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Identification Of Bycatch Sharks In The India Ocean Based On DNA Barcoding In Conservation Effort

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Abstract

The most potential shark fishing area in Indonesia is the Indian Ocean. One of the fishing ports that has bycatch shark is Benoa Harbor. The purpose of this research is to identify species to determine the conservation status of bycatch shark species from body tissue samples caught in the Indian Ocean in conservation efforts. The research was conducted from January to March 2021 at Pangkalan Pengawasan Sumber Daya Kelautan dan Perikanan (PSDKP) of Benoa Harbor. This study uses an exploratory method with survey techniques. The data used in this study are primary data in the form of shark fin samples in the study area and secondary data in the form of locations where sharks are caught by fishermen based on the PSDKP database. The results showed that sharks bycatch from body tissue samples caught in the Indian Ocean based on morphological identification and DNA barcoding techniques were *Prionace glauca*, *Isurus paucus*, *Sphyrna zygaena*, *Isurus oxyrinchus*, *Alopias superciliosus* and *Carcharhinus longimanus*. Bycatch sharks from body tissue samples caught in the Indian Ocean based on morphological identification and DNA barcoding techniques have close phylogenetic relationships with the Carcharhinidae, Sphyrnidae, Alopiidae and Lamnidae families. Bycatched shark from body tissue samples caught in the Indian Ocean based on morphological identification and DNA barcoding techniques has a conservation status, near threatened for *Prionace glauca* species, vulnerable for *Sphyrna zygaena* species and *Alopias superciliosus* species, endangered for *Isurus paucus* species and *Isurus oxyrinchus* species, critically endangered for *Carcharhinus longimanus* species.

Keywords: *shark; Indian Ocean; DNA; barcoding; phylogenetic*

Abstrak

Wilayah yang menjadi daerah penangkapan ikan hiu paling potensial di Indonesia adalah Samudra Hindia. Salah satu pelabuhan perikanan yang memiliki hasil tangkapan sampingan hiu adalah Pelabuhan Benoa. Tujuan dari penelitian ini adalah mengidentifikasi spesies untuk mengetahui status konservasi spesies ikan hiu hasil tangkapan sampingan (bycatch) dari sampel jaringan tubuh yang tertangkap di Samudra Hindia dalam upaya konservasi. Penelitian dilaksanakan dari bulan Januari sampai Maret 2021 di Pangkalan Pengawasan Sumber Daya Kelautan dan Perikanan (PSDKP) Pelabuhan Benoa. Penelitian ini menggunakan metode eksplorasi dengan teknik survei. Data yang digunakan dalam penelitian adalah data primer berupa sampel sirip ikan hiu di wilayah lokasi penelitian dan data sekunder berupa lokasi dimana ikan hiu tertangkap oleh nelayan berdasarkan database PSDKP. Hasil penelitian menunjukkan ikan hiu hasil tangkapan sampingan (bycatch) dari sampel jaringan tubuh yang tertangkap di Samudra Hindia berdasarkan hasil identifikasi morfologi dan teknik DNA barcoding merupakan spesies *Prionace glauca*, *Isurus paucus*, *Sphyrna zygaena*, *Isurus oxyrinchus*, *Alopias superciliosus* dan *Carcharhinus longimanus*. Ikan hiu hasil tangkapan sampingan (bycatch) dari sampel jaringan tubuh yang tertangkap di Samudra Hindia berdasarkan hasil identifikasi morfologi dan teknik DNA barcoding memiliki hubungan filogenetik yang dekat dengan famili Carcharhinidae, Sphyrnidae, Alopiidae dan Lamnidae. Ikan hiu hasil tangkapan sampingan (bycatch) dari sampel jaringan tubuh yang tertangkap di Samudra Hindia berdasarkan hasil identifikasi morfologi dan teknik DNA barcoding memiliki status konservasi, near threatened untuk spesies *Prionace glauca*, vulnerable untuk spesies *Sphyrna zygaena* dan spesies *Alopias superciliosus*, endangered untuk spesies *Isurus paucus* dan spesies *Isurus oxyrinchus*, critically endangered untuk spesies *Carcharhinus longimanus*.

Kata Kunci: *hiu; Samudra Hindia; DNA; barcoding; filogenetik*

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1. Pendahuluan

Geographically, Indonesia is located between two continents and two oceans, namely the continent of Asia and the continent of Australia and the Pacific Ocean and the Indian Ocean. This geographical position affects the high biodiversity in Indonesia. Indonesia as a maritime country with a water area larger than the land area, has a total area of Indonesia is 7.81 million km2 consisting of 2.01 million km2 of land, 3.25 million km2 of ocean and 2.55 million km2 of the Zona Ekonomi Eksklusif (ZEE) (Purwanto and Mangku, 2016). The Indonesian oceans are included in the Marine Mega Biodiversity area in the world, having 8,500 fish species, 555 seaweed species and 950 biota species associated with coral reef ecosystems (Appeltans *et al*., 2012). This shows the high biodiversity in Indonesian waters, one of which is the diversity of shark species.

Indonesian waters have a fairly high diversity of shark species. At least 116 species of sharks belonging to 25 tribes are found in Indonesian waters. The area that is the most potential shark fishing area in Indonesia is the Indian Ocean. Shark fishing activity in Indonesian waters lasts throughout the year, but there are certain months which are the highest catching season for shark commodities in Indonesian waters. Generally, sharks are caught in Indonesian waters as bycatch from various types of fishing gear such as longline fishing rods, gill nets, circle nets and so on. However, based on data on shark catches from 1975 to 2011 it showed a significant upward trend (Fahmi and Dharmadi, 2013).

Indonesia is one of the countries in the world with the most shark fishing activities. This puts Indonesia in the top position of the largest shark fishing country globally (Permana and Kusuma, 2020), where most of the sharks in Indonesia 72% are bycatch and 28% are the main catch targets (Fahmi and Dharmadi, 2013). Conditions like this can cause some species of shark to become extinct. Moreover, what is currently happening is that the catches of fishermen in fishing ports do not pay attention to protected or unprotected shark species (Dick and Jefferies, 2013). One of the fishing ports that has shark bycatch is Benoa Harbor.

Until now, Benoa Harbor has only grouped all types of by-catch sharks into one group, namely the shark or shark group in fisheries data, especially in the Pangkalan Pengawasan Sumber Daya Kelautan dan Perikanan (PSDKP) data at Benoa Harbor. Knowledge of specific types of shark is still very low due to the ability to identify fish or because it is only a bycatch. On the other hand, the lack of publications regarding the identification of sharks in the world also makes it difficult for fishermen and fisheries practitioners in the world to recognize the types of sharks that exist in the world, especially species that are rarely found or classified as rare, so that efforts to manage or protect these types have problems (Fahmi and Dharmadi, 2013). Another way of identification is by DNA barcoding technique which requires only a small amount of body tissue from the shark. DNA barcoding provides accuracy in species identification by focusing the analysis on small segments of mtDNA (Pramono *et al*., 2017).

The use of DNA barcoding in this study is intended to identify bycatch sharks based on the CO1 gene. CO1 is one of the genes in the mitochondrial genome (mtDNA) whose sequence is commonly used as a barcode. The use of mitochondrial DNA (mtDNA) as a target gene is increasingly being carried out for a species (Kyle and Wilson, 2007).

Maintaining the stability of biodiversity from pressures that causes a decline in ecosystem health conditions need to be done because biodiversity is often used as a measure of system health biological. Measures to conserve biodiversity will result in the protection and sustainability of goods and services that ecosystem provided. Likewise, efforts to maintain or maintaining ecosystem health is very useful in strengthening conservation biodiversity.

Therefore, this study aims to identify species for knowing the conservation status of bycatch shark species from body tissue samples caught in the Indian Ocean in conservation efforts are in accordance with the concept of the Poros Maritim Dunia (PMD), one of which is rehabilitation of environmental damage and biodiversity conservation.

2. Research methods

2.1 Time and location

The study was conducted in January-March 2021. Sampling was carried out in January 2021 at the Pangkalan Pengawasan Sumber Daya Kelautan dan Perikanan (PSDKP) Benoa Harbor, Jl. Benoa Harbor, South Denpasar District, Denpasar City, Bali Province. Sample data processing was carried out in February-March 2021 at the Biodiversitas Indonesia Laboratory (BIONESIA), West Denpasar District, Denpasar City, Bali Province. Sequencing was carried out in March 2021 at PT. Genetics Science Indonesia, Jl. Green Lake City Boulevard, Cipondoh District, Tangerang City, Banten Province. Map of bycatch shark fishing locations can be seen in Figure 1.

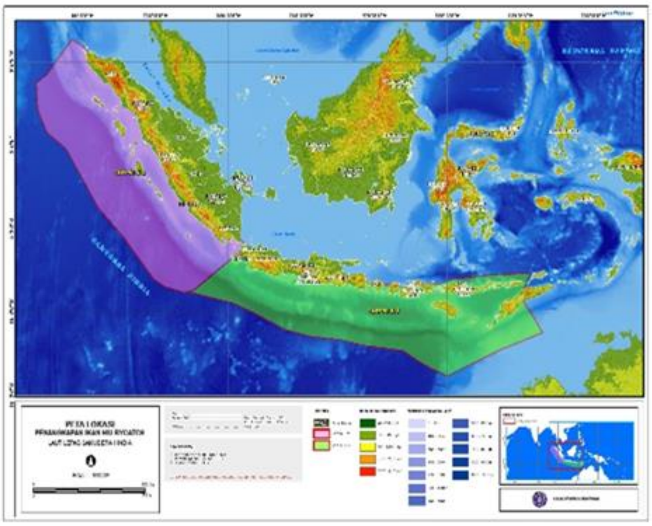

Figure 1. Map of bycatch shark fishing locations.

2.2 Data retrieval method

2.2.1. Data source

The data collected includes primary data in the form of samples of bycatch shark fins in the Indian Ocean and secondary data in the form of locations where sharks are caught based on the PSDKP Base database of Benoa Harbor. The data collection technique in this study is an observation technique. The source of data in this study is an infinite population, that is, a population that has data sources whose boundaries cannot be determined quantitatively.

2.2.2. Data collection technique

Primary data in the form of bycatch shark fin samples in the Indian Ocean were taken from the 3 largest fishing companies in Benoa Harbor, namely: 1) PT. Bandar Nelayan, 2) PT. Golden Tuna dan 3) PT. Perintis Jaya Internasional to be brought to the PSDKP Base in Benoa Harbor. While secondary data is in the form of locations where sharks were caught by fishermen based on the database of the PSDKP Base in Benoa Harbor.

2.3 Data analysis

2.3.1. Shark identification results

The stages of morphological identification, namely: 1) shark fins were collected, 2) visual observations were made to see the morphology of shark fins, 3) shark fins were lined up, 4) shark fins were documented, 5) observed shape, color and size of shark fin, 6) given the information DIB (first dorsal base), DIA (first dorsal anterior margin), DIH (first dorsal hight), DIP (first dorsal pasterior margin), PIB (pectoral base), PIA (pectoral anterior margin), PIH (pectoral height), PIP (pectoral posterior margin), 7) using a loupe to clarify observations, 8) using a digital scale to weigh the weight of shark fins before and after being taken for samples in the study, 9) performed shark fin morphometric measurements.

The stages of identification of DNA barcoding, namely: 1) DNA isolation, 2) strengthening / ensuring the target area of DNA barcoding using PCR, 3) electrophoresis and visualization, 4) sequencing of PCR products, 5) matching the resulting sequence with the information center (database) against identified species (Hubert and Hanner, 2015).

2.3.2. Phylogenetic tree analysis

The stages of phylogenetic tree analysis are: 1) sequence alignment, 2) reconstruction of phylogenetic trees, 3) evaluation of phylogenetic trees with statistical tests (distance matrix). The main purpose of this step is to determine whether one DNA sequence is homologous to another.

2.3.3. Species conservation

The 9 species conservation categories are as follows: extinct (EX), extinct in the wild (EW), critically endangered (CR), endangered (EN), vulnerable (VU), near threatened (NT), least concern (LC), data deficient (DD) and not evaluated (NE) (Rueda-Cediel *et al*., 2018).

3. Results and Discussion

3.1 Shark identification results

Shark bycatch in the form of fin samples obtained from PT. Bandar Nelayan, PT. Golden Tuna dan PT. Perintis Jaya Internasional in January 2021. Bycatch sharks are the fin samples from the visual observation showed that there were 7 different individuals, which were continued to the morphological identification stage. The results of morphological identification can be seen in Figure 2.

Nomor Sirip	Sampel Sirip	PIB (cm)	PIA (cm)	PIH (cm)	PIP (cm)	Berat (gr)
2	Lokasi penangkapan: Laut Lepas Samudra Hindia	16	45	40	38	425
3	Lokasi penangkapan: Laut Lepas Samudra Hindia	16	44	42	39	424
5	Lokasi penangkapan: Laut Bali, Perairan Selatan Bali	17	50	44	38	430
6	Lokasi penangkapan: Laut Arafura, Laut Aru, Laut Seram	18	54	44	40	432
8	Lokasi penangkapan: Laut Lepas Samudra Hindia	20	50	50	51	435
9	Lokasi penangkapan: Laut Lepas Samudra Hindia	19	54	48	49	438

No.	Sampel Sirip	DIB (cm)	DIA (cm)	DIH (cm)	DIP (cm)	Berat (gr)
1	Lokasi penangkapan: Laut Lepas Samudra Hindia	14	22	19	15	389
4	Lokasi penangkapan: Dobo, Merauke, Tual	16	24	20	17	390
7	Lokasi penangkapan: ZEEI Laut Arafura	15	21	20	16	392
10	Lokasi penangkapan: Laut Lepas Samudra Hindia	15	20	19	16	392
11	Lokasi penangkapan: Poumako, Dobo, Merauke	14	19	18	15	390
12	Lokasi penangkapan: Laut Lepas Samudra Hindia	15	20	21	16	389
13	Lokasi penangkapan: Laut Lepas Samudra Hindia	16	22	21	17	392
14	Lokasi penangkapan: Laut Lepas Samudra Hindia	15	21	19	16	390
15	Lokasi penangkapan: Laut Lepas Samudra Hindia	17	24	22	18	394

Figure 2. morphological identification results.

3.1.1. Morphological identification

The results of morphological identification in the field show that there are morphological similarities for numbers 1-7, number 8-9, number 10-11, number 12 is similar to number 10-11, number 13, number 14, number 15 with a total of 7 different samples to be identified. genetics are numbers 1, 8, 10, 12, 13, 14 and 15.

Based on these morphological characteristics, it shows that the bycatch sharks landed during the research at Benoa Harbor are 1) blue sharks, 2) longfin mako sharks, 3) fine hammerhead sharks, 4) sharks that have similar characteristics with the fine hammerhead shark, 5) the short-finned mako shark, 6) the big eye thresher shark and 7) the cowboy shark. According to Jatmiko *et al*. (2019), from 2013-2016, at least seven species of sharks were found, namely *Prionace glauca*, *Isurus oxyrinchus*, *Carcharhinus longimanus*, *Hemipristis elongata*, *Sphyrna* spp., *Carcharhinus falciformis* dan *Alopias* spp.

3.1.2. Genetic identification of DNA barcoding

The extraction stage is the initial stage of genetic identification using DNA barcode technique. Based on the results of electrophoresis, DNA extraction by the chelex method can be used for the extraction of shark fin DNA.

The results of the extraction stage are continued to the PCR stage. Based on the results of electrophoresis, the PCR results will not be optimal if there are errors in the optimization process of the PCR process. According to Walsh *et al*. (2013), DNA amplified by PCR cannot be seen with the naked eye, but can be identified by electrophoresis techniques. A positive electrophoresis result indicates that the PCR results are optimal. Optimization of the PCR process related to factors such as DNA template, primer JGLCO and primer FISHF1 as forward and primer JGHCO and primer FISHR1 as reverse, DNA polymerase, buffer, MgCl2 and dNTPS. The results of electrophoresis with a marker size according to the target, namely ± 600 bp.

The results of the PCR stage are continued to the electrophoresis stage. The results of the electrophoresis of a positive sample on March 15, 2021 can be seen in Figure 3. The results of the electrophoresis of a positive sample on March 16, 2021 can be seen in Figure 4. The results of the electrophoresis of a positive sample on March 17, 2021 can be seen in Figure 5.

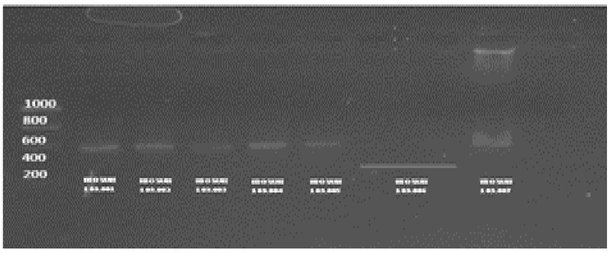

Figure 3. Positive sample on March 15, 2021.

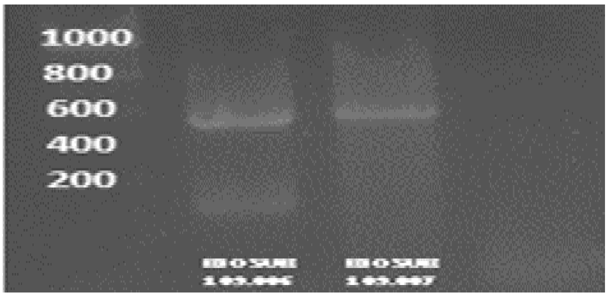

Figure 4. Positive sample on March 16, 2021.

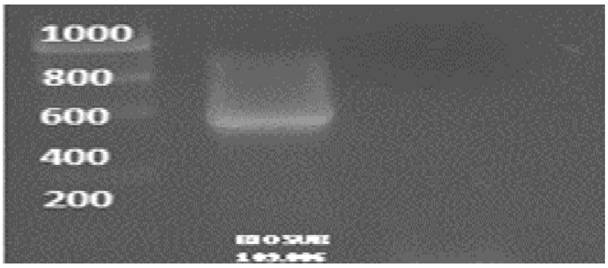

Figure 5. Positive sample on March 17, 2021.

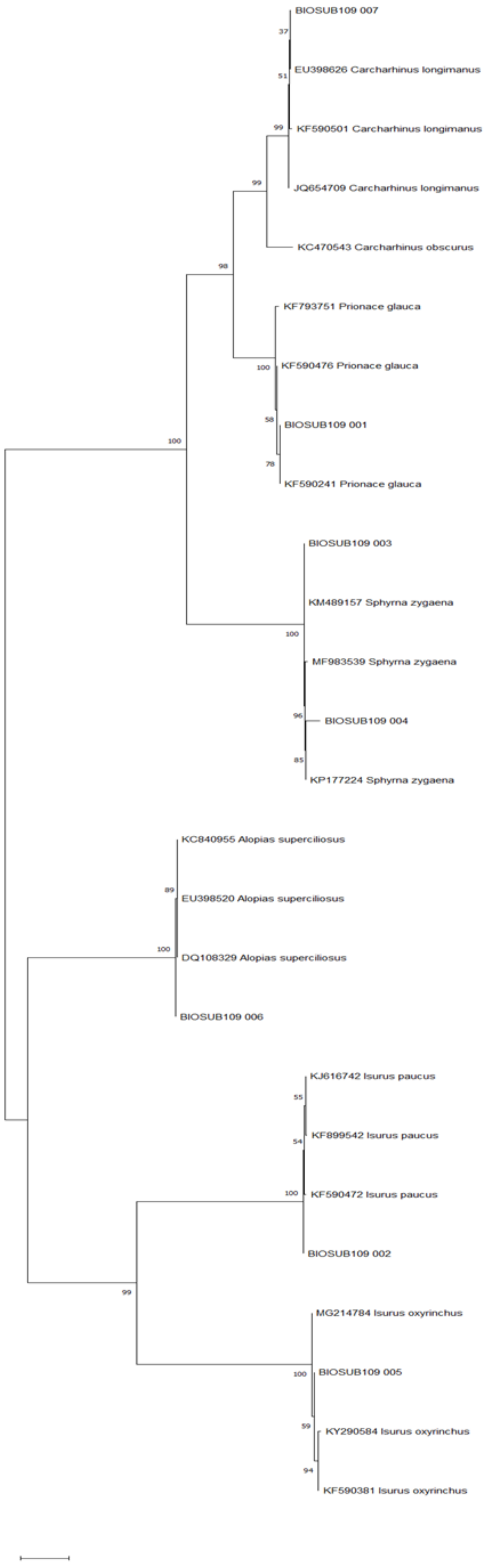

Figure 6. Results of phylogenetic tree.

3.2 Phylogenetic tree analysis

Sequence results from the BLAST process that have been aligned sequences are used to identify species from the research sample, then proceed with making a phylogenetic tree to see the relationship, distance between species and genetic diversity of sharks in the same family. The results of the phylogenetic tree can be seen in Figure 6.

The phylogenetic tree explained that the seven samples of sharks analyzed were from different families, namely Carcharhinidae, Sphyrnidae, Alopiidae and Lamnidae with similarity in groups reaching a value of 100 with species in GenBank. Seven samples from six species including BIOSUB109001 *Prionace glauca*, BIOSUB109002 *Isurus paucus*, BIOSUB109003 *Sphyrna zygaena*, BIOSUB109004 *Sphyrna zygaena*, BIOSUB109005 *Isurus oxyrinchus*, BIOSUB109006 *Alopias superciliosus*, BIOSUB109007 *Carcharhinus longimanus*.

3.3 Species conservation

Sharks from the species *Prionace glauca* are included in the near threatened category. Sharks from the species *Isurus paucus* are included in the endangered category. Sharks from the species *Sphyrna zygaena* are included in the vulnerable category. Sharks from the species *Isurus oxyrinchus* are included in the endangered category. Sharks from the species *Alopias superciliosus* are included in the vulnerable category. Sharks from the species *Carcharhinus longimanus* are included in the critically endangered category.

4. Conclusion

Bycatch sharks from body tissue samples caught in the Indian Ocean based on morphological identification and DNA barcoding techniques are *Prionace glauca*, *Isurus paucus*, *Sphyrna zygaena*, *Isurus oxyrinchus*, *Alopias superciliosus* and *Carcharhinus longimanus* species.

Bycatch sharks from body tissue samples caught in the Indian Ocean based on phylogenetic tree analysis belong to the families Carcharhinidae, Sphyrnidae, Alopiidae and Lamnidae.

Bycatch sharks from body tissue samples caught in the Indian Ocean based on morphological identification and DNA barcoding techniques have conservation status near threatened for *Prionace glauca* species, vulnerable for *Sphyrna zygaena* species and *Alopias superciliosus* species, endangered for *Isurus paucus* species and *Isurus oxyrinchus* species, critically endangered for *Carcharhinus longimanus* species.

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