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Population genetic structure of Blue Swimming Crab (*Portunus pelagicus*) in the Gulf of Thailand

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Abstract. *Supmee V, Sawusdee A, Sangthong P, Suppapan J. 2020. Population genetic structure of Blue Swimming Crab (*Portunus pelagicus*) in the Gulf of Thailand. Biodiversitas 21: 4260-4268.* The Blue Swimming Crab (*Portunus pelagicus*) is an important commercial fishery product in the Gulf of Thailand. To provide a strategy for management, information on genetic features is needed. In our study, the population genetic structure and demographic history of the *P. pelagicus* living in the Gulf of Thailand were analyzed based on the variation of the nucleotide sequence of the mitochondrial DNA in the control region (mtDNA *CR*). Ninety-seven samples were collected from 5 sampling sites: Rayong, Chonburi, Chumphon, Surat Thani, and Nakhon Si Thammarat provinces in the Gulf of Thailand. Forty-nine haplotypes were identified and 39 private haplotypes were found. An AMOVA showed no genetic structure among populations. The pairwise F_{ST} also indicated no statistically significant difference between all possible regional combinations. The topology of a minimum spanning network revealed a star-like topology that was not separated by geographic structure. The historical demographic analysis revealed a stable population size for a long period and followed by a very recent expansion. An absence of a population structure of the *P. pelagicus* was possibly caused by a high level of gene flow. The results of this study differ from previous studies that used genetic markers in nuclear DNA. Thus, to clear the genetic structure information of *P. pelagicus* in the Gulf of Thailand, we suggested that more sensitive markers to detect genetic structure should be used in further analysis.

Keywords: Blue Swimming Crab, control region, demographic history, genetic diversity, Thailand

INTRODUCTION

The Blue Swimming Crab (*Portunus pelagicus*) belongs to the family Portunidae. It is generally found inshore in sandy and muddy habitats as well as among seagrass beds throughout the Indo-West Pacific (Kembaren et al. 2018; Zainal 2013). It is an important commercial seafood product in Thailand (Nitiratsuwan et al. 2013). In 2017, the total catch volume of *P. pelagicus* in Thailand was 28,907 tons. Especially, in the Gulf of Thailand, the important fishing ground was fished about 22,123 tons (Fishery Statistics Analysis and Research Group 2017). Nowadays, numerous commercial product of *P. pelagicus* in the Gulf of Thailand is increasing (Oniam et al. 2018). Further, a large number of the small size of wild-caught crabs are increasing, indicating overexploitation of this species (Anantasuk and Nitiratsuwan 2015).

Population genetic structure is referred to as the pattern of genetic diversity across multiple demes (Reed and Frankham 2003). Genetic diversity is maintained by gene flow and correlated with population fitness (Garner et al. 2005). Then understanding the genetic structure is necessary for the conservation of the organism (Frankham et al. 2002). The population genetic structure of *P. pelagicus* in Thailand was studied by Klinbunga et al. (2010) and Klinbunga et al. (2007), and the result showed a restricted gene flow level of *P. pelagicus* in Thai waters. Many studies have revealed the genetic structure in various marine populations in the Gulf of Thailand such as *Donax spp.* (Manatrinon et al. 2012), *Paphia undulata* (Donrung et al. 2011), and *Penaeus merguiensis* (Wanna et al. 2004). However, the various report revealed that only one population of marine animals in the Gulf of Thailand has been found such as *Lates calcarifer* (Sodsuk et al. 2012), *Rachycentron canadum* (Phinchongsakuldit et al. 2013), and *Perna viridis* (Prakoon et al. 2010). It shows that the population genetic structure of marine species living in the Gulf of Thailand does not occur in all marine species.

The genetic feature of *P. pelagicus* in Thailand was studied using nuclear DNA marker by Klinbunga et al. (2010) (AFLP) and Klinbunga et al. (2007) (RAPD). However, in the past decade, mitochondrial DNA (mtDNA) has been extensively used for studies regarding the population genetic structure of many marine species (Guo et al. 2011; Durand et al. 2013; Taguchi et al. 2015; Lau et al. 2018) because it has a unique feature, e.g. haploidy, maternal inheritance, high copy number, non-recombination, and rapid mutation rate (Boore, 1999). Especially, the control region (mtDNA *CR*) is a high mutation area within the mtDNA molecule (Avise, 1994). The mutation rate in the mtDNA *CR* is approximately 5-10 times higher than other regions in the mitochondrial

genome and is approximately 25-100 times higher than nuclear genes (Boore, 1999). It has been used to examine population genetic structure in various marine crabs such as *Episesarma versicolor* (Supmee et al. 2012a), *P. trituberculatus* (Shan et al. 2018), and *Cardisoma guanhumi* (Amaral et al. 2015). Thus, mtDNA *CR* is one good DNA marker for studies of population structure. In our study, we used the mtDNA *CR* to study the genetic structure of *P. pelagicus* in the Gulf of Thailand as additional information in addition to the nuclear DNA marker such as RAPD and AFLP genetic markers. The objective of the study was to examine the population genetic structure and the demographic history of *P. pelagicus* living in the Gulf of Thailand based on the nucleotide sequence of mtDNA *CR*. This information would assist the management of *P. pelagicus* to aid policy design about fisheries and conservation management.

MATERIALS AND METHODS

Sampling

Ninety-seven of *P. pelagicus* were caught from 5 sampling sites: Rayong (*N*= 17), Chonburi (*N*= 19), Chumphon (*N*= 19), Surat Thani (*N*= 22), Nakhon Si Thammarat (*N*= 20) in the Gulf of Thailand (Figure 1). Fresh samples were stored in ice during transportation to a laboratory and kept at -20 ºC until required.

DNA extraction, PCR amplification, and nucleotide sequencing

Total genomic DNA was extracted from the muscle in the walking leg using the Genomic DNA Extraction Kit (Tiangen BioTech, China) followed by the manufacturer protocol. Based on the complete nucleotide sequence database in mtDNA *CR* of *P. pelagicus* from the National Center for Biotechnology Information (accession number: MN635711.1, Koolkarnkhai et al. 2019), primers were designed using Primer 3 program (Untergasser et al. 2012) to amplify the partial nucleotide sequence of the control region. The pair of primers was PPCR_H1 5'-TTG AGG GAA ACC AGA AAG ATT 3' and PPCR_L1 5'-CCA TGC GTT AAA ATA CAA ATT C 3'. The polymerase chain reaction (PCR) was conducted. The reaction mixture, a total volume of 50 ml, consisted of 10X *Taq* buffer 5 ml, 25 mM $MgCl_2$ 7.5 ml, 2 mM dNTPs mix 4 ml, 10 mM forward primer 2 ml, 10 mM reverse primer 2 ml, *Taq* DNA polymerase (RBCbiosciences, USA) 0.5 ml (2.5 unit), total DNA 5 ml (50-100 ng) and ultrapure water 24 ml. The reaction mixtures were amplified in Major Cycler, CYCLER, TAIWAN. The PCR cycling profile consisted of initial denaturation at 94ºC for 4 min, followed by 35 cycles of 94ºC for 40 sec, 52.5 ºC for 1 min, 72ºC for 1 min, and a final extension at 72ºC for 5 min. The correct size of the PCR products was checked by gel electrophoresis technique. The PCR products were purified by using Gel/PCR Purification Mini Kit (TiangenBioTech, China) according to the manufacturer's protocol. The purified DNA was sent to 1ST Base Laboratory, Malaysia for direct sequencing.

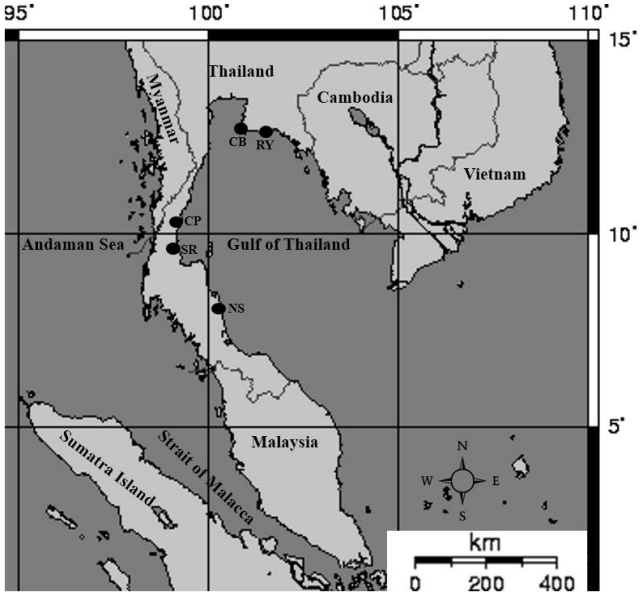

Figure 1. Map showing sampling areas of the *Portunus pelagicus* from the Gulf of Thailand: the upper Gulf of Thailand; Rayong (RY), and Chonburi (CB): the lower Gulf of Thailand; Chumphon (CP), Surat Thani (SR), and Nakhon Si Thammarat (NS)

Data analysis

Genetic diversity

Sequences obtained from each specimen were initially confirmed with the reference sequence of mtDNA *CR* from NCBI (accession number: MN635711.1, Koolkarnkhai et al. 2019). Multiple sequences were aligned using ClustalW version 2.0.12 (Larkin et al. 2007) and the ambiguous positions of the aligned sequences were adjusted manually. The genetic diversity analysis including the nucleotide diversity (π), haplotype diversity (h), and the number of polymorphic sites among all haplotypes per population and total population were calculated using DnaSP version 6.00 (Rozas et al. 2017).

Population genetic structure

The population genetic structure of *P. pelagicus* was determined based on 2 putative structures. The samples were separated into a single region according to the sampling sites (RY, CB, CP, SR, and NS) for the first structure. For the second structure, the specimens were determined according to the geographic-based regions: the upper Gulf of Thailand (RY and CB) and the lower Gulf of Thailand (CP, SR, and NS). An analysis of molecular variance (AMOVA) was performed with ARLEQUIN v. 3.5 (Excoffier and Lischer 2010) to compare levels of genetic diversity within and among putative populations. The associated *F*-statistic analogs including Φ_{CT}, Φ_{SC}, and Φ_{ST} were estimated at different hierarchical levels using 10,000 permutations. Pairwise F_{ST} was used to estimate the genetic distances between all possible combinations of populations using 10,000 permutations. A minimum spanning network (MSN) was constructed using ARLEQUIN based on the mean number of pairwise differences between all haplotypes of the mtDNA *CR* and drawn by hand.

Demographic history

The demographic history of *P. pelagicus* was examined by using 3 different approaches. Firstly, neutrality tests for each sampling locality and the entire Gulf of Thailand population were tested using Fu's *Fs* (Fu 1997) and Tajima's *D* (Tajima 1989) statistics based on 10,000 replicates. Secondly, the mismatch distribution analysis under the sudden expansion model was estimated as implemented in ARLEQUIN. The fit between observed and expected mismatch distribution was tested using the Harpending raggedness index (Harpending 1994). The sum of squared deviations (*SSD*) was performed to test the goodness-of-fit with 10,000 bootstrap replicates. Finally, Bayesian skyline analysis was calculated by using BEAST/BEAUTi ver. 1.7.2 (Drummond et al. 2012) to infer the temporal change in the effective population size (*Ne*). Due to a lack of the evolutionary rate of the mtDNA *CR* in crabs, we employed a mutation rate of 19 % per million years, as used by *Farfantepenaeus aztecus* (McMillen-Jackson and Bert 2003). The Bayesian skyline plot was generated by Tracer ver. 1.6 (Rambaut et al. 2014).

RESULTS AND DISCUSSION

Genetic diversity

Partial nucleotide sequences of mtDNA *CR* from 97 individuals of *P. pelagicus* were 411–417 base pairs. The average nucleotide compositions of A, T, G, and C were 41.3%, 34.0 %, 8.3%, and 16.4%, respectively. The A+T base content was 75.3% and G+C was 24.7 %. The results showed 59 polymorphic sites (36 singleton sites and 23 parsimony informative sites). In total, 49 haplotypes were identified. Nine haplotypes were shared among the sampling localities (H01, H02, H05, H06, H08, H12, H14, H16, and H19), and 1 haplotype was shared within the population (H21). Five populations had their specific characteristic haplotypes (private haplotype). Thirty–nine private haplotypes were found. CP and SR population possessed 9 private haplotypes, while RY, CB, and NS population carried 7 private haplotypes, respectively (Table 2). Haplotype diversity and nucleotide diversity ranged from 0.868 to 0.985 and 0.009 to 0.011, respectively. Overall, haplotype diversity and nucleotide diversity were 0.933 and 0.008, respectively (Table 1).

Population genetic structure

The AMOVA of the first putative structure was not statistically significant (Φ_{ST}=-0.018, p=0.920). Regarding the second structure tested (upper Gulf of Thailand x lower Gulf of Thailand), we found no significant genetic structure as well (Φ_{CT}=-0.002, p=0.799) (Table 3). Every pairwise F_{ST} of the geographic-based populations showed no significant differences for most pairwise comparisons (Table 4). The haplotype network showed a star-like network and did not indicate any distinct pattern of phylogeographic structure (Figure 2).

Demographic history

Neutrality tests, the Fu's *Fs*, and Tajima's *D* were performed for each locality and pooled populations (Table 5). Fu's *Fs* statistics of all populations were statistically significantly negative. The *Fs* statistic of the pooled population showed a statistically significant negative value (*Fs*=-26.121, p=0.000). Tajima's *D* statistics of populations of CP and NS populations were statistically non-significantly negative, and populations of RY, CB, and SR populations were statistically significantly negative. However, when all populations were pooled together, the *D* statistic was statistically significant (*D*=-2.221, p=0.000). According to the measured *SSD* from the goodness-of-fit test, the mismatch distribution observed from the pooled population did not fit a sudden expansion model (*SSD*=0.122, p=0.002). The Harpending raggedness indices revealed non-significant low values (*rg*=0.032, p=0.924) (Table 5). Mismatch distribution determined from the total sample was bimodal (Figure 3). The Bayesian skyline analysis revealed that the expansion time of the *P. pelagicus* population in the Gulf of Thailand had occurred around 3,000 years ago (Figure 4).

Table 1. Collecting localities, number of individuals per sampling site (*N*), number of polymorphic sites, number of haplotypes, haplotype diversity (*h*), and nucleotide diversity (π) of *Portunus pelagicus* living along the Gulf of Thailand coast

Collecting localities	*N*	No. polymorphic sites	No. haplotypes	Haplotype diversity (*h*) (mean±SD)	Nucleotide diversity (π) (mean±SD)
Rayong (RY)	17	26	15	0.985±0.025	0.010±0.002
Chonburi (CB)	19	26	13	0.930±0.047	0.011±0.002
Chumphon (CP)	19	24	16	0.982±0.022	0.011±0.001
Surat Thani (SR)	22	27	15	0.939±0.037	0.009±0.001
Nakhon Si Thammarat (NS)	20	22	11	0.868±0.057	0.009±0.002
Total	97	59	49	0.933±0.016	0.008±0.000

Table 2. Haplotype distributions of *Portunus pelagicus* from 5 localities in the Gulf of Thailand

Haplotype	RY	CB	CP	SR	NS	Total	Haplotype	RY	CB	CP	SR	NS	Total
H01	1	-	1	-	-	2	H26	-	-	1	-	-	1
H02	2	5	2	2	6	17	H27	-	-	1	-	-	1
H03	1	-	-	-	-	1	H28	-	-	1	-	-	1
H04	1	-	-	-	-	1	H29	-	-	1	-	-	1
H05	1	-	1	-	-	2	H30	-	-	1	-	-	1
H06	1	-	1	1	-	3	H31	-	-	1	-	-	1
H07	1	-	-	-	-	1	H32	-	-	1	-	-	1
H08	3	1	2	6	5	17	H33	-	-	1	-	-	1
H09	1	-	-	-	-	1	H34	-	-	-	1	-	1
H10	1	-	-	-	-	1	H35	-	-	-	1	-	1
H11	1	-	-	-	-	1	H36	-	-	-	1	-	1
H12	1	1	-	-	-	2	H37	-	-	-	1	-	1
H13	1	-	-	-	-	1	H38	-	-	-	1	-	1
H14	1	-	1	-	-	2	H39	-	-	-	1	-	1
H15	-	1	-	-	-	1	H40	-	-	-	1	-	1
H16	-	1	-	1	1	3	H41	-	-	-	1	-	1
H17	-	1	-	-	-	1	H42	-	-	-	1	-	1
H18	-	1	-	-	-	1	H43	-	-	-	-	1	1
H19	-	2	2	3	1	8	H44	-	-	-	-	1	1
H20	-	1	-	-	-	1	H45	-	-	-	-	1	1
H21	-	2	-	-	-	2	H46	-	-	-	-	1	1
H22	-	1	-	-	-	1	H47	-	-	-	-	1	1
H23	-	1	-	-	-	1	H48	-	-	-	-	1	1
H24	-	1	-	-	-	1	H49	-	-	-	-	1	1
H25	-	-	1	-	-	1	Total	17	19	19	22	20	97

Note: Stations codes are given in Table 1.

Table 3. A hierarchical analysis of molecular variance (AMOVA) based on nucleotide sequence in mtDNA *CR* of *Portunus pelagicus*

Source of variation	df	Sum of squares	Variance components	Percentage of variation	*p*-value
Single region					
Among populations	4	4.734	-0.032 Va	-1.81	Φ_{ST}= -0.018, (*p*= 0.920)
Within populations	92	166.050	1.804 Vb	101.81	
Total	96	170.784			
Upper and lower of Gulf of Thailand					
Among groups	1	1.041	-0.004 Va	-0.26	Φ_{CT}= -0.002, (*p*= 0.799)
Among populations within groups	3	3.693	-0.029 Vb	-1.66	Φ_{SC}= -0.016, (*p*= 0.845)
Within populations	92	166.050	1.804 Vc	101.92	Φ_{ST}= -0.019, (*p*= 0.923)
Total	96	170.784	1.770		

Note: *p* values in parentheses

Table 4. Population pairwise F_{ST} values based on nucleotide sequence in mtDNA *CR* of *Portunus pelagicus*

Population		Upper Gulf of Thailand		Lower Gulf of Thailand		
		RY	CB	CP	SR	NS
Upper Gulf of Thailand	RY	-				
	CB	-0.023 (0.828)	-			
Lower Gulf of Thailand	CP	-0.009 (0.468)	-0.004 (0.369)	-		
	SR	-0.028 (0.918)	-0.023 (0.855)	-0.006 (0.468)	-	
	NS	-0.025 (0.837)	-0.030 (0.936)	-0.014 (0.612)	-0.021 (0.864)	-

Note: *p* values in parentheses. Stations codes are given in Table 1.

Table 5. Neutrality test and parameter indices of mismatch distribution analysis based on nucleotide sequence in mtDNA *CR* of *Portunus pelagicus*

Collecting localities	Fu' s *Fs*	Tajima's *D*	Sum of squared deviations (*SSD*)	Raggedness index (*rg*)
Rayong	-9.601* (0.000)	-1.924* (0.014)	0.033 (0.092)	0.089 (0.190)
Chonburi	-6.085* (0.001)	-1.687* (0.029)	0.065 (0.059)	0.065 (0.404)
Chumphon	-10.141* (0.000)	-1.270 (0.095)	0.050 (0.051)	0.049 (0.384)
Surat Thani	-6.382* (0.002)	-1.735* (0.023)	0.129 (0.059)	0.024 (0.980)
Nakhon Si Thammarat	-3.670* (0.027)	-1.432 (0.060)	0.029 (0.752)	0.057 (0.693)
Total	-26.121* (0.000)	-2.221* (0.000)	0.122 (0.002)	0.032 (0.924)

Note: *significant differentiation (*p*<0.05)**,** *p* values in parentheses

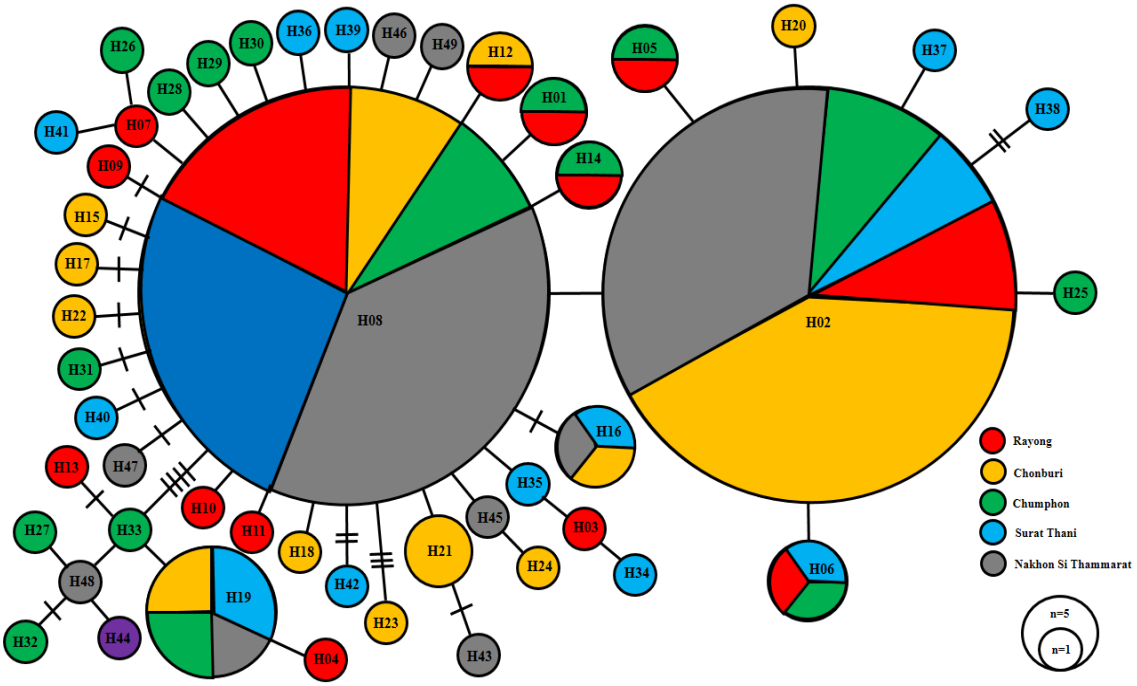

Figure 2. Minimum spanning network of mtDNA *CR* haplotypes of the *Portunus pelagicus*: Circles represent haplotypes and their size is proportional to the observed frequency. The color in the circle is the collecting site. The single line connecting directly between haplotype is one mutation step. The number of vertical bars on the connecting line is an increasing number of mutation steps

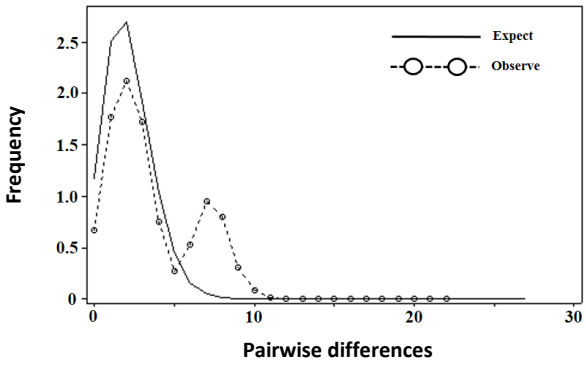

Figure 3. Mismatch distribution under a sudden population expansion model based on nucleotide sequence in mtDNA *CR* of *Portunus pelagicus*

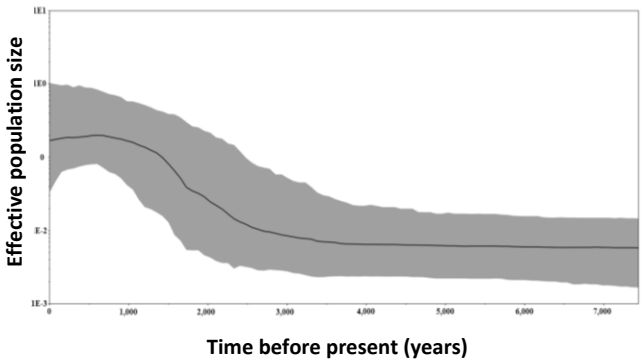

Figure 4. Bayesian skyline plots showing the historical demography of *Portunus pelagicus* in the Gulf of Thailand. The black line is the median estimation and the highlight is the limit of 95 % confidence interval

Discussion

Genetic diversity

In our study, it was found that A+T base content is higher than G+C base content, which is consistent with other reports that A+T base content is higher in the mitochondrial genome in animals (Fourdrilis et al. 2018). A total of 49 mtDNA CR haplotypes were analyzed and 39 haplotypes were private. The presence of many private haplotypes in all populations included in our study indicated the existence of a large female effective population size of the *P. pelagicus* in the Gulf of Thailand (Lewontin 1974). This haplotype frequency is thought to reflect a large effective population size that allowed for the retention of numerous unique haplotypes in the female. The nucleotide diversity of the *P. pelagicus* in our study was 0.008. Comparing with those of other crabs, the nucleotide diversity in the mtDNA *CR* of the *P. pelagicus* in the Gulf of Thailand was low. For example, the nucleotide diversity of the *P. trituberculatus* was 0.020 (Guo et al. 2011) and 0.025 (Hui et al. 2019), *C. guanhumi* was 0.027 (Amaral et al. 2015), and *E. mederi* was 0.023 (Supmee et al. 2012b). A high value of haplotype diversity but a low level of nucleotide diversity was presented within the *P. pelagicus* population in the Gulf of Thailand. This genetic variation pattern has been revealed in various crustacean species, such as *Fennneropenaeus chinensis* (Kong et al. 2010), *E.*

versicolor (Supmee et al. 2012a), and *Varuna litterata* (Suppapan et al. 2017).

Population genetic structure

The main factor to generate the genetic structure of marine animals in the Gulf of Thailand was the water circulation, the long-distance between habitat, and the reproductive behavior of marine species. Firstly, in the Gulf of Thailand, the seasonal currents showed a counterclockwise gyre in the upper gulf and clockwise gyre in the lower gulf during the southwest monsoon season. In the northeast monsoon season, the sea currents showed a clockwise gyre in the upper gulf and counterclockwise gyre in the lower gulf. (Sojisuporn et al. 2010). In the central gulf, the sea current revealed a clockwise gyre connected with the sea current of the lower and upper gulf in all monsoon season (Saramul 2017). The different flow patterns of sea current in the Gulf of Thailand act like geographic obstacles and prevent the gene flow between populations (Panithanarak 2017). The results of a study of population genetics using different genetic markers of marine animals in the Gulf of Thailand found that the genetic structure was caused by water circulation such as *P. pelagicus* (RAPD) (Klinbunga et al. 2010), *Donax spp.* (ISSR) (Manatrinon et al. 2012), and *P. monodon* (mtDNA *COI*) (Khamnamtong et al. 2009). Secondly, the long distance between the upper and lower Gulf of Thailand population causes the exchange of genes to be restricted during the planktonic larva. Donrung et al. (2011) examined the genetic structure of *P. undulata* in the Gulf of Thailand by using the ISSR technique as a genetic marker. The result revealed that the distance of 450 km between the upper and lower Gulf of Thailand prevented the gene flow of this species. Thirdly, the reproductive behavior of marine species affects the genetic structure. For example, the reproductive process of a mature female of *P. pelagicus* is migrated outward to the deep sea for spawning and returns to the original habitation after spawning (Hamid et al. 2016). This reproductive behavior was limited habitats of the *P. Pelagicus* and a population genetic structure was formed.

This finding of the present study revealed that most of the population genetic structure analysis showed a lack of population genetic structure of *P. pelagicus* in the Gulf of Thailand. These results suggested a high-level gene flow of *P. pelagicus* in the Gulf of Thailand. The low levels of genetic differentiation among the *P. pelagicus* populations were reported in many areas such as *P. pelagicus* population living along with the coastal areas of Malaysia that inferred from microsatellites marker (Chai et al. 2017) and *P. pelagicus* population living along the southeastern area of China that analyzed based on mtDNA *COI* sequences (Ren et al. 2018). Genetic homogeneity of the *P. pelagicus* population in the Gulf of Thailand was plausibly maintained by the long duration of the planktonic larva stage. *P. pelagicus* had a long larval phase at the age of 26-45 days and high dispersal ability (Efrizal 2016). Most marine species spend part of the stage of their life cycle in the open sea as free-moving gametes, larvae, or adults (Uthicke and Benzie, 2003). Especially, marine species with a long duration of the larval stages are believed to have high levels of genetic variation within populations (Russo et al. 1994). Additionally, a long-duration planktonic larval stage influences the opportunity for a high degree of gene flow as evidenced by an absence of genetic differentiation in several species, for example, *Neritina canalis* and *Neripteron dilatatus* (46 days) (Crandall et al. 2010), *Neosarmatium meinerti* (43 days) (Huang et al. 2018), and *Uca annulipes* (28 days) (Silva et al. 2010). Further, the long larva duration may enhance the mixing of the planktonic larva by the connection of the water circulation among the lower, central, and upper Gulf of Thailand. Thus, it made it possible to promote gene flow that extensively covered these 2 areas. Besides, a lack of geographic barriers in the Gulf of Thailand may not disrupt the gene flow of the *P. pelagicus* population between the upper and the lower Gulf of Thailand. Genetic homogeneity between populations from long-distance marine crabs was maintained by the high ability of larva dispersal. For example, Silva et al. (2010) examined the population structure of *U. annulipes* on the African east coast by using mtDNA *COI* as a genetic marker. The result revealed that *U. Annulipes* maintained a high level of gene flow along 3,000 km of the African east coast. Several reports of the different genetic marker technique revealed that only one population of marine animals in the Gulf of Thailand has been found such as *L. calcarifer* (microsatellites) (Sodsuk et al. 2012), *Hippocampus kuda* (mtDNA *CR*) (Panithanarak et al. 2010), and *Meretrix meretrix* (mtDNA *COI*) (Supmee et al. 2020).

In our result, the genetic structure of *P. pelagicus* in the Gulf of Thailand was different from the findings of Klinbunga et al. (2010) and Klinbunga et al. (2007) that revealed a restricted gene flow level of *P. pelagicus* between geographic samples in the Gulf of Thailand. Different results of the study were likely due to the use of different genetic markers. The studies were done by Klinbunga et al. (2010) and Klinbunga et al. (2007) used the genetic marker in the nuclear genome including RAPD and AFLP techniques to detect genetic structure, while our study employed the mitochondrial genome. In general, nuclear DNA markers have a high level of polymorphism and are powerful DNA markers for quantifying genetic variations within and between populations (Abdul-Muneer 2014). However, comparing the genetic markers between nuclear DNA and the mtDNA in the previous study, it was found that there were different results in each marine species. For example, the *Synechogobius ommaturus* population living along the coast of China showed a genetic structure by using the mtDNA *CR* marker (Song et al. 2010a) but revealed a weak genetic structure by using the AFLP marker (Song et al. 2010b). The genetic structure of *Haliotis asinine* in the Thailand coast was found by using an AFLP genetic marker (Praipue et al. 2010), while a lack of genetic structure was reported based on restriction analysis of mtDNA in *16S rRNA* (Klinbunga et al. 2003). Genetic differentiation of the *P. trituberculatus* population along the coast of China was revealed by using a microsatellite marker (Guo et al. 2013), but a weak genetic structure was found by using the mtDNA *CR* marker (Wu et al. 2009). Therefore, in the study of the population

genetic structure of marine animals, various genetic markers should be used for the combined analysis as the results of this study differ from those of previous studies. To clear the genetic structure information of *P. pelagicus* in the Gulf of Thailand, we suggested that more sensitive nuclear DNA markers to detect genetic structure should be used in further analysis.

Demographic history

A goodness-of fit-test was not fitted with a mismatch distribution under a sudden expansion model. That the observed mismatch distribution was bimodal indicated a stable population size for a long time (Rogers and Harpending 1992). However, the minimum spanning network showed a star-like topology with a lot of single mutation steps. This topology can be found in populations that have sudden expansion (Ferreri et al. 2011). Also, the Bayesian skyline plot showed a recent population expansion. Besides, neutrality test, Fu's *Fs*, and Tajima's *D* showed significantly negative deviation from the neutral state and indicated that a recent population expansion of *P. pelagicus* living in the Gulf of Thailand might have occurred (Omori and Wu 2017). Thus, all independent analyses of the demographic history test revealed that the demographic history of *P. pelagicus* in the Gulf of Thailand was a stable population size for a long period followed by the occurrence of its recent expansion.

In conclusion, this finding of the present study found that the *P. pelagicus* population in the Gulf Thailand was the single population. The low level of the genetic structure of *P. pelagicus* might be caused by a high level of gene flow from a lack of geographic barrier, a long larva duration stage, and high dispersion ability of *P. pelagicus*. However, our findings differ from those of Klinbunga et al. (2010) and Klinbunga et al. (2007) due to the use of different genetic markers. Therefore, for effective management of the genetic diversity of *P. pelagicus* in the Gulf of Thailand, additional studies should be performed using more highly sensitive genetic markers. Population history results show that the population of *P. pelagicus* in the Gulf of Thailand has been stable for a long time and has recently started to rapidly expand the population size.

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