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Phylogeography of the planktonic shrimp *Lucifer hanseni* Nobili 1905 in the Indo-Malayan Archipelago

MARY MAR NOBLEZADA[1,2,3], HIROOMI MIYAMOTO[4], WILFREDO L. CAMPOS[3], FATIMAH MD. YUSOFF[5] AND SHUHEI NISHIDA[1]

[1]Atmosphere and Ocean Research Institute, University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa 277-8564, Japan, [2]The Marine Science Institute, University of the Philippines Diliman, Velasquez St., 1101 Quezon City, Philippines, [3]OceanBio Laboratory, Division of Biological Sciences, College of Arts and Sciences, The University of Philippines Visayas, 5023 Miagao, Iloilo, Philippines, [4]Tohuku National Fisheries Research Institute, Fisheries Research Agency, 3-27-5 Shinhama, Shiogama 985-0001, Japan, [5]Department of Aquaculture, Faculty of Agriculture, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

Using partial sequences of two mitochondrial genes, cytochrome c oxidase subunit I (COI) and 12S ribosomal RNA (12S rRNA), and one nuclear gene, 28S ribosomal RNA (28S rRNA), we investigated population genetics of the holoplanktonic shrimp Lucifer hanseni *Nobili, 1905 in the Indo-Malayan Archipelago (IMA), encompassing Andaman Sea, Malacca Strait, Gulf of Thailand, Borneo Island, Philippines (hereafter collectively referred to as the Thailand-Malaysia-Philippine area: TMP), Celebes Sea (CS), and the waters near islands in the Western Pacific (WP) including Palau, Papua New Guinea and Solomon Islands. The samples from the TMP showed the highest number of haplotypes. Significant phylogeographic structure was found in the* L. hanseni *populations ($\Phi_{ST} = 0.832$ for COI, 0.159 for 12S rRNA, 0.783 for 28S rRNA). The total number of haplotypes was 46 in COI, 28 in 12S rRNA and 23 in 28S rRNA. The haplotype network analyses revealed two major clades for COI (subgroups: TMP + CS, WP) and for 12S rRNA and 28S rRNA (TMP, CS + WP). The CS and WP populations appeared isolated from the TMP populations. The samples from the CS showed low genetic diversity compared with the other samples at both haplotype and nucleotide levels, suggesting that the population CS experienced bottleneck events. This is the first demonstration of significant genetic structure of a holoplanktonic metazoan in IMA, which is suggested to be synergistically influenced by historical events (vicariance) and contemporary oceanographic circulations and corroborates the results of previous studies on other benthic/demersal animals with mero-planktonic phases.*

Keywords: *Lucifer hanseni*, Indo-Malayan Archipelago, phylogeography

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INTRODUCTION

The Indo-Malayan Archipelago is geologically dynamic and is considered a hotspot of marine biodiversity and speciation (Benzie, 1999; Carpenter & Springer, 2005). The region is also characterized by geographic and oceanographic complexity (Gordon & Fine, 1996), which played a major role in the evolution and ecology of various species (Benzie, 1999; Carpenter & Springer, 2005). Molecular and phylogeographic studies on marine organisms have shown that this region is of undeniable biogeographic and evolutionary importance for marine biodiversity (Barber *et al.*, 2000; DeBoer *et al.*, 2008). Concurrent patterns of maximum species richness across multiple taxa from larger organisms, e.g. corals, fish and invertebrates (Carpenter & Springer, 2005; Tittensor *et al.*, 2010) to minute zooplankton (Fleminger, 1986; Carpenter, 1998) have been found in this region.

The studies of molecular phylogeography of marine animals in the Indo-Malayan Archipelago commenced in the early 1990s and have focused mostly on larger organisms such as fish (McMillan & Palumbi, 1995) and macrobenthos (Benzie & Williams, 1997; Benzie, 1998; Williams & Benzie, 1998) with planktonic larval stages, but little attention has been paid to holoplankton. These studies have suggested a wide variety of population structures within and between biogeographic regions (Carpenter *et al.*, 2011). Sharp intraspecific genetic breaks have been observed for a number of species broadly distributed across the region (Williams & Benzie, 1998; Benzie, 1999; Barber *et al.*, 2002a, b), while apparent lack of such breaks has been shown for other species such as starfish *Acanthaster planci* (Benzie, 1999), pearl oyster (Lind *et al.*, 2012), mantis shrimp (Barber *et al.*, 2002a, b) and round scad mackerel (Borsa, 2003). The complex geological history of this region has been suggested as a major contributor to the observed phylogeographic patterns (Benzie, 1998), such as the tectonic movements (Hall, 2002), the recent emergence of the Philippines, and the formation and destruction of land bridges as a result of Pleistocene changes in sea level (Voris, 2000). The genetic structuring within this region may also have been impacted by oceanographic factors, as represented by the immense passage of water conveyed by

Corresponding author:
M.M. Noblezada
Email: mmpnoblezada@gmail.com

Table 1. Sampling stations of *Lucifer hanseni*.

Area	Station	Coordinates		Sampling gear
		Latitude	Longitude	
Gulf of Thailand	GT1	13.27°N	100.92°E	Hand scoop
	GT2	10.50°N	99.26°E	Hand scoop
	GT3	4.37°N	103.47°E	Hand scoop
Andaman Sea	AS20	7.85°N	98.37°E	Hand scoop
Malacca Strait	MS1	2.47°N	101.85°E	Hand scoop
Borneo Island,	MS2	1.33°N	103.60°E	Hand scoop
Malaysia	MB	6.87°N	116.61°E	Hand scoop
Philippines	PC	11.24°N	123.12°E	Horizontal tow
	PB	10.17°N	124.31°E	Horizontal tow
	PMB	9.30°N	125.20°E	Horizontal tow
Celebes Sea	C24	3.00°N	125.00°E	NORPAC
	C36	3.00°N	123.70°E	NORPAC
Western Pacific	WP4	5.30°N	134.30°E	IKMT
	WP6	5.30°N	136.00°E	IKMT
	WP14	5.30°N	146.80°E	IKMT
	WP20	5.00°S	161.00°E	IKMT
	WP22	10.00°S	162.20°E	IKMT

NORPAC (North Pacific) standard net, IKMT (Isaacs Kidd Midwater Trawl).

the Indonesian throughflow (ITF) (Lind *et al.*, 2012). Such geological and climatic changes could provide opportunities for population vicariance on a relatively small spatial scale (McManus, 1985), island-hopping (Williams & Benzie, 1997), range expansion by long-distance colonization across previously uncrossable barriers (Voris, 2000), and extinction and recolonization of continental shelf areas as a result of sea-level changes (Voris, 2000). Meanwhile, oceanographic factors could also drive diversification by either facilitating or restricting passive larval transport between populations and ultimately speciation in marine organisms in the region (Barber *et al.*, 2002a, b).

The planktonic shrimp *Lucifer hanseni* Nobili, 1905 belongs to the family Luciferidae (Decapoda, Sergestidae) distributed widely in the Indo-Pacific region (Dakin & Colefax, 1940; Huang & Fang, 1987; Grabe & Lee, 1992; Goswami & Shrivastava, 1996; Farfante & Kensley, 1997; Hashizume & Omori, 1998; Naomi *et al.*, 2006) and Atlanto-Mediterranean coast (Gurney, 1924; De Grave *et al.*, 2012), and particularly abundant in the coastal waters of the Indo-West Pacific region (Omori, 1977; Lin *et al.*, 1998; Tan *et al.*, 2004; Zhou & Xu, 2009). Because *L. hanseni* is an ecologically important component of the zooplankton, it is an ideal model to investigate intraspecific diversification, broad-scale patterns of genetic connectivity, and the extent of gene flow of zooplankton in this region. To our knowledge, there is no information available on genetic population structure of holoplanktonic metazoans in this region.

This study investigated the phylogeographic structure of *L. hanseni* in the Indo-Malayan Archipelago and the adjacent waters, encompassing the coastal waters of Andaman Sea, Malacca Strait, Gulf of Thailand, Borneo Island, Philippines, Celebes Sea, and the waters near islands in the Western Pacific including Palau, Papua New Guinea and Solomon Islands. In particular, we examined the genetic structure of *L. hanseni* using partial sequences of two mitochondrial genes, cytochrome *c* oxidase subunit I (*COI*) and 12S ribosomal RNA (12S rRNA), and one nuclear gene, 28S ribosomal RNA (28S rRNA). Both mitochondrial and nuclear DNA genes were utilized to gather more insight into the genealogical patterns evolving in genomes as a response to evolutionary history and processes that populations may have experienced (Hare, 2001; Hudson & Coyne, 2002; Brito & Edwards, 2009; Machida *et al.*, 2012).

MATERIALS AND METHODS

Sample collection and processing

Lucifer hanseni were collected in bulk zooplankton samples from 17 stations across the Indo-Malayan Archipelago using various types of gears (Table 1, Figure 1), fixed in 95–100% ethanol and identified under a stereomicroscope. The stations were grouped into seven areas based on geography: Andaman Sea (AS), Malacca Strait (MS), Gulf of Thailand (GT), Borneo Island (BI), Philippines (PH) (for descriptive convenience, these areas are hereafter collectively referred to as Thailand-Malaysia-Philippine area, TMP), Celebes Sea (CS), and waters near islands in the Western Pacific (WP) including Palau, Papua New Guinea and Solomon Islands.

DNA extraction, amplification and sequencing

DNA was extracted from individual specimens using a QIAGEN DNeasy blood and tissue kit (QIAGEN, Venlo, Limburg, the Netherlands). Partial nucleotide sequences of *COI*, 12S rRNA and 28S rRNA genes were amplified by polymerase chain reaction (PCR) using primers listed in Table 2 (Folmer *et al.*, 1994; Machida & Knowlton, 2010; Machida *et al.*, 2012). PCR amplifications were performed in 15-μL reactions containing 7.62 μL sterile H_2O, 1.5 μL 10× buffer, 1.2 μL 2.5 μM dNTPs (2.5 mM each), 1.8 μL each primer (5 μM), 0.08 μL *Z-taq* (TaKaRa Bio, Ohtsu, Japan), and 1.0 μL template. The thermal cycle profile consisted of 35 cycles of denaturation at 94°C for 5 s, annealing at 50°C for 5 s, and extension at 72°C for 30 s. The PCR amplifications were carried out in an ABI 9700 Thermal Cycler (Applied Biosystems, Foster City, CA, USA). PCR-amplified products were electrophoresed on 2% TBE L03 agarose gel (TaKaRa Bio), stained with ethidium bromide for DNA band characterization using ultraviolet transillumination to check if the target regions could be amplified. The PCR products were purified with ExoSap-IT (USB Corporation, Cleveland, OH, USA). Subsequently, the PCR products were used for direct cycle sequencing with dye-labelled terminators (Applied Biosystems). The primers used in the sequencing reactions were the same as those used in the initial PCR amplification. Labeled fragments were analysed on an ABI 3130 DNA Sequencer (Applied Biosystems).

Sequence and phylogenetic analysis

DNA was extracted from at least 20 individuals per station and PCR amplifications were carried out for all sampled individuals using selected primers for each gene. Only the non-ambiguous amplified DNA sequences were included in the analysis.

A total of 127 consensus sequences for *COI*, 257 for 12S rRNA, and 194 for nuclear 28S rRNA were generated after the assembly of forward and reverse sequences using Geneious version 5.3.4 (Drummond *et al.*, 2010).

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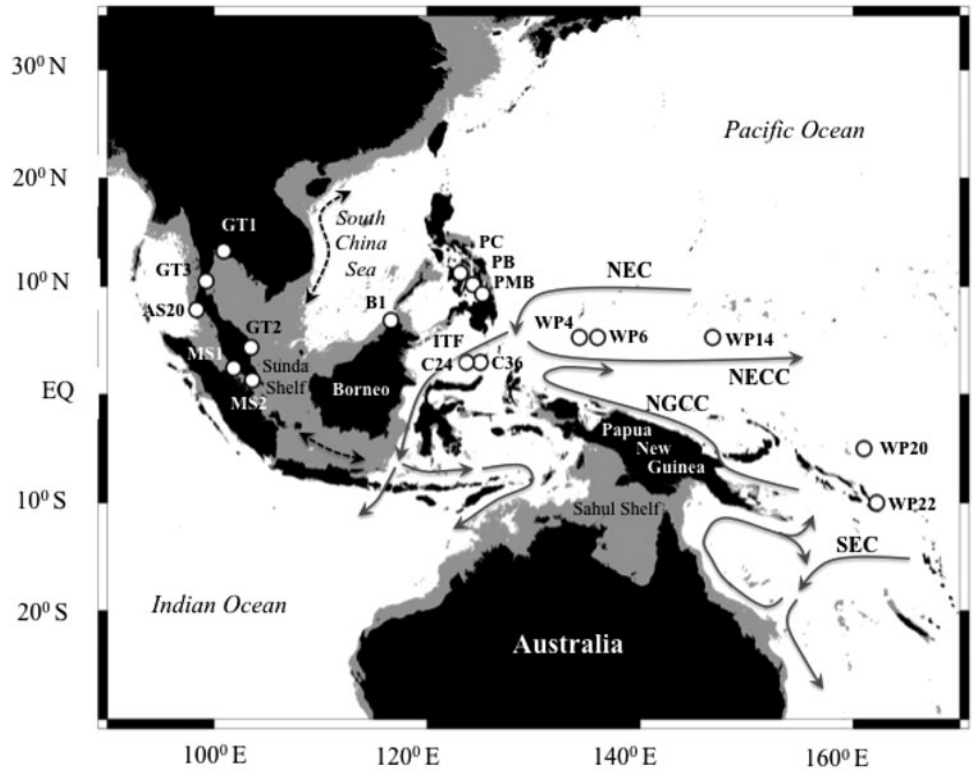

Fig. 1. Map showing sampling locations of *Lucifer hanseni* in the Indo-Malayan Archipelago. White dots represent collection sites presented in details in Table 1. Grey areas indicate the shoreline during the Pleistocene low sea-level stands (100 m below the present-day level, according to Voris, 2000). Black arrows indicate major ocean currents across the region, and dotted arrows show seasonally reversing currents. Indonesian Throughflow (ITF), North Equatorial Current (NEC), North Equatorial Counter Current (NECC), New Guinea Coastal Current (NGCC), and South Equatorial Current (SEC).

Table 2. List of PCR and sequencing primers used in the molecular analysis of *Lucifer hanseni*.

Primer	Sequence 5′–3′	
Mitochondrial region		
COI		Folmer *et al.* (1994)
HCO2198-COI	TAA ACT TCA GGG TGA CCA AA AAA TCA	
LCOI490-COI	GGT CAA CAA ATC ATA AAG ATA TTG G	
12S rRNA		Machida *et al.* (2012)
H13845-12S	GTG CCA GCA GCT GCG GTTA	
L13337-12S	YCT ACT WTG YTA CGA CTT ATC TC	
Nuclear region		
28S rRNA		Machida & Knowlton (2010)
28SF1	GCG GAG GAA AAG AAA CTA AC	
28SR1	GCA TAG TTT CAC CAT CTT TCG GG	

Subsequently the consensus sequences were aligned using Clustal X (Thompson *et al.*, 1997). The aligned multiple-sequence matrix of each gene after trimming was 379 bp long for *COI*, 279 bp for 12S rRNA, and 326 bp for 28S rRNA. DNA sequences were submitted to GenBank (accession numbers: LC003313–LC003491, LC003528–LC003595).

Phylogenetic analyses were performed using maximum likelihood (ML) and Bayesian analyses. The Akaike Information Criterion in MrModeltest 2.3 (Nylander, 2004) was used to identify an appropriate nucleotide substitution model. The best fit substitution models were GTR + I + G for *COI*, HKY + G for 12S rRNA, and GTR + I for 28S rRNA. The ML analysis was performed using 1000 bootstrap replications for nodal support in PhyML version 3.0 (Guindon & Gascuel, 2003). A Bayesian approach to phylogeny reconstruction was applied using MrBayes v. 3.1.2 (Ronquist & Huelsenbeck, 2003). The Markov Chain Monte Carlo chains were run for 10^6 generations with trees sampled every 100 generations. The first 25% of the generated trees were discarded as burn-in. The shrimp *Acetes chinensis*, a species of the sister family Sergestidae family was used as outgroup (accession number JN689221). Both are the only two classified families of superfamily Sergestoidea (Farfante & Kensley, 1997).

Population genetic analyses

Unless stated otherwise, all the analyses were conducted using Arlequin v. 3.5.1.2 (Excoffier *et al.*, 2007) and DNAsp 5.0. (Librado & Rozas, 2009). To explore genetic

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Table 3. Genetic diversity statistics and tests of selective neutrality for *Lucifer hanseni* (DNA regions: COI, 12S rRNA, 28S rRNA). Number of individuals sequenced (N_{ind}), number of haplotypes (N_H), number of polymorphic sites per population (N_P), haplotype diversity (H_D), and nucleotide diversity (π).

Area	No. of Stations	N_{ind}			N_H			N_P			H_D			π		
		COI	12S	28S	COI	12S	28S	COI	12S	28S	COI	12S	28S	COI	12S	28S
Gulf of Thailand (GT)	3	29	53	39	8	14	6	14	8	5	0.68	0.91	0.69	0.01	0.009	0.005
Andaman Sea (AS)	1	9	36	35	1	4	3	1	2	3	0.20	0.73	0.68	0.00	0.003	0.004
Malacca Strait (MS)	2	25	32	31	15	5	5	56	7	6	0.91	0.80	0.79	0.02	0.008	0.007
Philippines (PH)	3	18	29	43	10	2	5	36	2	4	0.90	0.44	0.71	0.02	0.003	0.003
Borneo Island (BI)	1	5	11	5	3	3	2	24	2	1	0.75	0.76	0.60	0.03	0.004	0.002
Celebes Sea (CS)	2	21	24	11	2	1	1	1	–	–	0.50	–	–	0.00	–	–
Western Pacific (WP)	5	20	72	30	13	7	6	53	4	4	0.94	0.80	0.78	0.06	0.004	0.004
Total	17	127	257	194	46	28	23									

diversity, the standard measures of genetic diversity including the number of haplotypes (H*n*), haplotype diversity (H*d*) and nucleotide diversity (π) for each area and clade identified were calculated. In order to gain appreciation of gene flow between populations and characterize the population structure, we estimated pairwise Φ_{ST} among and within areas and clades which were determined by phylogenetic analysis, and tested significance of these values with 10,000 random permutations and applied standard Bonferroni corrections.

In order to visualize the diversity and phylogenetic relationships among the different haplotypes and provide qualitative assessment of their geographic distributions, the median-joining (MJ) networks were constructed using Network v. 4.6.1 (http://www.fluxus-engineering.com/) (Bandelt *et al.*, 1999).

RESULTS

Mitochondrial *COI* and 12S rRNA

The *COI* sequences with length of 379 bp from 127 individuals collected from the seven areas yielded a total of 46 haplotypes (Table 3). Haplotype diversity within area ranged from 0.20 to 0.94, whereas nucleotide diversity ranged from 0.001 to 0.06 and the number of polymorphic sites ranged from 1 to 58. Phylogenetic analysis revealed two major clades: COI-A (with subgroups COI-A1 and COI-A2) and COI-B (Figure 2A). These clades were clearly shown in the phylogenetic tree (Figure 2A) and the haplotype network (Figures 3A and B), and with high bootstrap and posterior probability support in Bayesian and ML analyses. The largest group

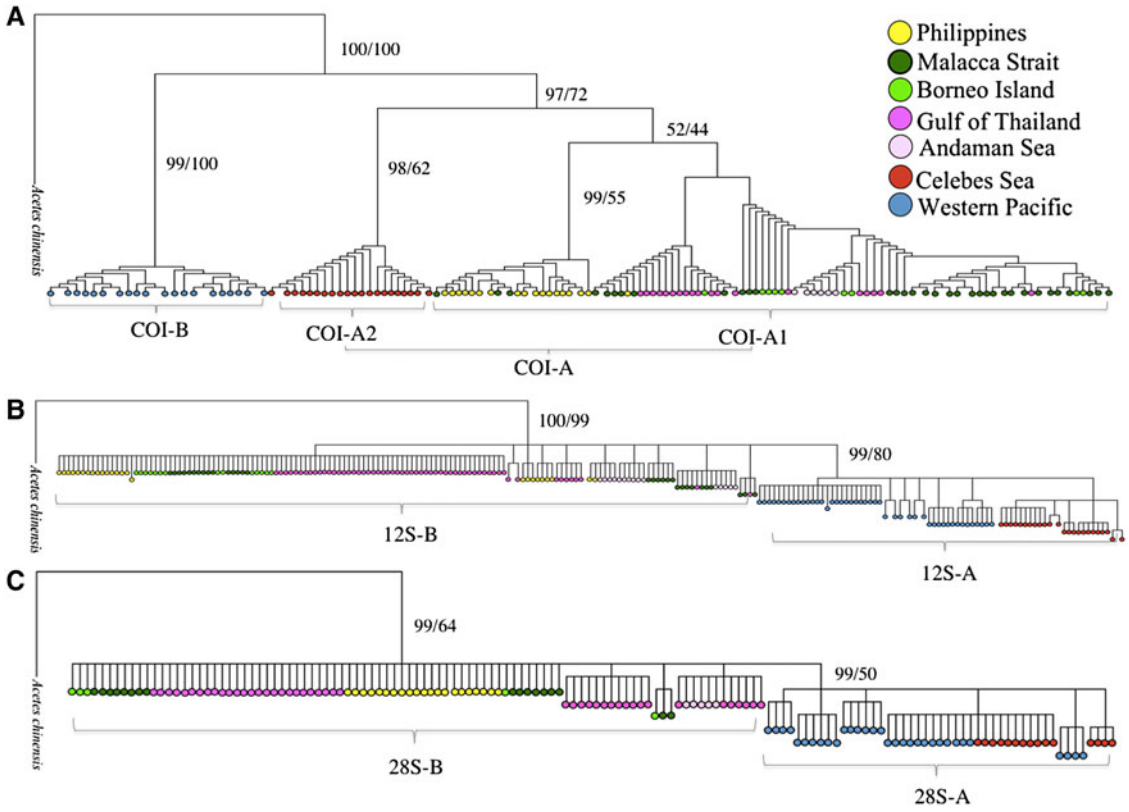

Fig. 2. Bayesian tree based on sequences of *COI* (A) 12S rRNA (B) and 28S rRNA (C) of *Lucifer hanseni*. Numerical values are Bayesian posterior probabilities (left) and maximum likelihood bootstrap values (right). For easy visualization, we have displayed the tree based on mitochondrial gene *COI* as a phylogram and those based on mitochondrial gene 12S rRNA and nuclear gene 28S rRNA as ultrametric trees.

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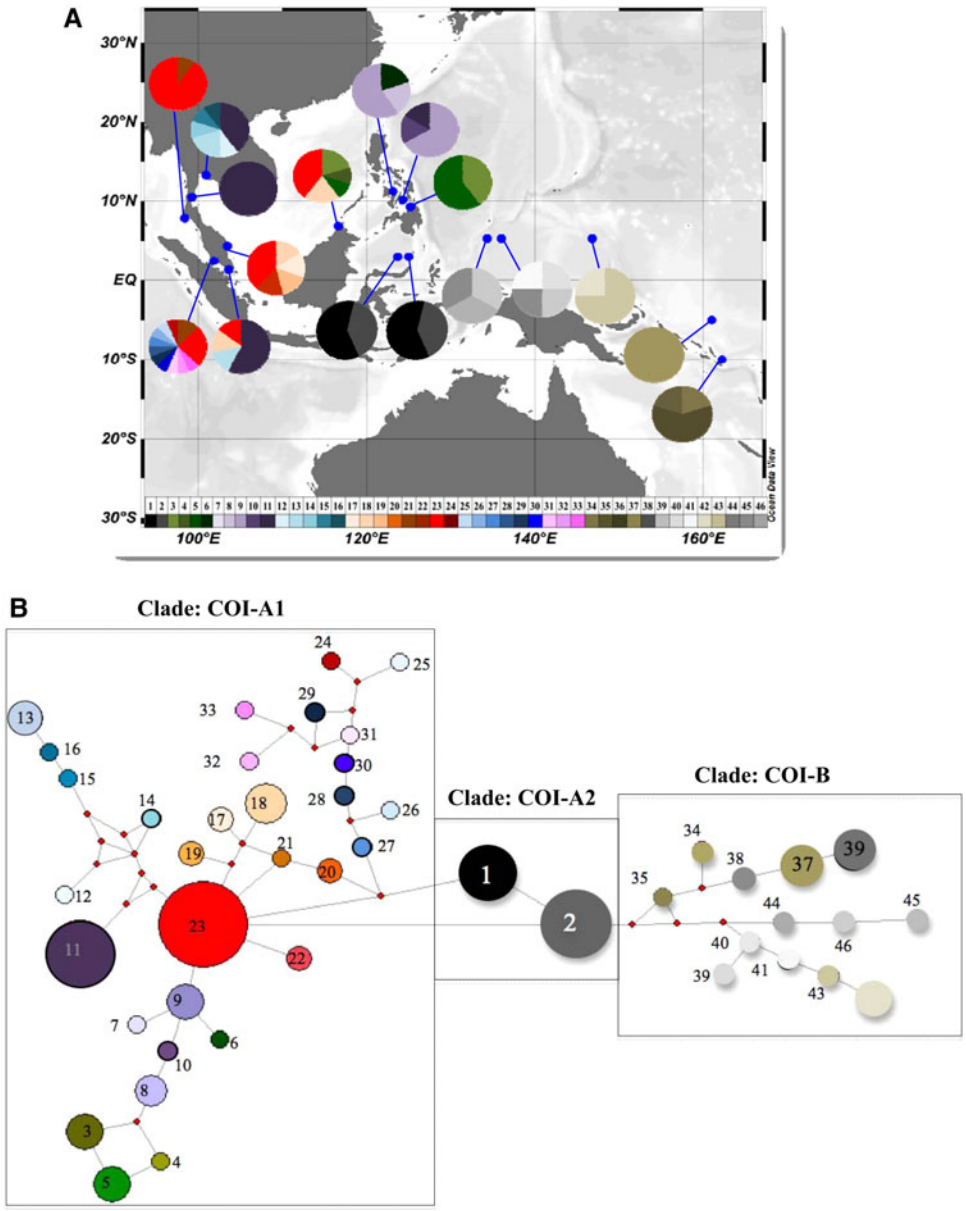

Fig. 3. Geographic distribution (A) and median joining network (B) of *COI* haplotypes of *Lucifer hanseni*. Shading indicates the frequency of occurrence of each haplotype. The numbers inside or at the side of each circle and the colour represent the unique haplotype corresponding to the legend. The circle size is proportional to the frequency of occurrence, ranging from 1–46. The black nodes denote the mutational step.

(COI-A) comprised 33 haplotypes and contained individuals from the TMP and CS (Table 4a) separated by one to five substitutions. Furthermore, this group exhibited the largest number of haplotypes, of which one to eight haplotypes were shared (COI: 2, 3, 5, 11, 13, 18 and 23) and the others were represented by a single individual. In contrast, only two haplotypes were found in the CS stations. These haplotypes were located near COI-B on the haplotype network but included in a highly supported monophyletic clade within COI-A on the phylogenetic tree. COI-B contained 13 haplotypes (Table 4) comprising individuals from the WP separated by one to three substitutions. All COI-B haplotypes appeared exclusive to each station.

Mitochondrial 12S rRNA exhibited 28 haplotypes from 257 sequences 279-bp long. Haplotype diversity within area ranged from 0.44 to 0.91, while nucleotide diversity ranged from 0.003 to 0.009 (Table 3). The occurrence of polymorphic sites was low, with values ranging from two to eight and no polymorphic sites in the CS populations. Two distinct clades (12S-A and 12S-B) were revealed with strong statistical support in the Bayesian and ML analyses (Figure 2B). Clade 12S-A comprised eight haplotypes and consisted of individuals from the WP and CS (Figures 4A, B, Table 4b). Meanwhile, clade 12S-B comprised individuals from TMP with 20 haplotypes (Figures 4A, B). The majority of the individuals found in the two clades were separated by a single substitution. Sixteen haplotypes were private (found in more than one individual, but only in one population). Sharing of haplotypes was apparent in 12S-B with eight haplotypes (12S: 1, 10, 12, 16, 17, 18, 19 and 20) shared either within stations or within regions, while four haplotypes (12S: 23, 24, 27, and 28) were shared in 12S-A clade (Figures 4A, B).

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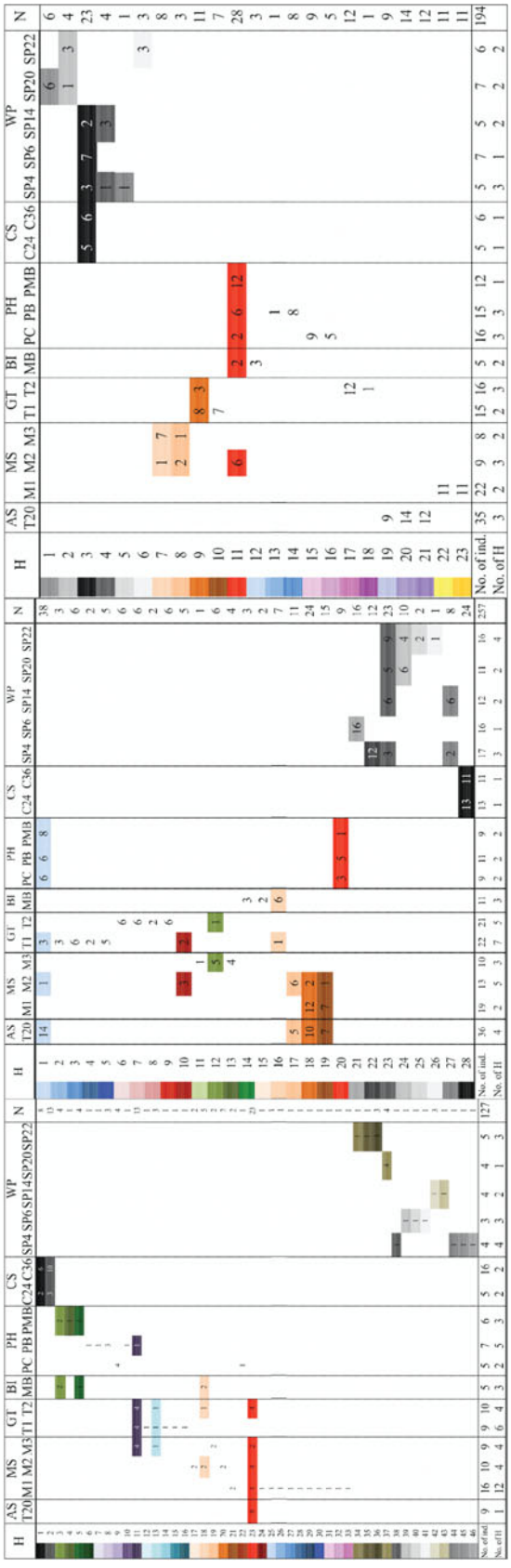

Table 4. Geographic distribution, frequency distribution and number of haplotypes of *Lucifer hanseni* for: (a) COI, (b) 12S rRNA, and (c) 28S rRNA. Shaded are haplotypes shared between stations and/or between areas.

Nuclear 28S rRNA

The sequences of the nuclear 28S rRNA yielded the lowest number of haplotypes (23, ranging from one to six) from 194 sequences 326-bp long (Table 3). The measure of genetic variability exhibited generally low diversity in both haplotypes and nucleotides compared with the mitochondrial genes (Table 3). The occurrence of polymorphic sites was also low, with values ranging from 1 to 7 sites. Phylogenetic reconstructions generated two clades, 28S-A and 28S-B, with high statistical support inferred by the Bayesian and ML analyses (Figure 2C). The haplotype network was similar to that of the mitochondrial 12S rRNA and separated only by a single substitution. 28S-A comprised populations from the CS and WP with six haplotypes, while 28S-B comprised 17 haplotypes that included individuals from the TMP (Figures 5A and B). Seven haplotypes were shared, three within 28S-A (28S: 2, 3 and 4), four within 28S-B (28S: 7, 8, 9 and 11), and 16 haplotypes were found to be private (Table 4c).

Genetic and phylogeographic structure

The analyses of pairwise population genetic differentiation (Φ_{ST}) revealed highly significant differences between areas (Table 5) and clades (Table 6). All comparisons remained significant even after Bonferroni corrections were employed. The CS and WP are strongly isolated and most differentiated from the TMP areas, while the latter are closely related. Furthermore the haplotype composition was different between stations. For example, just one sequence was shared in MS for COI, although 15 haplotypes were found. Also, the genetic differentiation was observed between north and south in WP region based on COI and 28S haplotype composition.

DISCUSSION

Marine holoplankton are often presumed to exhibit limited genetic structure and high gene flow among populations throughout their biogeographic range due to their extremely large population sizes, high dispersal potential and lack of obvious physical barriers in the pelagic realm (Palumbi, 1992; Norris, 2000). However, many empirical studies have detected genetic structure over a range of spatial scales including between basins, between gyres within ocean basin, regionally among coastal embayments and estuaries, and variation across different species even sometimes collected at the same site (Miyamoto *et al.*, 2010; Blanco-Bercial *et al.*, 2011; Chen & Hare, 2011; Goetze, 2011). These studies suggested that population genetic structure may be prevalent in marine zooplankton, contrary to prior predictions. It was suggested further that the observed genetic structures appeared to be linked to ecological and/or environmental requirements of the organisms as well as to biophysical and different oceanographic features.

This study presents the first phylogeographic and population-genetic analyses of a holoplanktonic metazoan species in the Indo-Malayan Archipelago, demonstrating the presence of a moderate to strong genetic population structure in *L. hanseni*. Possible factors contributing to the phylogeographic patterns observed in this study are discussed below with reference to the body of information from the previous studies.

The pattern of genetic structure along the TMP, which includes the Andaman Sea, Gulf of Thailand, Malacca Strait,

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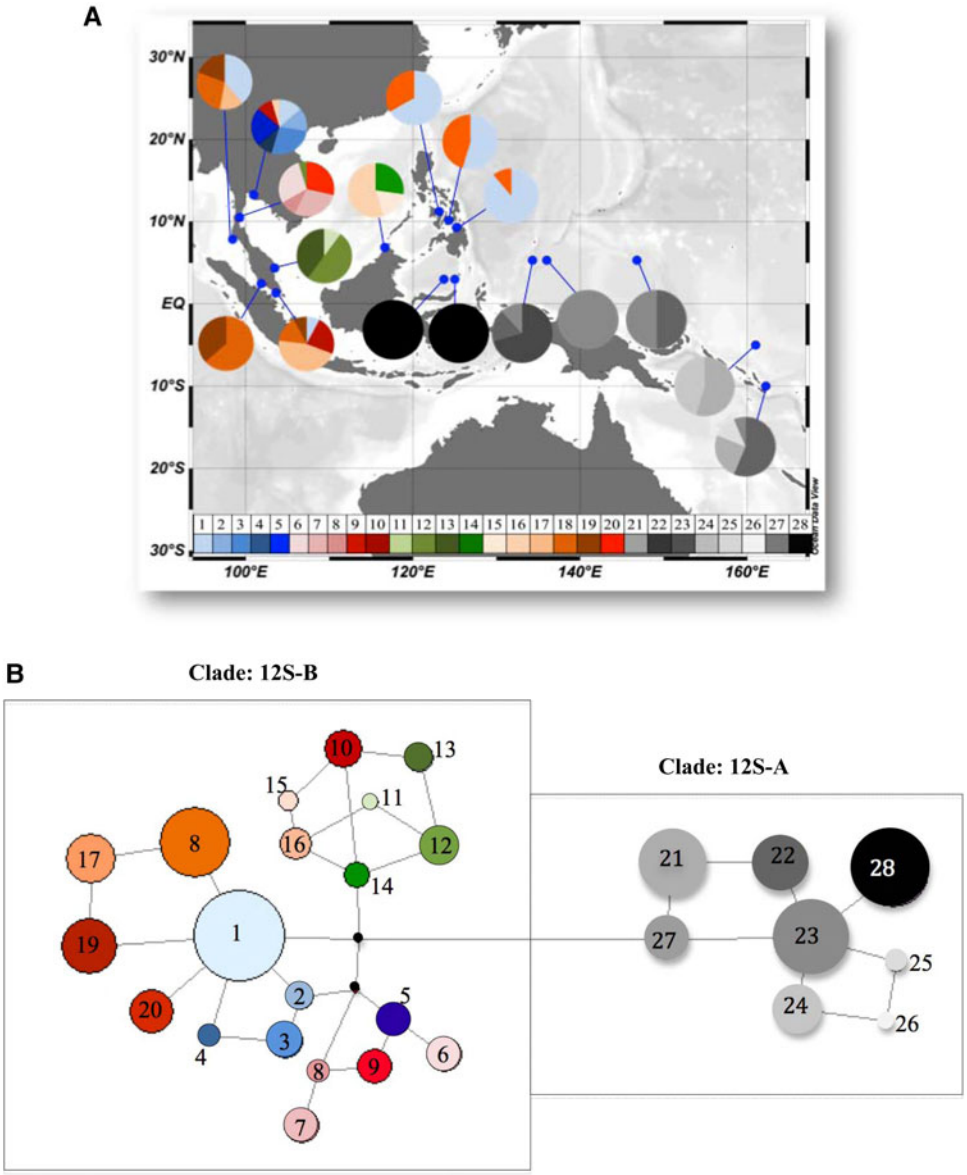

Fig. 4. Geographic distribution (A) and median-joining network (B) of 12S rRNA haplotypes of *Lucifer hanseni*. Shading indicates the frequency of occurrence of each haplotype. The numbers inside or at the side of each circle and the colour represent the unique haplotype corresponding to the legend. The circle size is proportional to the frequency of occurrence, ranging from 1–28. The black nodes denote the mutational step.

Borneo Island and the Philippines, imprints a mixed pattern of isolation (high number of haplotypes and high haplotype diversity) yet an ongoing gene flow (haplotype sharing). The signal of isolation was supported by the analysis of gene flow patterns through pairwise Φ_{ST} values (Table 5), which show differentiation among populations across the three genes. Also genetic differentiation appears to have occurred between the neighbouring stations within the region on the basis of haplotype frequency in each station. For *COI*, the present study might be unable to grasp all the haplotypes that occurred in a station, because the local diversity of haplotype was remarkably high. However, the results from all genes suggest that gene flow might be limited between stations within TMP even though they were close to each other geographically. This implies that TMP might be a high potential region for allopatric speciation. Furthermore, the mixed occurrence of haplotypes in the MJ network (Figures 3–5) and monophyletic clade trees (Figure 2) indicates the presence of gene flow within this area (Table 4), while the observed shared haplotypes between areas, although scattered, may suggest maintenance of the widespread ancestral haplotypes. Thus, it is possible that this area harbours remnants of the oldest as well as recent variations of this species. Such variations have been observed in other marine organisms in the area, consistent with the notion that this area contains a large number of marine organisms and high speciation opportunity (Benzie, 1998; Carpenter, 1998; Carpenter & Springer, 2005). The extensive, shallow, continental Sunda Shelf across the Gulf of Thailand is among the prominent marine biogeographic landmarks in the Indo-Malayan region (Kochzius *et al.*, 2009) and most likely played a significant role in shaping population genetic patterns in this species. After the last ice age, seawater filled the Gulf of Thailand and connected the Java and the Andaman Sea with the South China Sea (Woodruff, 2010).

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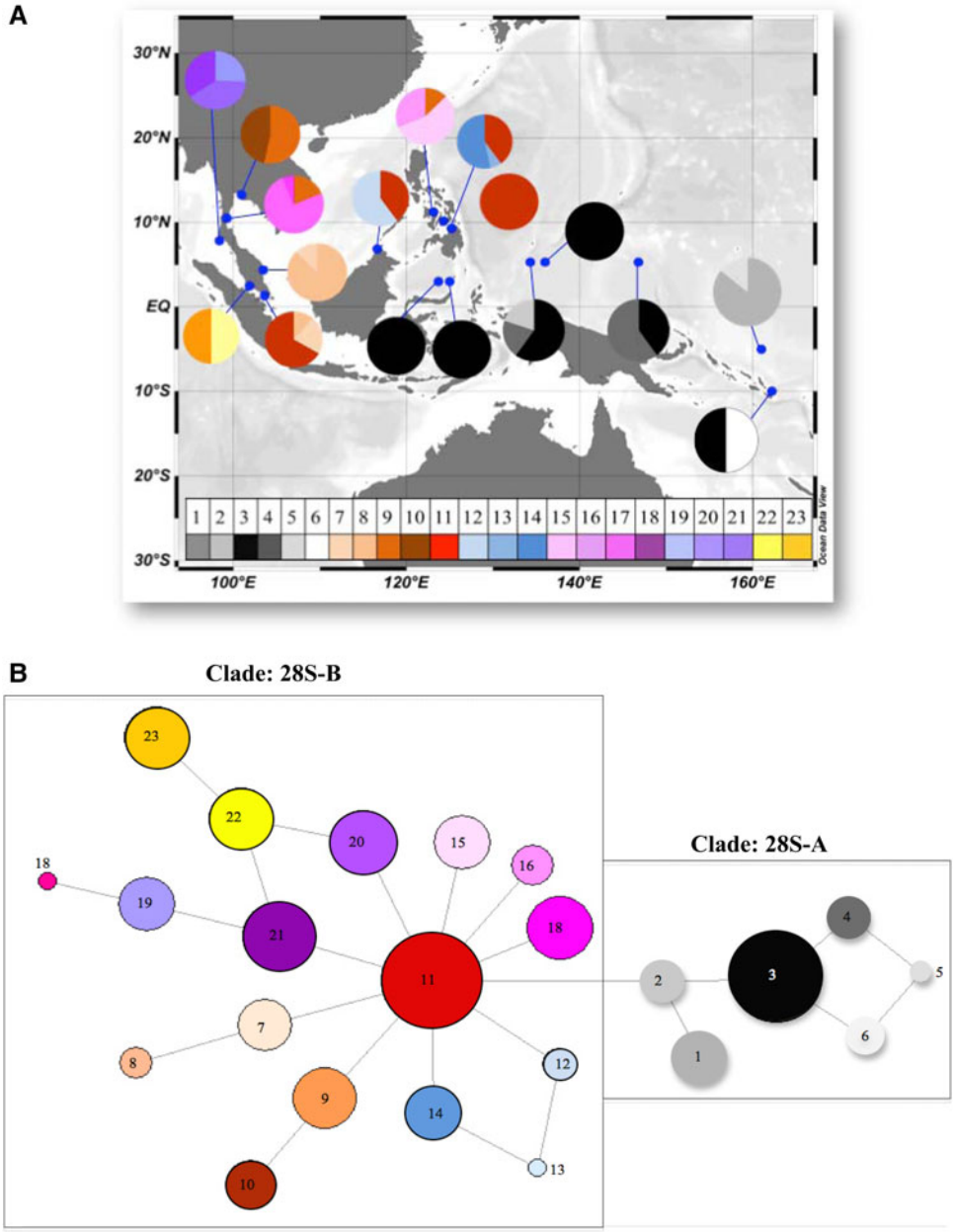

Fig. 5. Geographic distribution (A) and median-joining network (B) of 28S rRNA haplotypes of *Lucifer hanseni*. Shading indicates the frequency of occurrence of each haplotype. The numbers inside or at the side of each circle and the colour represent the unique haplotype corresponding to the legend. The circle size is proportional to the frequency of occurrence, ranging from 1–23.

This provided a favourable habitat for rapid re-colonization of previously exposed land with rising sea levels and could have intensified genetic differentiation of populations in the region through the founder effect (Hanebuth *et al.*, 2000). The latter is considered a significant contributor to genetic patterns in several marine species throughout this region (Lind *et al.*, 2012). The exposure of the Sunda Shelf during several Pliocene and Pleistocene glacial low sea-level stands may have consequently reduced the population size of the marine organisms. In contrast, the rising sea level in the interglacial periods initiated the colonization of new habitats, resulting in a demographic and spatial population expansion. Furthermore, the sharing of haplotypes in *L. hanseni* observed in the region may have been a result of a recent or ongoing migration process (range of dispersal or gene flow), which could have started only during the interglacial periods, while the contemporary oceanographic circulation may have contributed to the continuous connectivity of the populations in the region up to the present.

The CS population exhibited an interesting pattern with both unique haplotype and low nucleotide diversity (Table 3), while the other areas of TMP and WP exhibited moderate to high haplotype diversities. As mentioned above, the terrestrial area increased during the low sea level in the Pleistocene, suggesting that semi-enclosed seas, such as the Sulu and Celebes Seas, were more isolated from the adjacent seas during this period (Voris, 2000). It appears that the populations in these areas may have undergone a bottleneck and/or founder effect, which is attributable to the unique geographic and hydrographic feature of this area. The founder effect has been a popular hypothesis of

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Table 5. Pairwise Φ_{ST} distances between areas for *Lucifer hanseni*. Asterisks indicate significance level (P) calculated from 10,000 permutations: * for $P < 0.001$.

Area	*COI*							12S rRNA						28S rRNA					
	GT	AS	MS	P	BI	CS	WP	GT	AS	MS	P	BI	CS	WP	GT	AS	MS	P	BI	CS	WP
Gulf of Thailand (GT)	–							–							–						
Andaman Sea (AS)	0.76*	–						0.14*	–						0.44*	–					
Malacca Strait (MS)	0.51*	0.03	–					0.15*	0.07*	–					0.41*	0.14*	–				
Philippines (P)	0.79*	0.67*	0.55	–				0.15*	0.25*	0.23*	–				0.37*	0.38*	0.33*	–			
Borneo Island (BI)	0.58*	0.170	0.10*	0.36*	–			0.26*	0.19*	0.31*	0.43*	–			0.36*	0.40*	0.32	0.27	–		
Celebes Sea (CS)	0.90*	0.97*	0.72*	0.87*	0.84*	–		0.51*	0.59*	0.51*	0.69*	0.76*	–		0.83*	0.80*	0.69	0.84	0.75	–	
Western Pacific (WP)	0.73*	0.72*	0.78*	0.72*	0.66*	0.72*	–	0.15*	0.23*	0.21*	0.22*	0.35*	0.49*	–	0.72*	0.75*	0.67	0.77	0.960	0.120	–

Table 6. Pairwise Φ_{ST} distances between major clades for *Lucifer hanseni*. DNA regions: COI, 12SrRNA, 28SrRNA. * Indicates significant differences for $P < 0.001$.

Clade	Φ_{ST}	
	COI-A1	**COI-A2**
COI-A1	–	
COI-A2	0.59*	–
COI-B	0.72*	0.72*
	12S-A	12S-B
12S-A	–	
12S-B	0.77*	–
	28S-A	28S-B
28s-A	–	
28s-B	0.66*	–

the origin of species featuring geographic isolation and population differentiation in many organisms (Coyne & Orr, 2004), such as the reef sponge *Leucetta chagosensis* (Wörheide *et al.*, 2005) and stomatopod *Haptosquilla pulchella* (Barber *et al.*, 2002a, b).

The clear isolation of WP from TMP and CS was indicated by haplotype frequency of mitochondrial and nuclear genes. Furthermore the monophyletic tree of TMP and WP populations was supported by high bootstrap values, suggesting highly limited gene flow between them. The observed low gene flow and genetic divergence suggests that the highly divergent WP clade evolved due to limited genetic exchange over an extended period of time and limited dispersal, which was attributed to low water transport from Papua New Guinea to the Asian region and Indian Ocean even at high sea levels (Lind *et al.*, 2012). The low water transport is in turn attributable to the retroflection of the South Pacific waters by the Halmahera Eddy (Morey *et al.*, 1999; Carpenter *et al.*, 2011). The present results support the hypothesis of limited dispersal and connectivity because these regions belong to different current systems and the population might have been genetically segregated compared with those within the TMP area. The limited dispersal or gene flow in the areas, especially between CS and WP, has been reported in various marine organisms, e.g. stomatopod *Haptosquilla pulchella* (Barber *et al.*, 2002a, b), starfish *Protoreaster nodusus* (Williams & Benzie, 1998), giant clams *Tridacna crocea* and *Tridacna maxima* (DeBoer *et al.*, 2008), and gastropods *Nerita albicilla* and *Nerita plicata* (Crandall *et al.*, 2008). However, dispersal is still possible between the WP and CS via the ITF (Williams & Benzie, 1998), albeit at a reduced rate.

In holoplankton, despite lack of obvious geographic barriers and dispersal capabilities, many molecular studies have revealed abundant cryptic species diversity such as chaetognaths (Peijnenburg *et al.*, 2004; Miyamoto *et al.*, 2010) cnidarians (Dawson & Jacobs, 2001) and copepods (Blanco-Bercial *et al.*, 2011; Chen & Hare, 2011; Goetze, 2011). Among them, some species previously thought to be cosmopolitan were found to consist of multiple diversified genetic lineages. In the present study, the robust allopatric differentiation supported by mitochondrial and nuclear genes were found between WP and TMP. However a reproductive isolation might have not occurred between these populations because genetic distance was remarkably lower than those commonly observed between congeneric species of crustaceans (Herbert *et al.*, 2003).

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CONCLUSIONS

In conclusion, molecular data of both nuclear and mitochondrial DNA of the holoplanktonic shrimp *Lucifer hanseni* is characterized by high genetic diversity, exhibiting different genetic lineages within the Indo-Malayan Archipelago and its vicinities, which is consistent with major patterns reported on various marine organisms in the region. The phylogeographic structure concords well with the geographic regions effectively isolated during the lowering of sea level associated with Pleistocene glaciation and by contemporary oceanographic circulations. The most salient genetic patterns are (1) high genetic diversity centred in the TMP area suggesting a high speciation opportunity for this species, (2) dramatic decrease in genetic diversity in the CS populations, which is probably due to a founder/bottleneck effect by the lowering of sea level associated with Pleistocene glaciation, and (3) distinct and highly divergent WP populations attributed to the historical and prevailing oceanographic conditions. The results show that genetic differentiation and isolation can be attained even in species with high dispersal capability and large population size in a seemingly continuous habitat. How these identified key features affect the genetic structure and pattern of gene flow across the Indo-Malayan Archipelago merits further study. In particular, the analysis of additional samples from Indonesia may provide a more comprehensive perspective of the pattern of gene flow and structure across the region.

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Correspondence should be addressed to:
M.M. Noblezada
Atmosphere and Ocean Research Institute, University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa 277-8564, Japan
email: mmpnoblezada@gmail.com

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