



## Variasi genetik dan status populasi hiu: Studi mtDNA komparatif di perairan Aceh Jaya dan Aceh Barat

### Genetic variation and population status of sharks: A comparative mtDNA study in the waters of Aceh Jaya and West Aceh

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#### Abstrak

Analisis genetik berbasis DNA mitokondria (mtDNA) penting untuk mengungkap hubungan evolusi, mendukung taksonomi, dan menyediakan dasar bagi pengelolaan konservasi. Penelitian ini dilakukan di perairan Aceh Jaya dan Aceh Barat, dua wilayah dengan aktivitas penangkapan hiu yang tinggi namun masih minim data genetik. Sebanyak 12 individu hiu (6 per lokasi) dikoleksi dari pelabuhan pendaratan ikan, kemudian jaringan otot dorsal diambil, dipreservasi dalam etanol, dan dianalisis menggunakan metode ekstraksi DNA, sekuensing, serta bioinformatika. Identifikasi molekuler melalui perbandingan dengan GenBank menunjukkan tingkat kesesuaian sangat tinggi (Query Cover 99–100%, Identity 100%), dengan spesies yang terdeteksi antara lain *Carcharhinus falciformis*, *Sphyrna lewini*, dan *Hemigaleus microstoma*, yang mencerminkan distribusi luas di perairan barat Aceh. Komposisi nukleotida memperlihatkan dominasi basa Timin serta rasio A+T lebih tinggi dibanding G+C, sesuai karakteristik genom hiu. Analisis filogenetik menunjukkan klade monofiletik dengan dukungan bootstrap kuat (100%) dan menempatkan *S. lewini* sebagai taksa paling awal terdiferensiasi. Hasil penelitian ini mengkonfirmasi efektifitas DNA untuk identifikasi spesies yang akurat dan pemetaan keragaman genetik, sekaligus menggarisbawahi keprihatinan konservasi yang mendesak, karena sebagian besar spesies yang teridentifikasi diklasifikasikan sebagai Rentan hingga Sangat Terancam Punah dalam Daftar Merah IUCN.

**Kata kunci:** Aceh; DNA Barcoding; Filogenetik; Hiu; Variasi Genetik

#### Abstract

Mitochondrial DNA (mtDNA)-based genetic analysis is important for revealing evolutionary relationships, supporting taxonomy, and providing a basis for conservation management. This study was conducted in the waters of Aceh Jaya and West Aceh, two regions with high shark fishing activity but minimal genetic data. A total of 12 shark individuals (6 per location) were collected from fish landing ports, then dorsal muscle tissue was taken, preserved in ethanol, and analyzed using DNA extraction, sequencing, and bioinformatics methods. Molecular identification through comparison with GenBank showed a very high level of similarity (Query Cover 99–100%, Identity 100%), with detected species including *Carcharhinus falciformis*, *Sphyrna lewini*, and *Hemigaleus microstoma*, reflecting a wide distribution in the western waters of Aceh. The nucleotide composition showed a dominance of thymine bases and a higher A+T ratio than G+C, consistent with the characteristics of shark genomes. Phylogenetic analysis showed a monophyletic clade with strong bootstrap support (100%) and placed *S. lewini* as the earliest differentiated taxon. Our results confirm the efficacy of DNA barcoding for accurate species identification and genetic diversity mapping, while underscoring urgent conservation concerns, as most species identified are classified as Vulnerable to Critically Endangered on the IUCN Red List.

**Keywords:** Aceh; DNA Barcoding; Genetic Variation; Phylogenetics; Sharks

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#### 1. Introduction

Sharks, as apex predators in marine ecosystems, play a crucial role in maintaining ecological balance as they control their preys' population and expedite nutrient cycle (Heupel et al., 2014). Their presence serves as an indicator of ecosystem health indicator, showing the stability and productivity of the aquatic environment. Even though the variety of sharks' species is

globally high, most of them are facing an extreme threat that could cost their existence. Shark populations worldwide are declining drastically due to various anthropogenic pressures, as overfishing is the most threatening (Dulvy et al., 2021). The International Union for Conservation of Nature (IUCN) red list, as the the global standard to evaluate species conservation status, classifies more than third (37.5%) sharks' and stingrays' species are endangered, confirming the urgency of conservation action and an accurate data is needed to verify that evaluation (Nurastri & Marasabessy, 2021).

Genetic studies provide a crucial complement to ecological data, particularly for the specimens that are hard to differentiate morphologically or in proceeded product form found in markets (Liu et al., 2013; Joesidawati et al., 2025). Phylogenetic analysis using the mtDNA could reveal the interspecies or population evolution correlation, delivering fundamental knowledge to taxonomy understanding and conservation management unit (Larson et al., 2017). Moreover, genetic study also delivers crucial information about population status, such as genetic variety level and population structure, which reflects the population's health and vulnerability to extinction. The low variety genetic or isolated population that is critical could be a relevant extinction risks' indicator to the IUCN evaluation criteria (Clarke et al., 2015).

Indonesia, as a mega-biodiversity country, has a highly diverse elasmobranch (sharks and stingrays) (Dharmadi et al., 2009). However, the information about genetic structure and sharks' population status in most of the territory is highly limited. The aquatic area of Aceh Jaya and Aceh Barat are important coastal areas with significant fishery activities, including sharks' trapping (Fuadi et al., 2023). The availability of comprehensive data about sharks' genetic diversity and population status in both places is nearly not applicable, while the trapping pressure is still continuing. Therefore, this research is intended to complete the knowledge gap with genetic closure. With 12 shark sample sequences from two different locations, this research has an unique opportunity to perform deep comparative analysis about genetic variety and population structure of the species found in both locations.

The main goals of this research are (1) To identify shark species collected from both locations using a mitochondrial DNA marker, (2) to evaluate the intraspecific genetic variety and connectivity level or isolation among sharks' populations found in both locations (3) To analyze phylogenetic correlation among sharks' species found in both locations, to understand how these species are related to one another and their taxonomic position, (4) to interpret this genetic found in evaluation conservation status context for IUCN to inform relevant genetic knowledge that could support understanding about population status and a certain sharks' species extinction risk potential in both study locations.

## 2. Materials and Methods

### 2.1. Methods

This research was conducted in western Aceh, specifically in the districts of Aceh Jaya and West Aceh. These locations are chosen due to the significant potential of sharks' aquatic area and the existence of various shark species that are targeted or as bycatch species by the fishermen in this area. The collecting of sharks samples is executed directly at the main fish delivering harbour (Pelabuhan Pendaratan Ikan (PPI) from each aquatic area of each regency, which are PPI Righiah in Aceh Jaya and PPI Ujong Baroh in Aceh Barat. This approach allowed the researchers to collect fresh samples directly from the fishermen's catch. The premier sample collection is performed to 12 sharks in total; 6 of them are from each location. All the

samples are caught by the fishermen during the research. The selection of the amount is based on sample availability and the consideration to achieve adequate genetic representatives. The sample collection process occurs intensively in a month, to maintain optimal data scope in a certain period of time. The fish's body part taken to be analyzed is the muscle or flesh located around the dorsal fin of a shark. This part is chosen as it is known to have high mitochondria DNA concentration and a good quality for genetic analysis (Shivji et al., 2002). After collection, the samples are immediately preserved and brought to the biodiversity and marine genetic laboratory at Teuku Umar University as the preparation's first step, including DNA isolation. Then, deeper laboratory analysis for molecular assay, including DNA sequencing and bioinformatic analysis, performed at Indonesia's biodiversity foundation (Yayasan Biodiversitas Indonesia (BIONESIA))'s Laboratory in Denpasar, Bali. This facility provides infrastructure and adequate ability to perform complex genetic analysis which is necessary in this research.

### 2.2. Data collection procedure

The sharks' sampling procedure in the research area for DNA genetic barcoding analysis is performed according to the standard guide to guarantee the DNA's integrity. The body part that is taken is a small cut of muscle or flesh around the dorsal fin, to prevent the area that has important morphology character or parasitized. The sampling process is executed invasively by at least using the sterilized scalpel or punch biopsy to prevent cross contamination among sharks. The sample size is normally around 0.5-1 cm. Right after being caught, the tissue sample is located in a sterilized vial filled with absolute ethanol as preservation media (Bahri & Hafinuddin, 2023a). Ethanol is used to obstruct DNA degradation caused by enzymes and microorganisms, to preserve the sample quality until it is delivered to the laboratory. Vials are clearly labeled with the shark's individual information (date, location of catch, gender if applicable, and unique sample code) to maintain the data traceability. Samples are then secured in cold temperature, for instance in an ice box or portable freezer, during transportation to the laboratory.

### 2.3. Laboratory analysis

DNA extraction from shark's muscle tissue sample is the first crucial step to isolate high-quality genomic DNA. This process is normally executed using commercial tools (DNeasy Blood and Tissue Kit-Qiagen) (Saghai-Marooof et al., 1984). The tissue sample is broken down in a lysis buffer containing detergent and K proteinase to dissolve the membrane cells and degradate protein. Later, DNA is purified from cell debris and contaminants using spin columns or chemical precipitation, producing a perfect DNA for amplification. The concentration and purity of extracted DNA are validated using a spectrophotometer to maintain its quality before being executed to the next stage.

DNA amplification stage is executed through Polymerase Chain Reaction (PCR) technique to duplicate Cytochrome c Oxidase subunit I (COI) gene fragment which is a DNA barcoding standard label for animals. The selection of COI gene is based on its characteristic that has low intraspecific variety, but has high enough interspecific variety, so that it is effective to differentiate species (Bahri et al., 2023b). PCR reaction is provided in small volume consisting of DNA template, universal primer pair for fish COI gene (such as, primer of FishF1 and FishR1), dNTPs (deoxynucleotide triphosphates), PCR buffer, and polymerase DNA enzyme Taq. The PCR thermal condition is generally consisting of a primary denaturation stage to separate double strand DNA, repeated denaturation cycle, annealing (primer

attachment), and extension (DNA strand elongation), ending with the final extension (Bahri et al., 2023b).

Amplified PCR product later is visualized through DNA electrophoresis in agarose gel (generally 1.5%). Agarose gel is prepared by dissolving agarose powder in the TBE (Tris-Borat-EDTA) buffer and shaped using a well sample. The PCR product sample then mixed to load dye and filled into the well gel along with the DNA ladder (DNA size label). Gel is flowed through electric current (around 100-120V), moves the DNA fragment to flow through the gel based on its size. After electrophoresis, gel is colored using fluorescent color such as Ethidium Bromide or GelRed, then is visualized under the transilluminated UV light. The existence of DNA bands of the expected size (approximately 600-700 bp for COI gene) indicated the amplification success and the PCR product readiness for sequencing (Sambrook & Russell, 2001). Next, the sequencing process is carried out to the commercial service of PT. Genetika Science Indonesia.

#### 2.4. Data analysis

Molecular data analysis is a crucial stage after DNA sequences are collected, involving 2 main procedures: species identification and correlation reconstruction or phylogenetic. DNA sequence from the sequencing process is edited first then cut to reduce the noises or unqualified parts. To identify the species, purified DNA sequences will be compared to the GenBank database of National Center for Biotechnology Information (NCBI) through the algorithm Basic Local Alignment Search Tool (BLAST) (Bahri & Hafinuddin, 2023a). This process enables species identity determination based on similarity level (identity percentage) with the reference sequences that are available at GenBank. High similarity level, generally above 97-98%, often identifies the same species identity (Boratyn et al., 2013).

A phylogenetic tree is constructed using the MEGA application. The common method used includes Neighbor-Joining (NJ), Maximum Likelihood (ML), or Maximum Parsimony (MP) (Kumar et al., 2018). The NJ method, for instance, constructs the tree based on the genetic distance matrix, however the ML is seeking for the most possible tree based on the DNA evolution model. The tree's topology validity is evaluated through bootstrap analysis in a large amount of replication (e.g! 1000 times) to measure statistical support in each tree branching (Wang et al., 2022). Generated phylogenetic tree visualizes evolution correlation among individuals or species, helps to identify taxonomy groups, and to understand diversification patterns of the *Carcharhinus* sharks.

To analyze the population status based on the IUCN, genetical diversity data (such as, genetical diversity index) and population structure (such as, genetical differentiation level among locations) that are achieved will be implemented in the IUCN red list criteria context (IUCN Red List). This information is valuable to provide insights about population health, vulnerability to threats, and extinction risk potential. This closure provides sharks' population genetic evaluation to support the conservation attempt, as done in previous studies regarding sharks' and stingrays' population status based on genetic data (Bahri & Hafinuddin, 2023a).

### 3. Results and Discussion

#### 3.1. Result

##### 3.1.1. Identification

Based on the analysis table, it is shown that all the samples (From 1 to 12) were successfully identified with the highest level of credence. The percent identity (Per. Ident) for all samples reached 100%, indicating perfect genetic similarity amongst samples and the reference species from the database,

thus indicating the most accurate species identification and reliability. Meanwhile, Query Cover ranged between 99% to 100%. Most samples showed 100% coverage with the exception of two samples from Aceh Jaya (2 and 3) with coverage 99%. This number implies that most of or all of the sample's genetic sequence are successfully matched to the reference order, affirmed that most of the samples's genetic data were used in the identification process.

**Table 1**  
Sample identification of the shark species found

No	Sample Codel	Origin	Identified	Per. Ident	Query Cover
1	4898652	Aceh Jaya	<i>Carcharhinus falciformis</i>	100%	100%
2	4898655	Aceh Jaya	<i>Carcharhinus amblyrhynchos</i>	100%	99%
3	4898657	Aceh Jaya	<i>Carcharhinus limbatus</i>	100%	99%
4	4898653	Aceh Jaya	<i>Hemigaleus microstoma</i>	100%	100%
5	4898654	Aceh Jaya	<i>Sphyrna lewini</i>	100%	100%
6	4898656	Aceh Jaya	<i>Paragaleus randalli</i>	100%	100%
7	4618205	Aceh Barat	<i>Sphyrna lewini</i>	100%	100%
8	4618207	Aceh Barat	<i>Carcharhinus sorrah</i>	100%	100%
9	4618209	Aceh Barat	<i>Hemigaleus microstoma</i>	100%	100%
10	4618213	Aceh Barat	<i>Carcharhinus brevipinna</i>	100%	100%
11	4618215	Aceh Barat	<i>Carcharhinus falciformis</i>	100%	100%
12	4618219	Aceh Barat	<i>Carcharhinus amboinensis</i>	100%	100%

Some unique data patterns found include High Identification Consistency, where all the samples consistently showed 100% identity, indicating the effectiveness of the identification methods (likely DNA barcoding) with comprehensive database reference. In addition, there is a fairly high species diversity in the area, with various species such as *Carcharhinus falciformis*, *Sphyrna lewini*, and *Hemigaleus microstoma* found in both Aceh Jaya and West Aceh. Interestingly, some of the species repeated in the different regions, such as *Sphyrna lewini* and *Hemigaleus microstoma*, were found in two regions, implying geographic distribution or habitat similarities. Lastly, low variations on Query Cover (presence 99% in some samples) showed slight differences in the quality of comprehensiveness of the sequence data obtained, yet this does not reduce the accuracy of identification because Per. Ident remains 100%.

##### 3.1.2. Nucleotide composition

These nucleotide composition data present the percentages of thymine/uracil (T/U), cytosine (C), adenine (A), and guanine (G) of several shark species found in Aceh Jaya and West Aceh. Each sample contained a total of 634 nucleotides, demonstrating consistency in the size of the DNA/RNA fragments analyzed. In general, thymine/uracil (T/U) had the highest percentage among the four nucleotides, followed by cytosine (C) and adenine (A), while guanine (G) consistently had the lowest. This pattern was consistent across all species analyzed.

**Table 2**  
Nucleotide composition of the shark species found

Species	Location	T(U)	C	A	G	Total
<i>Hemigaleus microstoma</i>	Aceh Jaya	31.39	27.60	26.66	14.35	634
<i>Hemigaleus microstoma</i>	Aceh Barat	31.55	27.60	26.34	14.51	634
Average		31.47	27.60	26.50	14.43	634

<i>Sphyrna lewini</i>	Aceh Jaya	33.12	25.39	25.87	15.62	634
<i>Sphyrna lewini</i>	Aceh Barat	33.28	25.08	25.87	15.77	634
Average		33.20	25.24	25.87	15.69	634
<i>Carcharhinus falciformis</i>	Aceh Jaya	35.02	23.66	26.03	15.30	634
<i>Carcharhinus limbatus</i>	Aceh Jaya	35.17	23.66	26.18	14.98	634
<i>Carcharhinus amblyrhynchos</i>	Aceh Jaya	35.65	23.34	26.34	14.67	634
<i>Carcharhinus sorrah</i>	Aceh Barat	35.17	23.34	26.66	14.83	634
<i>Carcharhinus brevipinna</i>	Aceh Barat	33.75	25.08	26.03	15.14	634
<i>Carcharhinus falciformis</i>	Aceh Barat	35.33	23.19	26.18	15.30	634
<i>Carcharhinus amboinensis</i>	Aceh Barat	34.70	24.13	26.66	14.51	634
Average		34.97	23.77	26.30	14.96	634
<i>Paragaleus randalli</i>	Aceh Jaya	34.38	24.92	26.18	14.51	634

One notable feature was the variation in nucleotide composition between species, although the dominant pattern of T/U and the lowest G remained. For example, *Hemigaleus microstoma* had an average T/U of approximately 31.47%, while *Sphyrna lewini* was slightly higher at 33.20%, and the *Carcharhinus* group showed the highest T/U percentage with an average of 34.97%. These differences indicate significant genetic variation among these shark species. Another unique feature is the consistency of nucleotide composition within a single species despite different sampling locations. For example, for *Hemigaleus microstoma*, the nucleotide compositions from Aceh Jaya and West Aceh are very similar, indicating genetic stability within the species across adjacent geographic locations. The same is seen for *Sphyrna lewini*. Furthermore, despite interspecies variation, the percentage of adenine (A) showed relatively high stability across most samples, ranging from 25% to 26%. This could be a specific conservation characteristic of the genes analyzed. A final unique feature is the consistently low percentage of guanine (G) across all species and locations, which may reflect specific adaptations or evolutionary characteristics in the genomes of these sharks.

The percentage of nucleotide composition, specifically the G+C (Guanine + Cytosine) or A+T (Adenine + Thymine) ratio, is an important parameter in genomic analysis. A higher G+C ratio is often associated with greater thermal stability of DNA because the triple bond between G and C is stronger than the double bond between A and T. This can be an indicator of an organism's adaptation to high-temperature environments or other extreme conditions. Conversely, a high A+T ratio is often found in organisms with larger genomes or in non-coding regions (introns) that tend to be richer in A and T. In these data, it can be seen that the percentage of G is always the lowest, and overall, the percentage of A+T (T/U + A) tends to be higher than G+C (G + C). The average A+T for *Hemigaleus microstoma* was approximately 57.97% (31.47% + 26.50%), for *Sphyrna lewini* approximately 59.07% (33.20% + 25.87%), and for the *Carcharhinus* group approximately 61.27% (34.97% + 26.30%). Meanwhile, the average G+C ranged from 41.43% to 44.73%. This A+T dominance may indicate that the analyzed DNA/RNA fragments have a higher A+T tendency, which could be related to the specific genetic characteristics of the shark or the genetic function of the fragments studied.

### 3.1.3. Phylogenetic

The presented phylogenetic tree is a dichotomous dendrogram representing the evolutionary relationships between shark species, most likely based on sequence analysis of the Cytochrome Oxidase I (COI) gene. The use of the COI gene

as a molecular marker has proven effective in phylogenetic and DNA barcoding studies in fish, including sharks (Bahri et al., 2017). Each node in the tree represents a hypothetical common ancestor of the taxa branching from it. The length of the horizontal branches is proportional to the estimated genetic divergence, with a scale of 0.01 indicating 0.01 nucleotide substitutions per site. The bootstrap values indicated at each branch, particularly a value of 100, indicate very strong statistical support and high consistency of the clade topology, indicating the reliability of this phylogenetic reconstruction (Bahri et al., 2023b).

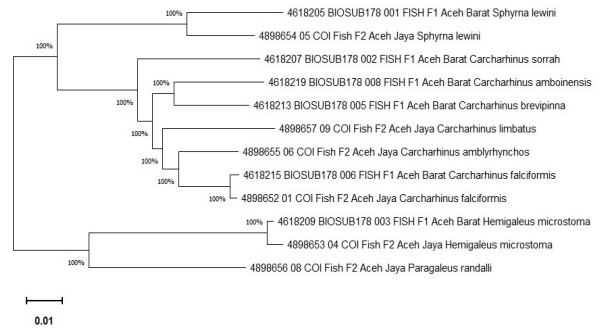


Figure 1. Phylogenetic tree of the shark species found

### 3.1.4. Population Status

The population status of the identified shark species raises serious concern for their survival in Aceh waters. Based on available data, most identified shark species are facing declining population trends, indicating significant pressure on their habitat and survival.

Table 3  
Population status of the shark species found

No	Identified	Population Status IUCN	Population Trend	Reference
1	<i>Carcharhinus falciformis</i>	Vulnerable	Decreasing	Rigby et al, 2021
2	<i>Carcharhinus amblyrhynchos</i>	Endangered	Decreasing	Simpfendorfer et al, 2020
3	<i>Carcharhinus limbatus</i>	Vulnerable	Decreasing	Rigby et al, 2021
4	<i>Hemigaleus microstoma</i>	Vulnerable	Decreasing	Sherman et al, 2022
5	<i>Sphyrna lewini</i>	Critically Endangered	Decreasing	Rigby et al, 2019
6	<i>Paragaleus randalli</i>	Vulnerable	Decreasing	Rigby et al, 2021
7	<i>Sphyrna lewini</i>	Critically Endangered	Decreasing	Rigby et al, 2019
8	<i>Carcharhinus sorrah</i>	Near Threatened	Decreasing	Simpfendorfer et al, 2021
9	<i>Hemigaleus microstoma</i>	Vulnerable	Decreasing	Sherman et al, 2022
10	<i>Carcharhinus brevipinna</i>	Vulnerable	Decreasing	Rigby et al, 2020
11	<i>Carcharhinus falciformis</i>	Vulnerable	Decreasing	Rigby et al, 2021
12	<i>Carcharhinus amboinensis</i>	Vulnerable	Decreasing	Simpfendorfer et al, 2021

Species such as *Carcharhinus falciformis* and *Carcharhinus limbatus* are categorized as "Vulnerable" by the IUCN, with a continuing declining population trend. This indicates that while not yet critically endangered, they face a high risk of extinction in the future without effective conservation efforts. Similarly, *Hemigaleus microstoma* is also listed as "Vulnerable" with a declining population trend. More concerning are the "Endangered" *Carcharhinus amblyrhynchos* and the "Critically

Endangered" *Sphyrna lewini*, both showing drastic population declines.

### 3.2. Discussion

The accuracy of shark species identification plays a crucial role in the conservation and management of marine resources. Molecular methods, specifically sequence genetic analysis based on the NCBI database, have revolutionized this process. Two key roles parameters in NCBI BLAST results are query cover and per.ident (percentage identity) have proven highly effective as benchmarks for shark identifications. Query cover shows the percentage of sample's DNA sequence length that matches the database's reference sequence. A high value (reached 100%) indicates that most of the sample successfully aligned, ensuring relevant genetic relation. Per.ident represents the percentage of identical nucleotide bases between the sample and the reference sequences in the aligned region. A high value (e.g., above 98-99%) is a strong indicator that the sequence belongs to the same or very closely related species. The combination of these two parameters ensures accurate identification, based on extensive and precise genetic sequence alignment. The application of query cover and per.ident is the most relevant for research in Indonesia, where shark identification faces challenges. A study by Bramasta et al. (2021) on shark identification in Bali using DNA Barcoding, relied on strict query cover and per.ident. Similarly, Alfitri et al. (2022) in recognizing sharked in smoked fish products had a high threshold for those parameters. Aisyah & Farhaby's study (2021) on molecular shark rays in Bangka Islands applied the same principles as well to validity of species determination. The use of these parameters increases the precision of understanding and provides a strong scientific basis for distinguishing shark species, supporting research and conservation in Indonesia.

Other studies regarding nucleotide composition of marine organisms, including sharks, often show significant variety according to the gene or genomic region analyzed. For example, a study of mitochondrial D-loop gene in Ikan Glodok (*Periophthalmus argentilineatus*) shows average nucleotide composition of T, A, C, and G of 25.77%, 27.35%, 21.46%, and 25.42%, accordingly (Arisuryanti, 2024). If we compare it with data from sharks in Aceh, it appears that sharks have a consistently higher percentage of T/U and a lower percentage of G. This difference highlights genetic diversity between species and the possibility of different evolutionary adaptations. Another study on shark identification using DNA barcoding in Bali also demonstrated success in identifying species based on nucleotide base sequences, although details of the percentage composition were not always presented explicitly (Toha et al., 2025). However, the consistency of base sequences within a single species, as seen in *Alopias pelagicus* and *Sphyrna lewini* in the study, aligns with the findings in these data, indicating stability of nucleotide composition within the same species across different locations. Overall, these nucleotide compositions provide important insights into genetic diversity and evolutionary relationships between species. The predominance of A+T and low G in these sharks may reflect unique genetic features or conservation in the analyzed genomic regions.

In terms of phylogenetic relationships, the tree clearly identifies several robust monophyletic clades. Two samples of *Sphyrna lewini* form a strongly supported clade (100), confirming their status as the same species with a most recent common ancestor. Similarly, two samples of *Hemigaleus microstoma* also form a strongly supported clade (100). The *Carcharhinus* clade is the most diverse, including *C. sorrah*, *C. amboinensis*, *C. brevipinna*, *C. limbatus*, *C. amblyrhynchos*, and *C. falciformis*. All these *Carcharhinus* species form a large, strongly supported

monophyletic group, indicating an origin from a common ancestor. Molecular identification studies of sharks in various regions of Indonesia also frequently find these *Carcharhinus* species (Bahri & Hafinuddin, 2023a). Intergenerically, the genera *Carcharhinus* and *Hemigaleus* show a closer relationship to each other than to *Sphyrna lewini* or *Paragaleus randalli*. *Paragaleus randalli* serves as the outgroup taxon for the *Carcharhinus* and *Hemigaleus* groups, indicating its position as the most distant relative within this tree. Overall, *Sphyrna lewini* is the earliest diversified taxon of all presented, followed by the group containing *Carcharhinus*, *Hemigaleus*, and *Paragaleus*. The evolutionary direction of this tree can be interpreted as a pattern of divergence from an unexplained common ancestor to the present-day taxa. The initial divergence occurred between the lineage leading to *Sphyrna lewini* and another lineage. Subsequently, the *Paragaleus randalli* lineage split. Finally, the *Carcharhinus* and *Hemigaleus* lineages diversified further, producing the various *Carcharhinus* and *Hemigaleus microstoma* species as a result of more recent branching. This pattern indicates that species topologically closer to the tip of the branch share a more recent common ancestor, while those branching earlier share an older common ancestor.

The population status results indicate that the species face a high risk of extinction in the near future. Other species, such as *Paragaleus randalli* and *Carcharhinus brevipinna*, are also listed as "Vulnerable" with declining population trends. Meanwhile, *Carcharhinus sorrah* is listed as "Near Threatened," meaning that while not yet threatened with extinction, it is approaching the criteria for the threatened category and requires conservation attention. Finally, *Carcharhinus amboinensis* is also classified as "Vulnerable" with a declining population trend. The population declines experienced by these various shark species underscore the urgency of comprehensive conservation measures, including sustainable fisheries management, habitat protection, and enforcement against illegal fishing, to prevent the extinction of these important species.

### 5. Conclusion

A comprehensive analysis of shark data from the waters of Aceh Jaya and West Aceh revealed a highly precise species identification, shown by Percentage of Identity (Per. Ident) of 100% and Query Cover reached 99-100%. These discoveries confirm the effectiveness of DNA barcoding methods and the comprehensiveness of the reference database. Species diversity that has been identified included *Carcharhinus falciformis*, *Sphyrna lewini*, and *Hemigaleus microstoma* in both places, indicating a wide geographic distribution. Nucleotide composition studies showed a consistent pattern with Thymine/Uracil (T/U) as the dominant base and Guanine (G) as the lowest. The high ratio of Adenine+Thymine (A+T) (57.97%–61.27%) to Guanine+Cytosine (G+C) (41.43%–44.73%) suggests shark-specific genetic characteristics or conservation in the analyzed genome segments. Phylogenetic analysis with a 100% bootstrap value supports a robust monophyletic clade, such as *Sphyrna lewini* and *Hemigaleus microstoma*, and places *Sphyrna lewini* as the earliest diversified taxon. However, the status of shark populations is very worrying; most species, including *Carcharhinus falciformis* and *Hemigaleus microstoma*, are listed as "Vulnerable," while *Carcharhinus amblyrhynchos* is "Endangered," and *Sphyrna lewini* is "Critically Endangered," with a drastic population decline. This situation emphasizes the urgency of comprehensive conservation measures to prevent the extinction of these vital species.

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