



Identification, relationship and population status of sharks and rays landed at Rigaih Fishing Port as an effort of genetic conservation to endangered biota

[Identifikasi, hubungan kekerabatan dan status populasi hiu dan pari yang didaratkan di Pelabuhan Perikanan Rigaih Sebagai upaya konservasi genetik biota terancam punah]

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ABSTRACT | Aceh Jaya is a potential habitat and migration water for various types of sharks and rays because of its location directly opposite the Indian Ocean. The objectives of this research include Identifying the types of sharks and rays found molecularly, analyzing kinship relationships, and describing the conservation status of the kinds of sharks and rays found. The research was carried out at the Lhok Rigaih Fishing Port, Aceh Jaya, throughout August. The samples obtained were ten individuals from sharks and rays and analyzed morphologically and molecularly. The research results found six individual sharks and four individual rays originating from 6 species of sharks and two rays that landed at the Rigaih Fishing Port. The molecular identification results show similarities with the morphological identification results, with an average similarity percentage reaching more than 95% and query cover more than 85%. The results of phylogenetic analysis show that the types *Sphyrna lewini* and *Rhynchobatus australiae* have plesiomorphic characters. References to the IUCN Red List standard units indicate that there are three types of sharks landed at the Rigaih Fishing Port with critical status (CR), four types with vulnerable group (VU), and one type with near extinction status (NT). The results of this research are significant because they are fundamental research and contribute to data collection on sharks and rays using morphological and molecular approaches, which have yet to be carried out.

Key words | Aceh Jaya, molecular, shark and ray fisheries, conservation status

ABSTRAK | Aceh Jaya menjadi salah satu wilayah perairan habitat dan migrasi potensial bagi berbagai jenis hiu dan pari karena lokasinya yang secara geografis berhadapan langsung dengan Samudera Hindia. Adapun tujuan pelaksanaan penelitian ini meliputi; Mengidentifikasi jenis-jenis ikan hiu dan pari yang ditemukan secara molekular, Menganalisis hubungan kekerabatan dan mendeskripsikan status konservasi dari jenis-jenis ikan hiu dan pari yang ditemukan. Penelitian dilaksanakan di Pelabuhan Perikanan Ikan Lhok Rigaih, Aceh Jaya sepanjang bulan agustus. Sampel yang diperoleh sebanyak 10 individu yang berasal dari jenis hiu dan pari dan dianalisis secara morfologi dan molekular. Hasil penelitian ditemukan 6 individu hiu dan 4 individu pari yang berasal dari 6 spesies hiu dan 2 spesies pari yang didaratkan di Pelabuhan Perikanan Rigaih. Hasil identifikasi molekular menunjukkan kesamaan dengan hasil identifikasi morfologi dengan rata-rata persentase kemiripan mencapai lebih dari 95% dan query cover lebih dari 85%. Hasil analisis filogenetik menunjukkan bahwa jenis *Sphyrna lewini* dan *Rhynchobatus australiae* memiliki karakter plesiomorfi. Berdasarkan referensi pada satuan baku IUCN Red List menunjukkan bahwa terdapat 3 jenis hiu yang didaratkan di Pelabuhan Perikanan Rigaih berstatus kritis (CR), 4 jenis berstatus rentan (VU) dan satu jenis berstatus mendekati kepunahan (NT). Hasil penelitian ini sangat penting karena menjadi penelitian dasar serta berkontribusi dalam pendataan hiu dan pari melalui pendekatan morfologi dan molekular yang selama ini masih sedikit dilakukan.

Kata kunci | Aceh Jaya, molekular, perikanan hiu dan pari, status konservasi

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INTRODUCTION

The capture fisheries sector has enormous potential in Indonesia because this region has a large area and vibrant fisheries biodiversity, but the possibility of being exploited is only around 62% (Bumulo & Panigoro, 2021). This condition explains that there is still much potential for capturing fisheries that must be utilized optimally. In addition, the capture fisheries business in Indonesia is also dominated by small fishermen who carry out many by-catch activities. (Amandra *et al.*, 2021). One type of by-catch that fishermen often catch is the shark and ray fishery. Catch activities pose a significant threat to marine biodiversity and are one of the most difficult challenges in supporting the growth of biodiversity conservation (Booth *et al.*, 2021). By-catch also can harm fishermen and communities because it threatens biodiversity, marine ecosystems, and fisheries' survival. One type of fish that experiences the most by-catch is the shark and ray fishery. Although shark and ray fishing activities in Indonesian waters are considered by-catch activities by fishermen, Indonesia is among the world's 20 most giant shark and ray-catching countries (Ayu *et al.*, 2021).

Aceh's territorial waters are between two Fisheries Management Areas (WPP): WPP 571 and WPP 572. Aceh Jaya waters are included in WPP 572, where this area is also included in the Regional Marine Protected Area (KKPD) through the Decree of the Governor of Aceh No. 532/1297/2018 concerning the Determination of Aceh Marine Protected Area Reserves. Geographically, the waters of Aceh Jaya are directly adjacent to the Indian Ocean, where fishermen find many sharks and rays fishery by-catch activities. The existence of both types of fish helps in regulating and maintaining the balance of marine ecosystems. The function of sharks and rays in marine ecosystems and coral reefs is essential so that an aquatic ecosystem remains healthy, maintained, and balanced. The population status and existence of sharks and ray fisheries today are of grave concern to fisheries marine environmentalists and observers worldwide. This is due to exploiting sharks and rays commodities that occur often and are not controlled. Indonesia is one of the largest exporters of shark and ray fins, along with Spain, Taiwan, and India (Nurastri & Marasabessy, 2021). This condition prompted the government to issue regulations on protecting eight species of sharks through the Regulation of the Minister of Marine Affairs and

Fisheries Number 5 of 2018. The eight types of sharks protected include Hammerhead Sharks (*Sphyrna lewini*, *S. mokarran*, and *S. zygaena*), Whale Sharks (*Rhincodon typus*), Lanjaman Sharks (*Carcharhinus falciformis*), Cowboy Sharks (*Carcharhinus longimanus*) and Rat Sharks (*Alopias superciliosus* and *A. pelagicus*). The Convention on International Trade in Endangered Species (CITES) index also reinforces the regulation (Dewana *et al.*, 2021).

Aceh waters are one of the potential areas for shark and ray fisheries habitat. However, by-catch activities for sharks and rays are still ongoing, which impacts environmental sustainability and the balance of the aquatic ecosystem because sharks and rays are one of the top aquatic predators. More data related to shark and ray fisheries in Aceh Jaya is still needed, so research related to identification and kinship analysis is essential to carry out as a basis for the next study stage. Research on shark and ray fisheries in Aceh Jaya waters has yet to be widely carried out or published nationally. One effective method for identifying and analyzing kinship relationships is using molecular markers. Molecules can answer several questions that cannot be answered ecologically. Through DNA (deoxyribonucleic acid) analysis, we can find further information can be used in sharks' and rays' conservation efforts (Madduppa *et al.*, 2019). This study was carried out to molecularly identify the types of sharks and rays found, analyze the evolutionary relationships of the sharks and rays found, and determine the conservation status of both species found in Aceh Jaya waters.

MATERIALS AND METHODS

The research was conducted at the Lhok Rigaih Fishing Port, Aceh Jaya Regency (Figure 1). The sampling period was carried out throughout August, and samples of 10 individual sharks and rays from various species landed at the port. The number of sample collections carried out takes into account the availability of data and the amount of research funds owned. The samples obtained were then prepared at the Marine Biodiversity and Genetics Laboratory, Faculty of Fisheries and Marine Sciences, Teuku Umar University. The molecular analysis consisted of extraction, amplification, electrophoresis, and DNA translation processes at the Aquatic Genetics and Biodiversity Laboratory, Banda Aceh. The tools and materials used in this research consist of field and laboratory equipment. The list of tools and materials

is attached in the Table below (Table 1).

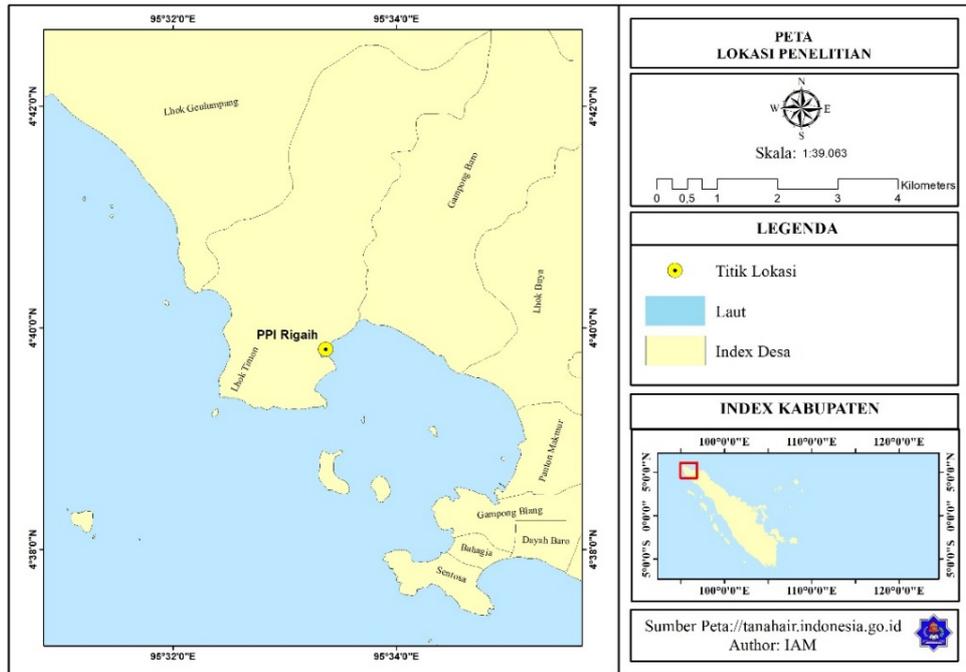


Figure 1. Map showing the sampling localities for fish from the Lhok Rigaih Fishing Port

Table 1. Research tools and materials

No.	Tools and Materials	Utilities
1.	Sample bottle 10 mL	Storing shark meat samples
2.	Latex gloves	Hand protection to prevent contamination
3.	Tweezers	To take sample meat
4.	Bottle marker	Sample naming label
5.	Stationery	Take notes in the field
6.	Alcohol 96%	Sample curing liquid
7.	Ruler	Fish length gauge
8.	Shark identification book	Guide to identifying shark species
9.	Camera	Shark breed documentation
10.	Mask	Protection from viruses and bacteria

Data Collection

Sampling was conducted at the Fishing Port, Lhok Rigaih, Aceh Jaya Regency. The samples collected were small parts of limbs from various sharks and rays landed at Fishing Port, with the number of samples collected as many as ten individuals. The body parts taken in the sample are parts of muscle tissue (meat), which contains much mitochondrial DNA. The stages of the sampling process include:

1. Samples were collected throughout August at the Lhok Rigaih fishing port.
2. The ten samples obtained were then put into a sample bottle filled with ethanol solution.
3. The samples are then prepared at the Marine Biodiversity and Genetics Laboratory to make them more sterile and controlled.
4. Samples were sent to the Genetics and Aquatic

Biodiversity Laboratory at Syiah Kuala University for molecular analysis.

Morphological Identification

The morphological identification of shark and ray species was carried out based on the literature of the Ministry of Marine Affairs and Fisheries guidebook in 2014 (Fahmi, 2021) and the literature of the Shark and Ray Quick Estimation Guidebook (Rigby & White, 2019). Identification is carried out by observing the morphology of shark and ray species found in PPI and documenting them according to guidelines. Furthermore, morphometric measurements and identification were carried out from morphological data obtained based on the literature of the guidebook for identifying shark and ray species.

Genetic Extraction

The extraction and isolation processes refer to the Dneasy Blood and Tissue Kit (Qiagen) DNA isolation and purification kit protocol. The Dneasy Blood and Tissue Kit (Qiagen) DNA isolation and purification kit protocol is considered adequate because it is easy to extract DNA from muscle tissue near the tip of the tail, which was cut with a size of 1 cm. Apart from that, this kit has also been carried out in many studies of fish species with optimal extraction results, so this kit is the right choice for researchers to extract the shark and ray samples.

Genetic Amplification and Electrophoresis

The amplification process on shark and ray DNA uses the Polymerase Chain Reaction (PCR) method. The primers used in shark samples were Primer Forward FishF2_t1 (5'-TGT AAA ACG ACG GCC AGT CGA CTA ATC ATA AAG ATA TCG GCA C3') and Primer Reverse FishR2_t1 (5'-CAG GAA ACA GCT ATG ACA CTT CAG GGT GAC CGA AGA ATC AGA AGA AGA A3') (Ward et al., 2005). Electrophoresis is a technique of separating molecules based on differences in their migration rates in an electric field. Electrophoresis aims to determine the quality of DNA contained in PCR products. DNA fragments were visualized using specific fluorescent dyes to describe the length of the DNA. The type of gel used is agarose, which can show the size of base pair fragments that can be seen using the help of ultraviolet light (Aisyah & Farhaby, 2021).

Genetic Translation

DNA translation is carried out to determine the sequence of nucleotides in DNA. DNA sequences deal with the inherited genetic information in mitochondria that form the basis of the development of all living things. Samples amplified through PCR are then translated (sequencing) to obtain the nucleotide sequence (Aisyah & Farhaby, 2021a).

Data Analysis

Molecular identification

The results of laboratory analysis of DNA samples are then processed computationally using the MEGA X software (Molecular Evolutionary Genetics Analysis). The MEGA application's computational analysis process includes trimming, reverse complement, and alignment. This analysis is carried out to visualize samples from laboratory analysis (Bahri et al., 2017). The nucleotide alignment information obtained is then matched with the information presented on the

GeneBank at NCBI (National Center for Biotechnology Information) through the Nucleotide BLAST (Basic Local Alignment Search Tool) (Madduppa et al., 2021).

Kindship and population status

Kinship (evolution) is a system of classification of living things based on the Phylogenetics approach between various taxons (Mabrouk et al., 2006). The classification system is a precious subject in tracing the kinship bonds of evolution by creating phylogenetic reconstructions. The phylogenetic approach attempted in this observation is the Neighbour-Joining Tree. Population status is narrated descriptively based on reference information on the formal pages of the International Union for Conservation of Nature (<https://www.iucnredlist.org/>).

RESULTS

Morphological Identification

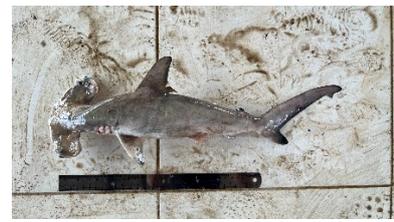
The result at the observation site found that as many as six sharks and four rays landed at Rigaih Fishing Port. The morphological identification results showed that the ten individuals came from 8 species consisting of 6 species of sharks and two species of rays. The eight species of sharks and rays obtained are also included in the international protection status of the IUCN Red List (The International Union for Conservation of Nature's Red List of Threatened Species) (Prehadi et al., 2015). Six sharks and rays were obtained: *Carcharhinus falciformis*, *C. amblyrhynchos*, *C. limbatus*, *Hemigaleus microstoma*, *Sphyrna lewini*, *Paragaleus randalli*. At the same time, the two types of rays obtained consist of *Rhynchobatus laevis* and *R. australiae*. Pictures of each type of shark and ray are attached to Figure 2.



Carcharhinus falciformis (Silky sharks)



Hemigaleus microstoma (Sicklefin weasel shark)



Sphyrna lewini (Scalloped hammerhead)



Carcharhinus amblyrhynchos (Grey reef shark)



Paragaleus randalli (Slender weasel shark)



Carcharhinus limbatus (Blacktip shark)



Rhynchobatus laevis (Smoothnose wedgefish)



Rhynchobatus australiae (Bottlenose wedgefish)

Figure 2. Morphological documentation of shark species found at Fishing Port, Lhok Rigaih, Aceh Jaya

Molecular Identification

The results of molecular identification carried out show similarities to the results of morphological identification. The *Carcharhinus falciformis* has a molecular identification value of 100.00% with a query cover value of 95%. *Hemigaleus microstoma* has a molecular identification value of 99.69% with a query cover value of 96%. *Sphyrna lewini* has a molecular identification value of 100.00% with a query cover value of 95%. *Carcharhinus amblyrhynchos* has a molecular identification value of 100.00% with a query cover value of 96%. *Paragaleus randalli* has a molecular identification value that reaches 100.00% with a query cover value of 94%. *Carcharhinus limbatus* has a molecular identification value of 99.51% with a query cover value of 92%. *Rhynchobatus australiae* has a molecular

identification value of 94.58% to 99.84%, with a query cover value of 70% to 87%. The last one *Rhynchobatus laevis* has reached a value reaches 99.69% with a query cover value of 90% to 91%. In general, the ten individuals were found to have a high level of molecular identification accuracy, where the average molecular identification value obtained was 100% and the average query cover value obtained was 95%. Identity percentage and query coverage are significant because they define sequence specificity. The higher similarity percentage indicates the level of accuracy of the data that is trusted; this proves that the sample obtained has been well identified, as evidenced by the high similarity percentage value (Partin et al., 2022). Data on types and percentages of molecular identification can be seen in Table 2.

Table 2. Results of molecular identification through the cloud-based application of the *National Center for Biotechnology Information*

No	Superorder	Genetic Ident	Per. Ident	Query Cover
1		<i>Carcharhinus falciformis</i>	100.00%	95%
2		<i>Hemigaleus microstoma</i>	99.69%	96%
3	Sharks (<i>Selachimorpha</i>)	<i>Sphyrna lewini</i>	100.00%	95%
4		<i>Carcharhinus amblyrhynchos</i>	100.00%	96%
5		<i>Paragaleus randalli</i>	100.00%	94%
6		<i>Carcharhinus limbatus</i>	99.51%	92%
7	Rays (<i>Batoidea</i>)	<i>Rhynchobatus laevis</i>	99.69%	90%
8		<i>Rhynchobatus laevis</i>	99.69%	91%
9		<i>Rhynchobatus australiae</i>	94.58%	70%
10		<i>Rhynchobatus australiae</i>	99.84%	87%

Kondship and Population Status

Phylogenetic analysis determines how a group trait is inherited during the evolutionary process. The results of the phylogenetic analysis showed a kinship pattern

among the ten shark individuals obtained. The kinship analysis used the Neighbor-Joining method, 2-parameter Kimura evolution model, and 1000x bootstraps replication from Mega X software (Bahri et

al., 2023). The Neighbor-Joining method has been widely applied to shark research carried out in previous research (Ulfah et al., 2023). The results of the phylogenetic analysis showed two large groups of samples obtained. The formation of the two groups occurred because both groups belonged to different superorders, where group I was a type of shark (Superorder: Selachimorpha) and group II was a type of ray (Superorder: Batoidea). However, both groups belonged to the same subclass, the subclass

Elasmobranchii. In group I, the type of *Sphyrna lewini* has a plesiomorphic character because it evolutionarily inherited primitive characters from its ancestors. While in group II, found the type of *Rhynchobatus australiae*. However, all types of sharks and rays found make up the phylogenetics of synapomorphy because it has evolved and formed new, more varied characters passed down by their ancestors.

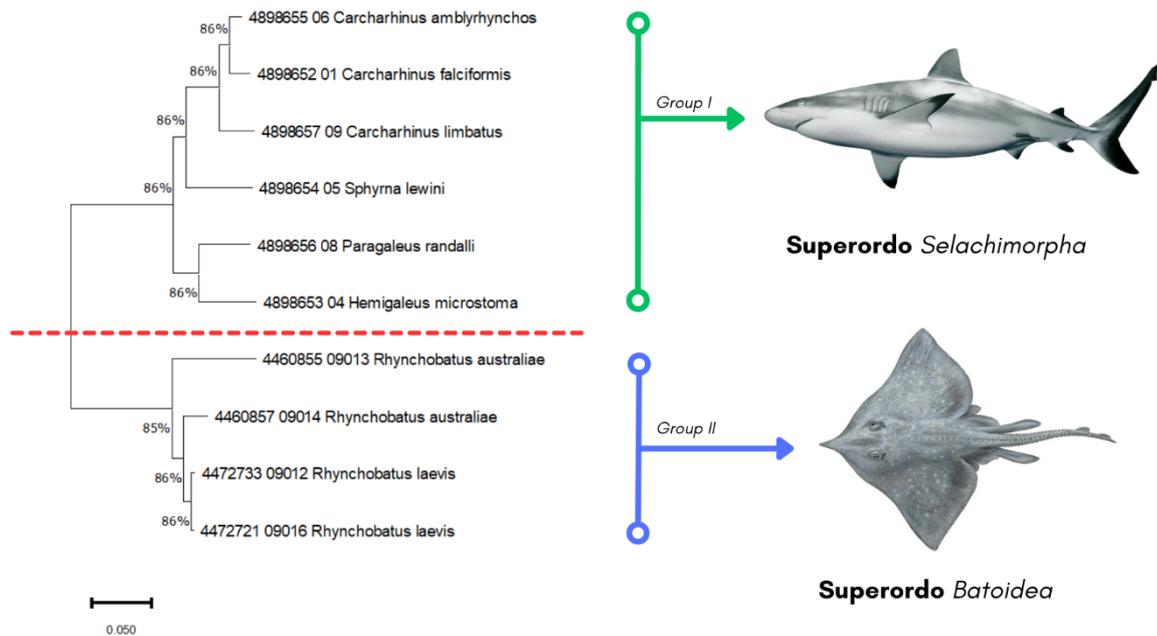


Figure 3. The phylogenetic tree between Superorder *Selachimorpha* and *Batoidea* (subclass *Elasmobranchii*) found in Rigaih Fishing Port, Aceh Jaya using a phylogenetic approach through the *Neighbor-Joining method*

References to the IUCN Red List standard unit show that there are four species of sharks landed at Rigaih Fishing Port with vulnerable status (VU), one type with critical status (CR), and one type with critical status (EN). While the two types of rays found both have critical population status (CR). This result is an important discovery. So far, the population status of

sharks landed and traded at Rigaih Fishing Port is unknown and scientifically recorded. This information can help local governments implement conservation policies against exploiting endangered shark species landed at Rigaih Fishing Port, Aceh Jaya Regency (Table 3). The level of population status of endangered biota based on the standard reference IUCN Red List is attached in Figure 3.

Table 3. Population status of sharks and rays landed at Rigaih Fishing Port, Aceh Jaya.

No	Sampling date	Superorder	Species Name	Number of Samples	IUCN Red List Population Status*
1	23/06/2023	Rays	<i>Rhynchobatus laevis</i>	1	CE
2	25/06/2023		<i>Rhynchobatus laevis</i>	1	CE
3	25/06/2023		<i>Rhynchobatus australiae</i>	1	CE
4	26/06/2023		<i>Rhynchobatus australiae</i>	1	CE
5	15/06/2023	Sharks	<i>Sphyrna lewini</i>	1	CE
6	21/06/2023		<i>Carcharhinus amblyrhynchos</i>	1	EN
7	08/08/2023		<i>Carcharhinus falciformis</i>	1	VU
8	21/06/2023		<i>Paragaleus randalli</i>	1	VU
9	22/06/2023		<i>Carcharhinus limbatus</i>	1	VU
10	15/06/2023		<i>Hemigaleus microstoma</i>	1	VU

*CE= Critically Endangered, EN= Endangered, VU= Vulnerable

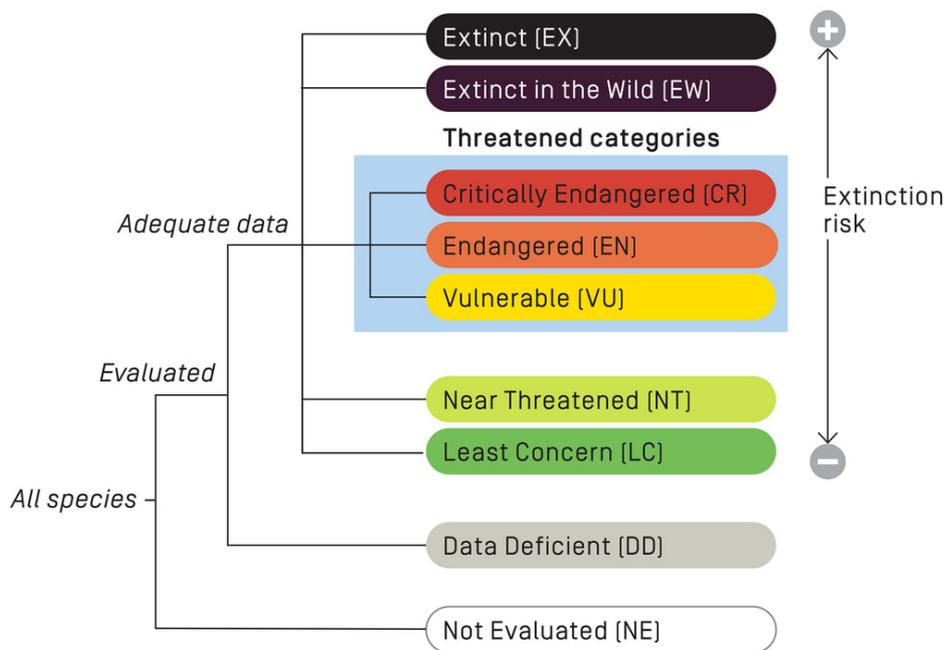


Figure 4. The IUCN red list of threatened Sharks and Rays

DISCUSSION

Morphological Identification

Carcharhinus falciformis (Silky sharks) is commonly found in pelagic and shallow waters. Silky sharks are the largest in the genus *Carcharhinus* and live solitary or in groups. This shark has become one of China's most exported target fish over the past two decades (Cardeñosa *et al.*, 2021). *Hemigaleus microstoma* (Sicklefin weasel shark) generally lives in shallow waters and is distributed along the waters of India, China, and Southeast Asia. It has a relatively small body size and spends much time on the bottom of the water by preying on biota that belong to the *cephalopod* class (Bineesh *et al.*, 2020). *Sphyrna lewini* (Scalloped hammerhead) has a long front dorsal fin body morphology, a shorter hind dorsal fin, and a long caudal fin tip. The back of the ventral fins is straight, has the shape of the head spread to the side, and has a slight curve into the middle of the head. It has a light gray or grayish-brown body color on the back and white on the ventral side; the tip of the pectoral fin is black, and there is a black spot on the lower caudal fin (Bahri *et al.*, 2023a).

Carcharhinus amblyrhynchos (Grey reef shark) is one of the most dominant reef sharks in Indo-Pacific waters, contributing up to 50% to the predatory biomass on coral reefs. Grey reef sharks have a wide geographic distribution and cover most Indo-Pacific

tropics (Momigliano *et al.*, 2017). Information about the *Paragaleus randalli* type (Slender weasel shark) remains minimal. This shark belongs to the family *Hemigaleidae* and is found in the western waters of the Indian Ocean outside Bahrain. This shark can reach a body length of up to 48 cm. *Carcharhinus limbatus* (Blacktip shark) has a sturdy, slender body with a long, pointed muzzle and relatively small eyes. The species reaches a maximum known length of 2.8 m (9.2 ft), although 1.5 m (4.9 ft) is more common, and a known maximum weight of 123 kg (Castro, 1996). The *Rhynchobatus* group is known as the stingray group in the family *Rhinidae*. This group of fish is found in tropical and subtropical regions of the Indo-Pacific. These species are very similar and often confused. The various species can generally be separated based on a combination of snout shape, number of vertebrae, proper color distribution of white spots, and presence/absence of black spots at the base of the pectoral fins. The largest species can reach about 3 m (10 ft). However, most minors account for less than a third of that size (Giles *et al.*, 2016).

Molecular Identification

Molecular identification helps confirm morphological doubts about the results of identification. The number of characters found through morphological approaches is generally limited, making it difficult to compare with other types with high similarity. In

contrast, molecular approaches have a more significant number of characters and are more specific, so they help identify even types that have a high degree of similarity. So, it is necessary to carry out the identification process through morphological and molecular methods to strengthen the identification results on the type of shark and ray obtained (Bramasta *et al.*, 2021). Identification of various types of sharks has also been widely carried out in Cilacap, Muncar, and Bali, identifying shark fins found in traditional fish markets (Prehadi *et al.*, 2015). Pieces of body parts that are not intact certainly complicate the identification process, so molecular approaches are beneficial in identifying various types of sharks based on the body parts of fins obtained (Sembiring *et al.*, 2015). Below is a table of identification results of shark species found using molecular markers.

Kindship and Population Status

The two groups of sharks and rays are evolutionarily related. Evolutionary relationships among sequences are described by placing separate group sequences in a tree (Aisyah & Farhaby, 2021b). The branches' relationship on the inside of the tree reflects the degree to which different sequences are interconnected. Phylogenetic trees provide information about classifying populations based on their evolutionary relationships. In the reconstruction of phylogenetic trees, molecular data are more widely used because they are considered more stable in the evolutionary process than morphological data (Dharmayanti, 2011). Phylogenetic tree images of 10 individual sharks and rays found at Rigaih Fishing Port, Aceh Jaya Regency.

Critically Endangered is a conservation status given to species at risk of imminent extinction (Aisyah & Farhaby, 2021c). Vulnerable is a conservation status given to species that are at risk of extinction in the wild in the future (Bahri *et al.*, 2023; Bahri *et al.*, 2023b). Based on the results of research that has been carried out, researchers see widespread fishing of sharks and rays in Aceh Jaya waters. This is proven by the many types of sharks and rays that are landed at the Lhok Rigaih fishing port every week. The current fishing activities are uncontrolled, and if this condition continues, it has the potential to damage the ecological balance and degrade various populations of sharks and rays in the waters of Aceh Jaya. This will undoubtedly impact the status of shark and ray populations that live and develop in Aceh Jaya

waters. Referring to improving the conservation and trade status of sharks and rays, it is necessary to carry out specific conservation and management. This management cannot be done by one institution alone but in collaboration with various parties using a co-management approach. The co-management approach requires the government to make stricter regulations in its technical management. On the other hand, there is a need for socialization in the private sector to reduce the demand for shark and ray fin consumption or socialization by prohibiting the sale of shark and ray fin soup. Meanwhile, people need to increase their awareness not to catch sharks and rays gradually but are encouraged to increase consumption of other types of fish that remain economically important. All these efforts are expected to contribute to maintaining the sustainability of shark and ray populations in Aceh Jaya waters.

CONCLUSION

Based on the results of a study on shark fisheries conducted at Rigaih Fishing Port, Aceh Jaya Regency, ten individuals of sharks from 8 species were found including *Carcharhinus falciformis*, *Hemigaleus microstoma*, *Sphyrna lewini*, *Carcharhinus amblyrhynchos*, *Paragaleus randalli*, *Carcharhinus limbatus*, *Rhynchobatus laevis*, *Rhynchobatus laevis*, *Rhynchobatus australiae*. Phylogenetically, the group of shark species obtained is divided into two large groups where two individuals are categorized as rays. However, in everyday life, the fishermen say that *Rhynchobatus laevis* and *Rhynchobatus australiae* are included in the type of shark. Based on the IUCN category index, the eight species of sharks and rays found are included in the endangered category and at risk of extinction, namely included in the critical (CE), precarious (EN), and vulnerable (VU) categories.

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researchers, practitioners, and implementers of shark fisheries conservation policymakers in Aceh Province.

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