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The first report of DNA barcoding of commercially important fish in Nias Islands, Indonesia

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Abstract. *Ramadhaniaty M, Manurung VR, Khairunnisa, Lubis F, Susetya IE. 2024. The first report of DNA barcoding of commercially important fish in Nias Islands, Indonesia. Biodiversitas 25: 742-753.* The Nias Islands are an archipelago in the western part of North Sumatera, encompassed by the Indian Ocean, and have become a hotspot for demersal and pelagic fishes. These geographical conditions endow the waters around Nias with large fishery resources, leading to their frequent utilization as fishing grounds for Sumatera Island and its surrounding areas. This research aimed to identify the commercially important fish species with the highest catch frequency in the waters of Nias, and this identification marked the initial stage of the sustainable utilization of fish resources. The method used was DNA Barcoding using genes targeting the COI Mitochondrial locus. Therefore, 43 samples were collected from several North and South Nias fish landing sites. The obtained species were classified into 3 groups based on the commodity type: demersal fish, pelagic fish, and Choncricthyes. There were 8 species of demersal fish (*Caesio caerulaurea, Halichoeres scapularis, Lethrinus ornatus, Mulloidichthys flavolineatus, Parupeneus barberinus, Scarus prasiognathos, Plectropomus leopardus,* and *Variola albimarginata*), displaying genetic distances ranging from 0.002-0.275. Pelagic fish consisted of 5 species, namely *Amblygaster clupoides, Caranx ignobilis, Caranx sexfasciatus, Ferdauia ferdau,* and *Scomberomorus commerson*, displaying genetic distances ranging from 0.002-0.267. The next commodities were Chondrichthyes with 9 species (*Carcharhinus sealei, Himantura leoparda, Neotrygon kuhlii, Paragaleus randalli, Pateobatis jenkinsii, Rhynchobatus cf laevis, Spyrna lewini, Taeniura meyeni,* and *Urogymnus granulatus*), displaying genetic distance ranging from 0-0.271. This value indicates that migratory species such as Chondrichthyes have quite extensive movements so that the genetic distance between species and between populations tends to be low.

Keywords: Biodiversity**,** Chondrichthyes, demersal, pelagic, rays, sharks

INTRODUCTION

The Nias Islands, classified under the 3T region category (Frontier (*Terdepan*), Outermost (*Terluar*), and Underdeveloped (*Tertinggal*)) (JDIH 2020), have been designated as a capture fisheries zone (Munthe 2019). This designation includes subzones for pelagic, demersal, and pelagic demersal fish subzones as per North Sumatera Province Regional Regulation Number 4 of 2019, which encompass the territories of North Nias District, West Nias District, South Nias District, Nias District and Gunung Sitoli City. The fisheries potential in the waters of the Nias Islands is highly diverse and unique, where these fish resources migrate towards Banyak Island and Simeulue Island, located north of Nias (Purwanto et al. 2021; Fadhilah et al. 2022). This migration is attributed to the extensive distribution of pelagic larval duration due to Indian Ocean currents (Ramadhaniaty et al. 2018).

However, despite the abundant fishery potential, it remains underexplored. This is evidenced by the limited inventory of fisheries data and the regional economic growth rate that is lower than the national economic growth rate (BPS 2022). Ecological issues, in addition to economic problems, also pose a concern. The 2004 and 2005 earthquakes led to environmental impacts and alterations in the Nias Islands and Aceh Singkil regions, specifically resulting in land subsidence and a decline in fishermen's catches within each area (Siregar et al. 2021). Also, in February 2023, there was an asphalt spill in the waters of North Nias, posing potential damage to fish habitat and coral reefs in the affected areas.

The high diversity and abundance of fish in Nias waters have increased fishing activity, potentially causing a decline in the fish population (Taurusman et al. 2020). The capture process exerts influence across various levels of biological habitats, from individual organisms to populations, demographic and genetic characteristics, and the dynamics of communities and ecosystems, thereby impacting trophic structures and energy flows (Stergiou et al. 2007). An ecosystem delineates a biological community's habitat where functional relationships occur between biotic components and their abiotic environment within a defined ecological boundary in a specific area. In this study, it is important to determine and understand the conservation status of each

species for anticipating and implementing sustainable fisheries management and utilization.

The initial step in managing fish resources involves accurately identifying species and forming the groundwork for subsequent steps. An inaccurate initial step can result in subsequent deviations, leading to either underestimating or overestimating results. This, in turn, impacts the optimal level of exploitation and subsequent management strategies (Ayunda et al. 2018). The morphological similarities among fish can lead to mislabeling, thereby influencing the utilization and management of the fisheries resources. The challenges posed by morphology-based identification techniques, compounded by a decrease in the number of taxonomists, have been met with the emergence of molecular identification techniques for species identification (Steinke et al. 2009; Zhang and Hanner 2011). Hebert et al. (2003) successfully introduced the DNA barcoding method, utilizing the gene sequence within mitochondrial DNA, specifically the Cytochrome Oxidase subunit I (COI), as a barcode for swift and precise species identification (Hebert et al. 2003). The COI gene serves as a commonly employed barcoding marker in animals due to its highly robust universal primer and wider phylogenetic signal range, surpassing other mitochondrial genes (Powers et al. 2018). Prior research on identifying key commercial fish species in Nias waters has not been conducted despite these waters being a primary hub for fishing activity in North Sumatra and its adjacent areas. Hence, conducting research utilizing a molecular method to identify commercial fish is crucial, serving as a fundamental fish data census and providing initial study material for establishing sustainable fisheries management in Nias waters.

Considering these discoveries and challenges, collaborative research involving academics from the Universitas Sumatera Utara, Universitas Syiah Kuala, and Universitas Teuku Umar is imperative. The primary objective is to identify the commercially important fish species with the highest catch frequency in the waters of Nias. This collaborative effort aims to ensure the optimal utilization of fisheries resources.

MATERIALS AND METHODS

Study area

This study was conducted in several fish landing sites in Nias Islands, North Sumatra, Indonesia, in August 2023 (Figure 1). The collecting sites were selected using a purposive sampling method, aiming for geographic representation. Specifically, two fish landing sites were selected in Northern Nias and Southern Nias (Table 1).

Fish collection and species identification method

Fish were collected from fish landing port and collectors by taking all samples based on three categories; demersal, pelagic and Chondrichthyes. However, the fish that were genetically analyzed were based on groups that were found dominantly and had important economic value in Nias waters. The demersal and pelagic fish were subsequently documented following the Fish Bold protocol (Steinke and Hanner 2011) to facilitate identification. Morphological identification was carried out using the Reef Fish identification book (Heemstra and Randall 1993), Allen (1999), White et al. (2013), Fish Base (Froese and Pauly 2023), and Last et al. (2016a,b). Species name, author, publication year, geographic distribution, and family were cross-checked using Fishbase and IUCN.

Table 1. Coordinates of the sites in Nias Islands, North Sumatra, Indonesia

Site	Coordinates	Administrative area	Part of Nias
1	0°02'14"S 98°16'01"E	Tello Islands	Southern Nias
2	0°33'55"N 97°49'12"E	Teluk Dalam	Southern Nias
3	0°56'28"N 97°24'22"E	Faekhuna'a	Northern Nias
4	1°23'46"N 97°10'17"E	Lahewa	Northern Nias

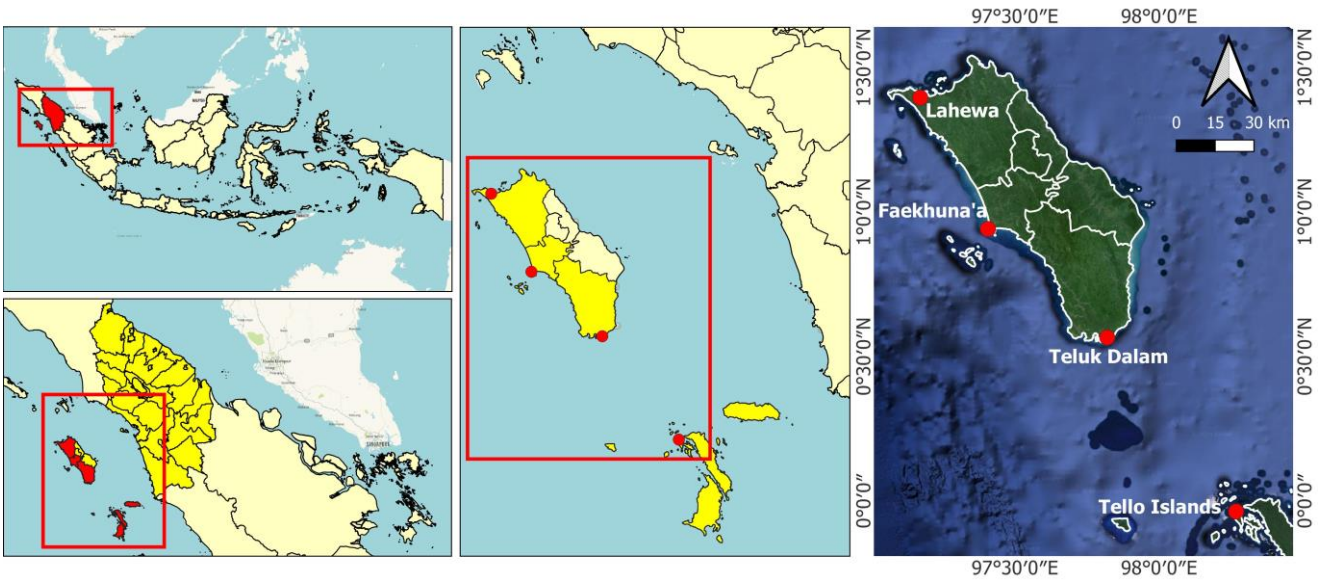

Figure 1. Collecting sites in Nias Islands, North Sumatra, Indonesia

Laboratory analysis

The fish tissue samples from pectoral fin (demersal and pelagic), shark and rays (meat and fin) underwent an extraction process to isolate DNA. The extraction method followed the Chelex 10% protocol. Subsequently, the extracted samples underwent analysis in the subsequent stage, namely Polymerase Chain Reaction (PCR). The PCR process used the BIONESIA laboratory protocol. The primers used in the amplification process for fish samples were FISH F1 (5'-TCA ACC AAC CAC AAA GAC ATT GGC AC-3') and FISH R1 (5'-TAG ACT TCT GGG TGG CCA AAG AAT CA-3') (Ward et al. 2005). The total PCR reaction volume was 26 µL, comprising a mixture of 2 µL DNA template obtained from the extraction, 1.25 µL of each primer at a concentration of 10 mM, 9 µL ddH2O, and 12.5 µL Ready-mix. The reaction mixture was amplified using the Applied Biosystems™ 2720 Thermal Cycler machine. The temperature and time profile of the PCR protocol used was as follows: Initial Denaturation at 94°C for 3 minutes, followed by Denaturation stage at 94°C for 30 seconds, Annealing at 50-55°C for 30 seconds, and Extension stage at 72°C for 60 seconds. The Denaturation to Extension stages were executed for 38 cycles, culminating in a final extension at 72°C for 2 minutes. Subsequently, the PCR outcomes were visualized using a 1% Agarose gel stained with Nucleic Acid Gel Stain (GelRed®). Samples undergoing successful amplification will be subject to nucleotide base translation using the Sanger dideoxy method at PT Genetika Science Jakarta (Limited Liability Company).

Data analysis

The sequenced samples' results were presented as sequence data in Ab1 files, which were subsequently analyzed using a computer. The acquired sequence data underwent editing and alignment procedures utilizing the ClustalW method within the MEGA X program. Each base arrangement was meticulously checked manually to ensure the high quality and accuracy of the utilized data. The data was cross-referenced with the database in the GenBank (NCBI) using the Basic Local Alignment Search Tool (BLAST) available on the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

In addition to employing the BLAST method, the data underwent analysis using a phylogenetic tree to assess the kinship relationships among samples. This method confirmed the BLAST results, validating the identification at the species level. The kinship tree was constructed using the Neighbor-Joining (NJ) method with 1000 bootstrap replications in the MEGA software. The genetic distance values were analyzed using the p-distance method to compare individual samples. Genetic diversity analysis was conducted utilizing DNAsp software, facilitating calculations for haplotype composition, Haplotype diversity (Hd), and nucleotide diversity (π). The intra and interspecies variation in mtDNA COI genes was assessed by tallying nucleotide counts. The haplotype diversity index was analyzed by the DNASP program (Rozas et al. 2003).

RESULTS AND DISCUSSION

Commercially important fish biodiversity in the Nias Islands

The fish sampling was focused on areas with the highest catch frequency, encompassing both fishing zones and fish landing areas. However, the collected number and variety may not comprehensively represent all fish species in Nias waters. This was influenced by the southern season, prompting fishermen to reduce their fishing activities, affecting the diversity and quantity of sampled fish. Therefore, 50 demersal fish samples underwent DNA Barcoding, utilizing the mitochondrial COI target gene. The amplification resulted in base lengths of 637 bp, identifying 22 species spanning 14 families. All species were validated using information from the NCBI BLAST website, exhibiting similarity ranging between 83.78% and 100% in similarity sequence data. The fish categories were divided into three groups: demersal fish (8 species), pelagic fish (5 species), and sharks-rays (9 species). The species composition is listed in Table 2. Sequences obtained from Nias waters were amalgamated with sequences available in NCBI (Table 3) to corroborate and validate the identification results. In recent decades, DNA Barcoding has emerged as a suitable tool to rectify morphological identification errors through molecular identification (Ramadhaniaty et al. 2023). This classification aims to explore biodiversity and interrelationships within the same commodity groups. Numerous samples of sharks and rays were procured from North Nias Regency, with rays prevailing as the dominant biota discovered.

The phylogenetic tree (Figures 2-4) combines data from Genbank (secondary) and primary sequence data from this study. The ID shown in the name in the tree is the accession number or sequence ID in Genbank (Table 3). Phylogenetic reconstruction is a general method to interpret and analyze phylogenetic relationships between species (Kapli et al. 2020). Organisms within a group are deemed closely related if they share numerous similar characteristics with their fellow members, indicating a common ancestry (Bhagabati et al. 2019). The three reconstructed phylogenetic trees exhibited bootstrap values ranging from 58 to 100 for demersal fish, 100 for pelagic fish, and 90 to 100 for Chondrichthyes. This value indicated stability within the sequence data, meeting the prerequisites for interpreting phylogenetic tree data. Goncalves et al. (2019) states that a bootstrap value ranging from 50% to 90% or higher is generally considered a strong branching pattern. Bootstrapping is conducted to apply differential weighting through resampling of the initial data. This process aims to generate consistent clades if the data are reliable, reflecting the true phylogeny while demonstrating minimal homoplasy.

Table 2. Species composition based on molecular identification (DNA Barcoding)

Family	Species	N	Accession number	Query cover (%)	Identity (%)
Clupeidae	*Amblygaster clupeoides* (Bleeker, 1849)	1	KF728074.1	100	94.5
Carangidae	*Caranx sexfasciatus* (Quoy & Gaimard, 1825)	5	KU199210.1	100	100
	Ferdauia ferdau	2	OQ387554.1	100	100
	Caranx ignobilis (Forsskål, 1775)	1	MF383170.1	100	99.85
Scombridae	*Scomberomorus commerson* (Lacepède, 1800)	2	OQ387544.1	100	100
Serranidae	*Variola albimarginata* (Baissac, 1953)	7	KM226315.1	100	100
	Plectropomus leopardus (Lacepède, 1802)	1	KM226310.1	100	99.85
Caesionidae	*Caesio caerulaurea* (Lacepède, 1801)	1	FJ237605.1	99	99.23
Mullidae	*Parupeneus barberinus* (Lacepède, 1801)	1	JQ350180.1	99	99.69
	Mulloidichthys flavolineatus (Lacepède, 1801)	1	OQ387397.1	100	99.85
Labridae	*Halichoeres scapularis* (Bennett, 1832)	1	JF435007.1	99	99.69
Lethrinidae	*Lethrinus ornatus* (Valenciennes, 1830)	1	MN870327.1	99	100
Scaridae	*Scarus prasiognathos* (Valenciennes, 1840)	2	OQ387530.1	98-99	83.78-100
Hemigaleidae	*Paragaleus randalli* (Compagno, Krupp & Carpenter, 1996)	4	JN313302.1	99	99.85
Carcharhinidae	*Carcharhinus sealei* (Pietschmann, 1913)	2	OQ386529.1	100	100
Rhinidae	*Rhynchobatus cf. laevis* (Bloch & Schneider, 1801)	1	KF899689.1	100	99.69
Sphyrnidae	*Sphyrna lewini* (Griffith & Smith, 1834)	1	MG816735.1	100	100
Dasyatidae	*Taeniura meyeni* (Müller & Henle, 1841)	1	KF899825.1	100	99.54
	Himantura leoparda (Manjaji-Matsumoto & Last, 2008)	1	JX263418.1	94	99.84
	Pateobatis jenkinsii (Annandale, 1909)	2	MG792095.1	89-99	89.8-99.39
	Neotrygon kuhlii (Müller & Henle, 1841)	4	KU497913.1	100	99.54-100
	Urogymnus granulatus (Macleay, 1883)	1	OR252866.1	100	99.85
Total		43			

Note : N: Number of samples

Table 3. Composition of external sequences downloaded from GenBank

Species	Accession number and reference
Caesio caerulaurea	OR113815, OR113822, OR113821(Huang et al. 2023)
Lethrinus ornatus	OQ851998 (Nuryanto 2023 (Unpublished), MN870266, MN870327 (Limmon et al. 2020)
Mulloidichthys flavolineatus	OQ387567 (Bemis et al. 2023), OR113991(Huang et al. 2023)
Parupeneus barberinus	OP720928, OP720929 (Smith et al. 2023), OQ387465 (Bemis et al. 2023)
Halichoeres scapularis	OR113935 (Huang et al. 2023), OQ385516, OQ386908 (Bemis et al. 2023)
Plectropomus leopardus	ANGBF3385-12 RESIC303-11, GBGCA3868-13 (BOLD)
Scarus prasiognathos	OK348025 (Xiao et al. 2022), OQ387530 (Bemis et al. 2023)
Caranx sexfasciatus	OR346255, OR113831, OR113832 (Asha et al. 2023 (Unpublished)
Caranx ignobilis	MW498555 (Abidin et al. 2021), OQ284063 (Ramees 2023 (Unpublished)), LC646711 (Kimura et al. 2022)
Ferdauia ferdau	OQ386627 (Bemis et al. 2023)
Scomberomorus commerson	MW590647 (Mahapatra 2021 (Unpublished)), MT701623, MT701622 (Alhababy et al. 2020 (Unpublished))
Amblygaster clupeoides	EF607313 (Zhang 2011)
Pateobatis jenkinsii	MG792096 (Arshaad et al. 2018 (Unpublished)), MK422144 9 Ravi et al. 2018 (Unpublished)), GU673708
Himantura leoparda	OQ628402 (Naylor et al. 2012), MG774913, MG774922 (Arshaad et al. 2018 (Unpublished))
Urogymnus granulatus	OR252866 (Baldaniya, A 2023 (unpublished))
Taeniura meyeni	OQ385125 (Loh et al. 2023), OR227126 (Jaquemet et al. 2023), GU673424
Rhynchobatus cf. laevis	DQ108192, DQ108197, DQ108198 (Ward et al. 2005)
Sphyrna lewini	FJ519447, FJ519452 (Wong et al. 2009)
Carcharhinus sealei	OQ385024, OQ385025, OQ386462 (Loh et al. 2023)

Commercially important demersal fish in Nias waters

There were 8 species of demersal fish collected from several fish landing sites in Nias. The phylogenetic tree reconstruction of demersal fish demonstrated a distinct clade separation among groupers, snappers, and parrotfish (Figure 2). Phylogenetic tree reconstruction of demersal fish shows 3 main clades, namely Scariadae with a bootstrap of 100, Serranidae with a bootstrap of 58 and Caesionidae, Mullidae and Lethridae with a bootstrap of 34. The most extensive clade encompasses all species from the Perciformes, while parrotfish originate from the order Labriformes. The separation of this clade can also be seen from the genetic distance value which shows a range of 0.274-0.287, which is the highest value among the others. Genetic distance

analysis is based on 2 variables, namely within species and between species, where the bolded values in the table indicate the genetic distance values within species. The observed clade separation corresponded to the outcomes depicting the greatest genetic distance within species, ranging from 0.262 to 0.287. Meanwhile, the lowest genetic distance between species was found between *Mulloidichthys flavolineatus* and *Parupeneus barberinus*, with a value of 0.154 (Table 4). The close distance between these two species is attributed to their classification as a subgroup of goatfish within the same family (Mullidae), indicating their close relationship. The genetic distance between demersal and reef fish ranged between 0.0002 to 0.005, whereas the genetic distance between species ranged from 0.262 to 0.287 (Table 4). The overall genetic distance value from this study was in the low category for both within and between species. This phenomenon was attributed to the cryptic phase observed in demersal fish, contributing to a widespread distribution of larvae, thus resulting in an even dispersal. Similar studies combining original sequences from the Philippines with sequences from Genebank also observed comparable trends. They reported that all demersal fish species exhibited genetic distances below 5% or 0.05 (Xiao et al. 2022).

Variola albimarginata was the most dominant fish species collected in this study due to their high abundance in all landing sites (Table 2). This demersal fish is a highly demanded commodity with substantial market demand and commercial significance, inhabiting numerous coral reef ecosystems within oceanic waters (Paxton et al. 2021). The geographical location of Nias waters within the Indian Ocean, bordered by a series of islands, renders the coral reef ecosystem in this region a hotspot for grouper larvae aggregation (Grüss et al. 2019). The molecular identification research that identified *Variola albimarginata* and *Plectropomus leopardus* from the western Indian Ocean was conducted in Simeulue and the North Coast of Aceh. In these studies, these two groupers were identified as highly abundant fish and displaying remarkably similar morphology, thereby necessitating meticulous morphological identification procedures to prevent mislabeling (Batubara et al. 2017; Fadli et al. 2021; Andriyono et al. 2022).

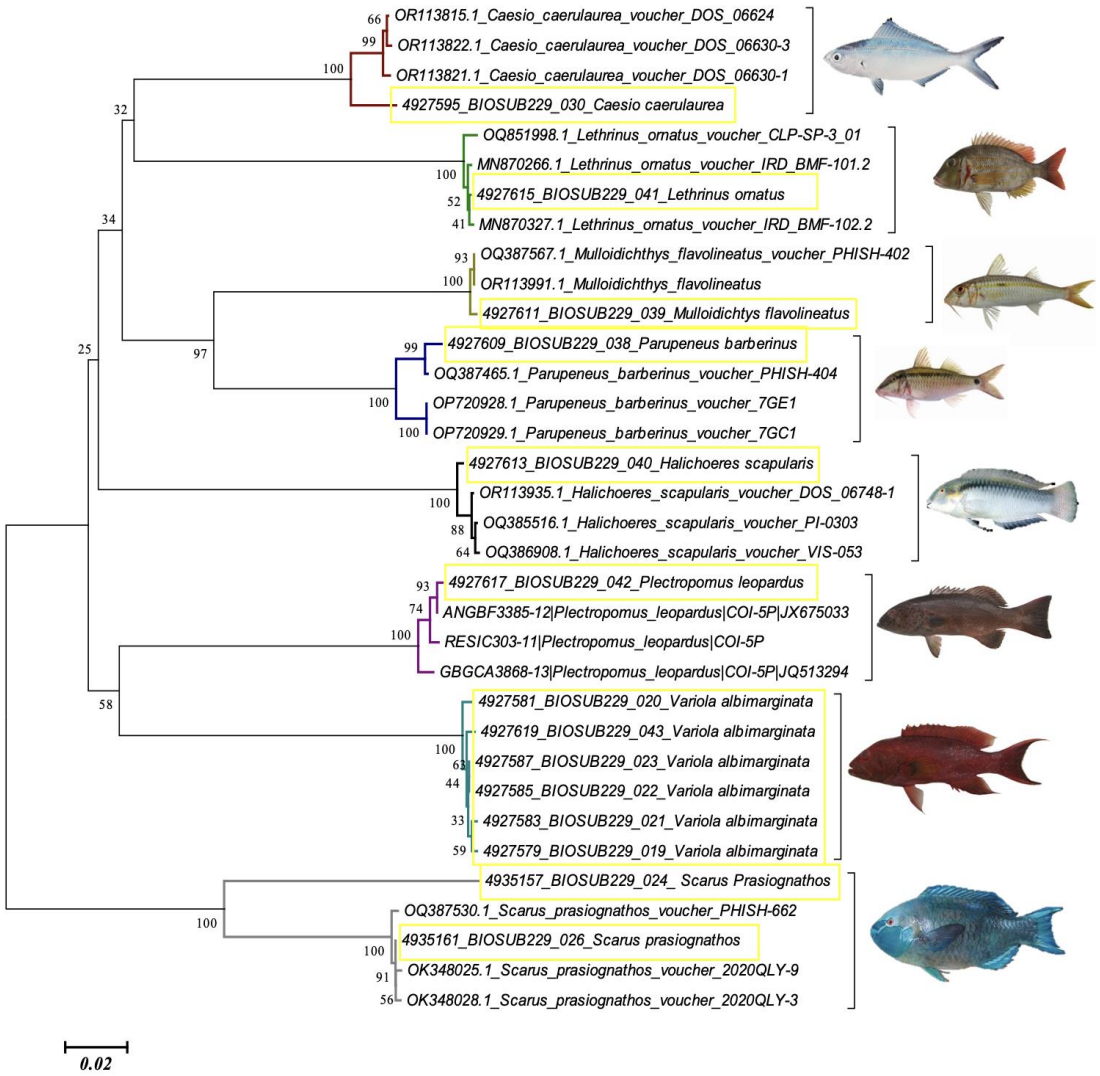

Figure 2. Phylogenetic tree reconstruction of demersal fish in Nias waters (Yellow shape are primary sequences)

Table 4. Genetic distance between and within demersal fish populations (Bold) in Nias waters, North Sumatra, Indonesia

Species	1	2	3	4	5	6	7	8
Caesio caerulaurea	**0.015**							
Halichoeres scapularis	0.217	**0.005**						
Lethrinus ornatus	0.196	0.232	**0.004**					
Mulloidichthys flavolineatus	0.214	0.241	0.221	**0.002**				
Parupeneus barberinus	0.184	0.237	0.211	0.154	**0.016**			
Scarus prasiognathos	0.275	0.287	0.262	0.264	0.274	**0.057**		
Plectropomus leopardus	0.238	0.226	0.248	0.224	0.228	0.273	**0.009**	
Variola albimarginata	0.212	0.249	0.250	0.253	0.232	0.280	0.216	**0.004**

Table 5. Composition of genetic diversity of demersal fish in Nias waters, North Sumatra, Indonesia

Species	Hd	π	Hap
Caesio caerulaurea	1	0.014	4
Lethrinus ornatus	0.833	0.003	3
Mulloidichtys flavolineatus	0.666	0.002	2
Parupeneus barberinus	0.833	0.012	3
Halichoeres scapularis	1	0.005	4
Plectropomus leopardus	1	0.008	4
Variola albimarginata	0.800	0.002	4
Scarus prasiognathos	1	0.003	4
All Species	0.988	0.178	28

Note: Hd: Haplotype diversity, π: Nucleotide diversity, Hap: Haplotype

The genetic diversity of demersal fish was in a very high category at 0.98 (Table 5), where the range of genetic diversity was from 0 to 1 (Ramadhaniaty et al. 2018). The species with the highest genetic diversity values were *Caesio caerulaurea*, *Halichoeres scapularis, Plectropomus leopardus,* and *Scarus prasiognathos,* targeted for demersal fish exploitation. While *Mulloidichtys flavolineatus* exhibited the lowest genetic diversity at 0.666, this fish was not the primary catch, thereby posing no significant threat to its population in nature (Martinez et al. 2018). The high and low genetic diversity influenced the adaptation and self-defense mechanisms of living organisms against threats within their habitat (Dudu et al. 2015; Shen et al. 2016). The higher the intraspecies genetic distance, the higher the genetic variation or diversity within the species. Conversely, low genetic variation occurs as a response to decreased species abundance within a population due to exploitation, subsequently limiting the ability to adapt (Madduppa et al. 2018). Ecological factors significantly contribute to the genetic diversity of organisms. Fish inhabiting ecosystems like coral reefs and seagrasses exhibit remarkable adaptability to intense fishing activities and dynamic environmental changes. In addition, demersal fish larvae spread and inhabit waters influenced by currents and other physical oceanographic phenomena, leading to this adaptation providing these fish with robust defenses in nature (Dalongeville et al. 2016).

The demersal and coral fish species discovered in this study exhibited lower numbers than those found in several other studies conducted along the island of Sumatera. Rizal and Jaliadi (2018) research in West Aceh discovered 12 families of demersal fish, including 12 species from the Lutjanidae family and 6 from the Serranidae family. However, the findings contrast with research conducted in Bintan Regency, where only 7 demersal fish species were identified (Lubis et al. 2021).

Commercially important pelagic fish in Nias waters

The phylogenetic tree of pelagic fish consisted of *Caranx sexfasciatus, Caranx ignobilis, Ferdauia ferdau, Scomberomorus commerson,* and *Amblygaster clupeoides*. A bootstrap value of 100 divided these fish into 3 clades: the first clade comprised the genus Caranx, the second consisted of the genus *Scomberomorus commerson*, and the last clade included *Amblygaster clupeoide* (Figure 3).

The largest clade within the Carangidae group exhibited the lowest genetic distance between species, specifically between *Caranx ignobilis* and *Caranx sexfasciatus* at 0.097. Conversely, the highest genetic distance between species was found between *Caranx ignobilis* and *Amblygaster clupeoides* at 0.267 (Table 6). The dominant pelagic fish found in this study belongs to the Carangidae family, consisting of three species and totaling eight individuals sampled. The Carangidae fish species utilize estuary habitats and seagrass ecosystems as spawning areas before eventually moving to open waters as pelagic fish (Sartori et al. 2021). The high density of seagrass ecosystems present in the bays along the Nias waters substantiates this. Nias waters harbor significant potential due to their proximity to three primary complex ecosystems (coral reefs, seagrass, and mangroves), enriching these waters with high aquatic productivity (Abdul Wahab et al. 2018).

Genetic diversity among pelagic fish exhibited a high range between 0.6 and 0.9, as indicated in Table 7 *Scomberous commerson,* known for its extensive migratory patterns, exhibited the highest diversity. Generally, fish undertaking long migratory movements tend to demonstrate elevated genetic diversity due to their heightened adaptability to dynamic environments. Multiple studies have consistently indicated that the life history traits of *Scomberous commerson*, such as its spawning behavior, extended pelagic larval stage, and proficient swimming capabilities, significantly contribute to the genetic heterogeneity observed across the studied population. This underscores the species' remarkable genetic diversity, attributed to its adaptable nature, unpredictable movements, and ability to cope with environmental dynamics (Fauvelot and Borsa 2011; Habib and Sulaiman 2016). These genetic diversity results were also in line with the high number of haplotypes in *Scoberous commerson* and *Caranx sexfasciatus*; this was

confirmed by Akbar et al. (2014) and Wahyudewantoro et al. (2023) that the number of diverse haplotypes influenced the genetic diversity in a population where the more diverse the haplotype types, the higher the genetic diversity of the population. In addition, the diversity of haplotypes shows that the species has good adaptability to environmental changes (Asril et al. 2022).

Commercially Chondrichthyes in Nias

The number of Chondrichthyes collected from two locations (South Nias and North Nias waters) totaled 9 species, comprising 6 species of rays and 3 species of sharks. Based on the reconstruction of the phylogenetic tree of Elasmobranch fish, there was a distinct clade separation between the stingray species *Pateobatis jenkinsii* and *Himantura leoparda*. The largest clade consisted of the Order Myliobatiformes. This clade separation aligned with the quite far genetic distance values between the three species, measuring 0.000 and 0.047. Meanwhile, *Carcharhinus sealei* and *Paragaleus randalli* were grouped in the same clade. The genus Carcharhinus was among the most economically important groups of shark found in tropical regions worldwide (White and Sommerville 2010). The dominant stingray species was *Neotrygon kuhlii*, while the shark species was *Paragaleus randalli*. Aquatic habitat conditions in the waters of South Nias and North Nias support the habitat of Elasmobranchs, especially stingrays that were generally adult-sized and targets for fishing. In addition, in the Andaman Islands of the Indian Ocean, fisheries have reported declines in the population of several shark and ray species, but the targeted fishery for Chondrichthyes persists (Tyabji et al. 2022).

The number of Chondrichthyes found in this study was relatively small compared to several other studies conducted along the island of Sumatera. The level of similarity in the genetic distance (pairwise distance) between *Taeniura meyeni* and *Urogymnus granulatus* indicated that both belong to the same main clade. Meanwhile, the sharks *Carcharhinus sealei* and *Paragaleus randalli* exhibited a similar genetic distance and belonged to the same main clade. *Spyrna lewini* clustered in the main clade, which differed greatly from the other species. This showed that morphometrics aligned with genetic data, so it could validate the differences between the 3 species, as seen in Table 8. The estimated genetic distance between our samples and reference sequences was used to indicate the most likely candidate species (Almerón-Souza et al. 2018). Genetic distance involves analyzing nucleotide sequences, where various species exhibit low genetic distances among themselves (Maduppa et al. 2020).

Table 6. Genetic distance between and within pelagic fish populations (Bold) in Nias waters, North Sumatra, Indonesia

Species	1	2	3	4	5
Amblygaster clupoides	**0.040**				
Caranx ignobilis	0.267	**0.003**			
Caranx sexfasciatus	0.255	0.097	**0.003**		
Ferdauia ferdau	0.253	0.155	0.144	**0.002**	
Scomberomorus commerson	0.239	0.236	0.222	0.222	**0.031**

Table 7. Composition of genetic diversity of pelagic fish in Nias waters, North Sumatra, Indonesia

Species	Hd	π	Hap
Caranx sexfasciatus	0.750	0.002	4
Caranx ignobilis	0.833	0.003	3
Ferdauia ferdau	0.400	0.001	2
Scromberomorus commerson	0.900	0.028	4
Amblygaster clupeoides	0.666	0.038	2
All species	0.94667	0.138	15

Note: Hd: Haplotype diversity, π: Nucleotide diversity, Hap: Haplotype

Table 8. Genetic distance between and within populations of Chondrichthyes (Bold) in Nias waters, North Sumatra, Indonesia

Species	1	2	3	4	5	6	7	8	9
Carcharhinus sealei	**0.003**								
Himantura leoparda	0.244	**0.047**							
Neotrygon kuhlii	0.252	0.205	**0.012**						
Paragaleus randalli	0.118	0.264	0.237	**0.000**					
Pateobatis jenkinsii	0.268	0.134	0.195	0.282	**0.015**				
Rhynchobatus cf laevis	0.225	0.237	0.241	0.244	0.245	**0.002**			
Spyrna lewini	0.125	0.242	0.253	0.146	0.271	0.235	**0.023**		
Taeniura meyeni	0.255	0.226	0.191	0.273	0.238	0.231	0.252	**0.004**	
Urogymnus granulatus	0.232	0.174	0.236	0.260	0.158	0.231	0.259	0.223	**0.002**

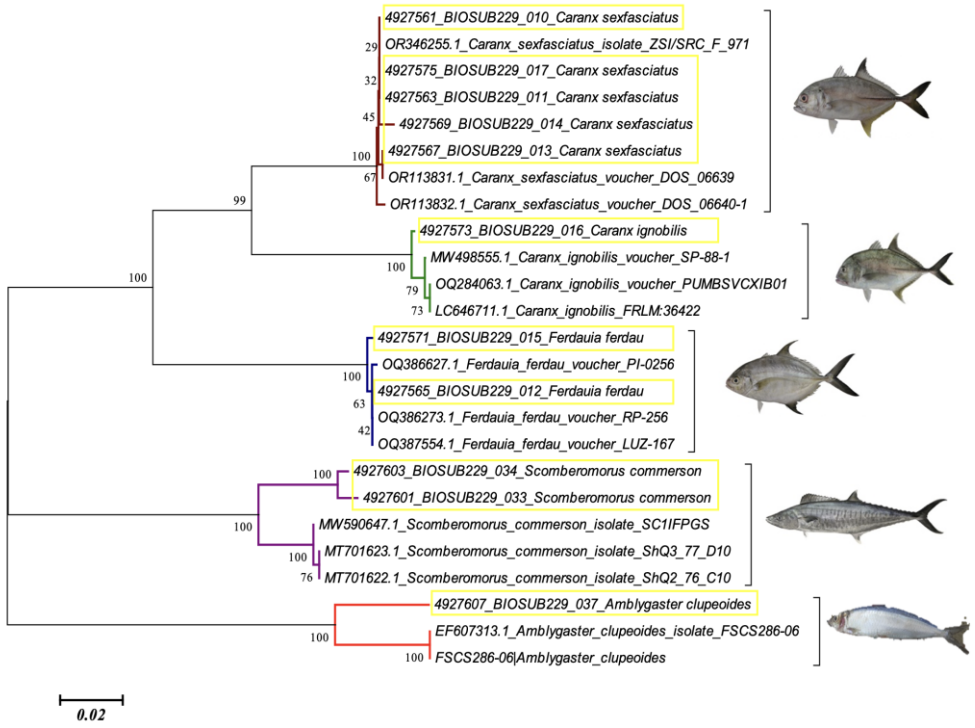

Figure 3. Reconstruction of the phylogenetic tree of pelagic fish in Nias waters, North Sumatra, Indonesia (Yellow shape are primary sequences)

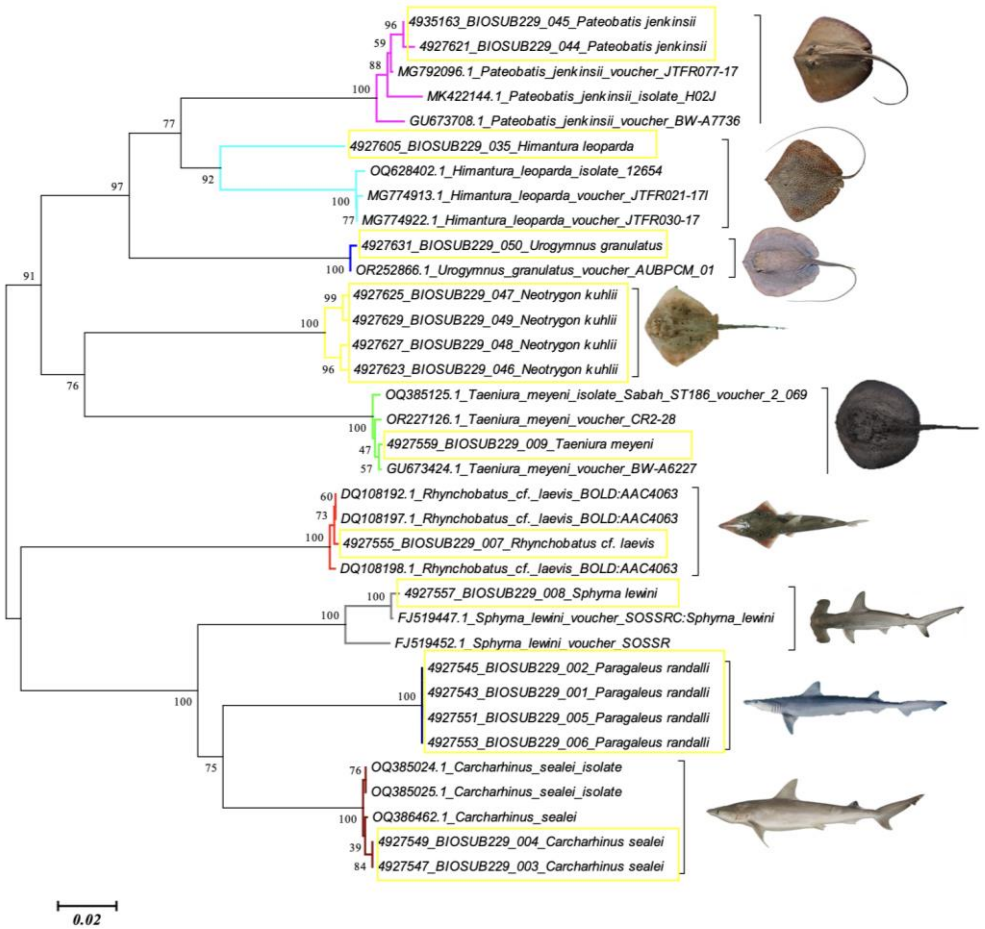

Figure 4. Reconstruction of the phylogenetic tree of shark-ray fish in Nias waters, North Sumatra, Indonesia (Yellow shape are primary sequences)

Table 9. Composition of genetic diversity of Chondrichthyes in Nias waters

Species	Hd	π	hap
Carcharhinus sealei	0.600	0.002	2
Paragaleus randalli	0	0	2
Spyrna lewini	1	0.146	3
Rhynchobatus cf. Laevis	0.833	0.002	3
Taeniura meyeni	1	0.004	4
Neotrygon kuhlii	0.833	0.008	3
Urogymnus granulatus	1	0.001	2
Himantura leoparda	1	0.045	4
Pateobatis jenkinsii	1	0.015	5
All Species	1	0.027	28

Note: Hd: Haplotype diversity; π: Nucleotide diversity; Hap: Haplotype

The genetic diversity observed in Chondrichthyes felt within the high category, specifically ranging from 0 to 1, as outlined in Table 9. There were 5 fish species with the highest diversity: *Spyrna lewini*, *Taeniura meyeni*, *Urogymnus granulatus*, *Himantura leoparda*, and *Pateobatis jenkinsii*. Based on the molecular analysis, among the nine types of Chondrichthyes studied, *Pateobatis jenkinsii* produced the highest number of haplotypes, specifically five haplotypes. High haplotype diversity is coupled with low nucleotide because marine species can be categorized by haplotype and nucleotide to interpret population history. This showed high genetic diversity compared to the other 8 species. Fish with high diversity tend to adapt more easily to environmental changes and are more susceptible to and resilient against diseases, thereby ensuring safer populations and less vulnerable to the threat of extinction (Ralls et al. 2018). The similarities among stock could result from homogeneous environmental factors and shared habitat characteristics (Hanif et al. 2019). The abundance of *Pateobatis jenkinsii* corroborates this found in fish marketplaces and the waters of North Nias.

Conservation status

The IUCN conservation status of fish found at the 2 fish landing sites of South Nias and North Nias are shown in Table 10. A total of 2 fish species, *Rhynchobatus cf laevis* and *Sphyrna lewini*, were classified as Critically Endangered (CR) in 2019. Manurung et al. (2022) found *Rhynchobatus australiae* in the waters of the Malacca Strait, categorized as Vulnerable (VU) at the IUCN status, indicating vulnerability within its population. *Rhynchobatus cf laevis* and *Sphyrna lewini* were discovered in the waters of Faekhuna'a, with both sharks being small. Additionally, the habitat of *Sphyrna lewini* was also found in Ujong Baroh PPI near the Indian Ocean, as reported by (Ramadhaniaty et al. 2023). This shark is the most frequently exploited among the Sphyrnidae family and ranks fourth most commonly found and sampled species (Feitosa et al. 2018). Genus Sphyrna identified in the present study are threatened with extinction (Martins et al. 2021). According to fishermen's information, the waters of Faekhuna'a served as a habitat for hammerhead sharks and was a primary catch zone. There's an assumption that *Sphyrna lewini* extends its habitat to the waters of West Aceh (Ramadhaniaty et al. 2023).

Table 10. Conservation status

Family	Species	Local name	Status	Location	
				South Nias	North Nias
Carangidae	*Caranx sexfasciatus*	-	LC	+	+
	Caranx ignobilis	-	LC	+	+
	Ferdauia ferdau	-	LC	+	+
Dorosomatidae	*Amblygaster clupeoides*	-	LC	+	
Scombridae	*Scomberomorus commerson*	Tenggiri	NT	+	+
Caesionidae	*Caesio caerulaurea*	-	LC	+	+
Labridae	*Halichoeres scapularis*	-	LC	+	+
Lethrinidae	*Lethrinus ornatus*	-	LC	+	+
Mullidae	*Mulloidichtys flavolineatus*	Bayam-bayam	LC	+	+
	Parupeneus barberinus	-	LC	+	+
Scaridae	*Scarus prasiognathos*	kakatua	LC	+	+
Epinephelidae	*Plectropomus leopardus*	Kakap	LC	+	+
	Variola albimarginata	Kakap	LC	+	+
Carcharhinidae	*Carcharhinus sealei*	-	VU	+	+
Hemigaleidae	*Paragaleus randalli*	-	VU	+	+
Dasyatidae	*Himantura leoparda*	-	VU	+	
	Neotrygon kuhlii	Pari totol biru	DD	+	+
	Pateobatis jenkinsii	-	VU	+	
	Taeniura meyeni	-	VU		+
	Urogymnus granulatus	-	VU		+
Rhinidae	*Rhynchobatus cf laevis*	Pari kekeh	CR		+
Sphyrnidae	*Spyrna lewini*	Hiu martil	CR		+

Paragaleus randalli and Scomberomorus commerson found in South Nias and North Nias were included in the Near Threatened (NT) category. Six fish species, namely *Carcharhinus sealei*, *Himantura leoparda*, *Pateobatis jenkinsii*, *Taeniura meyeni*, *Paragaleus randalli*, and *Urogymnus granulatus*, were categorized as Vulnerable (VU) in terms of population status. The habitats of *Himantura leoparda*, *Pateobatis jenkinsii*, *Taeniura meyeni*, and *Urogymnus granulatus* encompassed the waters of the Indian Ocean. The previous record of *Urogymnus granulatus* from the present region was based on an underwater image lacking detailed taxonomic notes (Kumar et al. 2022). Other species, almost Least Concern (LC) and Data Deficiet (DD), face widespread capture and trade across various water areas, posing a significant challenge to their populations (Ilham and Marasabessy 2021).

Carcharhinus sealei, also known as the silk shark, was quite common in the waters of North Nias. Currently, management of the silky shark fishery is largely carried out by four tuna Regional Fisheries Management Organizations (RFMOs), including the Indian Ocean Tuna Commission (IOTC), the International Commission for the Conservation of Atlantic Tuna (ICCAT), the Western and Central Pacific Fisheries Commission (WCPFC) and Inter American Tropical Tuna Commission (IATTC) (Peiris et al. 2021).

Furthermore, there were 12 species whose conservation status was Least Concern (LC), including *Caranx sexfasciatus*, *Caranx ignobilis*, *Ferdauia ferdau*, *Amblygaster clupeoides*, *Caesio caerulaurea*, *Halichoeres scapularis*, *Lethrinus ornatus*, *Mulloidichtys flavolineatus*, *Parupeneus barberinus*, *Scarus prasiognathos*, *Plectropomus leopardus* and *Variola albimarginata*. Meanwhile, Amblygaster clupeoides is similar in morphology to *Sardinella lemuru*; the possible causes of the variations were local fish migration and environmental factors (Hanif et al. 2019). All the mentioned fish species were classified as demersal and pelagic, commonly found in the waters of South and North Nias. Elasmobranch species classified as near threatened, vulnerable, and endangered were present in South Nias and North Nias. Many of these species are a cause for concern due to intense fishing pressure, unregulated and unreported fishing practices, and exploitation of their fins and meat (IUCN 2023). It's crucial to emphasize the necessity of implementing sustainable fisheries specifically tailored to protect and conserve these species in certain geographic areas.

Exploration of biodiversity that has been carried out found that economically important fish in Nias waters have a very high diversity consisting of demersal, pelagic and Chondrichthyes. Genetic analysis found low genetic distances for all species both within and between populations, indicating that oceanographic factors play a major role in larval transfer and species migration. The high diversity of haplotypes and nucleotides shows that all of these species are not under stress and are not indicated species resulting from inbreeding, so they have high genetic variation. The value of genetic variation can be initial information in improving the utilization of fish resources in Nias waters and in sustainable management of the species.

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