

Article

# Genetic Variations and Expansion of the Blue Swimmer Crab (*Portunus pelagicus*) in Southeast Asia

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**Abstract:** Blue swimmer crabs (*Portunus pelagicus*) primarily inhabit the coastal regions of Southeast Asia. This study aimed to examine their population genetic structure, phylogeography, and historical demography by applying partial sequences to the control region in the mitochondrial DNA. The analysis focused on 6 populations comprising 166 individual samples collected from Taiwan (Kezailiao Kaohsiung (KK) and Penghu (PH)), mainland China (Xiamen (XM) and Hong Kong (HK)), Vietnam (Hanoi (VN)), and Singapore (SGP). The estimated nucleotide diversity ( $\pi$ ) for all of the samples was 0.062, with values ranging between 0.018 (PH) and 0.045 (HK). Our study collected 116 haplotypes and classified them into lineages A and B. The XM, HK, VN, and SGP populations made up lineage A, and the PH and KK populations comprised lineage B. For each lineage, the results highlighted indications of demographic expansion. Lineage A can be traced back to the second-to-last interglacial period, while lineage B possibly originated in the last glacial period. There were significant differences in the  $F_{ST}$  values among the six populations, except for the pairs HK–XM and PH–KK. In conclusion, the phylogeography and genetic structure of *P. pelagicus* in Southeast Asia were potentially affected by the Pleistocene glacial cycles and human introduction. These findings further highlight the possible dispersal routes of *P. pelagicus* throughout Southeast Asia.

**Keywords:** *Portunus pelagicus*; historical demography; population structure; population expansion



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## 1. Introduction

Blue swimmer crabs (*Portunus pelagicus* (Linnaeus)), are found in the Indo-Pacific, Southeast and East Asia (i.e., Japan, Korea, China, Taiwan, and the Bay of Bengal), and the west, north, and east of Australia [1,2]. They tend to be dispersed across several kilometres by ocean currents, are generally not limited by demographic boundaries, and have high population exchange levels [3,4]. The species is particularly valued in the commercial fisheries of Southeast Asia, and a key factor in the successful and sustainable management of fishery resources is population genetic structure [5]. Research on population genetics [6] and historical demography [7,8] has offered valuable data on catches and age composition [9]. Studies on the population genetic structure of *P. pelagicus* have demonstrated that highly dispersed organisms tend to show low genetic distinction across a large geographic scale [10–12]. Moreover, the gene flow rate is high among marine populations [13]. However, given their high dispersal potential and the lack of obvious barriers to dispersal in the ocean, marine organisms are more genetically structured than expected, in contrast with the findings of previous studies on genetic structure [14–16].

However, habitats, local oceanic conditions, and historical events impede the dispersal of marine organisms [17], creating the potential for genetic distinction [18].

Marine organism research has applied a wide variety of methodologies to gain an understanding of population genetic structures [18]. Some studies have analysed the distribution and abundance of life-history stages, while others have explored marking and tagging, morphological characteristics, allozymes, or DNA markers [1,2]. A multi-pronged approach that integrates the outcomes of several methods could maximise the probability of accurately defining stocks [19]. Mitochondrial (mt) DNA is a key component in population genetic studies given its rapid evolution rate, lack of recombination, and maternal inheritance [14,15,20]; mtDNA—specifically, its control region—is an ideal marker of geographical patterns in genetic variations within and across crab populations [20–31]. However, research findings on the phylogeography and genetic structure of *P. pelagicus* in Southeast Asia have remained inconsistent.

Geological evidence indicates that land bridges connected the island of Taiwan to the Asian continent 2–3 times during the Pleistocene [20]. During the Late Pleistocene glaciation, sea levels in the area were about 140 m lower than at present [20]. As a result, the floor of the Taiwan Strait was exposed as a broad land bridge that was crossed by mainland fauna until the beginning of the Holocene 10,000 years ago [20]. The changing sea levels and temperature during the glacial period critically altered the marine environment and, with it, the distribution, connectivity, and genetic structure of marine organisms [32]. Paleoclimate changes influenced the modern biodiversity patterns of the continent and these islands [33]. The Pleistocene glacial period resulted in fluctuations in East Asia's sea levels which, in turn, repeatedly altered the connections between these islands or with the mainland [34,35]. More specifically, the Taiwan Strait was almost separated from the Pacific Ocean, the South China Sea was completely exposed, and the major islands of Taiwan were connected to the continents [31]. Studies have reported that diverse and unique biota emerging from complex geological and climatic factors contributed to ancient substitution and diffusion events [36]. After the end of the glacial period, the islands were once again isolated.

The abovementioned historical events critically influenced the genetic isolation, distribution, and structure of East Asian organisms [37]. Other significant factors included ocean currents [38] and past and current land barriers [39,40]. A genetic study using gene markers in the mtDNA cytochrome oxidase (COI) to determine the genetic structure of *P. pelagicus* around the South China Sea highlighted historical vicariance as an influencing factor [41]. The mtDNA D-loop has high variability, a high level of polymorphism in other crustacean species, and can be used to distinguish between populations [42–44]. Approximately 0.01–0.11 million years ago (Mya), the South China Sea was 100–120 m lower owing to global glaciation, and the Pacific and Indian oceans were separated by the Sundaland—an exposed land ridge [11,12,45]. This study explores the impact of these areas on the population structure of the subsamples of species distribution. This study aims to explore the population genetic structure of blue swimmer crabs in Southeast Asia by conducting sequence analyses on the mtDNA control region in the species.

## 2. Materials and Methods

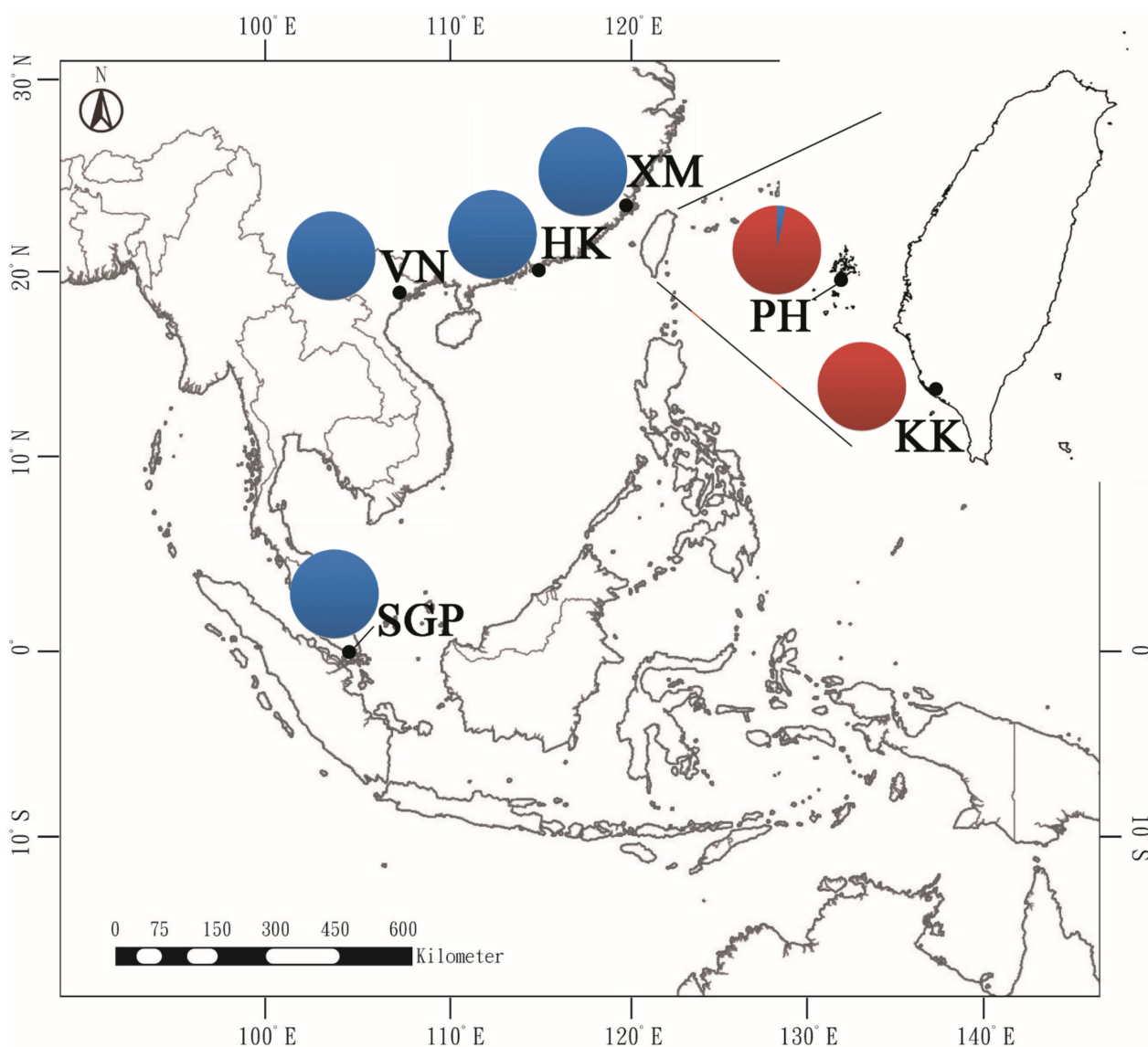
### 2.1. Sample Collection

This study is based on 166 individual specimens of *P. pelagicus* collected from 6 regions: Kezailiao Kaohsiung (KK) and Penghu (PH) in Taiwan, Xiamen (XM) and Hong Kong (HK) in mainland China, Hanoi (VN) in Vietnam, and Singapore (SGP). The specimens were collected using commercial crab traps or gillnets (Table 1) and immediately stored at  $-75^{\circ}\text{C}$  before DNA was extracted. This study was conducted under permits granted by wildlife conservation authorities in Vietnam, Singapore, China, and Taiwan (Figure 1).

**Table 1.** Codes of sampling sites, sample sizes ( $n$ ), number of haplotypes ( $n_h$ ), gene diversity ( $h$ ), nucleotide diversity ( $\pi$ ), Tajima's  $D$ , and Fu's  $F_s$  statistics for 6 populations of *Portunus pelagicus* in Southeast Asia.

Population	$n$	$n_h$	Lineage A	Lineage B	$h$ Diversity	$\pi$ Diversity	Tajima's $D$	Fu's $F_s$
XM	22	16	22	0	$0.970 \pm 0.022$	$0.045 \pm 0.00462$	$-0.87197$	1.271
PH	30	20	1	29	$0.961 \pm 0.021$	$0.023 \pm 0.00431$	$-1.53515$	$-1.445$
KK	36	23	0	36	$0.962 \pm 0.019$	$0.020 \pm 0.00114$	$-1.03393$	$-2.597$
HK	28	26	28	0	$0.995 \pm 0.011$	$0.046 \pm 0.00252$	$-1.15632$	$-4.889$
VN	27	15	27	0	$0.949 \pm 0.022$	$0.025 \pm 0.00194$	0.5638	1.662
SGP	23	17	23	0	$0.972 \pm 0.020$	$0.032 \pm 0.00152$	0.03936	$-0.197$
Lineage A	101	75			$0.993 \pm 0.003$	$0.042 \pm 0.00186$	$-1.59138^*$	$-24.23^*$
Lineage B	65	41			$0.979 \pm 0.007$	$0.020 \pm 0.00097$	$-1.30135$	$-11.305^*$
Total	166	116			$0.994 \pm 0.002$	$0.064 \pm 0.00120$	$-1.0188$	$-31.544^*$

\*  $p < 0.05$ . XM: Xiamen, PH: Penghu, KK: Kezailiao Kaohsiung, HK: Hong Kong, VN: Vietnam, SGP: Singapore.



**Figure 1.** Sampling areas and haplotype frequencies of *P. pelagicus* in Southeast Asia. Table 1 presents the numbers of lineages A and B at each sampling site.

## 2.2. DNA Extraction, PCR, and Sequencing

Genomic DNA was extracted from the pereopod muscle using a QIAamp DNA Mini Kit [46]. A fragment of the mtDNA control region was amplified and sequenced using YEN-F (5'- GCA AAT ACA CGC AAT AAC TCT CAT AC -3') and YEN-B (5'- TGT AAA TCC GTT ACG AAT AAT ATA GG -3') primers. Thermal cycling was performed using a GeneAmp 2400 thermal cycler (PerkinElmer, Norwalk, CT, USA). The PCR conditions were as follows: 39 cycles of denaturation at 95 °C for 50 s, annealing at 50 °C for 1 min, and an extension at 72 °C for 1.5 min. The first cycle included an initial denaturation step at 95 °C for 5 min, and the last cycle had a final extension at 72 °C for 10 min. The PCR product was separated on 1.5% agarose gels using electrophoresis. Purification was conducted using the Gene Clean II Kit (Bio101, Vista, CA, USA). Sequencing was performed using the ABI 377 DNA sequencer (Applied Biosystems, Inc., Foster City, CA, USA).

## 2.3. Analyses of Sequences

MegAlign (DNASTAR, Lasergene, Madison, WI, USA) was used to align all of the sequences, and DnaSP version 5.00 was used to estimate the numbers of variable and parsimony-informative sites, base composition, and haplotype and nucleotide diversities [47–50].

The neighbour-joining (NJ) and maximum likelihood (ML) methods were employed to conduct phylogeographic analyses of the control region using MEGA X [31,49,50]. Bootstrap analyses were conducted on 1000 replicates to evaluate the phylogenetic relationships of all haplotypes. MEGA was used to determine the optimal substitution model. The median-joining method with Network version 4.6.1.3 ([www.fluxus-engineering.com](http://www.fluxus-engineering.com) [accessed 1 February 2021]) was employed to construct a network of haplotypes [50]. Insight into the expansion of the historical demography was derived by using DnaSP to examine the frequency distributions of pairwise differences between sequences (mismatched distribution) [31]. Rough dates of population expansion were estimated using the formula  $T = \tau / 2u$  [7,51], where  $T$  = time since expansion,  $\tau$  = expansion time, and  $2u = \mu$  (mutation rate)  $\times$  generation time  $\times$  number of bases sequenced. The average divergence rate was 1.72–2.91% per million years, with a generation time of one year.

Pairwise  $F_{ST}$  statistics were estimated using ARLEQUIN version 3.5 (Excoffier and Lischer 2010) to examine genetic differentiation between sites [51,52]. Next, on the basis of the  $F_{ST}$  values, a dendrogram of the six sampling sites was constructed using the unweighted pair-group method with arithmetic means (UPGMA). Population structure was assessed by conducting an analysis of molecular variance (AMOVA [6]) using ARLEQUIN. The UPGMA tree highlighted various groupings for the six populations, of which one grouping reported the maximal value for  $\Phi_{CT}$ . The groupings significantly differed from a random organisation of similar groupings. Furthermore, they indicated geographic subdivisions that were the most probable [53,54]. The statistical results were tested for significance using 10,000 random permutations.

Population expansion or deviation from neutrality was analysed using three methods. Furthermore, to determine whether population expansion occurred in the past, deviations from neutrality were examined by estimating Tajima's  $D$  [55]. In addition, evidence of population expansion was analysed by conducting Fu's  $F_s$  test [8] using DnaSP. A mismatch analysis was also performed using DnaSP to check for population expansion and the frequency distributions in the nucleotide differences as a function of frequency [56].

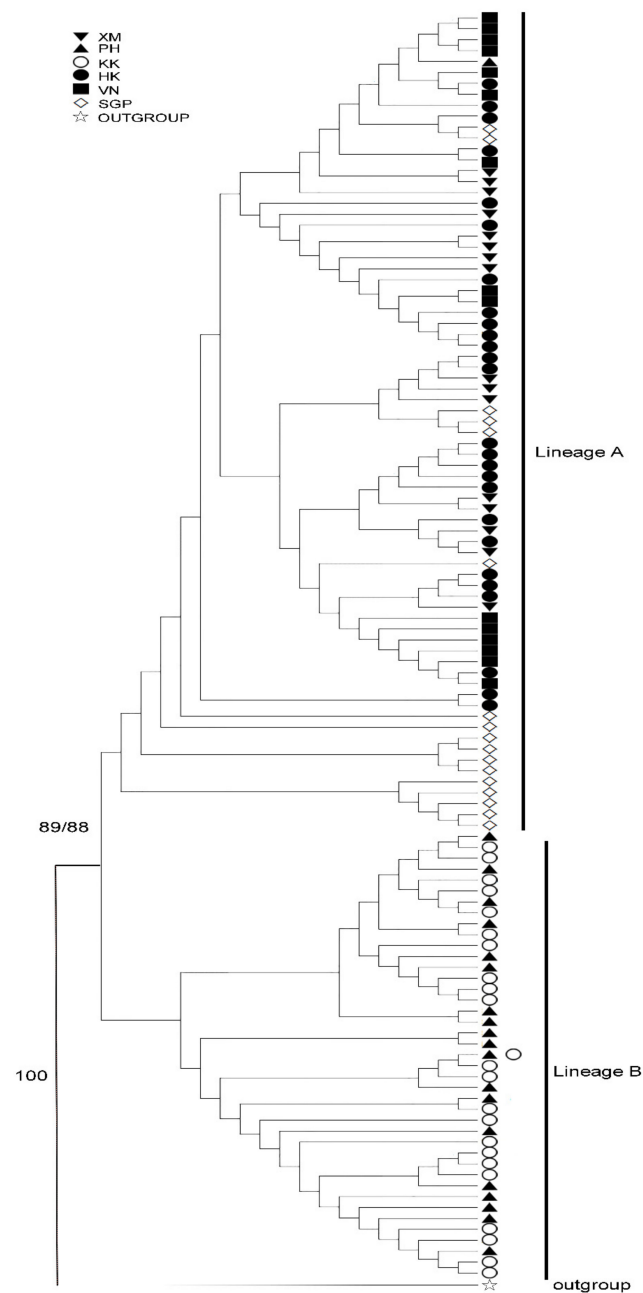
## 3. Results

The sequencing analyses of the 166 specimens revealed 92 variable sites and 199 parsimony-informative sites, thus detecting a total of 116 unique haplotypes (S1 Dataset). The D-loop fragment was estimated at 715 bp. All of the sequences were deposited in GenBank (accession numbers MZ512410–MZ512575), and no gap was detected. The nucleotide composition frequency indicated an AT bias (G + C content: 46.0%).

Table 1 presents the estimates for the haplotype ( $h$ ) and nucleotide ( $\pi$ ) diversities of all populations. The average value for haplotype diversity was between 0.949 and 0.995,

with values ranging between 0.949 (VN) and 0.995 (HK), while that for nucleotide diversity was 0.064, with values ranging from 0.020 (KK) to 0.045 (HK).

The study's data were best explained by the T92 model, which was subsequently applied to NJ and ML reconstructions and AMOVA analyses. Figure 1 illustrates the phylogenetic tree for all haplotypes. The tree and the network highlight two distinct lineages (A and B) (Figures 1 and 2). The individual specimens in KK belonged only to lineage B; those in HK, XM, VN, and SGP were only in lineage A; and specimens in PH were in either lineage A or B. For lineages A and B, the estimated values for haplotype diversity ( $h$ ) were 0.993 and 0.990, respectively, while those for nucleotide diversity ( $\pi$ ) were 0.040 and 0.031, respectively (Table 1). The  $\tau$  values for lineages A and B were  $19.439/2\mu$  and  $11.712/2\mu$  generations, respectively.



**Figure 2.** Neighbour-joining (NJ) tree based on mtDNA D-loop sequences with bootstrap values (NJ/ML, respectively) shown adjacent to the two corresponding lineages for *Portunus pelagicus*. The numbers at the nodes indicate bootstrap values (expressed as percentages) with 1000 replicates.

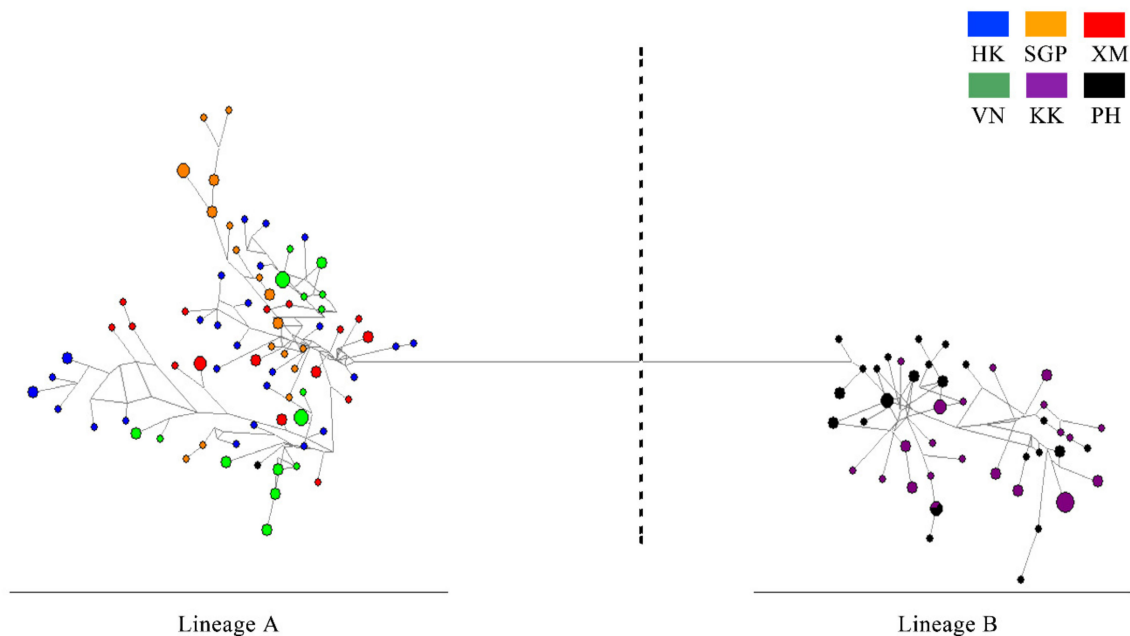


Table 2 reports the  $F_{ST}$  results. The tests showed significant values for all six populations, except for the relationships between HK and XM, and between PH and KK. Figure 3 illustrates the UPGMA tree for the six sampling areas. According to the intergroup variance component test results, five of the six designated grouping schemes (G1, G2, G3, G4, and G5) were statistically significant. The selection of the G2, G3, and G4 grouping schemes as the best grouping schemes was based on the statistics of  $\Phi_{CT}$  values (Figure 4).

**Table 2.** Matrix of pairwise  $F_{ST}$  (below diagonal) and  $p$ -values (above diagonal) among 6 populations of *Portunus pelagicus* in Southeast Asia.

	HK	XM	VN	SGP	PH	KK
HK	-	0.083	0.000	0.000	0.000	0.000
XM	0.037	-	0.000	0.000	0.000	0.000
VN	0.122	0.139	-	0.000	0.000	0.000
SGP	0.176	0.162	0.250	-	0.000	0.000
PH	0.588	0.571	0.703	0.447	-	0.090
KK	0.532	0.514	0.629	0.389	0.035	-

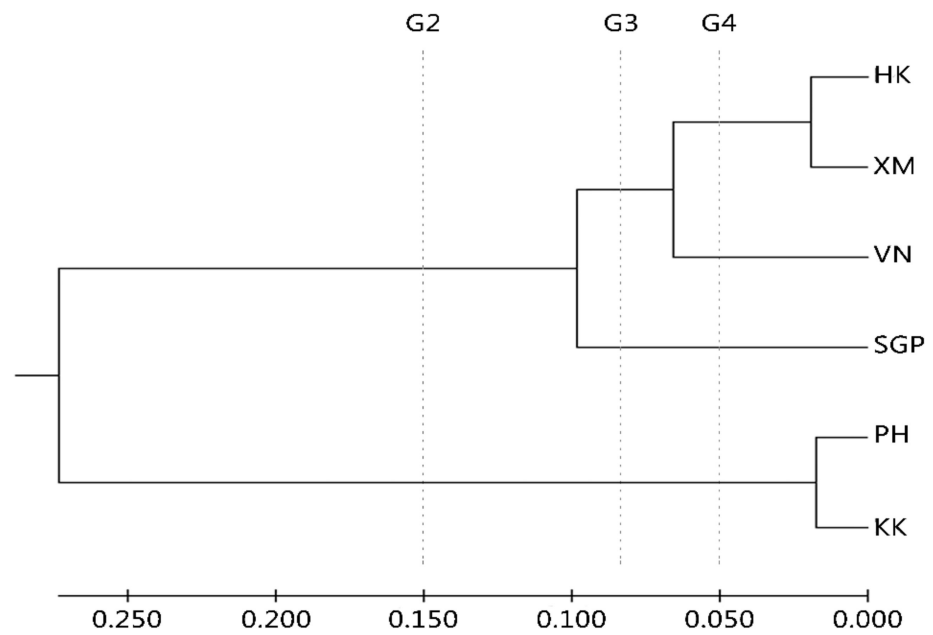
XM: Xiamen, PH: Penghu, KK: Kezailiao Kaohsiung, HK: Hong Kong, VN: Vietnam, SGP: Singapore.



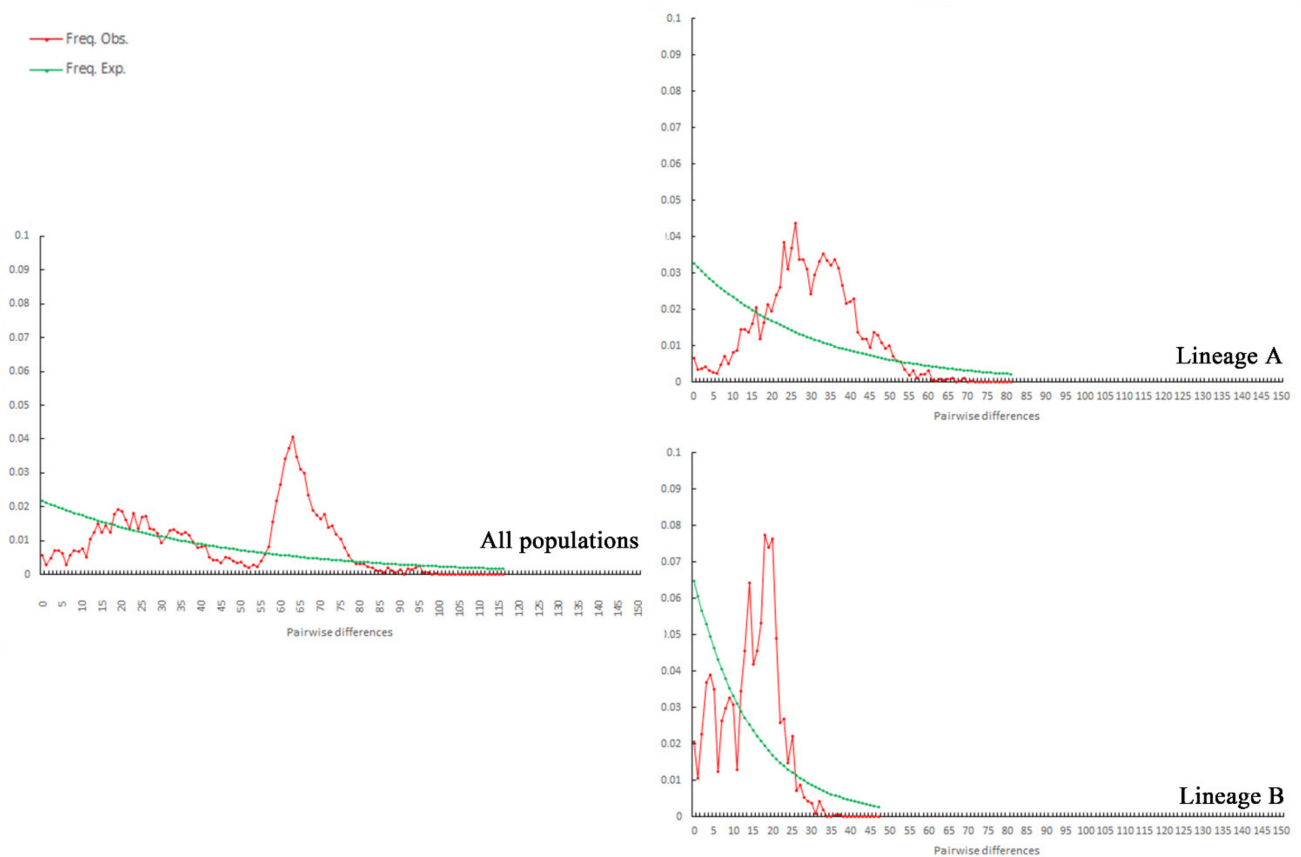
**Figure 3.** The haplotype network of *Portunus pelagicus* in all sampling sites.

The Tajima's  $D$  values were not significant for either lineage, for the six individual populations, or for the total population (Table 1). The Fu's  $F_s$  test results were significant for all populations (Table 1), both lineages, and for the total population. Statistically, a unimodal distribution was obtained from lineage A or B when measured by squared deviation ( $p > 0.05$ ). The distribution did not significantly differ from that estimated by the growth expansion model (Figure 5).

Table 3 shows the AMOVA results, and for all six populations, they reported a significant  $F_{ST}$  value of 0.41978. Specifically, there was significant heterogeneity in at least one of the pairwise populations.  $\Phi_{CT}$  values were significant for all groupings, of which group 2 reported the highest  $\Phi_{CT}$  value of 0.46573. These results support the conclusion that the six populations can be divided into two main groups: the first group comprises the HK, XM, VN, and SGP populations, while the second group comprises the PH and KK populations. Furthermore, the findings suggest the possibility of additional genetic discontinuity between populations, given the significant  $\Phi_{CT}$  values for different groupings.



**Figure 4.** UPGMA tree showing relationships between the 6 sampling sites.



**Figure 5.** The observed pairwise differences and the expected mismatch distributions under the sudden expansion model of swimming crabs: all populations; lineage A; lineage B.

**Table 3.** AMOVA results for 6 populations of *Portunus pelagicus* in Southeast Asia.

Population	Grouping	Source of Variation	Percentage of Variation	$\Phi$ Statistics	$p$ (More Extreme Value)
One group	Group 1 (HK, XM, VN, SGP, PH, KK)	AP	41.98	$\Phi_{ST} = 0.41978$	***
		WP	58.02		
Two groups	Group 1 (HK, XM, VN, SGP) Group 2 (PH, KK)	AG	46.57	$\Phi_{CT} = 0.46573$	***
		AP/WG	7.07	$\Phi_{SC} = 0.13235$	
		WP	46.36	$\Phi_{ST} = 0.53644$	
Three groups	Group 1 (HK, XM, VN) Group 2 (SGP) Group 3 (PH, KK)	AG	44.45	$\Phi_{CT} = 0.44445$	***
		AP/WG	4	$\Phi_{SC} = 0.07201$	
		WP	51.55	$\Phi_{ST} = 0.48446$	
Four groups	Group 1 (HK, XM) Group 2 (VN) Group 3 (SGP) Group 4 (PH, KK)	AG	43.42	$\Phi_{CT} = 0.43419$	***
		AP/WG	2.13	$\Phi_{SC} = 0.03759$	
		WP	54.45	$\Phi_{ST} = 0.45546$	

AG is the intergroup component of variance, AP/WG is the inter-population/intragroup component of variance, and WP is the intra-population component of variance. \* Significant at \*  $p < 0.05$ , \*\*\*  $p < 0.001$ , based on the permutation test.

#### 4. Discussion

This study analysed two lineages (A and B) of blue swimmer crabs (*P. pelagicus*) inhabiting Southeast Asia. The findings are consistent with those of previous research [57–60]. Greater nucleotide and haplotype diversities in populations with ancestral genotypes are an outcome of the long-term accumulation of mutations [61–63]. The study reported significantly higher nucleotide and haplotype diversities in lineage A ( $\pi = 0.044$ ;  $h = 0.992$ ) than in lineage B ( $\pi = 0.031$ ;  $h = 0.990$ ), indicating that lineage A was older than lineage B. Population age was estimated on the basis of an average mutation rate of 2.3%/My. The time of expansion was 597,718 and 360,125 years for lineages A and B, respectively. It appeared that lineage B had a smaller original population prior to an expansion or bottleneck given its steeper peak in the mismatch distributions (Figure 5) [7]. Furthermore, the pairwise distribution was more clearly displaced to the right of the distribution pattern, suggesting that lineage B's expansion could have been more recent [58–60].

As swimmer crabs progress through their life stages, they tend to migrate from inshore to offshore. However, given the limited distance of migration [38], larval dispersal remains the primary source of gene flow, with ocean currents playing an important role. This explains why the  $F_{ST}$  values between the KK and PH populations were at least twice as high as those obtained among the other populations. The mixing of populations and reduced genetic variation can also be attributed to disappearing habitats [17]. Since the Last Glacial Maximum, there has been a gradual expansion in blue swimmer crabs, depending on the sea level of the East China Sea.

While this study found two lineages (Figures 2 and 3), samples from the six populations did not always belong to both lineages. The XM, HK, VN, and SGP populations were only in lineage A, whereas the PH and KK populations comprised lineage B. Geographical conditions such as coastal currents traveling from mainland China to the South China Sea, the Chinese coastal stream flowing along Fujian to Vietnam [64], and the thermohaline circulation in Singapore's waters played a crucial role in the population structure. However, there was an isolated group in lineage B given the lack of obvious interactions between the KK samples in Taiwan and the coastal current in China for lineage A. *P. pelagicus* has an extended planktonic larval stage with potentially high larval dispersal, causing extensive gene flow between conspecific samples within a geographic mesoscale of 10–100 km [65]. In China, however, the population genetic structure had a limited occurrence and high gene flow along the distribution areas [41]. In Malaysia, microsatellite analyses have indicated low genetic differentiation between *P. pelagicus* populations [11], which is consistent with



findings from Moreton Bay, Queensland, where the species was found to travel as far as 20 km in one day [66,67]. This study found that blue swimmer crabs tend to be mixed within a mesoscale region (e.g., the Java Sea), but differ genetically at larger scales [68]. This finding is consistent with those of previous studies that reported that adult *P. pelagicus* travel within a geographic mesoscale on a daily basis, making them vagile [69].

Past and present habitat conformations have considerably influenced the genetic patterns of *P. pelagicus*. The exposed land mass of China and Southeast Asia contributed to lineage A, which has an older evolutionary age than lineage B. During the Last Glacial Maximum, the species in lineage A may have dispersed along the coast from China to Southeast Asia. As the sea levels declined, they first colonised the HK area, and subsequently spread to the SGP coasts with the rise in sea levels and the withdrawal of shorelines. The rising sea immersed the coastline, giving way to the PH and KK populations in lineage B. The warm currents of the South China Sea possibly dispersed lineage B to Penghu's waters.

The differing ages of the two lineages can be partially ascribed to the varying dispersal routes and times of origin, approximately one million years ago [1,41,43]. Lineage A—specifically the XM, HK, VN, PH, and SGP populations—carried the most prevalent allele. In other words, these populations likely share a common ancestor. For populations with ancestral genotypes, the long-term accumulation of mutations results in higher nucleotide and haplotype diversity [44–46]. The HK population may be the oldest population, because it showed significantly higher nucleotide ( $\pi = 0.046$ ) and haplotype ( $h = 0.995$ ) diversity compared to the other populations (Table 1). The KK population, on the other hand, may be the youngest. Our study also highlights that *P. pelagicus* originated in mainland China [16].

From the discussions thus far, it appears that the dispersal route for lineage B originated from the south of China (XM) to the north of areas at the same latitude (PH) and further toward southern Taiwan (KK). The SGP and VN populations in lineage A are to the extreme south of the sampling areas. The XM population in lineage B showed the lowest nucleotide and haplotype diversity. Furthermore, the relationship between the HK and SGP populations was not as close as that between the XM and HK populations (Figure 2). Thus, it is possible that lineage A had two distinct dispersal routes: one from the south of China to the south of the low-latitude areas (VN and SGP) and further north of Xiamen (XM), and the other one the south of China to the north of areas at the same latitude (PH and KK). Both lineages demonstrated demographic expansions, with lineage A starting in the second-to-latest interglacial period (597,718 years ago) and lineage B in the Last Glacial Maximum (360,125 years ago).

The distribution limit of lineage B probably extended to the Indian Ocean through the Strait of Malacca, given that the Sunda Shelf was blocked during the Last Glacial Maximum [17]. Thus, *P. pelagicus* found in the Indian Ocean possibly belong to another species, as indicated by Lai et al. [1]. More specifically, there was a sharp genetic break on the west side of the strait. Thus, the distribution of lineage B was limited to the South China Sea, and did not extend to the West Pacific (lineage A).

Studies have highlighted chaotic mismatch distributions only for populations in a long-term stable demographic equilibrium. However, recent rapid expansions or bottlenecks have resulted in unimodal (approximately Poisson) profiles with a steeper wave, indicating a smaller initial population prior to expansion [1,41]. This study revealed a steeper wave for lineage B (Figure 4).

## 5. Conclusions

This study found a strong genetic structure in Southeast Asia's blue swimmer crabs. It identified two main lineages (A and B), of which lineage A was the older. Both lineages had experienced population expansion since the Pleistocene glacial cycles in Southeast Asia (approximately 597,718 and 360,125 years ago for lineages A and B, respectively). Our research also examined the possible dispersal routes of the species, the findings of which indicated that lineage B could have experienced expansion more recently than lineage A.

Finally, land bridges formed during Pleistocene glacial maxima may have influenced the phylogeography and genetic structure of the species.

**Author Contributions:** Conceptualisation, Y.-M.L., C.-H.S., P.-C.C. and T.-D.T.; methodology, Y.-M.L., C.-H.S., P.-C.C. and T.-D.T.; software, Y.-M.L., C.-H.S. and P.-C.C.; validation, Y.-M.L., C.-H.S., P.-C.C., W.-C.K., Y.-C.L. and T.-D.T.; formal analysis, Y.-M.L., C.-H.S., P.-C.C., W.-C.K., Y.-C.L. and T.-D.T.; investigation, Y.-M.L., C.-H.S., P.-C.C., W.-C.K., Y.-C.L. and T.-D.T.; resources, Y.-M.L., C.-H.S. and P.-C.C.; data curation, Y.-M.L., C.-H.S. and P.-C.C.; writing—original draft preparation, Y.-M.L., C.-H.S. and P.-C.C.; writing—review and editing, Y.-M.L., C.-H.S., Y.-S.H. and P.-C.C.; visualisation, Y.-M.L., C.-H.S. and P.-C.C.; supervision, Y.-M.L., C.-H.S., P.-C.C. and T.-D.T.; project administration, Y.-M.L., C.-H.S., P.-C.C. and T.-D.T.; funding acquisition, Y.-M.L., C.-H.S., P.-C.C. and T.-D.T. All authors have read and agreed to the published version of the manuscript.

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