

Genetic diversity of bigeye thresher shark (*Alopias superciliosus* Lowe, 1841) landed in Palabuhanratu Fishing Port, Sukabumi, West Java, Indonesia

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Abstract. Kanedi MM, Wijayanti DP, Widowati I, Malik MDA, Yusmalinda NLA, Sembiring A. 2023. Genetic diversity of bigeye thresher shark (*Alopias superciliosus* Lowe, 1841) landed in Palabuhanratu Fishing Port, Sukabumi, West Java, Indonesia. *Biodiversitas* 24: 3488-3494. The bigeye thresher shark *Alopias superciliosus* (Lowe, 1841) is a highly migratory marine shark, widely distributed globally in tropical and temperate seas. The bigeye thresher shark is listed as a Vulnerable/VU species on the red list of the International Union for Conservation of Nature (IUCN) and listed on Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Genetics is a substantial essential approach to conservation, management, and sustainability. This study investigates the bigeye thresher shark's genetic diversity and structure populations *A. superciliosus* that landed in Palabuhanratu Fishing Port, Sukabumi, West Java, Indonesia. Samples were collected in Palabuhanratu (n=16) and secondary data from the Atlantic Ocean (n=212) and Indian Ocean (n=16) sequences were obtained from GenBank. A total of 16 sequences of *A. superciliosus* have been amplified using mitochondrial DNA control region (dloop) with 857 bp in size sequenced. The Genetic diversity result showed haplotype diversity value in Palabuhanratu ($H_d=0.86667$; $\pi=0.01048$) considered a high value. Overall, AMOVA and F_{ST} results showed significant differences in population structure between Palabuhanratu, Atlantic, and Indian Ocean with an F_{ST} value of 0.04420 (p-value <0.05). Based on the result, an effective strategy is needed to manage *A. superciliosus* sharks, both with the government and the private sector.

Keywords: *Alopias superciliosus*, control region, genetic diversity, Palabuhanratu

INTRODUCTION

The bigeye thresher shark *Alopias superciliosus* (Lowe, 1841) is a pelagic shark with a long whip-like tail, big eyes and horizontal grooves above the gills (Fernandez-Carvalho et al. 2015a). This species has a long life cycle, the maturity age of males reaches 9-10 years and 12-13 years for females (Tsai et al. 2020). This species has long-lived characteristics, slow growth, small offspring and takes a long time to reach maturity (Dulvy et al. 2014; Tsai et al. 2019). The combination of high pressure and their biological productivity makes this species vulnerable to overexploitation (Hadi et al. 2020).

The bigeye thresher shark is one of three sharks in the Alopiidae family, which has a global distribution, inhabiting tropical and subtropical areas, especially the open sea in the pelagic zone (Fernandez-Carvalho et al. 2015b; Rigby et al. 2019). The distribution of bigeye thresher sharks in the world is spread in almost all tropical seas, starting from the Atlantic Ocean, Indian Ocean and Pacific Ocean (Calle-Morán et al. 2023). Meanwhile, in Indonesia, the bigeye thresher shark can be found in the Indian Ocean from Sumatra to Nusa Tenggara, the Banda Sea, the Sulawesi Sea, the Makassar Strait and the Pacific Ocean (Dharmadi et al. 2017). This species is landed in large fishing ports,

such as Aceh, Cilacap, Palabuhanratu, Banyuwangi and Lombok, where fishermen operate around the Indian Ocean.

Alopias superciliosus are generally caught as by-catch in tuna longline fishing gear (Tsai et al. 2020). The bigeye thresher shark is listed as a Vulnerable/VU species on the Red List of the International Union for Conservation of Nature (IUCN) (IUCN 2019) and listed on Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (CITES 2019). Regulations regarding Thresher sharks or the Alopiidae family in Indonesia have been regulated in Permen KP Number 30/PERMEN-KP/2012, that fishermen are required to release caught sharks if they are still alive and record and report them to the port when the shark caught is dead (Ichsan et al. 2020). This species has been identified as the least productive of sharks since the fish is known as a slow-growing species (Young et al. 2016) and there is concern about its conservation status (Fernandez-Carvalho et al. 2015b).

Shark catches, including thresher sharks in West Java have decreased yearly. Based on statistical data from the Ministry of Maritime Affairs and Fisheries, shark catch production in West Java in 2020 reached 1.336,59 tons (KKP 2020). In 2021, it began to experience a decrease in production volume to 1.235,34 (KKP 2021). All parts of

the shark's body can be utilized, including fins for traditional medicine, skin for leather and liver oilfish, which is extracted for vitamins, carcasses are also rendered for fishmeal (Davidson et al. 2016; Muttaqin et al. 2019). The high market demand for sharks has led to an increase in shark poaching. High exploitation of the *A. superciliosus* impacts its population structure, reducing the fecundity of the species and genetic diversity (Cardeñosa et al. 2014; Carreón-Zapiain et al. 2020; Sukumaran et al. 2020; Hasan et al. 2021a). Elasmobranchs are included in a group of marine species most threatened by overexploitation (Bräutigam et al. 2015). Conservation and management of sharks are critical to protecting marine ecosystems, maintaining biodiversity and sustainable use of resources (Hasan and Islam 2020; Hasan and Widodo 2020).

One approach that can be taken includes assessing population structure based on a genetic level (Akbar and Aris 2018; Guzmán et al. 2021). This is important to determine the characteristics and connectivity of populations on genetic diversity by seeing whether there is a genetic transfer between populations so that it can be used as an effort for the survival of the population within it (Liu et al. 2018). Studies on genetic diversity and species structure are crucial for developing resource management and conservation strategies (Hadi et al. 2020). One of the numerous advantages of genetic information is that it will allow scientists and governments to determine what endangered and threatened species are in the wild so that in the future, action can be taken to protect these species (Alghozali et al. 2019; Hasan et al. 2021b).

This study aimed to know the genetic diversity and population structure *A. superciliosus* that landed in Palabuhanratu Fishing Port, Sukabumi, West Java, Indonesia. The implications of this study are used for the management and conservation of the species were examined.

MATERIALS AND METHODS

Study area

Samples were obtained from the Palabuhanratu Fishing Port, Sukabumi, West Java, Indonesia ($6^{\circ} 57''\text{S}$, $106^{\circ} 22''$ - $106^{\circ} 33''\text{E}$) (Figure 1). Samples collection was conducted from June until November 2022.

Identification and sample collection

Before taking a tissue sample, diagnostic morphological characters of the sample were analyzed following White et al. (2006). *Alopias superciliosus* has characteristics including, it has large eyes, there are strokes or indentations on the nape of its neck (behind the eyes), the body color tends to be darker, the shape of the pectoral and dorsal fins is slightly curved backward and a long tail almost as long as her body (Figure 2). Nineteen samples *A. superciliosus* were collected from Palabuhanratu Fishing Port, Sukabumi, West Java. Tissue samples by cutting off the edge of the gill slits using scissors or a cutter. The tissue samples were preserved using 96% ETOH and have been labeled. The tube filled with samples and ethanol is stored for further DNA analysis.

DNA extraction, PCR amplification, and DNA barcoding

The DNA extraction method used Chelex 10% (Walsh et al. 1991). Two mm long of tissue was chopped from the edge of the gill slits using a sterile tweezer and were putting into Chelex 10% solution. Before and after the specimen is taken, the tweezers are immersed in 96% alcohol and heated in a Bunsen lamp for sterilization to avoid contamination between specimens. Samples were then homogenized using Vortex for 15 seconds and centrifuged for 1 minute at 8000 rpm and followed by incubation in the heating block at 95°C for 45 minutes.

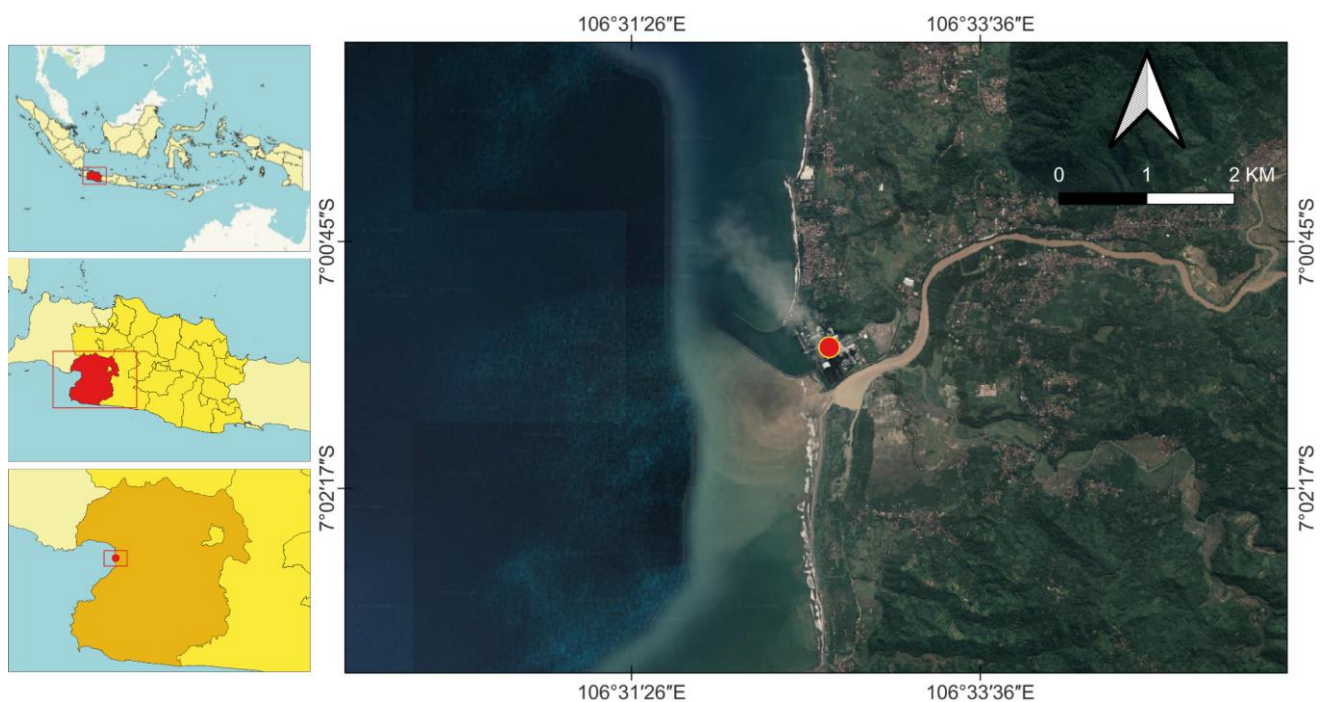


Figure 1. Sampling site in Palabuhanratu Fishing Port, Sukabumi, West Java, Indonesia



Figure 2. *Alopias superciliosus* landed in Palabuhanratu Fishing Port, Sukabumi, West Java, Indonesia

This study used primers with a target locus Control Region (Morales et al. 2018). Mitochondrial DNA Control Region (mtDNA CR) was amplified by Polymerase Chain Reaction (PCR), using forward primer Pro-L (5'-AGG GRA AGG AGG GTC AAA CT-3') and reverse primer LGL-282 (5'-AAG GCT AGG ACC AAA CCT-3') (Morales et al. 2018). PCR was conducted using Red Taq Mix (BIOLINE) following a cycle: 94°C initial denaturation for 5 minutes; 35 cycles of denaturation at 94°C 1 minute, primer annealing at 58°C for 30 seconds and extension at 72°C for 1 minute; and post extension 72°C for 7 minutes. PCR products were then visualized for DNA band using electrophoresis on a 1% agarose gel and EtBr (Ethidium Bromide) staining (Sembiring et al. 2015). PCR products were sequenced by PT. Genetika Science Indonesia using the Sanger method (Toha et al. 2020).

Data analysis

Each sample's forward and reverse DNA sequences were checked, aligned and edited using MEGA 11 (Tamura et al. 2021). Species identification from the sequences was made before data analyses using BLAST (Basic Local Alignment Search Tool) by matching them with the sequences from NCBI (National Center for Biotechnology Information) GenBank. The phylogenetic tree from the sequences was made using the Maximum Likelihood method, Kimura-2-parameter model and 1000 bootstrap replicate. Included in the analysis were bigeye thresher shark reference accessions with the following accession numbers: MF069493-MF069495 from the Atlantic and Indian Ocean (Morales et al. 2018) and the outgroups used were the other lamniform groups, *A. pelagicus* Nakamura, 1935, *A. vulpinus* Bonnaterre, 1788 and *Isurus oxyrinchus* Rafinesque, 1810 (Gen-Bank Accession No. KF412639, MF374733 and NC022691). The genetic distance between species was calculated and modeled using the Kimura-2-parameter. Analysis of genetic diversity in control region mtDNA sequence data used the DnaSP 6 application (Rozas et al. 2017). Genetic diversity was examined by determining the numbers of Segregating sites (S), haplotype number (h), Haplotype diversity (Hd) and nucleotide diversity (π). Genetic diversity, haplotype distribution, and genetic

differentiation analysis from between locations were analyzed using 1000 permutations in each significance test (p-value <0.05), which were analyzed using DNASp 6 (Rozas et al. 2017). Meanwhile, population genetic structure (F_{ST}) between locations (Palabuhanratu, Atlantic and Indian Ocean) was analyzed using Analysis of Molecular Variance (AMOVA) with 10000 replicates permutations in Arlequin Ver.3.5 (Excoffier and Lischer 2010). To test different hypothetical scenarios, the variation due to differences between groups (F_{CT}), between groups within each group (F_{SC}) and between all groups (total F_{ST}) was analyzed using Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992).

RESULTS AND DISCUSSION

DNA extraction, PCR amplification, and DNA barcoding

In this study, a total of 16 out of 19 samples were successfully amplified and sequenced. The final edited sequences had a length of 857 bp. All sequences were identified as *A. superciliosus* with Identifying values ranging between 95.57%-100%. All the sequences generated from the samples were deposited in the Genbank database (<http://www.ncbi.nlm.nih.gov>) with accession no. OQ623183-OQ623198. The results showed that 244 samples with 857bp mitochondrial control region gene sequences were obtained from sampling locations Palabuhanratu (n=16 samples), and additional data obtained from the Genbank on the Atlantic Ocean (n=212 samples) and Indian Ocean (n=16 samples) with accession number MF069493- MF069496.

Phylogenetic tree

Evolutionary relationships for the mtDNA control region fragment of the bigeye thresher shark from Palabuhanratu were co-analyzed with data from the Atlantic and the Indian Ocean with accession number MF069493-MF069496. The tree produced in this study (Figure 3) shows a bootstrap value with the Neighbor-Joining Tree method with Kimura 2 Parameters ranging from 99%. A bootstrap value of >80% indicates that the

species is very similar to the existing database, and the accuracy of the phylogenetic tree reconstruction can be trusted. The phylogenetic tree arrangement in this study of *A. superciliosus* samples formed one clade (Figure 3). The clade formed describes the same species group.

Genetic diversity and genetic structure

The results of Haplotype diversity (Hd) showed a value of 0.19439 and a nucleotide diversity (π) value of 0.00180, both were considered low values, compared with the result conducted by Trejo (2005). The genetic diversity value of each of the populations is shown in Table 1. The lowest Hd's was observed in the Atlantic Ocean (0.11893), while the highest (0.86667) was found in Palabuhanratu.

The result of the Analysis of Molecular Variance (AMOVA) of control region mtDNA from various sampling locations is shown in Table 2. Based on the study results, the overall Hd (Haplotype diversity) value for Palabuhanratu, Atlantic and Indian Ocean was 0.19439, with a Hd value for the Atlantic Ocean sample of 0.11893, the Indian Ocean sample of 0.23333 and the Palabuhanratu sample of 0.86667. Genetic population analysis using the Analysis of Molecular Variance (AMOVA) showed an FST value of 0.04420 (p-value <0.05) (Table 2). This index shows significant differences in population structure between Palabuhanratu, Atlantic and Indian Ocean.

Discussion

Genetic diversity is an important aspect to consider in shark management and conservation policy because the long-term survival of a species is highly dependent on the level of genetic diversity within and between populations (Domingues et al. 2017). This study shows that the *A. superciliosus* population in Palabuhanratu has a high haplotype diversity value compared to the Atlantic and Indian Ocean. According to Nei (1987), the haplotype diversity value of the Palabuhanratu population is in the high category (0.8-1). The Atlantic and Indian Ocean populations have haplotype diversity values in the low category (0-0.4). The IUCN classifies all thresher sharks as "Vulnerable" worldwide. It is well recognized that these animals have vulnerable life cycle parameters, which leaves them with a low potential to recover even from modest levels of exploitation (Fu et al. 2017).

The high haplotype diversity of the bigeye thresher shark is thought to be caused by large population size and interbreeding between individuals. Random mating can ensure the stability of allele frequencies from one generation to the next (Astarini et al. 2021). In addition, migration factors can also affect the value of haplotype diversity, this occurs due to cross-breeding and gene mixing between populations (Akbar and Aris 2018). The high genetic diversity indicates that the bigeye thresher shark population can adapt to environmental changes (Bramandito et al. 2018).

Gene mutations or variations within each species reveal the relationship between genetic adaptation to climate fluctuations and the species' contribution to environmental change (Ellegren and Galtier 2016). Because sharks are widespread and have a breeding season that allows them to

travel to calmer waters, sharks and rays have a life cycle with a wide range of distribution (Bineesh et al. 2017). Due to the process of adapting to different environments, this cycle causes shark species to show a high degree of variation (Ramadhaniyati et al. 2023).

Table 1. The value of genetic diversity of the bigeye thresher shark (*Alopias superciliosus*) based on three populations

Population	Hn	Hd	π	N
Atlantic Ocean	10	0.11893	0.00123	212
Indian Ocean	2	0.23333	0.00027	16
Palabuhanratu	10	0.86667	0.01048	16
All	18	0.19439	0.00180	244

Note: N: Number of sample, Hn: Number of Haplotype, Hd: Haplotype diversity, π : Nucleotida diversity

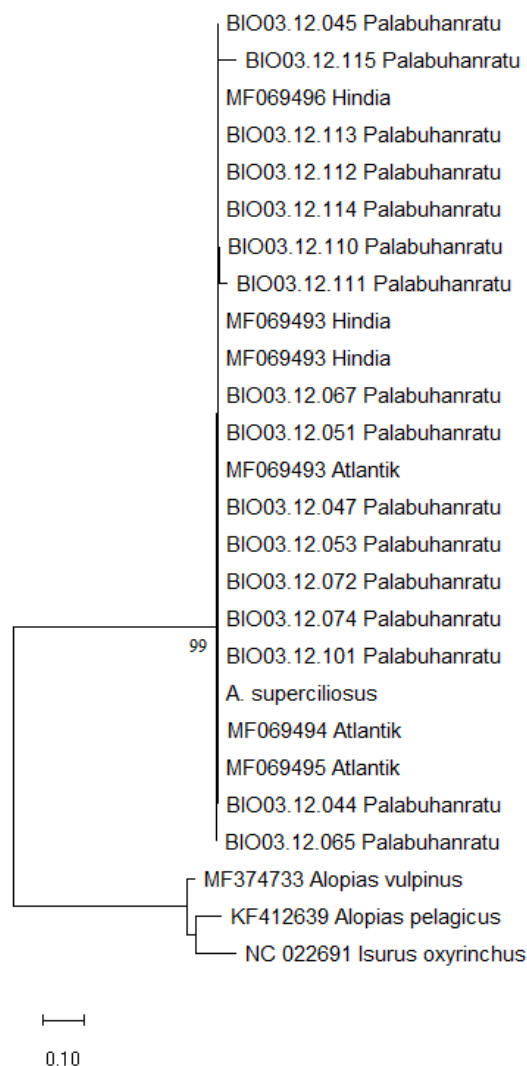


Figure 3. Phylogenetic tree of *Alopias superciliosus* landed in Palabuhanratu Port, Sukabumi, West Java, Indonesia

Table 2. Analysis of Molecular Variance (AMOVA) of *Alopias superciliosus* in Palabuhanratu, Atlantic and Indian Ocean

Source of variation	d.f	s.s	Variance component	Percentage of variation	FST (P-value)
All					
Among population	9	13.559	0.03422	4.42	Fst: 0.04420 (0.04497)
Within population	234	173.146	0.73994	95.58	
Total	243	186.705	0.77416		
Atlantic vs. (Indian Ocean + Palabuhanratu)					
Among groups	1	3.556	0.04257	5.29	Fsc: 0.02948 (0.04301)
Among populations within group	8	10.003	0.02248	2.79	Fst: 0.08081 (0.04203)
Within populations	234	173.146	0.73994	91.92	Fcr: 0.05288 (0.08700)
Total	243	186.705	0.80499		

The Fixation Index (F_{ST}) assesses the strength of the genetic population structure by describing genetic variation within individuals, within populations and between populations (Meirmans and Liu 2018). The analysis results showed that even F_{ST} result was low (0.04420), but the p-value was significant. Then, the percentage of genetic variation between populations tends to be low at 4.42% and the rate of variation within populations tends to be high at 95.58% (Table 2). Then, F_{ST} result when the data were grouped between Palabuhanratu and Indian Ocean versus Atlantic indicated a significant value (F_{ST} : 0.08081 and p-value= 0.04203). This result indicated that Palabuhanratu has the same population as Indian Ocean but is structured with Atlantic.

Furthermore, the obtained F_{ST} results indicate a high gene flow between Palabuhanratu and Indian Ocean. The high gene flow may be because the three populations influence each other genetic flow between populations. In addition, the high flow of genes into the population per generation also affects the genetic closeness of the two populations. Bigeye thresher shark (*A. superciliosus*) can migrate a great distance of more than 1500 miles (Briones-Mendoza et al. 2021) and is a good swimmer (Frumkin and Shimada 2020). Thus, seasonal changes in abundance and distribution are thought to reflect seasonal migrations in latitude associated with changes in water temperature (Morales et al. 2018). Large sharks have a higher thermal capacity, which makes them more inclined to move to higher latitudes (Matsunaga and Yokawa 2013).

A previous study of 64 individuals of *A. superciliosus* reported genetic structure between Atlantic and all Pacific populations based on pairwise F_{ST} tests, whereas AMOVA detected no overall genetic structure Trejo (2005). A single haplotype in the Atlantic by Trejo (2005) was also shared with some individuals from the Pacific population, including individuals from the Indian Ocean near South Africa. Similarly, the *A. superciliosus* sequenced reported by Morales et al. (2018), indicate a lack of population structure across the Atlantic and between the Atlantic and Indian Oceans. Therefore, even in an overall analysis that included two lineages, the species *A. superciliosus* had a single phenotype with high gene flow in the Atlantic.

The results showed that an effective strategy for managing *A. superciliosus* requires attention and cooperation regionally and internationally. This species has very low genetic diversity and few genetically heterogeneous

individuals. An effective strategy is needed to manage *A. superciliosus* sharks, with the government and the private sector. Based on the National Action Plan (RAN) regarding the Conservation and Management of Sharks and Rays in 2016-2020, several international and national regulations exist in efforts to manage sharks and rays. International regulations consist of the Indian Ocean Tuna Commission (IOTC), Commission for the Conservation of Southern Bluefin Tuna (CCSBT), Western and Central Pacific Fisheries Commission (WCPFC), Inter American Tropical Tuna Commission (IATTC), Convention on International Trade in Endangered Species (CITES). All of the international regulations above regulate the management of sharks and rays, one of the provisions is that every fishing vessel is prohibited from storing on board, transferring from or to other ships or landing shark fin catches. Meanwhile, at the national level, Indonesia has issued several regulations to manage sharks and rays, including PP No. 7 of 1999 concerning the conservation of living natural resources and their ecosystems, Minister of Maritime Affairs and Fisheries Regulation No. 57/PERMEN-KP/2014 concerning capture fisheries, in the fishery management area of the Republic of Indonesia.

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