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Genetic diversity of the endangered species *Sphyrna lewini* (Griffith and Smith 1834) in Lombok based on mitochondrial DNA

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Abstract. *Sphyrna lewini* is a shark with a wide distribution and one of the most endangered species. Although The International Union for Conservation of Nature (IUCN) classified this species as endangered and the species was already protected by law in Indonesia, *S. lewini* are currently high exploited because of their high economic value. This study conducted to analyze the genetic diversity and phylogenetic reconstruction the *S. lewini* landed in Lombok (n = 30) using a molecular approach based on the mitochondrial DNA. DNA amplification with the CO1 gene successfully identified 30 individuals of *S. lewini* with an average 625-bp nucleotide length. The value of haplotype diversity in Lombok (Hd) = 0.40 and nu π = 0.0018. The results of phylogenetic analysis using Neighbor-Joining (NJ) by Kimura 2-parameter model with 1000 bootstrap showed the connectivity of *S. lewini* landed in Lombok in 1 clade of 5 haplotypes. This study showed that low genetic diversity and significant differences in genetic structure between populations made this species vulnerable to extinction in Indonesia and required special treatment.

1. Introduction

The scalloped hammerhead shark *Sphyrna lewini* is circumglobally distributed in tropical waters [1]. *S. lewini* has low resilience to exploitation, due to its late sexual maturity (males mature at 10 years and females at 15), and long generation times (around 30 years) [2]. Adult sharks from both sexes are extremely mobile and are often found in the open ocean [3,4]. *S. lewini* is one of the threatened species of sharks. The status is endangered species under International Union for Conservation of Nature (IUCN) and is categorized in Appendix II of The Convention on International Trade in Endangered Species (CITES) with trade volume restrictions. In Indonesia, the status of *S. lewini* has been protected through Decree of the Minister of Marine Affairs and Fisheries of the Republic of Indonesia Number 5/PERMEN-KP/2018 on the prohibition of cowboy shark and hammerhead shark export from Indonesia.

Sharks are an important economic commodity in the fisheries sector. The presence of shark populations is increasingly threatened along with fishing and trading activities. Dent and Clarke [5] on the state of the global market for shark product reported that the volume of shark and ray caught in Indonesia in the period of 2000-2011 is the highest in the world reaching an average of 106.034 tons per year. While in the same period for shark fin trade, Indonesia at the third after China and Thailand,

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an average of 6.594 tons per year. Sharks decline in numerous shark populations around the world have generated considerable interest in better understanding and characterizing their biology, ecology, and critical habitats [6]. Many research has conducted to reveal the declining population of sharks with several approaches including genetic analysis.

Genetic diversity became one of the most concern of population studies over recent decades relation to species conservation. Genetic diversity can identify population healthy, resilience rate, and adaptation with environmental shifting [7]. Genetics plays an important role to conservation because of its function as tracking and maintaining population from damage [8,9]. The genetic method with a mitochondrial marker for all animal species, for one chain of the gene, is claimed to be enough for distinguishing one species to another [10]. It has been applying in several studies in sharks in Indonesia [11,12]. The previous study reported that the mitochondrial DNA (mtDNA) cytochrome oxidase 1 (COI) barcode region is often the marker of choice when looking to identify and evaluate the genetic diversity of species [11,12,13].

Several studies related to mtDNA cytochrome oxidase 1 (COI) of the *S. lewini* population also have been reported [14-16]. However, there have been limited the study of *S. lewini* in Indonesia. Therefore, the aim of this study was to analyze the genetic diversity and phylogenetic reconstruction of *S. lewini* in Lombok (n = 30) using a molecular approach based on the mtDNA.

2. Material and methods

2.1. Sample collection

Tissue samples were collected from *S. lewini* landed in the fishing port in Tanjung Luar, Lombok (n = 30). The data were collected according to guidelines of the Indonesian performance test. The tissue sample was stored in 96% ethanol until used for extraction of DNA.

2.2. Isolation of DNA, amplification, and sequencing

Total DNA was isolated using gSYNC DNA extraction kit [17]. A fragment of mitochondrial Cytochrome Oxidase subunit-I gene (COI) was amplified using the PCR technique (Polymerase Chain Reaction). Gene-specific primers for COI were amplified with the *forward* fish-BCL (5' TCA ACY AAT CAY AAA GAT ATY GGC AC '3) and *reverse* fish-BCH (5' TAA ACT TCA GGG TGA CCA AAA AAT CA '3) [11,18]. PCR was performed in conditions of 3 µL template DNA, 9 µL ddH2O, 1.25 µL forward and reverse primer and 12.5 µL *MyTaq HS Red Mix*. The PCR technique was conducted with the following program: 94 °C for 30 s (denaturation); 50 °C for 30 s (annealing); 72 °C for 45 s (extension); and 72 °C for 45 sec (final extension) on the thermo cycler with the 38 total cycles [11]. PCR products were separated by electrophoresis using 1.5 % agarose gels stained with ethidium bromide alongside a 100 base pairs (bp) molecular weight marker and visualized under UV light. Sequencing of unpurified PCR products was performed using the BigDye® Terminator v3.1 cycle sequencing kit chemistry and analyzed on an ABI 3730 at First Base, Malaysia.

2.3. Data analysis

Forward and reverse sequences were aligned and edited in Mega 6.06 [19]. The species identity was determined by comparing sequences to GeneBank NCBI (http://www.ncbi.nlm.nih.gov) databases enforcing a sequence homology threshold of >99% as previously applied [20]. Genetic distance (D) was calculated within and between populations. We used Genebank sequence of *S. lewini* from other population in Madagascar [21] to determine the connectivity between populations. A Neighbor-Joining (NJ) tree was constructed in Mega 6.06 [19] based on Kimura 2-parameter model, and 1.000 bootstrap replicates, we also used one sequence of *Rhincodon typus* (accession: EU398993) from Genebank as an outgroup. DnaSP 5.10 [22] was used to analyze a number of the haplotype (H), haplotype diversity (Hd) [23] and nucleotide diversity (π) [24]. Population differentiation was assessed using Arlequin 3.5 [25].

3. Results and discussion

3.1. Genetic diversity and population structure

The DNA amplification with the CO1 gene successfully identified 30 individuals of *S. lewini* with an average 625-bp nucleotide length. The value of haplotype diversity and genetic diversity in Lombok (Hd = 0.40; π = 0.0018). The haplotype diversity in Lombok was lower than the previous study in the Indo-Australian Archipelago and Eastern Pacific Ocean (Table 1). Duncan *et al.* [14] reported that the highest global haplotype diversity of *S. lewini* (Hd) 0.80. In contrast, the value of genetic diversity in Madagascar was the lowest (Hd=0.13; π =0.0002).

Table 1. Genetic diversity of *S. lewini* in Lombok and several regions in the world using mtDNA analysis.

Sample site	Genetic Diversity		Reference
	Hd	π	
Lombok, Indonesia	0.40	0.0018	*current study
Madagascar	0.13	0.0002	[21]
Indo-Australian Archipelago	0.61	0.0098	[26]
Western Atlantic Ocean	0.38	0.0013	[27]
Eastern Pacific Ocean	0.53	0.0011	[28]
Global	0.80	0.0013	[14]

Hd = haplotype diversity, and π = nucleotide diversity

For the population genetic analysis, the most commonly used methods of summarising structure within genetic variability are the F statistics (F_{ST}) developed by Sewall [29]. F statistics partition genetic variability as measured by levels of heterozygosity into components of within-population and between population variations. The value of F_{ST} between Lombok and Madagascar were genetically significant structure. The F_{ST} value confirmed that both populations are different due to the geographical isolation of the population. The percentage of variation values a higher difference between populations than the percentage of variation values within a population. (Table 2).

Table 2. Analysis of molecular variance (AMOVA) *S. lewini* based on control region sequence.

Source of Variation	d.f.	Percentage of Variation (%)	F_{ST}	P-value
Between Population	1	97.30	0.973	0.000 ± 0.000
Within Population	56	2.70		
Total	57			

Sample site: Lombok and Madagascar

The effects of declining shark population in terms of decreased genetic diversity have recently drawn attention. Many studies showed that application of genetic diversity is needed in fisheries management because of the harmful effect of inbreeding, loss of genetic diversity and evolutionary potential also changes in population structures [30,31,32]. The result of genetic analysis from *S. lewini* population in Lombok can indicate the population in this site was not in good evolving.

3.2. Phylogenetic analysis

Mitochondrial DNA is a pivotal tool in evolutionary and population genetics including molecular ecology. The control region of the mtDNA due to its elevated mutation rate, lack of recombination and maternal inheritance serve as a biomarker in phylogenetic studies. A functional marker system for population and evolutionary biology and recently, the mtDNA sequence analysis has been widely used as it provides rich sources of data to analyze genetic diversity and phylogeny [33]. The phylogenetic analysis of mtDNA showed the connectivity of *S. lewini* in Lombok and Madagascar in 2 different clades of 7 haplotypes (Lombok H=5; Madagascar H=2) (Figure 1).

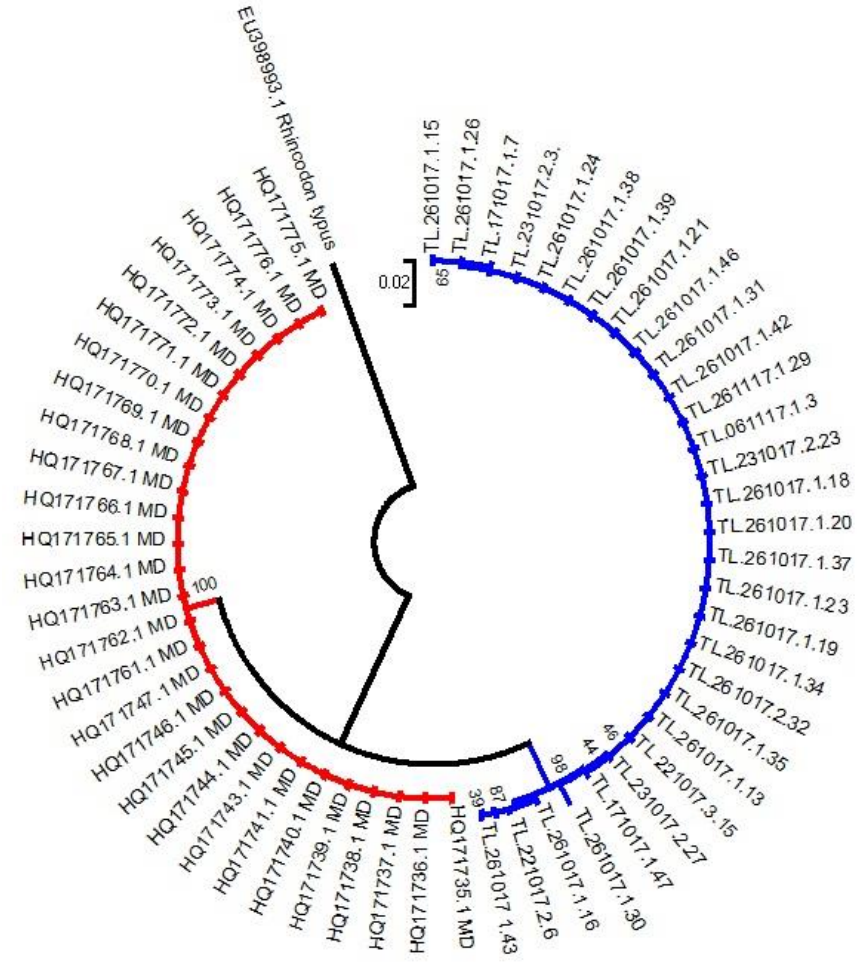

Figure 1. Phylogenetic tree of *S. lewini* from Lombok (TL) and Madagascar (MD) populations constructed using Neighbor-Joining (NJ) by Kimura 2-parameter model with 1000 bootstrap.

4. Conclusion

The value of haplotype diversity and genetic diversity in Lombok is low than the value at any regions in the world. The value of F_{ST} in between population in Lombok was genetically significant structure. The phylogenetic analysis showed the connectivity of *S. lewini* in 1 clade of 5 haplotypes. This study showed that low genetic diversity and significant differences in genetic structure between populations made this species vulnerable to extinction in Indonesia and required special treatment.

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