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# Population genetic structure of the bentfin devil ray (*Mobula thurstoni*) in the South Indonesia Sea with limited sample based on ND5 gene

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**Abstract.** *Wardana ED, Putra ING, Al Malik MD, Yusmalinda NLA, Ningsih EY, Pertiwi NPD, Salim MG, Kanedi MM, Putra MIH, Sembiring A. 2023. Population genetic structure of the bentfin devil ray (*Mobula thurstoni*) in the South Indonesia Sea with limited sample based on ND5 gene. Biodiversitas 24: 3743-3749.* Bentfin devil ray (*Mobula thurstoni*) is one of five Mobula rays with a worldwide distribution. This species has high economic value and play an essential ecological role. However, due to their biological characteristics, such as low reproductive rates and late maturity, these species are highly vulnerable to overfishing and slow to recover from depletion. Genetic information, such as this species's population structure and genetic diversity is limited. This study aimed to determine the genetic variation and population structure of *M. thurstoni* in the South Indonesia Sea based on mitochondrial DNA from ND5 gene. Twenty-two samples of *M. thurstoni* were successfully collected from three locations Palabuhanratu (n=5), Muncar (n=13), and Savu Sea (n= 4). The haplotype diversity (Hd) ranged from 0.782 to 1.00 (mean=0.839), and Nucleotide diversity ($\pi$) ranged from 0.004 to 0.018 (mean=0.007). Then, no significant population differences (P-value > 0.05) were observed between locations, indicating that *M. thurstoni* population in the South Indonesian Sea represents a single genetic population. Additionally, the haplotype distribution also indicated the presence of two shared haplotypes between locations. Consequently, co-management strategies for sustainable fishing and conservation must be implemented across these three locations to ensure the sustainability of this species in the nature habitat.

**Keywords:** Connectivity, genetic diversity, fisheries management

## INTRODUCTION

The Southern Indonesian Sea is heavily affected by the Indian Ocean because of the location flow circulation; therefore, the value of nutrient concentration is relatively high in Java waters causing the water quality in Java waters is still relatively good with the gas exchange between oxygen at sea level and atmosphere and comes from plants (Nontji 1987; Meirinawati and Iskandar 2019). As a result, the southern waters of Indonesia are the preferred habitat for mobulid rays, including mobula rays, because these waters are rich in plankton abundance and have suitable temperatures for this animal (Nontji 1987). Mobula rays have gill plates, called gill combs, which are hard sieves used to filter zooplankton from seawater (Paig-Tran et al. 2013). Although mobula rays play an important ecological role in marine ecosystems, their exploitation rate is high because they are economically crucial for the fisheries industry. It threatens to decrease their natural population substantially (Alfaro-Cordova et al. 2017; Notarbartolo et al. 2020). Mobulid rays can increase fishermen's income because they are included in export commodities, such as mobula rays (*Mobula mobular, M. tarapacana, M. thurstoni and M. kuhlii* (White et al. 2006; O'Malley et al. 2017). Many mobulids are distributed all over the world. However, differences in species and population composition reported quantities might reflect preferred distribution territories (Croll et al. 2015).

In the southern waters of Java, several species of mobula rays were found to be caught during tuna fishing (Novianto et al. 2015). It is estimated that 9% of all mobulids are caught as bycatch in drift gillnets for skipjack and tuna fishing in southern Indonesian waters (Mukharror et al. 2018). Fisheries supplying dry gill plates to the Asian market have been recorded in the Philippines, India, Indonesia, Sri Lanka, Malaysia, Mozambique, Palestine, and China, and have strongly impacted many migratory populations (Abudaya et al. 2014; Dent and Clarke 2015; Lewis et al. 2015). The head of the Marine and Fisheries Research and Human Resources Agency (BRSDM) said 13 percent of the world's shark and ray production comes from Indonesia (Setiawan 2022). Due to the high exploitation rate of mobulid rays and their population declined in the wild, the 17th Conference of Parties (COP) of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) in October 2016 agreed to include thresher shark (*Alopias* spp.), silky shark (*Carcharhinus falciformis*) and mobula ray (*Mobula* spp.) into CITES Appendix II. According to the Coastal and Marine Resources Management Center (BPSPL) by Law No. 31/2004 JO Law No. 45/2009 Article 88, *Mobula* spp. is a type of mobulid ray that is prohibited for export, and its utilization can only be done within the territory of the Republic of Indonesia. The International Union for Conservation of Nature (IUCN) classifies the bentfin devil ray (*Mobula thurstoni*) as an endangered species (EN), which means that its existence is dangerously threatened because it has experienced a population decrease (Marshall et al. 2022). Consequently, if the catching of mobula rays continues, the extinction of this species will occur. Thus, genetic variation and structure information is needed to understand better how mobula rays can adapt to their environment and how their genetic diversity affects current fishing pressure.

The study of molecular genetics can be the basis of species conservation because it can be an essential reference in maintaining and recovering populations (River et al. 2014; Pierson et al. 2016; Coates et al. 2018). Information on genetic diversity can help consider the sustainable management of marine species protection areas (Romiguier et al. 2014; Van Oppen and Coleman 2022). The efforts have been made by the government in terms of managing exports of mobulid rays (except manta rays that have been protected since 2014) products outside the territory of Indonesia through the Regulation of the Minister of Marine Affairs and Fisheries. Many fishermen still illegally catch and export mobula rays, including bentfin devil rays, due to their high economic value and international demand (Ward-Paige et al. 2013; Pardo et al. 2016; Hosegood 2020). Even though this species is a migratory species (Bessey et al. 2019; Bucair et al. 2022) the population of this species is decreasing over time (Lewis et al. 2015). As a result, the *M. thurstoni* can become extinct soon, especially in the South Indonesia Sea. Therefore, this study aimed to quantify the genetic diversity and population structure of *M. thurstoni* in southern Indonesian waters. The results can be used as a reference in conservation management and sustainable resource utilization, especially for *M. thurstoni* species.

## MATERIALS AND METHODS

### Study area

This study collected tissue samples of bentfin devil rays (*Mobula thurstoni*) from the south Indonesia Sea, consisting of several sites such as Palabuhanratu, Muncar, and Savu Sea, Indonesia (Figure 1). A total of twenty-two samples from different individuals were successfully collected; four samples were from the Savu Sea, thirteen from Muncar, and five from Palabuhanratu. The tissue samples from each location were preserved using 96% ethanol for subsequent analysis. No specific permission is required during sample collection since the samples were obtained from bycatch caught unintentionally in a fishery or retained due to nets being non-selective, which are not the primary target of the fishery, and trapped in drift gillnets.

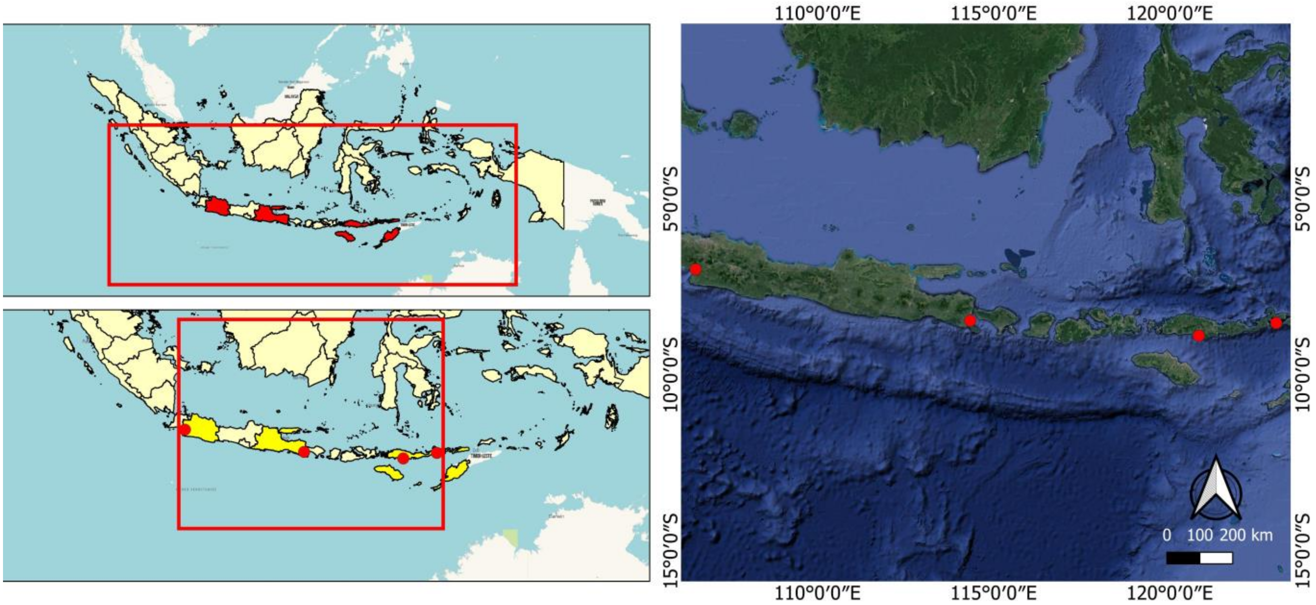

**Figure 1.** Sampling bentfin devil ray (*Mobula thurstoni*) locations at three locations, including Savu Sea, Muncar, and Palabuhanratu. The sampling activity is based on the landing site

### Lab works

DNA extraction was performed using the DNeasy Blood & Tissue Kit (Qiagen®, Hilden, Germany) following the manufacturer's protocol. Mitochondrial DNA targeting the NADH locus ND5 was amplified using forward primer MLF2 (5'-TGGTGCAACTCCAAGCTAAA-3') and reverse primer MNR4 (5'-TCAGGCGTTRAGGTATGATG-3') (Kashiwagi et al. 2012). The DNA amplification was carried out with a total volume of 26 µl which consisted of 2 µl DNA templates, 9 µl ddH2O, 2.5 µl primers, and 12.5 µl bioline ready mix. Polymerase Chain Reaction (PCR) was conducted in 38 cycles, initial denaturation at 95°C for 15 minutes, denaturation at 94°C for 1 minute, annealing at 55°C for 57 seconds, elongation at 72°C for 1 hour and 45 minutes, final elongation 72°C for 10 minutes, and stabilization process with a temperature of 24°C for 1 minute (Malik et al. 2022). The quality of the DNA was visualized using 1% agarose gel. The successful PCR products were sent to the DNA Sequencing Facility.

### Data analysis

The MEGA X application was used to organize and align nitrogenous base sequences (Kumar et al. 2018). Species identification was performed by comparing sequence data with the database on GenBank (www.ncbi.nlm.nih.gov) using BLAST (Basic Local Alignment Search Tool). Phylogenetic trees were constructed using the Neighbor-Joining (NJ) method (Kumar and Sudhindra 2000) with 1000 bootstraps in The Molecular Evolutionary Genetics Analysis (MEGA X) software. The phylogenetic tree was constructed using 22 samples of *M. thurstoni* and additional samples were obtained from National Center for Biotechnology Information (NCBI) GenBank database (Accession number: FJ235629, KM364993, FJ235628). The outgroup consists of three species of mobulid rays, namely *Mobula mobular* (KT203434), *Manta birostris* (KR703226), and *Manta alfredi* (KR703218), as well as sharks group including bigeye thresher shark, *Alopias superciliosus* (MF069502). These sequences were retrieved from the NCBI GenBank database. Genetic diversity, including the number of haplotypes, haplotype diversity (h), and nucleotide diversity (π), was calculated using DnaSP 6 (Rozas et al. 2017). Population genetic structure (FST) was analyzed using Analysis of Molecular Variance (AMOVA) with 10,000 permutation repeats in Arlequin version 3.5.2.2. (Excoffier and Lischer 2010).

## RESULTS AND DISCUSSION

### Phylogenetic tree and population structure

The phylogenetic results showed that all samples have resided in a single cluster, which indicates that all samples used are target species (Figure 2). According to AMOVA, no genetic structure was observed between locations (Fst = 0.02755 with P-value = 0.33010 or P-value > 0.05), indicating that the *M. thurstoni* population in the South Indonesian Sea is panmictic (Table 1). Haplotype distribution showed that the most common haplotype shared by the three locations was haplotype 4. Meanwhile, Palabuhanratu and Muncar shared haplotypes (5 haplotypes), and the rest was a unique haplotype found exclusively at each site (8 haplotypes) (Figure 3). Therefore, the total number of haplotypes found in this study was ten haplotypes.

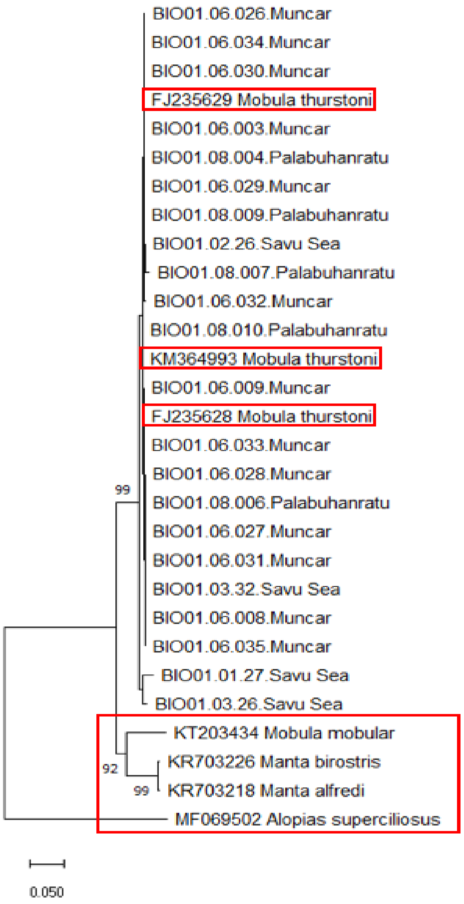

**Figure 2.** Neighbor-Joining (NJ) tree generated from mtDNA ND5 loci data from three locations, including Savu Sea, Muncar, and Palabuhanratu. The red line on the tree indicates the accession number was retrieved from the NCBI Genbank database

**Table 1.** Analysis of molecular variance (AMOVA) of bentfin devil ray (*Mobula thurstoni*) in three locations, including Savu Sea, Muncar, and Palabuhanratu

| Source of variation | DF | Sum of squares | Variance component | Percentage of variation |
|---|---|---|---|---|
| Among population | 2 | 118.470 | 1.42629 Va | 2.75 |
| Within-population | 19 | 956.712 | 50.35324 Vb | 97.25 |
| Total | 21 | 1075.182 | 51.77953 | |
| FST | 0.02755 | | | |
| P-value | 0.33010 | | | |

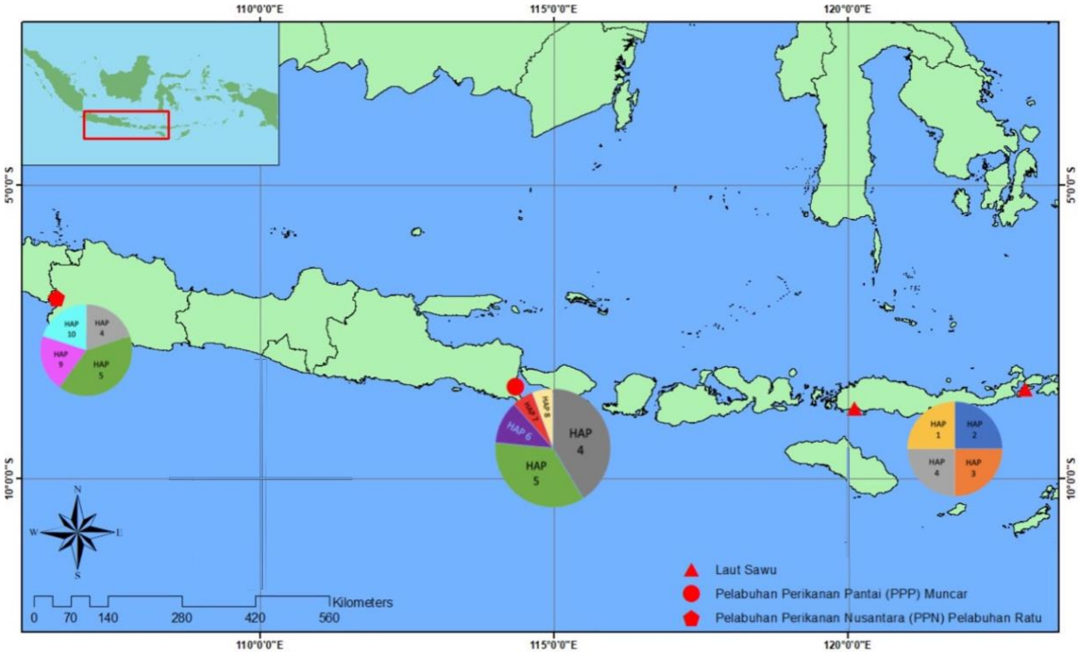

**Figure 3.** Haplotype distribution of bentfin devil ray (*Mobula thurstoni*) from three locations

**Table 2.** Genetic diversities parameters, including the number of haplotypes (Hn), haplotype diversity (Hd), and nucleotide diversity (π) of *Mobula thurstoni* in each location

| Population | n | Hn | Hd | π |
|---|---|---|---|---|
| Savu sea | 4 | 4 | 1 | 0.018 |
| Muncar port | 13 | 5 | 0.782 | 0.004 |
| Palabuhanratu port | 5 | 4 | 0.900 | 0.005 |
| Total | 22 | 10 | 0.839 | 0.007 |

## Genetic diversity

Haplotype diversity in the Savu Sea was 1.00, and in Palabuhanratu was found to be 0.900. The haplotype diversity obtained in Muncar is in the medium category at 0.782. Meanwhile, the overall results of haplotype diversity were high with a value of 0.839. The highest nucleotide diversity was found in the Savu Sea (0.018), and the lowest was in Muncar. Overall, nucleotide diversity is low, with a value of 0.007 (Table 2).

## Discussion

The results of molecular identification using the ND5 gene showed that all samples were the targeted species, *M. thurstoni*. Using the ND5 locus in mobulid research has been done by previous studies (Kashiwagi et al. 2012). The use of primers MLF2 and MNR4 in the PCR process of mobula rays research showed a more specific and more informative result of DNA sequence after being compared with several different loci. The results obtained by ND5 are more specific, and the resulting base length is longer and quite significant compared to other target loci (Malik et al. 2022). The results of matching DNA samples with data on Genbank (www.ncbi.nlm.nih.gov) show that all samples collected are the targeted species in this study, *M. thurstoni*. All sequence data have been uploaded to Genbank with accession numbers OQ025561-OQ025582.

### *Phylogenetic tree and population structure*

The phylogenetic tree constructed was supported by high bootstrap values on each branch in the population group. It shows that the generated relationship tree has a high level of accuracy, and then all values obtained were above the limit at an average of 90-100 (Felsenstein 1985). The results of the phylogenetic analysis found four different clades. All the samples used were found to belong to the *M. thurstoni* clade, which means that all the samples used came from the correct target species. The other clade formed is the outgroup used from *M. mobular*, *M. alfredi*, and *M. birostris*, which include one clade, meaning the distance is quite close. The outgroup clade with the farthest distance is from *A. superciliosus*, a shark species.

Analysis of the fixation index (FST) through the AMOVA test showed no genetic differentiation among populations from all locations. According to Excoffier et al. (1992), the results of the FST analysis are included in the low category because the value obtained is between 0.1 to 0.3. The low FST value explains that there is a high similarity of genes between populations. Mulyasari et al. (2010) demonstrated that many genetic similarities between populations could cause populations with a low level of differentiation. It is related to the geographical location between the three locations located on one flow path, namely in southern Indonesia, which may affect the high flow of genes into and between populations which causes genetic similarity between populations (Nontji 1987; Harris et al. 2020). There are no genetic differences between populations. As a result, the three locations can be considered as one genetic population. High migration rates in those three-sampling locations can also result in gene flow. According to the statement (Mayr 1970), it is mentioned that a population with a high level of relationship has many morphological and genetic similarities due to environmental influences.

The p-value analysis showed that the three locations had p-values above 0.05, indicating that they were not significant. Overall, the FST result is 0.02755, which means that the results of the population structure analysis from all locations fall into the low category, while the p-value result is 0.33010, which states that there is no significant population structure in all locations. *M. thurstoni* connectivity in all sampled locations indicates the presence of gene flow. This is supported by similar studies in this region showing no genetic structure was found for other mobulid rays (Malik et al. 2022; Phardana et al. 2022).

### *Genetic diversity*

From the results of genetic diversity analysis, it was found that from the Savu Sea, the number of haplotypes obtained was four haplotypes. Five haplotypes were obtained in Muncar Port, and four haplotypes were obtained in Palabuhanratu population. According to Nei (1987), the results of haplotype diversity obtained at the Savu Sea and Palabuhanratu locations are included in the high category because they reach a value of 0.900-1.000, while the other location, Muncar Port, is included in the medium category, namely 0.782. As for the results seen that all nucleotide diversity is high because it is above 0.005. As for the overall number of haplotypes found, are ten haplotypes from the 22 samples used. The haplotype diversity obtained is 0.839, which is included in the high category. The suspected factor influencing the high genetic diversity of *M. thurstoni* is the ability of *M. thustoni* to travel long distances or long migration, thus increasing the possibility of interbreeding between individuals in the species from various locations. According to the statement (Germanov and Marshall 2014; Sciara et al. 2015), the approximate movement distance of *M. mobular* and *Mobula alfredi* is 80-450 km (straight-line distance) around 33-1550 days.

The data from this study support the concept that *M. thurstoni* can rapid long-distance migrations. This interbreeding can increase the diversity of alleles from one generation onward or allow gene flow between populations. Large populations can have an impact on population stability. Increases in population sizes have equally important implications for population growth rates. This results in no species extinction (Moore et al. 2014). Populations with high genetic diversity are likely to survive longer because they can adapt and have different responses to environmental conditions. Although the value of diversity shows a high category, this cannot be used as a reference for not enforcing regulations on its capture. If the capture is carried out continuously, there will still be a possibility of extinction because the reproduction of this *M. thurstoni* species takes a long time, and also the survival period is relatively short (Dulvy et al. 2014). Although mobulidae may have different ecological behaviors (e.g. deep dive, distribution range), spawn characteristics that are consistent across species, such as similar ages to adulthood (i.e. ~7-10 years for *M. alfredi* (Dulvy et al. 2014), *M. mobular, M. thurstoni and M. birostris* (Nozu et al. 2017) and the number of their descent is low in their lifetime. The population growth rate of these species is extremely low (Pardo et al. 2016; Rambahiniarison et al. 2018). As a result, if the capture is carried out continuously, a population may decline, eventually leading to species extinction.

### *Conservation implication*

The continued catching of mobulid rays, especially the *M. thurstoni* species, can cause a population decrease, and mobulid rays are also individuals with reproductive rates that take a long time to reproduce, so extinction may occur if the *M. thurstoni* species is not strictly protected and is continuously caught. Because of this, the government is expected to reconsider the conservation of bentfin devil rays, especially *M. thurstoni*, because currently the status on the IUCN Redlist has entered the Endangered (EN) category, which means that it is threatened with extinction if appropriate conservation efforts are not made. Efforts include establishing regulations and the maximum number of catches in each fishing operation in several potential fishing areas such as the South Sea of Indonesia, including the Savu Sea and the South Java Sea. The results of this study can be used as a reference by the government through the Ministry of Marine Affairs and Fisheries to establish a genetic conservation area in terms of preserving mobula ray resources in the South Sea of Indonesia. Although this study found that *M. thurstoni* has high genetic diversity if fishing is carried out continuously for this species, it will threaten the sustainability of this species in nature.

In conclusion, the bentfin devil ray or *M. thurstoni* in the South Indonesia Sea is based on three locations indicated as single-stock or panmictic populations. As a result, conservation efforts from this result should be a priority through co-management between those locations to ensure the sustainability of this species in nature. Even though the number of samples was low, at least this research revealed preliminary results of population genetics from one of the migratory species in Indonesia and different scope from Malik et al. (2022). Then, additional samples from different populations are needed to study further a comprehensive population structure in Indonesia. In addition, advanced methods, such as microsatellite DNA (Barker 2002) or Restriction site-associated DNA sequencing (RAD-seq) analysis (Díaz-Arce and Rodríguez-Ezpeleta 2019), should be considered to substantiate these results.

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