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POPULATION GENETIC STRUCTURE OF SPINY LOBSTERS, *Panulirus homarus* AND *Panulirus ornatus*, IN THE INDIAN OCEAN, CORAL TRIANGLE, AND SOUTH CHINA SEA

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ABSTRACT

Populations of spiny lobsters worldwide are threatened by overfishing, while its supply from aquaculture is currently insufficient to meet the market demand. This current study investigated the genetic structure of two economically important spiny lobsters, *P. homarus* and *P. ornatus* sourced from the Indian Ocean and South China Sea. Fragments of the cytochrome oxidase subunit-I (COI) gene of the mitochondrial DNA of 71 *P. homarus* and 42 *P. ornatus* collected from 6 and 5 fish landing sites in Indonesia, respectively, were sequenced. Homologous sequences from the Indian Ocean and South China Sea available at GenBank were included in the analysis. No genetic differences were observed in *P. ornatus* populations from the two geographic regions ($x_{ST} = -0.005$) while a diminutive difference was found in the populations of *P. homarus* ($x_{ST} = -0.002$ and 0.009). These results, combined with a negative Tajima's D estimates, points to a deficit of nucleotide variation relative to the expectations from the mutation/drift equilibrium. Reconstruction of the phylogeny of *P. homarus* demonstrates that all Indonesian samples of *P. homarus* are grouped in one cluster and share the common cluster with GenBank data originated from Taiwan, Vietnam, India, Sri Lanka, Oman, and Iran. The phylogeny of *P. ornatus* indicates that there are two separated lineages existing in Indonesia.

KEYWORDS: lobster; *Panulirus*; mitochondrial DNA; COI; nucleotide sequence; phylogeography

INTRODUCTION

Spiny lobsters of the genus *Panulirus* have high economic value as they fetch high prices on the international seafood market, providing a substantial income to fishermen in Africa and Asia (Holthuis, 1991). An estimated 80,000 tons of spiny lobsters are produced annually, most of them wild caught, since aquaculture contributes less than 7% (Carpenter *et al.*, 2011) from the total production. Spiny lobster aquaculture is based on the collection of naturally settling puerulus larvae in the wild (Jones, 2012). The success of breeding lobster in captivity is limited, because of insufficient knowledge on reproductive biology, physiological requirements of larvae and juveniles, and because of the long planktonic phase and disease prob-

lems of the lobsters (Jones, 2012). Vietnam has become one of the major destinations for pueruli lobsters from Indonesia. *P. homarus* and *P. ornatus* are emerging as aquaculture products in the Indo-West Pacific region, especially in Vietnam and Indonesia (Jones, 2012). Spiny lobsters are at risk of overfishing because much of the fishery is largely unregulated, with lobsters of all types, sizes, and conditions, including berried females being caught (Hart, 2009).

Genetic data are needed to rationally manage sustainable fisheries of spiny lobster pueruli and adults. The genetic structure of *P. homarus* has been investigated at various geographical scales (Dao *et al.*, 2013; Farhadi *et al.*, 2013; Lavery *et al.*, 2014). Only slight population structure has been reported from the western and northwestern Indian Ocean (Farhadi *et al.*, 2013), which excluded the southernmost part of the eastern Indian Ocean. Meanwhile, strong genetic differences were reported at the scale of the range of the species in the Indo-Pacific (Farhadi *et al.*, 2013).

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Panulirus homarus rubellus, the subspecies endemic to the southwestern Indian Ocean, belongs to a morphologically and genetically distinct lineage which may deserve full-species status (Lavery *et al.*, 2014), while the population sampled in the Marquesas Islands was represented by a divergent mitochondrial lineage suggesting allopatric isolation. Little information on population genetic structure is available for the Indian Ocean, although a handful of sequences were included in a former publication (Lavery *et al.*, 2014).

The main objective of the present study was to investigate the population genetic structure of both *P. homarus* and *P. ornatus* in the Indian Ocean, the core of the central Indo-West Pacific region. It is expected that the result of this study could be used as a basis to inform fisheries and conservation management. In addition, the information regarding the population genetic structure is also a pre-requisite for selecting aquaculture broodstock of both species.

MATERIAL AND METHODS

Sampling Area

P. homarus samples were collected from fishermen at six fish landing sites in Indonesia (Yogyakarta, on the Indian Ocean coast of central Java (site-1); Muncar, on the Indian Ocean coast of eastern Java (site-2); Jembrana, in western Bali (site-3); Lombok; in the Lesser Sunda Islands (site-4); Takalar, of Southern Sulawesi (site-5); and Gorontalo (site-6) (Figure 1). *P. ornatus* were collected from all the landing sites excluding Yogyakarta (Five sites). The sample sizes are presented in Table 1. The last segment of the last pereopod of each individual lobster was collected and preserved in absolute ethanol.

DNA was extracted using 10% Chelexresin (Walsh *et al.*, 1991). A fragment of the cytochrome oxidase 1 (CO1) gene of the mitochondrial DNA was amplified using polymerase chain reaction (PCR) with universal primers *LC01490* and *HC02198* (Folmer *et al.*, 1994). After a successful PCR, nucleotide sequencing was done in both forward and reverse directions using the PCR primers, at the sequencing facility of the University of California, Berkeley. The sequence chromatograms were edited and aligned using ClustalW in MEGA6 (Tamura *et al.*, 2013). All sequences, trimmed to 612 bp (*P. homarus*) or 594 bp (*P. ornatus*) were deposited in GenBank. For *P. homarus*, previously published sequences from the South China Sea and Indian Ocean were included in the analysis (Lavery *et al.*, 2014; Ptacek *et al.*, 2001; Senevirathna & Munasinghe, 2013; Senevirathna & Munasinghe, 2014). For *P. ornatus*, two sequences from the South

China Sea were added (GenBank nos. HM446347, JF775573). Genetic diversity parameters, including haplotype diversity, nucleotide diversity, and Tajima's *D*-statistic (Tajima, 1989) were estimated using DnaSP (Librado & Rozas, 2009). The best substitution models were T92+G for the *P. homarus* nucleotide-sequence dataset and T92 for the *P. ornatus* one, both, selected using the "Find best DNA/protein model" option of the MEGA6 software (Tamura *et al.*, 2013). Population-pairwise fixation index between populations (x_{ST} ; T92 model) were estimated using ARLEQUINVER 3.5 (Excoffier & Lischer, 2010; Excoffier *et al.*, 1992). The maximum clade credibility (MCC) phylogeny was inferred in the Bayesian Markov Chain Monte Carlo (MCMC) analysis using the Bayesian Evolutionary Analysis by Sampling Trees (BEAST) software package version 1.7 (Drummond & Rambaut, 2007; Drummond *et al.*, 2012).

RESULTS AND DISCUSSION

Haplotype number, haplotype diversity, and nucleotide diversity for each sample of spiny lobsters *P. homarus* and *P. ornatus* are presented in Table 1. Fragment sizes in *P. homarus* and *P. ornatus* were, respectively, 612 bp and 594 bp, starting at nucleotide site homologous to, respectively, site no. 40 and site no. 62 of the *CO1* gene in *P. ornatus* (GenBank no. NC_014854; (Qian *et al.*, 2011)).

Both species had high haplotype diversity in all sampling locations. Tajima's neutrality test resulted in generally negative *D* values in both *P. homarus* and *P. ornatus* (Table 1), suggesting a deficit of nucleotide variability relative to the expectations from mutation/drift equilibrium. Pairwise x_{ST} estimates indicated no geographic structure for *P. homarus* and *P. ornatus* across the Indo-Malay archipelago (Table 2). Overall, x_{ST} was -0.002 for *P. homarus* (using the 382-bp *CO1* gene fragment as the marker) and -0.005 for *P. ornatus* at the scale of the Indo-Malay archipelago. It was 0.009 in *P. homarus* from Indonesia (i.e., South China Sea sample excluded; 612-bp *CO1* gene fragment). Neither value was significantly different. Overall x_{ST} over the three main populations of *P. homarus* from the northwestern Indian Ocean, India, Sri Lanka, and Indonesia was 0.057 ($P < 0.001$).

Reconstruction of the phylogeny of *P. homarus* is shown in Figure 2. This demonstrates that all Indonesian samples of *P. homarus* are grouped in one cluster and share in, based on GenBank data, the common cluster with lobsters originating from Taiwan, Vietnam, India, Sri Lanka, Oman, and Iran. The data from Madagascar and South Africa form a separate cluster with a posterior probability value of 1. The

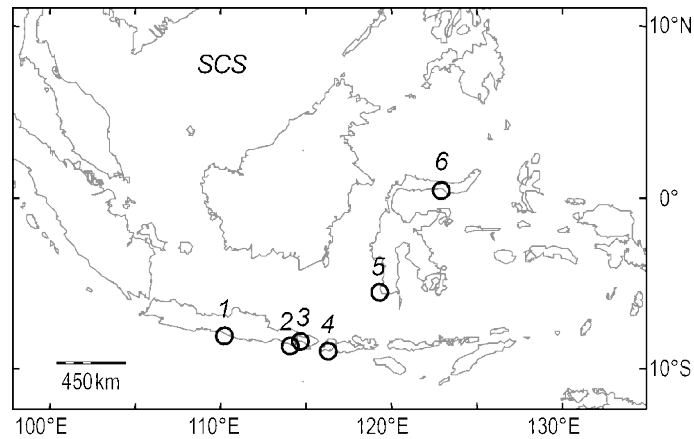


Figure 1. Sampling locations for *Panulirus homarus* and *P. ornatus* in the Indo-Malay archipelago. (1) Yogyakarta, southern Central Java; (2) Muncar, southern East Java; (3) Jembrana, Bali Strait; (4) Batu Nampar, Lombok; (5) Takalar, South Sulawesi; (6) Gorontalo, Tomini Bay; SCS South China Sea. Background map edited from images downloaded from Digital Vector Maps, San Diego (<http://digital-vector-maps.com/>).

MCMC tree of *P. ornatus* data is shown in Figure 3. The tree indicates that there are two separate lineages of *P. ornatus* existing in Indonesia (posterior probability = 1). The first group, represented by four samples from East Java, West Nusa Tenggara, and South Sulawesi, shares in the cluster with Indian sequence data, while the second is with a Pacific isolate.

The spiny lobster genus *Panulirus* White, 1847 is well known to have a high level of species diversity and wide geographic distribution (Ptacek *et al.*, 2010). Nineteen species have been described, three of which are divided into seven recognized subspecies (Holthuis, 1991; Sarver *et al.*, 1998). The phylogenetic history of this group has been determined. Using two mitochondrial genes of 16S rRNA and COI, two

Table 1. Genetic diversity parameters in spiny lobsters *P. homarus* and *P. ornatus* from the Indo-Malay archipelago, based on the nucleotide sequences of a fragment of the CO1 gene

Species and origin of sample	N	Parameters				
		h	HD±SD	Π±SD	D (Tajima)	
<i>P. homarus</i>	Yogyakarta	6	5	0.933 ± 0.122	0.01667 ± 0.00262	0.07920 NS
	Muncar	13	11	0.962 ± 0.050	0.00842 ± 0.00096	-1.02340 NS
	Jembrana	17	15	0.978 ± 0.031	0.01058 ± 0.00167	-1.68132 NS
	Lombok	17	17	1.000 ± 0.020	0.01238 ± 0.00208	-1.61781 NS
	Takalar	16	14	0.983 ± 0.028	0.01428 ± 0.00183	-1.07434 NS
	Gorontalo	2	2	1.000 ± 0.500	0.00163 ± 0.00082	NA
Total	71	49	0.981 ± 0.007	0.01190 ± 0.00096	-1.79075 NS	
<i>P. ornatus</i>	Muncar	11	9	0.945 ± 0.066	0.00637 ± 0.00162	-1.72985 NS
	Jembrana	9	9	1.000 ± 0.052	0.00608 ± 0.00069	-0.86291 NS
	Lombok	6	5	0.933 ± 0.122	0.00629 ± 0.00202	-0.88622 NS
	Takalar	10	9	0.978 ± 0.054	0.00655 ± 0.00150	-1.22780 NS
	Gorontalo	4	4	1.000 ± 0.177	0.01094 ± 0.00255	-0.07004 NS
Total	40	28	0.956 ± 0.023	0.00676 ± 0.00082	-1.97170 *	

Note: N: sample size; h: number of haplotypes; HD: haplotype diversity; $\hat{\pi}$: nucleotide diversity; D (Tajima): Tajima's (1989) statistic; NA: no computation made because of low sample size

Table 2. Population-pairwise x_{ST} estimates [16] estimated from partial CO1 gene sequences in rock lobsters *P. homarus* and *P. ornatus* across the Indo-Malay archipelago. For *P. homarus*, upper row values are x_{ST} estimates based on the 612-bp fragment; lower-row values are x_{ST} estimates based on a 382-bp fragment (see Materials and Methods). Probability of x_{ST} value under null hypothesis of genetic homogeneity estimated from 1,000 random permutations under ARLEQUIN [15]. SCS=South China Sea sample

Species, sample origin	Sample origin					
	Yogyakarta	East Java	Bali	West Nusa Tenggara	South Sulawesi	Gorontalo
<i>P. homarus</i>	East Java	0.173 ^{NS}				
	Bali	0.106 ^{NS}	-0.006 ^{NS}			
	West Nusa Tenggara	0.012 ^{NS}	0.016 ^{NS}	-0.014 ^{NS}		
	South Sulawesi	0.000 ^{NS}	0.037 ^{NS}	-0.003 ^{NS}	-0.043 ^{NS}	
	Gorontalo	-0.032 ^{NS}	-0.077 ^{NS}	-0.189 ^{NS}	-0.184 ^{NS}	-0.146 ^{NS}
SCS	-0.036 ^{NS}	0.055 ^{NS}	-0.004 ^{NS}	-0.050 ^{NS}	-0.036 ^{NS}	-0.097 ^{NS}
<i>P. ornatus</i>	Bali	-	0.032 ^{NS}			
	Lombok	-	-0.052 ^{NS}	0.058 ^{NS}		
	South Sulawesi	-	-0.036 ^{NS}	0.050 ^{NS}	-0.090 ^{NS}	
	Gorontalo	-	0.044 ^{NS}	0.137 ^{NS}	-0.093 ^{NS}	-0.019 ^{NS}
SCS	-	-0.194 ^{NS}	-0.130 ^{NS}	-0.092 ^{NS}	-0.095 ^{NS}	-0.121 ^{NS}

Note: nd: no data; NS: not significant;
*** P<0.001

major lineages were found with two groups within *Panulirus*. The first lineage included all species of *Panulirus* classified as Groups I and II; the second included all species classified as Groups III and IV. *P. homarus* and *P. ornatus* were grouped into group IV (Ptacek *et al.*, 2001). Based on morphologic and genetic data (Lavery *et al.*, 2014) recovered four subspecies of *P. homarus*, i.e. *P. homarus rubellus*, *P. homarus brown*, *P. homarus*, and *P. homarus megasculpta*. Besides those species, spiny lobsters known to inhabit Indo-West Pacific are *P. versicolor*, *P. polypagus*, *P. longipes*, and *P. penicillatus* (Humann & Deloach, 2012).

This is the accordingly published population data from Coral Triangle (CT) area of the most common species *P. homarus* and *P. ornatus* (Dao *et al.*, 2013; 2015) This study is to uncover the diversity of *P. ornatus*, the most desired species for the Chinese market (Hart, 2009). The sampling sites were at South-west corner of world CT.

A proportion of coastal marine Indo-West Pacific species show a marked phylogeographic differentiation on either side of the Indo-Pacific barrier, while others species show little or no differentiation distribution-wide (Carpenter *et al.*, 2011; Hubert *et al.*,

2012). The latter either inhabit the pelagic environment or have a long pelagic larval phase, for which estimates of genetic differentiation F_{ST} are generally ≤ 0.05 (Fauvelot & Borsa, 2011). The present results for *P. homarus* and *P. ornatus*, are finding the population genetic structure of spiny lobsters from the Indo-Malay archipelago, conform to the second category as expected for a lobster species with long pelagic larval stage, up to around 180 days (Goldstein *et al.*, 2008; Phillips & Matsuda, 2011; Dao *et al.*, 2015).

No genetic differences were observed either, at a geographic scale representing a substantial part of the geographic range of *P. homarus* (*P. homarus rubellus* and the Marquesas were excluded), i.e. from the north-western Indian Ocean to the South China Sea. A similar conclusion is valid for *P. ornatus*. This is confirmed by the available mitochondrial sequence dataset which gives no indication that *P. homarus* and *P. ornatus* are geographically structured into distinct populations, from the western Indian Ocean to the Indo-Malay archipelago. The apparent lack of population geographic structure in spiny lobsters across the Indo-Malay archipelago and at the much wider geographical scale of the Indian Ocean and the Indo-Malay archipelago can be explained by their long planktonic life and wide dispersal via ocean currents.



Figure 2. The maximum clade credibility (MCC) phylogeny for mitochondrial COI gene fragment of *P. homarus* from Indonesia analyzed with sequence data from Western Indian Ocean and South Chinese Sea as previously published (Farhadi *et al.*, 2013; Lavery *et al.*, 2014). The published sequence names are kept as published and added with the country origins. *Panulirus ornatus* sequence data from India (Acc No. JN418938) was co-analyzed as outgroup. Sequence codes with Bali, NTB, BW, and SS indicate the origin of samples were Bali; West Nusa Tenggara; Banyuwangi, East Java; and South Sulawesi; respectively. Phylogeny was inferred in the Bayesian MCMC analysis using BEAST software. The posterior probability values are shown.

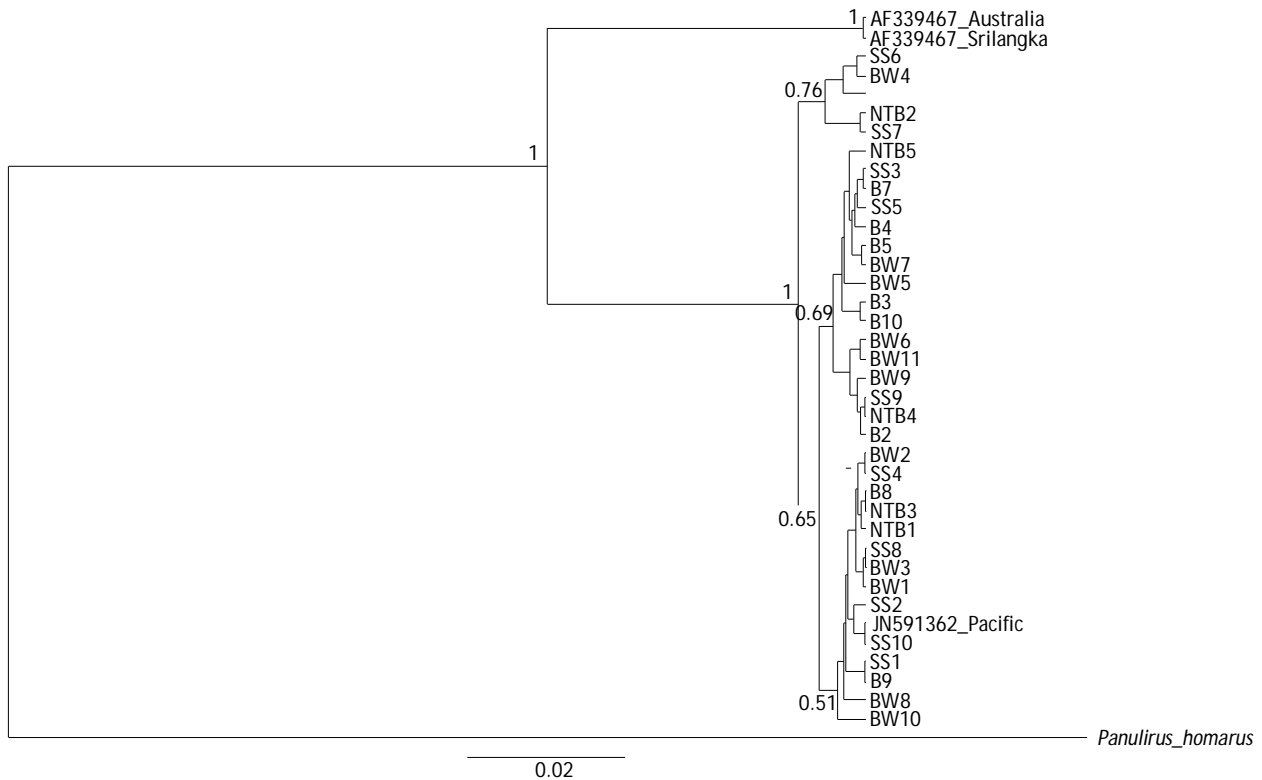


Figure 3. The maximum clade credibility (MCC) phylogeny for mitochondrial COI gene fragment of *P. ornatus* from Indonesia analyzed with sequence data from some countries in Australia, India, and Sri Lanka available in GenBank. Accession numbers and origin of each secondary data are indicated. Sequence codes and the method to reconstruct the tree are the same as used in Figure 2. The posterior probability values are shown.

Assuming that the CO1 gene is a neutral marker of population structure, negative Tajima's *D* values indicated an excess of rare haplotypes relative to the expectation from the mutation/drift equilibrium (Tajima, 1989). This would be consistent with the recent demographic expansion after a long contraction and consistent with the reduction of coastal habitat in the Indo-Malay region through the Pleistocene (Voris, 2000). Reduction in coastal habitat leading to the Indo-Pacific barrier during periods of lowest sea-level in the Pleistocene has promoted genetic differentiation between Indian and Pacific populations of a variety of species (Carpenter *et al.*, 2011). Although excesses of rare haplotypes may indicate a demographic effect of lower habitat availability on spiny lobsters *P. homarus* and *P. ornatus*, no Indian vs. Pacific differentiation was apparent at the mitochondrial locus CO1. While this may be explained by rapid rehomogenization following the rise in sea level, mediated by long planktonic larval duration, of populations from either side of the Indo-Pacific barrier, one cannot discard the hypothesis of possible rem-

nant genetic differentiation in the nuclear genome. Genome scans are warranted, as this may provide new insights into spiny lobster population structure, which the sole mitochondrial marker might not have accessed.

The phylogenetic reconstruction using Bayesian MCMC of *P. homarus* (Figure 2) supports the notion of allopatric speciation of *P. h. rubellus*, which inhabits Madagascar and South Africa waters, and *P. h. brown* in Marquesas is in the central Pacific (Lavery *et al.*, 2014). However, this seems invalid for the other two subspecies, *P. h. homarus* and *P. h. megasculpta*. The database sequence data that claimed to be both subspecies are distributed well in a single cluster of our sequence data as well as those published sequences from India and Sri Lanka (Senevirathna & Munasinghe, 2013; 2014).

The *P. ornatus* species also follows this pattern, as the limited data from India and the Pacific Ocean also show a monophyletic pattern in the phylogenetic result. However, the evidence of two distinct lineages

in Indonesia is interesting. The result of this study (Figure 3) also shows that there is a third distinct lineage of *P. ornatus*, represented by GenBank data from Australia and Sri Lanka (posterior probability = 1). Further population genetic study of this species must be stimulated to clarify this finding.

CONCLUSIONS

P. homarus and *P. ornatus* have high haplotype diversity in all sampling locations with no significant population differentiation. Moreover, the Indian Ocean and South China Sea seem to be a single population.

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