leopardus	19.17	17.43	0.00																	
Cephalopolis miniate	21.76	19.67	11.75	0.01																
Cephalopolis sonnerati	19.24	17.50	11.48	8.70	0.00															
Epinephelus areolatus	18.67	19.94	17.75	19.66	19.14	0.01														
Epinephelus coeruleopunctatus	15.96	18.85	15.18	18.34	17.15	17.60	0.00													
Epinephelus fasciatus	18.24	18.73	20.43	20.37	21.10	14.37	17.22	0.00												
Epinephelus flavocaeruleus	18.18	19.28	19.36	20.11	20.63	8.41	16.67	15.54	0.02											
Epinephelus fuscoguttatus	12.01	18.27	17.00	19.01	17.55	16.52	9.88	15.51	17.36	0.01										
Epinephelus longispinis	17.55	21.63	14.53	19.01	19.10	13.58	13.33	15.36	14.22	14.63	0.00									
Epinephelus macrospilos	19.28	20.50	18.16	19.48	18.58	16.81	15.60	15.51	17.39	16.23	16.33	0.00								
Epinephelus merra	18.18	20.27	17.39	20.90	19.74	15.77	15.00	12.86	15.26	16.28	15.90	15.19	0.00							
Epinephelus morrhua	15.63	19.19	18.93	20.25	17.13	15.28	15.40	15.50	17.68	12.01	15.89	18.17	16.37	0.00						
Epinephelus tauvina	16.60	18.24	17.15	21.16	18.98	15.60	15.27	12.97	15.15	15.13	15.24	16.06	12.90	14.68	N/A					
Hyporthodus octofasciatus	14.79	19.16	18.02	18.39	18.12	15.51	15.44	17.75	16.50	14.55	16.69	17.29	16.57	10.71	14.90	N/A				
Plectropomus areolatus	19.57	21.68	21.31	24.18	21.25	20.46	20.07	21.15	20.95	18.94	21.46	21.87	20.45	18.77	18.97	20.37	0.01			
Plectropomus leopardus	19.85	21.92	22.10	24.94	22.98	20.51	21.69	20.67	20.31	19.20	23.28	22.17	20.68	21.10	19.00	22.37	8.08	0.00		
Variola albimarginata	21.47	19.36	22.29	22.38	20.23	20.81	20.69	21.46	22.40	20.28	21.33	19.82	22.62	20.07	21.19	19.65	23.41	22.43	0.01	
Variola louti	23.92	23.55	22.75	22.01	20.67	23.80	24.11	23.01	25.08	23.75	23.69	22.21	23.63	22.55	22.45	20.49	24.81	24.30	9.58	0.00

N/A: not valid because of single sample species

Table 5. The mean and maximum intraspecific values for each type of grouper, compared to the closest relative distance. If the species is single, N/A is shown for intraspecific values

Species	Mean Intra-species (K2P%)	Max Intra-species (K2P%)	Nearest Neighbour	Distance to nearest neighbour (K2P%)
Anyperodon leucogrammicus	N/A	0.00	*Epinephelus fuscoguttatus*	12.01
Cephalopolis boenak	N/A	0.00	*Cephalopolis leopardus*	17.43
Cephalopolis leopardus	0.00	0.01	*Cephalopolis sonnerati*	11.48
Cephalopolis miniata	0.01	0.01	*Cephalopolis sonnerati*	8.70
Cephalopolis sonnerati	0.00	0.00	*Cephalopolis miniata*	8.70
Epinephelus areolatus	0.01	0.03	*Epinephelus flavocaeruleus*	8.41
Epinephelus coeruleopunctatus	0.00	0.00	*Epinephelus fuscoguttatus*	9.88
Epinephelus fasciatus	0.00	0.00	*Epinephelus tauvina*	12.97
Epinephelus flavocaeruleus	0.02	0.03	*Epinephelus areolatus*	8.41
Epinephelus fuscoguttatus	0.01	0.02	*Epinephelus coeruleopunctatus*	9.88
Epinephelus longispinis	0.00	0.00	*Epinephelus coeruleopunctatus*	13.33
Epinephelus macrospilos	0.00	0.00	*Epinephelus merra*	15.19
Epinephelus merra	0.00	0.01	*Epinephelus fasciatus*	12.86
Epinephelus morrhua	0.00	0.00	*Hyporthodus octofasciatus*	10.71
Epinephelus tauvina	N/A	0.00	*Epinephelus merra*	12.90
Hyporthodus octofasciatus	N/A	0.00	*Epinephelus morrhua*	10.71
Plectropomus areolatus	0.01	0.02	*Plectropomus leopardus*	8.08
Plectropomus leopardus	0.00	0.00	*Plectropomus areolatus*	8.08
Variola albimarginata	0.01	0.01	*Variola louti*	9.58
Variola louti	0.00	0.00	*Variola albimarginata*	9.58

Table 6. The comparison of grouper identification based on BLAST and BOLD databases

No.	Total sample	Species	Similarities (%)	
			BLAST	BOLD
1	1	*Anyperodon leucogrammicus*	100	100
2	5	*Cephalopolis leopardus*	99.84-100	99.84-100
3	3	*Cephalopolis miniata*	98.75-100	98.85-100
4	1	*Cephalopholis boenak*	99.53	99.53
5	4	*Cephalopolis sonnerati*	100	100
6	5	*Cephalopolis sonnerati* *Epinephelus areolatus*	99-100	100

Table 6.Continued

No.	Total sample	Species	Similarities (%)	
			BLAST	BOLD
7	5	*Epinephelus coeruleopunctatus*	100	100
8	2	*Epinephelus fasciatus*	100	99.84-100
9	4	*Epinephelus flavocaeruleus*	98.12-100	98.24-100
10	4	*Epinephelus fuscoguttatus*	98.12-100	98.22-100
11	4	*Epinephelus longispinis*	99.84-100	99.84-100
12	4	*Epinephelus macrospilos*	99.69-100	99.69-100
13	4	*Epinephelus merra*	99.84-100	99.84-100
14	5	*Epinephelus morrhua*	99.84-100	100
15	1	*Epinephelus tauvina*	100	100
16	1	*Hyporthodus octofasciatus*	100	100
17	4	*Plectropomus areolatus*	98.44-99.84	98.42-99.84
18	5	*Plectropomus leopardus*	100	100
19	5	*Variola albimarginata*	99.06-100	99.19-100
20	3	*Variola louti*	99.69-99.84	99.84-100

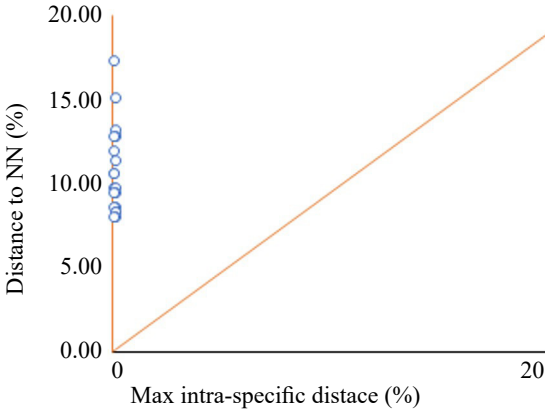

Figure 3. The maximum intraspecific divergence (K2P%) in the COI barcode region was plotted against the nearest neighbor distance (%K2P) for the 20 grouper morphospecies studied in this study. The dot above the diagonal line indicates the species with the barcode gap

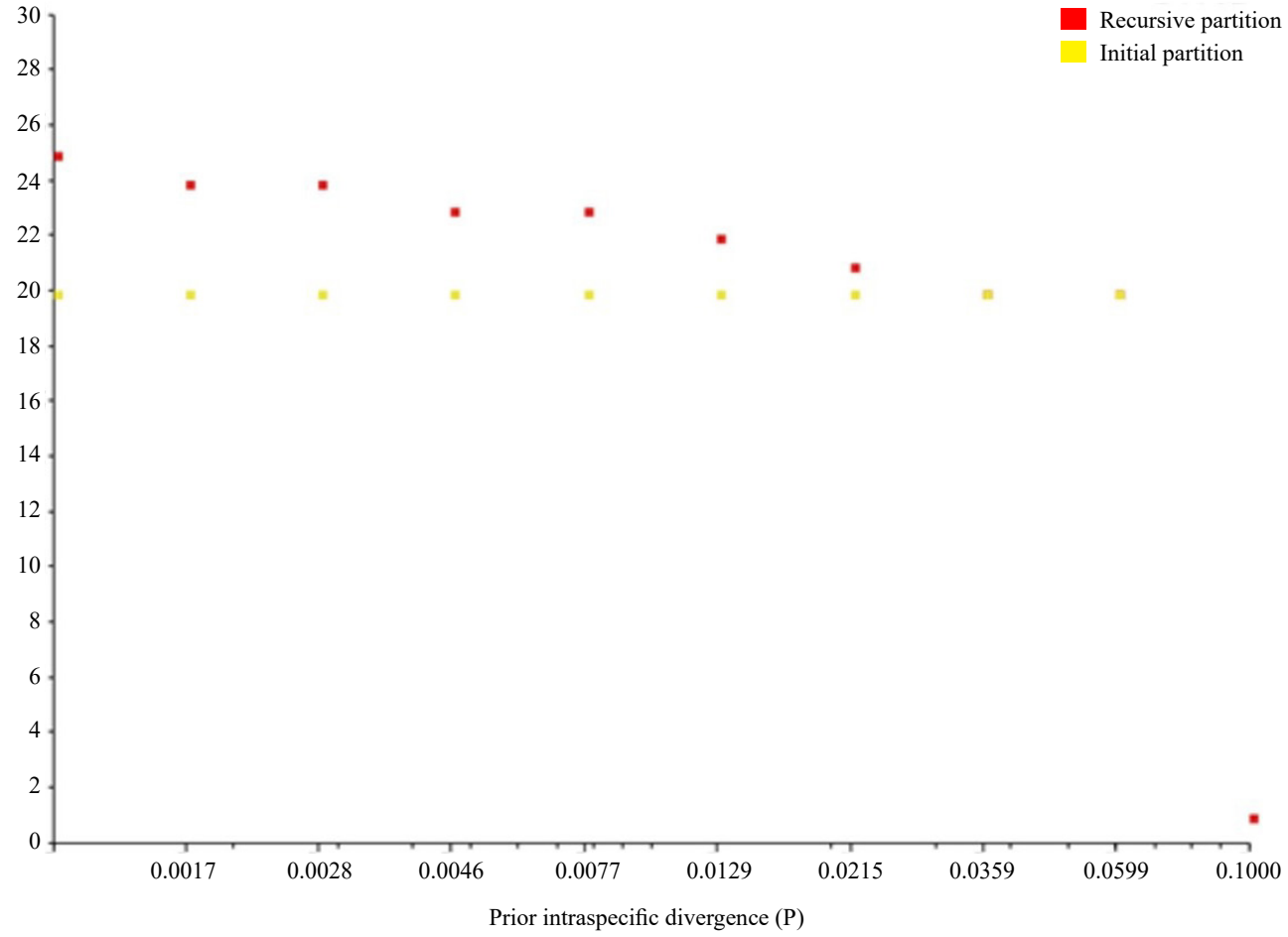

Figure 4. The number of genetically distinct OTUs according to the previous intraspecific divergence values generated by ABGD based on K2P

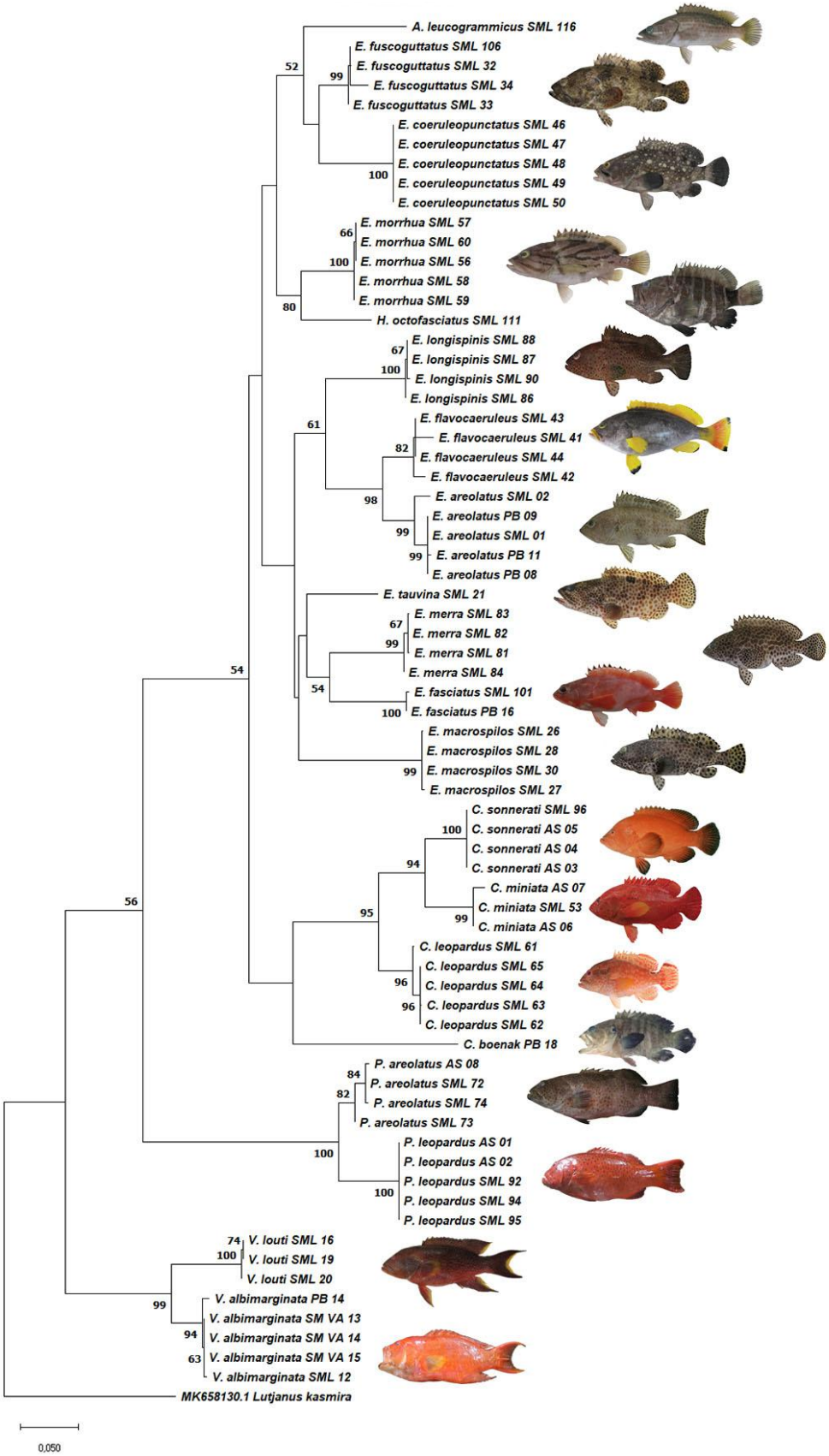

Figure 5. Phylogeny tree of 70 Simeulue and Banyak Islands grouper sequences using the Maximum Likelihood method (Felsenstein 1985; Hasegawa *et al.* 1985; Kumar *et al.* 2018)

4. Discussion

This study identified 20 grouper species belonging to six genera in Simeulue and Banyak Islands with several newly generated DNA barcodes in the Aceh region (*Anyperodon leucogrammicus, E. fuscoguttatus, E. macrospilos, E. morrhua, Hyporthodus octofasciatus,* and *Plectropomus areolatus*). This is slightly lower in species number but higher in genus than the study by Fadli *et al.* (2021), who found 26 species and four grouper genera, respectively. However, sampling was over a more extensive geographical coverage along the coastal areas of Aceh in the earlier study. Jefri *et al.* (2015), observed only seven species from the genus *Epinephelus* in several regions in Indonesia, including Central Java (Karimunjawa), West Nusa Tenggara (Lombok), East Java (Madura), Southeast Sulawesi (Kendari), Lampung, South Sulawesi (Tanakeke) and Papua (Numfor), compared to the 10 in the current study. Similarly, although the grouper diversity observed in this study is lower than in studies in the Philippines (27 species) (Alcantara & Yambot 2016) and India (36 species) (Basheer *et al.* 2017), both studies involved nationwide surveys.

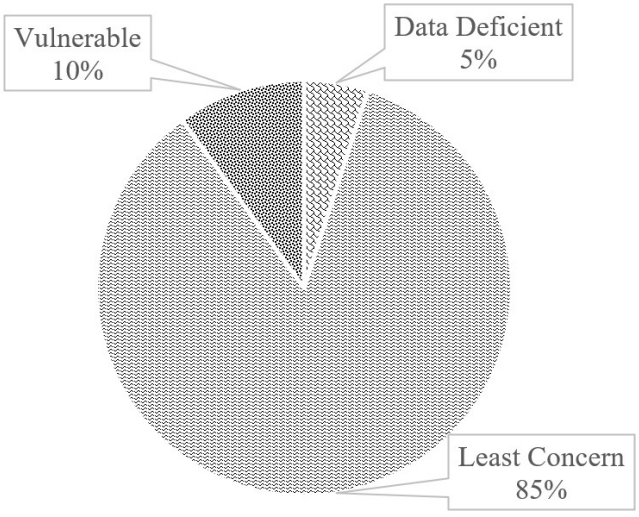

Figure 6. Percentage of grouper in each IUCN category

Comparison of available DNA sequences in GenBank, genetic distance, Barcoding Gap, and ABGD analysis in this research confirms the utility of the COI gene in the precise identification of 20 commercially essential grouper species from Simeulue and the Banyak Islands. Sequence similarity (98-100%) with current sequence information is one of the accurate confirmations of the success of the DNA Barcoding approach (Bhattacharjee *et al.* 2012; Cerutti-Pereyra *et al.* 2012; Alcantara & Yambot 2016). In this study, the similarity of the base sequences was compared using the Genbank data available in BLAST and the BOLD Identification System (IDS). This study also showed that the intraspecies genetic distance was less than 2%, i.e., 0.51%, as expected (Ward 2009). However, the intraspecific value of this study is higher than some previous studies reported for grouper in several regions: Aceh (0.16%) (Fadli *et al.* 2021), Papua (0.247%) (Tapilatu *et al.* 2021), Madura (0.189%)

Table 7. The list of grouper species studied in Simeulue and the Banyak Islands, their IUCN and CITES status (http://www.iucnredlist.org, accessed 20 June 2023, http://www.cites.org/, accessed 20 June 2023)

Genus	Species	Common name	Status CITES	Red list IUCN	Population trend
Anyperodon	*Anyperodon leucogrammicus*	Slender Grouper	Not evaluated	Least Concern	Unknown
Cephalopholis	*Cephalopholis boenak*	Chocolate Hind	Not evaluated	Least Concern	Stable
	Cephalopholis leopardus	Leopard Hind	Not evaluated	Least Concern	Unknown
	Cephalopholis miniata	Coral Hind	Not evaluated	Least Concern	Stable
	Cephalopholis sonnerati	Tomato Hind	Not evaluated	Least Concern	Stable
Epinephelus	*Epinephelus areolatus*	Areolate Grouper	Not evaluated	Least Concern	Unknown
	Epinephelus coeruleopunctatus	Whitespotted Grouper	Not evaluated	Least Concern	Stable
	Epinephelus fasciatus	Blacktip Grouper	Not evaluated	Least Concern	Unknown
	Epinephelus flavocaeruleus	Blue and Yellow Grouper	Not evaluated	Least Concern	Unknown
	Epinephelus fuscoguttatus	Brown-marbled Grouper	Not evaluated	Vulnerable	Decreasing
	Epinephelus longispinis	Longspine Grouper	Not evaluated	Least Concern	Unknown
	Epinephelus macrospilos	Snubnose Grouper	Not evaluated	Least Concern	Stable
	Epinephelus merra	Honeycomb Grouper	Not evaluated	Least Concern	Stable
	Epinephelus morrhua	Comet Grouper	Not evaluated	Least Concern	Stable
	Epinephelus tauvina	Greasy Grouper	Not evaluated	Data Deficient	Unknown
Hyporthodus	*Hyporthodus octofasciatus*	Eightbar grouper	Not evaluated	Least Concern	Unknown
Plectropomus	*Plectropomus areolatus*	Squaretail Coralgrouper	Not evaluated	Vulnerable	Decreasing
	Plectropomus leopardus	Leopard Coral Grouper	Not evaluated	Least Concern	Decreasing
Variola	*Variola albimarginata*	White-edged Lyre Tail	Not evaluated	Least Concern	Decreasing
	Variola louti	Yellow-edged Lyretail	Not evaluated	Least Concern	Stable

(Basith *et al.* 2021), West Papua (0.175%) (Ariyanti & Farajallah 2019), and India (0.044%) (Basheer *et al.* 2017). However, this percentage is lower than that of studies conducted in the following regions: Malaysia (1.27%) (Aziz *et al.* 2016) and the Philippines (0.68%) (Alcantara & Yambot 2016).

In addition, out of 20 observed species, ten grouper species showed 0 (zero) genetic distance within species (*C. leopardus, C. sonnerati, E. coeruleopunctatus, E. fasciatus, E. longispinis, E. macrospilos, E. merra, E. morrhua, P.leopardus*, and *V. louti*). The lower genetic diversity in a population indicates a higher exploitation rate of grouper in nature (Sala *et al.* 2001; Lante *et al.* 2011). Several fish genetic studies have exposed that overfishing may have reduced genetic diversity; for instance, in Aerolate grouper (*Epinephelus areolatus*) in six locations in Aceh (Banda Aceh, Sabang, Aceh Besar, Tapak Tuan, Lhokseumawe and Idi, Aceh) showing a genetic variation of 0.15% (Fadli *et al.* 2021). Nuryanto *et al.* (2018) reported that the grouper *Cromileptes altivelis* showed a genetic variation of 0.016 % in the Spermonde Islands. Overfishing is a significant threat to groupers globally (Sadovy de Mitcheson *et al.* 2013).

Additionally, *E. fuscoguttatus* and *P. leopardus*, two of the 20 grouper species discovered in this study, were categorized by the IUCN as vulnerable. Sadovy de Mitcheson *et al.* (2013) discovered that fish in the vulnerable category tend to be larger than other groupers. This also appears in this research, where it is known that *E. fuscoguttatus* and *P. leopardus* can reach a maximum length of 120 cm (Heemstra & Randall 1993; FRCI 2021; Froese & Pauly 2024). Due to its large size, this species has become a target for fishing in Simeulue and Banyak Islands coupled with high market demand, especially for the *P. leopardus*, which has the highest market value among other groupers (Fadli *et al.* 2021). With the Philippines, Indonesia is a significant grouper exporter (especially live fish, called "The Live Reef Food Fish Trade") to Hong Kong. The two main species exported to Hong Kong and China are *P. leopardus* and *E. fuscoguttatus* (Suhana 2020).

Overall, this study resulted in a comprehensive reference library of molecular COI sequences of various grouper species from Simeulue and the Banyak Islands, which had never been done before in the region. This research, therefore, provides the first molecular database of a commercially important grouper in the area that can be used to support the future management of grouper fisheries in Aceh.

In conclusions, the study offers the first DNA library of genetic information on commercially significant grouper from the waters of the Simeulue and Banyak Islands. In total, 20 grouper species from the Epinephelidae family have been successfully DNA-barcoded. Of the 70 sequences generated, 45 haplotypes were obtained and classified into 20 species with 6 genera each. *Epinephelus* was the most prevalent genus (54%), followed by *Cephalopholis* (19%), *Plectropomus* (13%), *Variola* (12%), *Hyporthodus* (1%), and *Anyperodon* (1 %). The conservation status of 20 species of grouper fish showed that seventeen species (85%) are rated as Least Concern, two species (10%) are rated as Vulnerable, and one species (5%) is rated as Data Deficient.

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