UDC 639

SPINY LOBSTER PANULIRUS VERSICOLOR FILOGENETIC AND GENETIC IN LOMBOK WATERS, WEST NUSA TENGGARA, INDONESIA

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ABSTRACT
This study aims to identify the phylogenetic spiny lobster *Panulirus versicolor* in Lombok waters, Indonesia and its association with *P. versicolor* spiny lobster from several regions of the Indian Ocean based on the cytochrome oxidase I (COI) gene. The researchers collected tissue samples from 13 *P. versicolor* spiny lobster in Lombok waters. 9 haplotypes were identified with haplotype diversity values (Hd) and nucleotides (Pi) respectively Hd = 0.859 and Pi = 0.00509. Research results exhibit *P. versicolor* spiny lobster population from the waters of Lombok is closely related to the spiny lobster population in some regions of the Indian Ocean. In general, *P. versicolor* spiny lobster population formed a monophyletic clone with spiny lobsters from several regions of the Indian Ocean with genetic distance values (P-distance from 0.001 to 0.004). The reconstruction of the haplotype network exhibited no genetic structure, which means that each population is not genetically isolated from others.

KEY WORDS
P. versicolor, COI, diversity, phylogenetic.

Spiny lobsters *P. versicolor* is one species of a tropical region with a complex life cycle. The initial phase of the *Panulirus* lobster consists of long periods of platonic pelagic larvae in the open ocean [1]. *P. versicolor* spiny lobster *phyllosoma* larvae are about 1-2 mm in size and the larval phase lasts for 6-7 months, before the *phyllosoma* larva morphed into *puerulus* [2]. Long larval phase, causing spiny lobsters has a wide spread and allows the supply of stocks between regions [3], resulting in gene flow through outbreeding between populations.

Lombok's waters are geographically influenced directly by the oceanographic process of the Indian Ocean and are the routes of mass water exit from the Pacific Ocean under ARLINDO (Indonesian Cross Flow) to the Indian Ocean. On the other hand, Lombok's waters are open geographically, so the chances of meeting or entering the spiny lobster *phyllosoma* larvae from some areas are very high.

Current tends to create barriers and directions from the spread of lobster *phyllosoma* larvae [4]. Therefore, it is important to identify the genetic and phylogenetic linkages of *P. versicolor* spiny lobsters from Lombok's waters and their relation to spiny lobsters from several other regions of the Indian Ocean. Phylogenetic knowledge is essential for understanding the evolutionary, adaptation, morphological, ecological and behavioral processes of species [5]. In addition, understanding the genetic and phylogenetic linkages is essential for marine conservation planning as it can identify routes of larval spread, or barrier to spreading [6].

Identification based techniques have been successfully used to identify genetic and phylogenetic linkages between populations [7]. Molecular markers are often used for population genetic studies of mtDNA (mitochondrial DNA) [7]. In this study, phylogenetic identification and genetic linkage used the COI gene (cytochrome oxidase c subunit I) which

is the protein region coding of the mitochondrial genome [8]. Several previous studies have used the COI gene to study the genetic population of spiny lobsters as conducted by Ptacek et al. 2001; da Silva et al. 2011; Jeena et al. 2015 [8,9,10]. The purpose of this study was to identify the phylogenetic and genetic linkages of *P. versicolor* spiny lobsters in Lombok waters and their relationship to *P. versicolor* spiny lobster from other regions of the Indian Ocean.

MATERIALS AND METHODS OF RESEARCH

A total of 13 individuals *P. versicolor* spiny lobster were collected from Lombok waters (Figure 1) and 8 sequences for some areas of the Indian Ocean downloaded from gene bank with accession numbers presented in Table 1.

Extraction of genomic DNA *P. versicolor* spiny lobster using KIT: Genomic DNA Mini Kit Animal Tissue (GENE AID). Amplification of CO1 gene using universal primer LCO1490: 5'-ggtcaacaaatcataaagatattgg-3 'and HCO2198: 5'-taaacttcagggtgaccaaaaaatca-3' [11]. The materials used for mastermix manufacture are LCOI and HCO2 primers 2.5 µL each, DMSO 1 µL, ddH2O 14 µL, Go Taq Green 25 µL and 5 µL DNA extract. The amplification process was carried out for 35 cycles, consisting of denaturation (94 ° C for 30 seconds), annealing (50 ° C for 30 seconds) and extension (72 ° C for 45 seconds). The PCR result undergoes electrophoreses process using 1% agarose gel with 50 mLTris Borate EDTA (TBE). Bi-directional sequencing was conducted using First Base CO (Malaysia) Big Dye © terminator chemistry (Perkin Elmer).

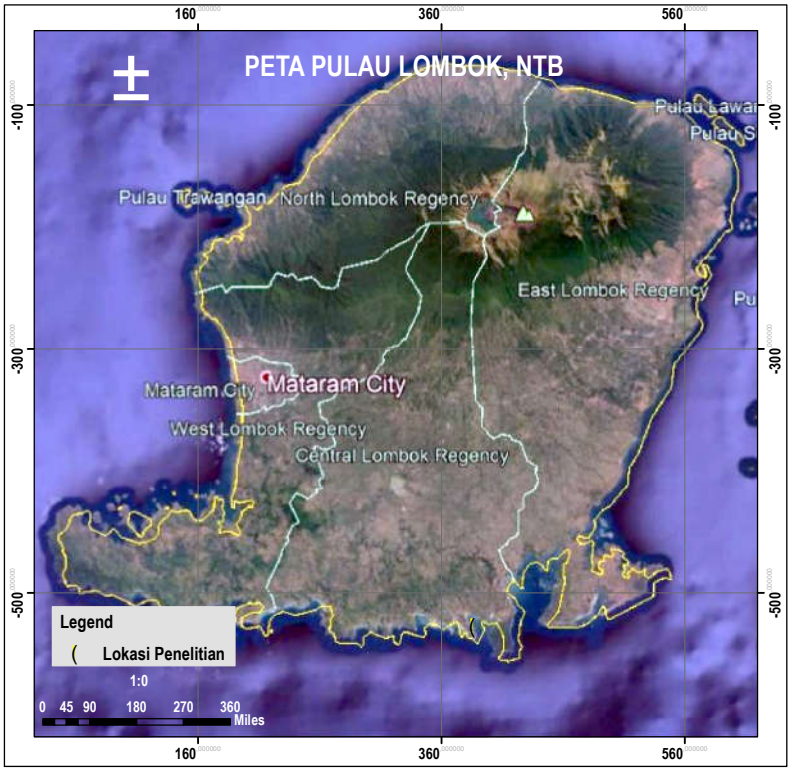

Figure 1 – Research Site Map

The sequenced results are edited and aligned using Mega5 [12]. Identification of species online used GenBank data at NCBI (National Center for Biotechnology Information) with BLAST (Basic Local Alignment Search Tool) method. Analysis of the diversity of haplotype (Hd) and nucleotides (Pi) used DnaSP 5.10 [13]. The nucleotide composition is estimated to be based on Kimura's parameter-2 model MEGA6. Phylogenetic reconstruction with Maximum Likelihood method [14], Kimura-2 model parameters and 1000 × bootstrap test utilizing MEGA 6.06 [12]. The haplotype network is built using Network 5.0.

Table 1 – *P. versicolor* spiny lobster Sequence from Genbank

Location	Accession number
Sri Lanka	KF548586
	KF548585
	KF548584
	KF548583
India	JQ229882
Persian Gulf & Oman Sea	KT001513
	KT001512
South Africa (South-west Indian Ocean)	KX275386

RESULTS AND DISCUSSION

COI gene amplification result fragment length of *P. versicolor* spiny lobster from Lombok waters using primer LCO1490 and HCO2198 i.e 750 bp (base pairs) (Figure 2). The primary use of LCO1490 and HCO2198 is based on Folmer et al. research (1994). The results exhibited that the primary pairs LCO1490 and HCO2198 consistently amplified the 710 bp of the COI genes throughout the invertebrate series and produced an informative sequence for phylogenetic analysis of species and higher taxonomic levels [11].

The analysis result of nucleotide composition exhibited that the average amount of adenine and thymine base was the highest (Table 2). These results are consistent with several studies reporting that the COI gene is rich in adenine and thymine in many invertebrates, including crustaceans (8-10). The different compositions of the purine and pyrimidine bases are related to the amino acids encoded by the codon.

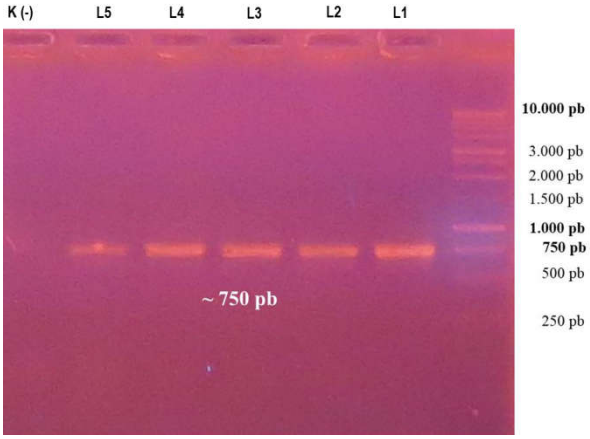

Figure 2 – Electrophoretic Result of *P. versicolor* spiny lobster Samples
(L = Lombok sample) K (-) = control

Table 2 – Mean composition of Nucleotide *P. versicolor* spiny lobster

Alkali (%)			
Adenine (A)	Timin (T)	Cytosine (C)	Guanine (G)
28.16	29.89	22.71	19.23

Analysis of genetic diversity using DNA SP 5.10. exhibits the diversity of haplotype (Hd) 0.859 and nucleotide (Pi) 0.00509. Hobbs et al. (2013) explain that there are 2 categories of haplotype diversity values. Values between ≥0 and <0.5 are in the low category, while> 0.5 and ≤1 are in the high category [15]. The high diversity of haplotype on spiny lobster is also reported in some previous studies (Table 3).

Analysis of genetic distance between population *P. versicolor* spiny lobster from Lombok waters with some region of Indian Ocean is presented in Table 4. Each population exhibited a close relationship with the mean value of P. distance of 0.004 (s.d 0.001). The

proximity of the genetic distance may be due to *P. versicolor* spiny lobster having the same origin.

Phylogenetic analysis was performed to determine the relation of *P. versicolor* spiny lobster between populations using Maximum Likelihood Trees (ML) method with 2-Parameter Kimura. Reconstruction of phylogenetic tree *P. versicolor* spiny lobster from Lombok waters with several areas in the Indian Ocean exhibited close kinship between regions by forming a large clade (Figure 3). Spiny lobsters *P. versicolor* from Lombok and Sri Lanka formed a monophyletic clade and no genetic structure formed between populations. Similar results were found by Chow et al. (2011), where the phylogenetic tree of the *P. penicillatus* lobster population from the Central Pacific forms a monophyletic clade with populations in Western Pacific [17]. In addition, Abdullah et al. (2014) also found no genetic structure in phylogenetic trees of *P. penicillatus* lobster populations from Aceh, Java, Maldives and Madagascar [18]. A wide spread allows no genetic structure in the phylogenetic tree of the *Panulirus* lobster between the observed areas.

Table 4 – Genetic Distance of Lobster *P. versicolor* from P. Lombok and Outgroup

		1	2	3	4	5
1	Lombok					
2	Sri Lanka	0,004				
3	Persian Gulf	0,004	0,003			
4	South Africa	0,004	0,004	0,004	0,003	
5	India	0,003	0,002	0,002	0,001	0,002

Table 3 – Genetic Diversity of Lobster Genus *Panulirus* Comparison

Species	Location	Haplotype (*Hd*)	Nucleotide (*Pi*)	Source
P. japonicus	Ohara	0.959	0.009	Inoue *et al*. 2007 [24]
	Hamajima	0.898	0.009	
	Goto	0.990	0.010	
P. homarus	Southern Sri Lanka	0.9921	0.0129	Senevirathna and Munasinghe 2014 [19]
	South India	0.9772	0.0117	
P. marginatus	Main Hawaiian Islands	0.977	0.026	Iacchei *et al*. 2014 [25]
	Northwest Hawaiian Islands	0.993	0.030	
P. penicillatus	Northwest Hawaiian Islands	0.8931	0.0083	
	Main Hawaiian Islands	0.8696	0.0064	

The results of haplotype network reconstruction using Network 5.0. exhibited a relatively close relationship between the haplotypes of spiny lobster populations *P. versicolor* in Lombok with populations from several regions of the Indian Ocean (Figure 4). There are 9 haplotypes with the highest frequency of 9 individuals, where the populations share haplotypes H1, H2, H6, and H8 while 5 haplotypes are unique for each population. H2 is the most dominant haplotype found in *P. versicolor* spiny lobster, where the length or the shortness of tissue formed exhibited the number of changes in the DNA sequence to form a different haplotype. The longer the tissue is formed, the more changes in the DNA sequence of the lobster spiny sample. The haplotype network exhibited no Clade between different geographical locations and no population is genetically isolated from others. While H11 and H10 are outgroups of the *P. homarus* species, they form a separate structure of *P. versicolor* spiny lobster. The haplotype distribution pattern can be used as an indicator for stock identification [19].

Research results exhibited that *P. versicolor* spiny lobster from Lombok waters has similar haplotypes with populations present in Sri Lanka, Persian Gulf, India and South Africa. This is because spiny lobsters have a wide spread supported by long larval periods lasting for 6 months and are platonic, allowing for supply stocks between regions or populations [16,17,20]. In addition, Palero et al. (2008) also explain that long periods of planktonic larvae such as crustacean larvae *phyllosoma* can be found in a wider geographic distribution [21].

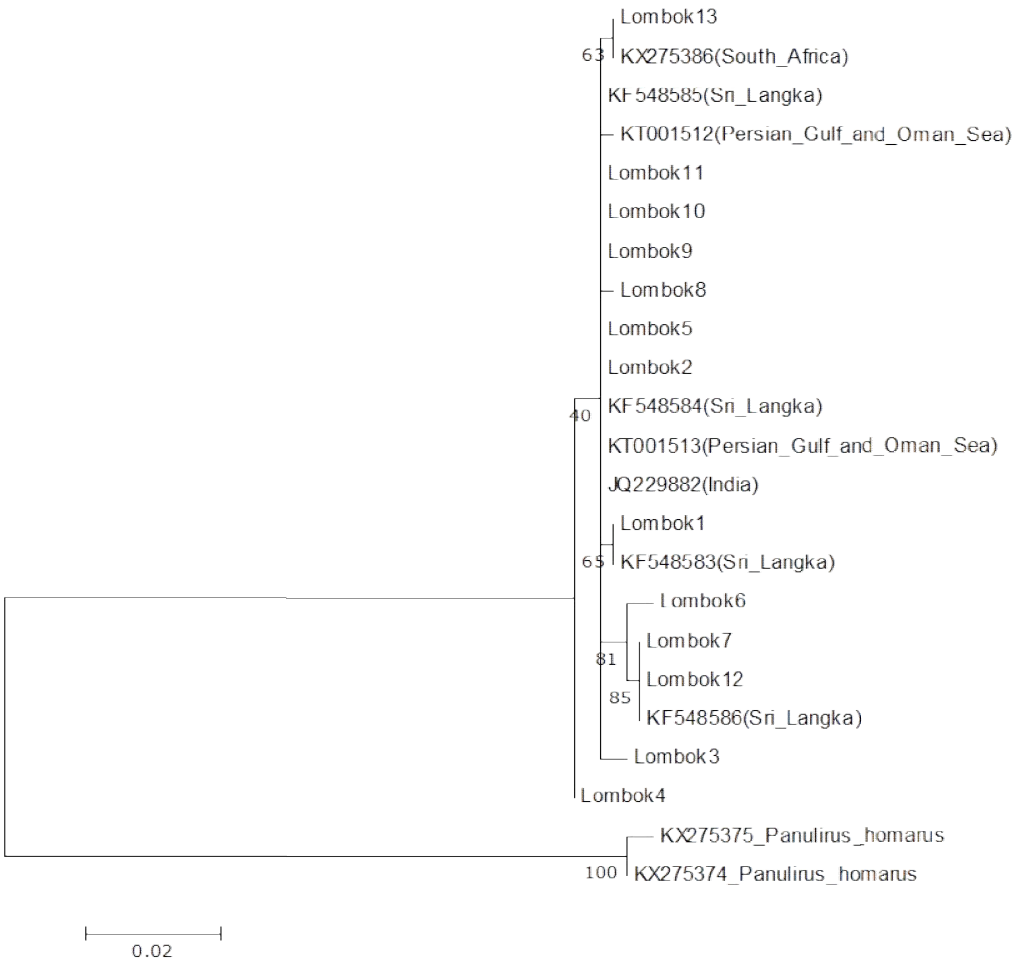

Figure 3 – *P. versicolor* spiny lobster Phylogenetic from Lombok Waters and Several Areas in the Indian Ocean using Maximum Likelihood Kimura 2-Parameter

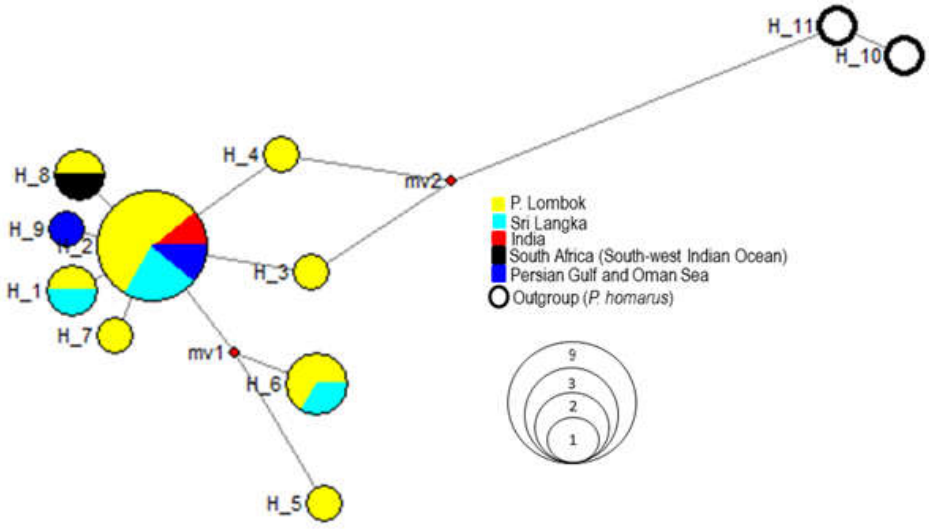

Figure 4 – *P. versicolor* spiny lobster Hemplotype Network from Lombok Waters and Several Regions in the Indian Ocean

On the other hand, the distribution pattern of *phyllosama* spiny lobster larvae is strongly influenced by the physical physiology of water and geographical formation.

Bradbury et al. (2008) explained that the transport and mixing of larvae in waters is influenced by the strength of water movement and the length of the larval period [22]. Current tends to create resistance and direction from spreading of spiny lobster *phyllosoma* larvae [4,18]. In addition, the pattern of distribution of some benthic invertebrate populations is less demographically or geographically isolated due to the presence of the barrier. Kennington et al. (2006) stated that the Barrier has caused significant differences in allele frequencies between locations [23].

CONCLUSION

P. versicolor spiny lobster population from the waters of Lombok is closely related to some populations in the Indian Ocean. This is supported by the value of P-distance 0.001-0.004. Reconstruction of population phylogenetic trees *P. versicolor* spiny lobster as a whole forms a monophyletic clade and haplotype tissue illustrates the close association between populations so as not to identify the genetic structure that occurs.

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