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Short Communication

DNA Barcoding of Shark and Ray Species from Bawean and Masalembu Waters East Java

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Abstract

Sharks and rays, as apex predators or mesopredators, help maintain marine biodiversity and ecosystem balance. Their ecological and economic value underscores the need for conservation, as they face threats from overfishing, habitat loss, and climate change, with many classified as vulnerable or endangered by the IUCN. This study investigates the genetic diversity and phylogenetic relationships of sharks and rays in Bawean and Masalembu Waters, East Java, using morphological identification and DNA barcoding. The specimens were obtained from fishermen operating in Bawean and Masalembu Waters. A total of 11 samples were analyzed from five shark species: Sphyrna lewini, Carcharhinus sealei, Stegostoma fasciatum, Galeocerdo cuvier, and Carcharhinus falciformis, and two ray species: Rhynchobatus australiae and Rhina ancylostoma. Results showed high genetic similarity within species, with some divergence observed between samples from the Bawean and Masalembu regions. For instance, populations of Sphyrna lewini from the two regions exhibited slight mitochondrial DNA sequence variations, indicating possible adaptations to local environmental conditions. Similarly, genetic differences in Rhynchobatus australiae suggest limited gene flow between populations, likely influenced by geographical barriers or habitat preferences. Phylogenetic analysis revealed seven distinct clades, highlighting evolutionary relationships such as the close grouping of S. lewini and G. cuvier, which suggests recent divergence. Several species identified, including S. lewini, G. cuvier, and C. falciformis, are protected, underscoring the need for stricter conservation and monitoring efforts to safeguard shark and ray populations. This study highlights the urgency of integrating genetic diversity into conservation strategies to ensure the long-term survival of these vital species.

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1. Introduction

Sharks and rays play a crucial role in marine ecosystems, with some species acting as apex predators that regulate prey populations, while others occupy mesopredatory roles, contributing to the structure and stability of the oceanic food web (Motivarash *et al*., 2019; Heupel *et al*., 2014). By controlling the abundance and health of species below them in the food chain, they contribute significantly to marine biodiversity, supporting ecosystems that are resilient to invasions by non-native species and environmental changes (Roff *et al*., 2016; Dedman *et al*., 2024). Beyond their ecological significance, sharks and rays also hold substantial economic value. They are pivotal to the fisheries sector, providing a source of livelihood for many coastal communities (Murphy *et al*., 2018). Additionally, they are a major draw for marine tourism, attracting divers and snorkelers worldwide, which further supports local economies (Prihadi, 2018; Mustika *et al*., 2020). The conservation of sharks and rays is thus vital, not only for ecological reasons but also for sustaining the economic benefits that many communities depend on.

Shark and ray populations are increasingly under threat from a variety of human activities. Overfishing is one of the primary threats, driven by the high demand for shark fins, meat, and other products. Many shark and ray species are caught as bycatch in fisheries targeting other species, exacerbating their decline (Dulvy *et al*., 2014b; Pollom *et al*., 2024). Habitat destruction, particularly in coastal areas, further threatens these species by degrading essential habitats such as mangroves, coral reefs, and seagrass beds (Nichols *et al*., 2019). Climate change also poses significant risks, altering ocean temperatures, sea levels, and the distribution of prey species, which can negatively impact shark and ray populations (Bouyoucos, 2020; Rummer *et al*., 2022).

The International Union for Conservation of Nature (IUCN) Red List provides a comprehensive overview of the conservation status of sharks and rays species. As of the latest assessments, many sharks and rays are listed as vulnerable, endangered, or critically endangered. For example, the scalloped hammerhead shark (*Sphyrna lewini*) is listed as critically endangered due to its significant population decline (Ayres *et al*., 2024). The sawfish family (Pristidae) is also critically endangered, with all species facing severe threats from habitat loss and bycatch (Dulvy *et al*., 2014b; Tanna *et al*., 2021). These listings highlight the urgent need for conservation measures to protect these vital marine species.

In the context of Indonesia, studies have focused on the rich diversity of sharks and rays in the region, revealing significant genetic diversity and endemism (Ramadhaniaty *et al*., 2023). A study by Hadi *et al*. (2020) examined the genetic structure of the critically endangered scalloped hammerhead shark (*S. lewini*) in Indonesian waters, finding distinct population segments that are crucial for effective management and conservation strategies. Similarly, a study by Malik *et al*. (2023) on the genetic diversity of manta rays in East Java identified unique genetic markers that can be used for population monitoring and conservation planning. DNA barcoding has been effectively used to identify various marine organisms, including octopus (Kholilah *et al*., 2021a; Kholilah 2021b), squid (Afiati *et al*., 2022), threadfin bream fish (Wora *et al*., 2024), cardinal fish (Putra *et al*., 2024), anchovy (Joesidawati *et al*., 2023a), spider crab (Ambariyanto *et al*., 2023), blue swimming crab (Joesidawati, 2023b), and redbelly yellow tail fusilier (Nursalim *et al*., 2022), providing valuable data for species identification and biodiversity assessment in similar studies.

These findings underscore the critical need for integrating region-specific conservation strategies that consider the genetic diversity and distinct population structures of shark and ray species in Indonesia. A key challenge in these efforts is the lack of detailed genetic information, particularly for species in the understudied marine areas of Bawean and Masalembu Waters, East Java. This knowledge gap complicates species identification due to morphological similarities and hinders the development of effective conservation policies informed by phylogenetic data. To address these issues, this study employs advanced molecular barcoding techniques. The aim of this study is to assess the genetic diversity and phylogenetic relationships of shark and ray species from Bawean and Masalembu Waters in East Java through molecular barcoding techniques. By analyzing tissue samples collected from the Bronjong Fish Landing Site, this research seeks to provide critical insights into the evolutionary relationships and genetic variability of sharks and rays species, which are essential for developing region-specific conservation strategies to ensure their long-term survival.

2. Materials and Methods

2.1 Materials

2.1.1 The equipments

The equipments used in this research included sterile scalpels for tissue extraction, ethanol for sam-

ple preservation, a -20°C freezer for sample storage. The tools used included a vortex mixer (Fisher Scientific, United States), a centrifuge (Mini Gyrozen, Fisher Scientific, United Satates), and a thermal cycler for PCR (Bio-Rad MJ Mini™ Personal Thermal Cycler, United States). Electrophoresis was performed with a standard gel electrophoresis setup, and the results were visualized under a UV transilluminator (220V Mini-300 serial 1709919A025of Major Science with tank specification B2; 0-150V,0-100mA of Thermo Scientific, United States). Sequencing was conducted using an ABI 3730xl DNA Analyzer (ThermoFisher Scientific, Massachusetts, United States).

2.1.2 The materials

This study analyzed tissue samples from 11 individuals representing six sharks and rays species collected in July 2024 from local fishermen at the Bronjong Fish Landing Site, East Java. Based on interviews with the fishermen, the samples were caught in two distinct locations: Bawean and Masalembu Waters, East Java, representing different fishing grounds and potential populations (Figure 1).

Each tissue sample was preserved in 96% ethanol immediately after collection and stored in a -20°C freezer until further analysis. The preserved samples were then transported to the Diponegoro Biodiversity Project (DBP) Laboratory at the Integrated Laboratory, Diponegoro University, Semarang, Indonesia, for molecular analysis.

The materials used in this study included 10% Chelex solution (Chelex® 100 sodium form 25 G, Sigma-Aldrich, PT. Genetika Science Indonesia) for DNA extraction, MyTaq™ HS Red Mix (Bioline, PT. Genetika Science Indonesia) for PCR amplification, and primers FishF1 and FishR1 (Ward *et al*., 2005). Agarose Biotechnology Grade 100g (1st BASE, PT. Genetika Science Indonesia) was used for gel electrophoresis, and Florosafe DNA Staining (1st BASE, PT. Genetika Science Indonesia) was applied for visualizing DNA bands.

2.1.3 Ethical approval

Ethical approval for this study was granted by the relevant ethics committee under ethical clearance

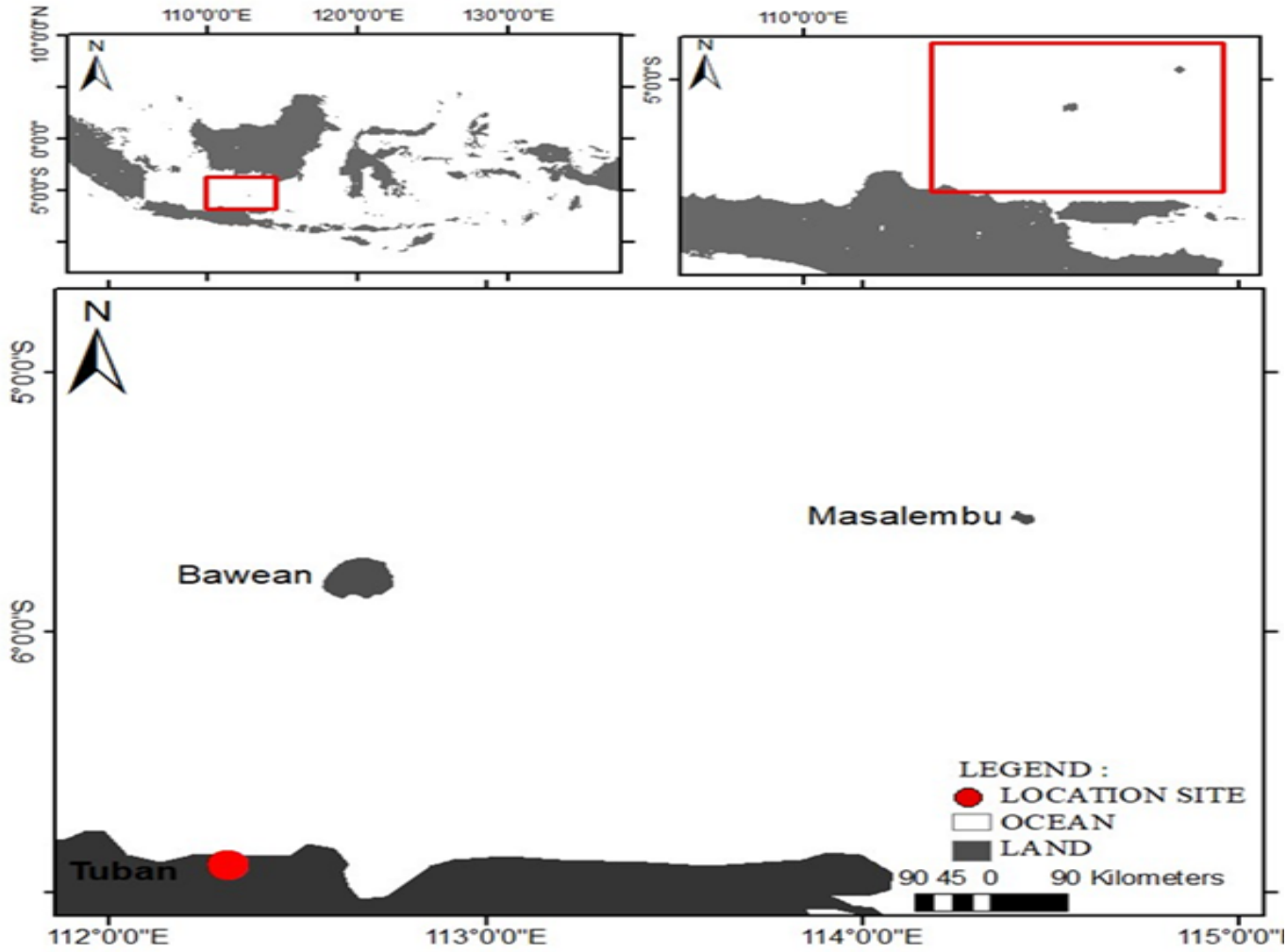

Figure 1. Speciment collection map from Bronjong Fish Landing Site and fishing ground of fisherman in Bawean and Masalembu Waters.

number 350/VII/071073/PGRI/LEMLIT/N/IX/2024, valid for a two-year period. This experiment was performed in accordance with all ethics and animal rights guidelines outlined by the Desert Research Center, following all applicable rules and regulations in conformity with the European Union directive for the protection of experimental animals (2010/63/EU). All specimens used in this study were obtained from local fishermen rather than being directly caught by the researchers. The collection and handling of samples adhered to ethical guidelines, with careful consideration for animal welfare principles.

2.2 Methods

Morfological identification was conducted at the Brondong and Masalembu Fish Landing Site using the field guide to look-alike sharks and rays species of the southeast asian region as a reference (Ali *et al*., 2013). Extraction was carried out using a 10% of chelex (Walsh *et al.,* 1991). Approximately 2 mm of the sample is inserted into the tube containing 10% chelex, then heated at 95°C for 45 minutes (Akbar and Aris, 2018). The Chelex was then vortexed and centrifuged to separate the pellet and the supernatant (the clear solution of DNA in the water solution above the pellet). A portion of the Cytochrome Oxidase Subunit I (COI) gene was amplified via PCR using the primers FishF1 and FishR1 (Sultana *et al*., 2018; Muttaqin *et al*., 2019). PCR reaction was carried out in 25 µL volumes, using 1 µL of template. Each reaction included 12.5 µL MyTeqTM Red Mix, 1 µL of each primer and 9.5 µL ddH2O. The thermocycling profile included an initial denaturation of 92°C for 5 min, 33 cycles of 92°C for 45s, 50°C for 60s, and 72°C for 1 min, with a final extension of 72° C for 10 min (**.** The PCR reactions were checked on 1% weight/volume (w/v) agarose gels (Dailami *et al.,* 2021), stained with Florosafe. After confirming the presence of a band, the sample was sent to PT. Genetika Science Indonesia for Sanger sequencing.

2.3 Analysis Data

The data obtained from sequencing facility for further analysis. DNA sequences were cleaned, edited, and aligned using ClustalW in the MEGA X program (Kumar *et al.,* 2018). The clean sequences were then compared to the open database NCBI (Table 1) (*The National Center for Biotechnology Information*; https://www.ncbi.nlm.nih.gov) using the BLAST program (*Basic Local Alignment Search Tool*; https://blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic tree was reconstructed using the maximum likelihood method with the Tamura-Nei 93 (TN93+G+I) model, selected based on the best model recommendations provided by the MEGA X program, and 1000 bootstrap replicates to ensure robustness.

3. Results and Discussion

3.1 Results

3.1.1 Species identification and genetic diversity

A total of 11 samples consisted of 5 shark species and 2 ray species (Figure 2) were collected in this study. The shark species identified were Hammerhead Shark (*Sphyrna lewini*), Blackspot Shark (*Carcharhinus sealei*), Zebra Shark (*Stegostoma fasciatum*), Tiger Shark (*Galeocerdo cuvier*), and Silky Shark (*Carcharhinus falciformis*), identified using the taxonomic keys and descriptions provided by Haroon and Kibria (2021). The ray species identified were Bottlenose Wedgefish (*Rhynchobatus australiae*) and Sharpnose Guitarfish (*Rhina ancylostoma*), identified using the guidelines from Last (2016).

All samples produced sequences with lengths of 654 base pairs. The Hammerhead Shark (*S. lewini*) was identified in four samples (DBP012125, DBP012128, DBP012132, and DBP012131) with sequence identities ranging from 88.24% to 100%. The Bottlenose Wedgefish (*R. australiae*) was identified in two samples (DBP012126 and DBP012133), both showing 100% identity. The Blackspot Shark (*C. sealei*) was identified in two samples (DBP012127 and DBP012134), each with 100% identity. The Zebra Shark (*S. fasciatum*), Tiger Shark (*G. cuvier*), and Silky Shark (*C. falciformis*) were each identified in one sample (DBP012129, DBP012130, and DBP012131 respectively), with identities ranging from 99.39% to 100%. Additionally, the Sharpnose Guitarfish (*R. ancylostomus*) was identified in one sample (DBP012135) with a sequence identity of 98.17% (Table 2).

3.1.2 Phylogenetic insights

The phylogenetic tree depicts seven clades, each representing the same species (Figure 3). The first clade represents *C. sealei* (DBP012127 and DBP012134) were compared with data from Indonesia, Malaysia, Philippines, and Brunei. In the second clade, *C. falciformis* DBP012131 was compared with samples from Indonesia and Australia. The third clade, *G. covier* DBP012130, was compared with samples from Malaysia, Thailand, and Mexico. In the fourth clade, *S. lewini* (DBP012125, DBP012128, and DBP012132) were compared with data from Indonesia, Malaysia, Australia, Philippines, and Colombia. The fifth clade, *S. fasciatum* (DBP012129), was compared with data from India, Australia, Red Sea, Saudi Arabia, and Madagascar. In the sixth clade, *R.*

Table 1. Molecular accession data by country and source.

No	Accession Number (ingroup/outgroup)	Country	Source
1.	EU398611.1	Indonesia	Ward *et al*., 2008
2.	EU398613.1	Indonesia	Ward *et al*., 2008
3.	EU399049.1	Indonesia	Ward *et al*., 2008
4.	FJ178399.1	Indo-West Pacific	Dugdeon *et al*., 2009
5.	HQ171777.1	Madagascar	Doukakis *et al*., 2011
6.	JN315433.1	Colombia	Caballero *et al*., 2012
7.	KC840952.1	Indonesia	Prehadi *et al*., 2015
8.	KF590366.1	Indonesia	Sembiring *et al*., 2015
9.	KF590372.1	Indonesia	Sembiring *et al*., 2015
10.	KF590375.1	Indonesia	Sembiring *et al*., 2015
11.	KF590377.1	Indonesia	Sembiring *et al*., 2015
12.	KF590378.1	Indonesia	Sembiring *et al*., 2015
13.	KF590484.1	Indonesia	Sembiring *et al*., 2015
14.	KF899640.1	Indonesia	Sembiring *et al*., 2015
15.	KF899641.1	Indonesia	Sembiring *et al*., 2015
16.	KM396944.1	Saudi Arabia	Spaet and Buremen, 2015
17.	MT357036.1	Brunei	Azri *et al*., 2020
18.	MT357044.1	Brunei	Azri *et al*., 2020
19.	MT357046.1	Brunei	Azri *et al*., 2020
20.	MT883980.1	Guam, Micronesia	Budd *et al*., 2021
21.	MT883981.1	Australia	Budd *et al*., 2021
22.	MT883983.1	Australia	Budd *et al*., 2021
23.	OQ385023.1	Malaysia	Loh *et al*., 2023
24.	OQ385024.1	Malaysia	Loh *et al*., 2023
25.	OQ385025.1	Malaysia	Loh *et al*., 2023
26.	OQ385053.1	Malaysia	Loh *et al*., 2023
27.	OQ385058.1	Malaysia	Loh *et al*., 2023
28.	OQ385071.1	Malaysia	Loh *et al*., 2023
29.	OQ385073.1	Malaysia	Loh *et al*., 2023
30.	OQ385074.1	Malaysia	Loh *et al*., 2023
31.	OQ386462.1	Philippines	Bemis *et al*., 2023
32.	OQ386529.1	Philippines	Bemis *et al*., 2023
33.	OR391899.1	Thailand	Khudamrongsawat *et al*., 2023 (unpublish)

(a) *S. lewini*
(DBP012125, DBP012128 and DBP012132)

(b) *R. australiae*
(DBP012126 and DBP012133)

(c) *C. sealei*
(DBP012127 and DBP012134)

(d) *S. fasciatum*
(DBP012129)

(e) *G. covier*
(DBP012130)

(f) *C. falciformis*
(DBP012131)

(g) *R. ancylostomus*
(DBP012135)

Figure 2. Photo of specimens *S. lewini* (a), *R. australiae* (b), *C. sealei* (c), *S. fasciatum* (d), *G. covier* (e), *C. falciformis* (f) and *R. ancylostomus* (g).

ancylostomus (DBP012135) was compared with samples from Malaysia. The seventh clade, *R. australiae* (DBP012126 and DBP012133), was compared with data from Indonesia, Malaysia, and India.

The genetic distance between 5 sharks and 2 rays species, with values ranging from 0.060 to 0.274 (Table 3). The lowest genetic distance is found between *C. falciformis* and *C. sealei* (0.060), suggesting a closer genetic relationship. On the other hand, the highest genetic distance is observed between *S. lewini* and *R. ancylostomus* (0.274), indicating large genetic distance. The genetic distance within species varied across the samples, with the highest genetic distance observed in *S. lewini* at 0.028, and the lowest in *R. australie* at 0.001. These values, alongside other species, are presented in Table 4, which highlights the genetic variation within each species.

Table 2. Percentage similarity with NCBI, species name based on the NCBI's sequences and Accession Number for each sequence obtained in this study.

No.	Sample code	Fishing Location	Blast Result Name	Accession Number	Length of Sequence (Bp)	Identity (%)	Accession Number References
1.	DBP012125	Masalembu	*S. lewini*	PQ135918	654	100	MT883980.1
2.	DBP012126	Masalembu	*R. australiae*	PQ135919	654	100	MW509724.1
3.	DBP012127	Masalembu	*C. sealei*	PQ135920	654	100	OQ385023.1
4.	DBP012128	Masalembu	*S. lewini*	PQ135938	654	88.24	MT883980.1
5.	DBP012129	Masalembu	*S. fasciatum*	PQ135921	654	100	FJ178399.1
6.	DBP012130	Masalembu	*G. covier*	PQ135922	654	100	OR391899.1
7.	DBP012131	Masalembu	*C. falciformis*	PQ135923	654	99.39	KF590366.1
8.	DBP012132	Bawean	*S. lewini*	PQ135924	654	100	MT883980.1
9.	DBP012133	Bawean	*R. australiae*	PQ135925	654	100	MW509724.1
10.	DBP012134	Bawean	*C. sealei*	PQ135926	654	100	OQ385025.1
11.	DBP012135	Bawean	*R. ancylostomus*	PQ135927	654	98.17	OQ385073.1

3.2 Discussion

3.2.1 Species identification and genetic diversity

The morphological and molecular identification of these species adds valuable data to the existing knowledge of shark and ray diversity in East Java, particularly in the understudied areas of Bawean and Masalembu Waters. This study complements previous research by Mardhotillah *et al.* (2024), which identified *Rhynchobatus australiae*, *R. laevis*, *R. springeri*, and *R. ancylostoma* based on morphology in the waters of Java Island, including Berondong. Molecular analyses in this study successfully confirmed the presence of *R. australiae* and *R. ancylostoma*, although additional samples are needed for molecular identification of other species. The presence of species such as the Hammerhead Shark (*Sphyrna lewini*) and the Silky Shark (*Carcharhinus falciformis*), both listed as threatened on the IUCN Red List, highlights the importance of these waters as critical habitats for vulnerable and endangered species. Furthermore, the identification of the Bottlenose Wedgefish (*Rhynchobatus australiae*) and Sharpnose Guitarfish (*Glaucostegus granulatus*) is particularly significant given their inclusion in Indonesia's list of protected species under the Ministry of Marine Affairs and Fisheries Regulation No. 1/2021. These findings support Indonesia's Rencana Aksi Nasional (RAN) Hiu dan Pari, which prioritizes conservation practices and sustainable management for sharks and rays, particularly species with high ecological and economic value. The discovery of these species underscores the importance of targeted conservation efforts and fisheries management in East Java to protect these ecologically and economically significant species while aligning with national conservation priorities.

The identification of shark species collected from East Java was carried out using DNA barcoding, as evidenced by the provided sequences and corresponding phylogenetic tree. The species identified include *S. lewini*, *R. australiae*, *C. sealei*, *S. fasciatum*, *G. cuvier*, *C. falciformis*, and *R. ancylostomus*. DNA barcoding has proven to be a highly accurate tool for species identification, with many samples showing 100% identity matches to known species in genetic databases. This high degree of accuracy demonstrates the effectiveness of DNA barcoding in distinguishing species based on their genetic makeup (Shen *et al.*, 2013). Leray *et al.* (2013) suggested that a minimum identity percentage of 97% is required to confirm spe

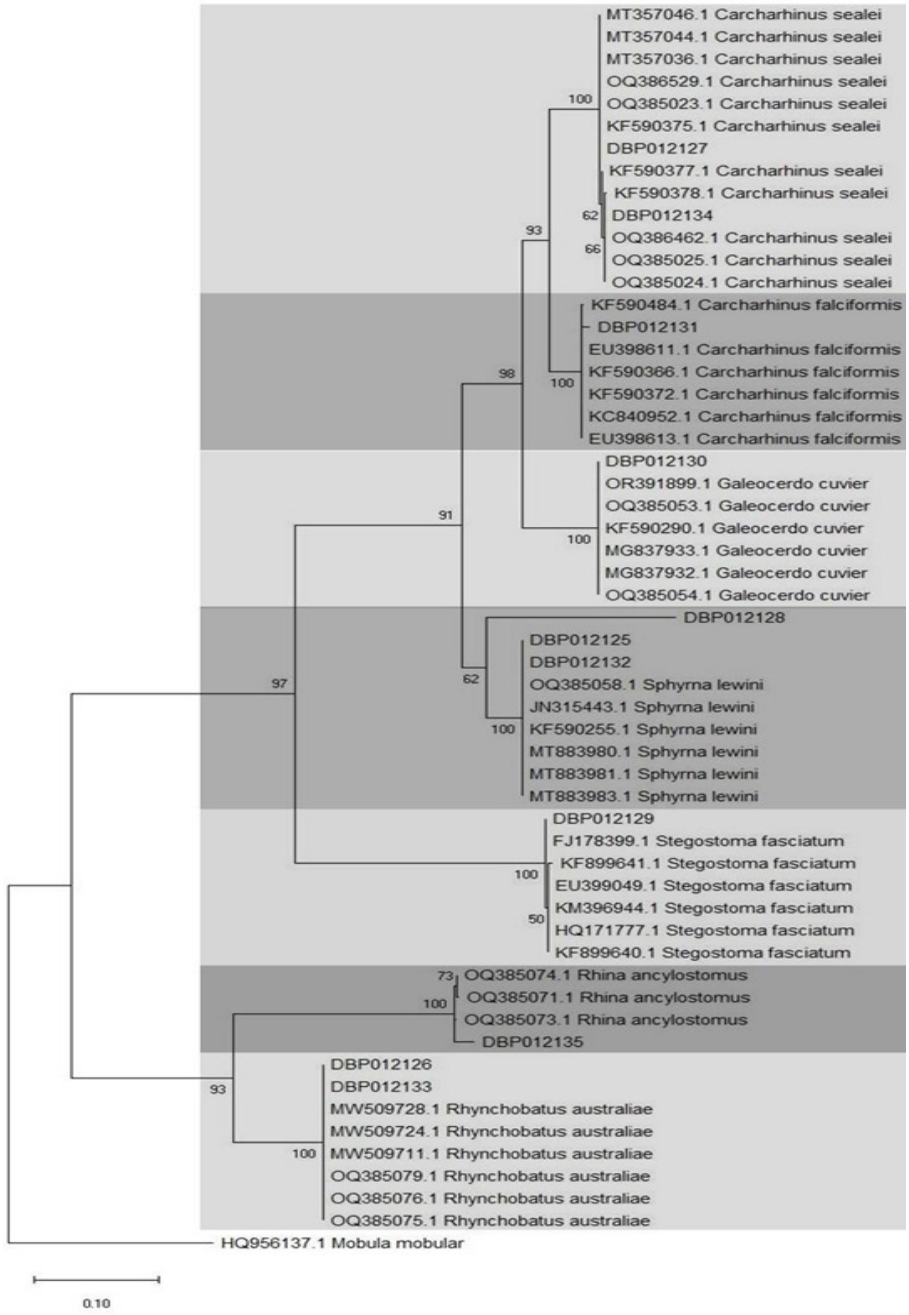

Figure 3. Phylogenetic tree of Shark and Ray with maximum likelihood method Tamura-Nei, 93 (TN93+G+I) model with 1000 bootstrap. Different shaded areas represent distinct clades corresponding to each species.

cies similarity, meaning that a 3% difference indicates a separate species. The results are consistent with the study conducted by Prehadi *et al*. (2015), with a similarity percentage of 99-100%. However, instances of lower identity matches, such as the 88.24% match for *Sphyrna lewini* (scalloped hammerhead). This is consistent with the findings of Ramadhaniaty *et al*. (2024), with a similarity range of 83.78-100%. This matter highlight some limitations and potential challenges in the barcoding process.

The success of molecular identification can be influenced by technical issues may also play a role based on. Errors during the DNA sequencing process, such as sequencing misreads or contamination, could reduce the accuracy of the results. Furthermore, the quality of the sample itself can have a significant impact. Poorly preserved samples, degraded DNA, or contamination during collection especially when samples are obtained from fishermen or handled improperly in the field may compromise the integrity of

the genetic material (Fields *et al*., 2015; Sultana *et al*., 2018). Chakraborty *et al*. (2006) emphasize the importance of meticulously controlling preservation methods and laboratory procedures to prevent errors that could compromise identity matching accuracy.

3.2.2 Phylogenetic insights

The phylogenetic tree further supports the identification, grouping the samples with reference sequences of the corresponding species with high bootstrap values. For instance, *C. sealei* samples (DBP012127 and DBP012134) clustered with other sequences of the same species, indicating genetic similarity and supporting the morphological identification shown in the photographs.

may be due to similar ecological conditions (Eckert *et al*., 2008). In the second clade, *C. falciformis* showed a low genetic difference (0-0.6%) compared to samples from Indonesia and Australia. This suggests that populations from Southeast Asia and Australia may share a recent ancestor, despite geographic separation. However, previous studies, such as Sembiring *et al*. (2023), reported significant population structuring of *C. falciformis* between Aceh and other regions in Indonesia, indicating regional differentiation within the country. The low genetic difference observed in this study may reflect potential gene flow or long-distance movement between broader regions (Sexton *et al*., 2014), while finer-scale studies highlight localized

Table 3. Genetic distance between species of sharks and rays collected from Bawean and Masalembu Waters.

Spesies	*S. lewini*	*R. australiae*	*C. sealei*	*S. fasciatum*	*G. cuvier*	*C. falciformis*
R. australiae	0.237					
C. sealei	0.128	0.225				
S. fasciatum	0.212	0.248	0.208			
G. cuvier	0.128	0.225	0.096	0.208		
C. falciformis	0.111	0.235	0.060	0.214	0.083	
R. ancylostomus	0.244	0.169	0.225	0.274	0.232	0.234

Table 4. Genetic distance within species of shark and ray collected from Bawean and Masalembu Waters.

No	Species	Genetic distance
1.	*S. lewini*	0.028
2.	*R. australiae*	0.001
3.	*C. sealei*	0.002
4.	*S. fasciatum*	0.002
5.	*G. cuvier*	0.000
6.	*C. falciformis*	0.002
7.	*R. ancylostomus*	0.012

In the first clade, *C. sealei*, with a low genetic difference (0-0.6%) compared to populations from Indonesia, Malaysia, the Philippines, and Brunei. This suggests strong genetic connectivity between these populations, indicating that *C. sealei* has a wide distribution in the region with few barriers to movement. The consistent genetic identity across different areas

population structures. These variations in genetic distances suggest differences in the evolutionary history and potential gene flow among the species (Nkurikiyimfura *et al*., 2024).

In the third clade, *G. covier* showed no genetic difference (0%) between samples from Malaysia, Thailand, and Mexico. This high genetic similarity-

suggests recent colonization or ongoing migration between these distant regions, which prevents genetic variation. The lack of difference is surprising and suggests further research is needed to understand the movement and population dynamics of *G. covier*. The fourth clade, representing *S. lewini*, showed the highest genetic difference, ranging from 0 to 12.7%, between samples from Indonesia, Malaysia, Australia, Philippines, and Colombia. This suggests that *S. lewini* populations are more genetically structured, likely due to geographic barriers or limited movement between populations. The high genetic difference, especially between Southeast Asian and South American populations, may indicate long-term isolation and adaptation to local environments (Ashe *et al*., 2015; Domingues *et al*., 2018). In the fifth clade, *S. fasciatum* had a low genetic difference (0-0.5%) between samples from India, Australia, the Red Sea, Saudi Arabia, and Madagascar. This suggests that *S. fasciatum* populations are genetically connected despite being spread across different regions, possibly due to long-distance movement or shared habitats (Alerstam *et al*., 2003). In the sixth clade, *R. ancylostomus* showed a moderate genetic difference (0-2.2%) compared to samples from Malaysia. This suggests some genetic variation between populations, likely due to geographic isolation or environmental differences in different parts of Malaysia (Eckert *et al*., 2008). In the seventh clade, *R. australiae* showed minimal genetic difference (0-0.2%) between samples from Indonesia, Malaysia, and India. This suggests that *R. australiae* populations in these regions are highly connected, likely through shared migratory routes or similar habitats (Webstera *et al*., 2002).

The genetic data underline the importance of adopting a holistic approach to the conservation of these species. Collaborative international efforts, habitat protection, and species-specific management plans are essential for maintaining genetic diversity and population stability. Addressing threats such as overfishing, habitat loss, and climate change will be critical for ensuring the long-term survival of these species in the wild.

4. Conclusion

This study successfully assessed the genetic diversity and phylogenetic relationships of shark and ray species from Bawean and Masalembu Waters, East Java, utilizing DNA barcoding on 11 samples. Despite the small sample size, the study identified five shark species and two ray species: *Sphyrna lewini*, *Rhizoprionodon australiae*, *Carcharhinus sealei*, *Stegostoma fasciatum*, *Galeocerdo cuvier*, *Carcharhinus falciformis*, and *Rhina ancylostoma*. Notably, several of these species, such as *S. lewini*, *G. cuvier*, and *C. falciformis*, are protected. Therefore, stricter conservation and monitoring efforts are essential to safeguard the shark and ray populations in Indonesia. This research underlines the urgent need for enhanced protection to ensure the sustainability of these ecologically important species.

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Authors' Contributions

MIJ: Conceptualization of the research, study design, and overall supervision of the project. Played a lead role in drafting the manuscript and revising the final version. NN: Conducted the data collection and analysis. Assisted in the initial writing of the results section and contributed to discussions of the findings. NK: Responsible for reviewing and refining the methodology section. Also played a key role in editing and formatting the manuscript. MEW: Focused on the literature review and background research. Provided technical support and expertise in this field study. NKDC: Contributed to data interpretation and writing the discussion. Also involved in coordinating revisions based on feedback from reviewers.

Conflict of Interest

The authors have declared that no competing interests exist.

Declaration of Artificial Intelligence (AI)

The author(s) acknowledge the use of ChatGPT for language refinement and grammatical correction in preparing this manuscript. All AI-generated content was rigorously reviewed, edited, and validated to ensure accuracy and originality. Full responsibility for the manuscript's final content rests with the author(s).

To ensure transparency and support the review process, a comprehensive delineation of the tool's application is furnished in the "Introduction" or "Materials and Methods" section of this manuscript in compliance with the publisher's ethical guidelines.

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