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Short Communication: Genetic diversity of scalloped hammerhead sharks (*Sphyrna lewini*) landed in Muncar Fishing Port, Banyuwangi

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Abstract. *Alghozali FA, Wijayanti DP, Sabdono A. 2019. Short Communication: Genetic diversity of scalloped hammerhead sharks (Sphyrna lewini) landed in Muncar Fishing Port, Banyuwangi. Biodiversitas 20: 1154-1159.* The majority of sharks caught in Indonesian fisheries were bycatch products from the tuna longline fisheries, but some regions in Indonesia fish the sharks as their main target. One of these regions is located in Muncar, Banyuwangi, which fishes the endangered Scalloped Hammerhead sharks (*Sphyrna lewini*) as target species. This research aimed to study the genetic diversity of the endangered Scalloped Hammerhead sharks landed in Muncar Fishing Port, Banyuwangi. Genetic analysis was done through PCR (Polymerase Chain Reaction) amplification and sequencing of the mitochondrial DNA COI (Cytochrome Oxidase subunit I) gene. Out of the 37 samples collected, 30 were successfully amplified and sequenced. The results showed moderate haplotype diversity ($Hd: 0,582 \pm 0,079$) and low nucleotide diversity ($\pi: 0,00392 \pm 0,0024$) with five haplotypes (h) and 26 polymorphic sites (S). Tajima's D neutrality model values indicated a population expansion event. Two different clades were determined through phylogenetic analysis and by GenBank sequences comparison. These results provided basic information and present status of the Scalloped Hammerhead sharks population genetically within the fishing ground (Makassar Strait-Kangean Islands).

Keywords: Genetic diversity, COI, scalloped hammerhead shark, *Sphyrna lewini*, Banyuwangi

INTRODUCTION

The Scalloped Hammerhead shark (*Sphyrna lewini*) is one of the three existing species of hammerhead sharks in Indonesia (White et al. 2006). The scalloped hammerhead is easily distinguished from other hammerhead shark species for having a distinctive indentation in the center of their head's front margin (Sadili et al. 2015). This species is circumglobal and widely distributed through all tropical and temperate waters all over the world. Although widely distributed, a genetic study revealed multiple subpopulations of this species (Duncan et al. 2006). The scalloped hammerhead shark can be found throughout the Indonesian waters and is the most abundant compared to the other hammerhead shark species (Harlyan et al. 2015). Adult scalloped hammerhead sharks can be found from the surface to a depth of 275 m of the continental and insular shelves (White et al. 2006).

There was an indication that the pups were abundant in their nursery ground and commonly found near the coastal area (Clarke 1971; Bejarano-Alvarez et al. 2010). This nursery ground at different times also serves as mating and pupping ground for adult sharks (Hazin et al. 2001). An adult female can produce between 14-41 pups in one reproduction cycle (Stevens and Lyle 1989; Hazin et al. 2001; Duncan et al. 2006; Bejarano-Alvarez et al. 2010). Adult males usually mature after reaching a length of 165-175 cm, and adult females mature at a longer length of 220-230 cm. While they mature at different body lengths, both

can reach a maximum length of 420 cm (White et al. 2006).

Like any other large shark species, the scalloped hammerhead shark fins are highly valued in the shark fins market. In the world's largest shark fin trading center in Hong Kong, their fins represent 4-5% of all the fins auctioned (Clarke et al. 2006). Genetic research in Indonesia claimed that the scalloped hammerhead shark fins are the second most traded fins in the traditional markets and shark-fin exporters (Sembiring et al. 2015). The large numbers of the scalloped hammerhead shark fins in the market were due to their habit to aggregate in large schools, which make them vulnerable to be caught as target and bycatch from trawls, purse seines, gillnets, longlines and inshore artisanal fisheries (Baum et al. 2007). The scalloped hammerhead shark had been listed as Vulnerable in the IUCN (International Union for Conservation of Nature) and the CITES (Convention on International Trade in Endangered Species) Appendix II (Baum et al. 2007). The Ministry of Maritime Affairs and Fisheries of Indonesia had banned the exports of all product forms derived from all hammerhead sharks species. The scalloped hammerhead is one of the most highly exploited shark species in Indonesia and is highly pressured by the capture fisheries industries, either as bycatch or target species (Fahmi and Dharmadi 2013; Gautama et al. 2018).

Muncar Fishing Port is located in Banyuwangi, East Java, and is one of the many fishing ports in Indonesia with the highest shark landing (Simeon et al. 2015). One of the most common shark species landed in Muncar Fishing Port

is the scalloped hammerhead shark, *Sphyrna lewini* (Harlyan et al. 2015). Sharks late maturity, long gestation period and slow growth rate, combined with the high pressure from the capture fisheries industries may result in the decrease of their population (Dulvy et al. 2014). Low population size will then results in low genetic diversity (Bazin et al. 2006). This will render the species within a particular population to be vulnerable to diseases, parasites, predators and environmental changes (Amos and Hardwood 1998; Reed and Frankham 2003). Genetic studies provide important information, therefore it is used as tools to help solve problems in species management and conservation (Engelhardt et al. 2014; Larson et al. 2017). One of many benefits from genetic information is that scientists, NGOs, and governments will be able to tell how threatened and endangered the species is in the wild, and how urgent it is to take action towards the species' conservation. This research aims to study the genetic diversity of the endangered scalloped hammerhead sharks (*S. lewini*) landed in Muncar Fishing Port, Banyuwangi.

MATERIALS AND METHODS

Study site

The study site was located in Muncar Fishing Port, Banyuwangi District, East Java Province, Indonesia. The exact location was the Muncar Fishing Port situated on the eastern side of the province (Figure 1).

Sample collection

Muscle tissue samples of 37 sharks were collected from Muncar Fishing Port, Banyuwangi. Shark species was

visually identified following White et al. (2006) before sampling to avoid misidentification (Figure 2). Samples were taken using a knife and tweezers which had been sterilized with 96% ethanol and rinsed with sterilized sea water to prevent contamination. Samples were then preserved in vial tubes containing 96% ethanol and stored at room temperature (Abercrombie et al. 2005). Fishing ground coordinates and locations were collected through interview with the fishermen to assume the origin of the samples. Only one fisherman was interviewed due to the shared fishing ground locations of all the shark fishing vessels in Muncar, and they are all under the same fisheries management.



Figure 2: *Sphyrna lewini* landed in Muncar Fishing Port, Banyuwangi, East Java, Indonesia



Figure 1: Location of study and sampling site in Muncar Fishing Port, Banyuwangi, East Java, Indonesia (8°26'30.73"S, 114°20'41.24"E)

DNA extraction, PCR amplification, and DNA barcoding

Genomic DNA was extracted from samples using a modified Chelex 100 method (Walsh et al. 1991; Galal-Khallaq et al. 2014). Approximately 1 mg muscle tissue sample was placed in an Eppendorf tube containing 500 μ L 10% Chelex solution combined with 7 μ L proteinase K (10 mg/mL). Samples were then incubated in a heating block at 55°C for 90 minutes to release DNA, followed by 100 °C for 20 minutes to deactivate the proteinase K. Aliquots of DNA were moved into a new tube and stored at 4 °C for further analysis.

Mitochondrial DNA COI (Cytochrome Oxidase subunit I) partial gene fragments were amplified using PCR (Polymerase Chain Reaction) method. A 26 μ L reaction mixture containing 1 μ L of DNA template, 12.5 μ L of KAPA Taq PCR kit (25 μ M MgCl₂, 5 U/ μ L Taq Polymerase, 10x Taq Buffer, and 10 μ M dNTPs), 1.25 μ L 10 mM of forward primer, 1.25 μ L 10 mM of reverse primer and 10 μ L of distilled water. The primers used were Fish BCL: 5'-TCA ACY AAT CAY AAA GAT ATY GGC AC-3' (forward) and Fish BCH: 5'-ACT TCY GGG TGR CCR AAR AAT CA-3' (reverse) (Baldwin et al. 2009). The mixture was run in a thermal cycler using the following PCR cycle: 95°C initial denaturation for 5 minutes; followed by 38 cycles of denaturation at 94 °C for 30 seconds, primer annealing at 48 °C for 30 seconds and extension at 72 °C for 45 seconds; and a final extension step at 72 °C for 5 minutes. PCR products from the samples were then visualized for DNA band via electrophoresis on a 1% agarose gel and EtBr (ethidium bromide) staining (Sembiring et al. 2015). PCR products were then sent to Genetika Science Indonesia to be purified and sequenced.

Table 1. *Sphyrna lewini* DNA sequences and sequences from NCBI GenBank

Location	Species	Accession Code
Hawaii	<i>Sphyrna lewini</i>	MG816735
Papua New Guinea	<i>Sphyrna lewini</i>	MF508692
California	<i>Sphyrna lewini</i>	GU440527
Philippine	<i>Sphyrna lewini</i>	KF009669
Australia	<i>Sphyrna lewini</i>	KU366619
Indonesia	<i>Sphyrna lewini</i>	KF793757
Indonesia 2	<i>Sphyrna lewini</i>	KF793742
Madagascar	<i>Sphyrna lewini</i>	HQ171776
Myanmar	<i>Sphyrna lewini</i>	MH235723
Saudi Arabia	<i>Sphyrna lewini</i>	KM396950
Brazil	<i>Sphyrna lewini</i>	JQ365585
Mexico	<i>Sphyrna lewini</i>	MG838000
H1 Sample	<i>Sphyrna lewini</i>	LC422406
(S: 1, 5, 7, 17, 19, 27, 29, 30)		
H2 Sample	<i>Sphyrna lewini</i>	LC422407
(S: 2-4, 9-12, 14-16, 18, 21-23, 25-26, 28, 31)		
H3 Sample (S: 6, 8)	<i>Sphyrna lewini</i>	LC422408
H4 Sample (S20)	<i>Sphyrna lewini</i>	LC422409
H5 Sample (S24)	<i>Sphyrna lewini</i>	LC422410

Data analysis

Forward and reverse sequences of each sample was checked, aligned and edited using MEGA 5.2 (Tamura et al. 2007). Species identification from the sequences was made before data analyses using BLAST (Basic Local Alignment Search Tool) by matching them with the sequences from NCBI (National Center for Biotechnology Information) GenBank. Phylogenetic tree from the sequences was made using the Maximum Likelihood method, Kimura-2-parameter model and 1000 bootstrap replicate. Several sequences from NCBI GenBank were included in the phylogenetic tree for comparison (Table 1). Genetic distances between each sequence were analyzed using Pairwise Distance method and Kimura-2-parameter (Kimura 1980; Tamura et al. 2011). Genetic diversity was examined by determining the numbers of segregating sites (S), haplotypes number (h), haplotype diversity (Hd) and nucleotide diversity (π). Tajima's D and Fu's FS were calculated for neutrality test (Librado and Rozas 2009). Fishing ground coordinates were processed and made into a fishing ground map using ArcMap 10.3.

RESULTS AND DISCUSSION

DNA extraction, PCR amplification, and DNA barcoding

A total of 30 out of 37 samples were successfully amplified and sequenced. The final edited sequences had length of 574 bp. All sequences were identified as *Sphyrna lewini* with Identify values of 99-100% and were submitted to the DDBJ (DNA Data Bank of Japan) under the accession number of LC422406-LC422410 (Table 2).

Phylogenetic tree

Two clades were determined from the constructed phylogenetic tree. Clade 1 consisted of all but one sample, S24 Fish BCL which forms clade 2 (Figure 3). The S24 Fish BCL sample also had the highest genetic distance values against other samples with 0.041 and 0.043 respectively.

Genetic diversity and neutrality test

Data analysis results showed five different haplotype (h) and 26 polymorphic sites (S) (Table 3). Haplotype (Hd) and nucleotide diversity (π) of all 30 samples were 0.582 ± 0.079 and 0.00392 ± 0.0024 respectively. Tajima's D neutrality test resulted in a significant negative value of -2.346 ($P < 0.05$) while Fu's FS neutrality test resulted in a not significant positive value of 1.721 ($P > 0.05$) (Table 4).

Discussion

The results of BLAST analysis showed that all samples were identified as *Sphyrna lewini* with Identify values of 99-100%. Two clades were determined by looking at the bootstrap values and the genetic distances between samples. S24 Fish BCL sample which forms clade 2 exhibit a strong branch position in the phylogenetic tree with a bootstrap value of 100%. Hillis and Bull (1993) and Nei

and Kumar (2000) mentioned that a bootstrap value higher than 95% is considered good and provide strong support in a phylogenetic tree. Felsenstein (1985) claimed that bootstrap value is only seen as repeatability and not as an accuracy. Therefore, while clade 2 exhibits strong support in the tree, clade 1 cannot be considered inaccurate. At the same time, the low genetic distances between samples indicate that clade 1 and 2 are closely related (Nei 1972).

The phylogenetic tree showed that *Sphyrna lewini* from clade 2 shares genetic relations with *S. lewini* from Madagascar, Myanmar, and Saudi Arabia, while *S. lewini* from clade 1 share genetic relations with *S. lewini* from Hawaii, Papua New Guinea, California, Philippine and Australia. The high number of pups (14-41) per litter produced by a scalloped hammerhead shark per reproduction cycle and the high connectivity of coastal nursery grounds can possibly be the reason as to why genetics from different clades can be found in Indonesia (Bejarano-Alvarez et al. 2010; Duncan et al. 2006; Hazin et al. 2001; Stevens and Lyle 1989). The scalloped hammerhead sharks ability to cover a distance of about 627 km might also be the reason they can be found in countries with tropical climate (Baum et al. 2007; Bessudo et al. 2011a, b).

Genetic diversity values exhibit moderate haplotype and low nucleotide diversity (Hobbs et al., 2013; Nei, 1987). Generally, migrational species will have high genetic diversity, but either natural or fisheries based high mortality rate in *Sphyrna lewini* can decrease their genetic diversity (Bessudo et al. 2011b; Fetzner Jr and Crandall 2001). Tajima's D neutrality test indicates a population expansion event. This also leads to the possibilities of a population bottleneck event before the population expansion, which was characterized by the appearance of more abundant haplotypes with fewer samples (Schmidt and Pool 2002). However, further researches are needed to verify this hypothesis. The *S. lewini* populations in Indonesia is threatened due to the high exploitation rate and fisheries pressure (Gautama et al. 2018). The capture of immature adults and juvenile can also lead to the reduction of their population size from the decrease in population recruitment (Diekert and Rouyer 2011). This will

eventually lower the genetic diversity of *S. lewini* in Indonesia (Fetzner and Crandall 2001).

Table 2. BLAST Results

Sample code	BLAST Result	Identify (%)	Accession Code
S1 Fish BCL	<i>Sphyrna lewini</i>	100	MF508691
S2 Fish BCL	<i>Sphyrna lewini</i>	100	MF508688
S3 Fish BCL	<i>Sphyrna lewini</i>	100	MF508688
S4 Fish BCL	<i>Sphyrna lewini</i>	100	MF508688
S5 Fish BCL	<i>Sphyrna lewini</i>	100	MF508691
S6 Fish BCL	<i>Sphyrna lewini</i>	100	MF508690
S7 Fish BCL	<i>Sphyrna lewini</i>	100	MF508691
S8 Fish BCL	<i>Sphyrna lewini</i>	100	MF508690
S9 Fish BCL	<i>Sphyrna lewini</i>	100	MF508688
S10 Fish BCL	<i>Sphyrna lewini</i>	100	MF508688
S11 Fish BCL	<i>Sphyrna lewini</i>	100	MF508688
S12 Fish BCL	<i>Sphyrna lewini</i>	100	MF508688
S14 Fish BCL	<i>Sphyrna lewini</i>	100	MF508688
S15 Fish BCL	<i>Sphyrna lewini</i>	100	MF508688
S16 Fish BCL	<i>Sphyrna lewini</i>	100	MF508688
S17 Fish BCL	<i>Sphyrna lewini</i>	100	MF508691
S18 Fish BCL	<i>Sphyrna lewini</i>	100	MF508688
S19 Fish BCL	<i>Sphyrna lewini</i>	100	MF508691
S20 Fish BCL	<i>Sphyrna lewini</i>	99	MF508688
S21 Fish BCL	<i>Sphyrna lewini</i>	100	MF508688
S22 Fish BCL	<i>Sphyrna lewini</i>	100	MF508688
S23 Fish BCL	<i>Sphyrna lewini</i>	100	MF508688
S24 Fish BCL	<i>Sphyrna lewini</i>	100	KP177306
S25 Fish BCL	<i>Sphyrna lewini</i>	100	MF508688
S26 Fish BCL	<i>Sphyrna lewini</i>	100	MF508688
S27 Fish BCL	<i>Sphyrna lewini</i>	100	MF508688
S28 Fish BCH	<i>Sphyrna lewini</i>	100	MF508691
S29 Fish BCL	<i>Sphyrna lewini</i>	100	MF508688
S30 Fish BCL	<i>Sphyrna lewini</i>	100	MF508691
S31 Fish BCL	<i>Sphyrna lewini</i>	100	MF508691

Table 4. Genetic diversity indices and neutrality test values

n	S	h	Hd ± Sd	π ± Sd	D*	FS**
30	26	5	0.582 ± 0.079	0.00392 ± 0.0024	-2.346	1.721

Note: *P<0.05, **P>0.05

Table 3. Haplotype and number of polymorphic sites

Haplotype	Polymorphic Sites																										
	7	3	7	1	1	1	1	1	1	2	2	2	3	3	3	3	3	3	4	4	4	4	4	4	5	5	5
H1	C	A	T	G	A	T	C	T	T	C	C	T	C	C	T	T	C	T	C	C	T	C	A	T	C	C	
H2	T
H3	.	.	C	T	T
H4	T	T
H5	T	C	.	A	C	G	T	C	C	T	T	C	T	T	C	C	A	C	T	.	C	T	C	C	T	T	

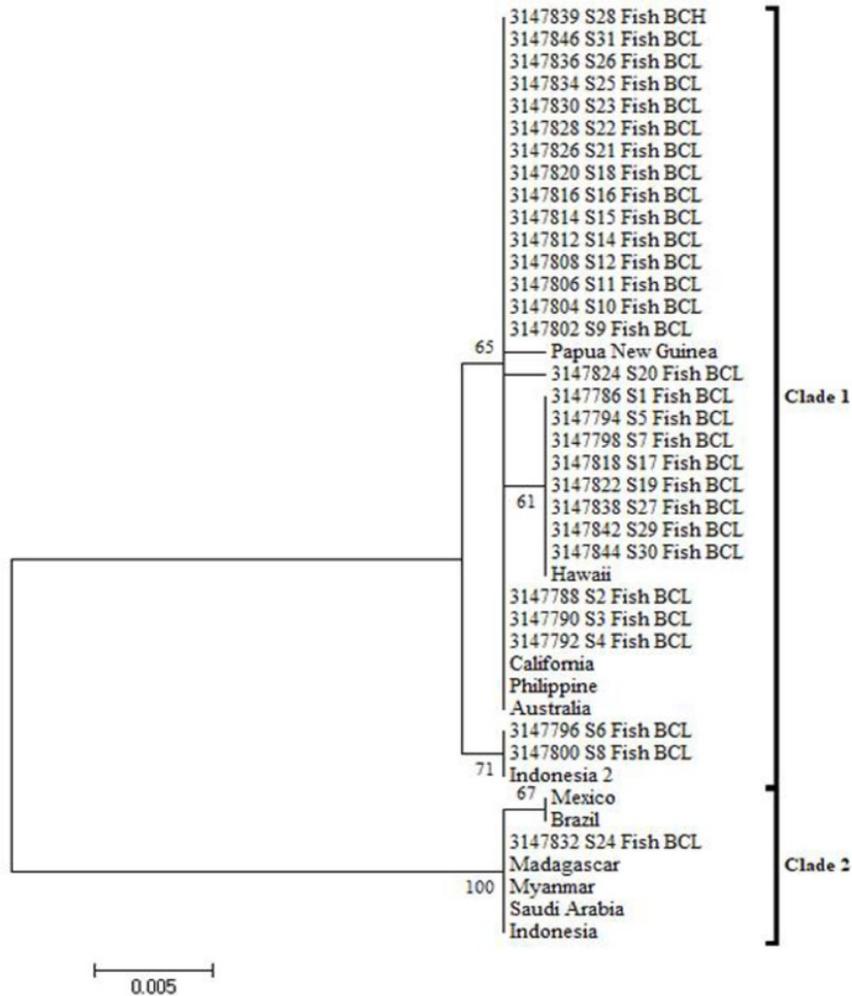


Figure 3. Phylogenetic tree of *Sphyrna lewini* landed in Muncar Fishing Port, Banyuwangi, East Java, Indonesia

The genetic diversity of *Sphyrna lewini* landed in Muncar can be considered low moderate from the analyzed indices. The possibility of a bottleneck event before the population expansion of *Sphyrna lewini* from the fishing grounds (Makassar Strait-Kangean Island) can no longer be ignored. Further researches with a larger area of sampling to obtain more robust data may provide more information on the genetic diversity of *Sphyrna lewini*. Therefore, the genetic diversity of this species could be fully understood, as well as the species population condition in Indonesia. Temporal and spatial researches of their migration, mating and pupping season are also crucial to implement effective actions in the field of this species conservation.

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