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DNA-barcoding as molecular marker for seafood forensics: Species identification of locally consumed shark fish products in the world's largest shark fishery

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Abstract. Indonesia is the heart of the Coral Triangle—the global epicenter for marine biodiversity, and home to many endemic, threatened and protected species. There is a need for rapid, low-cost methods to better identify and tackle seafood fraud in Indonesia because Indonesia is also the world's largest shark fishing nation. Levels of domestic consumption of sharks and their relatives are thought to be significant, with different drivers of consumption across different products and geographies, including active and passive consumption, where passive consumers being unaware of the species origin of their seafoods. This study applies a COI DNA barcoding method to identify seafood products sold in local markets, with the aim of better understanding of the species composition about non-fin shark products in the domestic trade, and assessing the reliability of the COI method for seafood forensics. The results showed that all sampled seafood products were successfully identified to the species level, with an accuracy of 97-100%. Samples varied from different products including satay, salted meat, and meat curry, which are mostly sold in traditional markets. The magnitude, distribution and drivers of domestic consumption of shark product need to be understood in order to guide the design of future conservation.

Keywords: Aceh, COI, cooked food, local market, local consumption, shark

1. Introduction

Sharks and their relatives (Class Chondrichthes, herein 'sharks') are intrinsically vulnerable to overexploitation due to their conservative life history traits and ecological sensitivity to fishing pressure [1]. In recent decades, there has been rapid global expansion of exploitation and trade of sharks to meet growing demand for a range of consumer goods – from fins to meat to cartilage [2]. As a result, drastic population declines have been observed for several species [3, 4] and it is now estimated that one-in-four chondrichthyan species is threatened with extinction. This makes sharks among the most threatened vertebrate species group in the world [5].

In order to offer targeted and effective management and conservation of shark and ray stocks, there is a need to better understand their trade and utilisation patterns. To date, much of the research in the shark trade has focused on the market for fins, however it is increasingly acknowledged that the market for non-fin products, in particular shark meat, is significant and represents a major knowledge gap [6, 7].



In Indonesia, shark products are generally traded as partially processed (i.e. dissected and dried or frozen) body parts, often with high morphological similarities between different species and high variations of derivative products. At the consumer level in Indonesia, there are few species-specific preferences for shark products, limiting the market demand for verifiable, sustainably-sourced products. This limits the ability of law enforcement officers, traders and consumers to verify that shark products are from legal and sustainable sources, leading to seafood fraud by intentional species substitution as well as false labelling. In turn, this facilitates seafood fraud, with intentional species substitution and/or false labelling. Seafood mislabelling can compromise sustainable fisheries management and facilitate Illegal Unreported and Unregulated (IUU) fishing); threaten protected and endangered species; and threaten the health and well-being of consumers [8, 9].

Over the last decade, development in DNA barcoding have created an opportunity to standardize species identification of wildlife products. The COI gene is a standardized gene region, which allow for rapid and accurate species identification, making it suitable for routine application to wildlife forensics [10, 11]. DNA barcoding has also been used to determine the species of other processed high value species such as tuna [12]. Previous researches related to shark species identification have been based on multiplex polymerase chain reaction (PCR) assays as well as species specific primers [13-15]. DNA barcoding is used to successfully discriminate many regional shark species which are landed in Indonesia, Australia or Brazil [16-18].

In this study, we use COI DNA barcoding to conduct seafood forensics on a range of locally-sold shark products in Indonesia, in order to 1) Understand the species of origin of these products, and 2) Test the application of COI DNA barcoding as a reliable, rapid, low-cost technique for verifying seafood products in trade and identifying fraud. Such methods can help to ensure that Indonesian fishers and consumers get a fair value for their seafood products, and improve compliance with fisheries and wildlife laws.

2. Materials and Methods

We collected samples of locally-traded non-fin shark products from several sites in two case study provinces: Aceh and West Nusa Tenggara. These provinces were chosen as shark and ray fisheries in Aceh and West Nusa Tenggara are among the largest in Indonesia, contributing up to 25% of national production [19]. Sampled products were genetically tested to verify their species of origin using DNA barcoding with standardized COI gene region methods [10]. We also obtained qualitative and quantitative information on the nature and magnitude of local trade and consumption of non-fin products, which was conducted through field observation and semi-structured interviews with key informants.

2.1. Sample collection

We opportunistically collected samples of non-fin products from local markets in five cities across Aceh and West Nusa Tenggara. A total of 40 samples were collected, from meat (raw and cooked), cartilage, skin and fins (table 1). All samples were labelled and kept refrigerated before transportation to the laboratory and stored at -20°C.

2.2. DNA extraction, amplification and sequencing (modified from [11])

DNA extraction was performed on 100 mg of fish muscle using the commercial DNA isolation kit with a slight modification. The extracted DNA samples were stored at -20°C until the next step in the analysis. All PCR analyses in this study were performed with the following PCR protocol: 25 µL reaction volume containing 1-2 mg of DNA µL⁻¹, 1.0 µM of primers and the master mix kit according to the instruction given by the company. The DNA barcoding (COI) fragment would be 655 bp long and was amplified using universal fish barcoding primer pairs [20] as Fish F1/Fish-R1 or Fish-F2/Fish-R2. The cycler conditions consisted of 35 cycles of 1 minute each at 94°C, 1 min at 54°C and 1 min at 72°C, followed by a final extension of 10 min at 72°C. Furthermore, the DNA mini barcode fragments (<300-

bp) of the COI gene [21] were amplified to determine the optimal method to use for species identification of various fish products (cooked under high temperature, with high fat or oil content as well as cured).

Table 1. Shark and ray samples collected in this study.

Province	City	Type of product	Number of samples
Aceh	Aceh Barat Daya	Meat	2
		Skin	4
	Aceh Besar	Meat	2
		Meat	4
	Aceh Timur	Skin	2
West Nusa Tenggara	East Lombok	Cartilage	3
		Fins	3
		Meat	8
		Skin	5
	Mataram	Smoke meat (satay)	3
		Meat	2
	Skin	2	
Total			40

Following a PCR analysis, 5 μ L of the PCR products were visualized on 1% agarose gel, and the expected amplicons were compared with the standard marker of 100 bp DNA ladder before the bi-directional sequencing process was performed. The resulting sequences were compared based on quality parameters, success rates, and genetic identifications.

2.3. Sequences data analysis

The obtained sequences were aligned using ClustalW integrated in MEGA version 6 [22]. Moreover, all of the sequences were compared to sequences available in databases by BLAST analysis (GenBank/NCBI). Sequence similarity of at least 98% was used as the threshold to determine the potential species identification [23, 24]. The genetic distances among sequences were calculated using the Kimura 2-parameter model [25] and the construction of the phylogenetic tree was carried out in Mega 6.0 [22] with the Neighbor-joining (NJ) method [26] using 1000 bootstrap replications. These methods can be efficiently used to authenticate shark or ray species in processed seafood samples within 24 hours by researchers or authorities to avoid seafood species substitution.

2.4. Field observation and semi-structured interview

Data were gathered through direct observation. Semi-structured interviews with key informants and interview administered questionnaires. Questions were focused on understanding the nature and magnitude of local sharks and rays product. The key informants approached for data collection were collectors (engaged in buying and collecting shark and ray products directly from fishers) and sellers (sellers are people who sell shark and ray products directly to consumers). Collectors or middlemen could be engaged in processing or selling to other processing parties while sellers could be trading locally or internationally. All respondents were required to be persons involved in the industry for more than five years, in order to get accurate information about patterns of change and trends in shark and ray fishing, trade and consumption patterns. A total of 48 key informants were interviewed, including, 17 collectors and 31 sellers from Aceh and West Nusa Tenggara.

3. Results and Discussion

3.1. *Species identification*

A total of 40 samples of sharks and rays from 5 cities in the two provinces of Aceh and West Nusa Tenggara were successfully collected, and their DNAs were isolated and amplified using DNA barcoding marker. Samples were various shark and ray derivative products that were sold in local traditional markets. For all 40 samples in this study, we attempted to amplify and sequence with 655 bp of COI gene and 295 bp of universal mini barcode marker. In the beginning, we applied 655 bp primers (F1/R1 and F2/R2) to all of the samples, however, some samples which have been processed at very high temperature or dried could not produce any amplicons. Therefore, for those samples, we applied universal DNA mini barcode primer which has been published previously [21] (table 2).

Some samples were not brought into the sequencing step due to DNA degradation during purification. Fourteen (14) samples were successfully brought into the sequencing process and have been successfully identified into species levels. Samples that were not succeeded into sequencing process came from dried skin, meat, satay, and bones.

Eight shark and ray species have been identified, namely *Rhynchobatus australiae*, *Isurus oxyrinchus*, *Alopias pelagicus*, *Rhynchobatus cf. laevis*, *Carcharhinus falciformis*, *Sphyrna lewini*, *Carcharhinus sorrah* and *Galeocerdo cuvier*. Meat sample from Aceh Timur (BL2) has been successfully identified as *A. pelagicus* species of shark. *I. oxyrinchus* was identified from shark skins from Aceh Barat Daya (C2) and Mataram (A1). Four samples of skin from East Lombok (GI2), meat from Aceh Timur (GD2), meat from mataram (SB2) and skin from East Lombok (KT1) have been identified as *Rhynchobatus*. There were 4 samples belonging to the species *C. falciformis* (J2, G1, F1, G3). The traded shark products varied from meat/carcass to skin (table 3).

The average of similarity percentage from all samples was 99% of the similarity of DNA sequence to the DNA sequence in the database with some samples having a perfect match or 100% similarity. Based on this study results, DNA barcoding has been successfully applied in authentication process of various shark products with some of them containing highly degraded genomic DNA or without morphological traits. A phylogenetic tree has been made using Kimura 2 Parameters (K2P) and neighbor joining (NJ) tree model (figure 1) with correlation into identification results from table 3. All 7 samples clustered into their group with other of their top BLAST related sequences. These eight species – specific clades spanned three order of shark: the Carcharhiniformes, Lamniformes, and Rhinopristiformes.

3.2. *Local consumption*

Utilisation of sharks and rays in Aceh and West Nusa Tenggara has been passed on for generations, with sharks caught by fishers and consumed locally as food. Shark and ray fishing in Indonesia began to increase drastically in the 1950s following the demands for shark fins as a high-value export commodity to countries such as China (including Hong Kong) and Taiwan. The high price of shark and ray fins drove fishers to increase their sharks fishing effort, and make sharks and rays their target species.

After being caught by fishers, sharks are purchased by collectors and sold to local sellers or traded to other locations in Indonesia. All shark and ray parts including fins, meat, skin, cartilage, liver oil and teeth are used and processed into various derivative products, each of which have their own specific buyers. After being dissected and skinned, shark and ray products are grouped by body parts. The meat is distributed and marketed in different villages/ areas to be processed further (table 4).

Table 2. Summary of work results on shark and ray samples.

No	Sample code	DNA isolation	PCR/Amplification	Sequence process	Sequence result
1	A1	√	√	√	√
2	A2	√	√	√	X
3	B1	√	√	X	X
4	B2	√	√	X	X
5	C1	√	√	X	X
6	C2	√	√	√	√
7	D1	√	√	X	X
8	D2	√	√	X	X
9	DK	√	√	X	X
10	DL	√	√	X	X
11	KT1	√	√	√	√
12	KT2	√	√	X	X
13	GI1	√	√	X	X
14	GI2	√	√	√	√
15	GB1	√	√	√	X
16	GB2	√	√	√	X
17	BL1	√	√	X	X
18	BL2	√	√	√	√
19	SR1	√	√	X	X
20	SR2	√	√	√	X
21	SB1	√	√	X	X
22	SB2	√	√	√	√
23	GD1	√	√	X	X
24	GD2	√	√	√	√
25	E1	√	√	X	X
26	E2	√	√	X	X
27	E3	√	√	√	√
28	Fa	√	√	√	√
29	Fb	√	√	X	X
30	F1	√	√	√	√
31	G1	√	√	√	√
32	G3	√	√	√	X
33	H1	√	√	√	√
34	H3	√	√	√	√
35	I1	√	√	X	X
36	I2	√	√	√	X
37	J1	√	√	X	X
38	J2	√	√	√	√
39	MY1	√	√	X	X
40	MY2	√	√	X	X

Notes: √ = succeed, X = failed.

Table 3. Identification results based on BLAST-n NCBI.

No	Sample name	Type of product	Species	Query coverage (%)	Homology (%)	E value	Accession number
1	GI2	Meat	<i>Rhynchobatus australiae</i>	100	99	0	JN022595 .1
2	GD2	Meat	<i>Rhynchobatus australiae</i>	100	99	0	JN022595 .1
3	C2	Skin	<i>Isurus oxyrinchus</i>	100	100	0	KP19333 1.1
4	BL2	Meat	<i>Alopias pelagicus</i>	100	100	0	KF02087 6.1
5	SB2	Fins	<i>Rhynchobatus cf.laevis</i>	84	95	1,00E-151	EU39901 0.1
6	A1	Skin	<i>Isurus oxyrinchus</i>	100	99	0	KP19333 1.1
7	KT1	Skin	<i>Rhynchobatus australiae</i>	100	99	0	MF50869 6.1
8	J2	Meat	<i>Carcharhinus falciformis</i>	100	100	2,00E-103	MG83790 9.1
9	G1	Skin	<i>Carcharhinus falciformis</i>	100	100	2,00E-104	MG83790 9.1
10	H3	Fins	<i>Sphyrna lewini</i>	100	100	0	KP17730 6.1
11	F1	Meat	<i>Carcharhinus falciformis</i>	100	100	2,00E-103	MG83790 9.1
12	E3	Meat	<i>Carcharhinus falciformis</i>	98	99	2,00E-109	MG83790 9.1
13	FA	Meat	<i>Carcharhinus sorrah</i>	100	100	2,00E+103	MF13519 1.1
14	H1	Fins	<i>Galeocerdo cuvier</i>	100	99	0	MG83793 3.1

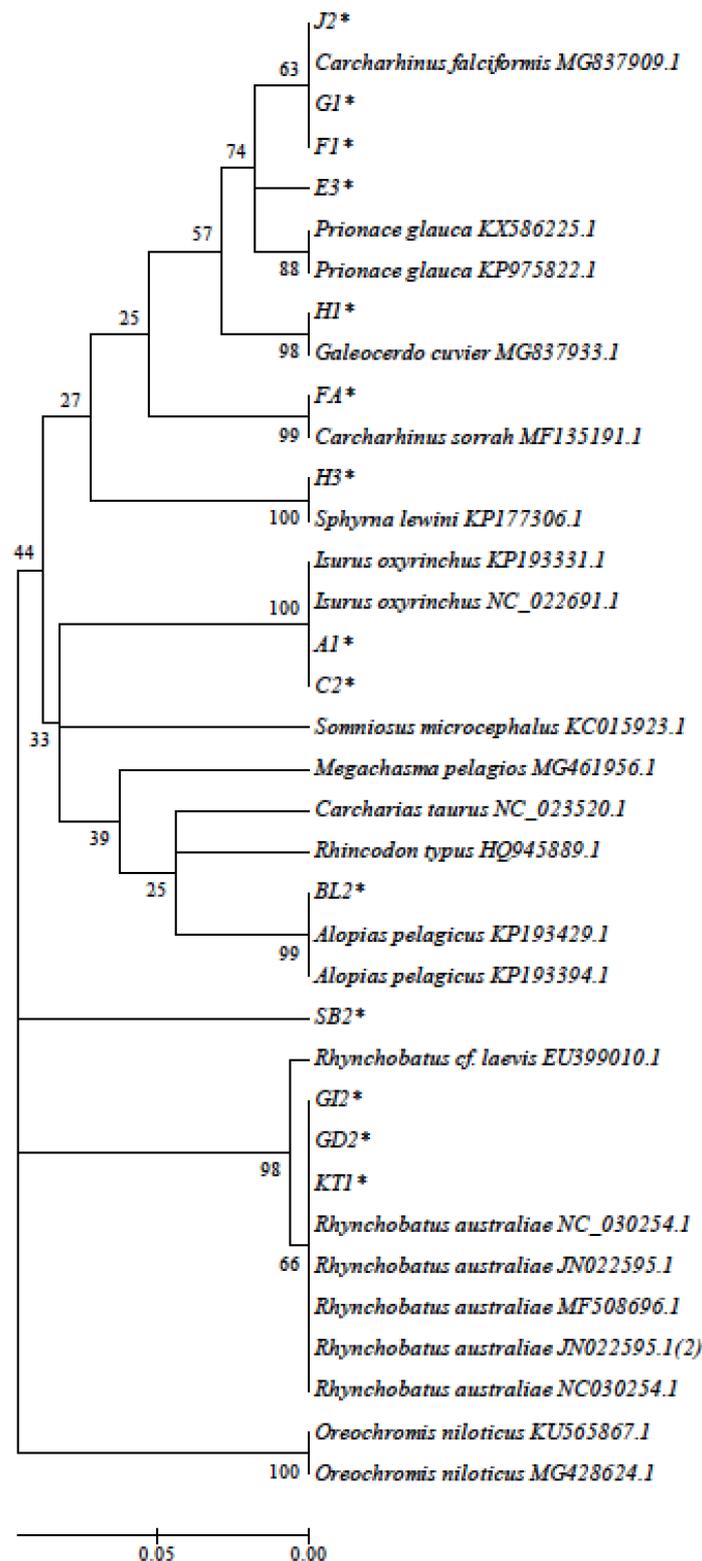


Figure 1. Phylogenetic tree, measuring of genetic relationship between samples and database results (Kimura 2P/NJ).

Table 4. Types and utilization of non-fin commodities in Aceh and West Nusa Tenggara.

Product	Utilization	Primary market(s)
Meat	Local food	Domestic market
Skin	Food (cracker)	Domestic market
	Fashion material (Wallet, belt)	National market (medan, Surabaya) and international export
Liver oil	Medical supplements	International export
Cartilage	Medical supplements	International export
	Cosmetics	
Gill plates	Medical supplements	International export
Teeth	Souvenirs	Bali
Offal	Livestock feed	Java

The use of the COI DNA barcode allowed us to identify all sharks product at the specific level and offers a reliable and efficient tool for identification of derivative shark product which unknown origin. It is noteworthy that the success of DNA barcoding is related to many factors, such as high quality of DNA and appropriate barcodes. For processed derivative sharks product with degraded DNA, mini barcodes usually exhibit a higher success rate in species identification as compared to full-length barcodes [27].

We found eight species of Elasmobranchii among the samples acquired in West Nusa Tenggara and Aceh. Unfortunately, our DNA result does not represent elasmobranch species caught in West Nusa Tenggara and Aceh and is estimated to represent less than 10% of species caught in West Nusa Tenggara and Aceh. Other studies on shark fisheries in Tanjung Luar, East Lombok, at least 42 of shark species have been landed in Tanjung Luar from January 2014 – December 2015 [28], meanwhile 20 species of sharks were landed in Banda Aceh and Sibolga [29].

Table 5. Conservation status of the species found in this study.

No	Common name	Species	IUCN Status	CITES Appendix
1	Whitespotted wedgefish	<i>Rhynchobatus australiae</i>	Vulnerable	Not
2	Shortfin mako	<i>Isurus oxyrinchus</i>	vulnerable	Not
3	Pelagic thresher	<i>Alopias pelagicus</i>	Vulnerable	II
4	Smoothnose wedgefish	<i>Rhynchobatus laevis</i>	Vulnerable	not
5	Silky shark	<i>Carcharhinus falciformis</i>	Vulnerable	II
6	Scalloped hammerhead	<i>Sphyrna lewini</i>	Endangered	II
7	Spottail shark	<i>Carcharhinus sorrah</i>	Near threatened	Not
8	Tiger shark	<i>Galeocerdo cuvier</i>	Near threatened	Not

C. falciformis, *S. lewini*, *G. cuvier*, *A. pelagicus* are the most common sharks landed in West Nusa Tenggara and Aceh. Those species are caught by surface and bottom longline with fishing grounds commonly in Indian Ocean water. Of those, 6 species found in sharks product in this study are threatened with extinction according to the IUCN Red List of Threatened Species (i.e. Vulnerable, Endangered), many of which continue to be landed in Aceh and West Nusa Tenggara and some of them consumed locally in various shark and ray products (table 5).

Socio-economically, the dependence level of the people in Aceh and West Nusa Tenggara on sharks and rays is very high. Sharks and rays that have been processed are consumed with rice by coastal communities as protein sources. It is a habit that has been passed down from generation to generation until now. Aside from being a cheap, easy and delicious source of food, people believe that consuming shark and ray meats can have positive impacts on their strength and health.

Levels of domestic consumption of shark and ray products in Aceh and West Nusa Tenggara are likely to be significant, but the drivers of consumption vary across different products, geographies and

demographics, with the largest domestic markets focused around meat. There is no specific species preferences of local consumers to consume shark products. Controlling the local demand and trade needs to be considered to prevent further exploitation of vulnerable species such as hammerhead shark (*S. lewini*). Besides, it should be supported by fisheries regulations to limit fishing pressure.

Finally, an important issue in the conservation of these species is how people should engage in more sustainable consumption practices. In this term, clear labelling of shark and ray products that are traded will impose a major boost in increasing the awareness and conservation efforts. Many peoples who were interviewed claimed that they have tried to eat sharks product, but they did not know that those products were of threatened species. It shows that the majority of the population in that province who buys shark and ray products is not aware of the impact of their consumption habits. Another issue found in this study was that all shark and ray products did not use a prior label that describes the source of the product, even though all consumers must know that the products they consume are from sharks.

4. Conclusion

Local consumption of shark and ray products in Aceh and West Nusa Tenggara are likely to be significant, especially meat. The species that were processed into shark meat product vary from non-vulnerable to vulnerable species. Hammerhead sharks (*S. lewini*), silky sharks (*C. falciformis*), and thresher sharks (*A. pelagicus*) are categorized as vulnerable or endangered species based on IUCN, and categorized as commonly caught and consumed in Aceh. There is no preference in specific shark or ray species consumed in those areas. Controlling local demands and trades through clear labeling needs to be considered to prevent more exploitations.

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