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Short Communication: Genetic variation of oceanic manta ray (*Mobula birostris*) based on mtDNA data in the Savu Sea, Indonesia

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Abstract. *Malik MDA, Putra MIH, Topan E, Pertiwi NPD, Artiningsih EY, Sari SK, Lewis S, Prabuning D, Sembiring A. 2022. Short Communication: Genetic variation of oceanic manta ray (*Mobula birostris*) based on mtDNA data in the Savu Sea, Indonesia. Biodiversitas 23: 1700-1706.* The Savu Sea, one of Indonesia's top conservation priorities, is home to various marine charismatic species, including the oceanic manta ray (*Mobula birostris*), whose conservation status is currently endangered and is protected by the Indonesian government. However, due to domestic and global demand for its fishery products, as well as shortcomings in fisheries management, this species is still poached and bycaught in the Savu Sea. Understanding their population structure is important to achieve effective conservation and fisheries management strategies that will have a positive impact on preserving their population in this area. This study aims to reveal the genetic variation of oceanic manta rays in the Savu Sea. Thirty samples from three locations in the Savu Sea were successfully preserved from East Flores (24), West Manggarai (4), and Rote Ndao (2) and then analyzed using ND5 locus from Mithocondiral DNA (mtDNA). The result indicated a close genetic relationship between three locations (East Flores, West Manggarai, and Rote Ndao) based on the phylogenetic tree and Analysis of Molecular Variance (AMOVA) result with value of 0.05158 (P-value = 0.62268) indicated as a single population. In conclusion, the findings of this study provide some insight into the possibility of manta ray populations in the Savu Sea having strong connectivity between areas, which is critical information for regulators and managers to integrate conservation and management strategies within the Savu Sea.

Keywords: Connectivity, fisheries management, genetic

INTRODUCTION

The Savu Sea is located in the coral triangle area known as mega marine biodiversity (Veron et al. 2009; Foale et al. 2013). This area is an essential habitat for nursery and migratory corridor for marine megafauna (Putra and Mustika 2020; Sahri et al. 2021). The region is known for its complex, high-energy currents, and temperature variation and is an essential habitat for charismatic species such as mobulids rays (Devantier et al. 2008; Lewis et al. 2015). The Savu Sea is also a crucial home for an oceanic manta ray (*Mobula birostris*) which provides abundant food for this species (Putra et al. 2016; Putra and Mustika, 2020; Putra et al. 2020).

In terms of spatial distribution, the Savu Sea with an estimated total area of 23,412 square kilometers is home to the oceanic manta ray (Putra et al. 2020; Figure 1). However, this species has been reported as one of the species targeted by local fisheries, specifically the Lamakeran community (Dewar 2002; Lewis et al. 2015). This community has been fishing for manta rays for generations and has increased their efforts in the last decade as demand for their gill plates grows in Asian markets (Dewar 2002; Lewis et al. 2015). These fishing pressures have had a significant impact on the potential decline in their population in the region, with evidence of a 75 percent reduction in annual landings (Lewis et al. 2015).

As a species that has a broad migration pathway (Couturier et al. 2012), efforts to conserve oceanic manta rays focus on international agreements such as the Convention on International Trade in Endangered Species (CITES) and the Convention on Conservation of Migratory Species (CMS) (Stewart et al. 2016). However, the effectiveness of efforts to preserve oceanic manta ray populations in a wide-scale or international approach is still questionable (Stewart et al. 2016). Regional and national strategies such as establishing Marine Protected Areas (MPAs) can effectively manage the oceanic manta ray (Davidson et al. 2015; Graham et al. 2016).

The molecular genetic technique could help identify the population structure (e.g. genetic variation) among populations and measure the population distinct which is valuable to determine appropriate scale for spatial management effectiveness (Pujolar et al. 2013; Hays et al. 2014). Thus, understanding the population structure of oceanic manta rays at the local, national, and worldwide levels can enhance the development of appropriate scale management strategies to ensure their sustainability (Graham et al. 2012; Hearn et al. 2014).

This study focuses on the genetic diversity and distance from oceanic manta ray populations in the Savu Sea, specifically that inhabit the East Flores MPA (Solor) and the West Manggarai and Rote Ndao of the Savu Sea MPA. This study will aid policy makers in harmonizing policies between the two MPAs in order to benefit marine organisms with extensive corridors, such as the oceanic manta ray.

MATERIALS AND METHODS

A tissue sample of oceanic manta rays were collected from 30 samples from three different locations, including East Flores (24), Rote Ndao (2), and West Manggarai (4) between 2014 and 2020 (Figure 1). This DNA information was acquired in partnership with the East Flores local government and the Central Government (Ministry of Marine and Fisheries Affairs) to better understand the manta ray population in Lamakera and its surroundings. Meanwhile, DNA samples are mostly collected from dead animals that have been landed from small-scale fishing activities in East Flores and West Manggarai. Small-scale fisheries in these two sites typically only capture within a 12-kilometer radius of their fishing base. These animals are largely landed in Lamakera as part of the region's target fisheries, with relatively little produced as bycatch from gillnet fishery (Dewar 2002; Lewis et al. 2015). Despite the fact that manta rays have been protected since 2014, Lamakera fishermen continue to illegally catch them. In fact, they are presently utilizing gillnets to create an alibi for catching manta rays as bycatch in tuna operations. Then, this project is an effort to monitor the capture of manta rays in this area, as well as to understand further information their population status for effectively designing conservation and management strategies (Booth et al. 2020; 2021). Meanwhile, DNA samples were collected in Rote from individuals using a biopsy stick that was inserted ventrally to obtain a tissue sample. These techniques and tools are based on previous research and adhere to the code of ethics (Kashiwagi et al. 2015; Hinojosa-Alvarez et al. 2016). Samples were collected from the muscle tissue and preserved in 96% ethanol on tube 2.5 mL.

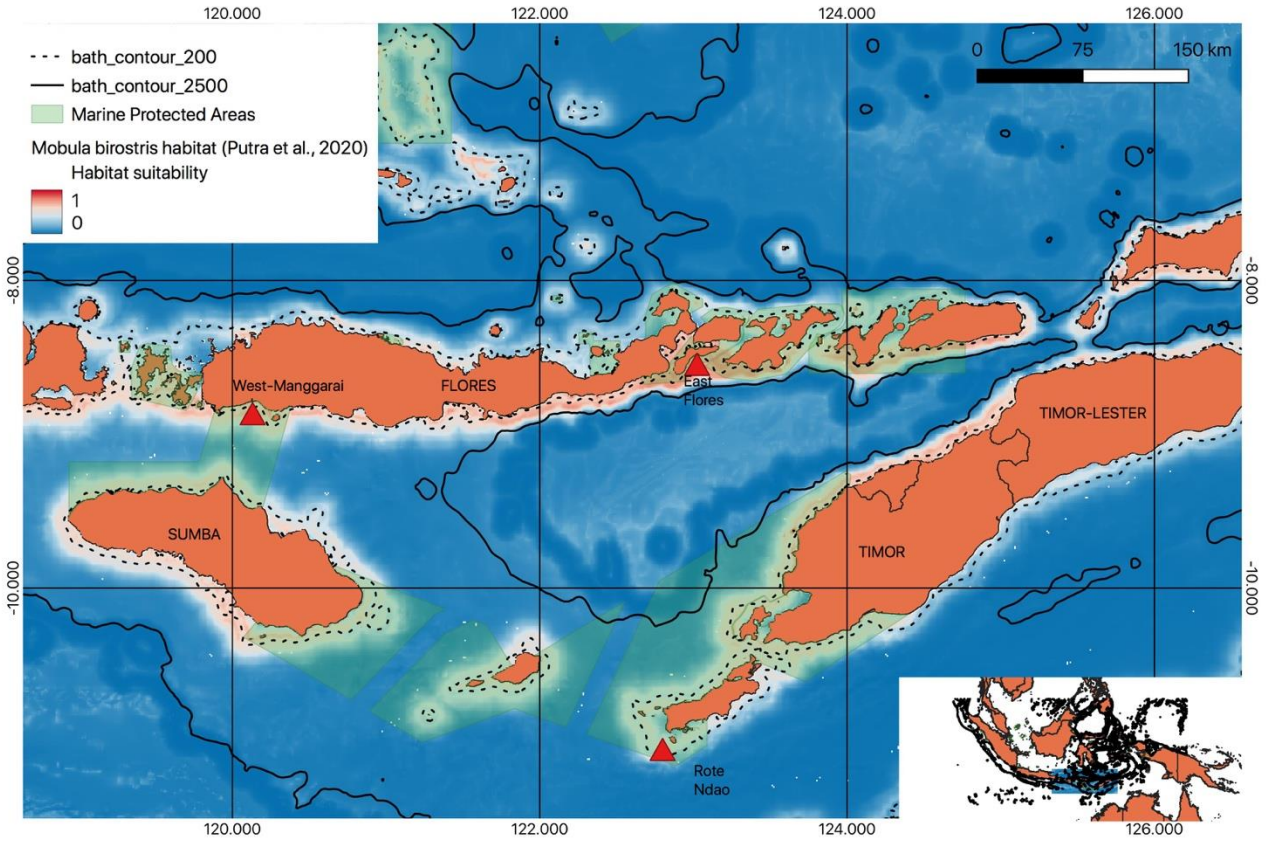

Figure 1. Sampling locations of oceanic manta rays (*Mobula birostris*) at three locations in the Savu Sea. Habitat map generated from previous publication (Putra et al. 2020)

Mitochondrial DNA was extracted using a 10% Chelex solution (Walsh et al. 1991). A portion of the mitochondrial ND5 locus was amplified via Polymerase Chain Reaction (PCR) methods, using forward primer MLF2 (5'-TGGTGCAACTCCAAGCTAAA-3') and reverse primer MNR4 (5'-TCAGGCGTTRAGGTATGATG-3') (Kashiwagi et al. 2012). The PCR reaction was carried out in 25 µL volumes, using 1 µL of template. Each reaction included 2.5 µL 10x PCR buffer (Applied Biosystems), 2 µL 25 mM MgCl2 solution, 2.5 µL 8 mM dNTPs, 1 µL of each primer at 10 mM, 0.25 µL AmplyTaq Red™ (Applied Biosystems), and 14.875 µL ddH2O. The thermocycling profile included an initial denaturation of 95°C for 3 min, 38 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min, with a final extension of 72°C for 5 min. PCR reactions were checked on 1% agarose gels stained with Gel Red® Biotium. PCR product was then sequenced using both forward and reverse primer with Big Dye Chain Termination protocol.

Sequences were edited and aligned using the CLUSTALW algorithm in MEGA X (Kumar et al. 2018). Species identification was performed using BLAST (Basic Local Alignment Search Tool)-comparing data to sequence database in genbank (www.ncbi.nlm.nih.gov), and also using phylogenetic tree analysis. Mitochondrial ND5 sequence of *Mobula birostris* was taken from Kashiwagi et al. (2012) and White et al. (2018) in National Center for Biotechnology Information (NCBI) Genbank (KR703226, KR703223, KR703232, KX151648) and used to confirm species. Sequences of Chilean devil ray (*Mobula tarapacana*) taken from Poortvliet et al. (2015) in NCBI Genbank (KM364986) were used as outgroup. A phylogenetic tree was constructed using Neighbor-Joining (NJ) analysis in MEGAX with 1000 bootstrap replication (Falsenstein 1985). Genetic diversity, including the number of haplotypes, haplotype diversity (*h*), and nucleotide diversity (π), were calculated using DnaSP 6 (Rozas et al. 2017). In addition, population genetic structure (FST) was analyzed using Analysis of Molecular Varience (AMOVA) with 10000 permutation replicates in Arlequin Ver.3.5 (Excoffier and Lischer 2010). AMOVA analysis used the significance level of 5% (p-value < 0.05).

RESULTS AND DISCUSSION

A total of 30 samples of oceanic manta ray from three locations (East Flores, West Manggarai, and Rote Ndao) were identified molecularly using mtDNA with ND5 loci. The use of ND5 loci in mobulid research has been carried out by previous studies (Kashiwagi et al. 2012; Hinojosa-Alvarez et al. 2016). Then, the use of ND5 loci can differentiate better than COI loci (Moritz and Cicero 2004; Hickerson et al. 2006), especially between *Mobula birostris* and *Mobula alfredi* (Kashiwagi et al. 2012). In addition, all sequence samples have been deposited on the NCBI GenBank database with accession number OM743321 - OM743350.

Through the result from oceanic manta rays in the Savu Sea, both molecular and morphological identification were indicated a similar result of species name. The detection of oceanic manta rays using a molecular approach is needed to ensure the sample is the correct species. Identification uses visual as the only method to ensure species of *Mobula* spp. are not sufficient due to the rapid change (within minutes) of the external color morphology, especially along dorsal surface (Ari 2014). As a result, the use of only visual measures is insufficient to ensure correct identification of *Mobula* spp. Then, the use of molecular (barcoding) can be a solution to validate the *Mobula* spp. from morphology identification.

Phylogenetic tree

Phylogenetic tree indicated that all thirty samples collected were oceanic manta ray and showed five different groups/clades in the tree (Figure 2). This clade was constructed based on haplotype results that grouped on the sample list. However, the clade was not indicated a different population because several samples from different locations are grouped into the same clade. This clade was more of an indication of different haplotypes (different sequences of genetic variation). Within the phylogenetic tree, we can also see that the samples from different sampling locations were mixing into several clades. There is no pattern of location on each clade.

The strong genetic relationship between the three locations (East Flores, West Manggarai, and Rote Ndao) of the oceanic manta ray was indicated based on the Analysis of Molecular Varience (AMOVA) results with value of 0.05158 (P-value = 0.62268), which indicated that oceanic manta rays between three locations are a single group (Table 1). The haplotype shared (Table 2) and haplotype distribution (Figure 3) also indicated that the oceanic manta rays found in the three locations are related. There are three haplotypes (H1, H2, and H3) that have a relationship between locations, and H2 is present in each location.

This is plausible because oceanic manta rays from East Flores, Rote Ndao, and West Manggarai interact and share habitats with each other. Studies in the Indo-Pacific (particularly Raja Ampat, Indonesia) have found that the majority of manta rays don't really travel large distances (over deep waters) and all tagged mantas remained close to their respective tag deployment location (Stewart et al. 2016). Stable isotopes and genetic data also indicate that such long-distance movements are likely rare and do not result in significant gene flow or individual interpopulation exchange (Stewart et al. 2016). There is evidence suggesting oceanic manta rays preferred coastal waters in the Gulf of Mexico, they spend the majority of their time in shallow areas of around 50 meters, which represent thermally dynamic and productive waters (Graham et al. 2012). Regardless of this, the strong connection between these three locations serves as a strong argument for ensuring the integration of spatial and fishery management for the Savu Sea.

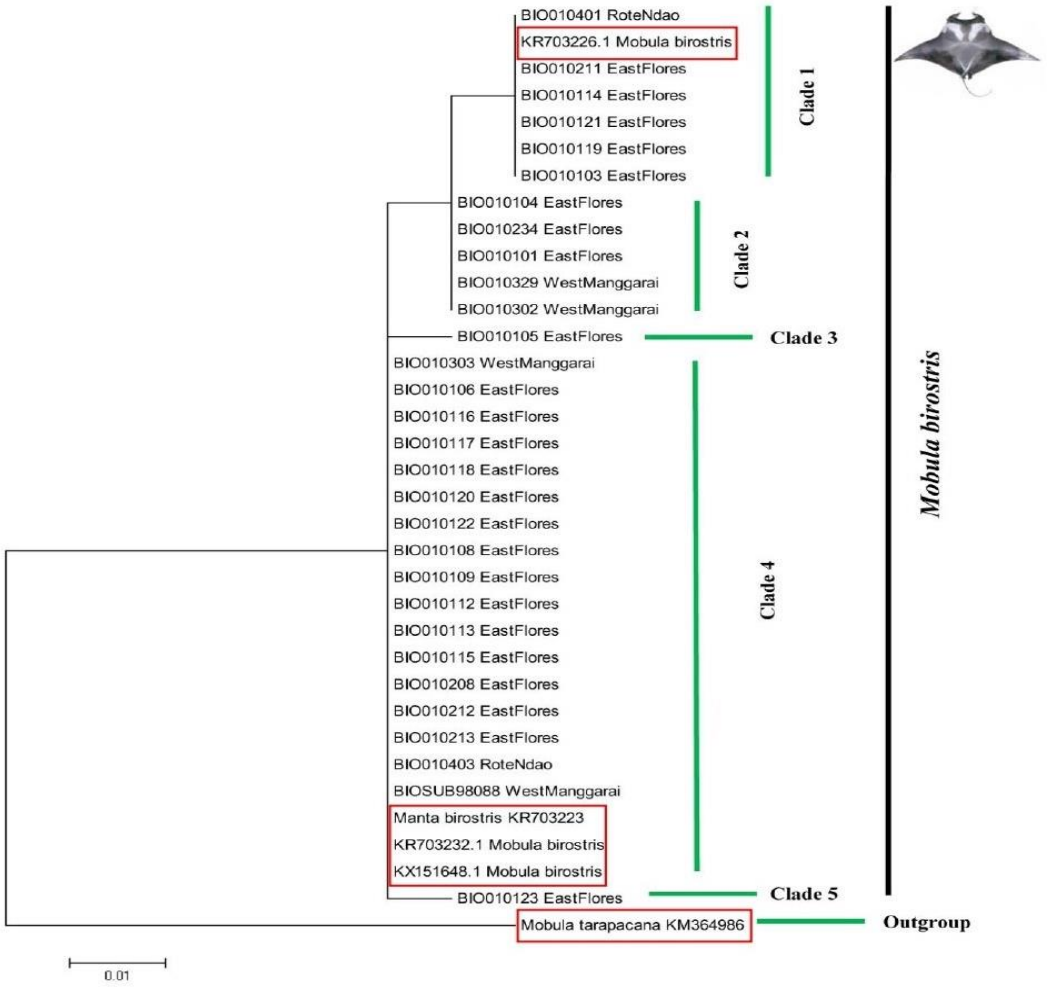

Figure 2. Neighbor-Joining (NJ) generated from mtDNA ND5 loci data from three locations (East Flores, West Manggarai, and Rote), the red squares indicated the sequences from NCBI GenBank

Table 1. Analysis of molecular variance (AMOVA) of oceanic manta ray (*Mobula birostris*) in the Savu Sea, Indonesia

Source of variation	DF	Sum of squares	Variance component	Percentage of variation
Among population	2	78.200	2.55214 Va	5.16
Within-population	27	1404.833	52.03085 Vb	105.16
Total	29	1483.033	49.47872	
FST	0.05158			
P-value	0.62268 ± 0.01332			

Table 2. Distribution of three shared haplotypes of oceanic manta ray (*Mobula birostris*) between locations

Haplotype	Sample location			Total sample
	West Manggarai	East Flores	Rote Ndao	
1	1	1		2
2	1	1	1	3
3		1	1	2

Table 3. Genetic diversity of *Mobula birostris* in each location

Population	n	Hn	Hd	π
East Flores	24	5	0.623	0.006
West Manggaarai	4	2	0.667	0.004
Rote Ndao	2	2	1.000	0.013
Total	30	5	0.629	0.006

Note: n: Number of samples, Hn: Number of haplotype, Hd: Haplotype diversity, π: Nucleotide diversity

Genetic diversity

The results of genetic diversity from ND5 loci (Table 3) of oceanic manta rays in the Savu Sea has a value is 0.629 (h=0.629). The different result happened in other species of mobulids such as Benfin devil ray (*M. thrustoni*) (h=0.222) from COI locus (Domingues et al. 2019). However, both result genetic diversity of oceanic manta ray on this research (ND5 loci) and Benfin devil ray (COI) from Domingues et al. (2019) could not be actually compared because of different markers or loci (Hoffman et al. 2009). Otherwise, nucleotide diversity of oceanic manta rays in the Savu Sea value is deemed to be low (π=0.006), which

stated that although the samples have a high genetic variation (haplotype), the variation within each individual sample is not very significant (probably less than ten base differences on each haplotype).

Oceanic manta rays are exposed to fisheries in Indonesia, including the Savu Sea, both in artisanal fisheries (Lewis et al. 2015) and large-scale commercial fisheries (Dharmadi and Satria 2015). In addition, the oceanic manta ray became the most charismatic species for tourism activities like manta watching in Komodo National (Hani et al. 2019) which is near West Manggarai. Then, unregulated and lack of management intervention (e.g., carrying capacity and code of conduct) on manta tourism could potentially increase habitat degradation and stressor for oceanic manta ray. Unmanaged tourism activities are known as one of the factors that could be decreasing biological diversity, including through the genetic level (Hall 2010). While fishing pressure might decrease nucleotide diversity that might cause a loss of the target species (Hauser et al. 2012; Madduppa et al. 2018), including oceanic manta ray in the Savu Sea.

However, this result can only show a small portion of oceanic manta ray population, because of the insufficient number of samples from each population, mainly from West Manggarai and Rote Ndao. Hale et al. (2012) concluded that a small sample would have significant error and high bias when estimating expected heterozygosity between samples or populations. Therefore, additional data to reveal the actual population signal of oceanic manta ray is needed for future research, with the addition of the newest genetic technology such as microsatellite (Kim and Sappington 2013; Putman and Carbone 2014) or Restriction-site-associated DNA (RAD) (Davey and Blaxter 2010; Mastretta-Yanes et al. 2015).

Conservation implication

The oceanic manta ray is a fully protected species in Indonesia, according to national regulations (No.4/KEPMEN-KP/2014). This includes both living individuals (their entire life cycle) and dead. The products of this species are prohibited to be utilized extractive. Despite the fact that it is still permitted for research and development purposes. Tourism is still permitted under the limitations of carrying capacity and with respect to a strict code of ethics. Despite this regulation, demand for oceanic manta ray products for the Asian medical market is increasing (O'Malley et al. 2017), while meat consumption is primarily for domestic purposes (Lewis et al. 2015). The Savu Sea, notably in East Flores, has become the world's most extensive artisanal fishing place for oceanic manta rays (Lewis et al. 2015). Therefore, strong law enforcement, fishery management, and habitat protection are required to preserve this species.

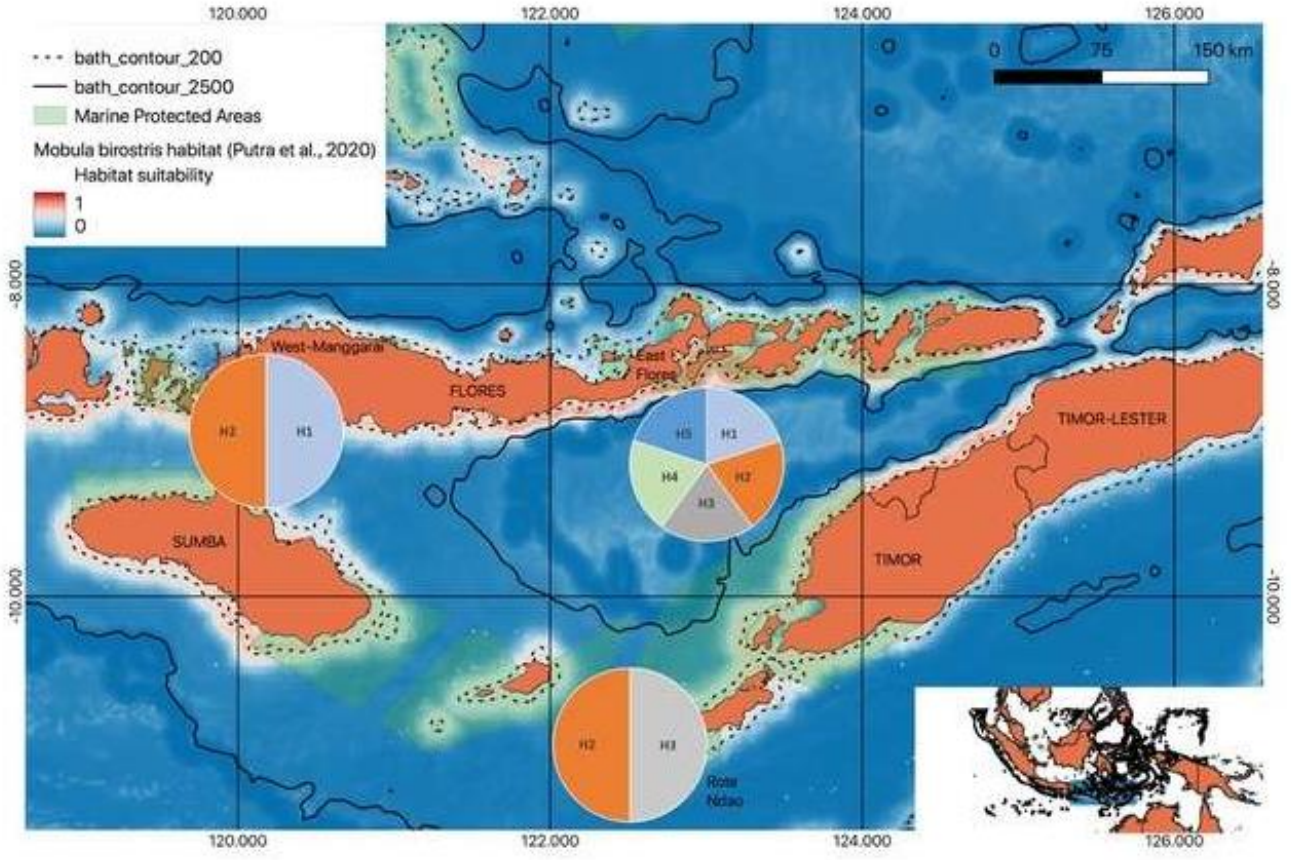

Figure 3. Distribution of 5 haplotypes of oceanic manta rays (*Mobula birostris*) from three locations. Habitat map generated from previous publication (Putra et al. 2020)

Due to inadequate data, most MPAs in Indonesia are currently designed with a coastal ecosystem approach and rarely include mobile species in MPA designs (Putra et al. 2020). The threat to their population grew increasingly serious. According to a study conducted by Putra et al. (2020), 52% of mobulid habitat (including oceanic manta rays) in the Savu Sea overlapped with fishing activity. Furthermore, fishing gear used by fishermen in the Savu Sea, such as gillnets, frequently catches mobulids as bycatch (Lewis et al. 2015). Therefore, the Savu Sea need to develop a management strategy that promotes species with a wide range of movement, such as oceanic manta rays.

Importantly, our findings reveal a strong genetic relationship between the East Flores MPAs and the West Manggarai and Rote Ndao MPAs (Savu Sea MPAs). The results of this study can serve as baseline data for the Government of Indonesia, especially in the Savu Sea to integrate regulations and management strategies between the two MPAs in order to effectively manage manta ray populations in the Savu Sea. As a result of this, the priority effort to protect this species, including both fisheries management and conservation activities should be worked together and side by side between East Flores MPAs and Savu Sea MPAs.

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