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Genetic diversity of purple-legged shovel-nosed lobster *Thenus unimaculatus* in Thailand

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ABSTRACT

Thenus unimaculatus or a purple-legged shovel-nosed lobster is one of the three *Thenus* species found in Thailand. Shovel-nosed lobsters have been increasingly consumed and this could give a great threat to *T. Unimaculatus*. This species has a narrow range of distribution, found only in the Andaman Sea. Genetic diversity study of the purple-legged shovel-nosed lobster is therefore needed for evaluating its current genetic status. In this study, 71 specimens of *T. unimaculatus* were collected from five provinces along the Andaman Sea coastline. 12S ribosomal RNA (12S rRNA) and cytochrome C oxidase subunit I (COI) mitochondrial genes were PCR amplified. The 12S rDNA and the COI nucleotide sequence alignments of 60 and 70 samples, respectively, were successfully prepared, suggesting that the parsimony informative sites of COI gene was higher than that of 12S rDNA. Phylogenetic analysis using Maximum Likelihood method revealed that populations of *T. unimaculatus* in Thailand are fairly homogenised. This blended genetic diversity among the five provincial populations indicates that there is a circulation of the planktonic larvae, resulting from two different patterns of sea currents of the Andaman Sea.

Keywords: the Andaman Sea, genetic diversity, mitochondrial DNA, Thailand, *Thenus unimaculatus*

INTRODUCTION

A purple-legged shovel-nosed lobster or *Thenus unimaculatus* is one of the three shovel-nosed lobster species found in Thailand: *T. unimaculatus*, *T. indicus* or common shovel-nosed lobster, and *T. orientalis* or spotted-leg shovel-nosed lobster (Iamsuwansuk *et al*., 2012). *Thenus unimaculatus* was proposed by Burton and Davie (2007) as a newly recognised species according to its distinctive nucleotide sequence of the mitochondrial cytochrome C oxydase subunit I (COI) gene and its unique purple spots on a carapace and purple stripes at the propodus of pereiopods. In Thailand, the purple-legged shovel-nosed lobster can be found only along the coastline of the Andaman Sea and it has been increasingly caught as authentic seafood and also exported as a seafood product.

Regarding to recent population genetic

studies of shovel-nosed lobsters in Thailand, which revealed a narrow genetic diversity of *T. indicus* populations in the Andaman Sea (Iamsuwansuk and Denduangboripant, 2011; Iamsuwansuk, 2012), the situation of *T. unimaculatus* populations may be alike and this could lead to a great risk if overexploitation happens. Therefore, a genetic diversity study of *T. unimaculatus* is needed for better understanding of its current genetic status. Seventy-one samples of purple-legged lobster were collected from five provinces along the Andaman Sea and two mitochondrial DNA sequences, 12S ribosomal RNA gene (or 12S rDNA) and COI gene, were analysed phylogenetically. Maximum likelihood phylogenetic tree suggested the mixing of genetic diversity of *T. unimaculatus* populations, which possibly resulted from the planktonic larvae circulating along the Andaman Sea.

MATERIALS AND METHODS

Thenus unimaculatus samples were collected from five provinces along the Andaman sea – Ranong, Pangnga, Phuket, Trang, and Satun – between September to November of 2012. No specimen was sampled from Krabi province because there was not any shovel-nosed lobster fishery in that area. Each sample was identified to be *T. unimaculatus* following the morphological description of Burton and Davie (2007). All samples were maintained in 95% ethyl alcohol before the merus segment tissues from their walking legs were taken to extract genomic DNA.

Two mitochondrial genes were amplified using polymerase chain reaction (PCR) technique with primers COI1490 (5’-GGTCAACAAATCATAAAGATATTGG-3’) and COI2198 (5’-TAAACTTCAGGGTGACCAAAAAATCA-3’) (Folmer *et al*., 1994) for the COI gene and primers 12SCRF (5’-GAGAGTGACGGGCGATAT-3’) and 12SCRR (5’-AAACCAGGATTAGATACCCTATTAT-3’) for isopod 12S rDNA amplification (Wetzer, 2001). The PCR conditions of the COI gene and the 12SDNA were modified from Folmer *et al*. (1994) and from Wetzer *et al*. (2003), respectively.

Any successfully amplified PCR products were then purified and sent to a commercial nucleotide sequencing service. MEGA version 5.0.2 program (Tamura *et al*., 2011) was used to check and correct the sequences and to prepare nucleotide sequence alignments of both genes. The best evolutionary model of DNA substitution was selected using JModelTest version 2.1.3 program (Darriba *et al*., 2012) using Bayesian Information Criterion (BIC). Maximum Likelihood (ML) approach implemented in PAUP* phylogenetic program version 4.10b (Swofford, 2002) was set to TIM2+I model (frequencies A:C:G:T = 0.2731:0.2109:0.1750:0.3411 with p-invariance = 0.8120) for COI gene and set to HKY+G model (frequencies A:C:G:T = 0.3375:0.1083:0.1802:0.3740 with gamma shape = 0.2100) for 12S rRNA gene as suggested. Bootstrap analysis was performed for each dataset with 500 pseudoreplicates. Nucleotide sequence of *T. orientalis* was retrieved from GenBank database and used as an outgroup for the analysis of COI gene following our previous study (Iamsuwansuk *et al.*, 2012). For 12S rDNA, *T. indicus* sequence was used instead since no 12S rRNA sequence of *T. orientalis* is available in GenBank.

RESULTS AND DISCUSSION

In this study, 71 specimens of *T. unimaculatus* were collected and their genomic DNA was extracted. Sixty and 70 PCR products from the 12S rRNA and the COI gene were successfully amplified and sequenced, respectively. This resulted in a 325-basepair sequence alignment of the 12S rRNA gene and a 549-basepair alignment of the COI gene. Comparison between the COI gene and the 12S rDNA alignments of *T. unimaculatus* specimens revealed that the former nucleotide alignment had an average sequence dissimilarity value within ingroups as 0.59 ± 0.12 %, similar to the latter alignment which had 0.60 ± 0.18 %. However, the COI gene had higher parsimony informative sites within ingroups than the 12S rRNA gene, which are 17 out of 549 sites (equally to 3.097%) compared with 8 out of 325 sites (2.462%).

According to the results of ML phylogenetic analyses of the COI gene (Fig. 1a) and the 12S rRNA gene (Fig. 1b), both ML trees showed similar topology that all of *T. unimaculatus* sequences were grouped together and separated from the outgroups. There was no monophyletic clade of the samples regarding to their provincially collecting areas on both ML phylogenies. Although there were several groupings of the samples found on each tree, none of them was statistically supported by high bootstrap value (data not shown). These five populations of purple-legged shovel-nosed lobster in Thailand seemed to have fairly low genetic diversity among them and may have been homogenised recently. This blended genetic diversity among *T. unimaculatus* populations probably result from a circulation of their planktonic larvae in the Andaman sea (Fig. 2), and not from a commonly short-ranged migration of the adults.

According to Rizal *et al.* (2012), although the sea current in the Malacca Strait is always directed north-westward towards the Andaman Sea, the circulation patterns of sea currents of the Andaman sea are different between the north-eastern (NE) and the south-western (SW) monsoon seasons. During the NE monsoon season (December- February) (Fig. 2 left), there are two main surface currents occurring in the Andaman Sea: one starts from the north of the Andaman sea, moving toward the south and leaving through the wide area between the Andaman Islands and Sumatra to the Indian Ocean; the other occurs at the Malacca strait, spreading to the borderline between Thailand and Myanmar with an anticlockwise gyre at the north of Sumatra. On the other hand, the water masses enter the Andaman sea during the SW monsoon season (June-September) (Fig. 2 right) and the surface

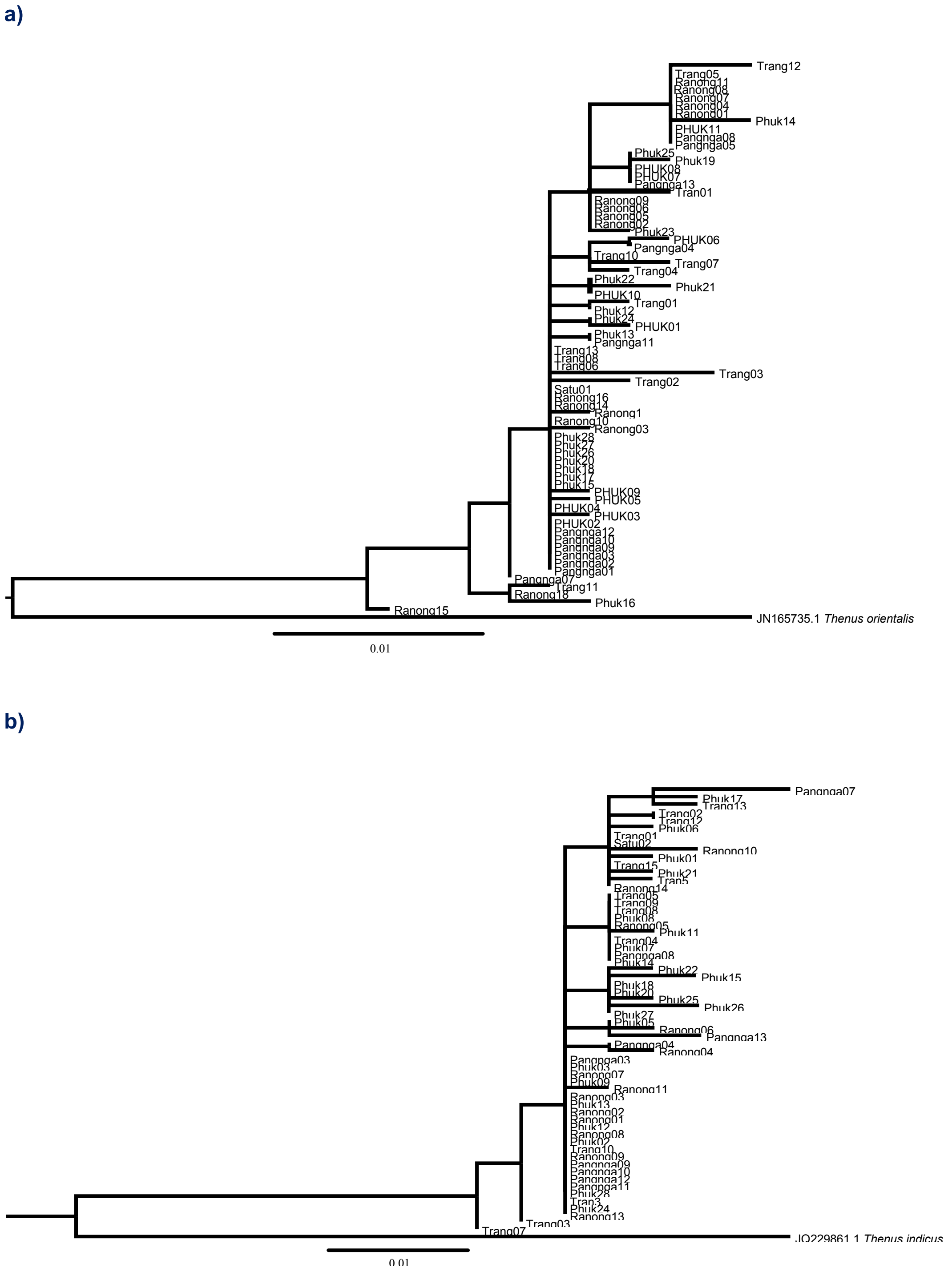

Figure 1 Maximum likelihood trees of nucleotide sequences of a) COI gene and b) 12S rDNA of *Thenus unimaculatus* in Thailand collected from five provinces along Andaman sea. *T. orientalis and T. indicus* were used as outgroups for these analyses, respectively.

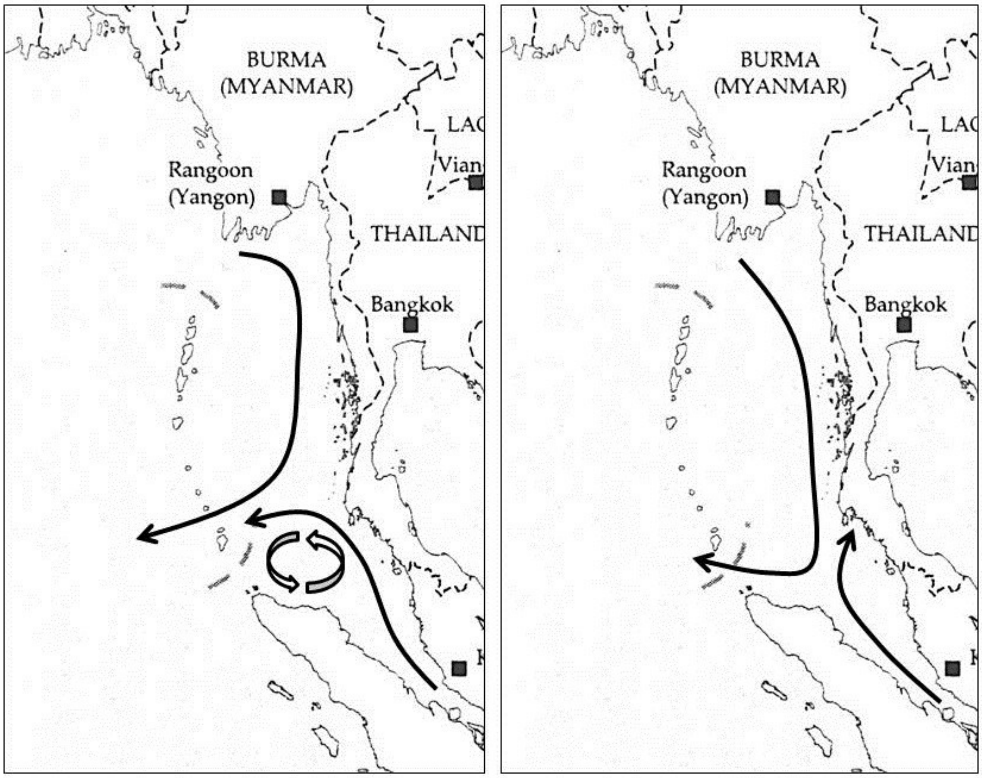

Figure 2 Different circulating patterns of surface currents of the Andaman sea during north-eastern (left) and south-western (right) monsoon seasons. The water current directions are simplified from Rizal *et al*. (2012) and the background map is adapted from Southeast Asia map of www.southchinasea.org.

current is concentrated between the south of the Nicobar Islands and Sumatra, and is recirculated along the north of Sumatra to the Indian Ocean; the second current in SW monsoon season also moves into the Andaman Sea but closer to the coast of the Malay Peninsula and flows towards Phuket island. Thus, planktonic larvae of *T. unimaculatus* in the Andaman sea would be circulated differently between the two seasons, resulting in the mixing of genetic diversity found in this study. We need to perform more sophisticated population genetic analyses to verify this hypothesis.

CONCLUSION

In conclusion, we collected 71 purple-legged purple-legged shovel-nosed lobsters from five provinces in Thailand along the Andaman sea and extracted their genomic DNA. Two mitochondrial genes, 12S rDNA and COI genes, of the samples were amplified and sequenced for phylogenetic analyses. COI gene showed a little higher genetic variation than 12S rDNA gene. Though having low numbers of parsimony informative sites, these regions were still suitable candidates for estimating genetic diversity of *T. unimaculatus*. Maximum Likelihood trees of both genes suggested that *T. unimaculatus* in Thailand were mixed between populations, causing by two different patterns of sea surface currents in the Andaman sea, which have a direct effect to the distribution of planktonic larvae of the lobsters. For future study, more *T. unimaculatus* samples from Pangnga and Satun provinces will be collected and population genetic analyses will be performed to study the genetic relationships within and among the populations.

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