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The Molecular Study on the Identity, Genetic Composition, and Phylogenetic Relationships of *Carcharhinus* (Blainville, 1816) Sharks in the Western Waters of Aceh

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ABSTRACT The shark genus *Carcharhinus* faces high exploitation pressure in the Western Waters of Aceh, while comprehensive genetic data to support conservation remains limited. This study aimed to identify species, analyze nucleotide composition, and reconstruct the phylogenetic relationships of *Carcharhinus* sharks using the Cytochrome c oxidase subunit I (COI) gene as a molecular marker. A total of 14 tissue samples were collected from three fish landing sites: Aceh Jaya, West Aceh, and Southwest Aceh. Standard procedures included DNA extraction, PCR amplification, sequencing, and bioinformatic analysis using BLAST for identification and MEGA for phylogenetic analysis via the Neighbor-Joining method. Molecular identification successfully confirmed the presence of six species: Carcharhinus falciformis (n=7), C. sorrah (n=3), C. brevipinna (n=1), C. amboinensis (n=1), C. amblyrhynchos (n=1), and C. limbatus (n=1), with identity matches ranging from 99.23-100%. Nucleotide composition analysis revealed a consistent bias towards Adenine and Thymine bases (AT-rich), a common characteristic of the elasmobranch mitochondrial genome. The reconstructed phylogenetic tree demonstrated that each species formed a solid monophyletic group with high bootstrap support. The analysis also confirmed C. brevipinna and C. amboinensis as sister taxa. No clear geographic population structure was found for widespread species, indicating high population connectivity within these waters. This molecular baseline data is crucial for supporting evidence-based fisheries management and conservation strategies for threatened shark species in Indonesia.

Keywords: Aceh Waters; Carcharhinus; conservation; molecular; phylogenetics

INTRODUCTION

Shark fisheries in Indonesia play a vital global role, significantly contributing to the world's total catch (Dharmadi *et al.*, 2021). This sector is the economic backbone of coastal communities, driven by the demand for shark products, especially fins, in the international market (Wulandari *et al.*, 2021). However, overexploitation poses a serious threat to shark populations; more than one-third of global shark and ray species are endangered, according to the IUCN (Dulvy *et al.*, 2021). Sharks, as apex predators, are essential for maintaining trophic balance and maintaining the biodiversity of marine ecosystems (Chin *et al.*, 2020; Dedman *et al.*, 2024). Their population decline can trigger trophic cascade effects and reduce ecosystem resilience (Ferretti *et al.*, 2015). Research on shark population dynamics in Indonesia continues to develop (Pramudji *et al.*, 2021). The government has demonstrated its commitment to conservation by implementing sustainable fisheries regulations (Dharmadi *et al.*, 2016; Shah, 2021). However, policy implementation and increasing fishermen's awareness remain the primary challenges in achieving effective management. Therefore, a comprehensive study of sharks' fishery status and ecology is urgently needed to support evidence-based management strategies.

Carcharhinus is a diverse group of sharks that inhabit various habitats from shallow waters to deep seas (Iqbal *et al.*, 2020; Jabado *et al.*, 2022). Species such as the lanjaman shark (C. falciformis) and the blackfin reef shark (C. melanopterus) are important predators that maintain the health and community structure of marine ecosystems (Chin *et al.*, 2020; Varela *et al.*, 2022). Their ecological role is vital in controlling prey populations and nutrient transfer. However, the *Carcharhinus* population faces a serious threat from overexploitation, driven by the high demand for global shark fins (Shiffman & Philipp, 2016). Many species are caught as primary or bycatch (Fahmi & Sumiarsa, 2017). Studies in Indonesia show alarming exploitation in several species of *Carcharhinus* (Utami *et al.*, 2021; Pramudji *et al.*, 2021). The conservation status of most *Carcharhinus* species is currently categorized as vulnerable to endangered by the IUCN (Dulvy *et al.*, 2021). Habitat degradation and climate change also exacerbate population decline by affecting the distribution and interaction of catches (Varela *et al.*, 2016; Varela *et al.*, 2022).

The waters of West Aceh, including Aceh Jaya, West Aceh, and Southwest Aceh, are areas rich in shark resources that serve as migratory routes and vital fishing areas (Saputra *et al.*, 2023; Harahap *et al.*, 2024). However, the sustainability of shark populations is seriously threatened. Shark landings, often young or immature individuals, continue with alarming intensity, indicating excessive fishing pressure (Yusri *et al.*, 2022). Comprehensive genetic information in this region is still limited, although previous studies have identified several shark species (Saputra *et al.*, 2023). Maintaining shark habitats in the western waters of Aceh is crucial as a nursery and spawning area (Saputra *et al.*, 2023). Habitat from fishing practices and pollution exacerbates the survival of shark populations. Given sharks' slow growth and reproduction rates, habitat protection and sustainable management are imperative to ensure their survival (Dulvy *et al.*, 2021).

Genetic research has revolutionized shark taxonomy and conservation in Indonesia. Molecular identification, such as DNA barcoding using COI genes, is highly effective for accurate species identification from various samples

(Astuti *et al.*, 2017; Abdullah *et al.*, 2022; Sahaba *et al.*, 2022). Nationally, shark genetic research verifies identity, detects protected species, and understands genetic diversity (Ferizal *et al.*, 2025). Phylogenetic analysis reconstructs evolutionary relationships, which are crucial for understanding diversification and identifying conservation units (Kottillil *et al.*, 2023). Genetic distance calculations measure intraspecific variation as a key indicator of a population's genetic health (Utama *et al.*, 2018). The urgency of this research arises from the need for comprehensive genetic data on *Carcharhinus* sharks in the West Waters of Aceh, which are currently minimal, becoming an important basis for management and conservation.

This study aims to fill the gap in genetic information on *Carcharhinus* sharks in the West Waters of Aceh. The problems to be answered include how the identity of the *Carcharhinus* shark species caught in the western waters of Aceh can be determined through molecular analysis, how the phylogenetic relationships between *Carcharhinus* shark species found in the region can be understood in the context of similar species from other locations, and how the level of variation and intraspecific genetic distance of *Carcharhinus* sharks in the waters of the West of Aceh can be quantified. Thus, the primary objective of this study is to identify the types of Carcharhinus sharks caught in the western waters of Aceh using a molecular approach, analyze the phylogenetic relationships of the Carcharhinus shark species found, and calculate the intraspecific genetic distance of these sharks. The benefits of this research are very significant for shark fisheries conservation networks in Aceh and Indonesia. Accurate species identification data will serve as the foundation for effective species-based management and law enforcement. Phylogenetic analysis will provide an in-depth understanding of evolutionary diversity and population connectivity, which is crucial for proper conservation unit planning. Genetic distance information will reveal the level of genetic diversity,enabling population risk assessment and the development of genetically informed protection strategies. Cumulatively, this research produced a robust genetic database that contributes to sustainable shark conservation efforts and strengthens local, regional, and national shark conservation networks, ensuring the adequate protection of these key predators in Indonesia's marine ecosystems.

MATERIALS AND METHODS

Materials

Place and time of research

This research was conducted in the western waters of Aceh, including Aceh Jaya, West Aceh, and Southwest Aceh Regencies. These locations were chosen due to the significant potential for shark fisheries and the presence of various shark species that are targeted and caught as bycatch by fishermen in the area. Shark sampling was conducted directly at the main fish landing ports (PPIs) located in each of the water districts, namely PPI Rigiah in Aceh Jaya, PPI Ujong Baroh in West Aceh, and PPI Labuhan Haji in Southwest Aceh. This approach allows researchers to obtain fresh samples that are representative of the catch of local fishermen.

Primary sample collection was carried out on a total of 14 shark individuals, including three individuals from Aceh Jaya, 4 individuals from West Aceh, and 7 individuals from Southwest Aceh. All samples are from the genus *Carcharhinus*, which fishermen landed during the study period. The number of individuals was selected based on the availability of samples and consideration to obtain adequate genetic representation. The sampling process is intensive over a one-month period, ensuring optimal data coverage over a measurable time frame. The body parts of the fish taken for analysis are the muscle or flesh tissue located around the shark's dorsal fin. This section was chosen because it is known to have a high concentration of mitochondrial DNA and good quality for genetic analysis, as it is commonly used in phylogenetic studies and species identification (Shivji *et al.*, 2002). After col-

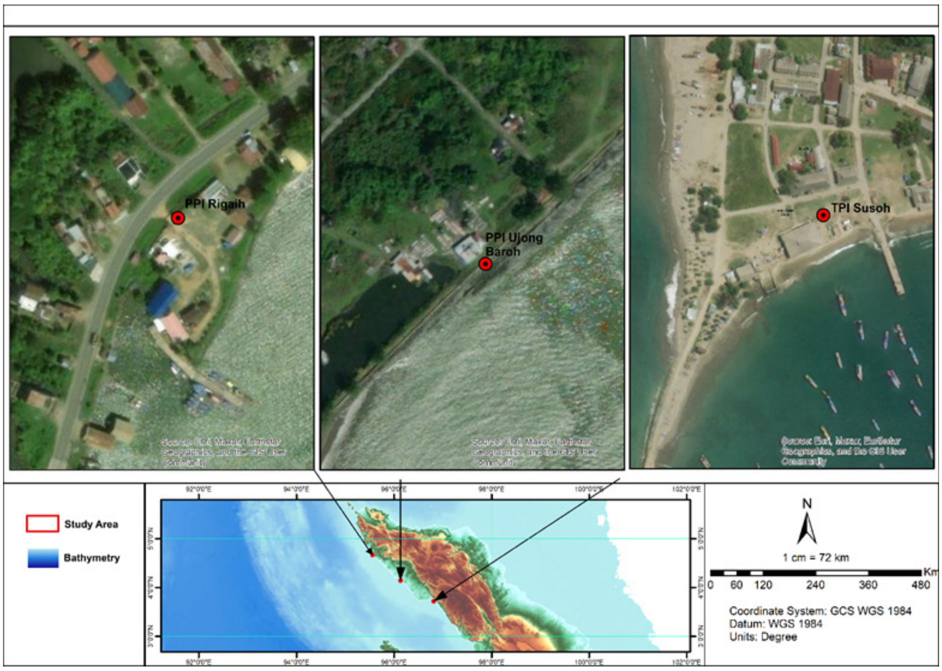

Figure 1. The sampling locations of the genus *Carcharhinus* include the waters of Aceh Jaya, West Aceh, and Southwest Aceh.

lection, the samples are immediately preserved and taken to the Laboratory of Marine Biodiversity and Genetics of Teuku Umar University for the initial preparation stage, including DNA isolation. Furthermore, more in-depth laboratory analyses of molecular assays, including DNA sequencing and bioinformatics analysis, were conducted at the Indonesian Biodiversity Foundation (BIONESIA) Laboratory in Denpasar, Bali. The facility offers sufficient infrastructure and expertise to conduct the complex genetic analysis required for this study.

Methods

Data collection procedure

Shark sampling procedures in DNA genetic analysis, including barcoding, follow standard guidelines to ensure DNA integrity. After fishermen land the shark, initial morphological identification is done to classify the genus. The body part taken is a small piece of muscle or flesh tissue around the dorsal fin, avoiding areas that have important morphological or parasitic characters-sampling minimally invasively using sterile scalpels or biopsy punches to prevent cross-contamination between individuals (Hanner *et al*., 2016). The sample size is usually about 0.5 - 1 cm. Immediately after retrieval, tissue samples are placed in s terile vials that have contained absolute ethanol (96-100%) as a preservation medium (Clarke *et al*., 2020). Ethanol inhibits DNA degradation by enzymes and microorganisms, maintaining the quality of samples until they arrive in the laboratory. Vials are clearly labeled with individual shark information (date, catch location, gender if possible, and unique sample code) to ensure data traceability. The sample is then stored at a cold temperature, such as in an ice box or portable freezer, during transportation to the laboratory (Clarke *et al*., 2020).

Laboratory analysis

DNA extraction from shark muscle tissue samples is a crucial first step to isolating high-quality genomic DNA. This process is generally performed using commercial kits, such as the DNeasy Blood & Tissue Kit (Qiagen), or by the CTAB (Cetyl Trimethylammonium Bromide) modification method (Saghai-Maroof *et al*., 1984; Abdullah *et al*., 2022). Tissue samples are broken down in lysis buffers containing detergents and proteinase K to dissolve cell membranes and degrade proteins. The DNA is then purified from cell remnants and contaminants using spin columns or chemical precipitation, resulting in DNA that is ready for amplification. The concentration and purity of the extracted DNA are validated using a spectrophotometer to ensure its quality before entering the next stage.

The DNA amplification stage is carried out through the Polymerase Chain Reaction (PCR) technique to multiply the fragment of the Cytochrome Oxidase subunit I (COI) gene, which is a standard DNA barcoding marker in animals. The selection of COI genes is based on their characteristics of having low intraspecific variation but relatively high interspecific variation, making them effective for differentiating species (Hebert *et al*., 2003; Abdullah *et al*., 2022). The PCR reaction is prepared in small volumes containing template DNA, universal primary pairs for fish COI genes (e.g., FishF1 and FishR1 primers), dNTPs (deoxynucleotide triphosphates), PCR buffers, and the DNA polymerase enzyme Taq. PCR thermal conditions generally include the initial denaturation stage to separate the double DNA strands, repeated cycles of denaturation, annealing (primary attachment), and extension (elongation of the DNA strand), ending with final extension (Wong & Hanner, 2012). Cycling parameters were an initial denaturation at 95 °C for 2 min followed by 35 cycles of denaturation (94 °C for 30 s), annealing (50 °C for 30 s), and extension (72 °C for 45 s) with the final extension step at 72 °C for 2 min (Prehadi *et al*., 2015)

The amplified PCR product was then visualized via DNA electrophoresis on agarose gel (generally 1.5%). Agarose gel is prepared by dissolving agarose powder in a TBE buffer (Tris-Borate-EDTA) and molded with a sample well. The PCR product sample is mixed with loading dye and loaded into a gel well along with a DNA ladder (DNA size marker). The gel is subjected to an electric current (e.g., 100-120V), causing DNA fragments to move across the gel based on size. After electrophoresis, the gel is stained with a fluorescent dye such as Ethidium Bromide or GelRed and visualized under the transilluminator's UV light. The presence of DNA bands of the expected size (about 600-700 bp for the COI gene) indicates the successful amplification and readiness of the PCR product for sequencing (Sambrook & Russell, 2001).

Data analysis

Molecular data analysis is a crucial stage after acquiring DNA sequences, involving three main procedures: species identification, analysis of genetic distance variations, and reconstruction of kinship or phylogenetic relationships. The DNA sequences are first edited and cut to remove noise or sub-quality parts. For species identification, the purified DNA sequence will be compared to GenBank's National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST) algorithm (Altschul *et al*., 1990; Abdullah *et al*., 2022). This process allows the determination of species identity based on the degree of similarity (percentage of identity) to the reference sequences already in GenBank. A high degree of similarity, generally above 97-98%, often indicates the identity of the same species (Ward *et al*., 2009).

Genetic distance variation analysis was conducted to measure the degree of genetic differences between individuals and species. The sequence data were aligned using programs such as ClustalW or Muscle in the MEGA (Molecular Evolutionary Genetics Analysis) application (Kumar *et al*., 2018). Genetic distance was then calculated using appropriate evolutionary models, such as the Kimura 2-Parameter (K2P) model, a standard method for COI barcoding DNA data (Kimura, 1980; Wardiatno *et al*., 2023). The results of these genetic distance calculations provide quantitative information about genetic diversity within a single species (intraspecific) and between species (interspecific), which is important for understanding population structure and species boundaries.

The phylogenetic tree was reconstructed using the MEGA application. Commonly used methods include Neighbor-Joining (NJ), Maximum Likelihood (ML), or Maximum Parsimony (MP) (Prehadi *et al*., 2015; Kumar *et al*., 2018). The NJ method, for example, builds trees based on a genetic distance matrix, while ML looks for the most likely trees based on DNA evolutionary models. The validity of the tree topology was evaluated using bootstrap analysis with many replications (e.g., 1000 times) to measure the statistical support for each tree branch (Felsenstein, 1985). The resulting phylogenetic tree visualizes evolutionary relationships between individuals or species, aids in identifying taxonomic groups, and helps understand the diversification patterns of *Carcharhinus* sharks.

RESULTS AND DISCUSSION

Results

Identification

Carcharhinus, commonly known as the gray shark or requiem shark, is one of the most dominant and widespread shark genus in the world's waters. The genus includes some of the most recognizable species, such as the bull shark (*C. leucas*), the blackfin shark (*C. limbatus*), and the silk shark (*C. falciformis*) (Bonfil, 2008). Physically, the Carcharhinus shark displays the classic body shape morphology of marine predators: cylindrical, slender, and highly hydrodynamic. Their distinctive features include a pointed snout, five-gill slits, two dorsal fins, where the first fin is much larger than the second, as well as eyes equipped with a nitrication membrane (third eyelid) for protection when attacking prey. Their teeth are usually wide triangles with fine serrations, very efficient for cutting and gripping. The distribution of this genus is very global, inhabiting tropical waters and temperate climates throughout the oceans. They are highly adaptive predators that can be found in a variety of habitats, from shallow coastal waters and bustling coral reefs to continental shelves and deep open oceans. These coastal habitats often serve as nursery areas that are crucial for the survival of shark hatchlings (Heupel *et al*., 2007).

Some species, especially bull sharks, have an incredible physiological tolerance to enter brackish water and freshwater systems, often traveling long distances upstream. As apex predators or key predators, their diet is highly varied, including bony fish, squid, crustaceans, and even smaller sharks and rays. The reproductive system of Carcharhinus sharks is viviparous, which means they give birth to fully developed offspring. Embryos obtain nutrients through the placenta of the yolk sac, an adaptation like that of mammals (Galván-Tirado *et al*., 2015). Despite having a crucial ecological role in maintaining the balance of marine ecosystems, many species in this genus face grave threats from overfishing, both as targets and bycatch, as well as habitat degradation. As a result, the conservation status of many Carcharhinus sharks is now a global concern, with some species already categorized as endangered (Aisyah *et al*., 2020).

Based on the identification results, a total of 14 shark samples has been analyzed from three regions in Aceh, namely Southwest Aceh, West Aceh, and Aceh Jaya. All samples were identified as members of the genus *Carcharhinus*. The identification process shows the percentage value of identity (Per. Ident) ranges from 99.23% to 100%, and Query Cover values range from 87% to 100%.

Figure 2. Shark genus *Carcharhinus* (Blainville 1816) which was the object of the study (*Source: personal documentation*).

From the analysis, six different species of *Carcharhinus* were identified. The species *Carcharhinus falciformis* (Silk shark) was identified in seven samples, *Carcharhinus sorrah* (Monas shark) in three samples.In contrast, the other four species were *Carcharhinus brevipinna* (Silk shark), *Carcharhinus amboinensis* (Bull shark), *Carcharhinus amblyrhynchos* (Grayfin reef shark), and *Carcharhinus limbatus* (Blackfin sharks), which were each identified in one sample. Based on the region of origin, the species *Carcharhinus falciformis* was found in all three locations. The species *Carcharhinus sorrah* is found in Southwest Aceh and West Aceh. Meanwhile, *Carcharhinus brevipinna* and *Carcharhinus amboinensis* are only found in West Aceh, while *Carcharhinus amblyrhynchos* and *Carcharhinus limbatus* are only found in Aceh Jaya. The uniqueness of this identification results lies in the level of species diversity detected from a relatively small number of samples. The discovery of six

Table 1. The molecular identification results of sharks of the genus *Carcharhinus* used the *Basic Local Alignment Search Tool* (BLAST) approach from each research site.

Yes	Sample Code	Region of Origin	Unidentified	Per. Ident	Query Cover	Status IUCN
1	4659875	Southwest Aceh	Carcharhinus sorrah	100%	98%	NT
2	4679264	Southwest Aceh	Carcharhinus sorrah	99.84%	98%	NT
3	4659876	Southwest Aceh	Carcharhinus falciformis	100%	100%	VU

4	4679266	Southwest Aceh	Carcharhinus falciformis	100%	95%	VU
5	4679267	Southwest Aceh	Carcharhinus falciformis	100%	100%	VU
6	4679268	Southwest Aceh	Carcharhinus falciformis	99.82%	87%	VU
7	4679270	Southwest Aceh	Carcharhinus falciformis	100%	100%	VU
8	4618207	West Aceh	Carcharhinus sorrah	100%	98%	NT
9	4618213	West Aceh	Carcharhinus brevipinna	100%	100%	VU
10	4618215	West Aceh	Carcharhinus falciformis	100%	100%	VU
11	4618219	West Aceh	Carcharhinus amboinensis	99.83%	90%	VU
12	4898652	Aceh Jaya	Carcharhinus falciformis	100%	97%	VU
13	4898655	Aceh Jaya	Carcharhinus amblyrhynchos	100%	97%	EN
14	4898657	Aceh Jaya	Carcharhinus limbatus	99.23%	99%	VU

different species out of a total of 14 samples showed significant variation in the genus *Carcharhinus* in those waters. In addition, the data also showed the dominance of the *Carcharhinus falciformis* species which accounted for 50% of the total samples. On the other hand, the existence of four species, each represented by only one individual, indicates the presence of species with a lower frequency of occurrence in the sampling area.

Nucleotide composition

Nucleotide composition analysis was carried out on DNA fragments along 653 base pairs (bp) from 14 shark samples of the genus *Carcharhinus*. The analysis results showed a consistent pattern of nitrogenous base composition throughout the tested samples. In general, the nucleotide composition is dominated by Thymine (T) with the highest average percentage, followed by Adenine (A), Cytosine (C), and Guanine (G) with the lowest average percentage. The average composition for the entire sample showed Thymine (T) around 35.04%, Adenine (A) 26.32%, Cytosine (C) 23.12%, and Guanine (G) 15.32%. The uniqueness of the data pattern obtained from the results of this analysis is the existence of a clear composition bias, where the base content of Adenine and Thymine (A+T) is significantly higher than the content of Guanine and Cytosine (G+C). This phenomenon, known as AT-rich, is a common characteristic of mitochondrial DNA sequences in many marine organisms. Another unique pattern is the very high level of consistency of nucleotide composition within a single species (intraspecific consistency). For example, all three samples of *Carcharhinus sorrah* had almost identical percentages of T, C, A, and G. Similarly, in seven samples of *Carcharhinus falciformis* which showed very minimal variation. This consistency contrasts with the slight interspecies variation (interspe-

Table 2. Nucleotide composition analysis was carried out on DNA fragments along 653 base pairs (bp).

No	Kind	Location	T(U)	C	A	G	Total
1	Carcharhinus sorrah	Southwest Aceh	35.07	22.97	26.65	15.31	653
2	Carcharhinus sorrah	Southwest Aceh	35.07	22.97	26.49	15.47	653
3	Carcharhinus sorrah	West Aceh	35.07	22.97	26.65	15.31	653
	Avg.		35.07	22.97	26.60	15.36	653
4	Carcharhinus falciformis	Southwest Aceh	35.22	22.82	26.19	15.77	653
5	Carcharhinus falciformis	Southwest Aceh	35.22	22.82	26.19	15.77	653
6	Carcharhinus falciformis	Southwest Aceh	35.22	22.82	26.19	15.77	653
7	Carcharhinus falciformis	Southwest Aceh	35.38	22.82	26.49	15.31	653
8	Carcharhinus falciformis	West Aceh	35.22	22.82	26.19	15.77	653
9	Carcharhinus falciformis	Southwest Aceh	35.22	22.82	26.19	15.77	653
10	Carcharhinus falciformis	Aceh Jaya	34.92	23.28	26.03	15.77	653
	Avg.		35.20	22.88	26.21	15.71	653
11	Carcharhinus brevipinna	West Aceh	33.69	24.66	26.03	15.62	653
12	Carcharhinus amboinensis	West Aceh	34.61	23.74	26.65	15.01	653
13	Carcharhinus amblyrhynchos	Aceh Jaya	35.53	22.82	26.34	15.31	653
14	Carcharhinus limbatus	Aceh Jaya	35.07	23.43	26.19	15.31	653
	Avg.		35.04	23.12	26.32	15.52	653

cific variation), which, although small, is the basis for molecular differentiation between closely related species.

Phylogenetics

The reconstructed phylogenetic tree illustrates the evolutionary kinship relationship between shark samples of the genus *Carcharhinus* originating from the waters of Southwest Aceh, West Aceh, and Aceh Jaya. The results of the analysis showed the formation of several main groups (clades) supported by a very high trust value (bootstrap), which is 100%. In terms of interspecies kinship, *Carcharhinus falciformis* from all three regions shows a very close relationship with *Carcharhinus amblyrhynchos* from Aceh Jaya, where the two form a monophyletic group. This group is related to another group consisting of *Carcharhinus brevipinna* and *Carcharhinus amboinensis*, both of which are native to West Aceh and are close relatives of each other (sister taxa). The species *Carcharhinus limbatus* from Aceh Jaya separated earlier from these groups, indicating a more distant kinship.

Meanwhile, three samples of *Carcharhinus sorrah* from Southwest Aceh and West Aceh form the most basal monophyletic group, indicating that this species is the earliest lineage separated from a common ancestor compared to the other species in this analysis. Regarding kinship between regions, this analysis shows the absence of specific genetic groupings based on the area of origin for the same species. For example, seven individuals of Carcharhinus falciformis originating from Southwest Aceh, West Aceh, and Aceh Jaya do not form separate sub-groups based on their geographical location but rather merge into the same clade. A similar pattern was also seen in the species *Carcharhinus sorrah*, where individuals from Southwest Aceh and West Aceh clustered together without regional separation. This indicates that the populations of these species in the three waters are very closely related and do not show a geographically differentiated population structure based on the analyzed sequence data.

Discussion

Identification

Identification using molecular methods is important, given the lack of data and information available to identify shark species morphologically. Molecular identification using DNA markings successfully confirmed the existence of six species of sharks of the genus *Carcharhinus* in Aceh waters, which include *Carcharhinus falciformis*, *C. sorrah, C. brevipinna, C. amboinensis, C. amblyrhynchos, and C. limbatus*. These findings indicate a high level of species diversity for the genus *Carcharhinus* in the western coastal region of Sumatra. Previous research by Bahri *et al*. (2023) in the waters of West Aceh also reported the findings of several similar species, such as *C. sorrah, C. brevipinna, C. amboinensis, and C. falciformis*, which reinforces the validity of the data that this region is an important habitat for diverse species of *Carcharhinus* sharks. The results showed that *Carcharhinus falciformis* (Silk shark) was the most dominant species, accounting for 50% of the total identified samples. The dominance of *C. falciformis* in catches has also been reported in various other areas of Indonesian waters, such as in the western waters of Sumatra and the Indian Ocean, indicating that this species has a wide population distribution and is often a target or bycatch in longline fisheries (Sime-

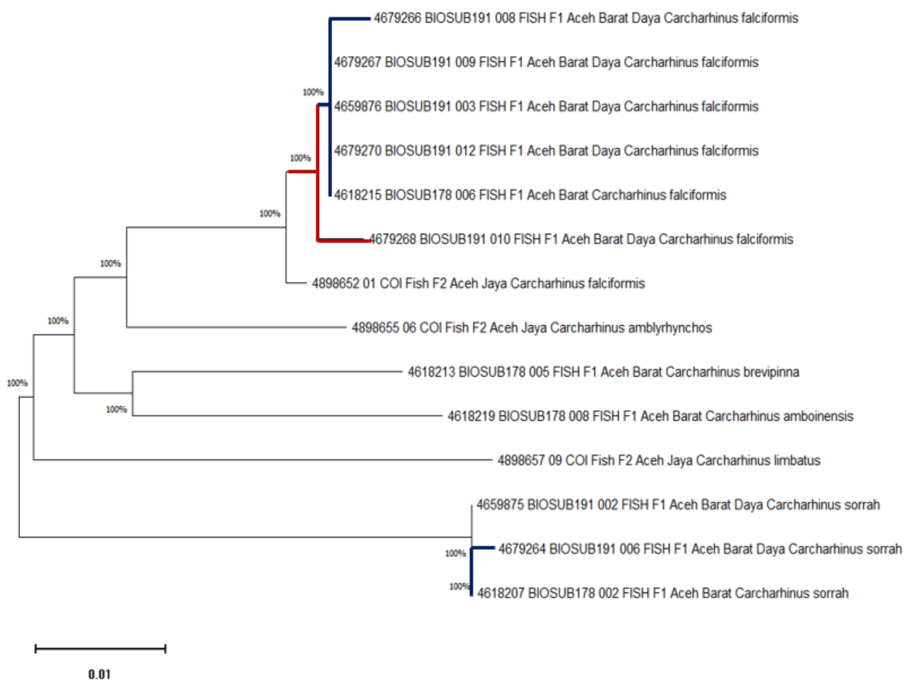

Figure 3. The results of the analysis of kinship relationships (phylogenetic) using Neighbor-Joining from sharks of the genus at each research location.

on *et al*., 2020).

The discovery of four species represented by only one individual (singleton) indicates that although these species exist, the population may not be as large as *C. falciformis*, or that the distribution area is not concentrated at the sampling site. The findings of species such as *C. amboinensis* and *C. amblyrhynchos* are also consistent with other studies in Indonesia, for example, by Wiryawan *et al*. (2024), who also reported the landing of *C. amblyrhynchos* at the Tanjung Luar Fishing Port, West Nusa Tenggara. The most significant impact of this identification result is on the conservation aspect. According to the IUCN Red List, some identified species have a vulnerable conservation status. *Carcharhinus falciformis* is categorized as Vulnerable globally due to massive fishing pressure for its fin trade (IUCN, 2021). Similarly, *C. amblyrhynchos* and *C. limbatus* face significant threats and are listed in the Near Threatened category. The presence of these threatened species in Acehnese waters underscores the region's vital role as a key habitat that requires urgent management attention and conservation strategies. Accurate data on the composition of such species is fundamental in formulating sustainable fisheries policies, monitoring the implementation of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) regulations, and efforts to reduce shark mortality as bycatch.

Nucleotide composition

Nucleotide composition analysis on 653 bp subunit I (COI) *Cytochrome c oxidase I* (COI) gene fragments from six shark species of the genus *Carcharhinus* showed a consistent and informative pattern. The data systematically revealed a significant bias in the composition of nitrogenous bases, where the content of Thymine (T) and Adenine (A) was consistently higher than that of Cytosine (S) and Guanine (G). The overall average shows Thymine as the most dominant base (around 35.04%), followed by Adenine (26.32%), Cytosine (23.12%), and Guanina as the lowest (15.52%). This phenomenon of high A+T proportions is characteristic of the mitochondrial genome in vertebrates, including elasmobranchii, indicating selective pressures or molecular mechanisms that support this composition. This pattern is not only uniform among individuals within a single species, as in *C. sorrah* and *C. falciformis*, but is also consistent among different species within the genus *Carcharhinus*, demonstrating the evolutionary stability of these characteristics on the COI gene markings.

Nucleotide composition analysis of the mitochondrial gene Cytochrome c oxidase I (COI) from shark populations of *the genus Carcharhinus* in the waters of Aceh Jaya, West Aceh, and Southwest Aceh consistently showed a strong compositional bias towards Adenin and Thymine (AT-bias), with total AT (A+T) content exceeding 61%. These genomic characteristics are not anomalies, but rather a reflection of conserved evolutionary features in the elasmobranch mitochondrial genome, which were formed because of asymmetric mutation pressures over millions of years, rather than as a direct indicator of the current ecological conditions of the population. The most significant implication of this baseline data lies in its potential as a foundation for more in-depth analysis of population genetics, where variations in nucleotide sequences, not just their composition, can be used to measure key parameters of population health, such as genetic diversity and population structure. Low levels of diversity may indicate a *genetic bottleneck* effect or overfishing pressure. At the same time, an understanding of population structure is essential to identify whether populations in all three locations are interconnected or are separate management units (Momigliano *et al*., 2017). Further, these sequence data are vital for practical conservation applications through *DNA barcoding* methods, which allow for the accurate identification of morphologically elusive fishery products, an essential tool for monitoring trade and supporting law enforcement against protected species in Indonesian waters. Thus, this nucleotide composition percentage value, although intrinsically a reflection of evolutionary history, constitutes an invaluable scientific foundation for studying the condition, connectivity, and conservation status of vulnerable *Carcharhinus* shark populations on the West Coast of Aceh.

Analysis of the nucleotide composition of the different shark species of the genus *Carcharhinus* presented in the table, it was seen that the GC (Guanin-Cytosine) content in their mitochondrial DNA sequences was relatively consistent, ranging from 38% to 40%. For example, *Carcharhinus sorrah* has an average GC content of 38.33%, while *Carcharhinus brevipinna* has a slightly higher value of 40.28%. This small difference indicates a very close kinship among the sample individuals, which confirms their placement in the same genus. Structurally, this relatively low percentage of GC has some significant impacts. Because the G-C bond is stronger (three hydrogen bonds) than the A-T bond (two hydrogen bonds), DNA with a lower GC content tends to be less stable. This can affect the rate of mutation, where sequences with higher A-T content may be more susceptible to genetic changes, potentially accelerating the rate of mutations in the mtDNA of this species (Sahaba *et al*., 2021; Bahri *et al*., 2023). Nonetheless, the consistency of the high GC content underscores the stability and strong genetic kinship among the individuals studied.

Phylogenetics

This molecular data-based phylogenetic reconstruction presents a tree topology that illustrates the evolutionary relationships between shark species in the genus *Carcharhinus* from the waters of Southwest Aceh and Aceh Jaya. The results of the analysis show that each species forms a well-defined monophyletic group, which is validated by strong statistical support values. One significant finding was the consolidation of all individuals of *Carcharhinus falciformis* into a single clade with full support, despite coming from two different geographic regions. This phenomenon indicates a high level of gene flow or a relatively recent evolutionary divergence event between populations, so substantial genetic differences have not accumulated. Further topological analysis reveals the details of interspecific kinship relationships. The species *Carcharhinus sorrah* was identified to form an exclusively monophyletic clade, as did other species such as *C. limbatus*, which also occupied its own terminal branches, which confirmed the validity and resolution of the molecular markers used. Another important thing is the placement *of Carcharhinus brevipinna* and *Carcharhinus amboinensis* as sister taxa. This position, characterized by the branching of a single internal node, definitively suggests that the two species share a Most Recent Common Ancestor that the other taxa do not have in this analysis, reflecting the closest phylogenetic kinship.

Comparatively, the results obtained from this analysis show high consistency with the phylogenetic framework of the genus *Carcharhinus* that has been published in global-scale studies. The pattern of monophyletic grouping at the species level, in which each species forms an exclusive clade, is a common finding and supports the effectiveness of molecular markers such as COI for shark taxonomic identification, as reported by Ward *et al*. (2005) and affirmed in the broader phylogeny of elasmobranchii by Naylor *et al*. (2012). More specifically, the sibling taxon relationships between *C. brevipinna and C. amboinensis* identified in this study are directly supported by previous molecular findings that also highlight the high genetic and morphological similarities between the two species (Vélez-Zuazo & Agnarsson, 2011). Thus, this analysis not only serves to validate the taxonomic identity and genetic structure of shark populations in Aceh but also reinforces that local evolutionary patterns reflect the evolutionary history of this genus on a broader biogeographic scale.

CONCLUSION AND RECOMMENDATION

Conclusion

Based on the results and discussion, it can be concluded that the molecular approach using COI DNA markings is very effective for the study of the diversity and evolution of Carcharhinus sharks in the western waters of Aceh. This study succeeded in accurately confirming the existence of six different species of Carcharhinus, namely C. falciformis, C. sorrah, C. brevipinna, C. amboinensis, C. amblyrhynchos, and C. limbatus. The high level of diversity of this limited number of samples underscores the importance of the West Aceh Waters as a key habitat. The dominance of C. falciformis in catches and the identification of several species with vulnerable (VU) and Near Threatened (NT) conservation status by the IUCN confirm the urgency of implementing specific management and conservation strategies to protect populations in this region. Nucleotide composition analysis showed the presence of a base composition bias (AT-rich), which is a stable and conserved characteristic of the elasmobranch mitochondrial genome. Phylogenetic reconstruction successfully maps evolutionary kinship relationships with high statistical validity, in which each species forms a distinctly monophyletic group. These findings not only confirm the relationship between species, such as placing *C. brevipinna* and *C. amboinensis* as sister taxons but also provide important insights into population structure.

Recommendation

The absence of genetic grouping based on geographic location in the same species indicates the presence of high population connectivity and gene flow between the three aquatic areas, a fundamental information for defining an effective fisheries management unit.

AUTHORS' CONTRIBUTION

SB: Established the idea, conducted research, performed the data analysis, corresponding author, manu- script preparation, editing manuscript and funding. BH: include ideas, doing research, data analysis, co-authors, manuscript preparation, editing manuscript, and funding. AR: are Team research, edit manuscript.

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