Genetic Diversity and Population Structure of *Panulirus Homarus* Populations of Southern Sri Lanka and South India Revealed by the Mitochondrial COI Gene Region

Jayan Senevirathna, and Dona Munasinghe

***Abstract*—** This study used data from mitochondrial marker to identify population stock structure pattern of *Panulirus homarus* in the Southern Sri Lanka and South India. Genetic variation in DNA sequences was examined from the 5' end of the mitochondrial COI gene region. High levels of haplotype diversity (h = 0.9921) was detected, indicating a high level of genetic diversity. A total of 60 polymorphic sites were determined, and 42 haplotypes were defined. The results of AMOVA analysis indicated that 100.64% of the genetic variation contained within populations and -0.64% occurred among populations. F_{st} value was suggested that no significant different between two populations. Tests of neutral evolution (Tajima's D) and analysis of mismatch distribution (SSD) suggested that *P. homarus* might have undergone a population expansion in the Indian Ocean. The knowledge on genetic diversity and genetic structure will be crucial to establish appropriate fishery management stocks for the species.

Keywords*—Panulirus homarus*, Population genetic, Mitochondrial marker, mitochondrial COI

I. Introduction

SIX spiny lobster species have been recorded in Sri Lanka [14] as *Panulirus homarus*, *P. ornatus*, *P. versicolor*, *P. longipes*, *P. polyphagus* and *P. penicillatus*, but currently only five species except *P. polyphagus* are found in the southern coastal belt of Sri Lanka. Among them *Panulirus homarus* is the most widely distributed species and is the commercially most important spiny lobster species. *P. homarus* occurs in shallow, inshore rocky reef habitats and three subspecies are known [13]. *P. homarus megasculpta* is found along the Somalian and Arabian coasts, *P. homarus homarus* is distributed throughout the Indo-South Pacific region and *P. homarus rubellus* occurs along the south-east coast of Africa and Madagascar [3], [13], [10].

P. homarus has been identified as a potential candidate for aquaculture [16]. As natural stocks are depleting rapidly, to fulfill the demand there is an attempt to establish culture systems in Sri Lanka with the support of the Government (Personal communication with NAQDA officers). Sri Lanka is an island which located very close proximity to Indian sub continent, thus, there is possibility to mixing up populations of marine species, especially which of those have planktonic larval stages. As *P. homarus*, is mostly distributed among the southern part of the island [15], it is necessary to gather information on the natural stocks for effective management of fisheries as well as for selection of suitable trait for farming.

Mitochondrial markers have been used to resolve taxonomic problems of crustaceans at different taxonomic levels [18], [6], [1]. Especially, barcoding region, Cytochrome Oxidase I (COI) gene region has been used successfully to examine genetic variation at population level of the crustacean species [5], [8] including lobster species [4], [17].

In this paper we report first genetic study on wild scalloped spiny lobster, *P. homarus* in Sri Lanka. This study was conducted to determine population genetic variation between southern Sri Lankan population and south Indian population using mitochondrial COI gene region.

II. Materials and methods

Adult spiny lobsters were collected from four locations of southern coastal belt of Sri Lanka (Kirinda (7), Godawaya (6), Waligama (6), and Hikkaduwa (8) and fixed in 100% ethanol, at the Research Laboratory of Department of Zoology, University of Ruhuna, Matara, Sri Lanka. GenBank published data of *P. homarus* in South Indian population were downloaded and used for the analysis. GenBank information of South Indian *P. homarus* populations were from Kerala (JQ229885.1, JQ229884.1, JQ229886.1, JQ229916.1, JQ229917.1, JQ229918.1, JQ229919.1, JQ229920.1, JQ229921.1), from Andhra (JQ229887.1, JQ229888.1, JQ229922.1, JQ229923.1, JQ229924.1, JQ229925.1, JQ229926.1) and from Tamil Nadu (JQ229883.1, JQ229884.1, JQ229910.1, JQ229911.1, JQ229912.1, JQ229913.1, JQ229914.1, JQ229915.1.) The distribution of lobster collection sites of both Southern Sri Lanka and South India populations were showed in figure 1.

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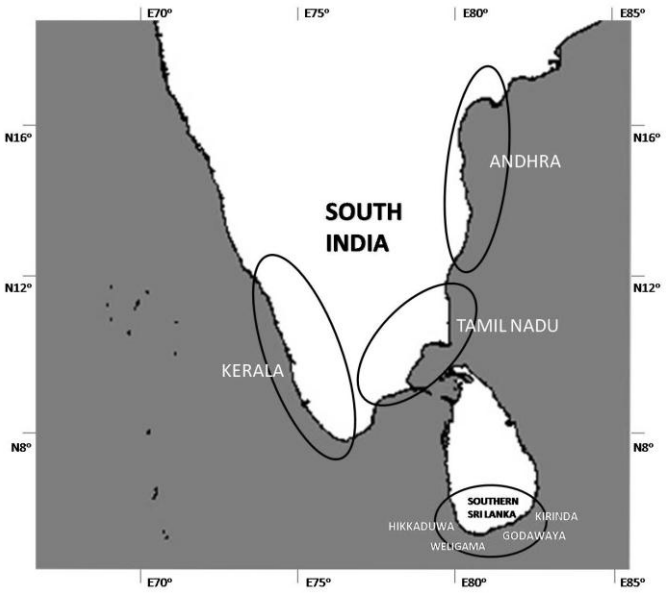

Fig. 1 Map of the *P. homarus* sampling locations and regions

Molecular analyses were conducted at the Paul Herbert centre for DNA Barcoding and biodiversity studies, Aurangabad, India. Genomic DNA was extracted from a leg muscle tissue of each individual by using Promega wizard genomic DNA isolation kit. Amplification of partial COI gene region (658 bp) was attempted using invertebrate universal primers, LOC1490 and HCO2198 [11]. PCR reactions were performed using the reaction master mix consisted of 9.65 µl 10% trehalose, 1.25 µl 10X PCR buffer 'B', 0.12 µl MgCl2, 1µl 2.5 mM dNTP, 0.5 µl 10 mM (LCO) forward primer, 0.5 µl 10 mM (HCO) reverse primer and 0.1 µl taq polymerase (5 units). The PCR reaction profile was comprised of an initial step of 3 min at 94 ^{0}C and 5 first cycles stage of 30 sec at 94 ^{0}C, 40 sec at 45 ^{0}C, and 1 min at 72 ^{0}C and second cycles stage of 30 sec at 94 ^{0}C, 40 sec at 53 ^{0}C, and 1 min at 72 ^{0}C with a final extension at 72^{0}C for 3 mins. These samples were processed for sequencing in ABI 3130 Genetic analyzer capillary sequencer.

Sequences were edited and aligned using Codon Code software [7] and MEGA 5.0 [20]. To estimate hypothesized patterns of spatial genetic structure, a hierarchical analysis of molecular variance (AMOVA) was used to partition variance components attributable to population variance and to individuals within the populations. The isolation-by-distance effects on population genetic structure were estimated by pairwise F_{st} statistics [21]. The neutrality analysis (Tajima's D test) and the shapes of the mismatch distributions by sum of squared deviations (SSD) were used to deduce whether a population has undergone sudden population expansion. All the analysis were implemented in ARLEQUIN version 3.0 [9]. All the generated sequences of Sri Lanka were published on GenBank (Blankt Submission ID: from 1665746).

III. Results and Discussion

A fragment of 493 bp of *P. homarus* mtDNA COI region was PCR amplified and sequenced from all 27 samples from Sri Lanka. For South Indian population, 23 GenBank published COI sequences were downloaded for analysis. In total, 60 variable sites were observed and 42 haplotypes were detected. The number of haplotypes, number of polymorphic sites, the values of haplotype diversity (Hd), and nucleotide diversity (p) within each population and all simples were presented in Table I.

The two populations have shared only H6, H9 and H18 haplotypes and rest of the 39 haplotypes were unique to their own population. The high level of mtDNA polymorphism, represented by the high nucleotide and haplotype diversity, may be related to a combination of high mutation rate and large population size. The haplotype distribution pattern suggested that those unique haplotypes could be used as indicators of stock identifications.

TABLE I
LOCATION OF SITES WHERE *P. HOMARUS* WERE SAMPLED FROM ALONG THE INDIAN OCEAN, NUMBER OF INDIVIDUALS PER SAMPLING SITE (N), DISTRIBUTIONS OF 42 HAPLOTYPES AND SUMMARY OF STATISTICS OF GENETIC VARIABILITY.

Locality	N	No. haplotype	Haplotype Distributions	No. polymorphic sites	Haplotype diversity (Hd)	Nucleotide diversity (p)
Southern Sri Lanka	27	21	H6(1), H9(2), H18 (3), H22(2), H23(3), H24(2), H25(1), H26(1), H27(1), H28(1), H29(1), H30(1), H31(1), H32(1), H33(1), H34(1), H35(1), H36(1), H37(1), H38(1), H39(1)	32	0.9772	0.0129
South India	23	21	H1(1), H2(1), H3(1), H4(2), H5(1), H6(1), H7(1), H8(1), H9(1), H10(1), H11(1), H12(1), H13(1), H14(1), H15(1), H16(1), H17(1), H18(2), H19(1), H20(1), H21(1)	37	0.9921	0.0117
Total	60	42		60	0.9921	0.0117

The analysis of AMOVA was used based on haplotype frequencies to test for large-scale patterns of genetic structure (Table II). The test was revealed that 100.64% of the genetic variation occurred within the populations, whereas -0.64% of the genetic variation occurred among populations (Table II). The AMOVA analysis for the COI gene region showed that the molecular variations observed in *P. homarus* were mainly occurred within the populations, which may imply a weak and unstable regional genetic structure in this species. The average F_{st} value was -0.00639 ($P < 0.01$), indicating no genetic differentiations between the two geographic locations and a single stock in the South Indian Ocean , which may cause due to infinite gene flow among population. The ocean water current pattern of this region may distribute planktonic larvae all alone the continent of the two locations and share the gene pool as other marine species [12]. The genetic isolations of species are facts to understand oceanic current patterns and larval distribution. Planktonic larval duration (PLD) is a key factor in shaping patterns of dispersal and degree of connectivity between populations of marine species [19].

Table II
Analysis Of Molecular Variance (Amova) Of Coi Gene Region Sequences Of *P. Homarus* In Southern Sri Lanka And South India

Source of variation	df	Sum of squares	Variance components	Percentage of variation	P
Among populations	1	2.569	- 0.0194	- 0.64	0.00
Within populations	48	146.411	3.0502	100.64	
Total	49	148.980	3.0309		
Fixation Index (F_{ST}) = -0.00639					

The Tajima's D values for both populations were negative values (Sri Lanka = -0.859 and India = -1.634), which measures the difference between the number of segregating sites and the pairwise genetic distance, for the overall population showed significant departure from neutrality, as may be expected when a population is under selection or expansion. The results of mismatch analysis of SSD were (Sri Lanka = 0.0049 and India = 0.0013) supported the occurrence of a population expansion and also suggested that *P. homarus* in the Indian Ocean might have experienced a rapid population expansion in the recent past. It should be recalled that traces of ancient demographic expansions have been observed in species experiencing population decline at present time [22].

The results of the present study conducted using sequences of mtDNA COI gene region revealed that genetic heterogeneity exits in *P. homarus* population in the south India Ocean and the limited connectivity among the two regional populations. However, *P. homarus* is a marine species with high potential for dispersal, suggesting that this species might have very little intraspecific population structuring over large geographic areas [2]. The analysis of a larger length of the COI gene region DNA fragment or other variable DNA marker such as nuclear gene regions, as well as additional population samples would help to understand the lack of genetic differentiation and a comprehensive population genetic structure. Genetic recognition of population stocks of *P. homarus* also represents species deviation. These data can be useful for further studies and management plans to develop sustainably mariculture of this species and conservation from over-exploitation.

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