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SHARK SPECIES ON EXPORT PRODUCTS FROM EAST JAVA AND BALI BY DNA BARCODING BASED ON INTERNAL TRANSCRIBED SPACER-2 (ITS-2) LOCUS IN MITOCHONDRIAL

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Abstract

DNA barcoding method is the mitochondrial marker for all animal species, and it is claimed as distinguishing feature from one species to another Species identification in shark products is often difficult to perform as they have morphological similarities with many other species and it is even more difficult as they are parts separated from the body for the storage. This research is aimed to know which species of sharks identified in the export products from East Java and Bali by DNA barcoding method. The samples of sharks (meat, fins, skin and bones) used were 90 samples acquired in Surabaya from the export products of East Java and Bali from 2015 to 2017. The DNA barcoding method uses universal primer through nested PCR (Polymerase Chain Reaction) process which is able to amplify the DNA until around 1,340 bp based on Internal Transcribed Spacer-2 (ITS-2) locus of mitochondria. Based on the result of phylogenetic analysis and the classification list by IUCN, from 90 samples of sharks acquired from export products in East Java and Bali, species identified were: 1.11% *Daenia sp* categorized as NE (not evaluated); 4.44% *Sphyrna zygaena* categorized as NT (not Threatened); 3.33% *Sphyrna lewini* categorized as NT (not Threatened); 10% *Rhizoprionodon taylori* categorized as LC *(*least concern); 24% *Charcarhinus brevipinna* categorized as NT (not Threatened); 2.22% *Charcarhinus obscurus* categorized as NT (not Threatened); 3.33% *Charcarhinus falciformis* categorized as LC (least concern); 1.11% *Charcarhinus plumbeus* categorized as NT (not Threatened); 27.78% *Charcarhinus longimanus* categorized as VU (vulnerable); 1.11% *Neutrygon kuhlii* categorized as NE (not evaluated); 1.11% *Charcarhinus Taurus* categorized as VU (vulnerable); 3.33% *Rhizoprionodon longurio* categorized as DD (data deficient); 1.11% *Rhizoprionodon porosus* categorized as *DD* (data deficient); 1.11% *Eusphyra blochii* categorized as NT (not Threatened); 4.44% *Chiloscyllium griseum* categorized as DD (data deficient); 5.56% *Rhizoprionodon oligolinx* categorized as LC (least concern); 1.11% *Prionace glauca* categorized as NT (not Threatened); 1.11% *Rhizoprionodon lalandii* categorized as DD (data deficient). Generally all species found in this research were special fish from Indo-Australian archipelago and included in IUCN red list. The government policy to prohibit export of these species was the right decision to prevent the species extinction.

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1. Introduction

Indonesia spreads for nearly 5,000 kilometers across the equator from the East to the West with the most diverse seagrass meadow, the greatest expanses of mangroves and extensive coral reef communities [7]. As a part of the Indo-Australian archipelago which contains about 30% of the one thousand shark and stingray species in the world [3]. Sharks are predators which play a crucial ecological role in structuring marine ecosystems and food webs. On the other hand, biological characteristics such as late maturation (8-13 years) and low fecundity as well as their meat and fins which is highly valuable commercially make them vulnerable to overexploitation and population decline [2]. Among species of sharks listed in Appendix II CITES, four of them are found in Indonesia. They are: (1) *Sphyrna leweni*, (2) *Sphyrna zygaena*, (3) *Sphyrna mokarran*, (4) *Carcharhinus longimanus* [5]. Even though Indonesia is recorded as the country with the most sharks and stingrays production and believed to have the most diverse sharks and stingrays species in the world, there are almost no studies or publications on the biological aspects or sharks and stingrays' composition in Indonesia. The science on identifying the type of sharks and stingrays in Indonesia is needed to follow the great exploitation of the species population as well as to acquire accurate data for making the policy about the resources management. Population and species genetic analysis is an important starting point to species conservation and sustainable exploitation.

DNA barcoding method is the mitochondrial marker for all animal species, and it is claimed as distinguishing feature from one species to another [3; 4]. The DNA barcoding method uses universal primer through PCR (*Polymerase Chain Reaction*) process which is able to amplify the DNA until around 1,340 bp based on Internal Transcribed Spacer-2 (ITS-2) locus of mitochondrial. DNA barcoding method has been used to identify over 207 species of fish in Australia including 143 species of teleostean, 61 species of shark and stingrays, and 3 species of chimaerid [5].

An international organization working in the field of biological species protection and conservation, IUCN or International Union for Conservation of Nature and Natural Resources, evaluates all biological species and the result of the evaluation is classified into nine categories: (1) Extinct (EX). It is for species which cannot be found any longer; (2). Extinct in the wild(EW). This category is for species which is not found in its original habitat but it is known only to survive in captivity, such as natural conservation, wildlife reserve, or any other preserving facility; (3) Critically Endangered (CR). This category is for the species in extremely high risk of extinction in the wild; (4). Endangered (EN). This category believed to have high risk of extinction in the wild; (5). Vulnerable (VU).

It is the category of species with high risk of endangerment in the wild; (6). Near Threatened (NT). It is for the species which is likely to become endangered in the near future if there is no management efforts made; (7). Least Concern (LC). This category is for the species which is likely to be threatened even though it does not qualify for a more at-risk category; (8). Data Deficient (DD); (9). Not Evaluated (NE). This research is aimed to know which species of sharks identified in the export products from East Java and Bali by DNA barcoding method.

2. Material And Method

The samples of sharks (meat, fins, skin and bones) used were 90 samples acquired in Surabaya from the export products of East Java and Bali from 2015 to 2017. DNA extraction was conducted to 25 mg of sample (meat, fins, skin and bones) using DNA extraction kit (*QIAamp® DNA mini kit* Qiagen) with product instruction. The last extraction volume was 50 µl which was used as DNA template. The final eluted volume of DNA was stored at -20°C until used [1]. The process of amplification in this research was only in the first round. In the first round amplification, PCR reagents mix inserted in *eppendorf* tube contained 12.5 µl GoTaq® Green Master mix, 0.5 µl distilled water, 1 µl forward primer (FISH 5.8SF : 5'-TTAGCGGTGGATCACTCGGCTCGT-3') and 1 µl reverse primer (FISH28SR : 5'-TCCTCCGCTTAGTAATATGCTTAAATTCAGC-3') from Internal Transcribed Spacer-2 (ITS-2) locus in mitochondrial and 5 µl DNA template [1].

The amplification result of PCR process then tested by electrophoresis on 2% agarose gel dipped in the tank containing buffer TBE. DNA marker was also added in the agarose gel to measure PCR produced DNA. Electrophoresis was set at constant voltage at 100 volt for 30 minutes. Electrophoresis then stopped and the gel extracted for observation transluminator-UV. The sample was determined ITS-2 positive from the bands with 1,340 bp in length [1].

PCR product acquired then purified through a procedure with kit qiagen [1]. After the purification, there was labeling and sequencing using ABI Prism 310 [1]. Phylogenetic analysis was conducted with Genetix Mac Ver. 10.0 software and the results compared to sharks sequence data recorded in GenBank.

3. Result and Discussion

Species identification in shark products is often difficult to perform as they have morphological similarities with many other species and it is even more difficult as they

Table 1: Identification results and IUCN status on shark export products in East Java and Bali by DNA barcoding method.

No.	Shark species	IUCN Status	Identified sample (%)	
1	*Daenia sp*	NE	1	(1.11)
2	*Sphyrna zygaena*	NT	4	(4.44)
3	*Sphyrna lewini*	NT	3	(3.33)
4	*Rhizoprionodon taylori*	LC	9 (10)	
5	*Charcarhinus brevipinna*	NT	24	(26.67)
6	*Charcarhinus obscurus*	NT	2	(2.22)
7	*Charcarhinus falciformis*	LC	3	(3.33)
8	*Charcarhinus plumbeus*	NT	1	(1.11)
9	*Charcarhinus longimanus*	VU	25	(27.78)
10	*Neutrygon kuhlii*	NE	1	(1.11)
11	*Charcarhinus taurus*	VU	1	(1.11)
12	*Rhizoprionodon longurio*	DD	3	(3.33)
13	*Rhizoprionodon porosus*	DD	1	(1.11)
14	*Eusphyra blochii*	NT	1	(1.11)
15	*Chiloscyllium griseum*	DD	4	(4.44)
16	*Rhizoprionodon oligolinx*	LC	5	(5.56)
17	*Prionace glauca*	NT	1	(1.11)
18	*Rhizoprionodon lalandii*	DD	1	(1.11)
Total			90 (100)	

NE : not evaluated; VU : vulnerable; NT : near threatened; LC : least concern; DD : data deficient.

are parts separated from the body for the storage [6]. From this research result, based on the method developed by [1] using universal primers, DNA of all shark species can be identified with band length about 1.340 b (Figure 1.). DNA barcoding with PCR will provide better results if the samples are fresh and not damaged from being stored too long.

Based on the result of phylogenetic analysis and the classification list by IUCN, from 90 samples of sharks acquired from export products in East Java and Bali, species identified were: 1.11% *Daenia sp* categorized as NE (not evaluated); 4.44% *Sphyrna zygaena* categorized as NT (not Threatened); 3.33% *Sphyrna lewini* categorized as NT (not Threatened); 10% *Rhizoprionodon taylori* categorized as LC *(*least concern); 24%

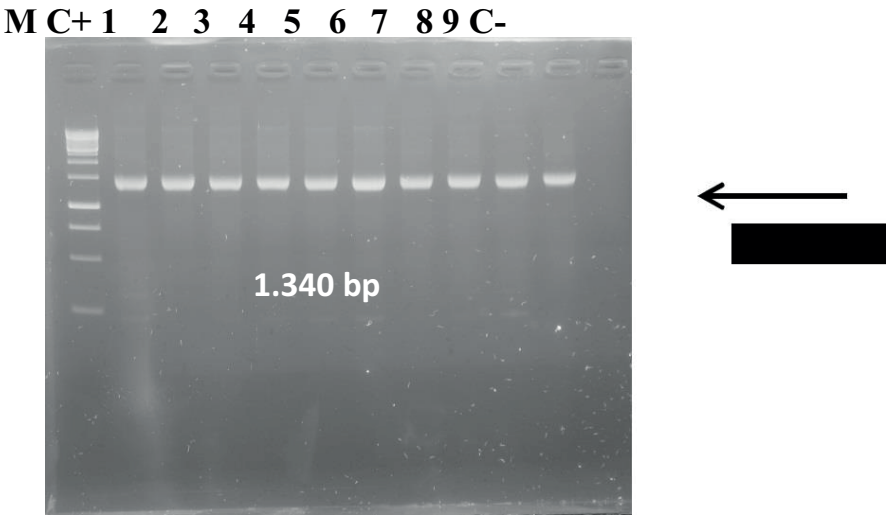

Figure 1: Electrophoresis result with 2% agarose gel from a shart sample (M: marker; C-: Negative control; C+:Positive control 1-9 : sample).

Charcarhinus brevipinna categorized as NT (not Threatened); 2.22% *Charcarhinus obscurus* categorized as NT (not Threatened); 3.33% *Charcarhinus falciformis* categorized as LC (least concern); 1.11% *Charcarhinus plumbeus* categorized as NT (not Threatened); 27.78% *Charcarhinus longimanus* categorized as VU (vulnerable); 1.11% *Neutrygon kuhlii* categorized as NE (not evaluated); 1.11% *Charcarhinus Taurus* categorized as VU (vulnerable); 3.33% *Rhizoprionodon longurio* categorized as DD (data deficient); 1.11% *Rhizoprionodon porosus* categorized as *DD* (data deficient); 1.11% *Eusphyra blochii* categorized as NT (not Threatened); 4.44% *Chiloscyllium griseum* categorized as DD (data deficient); 5.56% *Rhizoprionodon oligolinx* categorized as LC (least concern); 1.11% *Prionace glauca* categorized as NT (not Threatened); 1.11% *Rhizoprionodon lalandii* categorized as DD (data deficient). In 2000, IUCN red list tested 17,000 spesies. In 2015, IUCN analyzed 79,800 spesies. For 2020, IUCN has targeted to analyze 160,000 spesies. Generally all species found in this research were special fish from Indo-Australian archipelago and included IUCN red list. The government policy to prohibit export of these species was the right decision to prevent the species extinction.

4. Conclusion

Species of sharks acquired from the export products in East Java and Bali were mostly sharks which have high risk of endangerment based on the IUCN red list.

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