Zoological Studies **60:** 8 (2021)
doi:10.6620/ZS.2021.60-08

Zoological Studies

Open Access

Mitochondrial Markers Identify a Genetic Boundary of the Green Tiger Prawn (*Penaeus semisulcatus*) in the Indo-Pacific Ocean

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Received 25 September 2020 / Accepted 31 December 2020 / Published 18 March 2021
Communicated by Ka Hou Chu

A population genetics study of the commercially important Green Tiger Prawn (*Penaeus semisulcatus*) was conducted in the Indo-Pacific Ocean with a focus on the Indo-Malay Archipelago waters of the South China Sea (SCS), Sulu Sea (SLS), Celebes Sea (CLS) and the Strait of Malacca (SOM), the latter being the main waterway that connects the Indian Ocean with the Pacific Ocean. A 548-base-pair region of mitochondrial *COI* and 571 base pairs of the control region (CR) were analysed in 284 specimens from 15 locations. Genetic divergences (Tamura 3-parameter) for *COI* ranged from 0.1% to 7.2% and CR 2.3% to 21.7%, with Bagan Pasir (BGP) in central SOM being the most genetically different from other populations (*COI*: 3.3–4.2%; CR: 7.1–16.5%). All populations were differentiated into two lineages with a genetic break in the vicinity of BGP; Lineage I comprised populations south of this site (SCS, SLS, CLS and part of SOM) and Lineage II comprised populations north of BGP (part of the SOM). Specifically, most individuals of Bagan Pasir (BGP) and another site just south of it, Batu Pahat (BPT), clustered in Lineage I, while all SOM populations to the north of these sites clustered in Lineage II. The BGP population is believed to be a mixed gene pool between the two lineages. The results could be attributed to the fluctuations of Pleistocene sea levels and a possible influence of the One Fathom Bank in SOM. High genetic diversity was recorded, π (Lineage I: *COI*: 3.4%; CR: 7.4%) (Lineage II: *COI*: 3.8%; CR: 12.6%) and, *h* (Lineage I: *COI*: 0.81; CR: 1.0) (Lineage II: *COI*: 0.57; CR: 0.99). Demographic statistics revealed that both lineages underwent a sudden expansion and consequent stabilisation in genetic variability. The findings of this study have wide implications for fisheries in the Indo-Pacific. The increased sampling effort within a narrower geographical scale by the current study permitted a precise locality of the genetic break for this species within the Indo-Pacific Ocean to be identified. The substantial genetic diversity within both lineages should be considered in fishery management and aquaculture development programs of this species in this region.

Key words: *Penaeus semisulcatus*, Cytochrome oxidase subunit I (*COI*), Control region (CR), Population genetics, Indo-Pacific.

Citation: Abdul Halim SAA, Othman AS, Akib NAM, Jamaludin NA, Esa Y, Nor SAM. 2021. Mitochondrial markers identify a genetic boundary of the Green Tiger Prawn (*Penaeus semisulcatus*) in the Indo-Pacific Ocean. Zool Stud **60:**8. doi:10.6620/ZS.2021.60-08.

BACKGROUND

Knowledge of the population genetics of a species—including its distribution and changes in genotype and phenotype frequencies and the underlying causes—provide information that can answer various biological questions. Understanding population genetic diversity and structure is essential for the management and conservation of genetic resources in marine organisms and for productive fisheries conservation and sustainable population harvesting (Park and Moran 1994; Hillis et al. 1996; Thorrold et al. 2002; Xiao et al. 2012; Cui et al. 2018). Such population genetic data can contribute additional information for taxonomic and evolutionary historical analyses (Benzie et al. 1995; Uthicke and Benzie 2003; Cheng et al. 2018), as well as information on demographic history, such as effective population size (Fu 1997) and changes in population size (Rogers and Harpending 1992), critical for fisheries and aquaculture management, particularly of ecologically and commercially important groups such as the penaeids. Identification of reproductively isolated and genetically differentiated populations of existing resources within an exploited species is critical for planning conservation strategies (Brooker 2009; Schröder and Walsh 2010).

Due to their ecological and economic importance, the family Penaeidae has been the subject of considerable biological and genetic research. Dall et al. (1990) hypothesised that the genus *Penaeus* within family Penaidae, which is comprised of some of the most economically important species, arose in the Indo-Pacific based on the principle that biogeographic centres of origin have the highest species diversity and the deepest morphological differentiation (Briggs 1999). This was supported by the study of Baldwin et al. (1998), which showed that the highest mtDNA diversity and deepest mtDNA lineages for *Penaeus* are found in this region, based on 14 species. They further postulated that the genus *Penaeus* radiated westward into the eastern Atlantic and eastward into the eastern Pacific/western Atlantic. This bidirectional migration theory has received considerable support. Phylogenetic studies of *P. kerathurus* (eastern Atlantic) (Lavery et al. 2004) and *P. japonicus* (Indo-Pacific) (Tzong et al. 2004; Tsoi 2006; Shih et al. 2011) corroborated the hypothesis of the westward movement from the Indian Ocean into the eastern Atlantic. Similarly, several studies have documented a close relationship between the *P. monodon* lineages of the eastern Pacific/western Atlantic and the Indo-Pacific region, indicating the eastward radiation across the Pacific (Benzie et al. 2002; You et al. 2008; Waqairatu et al. 2012; Munasinghe 2014; Abdul-Aziz et al. 2015).

The green tiger prawn or grooved tiger prawn, *Penaeus semisulcatus* De Haan, 1844, is one of the commercially important species in the family Penaeidae. It is widely distributed in the Indo-West Pacific from the Red Sea, east and southeast Africa to Japan, Korea, the Malay Archipelago and northern Australia (Holthius 1980; Dall et al. 1990; Chan 1998). The species is widely distributed in the seas of the Indo-Malay Archipelago (IMA); Strait of Malacca, (the main channel connecting the Indian Ocean with the Pacific Ocean), South China Sea, Celebes Sea and Sulu Sea, the latter three are within the Pacific Ocean. A number of population genetic investigations of penaeids prawn in the Indo-Pacific region have been done—*e.g.*, for the tiger prawn, *P. monodon* (Duda and Palumbi 1999; Benzie et al. 2002; You et al. 2008; Waqairatu et al. 2012; Abdul-Aziz et al. 2015); kuruma prawn, *P. japonicus* (Tzong et al. 2004; Tsoi et al. 2007); brown tiger prawn, *P. esculentus* (Ward et al. 2006); banana prawn, *P. merguiensis* (Hualkasin et al. 2003; Wanna et al. 2004); and Indian prawn, *P. indicus* (De Croos and Pálsson 2010; Alam et al. 2015); these have displayed strong phylogeographic structuring among populations in the Indo-West Pacific region that have revealed a clear distinction between populations in the Indian Ocean and Pacific Ocean. This suggests that a similar pattern of population structuring may also occur in the widespread *P. semisulcatus* species. However, population genetics studies of the *Penaeus* are limited, particularly in the region. Most penaeid studies in the region have focused on the giant tiger prawn, *P. monodon*, and banana prawn, *P. merguiensis* (Daud 1995; Aziz 2011; Aziz et al. 2011; Nahavandi et al. 2011a b). Indeed, broad-scale phylogeographic division of this species between the Indian Ocean and Pacific Ocean has also been shown, but the genetic boundary remains obscure, primarily due to the lack of detailed coverage of the connecting populations between the two oceans (Alam et al. 2016).

Thus, the aim of this study was to identify the potential location of this genetic break in the Indo-Pacific Ocean, based on the mitochondrial cytochrome *c* oxidase I (*COI*) and control region (CR) genes, on a narrower geographical scale in the IMA. The *COI* gene (Folmer et al. 1994) is widely used because it is robust, is efficiently amplified using a universal primer, and has been successfully applied to reconstruct phylogenetic relationships among several *Penaeus* species, including the Indian white prawn, *P. indicus* (De Croos and Pálsson 2010; Alam et al. 2015); banana prawn, *P. merguiensis* (Hualkasin et al. 2003); kuruma prawn, *P. japonicus* (Tsoi 2006); and Chinese white prawn, *P. chinensis* (Li et al. 2009). The mitochondrial control region (CR) or D-loop is a non-coding protein and

commonly used for population studies due to its highly variable and rapid evolution (Upholt and Dawid 1977; Walberg and Clayton 1981; Chu et al. 2003). This gene has also found applicability when elucidating population variability and relationships in several *Penaeus* species: giant tiger prawn, *P. monodon* (Zhou et al. 2009; Alam et al. 2016); kuruma prawn, *P. japonicus* (Tsoi 2006; Tsoi et al. 2007); Chinese white prawn, *Penaeus chinensis* (Cui et al. 2007; Xiao et al. 2010); and pink prawn, *Penaeus duorarum* (McMillen-Jackson and Bert 2004b).

Although several population genetic studies have been reported on *P. semisulcatus* based on mitochondrial DNA and nuclear markers (Munasinghe and Senevirathna 2015; Alam et al. 2017; Jahromi et al. 2019), none have been within the IMA waters. Thus, the present study aimed to characterize the genetic diversity and population structure of *P. semisulcatus* populations within the IMA (Strait of Malacca, South China Sea, Sulu Sea and Celebes Sea) inferred from mtDNA *COI* and control region (CR) genes. This represents the first detailed population genetics study of the green tiger prawn, *P. semisulcatus*, in IMA waters. The combined application of the *COI* (DNA barcoding) gene and control region could provide useful insights into potential cryptic diversity and population structuring.

MATERIALS AND METHODS

Sample collection and species identification

Specimens of *P. semisulcatus* were collected from landing sites of 15 locations in the IMA, focusing on the Strait of Malacca, South China Sea, Celebes Sea and Sulu Sea in 2016 and early 2017 (Table 1 and Fig. 1). The prawn specimens were identified through assistance of a taxonomist and were identified using the following references; A guide to the Australian penaeid prawns (Grey et al. 1983), A guide to penaeid shrimps found in Thai waters (Chaitiamvong and Supongpan 1992) and FAO Species Identification Guide for Fishery Purposes (Chan 1998). All specimens were immediately iced or frozen after collection and later stored at -20°C or preserved in 99% ethanol until DNA extraction.

DNA Extraction and PCR Amplification

Total DNA were extracted from the pleopod tissue of the preserved samples based on the 2xCTAB method (Doyle 1991). The *COI* and CR gene fragments were amplified using a penaeid-specific primer COIf (5'-TAA CCT GCA GGA GGA GGA GAY CC-3') (Palumbi and Benzie 1991) and COI-P4 (5'-AGG AAA TGT TGA GGG AAG AAA GTA A-3) (Tong et al. 2000) for *COI* gene and 12S (5'-AAG AAC CAG CTA GGA TAA AAC TTT-3') and PCR-1R (5'-GAT CAA AGA ACA TTC TTT AAC TAC-3') (Chu et al. 2003) for the control region. The PCR amplifications for each of the two gene fragments were performed in a reaction mixture containing 1.5 µL DNA template, 0.5 µL of each primer, 2.5 µL of 10x *i*-Taq™ plus PCR Buffer, 2.0 of 25 mM $MgCl_2$, 1.0 µL of dNTP, 0.25 µL of *i*-Taq™ plus DNA Polymerase and 16.75 µL of distilled water (ddH_2O), adding up to 25 µL. The PCR cycling conditions for *COI* and CR was conducted according to the following thermal cycling profile: 4 min at 94°C, 35 cycles of 30 s at 94°C for denaturation, 50 s at 50°C (*COI*) and 48.8°C (CR) for annealing and 1 min at 72°C for extension followed by a final step of 7 min at 72°C for the complete fragment extension. The PCR products were sent to the service provider, First BASE Laboratories Sdn. Bhd., for sequencing in both the forward and reverse directions with an automated sequencer (ABI3730XL, Applied Biosystems USA).

Data Analysis

Nucleotide alignment

Both forward and reverse *COI* and CR sequences were aligned using ClustalW in MEGA 7 (Nei and Kumar, 2000; Kumar et al. 2016). The aligned sequences for *COI* were translated into proteins to ensure accurate alignment and to detect stop codons, if present. The haplotype distribution for sampled data was summarized using DnaSP 5.10 (Librado and Rozas 2009). Sequence data for both genes were analysed based on the Maximum Likelihood (ML) method in MEGA 7 (Kumar et al. 2016) to produce phylogenetic trees with a confidence level of 1000 bootstrap replicates. Prior to this, the Model Test was conducted to determine the best model for tree construction using MEGA 7 (Kumar et al. 2016). The Bayesian Information Criterion method was run to construct a Bayesian Inference tree phylogeny with the Markov Chain Monte Carlo (MCMC) algorithm (Ronquist et al. 2012). PartitionFinder2 (Lanfear et al. 2017) was used to determine the best-fit partitioning schemes and models of molecular evolution for phylogenetic analysis. The Bayesian Inference (BI) tree was run in MrBayes 3.2.6 (Ronquist et al. 2012) on CIPRES Science Gateway (Miller et al. 2010) together with gene partitions, 1 million MCMC chains and 50% burn in. The output files generated from the analyses were examined in Tracer v.1.6 (Rambaut et al. 2014) to assess the MCMC chain. The generated tree was displayed in FigTree v.1.4.3 (Rambaut 2016) and the complete

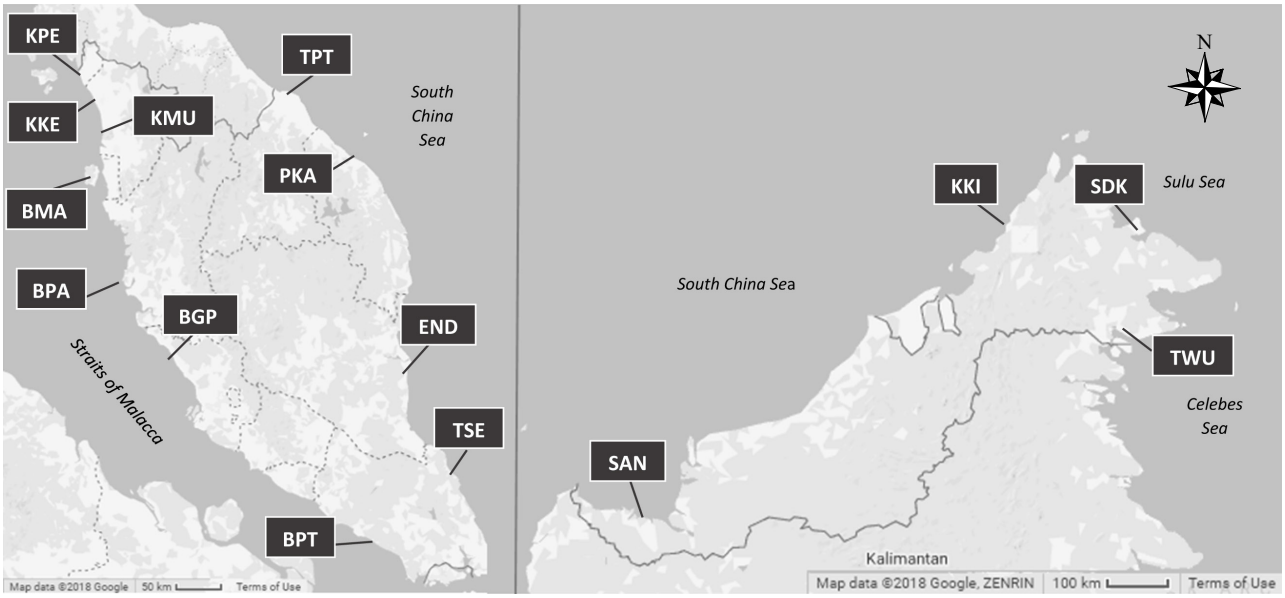

Fig. 1. Sampling locations of fifteen *Penaeus semisulcatus* populations amplified for mtDNA *COI* and control region gene analysed in the present study.

Table 1. Sampling location, sample size and number of haplotypes of 15 *Penaeus semisulcatus* populations based on mtDNA *COI* and CR

No	Population	Abbreviation	N		No. of haplotypes	
			COI	CR	*COI*	CR
Group 1: Straits of Malacca (SOM), West Coast of Peninsular Malaysia						
1.	Kuala Perlis	KPE	18	17	3	16
2.	Kuala Kedah	KKE	19	19	7	16
3.	Kuala Muda	KMU	19	17	10	17
4.	Batu Maung	BMA	18	14	5	14
5.	Bagan Panchor	BPA	15	15	8	14
6.	Bagan Pasir	BGP	23	20	11	20
7.	Batu Pahat	BPT	23	20	7	20
Group 2: South China Sea (SCS), East Coast of Peninsular Malaysia and Sabah and Sarawak, Malaysian Borneo						
8.	Tumpat	TPT	20	19	13	18
9.	Pulau Kambing	PKA	19	16	13	16
10.	Endau	END	20	17	13	17
11.	Tanjung Sedili	TSE	20	20	10	20
12.	Santubong	SAN	11	11	5	11
13.	Kota Kinabalu	KKI	20	20	11	20
Group 3: Sulu Sea (SLS), Sabah, Malaysian Borneo						
14.	Sandakan	SDK	19	18	11	18
Group 4: Celebes Sea (CLS), Sabah, Malaysian Borneo						
15.	Tawau	TWU	20	20	12	20
TOTAL			284	263	90	246

mitochondrial sequence of the giant tiger prawn, *P. monodon* (AF217843), sequence was selected from GenBank as an outgroup to root the phylogenetic trees. In addition, the Minimum Spanning Network (MSN) version 5.0.0.3 (Polzin and Daneshmand 2018) was used to display the haplotype relationships based on median joining or MJ network algorithm (Bandelt et al. 1999) together with reduction options.

Genetic diversity and Demographic history

The genetic diversity of these populations were examined using two estimators: haplotype diversity (Hd) and nucleotide diversity (p) in Arlequin 3.1 (Excoffier et al. 2005). Tajima's D (Tajima 1989) and Fu's Fs (Fu 1997) analyses were examined to evaluate the deviation from neutrality of the observed variation in the same software. Mismatch distribution analysis and goodness of fit assessed by the Harpending's raggedness index, *Hri* (Harpending 1994), were performed to evaluate demographic patterns in the major regional groups as identified by the median joining network and phylogenetic trees using DnaSP 5.10 (Librado and Rozas 2009). A non-significant result of *Hri* index analysis indicates an expanding population (Harpending 1994).

Population structure analysis

Analysis of Molecular Variance (AMOVA) was conducted to evaluate the genetic variation at different hierarchical levels using Arlequin 3.1 (Excoffier et al. 2005). Isolation by Distance (IBD) analysis (Mantel Test) was also tested to assess any significance in isolation by distance relationships (Bohonak 2002) using the same software. To determine groups of populations that are geographically homogenous and maximally differentiated from each other, and the genetic barriers between these groups a Spatial Analysis of Molecular Variance (SAMOVA) was performed in SAMOVA 2.0 (Dupanloup et al. 2002). The optimal number of groups, k (with maximum was $k = 12$), was chosen based on the number that has the highest among group (F_{CT}) variance. Significant values were adjusted for family-wise error rate using the False Discovery Rate Procedure at $\alpha = 0.05$ (Verhoeven et al. 2005).

RESULTS

A total of 284 individuals for *COI* and 263 individuals for CR specimens from 15 localities were successfully amplified. Sample size per site ranged from 11 to 23, with an average of 19 individuals per population available for further genetic analysis (Table 1).

Nucleotide composition

The sequence analysis for *COI* generated a 548-base-pair region from the 284 specimens with 89 variable sites—58 parsimony informative and 31 singleton—generating 90 haplotypes (Table 1). The average nucleotide composition for the *COI* gene was A: 27.7%, T: 37%, G: 17.4%, C: 17.9%. Sequence analyses for CR generated a 571 base pair region from the 263 specimens with 252 variable sites, 194 parsimony informative and 58 singleton sites, generating 247 haplotypes. The average nucleotide composition was, A: 41.7%, T: 41.6%, G: 7.7%, C: 9%. Most unique sequences have been deposited in GenBank with accession numbers for *COI* (MG020144–MG214725) and CR (MG214707–MG544283).

Haplotype distribution

Haplotypes 1 (22.9%) and 27 (27.8%) were the most dominant for the *COI* gene. Haplotype 1 was represented by all populations from the Strait of Malacca (SOM) except for BPT (southern SOM), and a few specimens from BGP. Haplotype 27 represented specimens from the South China Sea (SCS), Sulu Sea (SLS) and Celebes Sea (CLS), and included the SOM population of BPT and several individuals of BGP (SOM). Sixty-three haplotypes were private or population-specific, while the other 27 haplotypes were shared by more than one population. However, in CR gene, apart from a few exceptions, each of the 247 mtCR haplotype generated was restricted to only a single population with none being dominant. Of these 247 haplotypes, only seven are shared between two or more populations, which were mainly limited to the coast of the Straits of Malacca.

Phylogenetic relationships among haplotypes

The best-fit Model Selection for ML for both genes was Tamura 3-parameter (T92+G) with a discrete gamma distribution. Two lineages were generated, Lineage I and Lineage II, in both *COI* (Fig. 2A) and CR (Fig. 2B) (only selected representative haplotypes are shown) genes with high support (BP > 92%). Lineage I is composed of the SCS, SLS and CLS populations and the unexpected inclusion of BPT (southern SOM) and most of the BGP specimens (central SOM). Lineage II is composed of all SOM populations to the north of BGP and the rest of the BGP specimens (of SOM) that were not clustered in Lineage I.

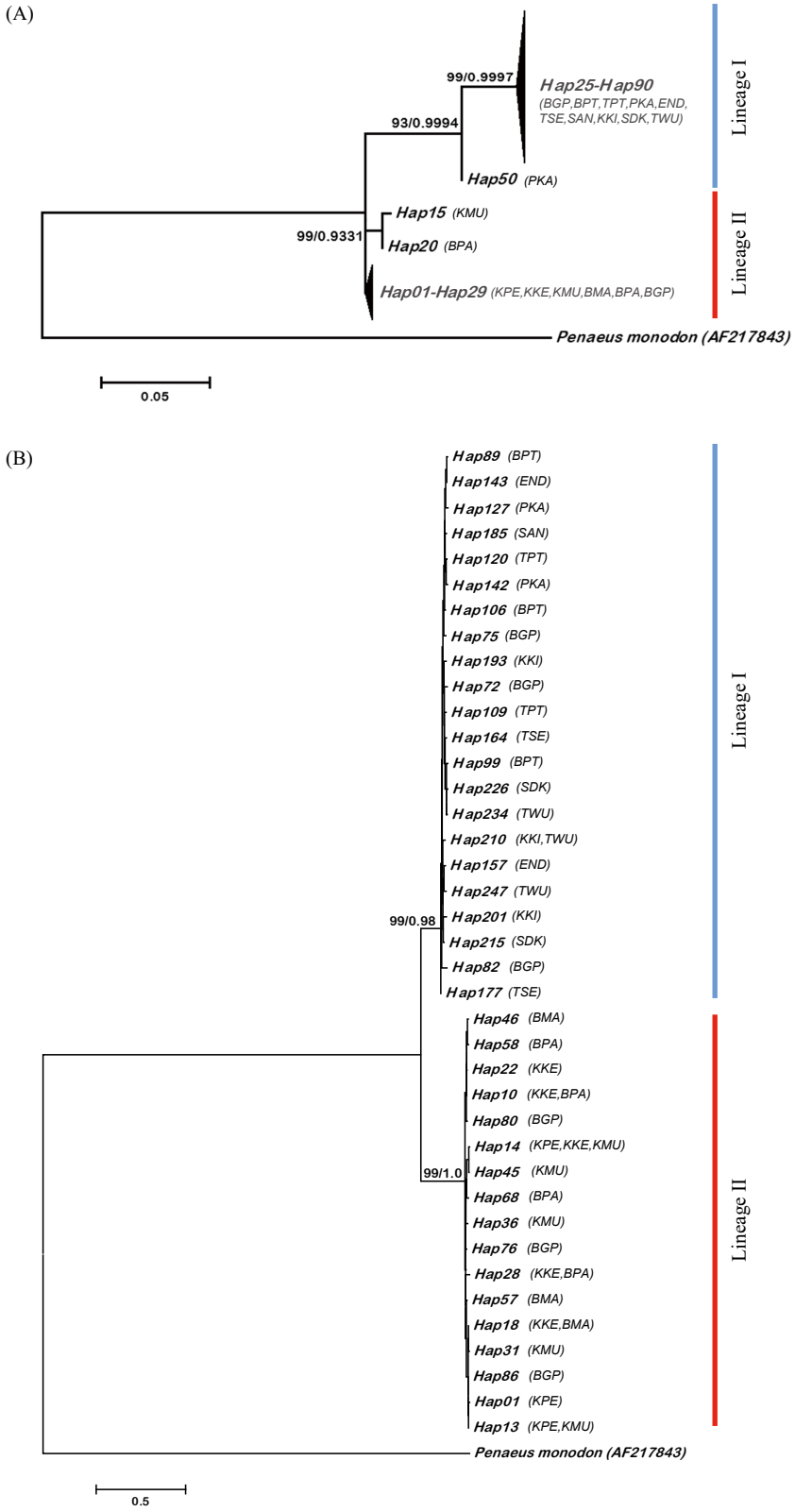

Fig. 2. (A) Maximum Likelihood tree of *P. semisulcatus* rooted with *P. monodon* (AF217843) from GenBank and Bayesian Inference (BI) analyses of mtDNA *COI* gene. (B) Maximum Likelihood tree of *P. semisulcatus* rooted with *P. monodon* (AF217843) from GenBank and Bayesian Inference (BI) analyses of mtDNA CR gene. The bootstrap support and posterior probability values are presented at the nodes. Population abbreviations are as defined in table 1.

The minimum spanning network (MSN) generated two discrete groups (Lineage I and Lineage II) (Fig. 3) for *COI* and CR haplotypes (only representative haplotypes are shown) (Fig. 4), with a reticulate pattern within each lineage in the latter, concordant with the phylogenetic tree analysis. The data shows the genetic isolation of the SOM populations from SCS, SLS and CLS populations with two distinct exceptions as in the earlier analyses, supporting a strong population structure of this species, except for the admixed gene pool at BGP.

Genetic diversity within and among populations

Based on *COI*, BGP had the highest within-population genetic diversity (4%), while KPE had the lowest (0.1%). Between-population genetic diversity ranged from 0.1% to 7.2% (Table 2). The BGP population of SOM was genetically differentiated from the northern populations (KPE, KKE, KMU, BMA and BPA) of SOM at a mean of 4%, while its genetic distance from the eastern seas of SCS, SLS and CLS populations was 3%. The BPT population of SOM was most genetically distant from the northern SOM populations, with an average of 7%. However, the BPT population was genetically similar to the SCS, SLS and CLS populations, with an average of 0.3%.

As in *COI*, a parallel trend was observed in CR (Table 2), although with higher absolute values. Within-population genetic diversity ranged from 2.3% to 9.7, while between-population diversity ranged from 2.3% to 21.7%. The BGP population of SOM was the highly differentiated from the northern SOM populations (KPE, KKE, KMU, BMA and BPA) at an average distance of 16%, while its distance from the eastern seas of SCS, SLS and CLS populations was only 7%. The BPT population was even more genetically distant from the northern populations of SOM, at a mean of 21%, while it was genetically similar to SCS, SLS and CLS populations at a mean of 3%. Thus, in summary, the genetic diversity between the SCS, SLS and CLS populations (Lineage I) and the northern SOM

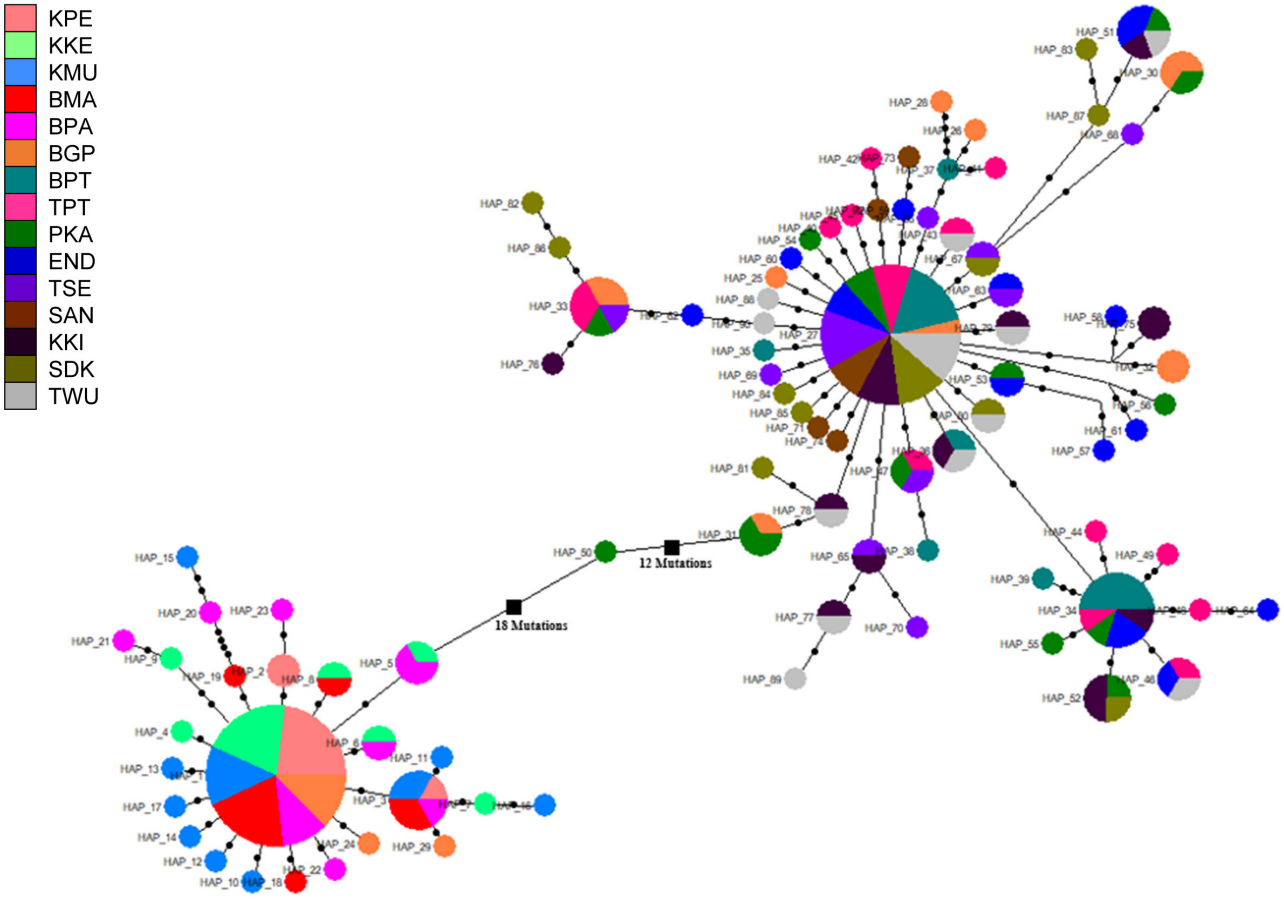

Fig. 3. Median joining-network of mtDNA *COI* haplotypes in *Penaeus semisulcatus*. The sizes of the circles are proportional to haplotype frequencies, colour coded corresponding to locations and black squares on the lines linking haplotypes represent the number of mutations.

populations (Lineage II) were high for both genes; *COI* (7%) and CR (21%). However, the two more southern populations of SOM—BGP and BPT—were more genetically close to SCS, SLS and CLS.

As derived from *COI* gene, the within population haplotype diversities were high for most populations (h: 0.307–0.911), except for KPE, KKE and BMA (all in SOM) at ($h < 0.5$) (Table 3). The nucleotide diversity was moderate, at 0.4% to 2.7%, except in the BGP population, with very high value of (p: 22%). Based on the lineages, the combined populations had high haplotype and nucleotide diversities ranging from (h: 0.567–0.813) and (p: 3.4%–3.8%), respectively.

On the other hand, based on the CR gene, the haplotype and nucleotide diversities were high: (h: 0.965–1.000) and (p: 2%–8%), respectively. Based on lineages, haplotype and nucleotide diversities were also very high ranging from (h: 0.993–1) and (p: 7.4–12.6%), respectively. Thus, according to the four basic classifications of demographic history by Grant and Bowen (1998), both Lineages I and II in general fall into category 4, with high haplotype ($h > 0.5$) and nucleotide ($p > 0.5\%$) diversities, indicating a long evolutionary history in a large stable population or high level of divergence among haplotypes that may be attributed to secondary contact between differentiated lineages.

Historical demographic pattern

Tajima's D and Fu's Fs revealed negatively significant deviations from the mutation drift equilibrium in Lineage I and Lineage II (except the Tajima's D for CR in Lineage II), thus making it consistent with the molecular response to population expansion (Table 3 and Fig. 5). Overall, most of the sites fit a sudden population expansion model, with negatively significant Fu's Fs and Tajima's D for *COI*. However, the Tajima's D for CR was not significant, which is not consistent with Fu's Fs. The discrepancy between Tajima's D and Fu's Fs is likely due to the superiority of Fu's Fs over Tajima's D in detecting significant changes in population (Fu 1997). A unimodal peak and non-significant Harpending's raggedness index were observed in both lineages (Fig. 5), which strengthen the evidence that both lineages underwent sudden expansion.

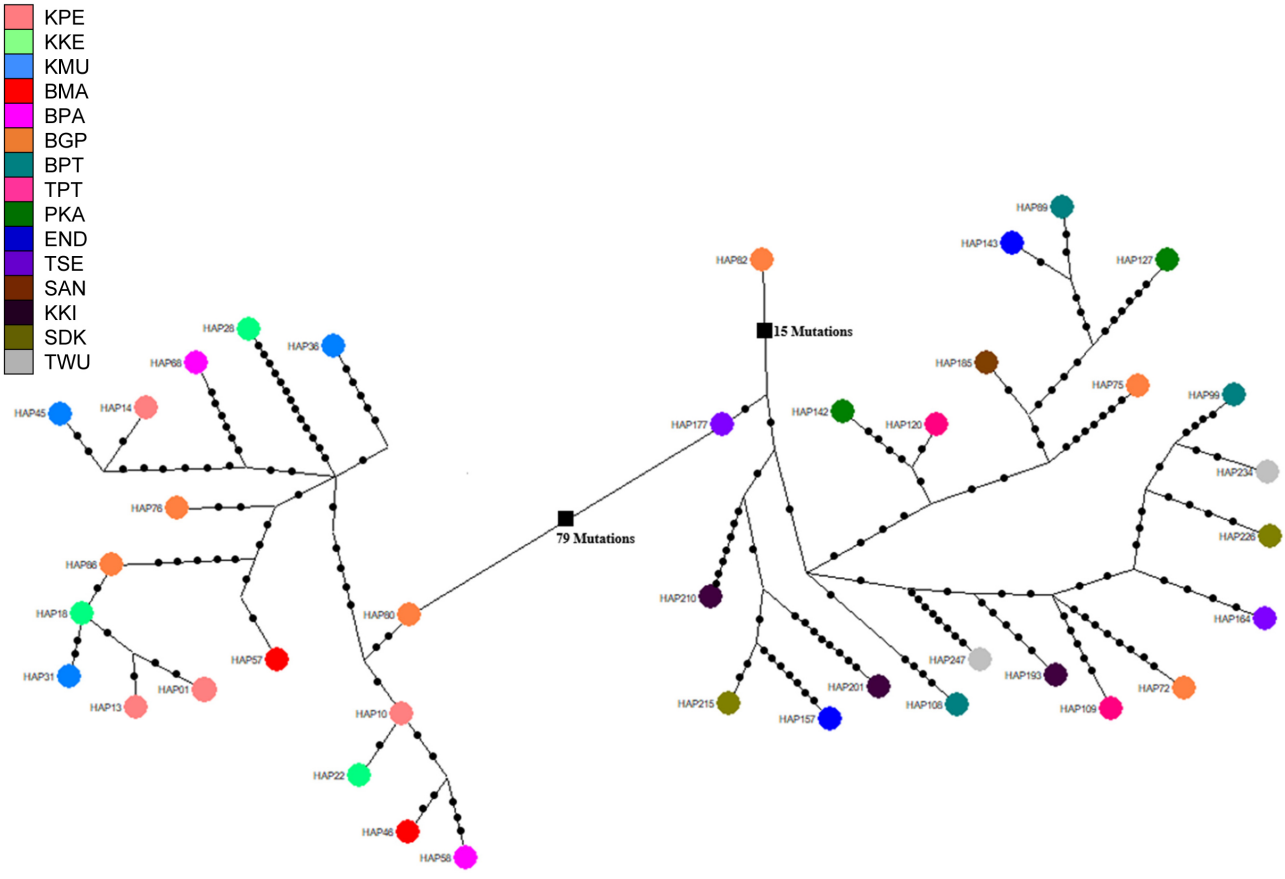

Fig. 4. Median joining-network of mtDNA CR haplotypes in *Penaeus semisulcatus*. The sizes of the circles are proportional to haplotype frequencies, colour coded corresponding to locations and black squares on the lines linking haplotypes represent the number of mutations.

Population genetic structure

In agreement with the earlier analyses, the SCS, SLS and CLS populations (Lineage I) were found to be genetically distinct from the SOM populations (Lineage II), except for the BGP and BPT populations (*COI*: F_{ST} = 0.912–0.984, CR: F_{ST} = 0.816–0.846, $p < 0.05$) (Table 4). The majority of non-significant values after FDR corrections were confined to within individual seas. Mantel tests indicated statistically non-significant correlation between genetic differentiation (F_{ST}) and geographical distance among populations within both lineages, for both genes: Lineage I (*COI*: r = 0.051, P = 0.585; CR: r = 0.227, P = 0.117) and Lineage II (*COI*: r = 0.317, P = 0.849; CR: r = 0.265, P = 0.837), which indicate gene flow, were not restricted by distance.

In SAMOVA, the best population configuration is defined by the highest F_{CT}. However, in this study, SAMOVA revealed that F_{CT} increased with partitioning of populations into the maximum group in all analyses. It should be noted that population structure analysis with one or more single population groups cannot deduce the group structure (Heuertz et al. 2004). Thus, the best population configuration with the highest F_{CT} was selected when the analysis had more than a single population (Tsukagoshi et al. 2011).

Based on the SAMOVA, k = 2 was selected for both genes as the best population configuration defined by the highest F_{CT} (*COI*: 90.05%, p-value: 0.0000; CR: 82.34%, p-value: 0.0000) for AMOVA analysis (Table 5). Two analyses were conducted for the AMOVA. For the *COI*, the first AMOVA analysis—which divided the 15 populations into four groups based on the seas (Group 1: KPE, KKE, KMU, BMA, BPA, BGP, BPT (SOM); Group 2: TPT, PKA, END, TSE, SAN, KKI (SCS); Group 3: SDK (Sulu Sea); Group 4: TWU (Celebes Sea)—showed significant difference among groups/seas (F_{CT}: 55.41%, $p < 0.05$). In the second approach, populations were divided into two groups based on SAMOVA, k = 2) (Group 1: BGP, BPT, TPT, PKA, END, TSE, SAN, KKI, SDK, TWU; Group 2: KPE, KKE, KMU, BMA, BPA) and resulted in even higher

Table 2. Genetic diversity within (bold) and among populations of *P. semisulcatus* based on *COI* (below diagonal) and CR (above diagonal) genes

Populations	KPE	KKE	KMU	BMA	BPA	BGP	BPT	TPT	PKA	END	TSE	SAN	KKI	SDK	TWU
KPE	**0.028**	0.026	0.029	0.025	0.031	0.163	0.211	0.211	0.212	0.214	0.213	0.214	0.214	0.211	0.215
KKE	**0.001**	**0.025**	0.028	0.023	0.030	0.162	0.210	0.211	0.211	0.213	0.213	0.213	0.213	0.210	0.215
KMU	0.002	**0.002**	**0.028**	0.026	0.032	0.166	0.213	0.213	0.213	0.216	0.215	0.215	0.215	0.213	0.217
BMA	0.001	0.001	**0.003**	**0.023**	0.029	0.162	0.211	0.211	0.211	0.213	0.212	0.213	0.213	0.210	0.215
BPA	0.002	0.003	0.004	**0.001**	**0.035**	0.165	0.212	0.213	0.213	0.215	0.214	0.215	0.215	0.212	0.217
BGP	0.041	0.041	0.042	0.041	**0.004**	**0.097**	0.072	0.072	0.073	0.072	0.072	0.073	0.073	0.071	0.074
BPT	0.070	0.070	0.071	0.070	0.070	**0.040**	**0.027**	0.027	0.028	0.026	0.026	0.027	0.026	0.026	0.028
TPT	0.071	0.071	0.072	0.071	0.071	0.034	**0.002**	**0.027**	0.028	0.027	0.027	0.028	0.027	0.026	0.029
PKA	0.068	0.068	0.068	0.068	0.068	0.033	0.004	0.005	**0.030**	0.027	0.028	0.028	0.028	0.027	0.030
END	0.070	0.070	0.071	0.070	0.070	0.034	0.003	0.004	**0.007**	**0.026**	0.026	0.027	0.026	0.025	0.028
TSE	0.070	0.070	0.070	0.070	0.070	0.033	0.002	0.003	0.005	**0.004**	**0.026**	0.027	0.026	0.026	0.028
SAN	0.069	0.069	0.070	0.069	0.069	0.033	0.002	0.003	0.004	0.003	**0.002**	**0.028**	0.028	0.027	0.029
KKI	0.071	0.071	0.071	0.070	0.071	0.034	0.003	0.004	0.005	0.004	0.003	**0.002**	**0.026**	0.026	0.028
SDK	0.071	0.070	0.071	0.070	0.071	0.034	0.003	0.004	0.005	0.004	0.003	0.003	**0.004**	**0.025**	0.028
TWU	0.070	0.070	0.070	0.069	0.070	0.033	0.003	0.004	0.005	0.004	0.003	0.002	0.003	**0.003**	**0.030**

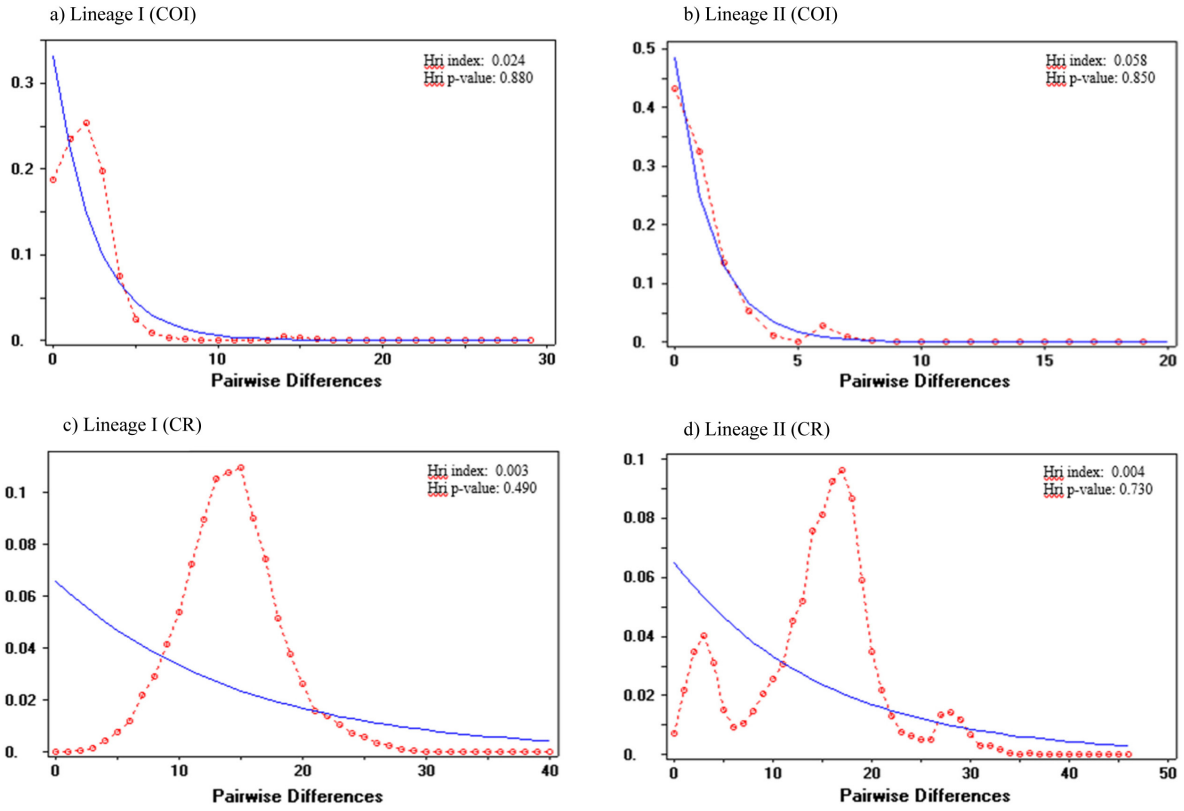

Fig. 5. Mismatch distributions of *Penaeus semisulcatus* in *COI* (a & b) and CR (c & d) for both Lineages I and II and goodnesss fit tests were tested using raggedness index.

Table 3. Demographic parameters of *Penaeus semisulcatus* based on mtDNA *COI* and CR in 15 populations sampled from the Strait of Malacca, South China Sea, Sulu Sea and Celebes Sea. Bold values indicate significance ($p < 0.05$) after the FDR procedure $\alpha = 0.05$

Populations	Haplotype diversity (*h*)		Nucleotide diversity (p%)		Tajima's D		Fu's Fs	
	COI	CR	COI	CR	COI	CR	COI	CR
KPE	0.307	0.993	0.4	2.7	-1.096	-0.398	-1.096	**-4.406**
KKE	0.544	0.965	0.9	2.5	**-2.162**	-0.817	**-4.343**	-2.979
KMU	0.784	1.000	2.1	2.7	**-2.149**	-0.641	**-5.314**	**-6.706**
BMA	0.484	1.000	0.7	2.3	**-1.742**	-0.264	**-2.380**	**-5.314**
BPA	0.791	0.991	2.4	3.1	**-2.163**	-0.761	**-2.876**	-2.814
BGP	0.866	1.000	22.0	8.0	1.864	0.695	4.551	**-2.642**
BPT	0.652	1.000	1.2	2.8	**-1.820**	-1.223	**-2.896**	**-8.909**
TPT	0.884	0.994	2.4	2.9	**-2.048**	-1.243	**-9.061**	**-5.461**
PKA	0.906	1.000	4.1	2.9	**-2.011**	-1.183	**-5.955**	**-5.682**
END	0.911	1.000	2.7	2.5	**-1.765**	-1.131	**-8.400**	**-7.055**
TSE	0.711	1.000	1.3	2.7	-1.640	-1.215	**-7.696**	**-9.081**
SAN	0.618	1.000	1.0	2.9	**-1.791**	-0.887	**-2.310**	-2.748
KKI	0.842	1.000	2.1	2.7	-1.361	-1.453	**-6.358**	**-9.055**
SDK	0.790	1.000	2.0	2.7	-1.578	-1.063	**0.042**	**-7.575**
TWU	0.811	1.000	1.9	3.0	**-1.981**	-1.166	**-9.088**	**-8.323**
LINEAGE I	0.813	1.000	3.4	7.4	**-2.428**	**-1.843**	**-27.260**	**-23.938**
LINEAGE II	0.567	0.993	3.8	12.6	**-2.430**	-1.223	**-29.041**	**-24.184**
TOTAL	0.727	0.010	3.1	3.1	-1.563	-0.850	-4.692	-5.984

and significant variations among groups (F_{CT} = 87.92%, $p < 0.00001$), indicating that the Group 1 populations and Group 2 populations (which were mainly composed of northern SOM populations) were genetically distinct from each other. For CR, the first AMOVA was grouped similarly to the *COI* and showed significant difference among groups (F_{CT} = 42.54%, $p < 0.05$). In the second approach with similar grouping to *COI*, an even higher significant variation among groups was revealed (F_{CT} = 79.76%, $p < 0.05$) and significant among populations within group (F_{SC} = 0.74%, $p < 0.0000$).

Table 4. Pairwise F_{ST} among 15 populations of *Penaeus semisulcatus* based on *COI* (above diagonal) and CR (below diagonal) genes. Bold values indicate significant ($p < 0.05$) F_{ST} values

Group		SOM					SCS							SLS	CLS
	Populations	KPE	KKE	KMU	BMA	BPA	BGP	BPT	TPT	PKA	END	TSE	SAN	KKI	SDK	TWU
SOM	KPE	*	0.000	0.014	0.001	0.004	**0.478**	**0.978**	**0.963**	**0.940**	**0.960**	**0.978**	**0.984**	**0.967**	**0.969**	**0.970**
	KKE	-0.031	*	0.006	-0.015	-0.017	**0.475**	**0.972**	**0.957**	**0.933**	**0.953**	**0.971**	**0.975**	**0.960**	**0.963**	**0.963**
	KMU	0.033	**0.062**	*	-0.015	-0.012	**0.462**	**0.959**	**0.943**	**0.919**	**0.940**	**0.957**	**0.957**	**0.947**	**0.949**	**0.950**
	BMA	-0.029	-0.031	0.041	*	-0.004	**0.470**	**0.974**	**0.958**	**0.935**	**0.955**	**0.973**	**0.978**	**0.962**	**0.965**	**0.965**
	BPA	-0.026	-0.024	0.035	-0.010	*	**0.429**	**0.957**	**0.939**	**0.912**	**0.935**	**0.955**	**0.953**	**0.943**	**0.945**	**0.946**
	BGP	**0.568**	**0.582**	**0.571**	**0.567**	**0.551**	*	**0.373**	**0.342**	**0.288**	**0.334**	**0.353**	**0.290**	**0.346**	**0.341**	**0.342**
	BPT	**0.831**	**0.838**	**0.831**	**0.842**	**0.823**	**0.113**	*	-0.011	0.013	0.010	0.017	0.050	0.009	0.006	0.017
SCS	TPT	**0.829**	**0.836**	**0.829**	**0.840**	**0.820**	**0.116**	-0.009	*	0.001	-0.016	0.016	0.044	0.005	0.006	0.020
	PKA	**0.827**	**0.835**	**0.827**	**0.839**	**0.817**	**0.102**	-0.008	-0.011	*	-0.011	0.019	0.022	0.006	-0.011	0.012
	END	**0.839**	**0.846**	**0.839**	**0.851**	**0.830**	**0.117**	-0.009	0.001	-0.013	*	0.034	0.063	0.008	-0.005	0.032
	TSE	**0.835**	**0.841**	**0.835**	**0.845**	**0.826**	**0.124**	0.000	0.010	0.008	0.002	*	0.015	0.010	-0.006	-0.011
	SAN	**0.830**	**0.839**	**0.830**	**0.844**	**0.819**	**0.091**	-0.018	0.008	-0.014	0.001	0.003	*	0.045	0.031	0.002
	KKI	**0.834**	**0.841**	**0.834**	**0.845**	**0.826**	**0.124**	-0.013	0.020	0.007	-0.005	0.013	0.002	*	0.003	-0.010
SLS	SDK	**0.835**	**0.842**	**0.835**	**0.846**	**0.826**	**0.115**	-0.007	0.009	-0.010	-0.015	0.016	0.003	0.002	*	-0.001
CLS	TWU	**0.825**	**0.832**	**0.825**	**0.835**	**0.816**	**0.124**	-0.002	0.019	0.017	0.011	0.022	0.004	-0.009	0.013	*

Table 5. AMOVA results for hierarchical genetic subdivision for percentage of variation and *F*-statistics of the mtDNA *COI* and control region (CR) genes

Grouping	Variance components	% Variations	*F*-statistics	*P*-value
Based on mtDNA *COI*				
i) All geographical seas	Among Groups	55.41	F_{CT}: 0.5541	0.0029
Group 1: (KPE,KKE,KMU,BMA,BPA,BGP,BPT)	Among populations within group	30.44	F_{SC}: 0.6828	0.0000
Group 2: (TPT,PKA,END,TSE,SAN,KKI)	Among populations within total	14.14	F_{ST}: 0.8586	0.0000
Group 3: (SDK)				
Group 4: (TWU)				
ii) All SAMOVA (k = 2)	Among Groups	87.92	F_{CT}: 0.8792	0.0000
Group 1: (BGP,BPT,TPT,PKA,END,TSE,SAN,KKI,SDK,TWU)	Among populations within group	2.89	F_{SC}: 0.2396	0.0000
Group 2: (KPE,KKE,KMU,BMA,BPA)	Among populations within total	9.18	F_{ST}: 0.9082	0.0000
Based on mtDNA control region				
i) All geographical seas	Among Groups	42.54	F_{CT}: 0.4254	0.0068
Group 1: (KPE,KKE,KMU,BMA,BPA,BGP,BPT)	Among populations within group	28.09	F_{SC}: 0.4888	0.0000
Group 2: (TPT,PKA,END,TSE,SAN,KKI)	Among populations within total	29.37	F_{ST}: 0.7063	0.0000
Group 3: (SDK)				
Group 4: (TWU)				
ii) All SAMOVA (k = 2)	Among Groups	79.76	F_{CT}: 0.7976	0.0020
Group 1: (KPE,KKE,KMU,BMA,BPA)	Among populations within group	0.74	F_{SC}: 0.0366	0.0000
Group 2: (BGP,BPT,TPT,PKA,END,TSE,SAN,KKI,SDK,TWU)	Among populations within total	9.18	F_{ST}: 0.9082	0.0000

DISCUSSION

High genetic diversity and historical demographic pattern

High levels of genetic diversity was evident in the green tiger prawn, *Penaeus semisulcatus*, in the studied regions, as reflected in both Lineage I (*h*: *COI* = 0.813; CR = 1.0) (p: *COI* = 3.4%; CR = 7.4%) and Lineage II (*h*: *COI* = 0.567; CR = 0.993) (*p*: *COI* = 3.8%; CR = 12.6%). Similar patterns of genetic diversity have been observed in other penaeid prawns: giant tiger prawn, *P. monodon* (*h* = 0.992, *p* = 4.3%) (You et al. 2008); spear prawn, *Mierspenaeopsis hardwickii* (*h* = 0.996, *p* = 0.77%) (Tzong 2007); and Indian white prawn, *P. indicus* (*h* = 0.88, *p* = 3.47%) (De Croos and Pálsson 2010). Thus, despite its commercial value, the genetic variability is maintained, suggesting a large and stable population. The current study, based on the criteria proposed by Grant and Bowen (1998), suggests that Lineage I and Lineage II were formed either through secondary contacts between previously differentiated allopatric lineages or a long evolutionary history in a large stable population ($h > 0.5$ and $p > 0.5\%$), in agreement with Category 4. This interpretation is supported by demographic analyses of Tajima's D, Fu's Fs, Harpending Index (*Hri*) and mismatch distribution that together support a population expansion hypothesis for *P. semisulcatus*.

Sudden population expansion can influence a species genetic diversity and also the relationships among haplotypes (McMillen-Jackson and Bert 2004a). During rapid population growth, lineage sorting (the stochastic loss of haplotypes and lineages) slows, as production of mutations overrides the effect of removal of alleles through genetic drift (Avise et al. 1984 1987), with a concomitant increase in haplotypes and lineages and hence genetic diversity. It appears that this diversity was consequently maintained in the populations of *Penaeus semisulcatus* in the IMA and stabilised in time, as recorded in the current study. This demographic expansion was reflected by the star-like genealogy and unimodal mismatch distribution (Rogers and Harpending 1992; Slatkin and Hudson 1991) in the mtDNA *COI* analysis. The unimodal mismatch analysis pattern for both lineages have been observed in other prawn species in the Indo-Pacific region: kuruma prawn, *P. japonicus* (Shih et al. 2011); pink prawn, *P. duorarum* (McMillen-Jackson and Bert 2004b); brown prawn, *P. aztecus* and white prawn, *P. setiferus* (McMillen-Jackson and Bert 2003); Chinese prawn, *P. chinensis* (Li et al. 2009; Xiao et al. 2010); Indian white prawn *P. indicus* (De Croos and Pálsson 2010); and giant tiger prawn, *P. monodon* (Benzie et al. 2002; Waqairatu et al. 2012).

Genetic boundary of *Penaeus semisulcatus* in the Strait of Malacca separates the Indian Ocean from the Pacific Ocean populations

Two distinct phylogenetic groups (Lineage I and Lineage II) were evident based on the mitochondrial *COI* and control region of *P. semisulcatus*. Lineage I comprised populations from the South China Sea, Sulu Sea, Celebes Sea including all Batu Pahat - BPT and most of Bagan Pasir - BGP (Straits of Malacca) individuals. On the other hand, Lineage II is composed of several specimens of Bagan Pasir and all populations to the north of it. Population structuring within the Indo-Pacific has been well depicted in other penaeid prawns, *P. monodon* and *P. merguiensis* (Daud 1995; Aziz et al. 2011) which also displayed at least two different phylogenetic groups between the Straits of Malacca and South China Sea specimens. The current study found deep genetic division between the populations to the north and south of Bagan Pasir, a site located in central SOM, the former being genetically aligned to the Indian Ocean and the latter to the Pacific Ocean. Previous studies have documented deep phylogeographic structuring in the Indo-Pacific Ocean between the Indian Ocean and Pacific Ocean populations involving broad geographically separated penaeid populations but with minimal focus within the SOM where only one or very few samples were sampled within the IMA (Daud 1995; Aziz 2011; Aziz et al. 2011; Jahromi et al. 2019). Highly differentiated populations were documented between the Andaman Sea (an extension of the Indian Ocean) from the Gulf of Thailand, South China Sea and Java Sea (part of the western Pacific Ocean) in the giant tiger prawn, *P. monodon* (Klinbunga et al. 1998 1999; Tassanakajon et al. 1998; Supungul et al. 2000; Klinbunga et al. 2001). A similar pattern of genetic differentiation involving other Indo-Pacific *P. monodon* populations have also been reported (Duda and Palumbi 1999; Benzie et al. 2002; You et al. 2008; Waqairatu et al. 2012; Abdul-Aziz et al. 2015), as has the kuruma prawn, *P. japonicus* (Tzong et al. 2004; Tsoi 2006; Shih et al. 2011). Abdul-Aziz et al. (2015) reported that *P. monodon* populations were genetically distinct among six Indonesian regions: Aceh, Bali, Cilacap (Central Java), Grajagan (East Java), Sumbawa (West Nusa Tenggara) and Timika (Papua). You et al. (2008) covered a wide geographical range of *P. monodon* populations in the Indo-Pacific area: four populations from Taiwan (Ta-NE, Ta-E, Ta-W and Ta-S), two from Thailand (Th-E and Th-W), three from Vietnam (VND, VNS and VNC), two from the Philippines (PHB and PHR), two from Australia (Aus-E and Aus-N), one from Madagascar (MG) and

one from Kenya (KY). They found that the West Indian Ocean populations were genetically differentiated from the West Pacific populations. In concordance, a study on the banana prawn, *P. merguensis*, Hualkasin et al. (2003) and Wanna et al. (2004) identified two lineages composed of specimens mainly from the Gulf of Thailand and the East Pacific and another lineage confined to the Andaman Sea. This common pattern among sympatric species provides strong evidence that, in a broad sense, species structuring is shaped by similar vicariant histories (Avise 1992) within the Indo-Pacific. However, for most of these studies, populations had been sourced from widely distant locations and the detailed sampling of intervening populations such as Strait of Malacca were not conducted. Thus, the precise genetic boundary between these two major oceans was not determined.

Although there has been limited documentation on the population genetics of the green tiger prawn, *P. semisulcatus*, a parallel Indian Ocean vs Pacific Ocean demarcation was documented by Alam et al. (2017). The authors investigated the genetic structuring of populations from Bangladesh, Sri Lanka, Iran (GenBank sequences), India (GenBank sequences), Philippines (GenBank sequences), China (GenBank sequences) and Malaysia (a single GenBank sequence from Penang, northwest Peninsular). They observed two distinct lineages, one in the Western Pacific Ocean (Philippines and China) and the other in the Indian Ocean (Bangladesh, Sri Lanka, Iran, India and Malaysia), suggesting that the point of genetic division lies between the Philippines and Penang (Alam et al. 2017).

Bagan Pasir: A mixture of gene pools of the two Lineages?

While numerous studies on penaeids and other marine species (Carpenter et al. 2011) unequivocably delineate the Indian Ocean from the Pacific Ocean, the most population genetic borders are largely undetermined. However, the genetic boundary in *P. monodon* was narrowed down to the Strait of Malacca (SOM), as inferred through microsatellite loci, which showed a cluster composed of three South China Sea populations (Pacific Ocean) and another composed of SOM populations involving a single population each in the north and south of the SOM, suggesting genetic homogeneity in this waterway (Aziz et al. 2011).

The Strait of Malacca waterway connects the Indian Ocean and the Pacific Ocean between southeast of the Andaman Sea and the southwest of the South China Sea (Leifer 1978). While the genetic structure of *P. monodon* (Aziz et al. 2011) parallel the current study as in the broad delineation of the Indian Ocean, (Strait of Malacca) from the western Pacific Ocean (South China Sea and neighbouring waters), the genetic boundary clearly differs between the two species. The homogeneity along the Strait of Malacca and its heterogeneity with South China Sea population conforms to a divide at the southern tip of Peninsular Malaysia in *P. monodon*. The southern entrance to the Strait at the southern tip of Peninsula Malaysia has numerous islets, some fringed by reefs and sand ridge accumulations of materials brought down by rivers from Sumatra, which presumably hindered trans-ocean crossing by the epibenthic tiger prawn in contemporary times. However, the same cannot be concluded in the present study in the case of *P. semisulcatus*. The unexpected inclusion of the southern SOM population of Batu Pahat (BPT) in Lineage 1 (Pacific Ocean-South China Sea, Sulu Sea and Celebes Sea) and a mixed Bagan Pasir gene pool (central SOM) comprised of Lineage 1 and Lineage 2 suggest that the physical obstacle at the tip of Peninsular Malaysia for *P. monodon* has not been effective in preventing the northward movement of *P. semisulcatus*, as significant phylogeographic structuring occurred further north in central SOM.

Thus, it is postulated based on the high genetic variability and haplotypic signatures derived from both lineages that the barrier lies at or within the vicinity of Bagan Pasir (BGP) in central SOM. Samples at this landing site originate from three neighbouring coastal fishing grounds; 1. the Klang Strait 2. a mud shoal called Angsa Bank and 3. Permatang Sedepa, more commonly known as One Fathom Bank. These areas are major contributors to the annual total fish catch (Sulaiman et al. 2014). Thus, the highly variable Bagan Pasir population could also represent a conglomerate of heterogeneous gene pools of the three fishing grounds. However, while this may explain the high diversity, it does not explain why the northern and southern populations are distinctly heterogeneous. One Fathom Bank is one of the largest sand banks in the Strait of Malacca and is characterised by a sudden dip in the sea. Its location is near the meeting point of the Indian Ocean and South China Sea fronts contributing to high biological productivity due to changing movements of currents and nutrients in these water bodies (Sulaiman et al. 2014). The sudden decrease in depth around One Fathom Bank could act as a physical barrier to migration of certain organisms between the north and south of it. The sand waves, ripples and sand banks at One Fathom Bank (Sulaiman et al. 2014) may also act as physical barriers that restrict the movement of this species leading to differentiated populations. In contrast, the tiger prawn, *P. monodon*, appears to move freely across this bank based on microsatellite

markers (Aziz et al. 2011). The reason (s) for this difference is unknown since both populations have a similar ecology and behaviour (Chan 1998). A more holistic study is required to identify the factors for this discordance and the precise point of delineation. These will include research on other penaeid species in this area which is currently lacking, more detailed studies of *P. semisulcatus* populations between Bagan Pasir (BGP) and Batu Pahat (BPT), monitoring of its larval behaviour and dispersal, analysing contemporary and past history of coastal currents as well as other ecological parameters to understand the population structuring in this oceanic realm.

The genus *Penaeus* is hypothesised to have originated or arose in the Indo-Pacific region and radiated eastward and westward, forming two groups (western Pacific and Indian Ocean), during the Tertiary and Pleistocene periods (Baldwin et al. 1998). The lowering of sea level to below 120 m in the glacial periods during the Pleistocene Ice Age exposed most parts of Malay-Peninsula, Sumatra, Java and Borneo to Palawan and led to vicariant events for many marine species (Voris 2000) due to restricted genetic connectivity of populations across the Sunda Shelf during these periods. Barriers to gene flow that structured prawn species have been recognised in the Malay-Indo Archipelago (Benzie et al. 1992; Dall et al. 1990; Daud 1995; Sodsuk 1996). However, subsequently, during the interglacial period, the sea level rose, and the Indian Ocean and South China Sea were consequently connected via the Straits of Singapore (Bird et al. 2006), which in contrast to distinct structuring in several prawn species, homogenised previously isolated populations. Several recent studies have documented intra-specific genetic homogeneity between the Strait of Malacca and South China Sea populations (Japanese threadfin bream, *Nemipterus japonicus* (Lim et al. 2014); black scar oyster, *Crassostrea iredalei* (Zainal Abidin et al. 2014); mud crab, *Scylla olivacea* (Rosly et al. 2017); and blood cockles, *Anadara granosa* (Chee et al. 2011). A study on the Indian mackerel, *Rastrelliger kanagurta* (Akib et al. 2015), found high connectivity up to the Gulf of Oman in the west Indian Ocean.

The dispersal ability of the adult green tiger prawn has been reported to be typically low (Farmer and Al-Attar 1981; Somers and Kirkwood 1984; Niamaimandi et al. 2010). While adult movement is limited, Rothlisberg et al. (1983) suggested that the larva could move up to 100 km between the offshore spawning grounds to inshore nursery habitats and enhance gene flow among populations. Furthermore, the ocean currents of the South China Sea, Sulu Sea, and Celebes Sea are very much subjected to seasonal monsoon cycles, which could facilitate the transportation of the green tiger prawn larvae across these seas. Similarly, the Strait of Malacca is strongly influenced by the northeast and the southwest monsoons (Thia-Eng et al. 2000) and ocean currents. In the case of the green tiger prawn, *P. semisulcatus*, the genetic connectivity among the four seas investigated as expected by the influences of Pleistocene and larval drift was impeded at a genetic barrier in the central Strait of Malacca.

Taxonomy ambiguity or biological factors that lead to genetic structuring

Taxonomic misidentifications could also lead to erroneous conclusions about population genetics assessment. For instance, several studies on the kuruma prawn, *P. japonicus* (Tzong et al. 2004; Tsoi 2006; Shih et al. 2011), over a wide geographical coverage reported significant genetic differentiation in the Indo-Pacific region, representing two varieties (I and II) of this species. However, in a more recent study, Tsoi et al. (2014) verified that variety I was actually *P. japonicus*, which is endemic to the East China Sea and northern South China Sea, while variety II was *P. pulchricaudatus*, which is widely distributed in the South China Sea, Australia, the Red Sea, the Mediterranean and the western Indian Ocean. Cases of cryptic diversity within this marine region have been recorded involving the Indian white prawn, *P. indicus* (Alam et al. 2015); Japanese thread bream, *Nemipterus japonicus* (Lim et al. 2014), Blacktip grouper, *Epinephelus fasciatus* (Kuriiwa et al. 2014); and Malaysian oysters, *Crassostrea belcheri* and *C. iredalei* (Suzana et al. 2011).

The high genetic distinction between the two clusters calls for taxonomic studies to be conducted to ascertain the taxonomic status of the two gene pools (*COI*: 7%, CR: 21%). The genetic distances observed in the current study are in accordance with inter-specific variation among marine prawn species. For example, five species of the genus *Parapenaeopsis—P. stylifera*, *P. coromandelica*, *P. hardwickii*, *P. sculptilis*, *P. uncta*—showed sequence divergences of 7.65 to 20.3% for *COI* gene and 2.4 to 12.0% for the 16S rDNA gene (Chowdhury et al. 2018). Furthermore, two juvenile pink prawn, *Penaeus brasiliensis* and *P. paulensis*, showed values between 4.3–18.1% based on the *COI* gene (Teodoro et al. 2016) and *Metapenaeus dobsoni* populations using RAPD, 5 to 18% (Mishra et al. 2009). Jahromi et al. (2019) recently reported the presence of two morphotypes of *P. semisulcatus* in the Persian Gulf namely the banded and non-banded antennae morphotypes genetically distant by 17.3%. The non-banded is restricted to the Persian Gulf waters

while the banded morphotype is found not only in the Persian Gulf but also reaches the Malaysian waters with albeit sampling restricted to only a single location north of the Strait of Malacca (Penang) (Jahromi et al. 2019). All haplotypes based on the *COI* gene of banded antennae of *P. semisulcatus* from Iran and Malaysia (Penang) were clustered in one lineage while non-banded antennae morphotype clustered in another distinct lineage, supporting the differentiation between the two morphotypes. In comparison, the genetic distance within each of the lineage was similar to that in the current study (0.0 to 0.3%). Thus, their study highlighted the prolific hidden diversity within this species (or potentially species complex). To extend the analysis, we conducted a comparative analysis of data from Jahromi et al. (2019) and the current data which further supported the genetic delineation of banded and non-banded (16%.) morphotypes. Furthermore, the banded populations from Iran and Malaysia (Jahromi et al. 2019) showed a close relationship with Lineage II (1.4%), geographically overlapping populations with Jahromi et al. 2019), while being genetically distant from Lineage I, in parallel with our findings.

The mitochondrial markers in this study successfully elucidated the population structuring and genetic diversity of the green tiger prawn, *P. semisulcatus*. However, information generated by these generally matrilineal markers are restricted to maternal inheritance. Over the past two decades, the complementary use of biparental nuclear markers, particularly microsatellites, have gained wide importance for a more holistic assessment of the population genetic variability of marine prawns and factors influencing them (Tsoi et al. 2007; Waqairatu et al. 2012; Abdul-Aziz et al. 2015; Song et al. 2018; Jahromi et al. 2019). In comparison to the mitochondrial DNA gene, the usage of microsatellites as molecular markers is more advantageous due to its abundance in genomes, small locus size facilitating polymerase chain reaction (PCR)-based genotyping, evenly distribution, codominant nature of Mendelian inheritance and high levels of polymorphism (Liu 2007). Furthermore, while mitochondrial DNA provides important insights into the population history of a population or species, it is limited to explaining contemporary factors, which could be elucidated through the use of microsatellite markers. In addition, since both sexes contribute to the genetic diversity of the progeny in biparental markers, sex biased dispersal could be assessed from the genetic output between the two sexes. However, the major disadvantage of microsatellite markers is the requirement for prior *de novo* development of markers for the species under investigation, although cross species amplification may sometimes work to a limited degree. Consequently, this could be challenging due to the cost and time needed to design them (Chambers and MacAvoy 2000; Jamaluddin 2017). Besides, a high number of samples is required for precise analysis of microsatellite variation. Chai et al. (2017) observed low microsatellite variations among blue swimmer crab (*Portunus pelagicus*) populations throughout the coastal areas of Malaysia, attributed to insufficient sample sizes. However, in recent years, advances and reductions in cost associated with Next Generation Sequencing analysis have facilitated microsatellite marker development, circumventing most of these challenges (Liu 2007; Gardner et al. 2011). Our future management programme for this study will be to include novel biparental microsatellite data for population genetics of this species.

This study contributes to the growing evidence of inherently high genetic variability in non-morphologically differentiated prawn species on a wide geographical scale. However, a morphological re-evaluation of the specimens should be conducted to confirm the taxonomic status. The understanding of the genetic structure of this species will be highly beneficial for fishery management and aquaculture development programs through identification of reproductively isolated and genetically differentiated populations.

CONCLUSIONS

Understanding population genetic diversity and structure is vital for managing productive fisheries, conserving genetic resources and sustainably harvesting populations of of marine organisms. The current study highlights substantial genetic diversity in the green tiger prawn, *P. semisulcatus*, with significant genetic differentiation between its two main lineages (I and II); Lineage I mainly comprised populations from the Pacific Ocean-South China Sea, Sulu Sea and Celebes Sea, while Lineage II was composed of northern to central populations in the Strait of Malacca. This genetic structure is likely attributed to a combination of factors, including historical vicariant events, dynamics of ocean currents and biological factors. The Bagan Pasir population is considered a mixture of gene pools of the two lineages, and probably represents the area (or vicinity) of demarcation of the two lineages. More intensive studies of *P. semisulcatus* along the Strait of Malacca using a greater number of molecular markers—including nuclear markers such as microsatellites—would provide further clues to the precise site of divide. This study is an important contribution to fishery management and aquaculture development programs because it identifies genetically variable and

differentiated populations.

Acknowledgments: We thank the Universiti Sains Malaysia for funding this project under the FRGS research grant (203/PBIOLOGI/6711455) - 'Elucidating the molecular taxonomy and phylogenetics of the shrimp, Superfamily Penaeoidea and population genetic structure of the banana prawn, *Penaeus merguensis* in Malaysia'. We are grateful to SEAFDEC and the Department of Fisheries, Malaysia, for assisting us with the sampling and also our colleagues and their respective institutions—Norli Fauzani binti Mohd Abu Hassan Alshari, Siti Zuliana binti Ahmad, Mohamad Firdaus bin Mohamad Ridzwan, Dr Nur Fadli and Dr Jamsari Amirul Firdaus bin Jamaluddin (members of Molecular Ecology Lab 308)—for helping with the collection of specimens, and Mr Abdul Rahman Abdul Majid (Al Hana Enterprise) for sharing his vast experience with species identification.

Authors' contributions: SAAAH collected and processed the samples, performed the molecular analysis and drafted the manuscript. NAJ collected and processed the samples. All authors revised and approved the manuscript.

Competing interests: All authors declare that they have no conflict of interests.

Availability of data and materials: Sequences generated in the study have been deposited in GenBank sequence database with accession number in the text. Raw data can also be provided upon request.

Consent for publication: Not applicable.

Ethics approval consent to participate: Not applicable.

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