

Population Genetic Structure of the Blue Swimming Crab (*Portunus pelagicus*) along the Andaman Sea Coast of Thailand

(Struktur Genetik Populasi Ketam Renang Biru (*Portunus pelagicus*) di sepanjang Pantai Laut Andaman, Thailand)

JUTHAMAS SUPPAPAN¹, APIRAK SONGRAK², WORAWITOO MEESOOK³ & VERAKIAT SUPMEE^{3,*}

¹Master of Education in Science Program, Faculty of Education, Nakhon Si Thammarat Rajabhat University, Nakhon Si Thammarat, Thailand

²Department of Fishery Technology, Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Trang Campus, Trang, Thailand

³Department of Science, Faculty of Science and Technology, Rajamangala University of Technology Srivijaya, Nakhon Si Thammarat Campus, Nakhon Si Thammarat, Thailand

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ABSTRACT

The blue swimming crab (*Portunus pelagicus*) is a valuable commercial species. In Thailand, the consumption of the blue swimming crab is increasing. To construct an effective sustainable management plan, genetic information is required. Knowledge of natural stock genetic information can help policymakers design fishing and conservation management policies to maintain genetic diversity. In our study, we examined the genetic structure of the blue swimming crab population on the Andaman Sea coast of Thailand. Partial sequences of the mitochondrial DNA control region (mtDNA CR) with a size of 369-371 base pairs were studied in 150 individuals collected from five sampling sites along Thailand's Andaman Sea coast. Seventy-one haplotypes were characterized by 127 polymorphic sites, indicating high haplotype diversity but low nucleotide diversity across five localities. The multiple tests of population genetic analysis showed a lack of genetic structure. The absence of genetic structure was possibly caused by a high level of gene flow due to the larval blue swimming crab's high dispersal ability. Demographic history findings indicate that the blue swimming crab population has expanded. The findings of our study can be used to guide the management of the blue swimming crab in this region of the Andaman Sea to conserve genetic diversity.

Keywords: Andaman Sea; genetic diversity; marine crab; mitochondrial DNA

ABSTRAK

Ketam renang biru (*Portunus pelagicus*) adalah spesies komersial yang berharga. Di Thailand, pengambilan ketam renang biru semakin meningkat. Untuk membina plan pengurusan mampan yang berkesan, maklumat genetik diperlukan. Pengetahuan tentang maklumat genetik stok semula jadi boleh membantu penggubal dasar membentuk dasar pengurusan perikanan dan pemuliharaan untuk mengekalkan kepelbagaian genetik. Dalam penyelidikan ini, kami mengkaji struktur genetik populasi ketam renang biru di pantai Laut Andaman, Thailand. Jujukan separa kawasan kawalan DNA mitokondria (mtDNA CR) dengan saiz 369-371 pasangan asas telah dikaji dalam 150 individu yang dikumpulkan dari lima tapak persampelan di sepanjang pantai Laut Andaman, Thailand. Tujuh puluh satu haplotip dicirikan oleh 127 tapak polimorfik, menunjukkan kepelbagaian haplotip yang tinggi tetapi kepelbagaian nukleotida rendah merentas lima lokaliti. Pelbagai ujian analisis genetik populasi mendedahkan kekurangan struktur genetik. Ketiadaan struktur genetik mungkin disebabkan oleh tahap aliran gen yang tinggi disebabkan oleh keupayaan penyebaran tinggi ketam renang biru larva. Penemuan sejarah demografi menunjukkan bahawa populasi ketam renang biru telah berkembang. Penemuan kajian kami boleh digunakan sebagai bimbingan pengurusan ketam renang biru di wilayah Laut Andaman ini untuk memulihara kepelbagaian genetik.

Kata kunci: DNA mitokondria; kepelbagaian genetik; ketam marin; Laut Andaman

INTRODUCTION

The blue swimming crab (*Portunus pelagicus*) is a commercial marine crab in Thailand (Nitiratsuwat, Tanyaros & Panwanitdumrong 2013). It is distributed along the coast, around seagrass sites with the sandy ground (Kembaren et al. 2018). Most crab yields come from natural catches since commercial farming has not been successful and is costly. In addition to the blue swimming crab being an aquatic crab commonly consumed in the country, they are an important raw material for the ongoing industrial sector in processing and export. In Thailand, the blue swimming crab is distributed throughout the Andaman Sea and Gulf of Thailand coast. Especially at the total distance of about 900 kilometers along the Andaman Sea coast, which is Thailand's crab fishing ground. According to fisheries statistics for the Andaman Sea, crab yields amounted to 8,600 metric tons in 2010. In 2020, it rose to 35,900 metric tons, indicating that the Andaman Sea's blue swimming crab catches over the past ten years had increased (Fishery Statistics Analysis and Research Group 2022, 2012).

Genetic variation changes cause population genetic structure. Various factors affect the genetic structure, such as mutation, disruption of gene flow, or environmental factors (Ayala 1982; Slatkin & Hudson 1991). The structural pattern of population genetics indicates survival ability; organisms can adapt to the source of the habitat, expressing themselves in the form of genetic diversity appropriate for the cohort (Tudela, Garcya-Marynn & Pla 1999). An improved understanding of population genetic structure is critical for effective fisheries management and the genetic conservation of resources in exploited marine organisms (Bert et al. 2007). Stock enhancement and cultivation have led to a decrease in the genetic diversity of the population. Admixed populations will undergo harmful genetic changes if genetic variation, fitness, and effective population size decrease because maintaining the genetic diversity of admixed populations and their wild population components first necessitate managing both genetic variability and genetic composition in brood stocks and broods (Bert et al. 2007). The genetic risks of aquaculture, according to Waples, Hindar and Hard (2012), include loss of genetic diversity within and among populations, as well as loss of fitness caused by the use of low genetic diversity and small numbers of brood stock. This situation can lead to inbreeding, which has implications for fry quality as symmetrical survival of 'families' causes stock quality to decline. The primary goals of genetic management are to maintain genetic variability

and avoid inbreeding to maximize captive populations' long-term viability. To achieve the goal of maintaining genetic variability, colony managers must be able to assess genetic variability. Additionally, understanding population connectivity via genetic structure has significant conservation implications because genetic assessment is a criterion in determining the appropriate units and spatial scale for conservation and management (Waples & Gaggiotti 2012). Therefore, information on population genetic structure has the potential to guide fisheries management for specific genetic groups (Roldan et al. 2000).

The Andaman Sea's primary currents go in opposite directions. The first current starts from the north in Ranong province to the south in Phang Nga province and flows into the Indian Ocean. The second current originates from the south in Satun province to the north in Phang Nga province and flows into the Indian Ocean (Brown 2007; Chatterjee et al. 2017; Kiran 2017). The different directions of the current are likely to be a factor in the disruption of the gene flow of aquatic animals living along the Andaman Sea. There have been many reports that marine life living along the Andaman Sea has emerged as population genetic form structures divided into the lower Andaman Sea and the upper Andaman Sea, such as the giant clam (*Tridacna maxima*) (Kittiwattanawong 1997), the greenback mullet (*Liza subviridis*) (Supmee et al. 2017), and the sandfish (*Holothuria scabra*) (Ninwichian & Klinbunga 2020). In our study, we hypothesized that the direction of current flow in the Andaman Sea separates the blue swimming crab population into two groups.

Currently, the study of population genetics in many animals is commonly analyzed based on nucleotide sequences from mitochondrial DNA (mtDNA) (Marini et al. 2021; Sun et al. 2021). The nucleotide sequence in mitochondrial DNA in the control region (mtDNA CR) is suitable because it is a region with a higher mutation rate than another region of mtDNA, making it appropriate for evaluating genetic variability within the population. In addition, maternal heredity is haploid, so samples are used to study in smaller numbers than other genome-examined genetic markers in the nucleus (Avisé 2000; Boore 1999). Over the years, nucleotide sequences from mtDNA CR have been used to study the population genetic structures in many marine animals, such as the Chinese pomfret (*Pampus chinensis*) (Sun et al. 2021), the invader brine shrimp (*Artemia franciscana*) (Subramani, Gunasagaran & Natesan 2021), and the blue swimmer crab (*P. pelagicus*) (Lu et al. 2022). Therefore, in our study, nucleotide sequences in mtDNA CR were used to

analyze the genetic diversity of the blue swimming crabs in the Andaman Sea. The results of this study can be used as a guide to consider effective management of the blue swimming crab populations in nature and maintain genetic diversity.

MATERIALS AND METHODS

SAMPLE COLLECTION AND DNA EXTRACTION

One hundred-fifty blue swimming crab samples were

obtained from fishermen that caught them in Satun, Trang, Krabi, Phang Nga, and Ranong using commercial crab traps (Figure 1). Fresh crab samples were packed on ice and transported to the laboratory for further DNA extraction. Total genomic DNA was extracted from the claw muscles with a tissue genomic DNA extraction mini kit (TIANGEN, Taiwan) according to the manufacturer's protocol.

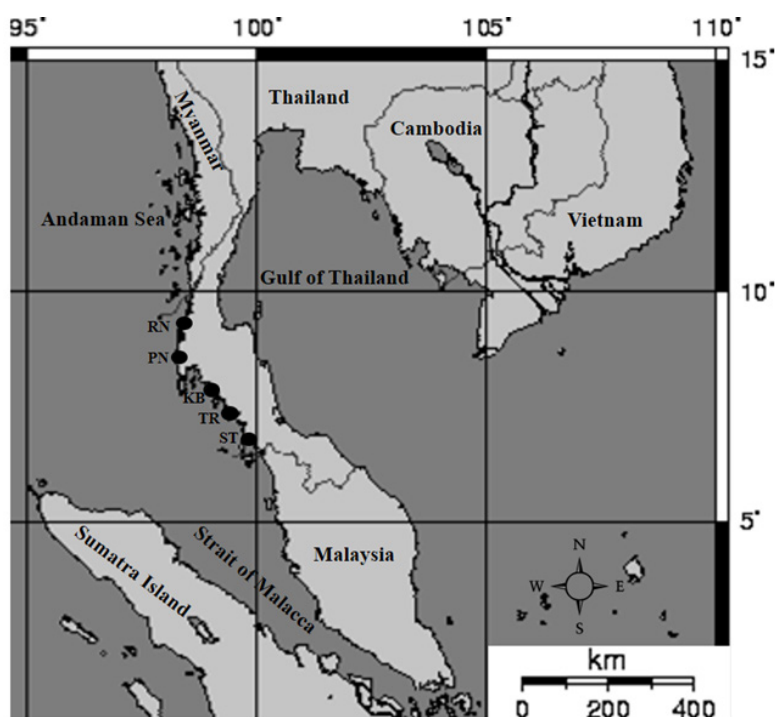


FIGURE 1. Sampling sites for the blue swimming crab along the Andaman Sea coast of Thailand

ST= Satun, TR= Trang, KB= Krabi, PN= Phang Nga, RN= Ranong

(Source: Wikimedia Common; https://commons.wikimedia.org/w/index.php?title=File:Straits_of_Malacca.png&oldid=471830265)

PCR AMPLIFICATION AND NUCLEOTIDE SEQUENCING

The primer pair (PPCR_H1 5'-TTG AGG GAA ACC AGA AAGATT 3' and PPCR_L1 5'-CCA TGC GTT AAA ATA CAA ATT C 3') (Supmee et al. 2020a) was used to amplify target DNA from the mtDNA *CR* with the Polymerase chain reaction (PCR) method. PCR reaction was conducted in total volume of 50 μ L. PCR reagent consisted of 10X Taq buffer 5 μ L, 25 mM MgCl₂ 7.5 μ L, 2 mM dNTPs mix 4 μ L, 10 μ M forward primer 2 μ L, 10

μ M reverse primer 2 μ L, Taq DNA polymerase (Thermo Scientific, USA) 0.5 μ L (2.5 units), DNA template 5 μ L (50-100 ng) and ultrapure water 24 μ L. PCR was performed with a Major Cycler, CYCLER-25 (Taiwan) machine consisting of 3 stages: (1) first denaturation at a temperature of 94 $^{\circ}$ C for 4 min, (2) 35 cycles of denaturation at 94 $^{\circ}$ C for 40 s, annealing at 52.5 $^{\circ}$ C for 1 min, and extension at 72 $^{\circ}$ C for 1 min (3) final extension at 72 $^{\circ}$ C for 10 min. PCR product was checked with gel

electrophoresis technique in 1% agarose gel (1×TAE). The correct PCR product size was also purified with the Gel/PCR Purification Mini Kit (Tiangen Biotech, China) and then taken to the nucleotide sequence at the service unit (1st Base Laboratory, (Selangor, Malaysia)) by direct sequencing method.

DATA ANALYSIS AND GENETIC DIVERSITY

The service unit's nucleotide sequences were verified. The correct nucleotide sequences were aligned with the ClustalW ver. 1.83 program (Thompson, Higgins & Gibson 1994) and then edited. Genetic diversity values, including the number of polymorphic sites, number of haplotypes, nucleotide diversity (π ; Nei 1987), and haplotype diversity (h ; Nei 1987) were examined with the DnaSP version 6.00 (Rozas et al. 2017).

POPULATION GENETIC STRUCTURE

Population genetic structure was examined with the analysis of molecular variance (AMOVA) to compare the degree of genetic diversity within and between populations using the ARLEQUIN version 3.5.1.2 program (Excoffier & Lischer 2010). The significance of the Φ -statistic was tested by repeating 10,000 permutations. The hierarchical analysis of population genetic structure was examined by dividing the population from the putative geography. First, the population was separated by a single region (Satun, Trang, Krabi, Phang Nga, and Ranong). Second, the population was determined in the lower Andaman Sea (Satun, Trang, and Krabi) and the upper Andaman Sea (Phang Nga, and Ranong). The genetic distance among the populations was analyzed with pairwise F_{ST} methods using the ARLEQUIN version 3.5.1.2 program (Excoffier & Lischer 2010), using 10,000 permutations. A phylogenetic tree was constructed using the neighbor-joining (NJ) method, which was implemented in MEGA version 7.0 (Kumar, Stecher & Tamura 2016), based on the matrix of Kimura 2-parameter distance. The bootstrapping method, which uses 1,000 repeats, was used to determine the statistical robustness of the tree topology.

DEMOGRAPHIC HISTORY ANALYSIS

Three independent analyses were used to assess the demographic history of the blue swimming crab. First, the selective neutrality test was analyzed by Tajima's D (Tajima 1989) and Fu's F_s (Fu 1997) tests with ARLEQUIN version 3.5.1.2 (Excoffier & Lischer 2010) using 10,000 permutations. Second, the sudden expansion model test was examined with mismatch

distribution. The goodness-of-fit test was analyzed using the Harpending Raggedness index (Harpending 1994) and the sum of squared deviations (SSD) with ARLEQUIN version 3.5.1.2 (Excoffier & Lischer 2010) using 10,000 bootstrap replicates. Third, the parameters θ_0 and θ_1 were utilized to estimate the size of the population when θ_0 is the population size before expansion, and θ_1 is the population size after expansion.

RESULTS

GENETIC DIVERSITY

The nucleotide sequence in the mtDNA *CR* of the blue swimming crab taken to analyze the genetic diversity had a nucleotide sequence of 369-371 base pairs. According to alignment, there were 369 aligned sites consisting of 127 polymorphic sites (34 singleton sites, 93 parsimonious informative sites), defining 71 haplotypes. All haplotypes were deposited in the GeneBank database with accession numbers ON922639-ON922709. The haplotypes that were found consisted of 12 shared haplotypes (4 shared haplotypes between populations and eight haplotypes within a population) and 59 private haplotypes (Table 2). Haplotype diversity values ranged from 0.857-0.947, and nucleotide diversity was in the range of 0.032-0.048. The haplotype diversity value of the entire population was 0.927, and the nucleotide diversity of the total population was 0.042 (Table 1). Genetic diversity values (the number of polymorphic sites, the number of haplotypes, haplotype diversity (h), and nucleotide diversity (π)) are shown in Table 1.

POPULATION GENETIC STRUCTURE

The population genetic structure of blue swimming crabs collected in samples along the Andaman coastline was analyzed. According to an analysis of molecular variance, the Φ_{ST} statistics exhibited no significant difference when assuming a model population structure pattern in the single region ($\Phi_{ST} = 0.012$, $p = 0.081$), indicating that there was no population segmentation between the sampling sites. The Φ_{CT} statistic parameter showed no significant differences ($\Phi_{CT} = -0.024$, $p = 0.062$), indicating no population genetic structure between crab populations in the lower and upper Andaman Sea (Table 3). The pairwise F_{ST} genetic distance analysis showed no significant difference when comparing all sampling areas, confirming that there was no population genetic structure of the blue swimming crabs in the Andaman Sea (Table 4). In the blue swimming crab populations from the Andaman Sea, the phylogenetic tree failed to identify any different lineages of haplotypes (Figure 2).

TABLE 1. Collecting sites, number of individuals per collecting site (N), and genetic diversity indices for the blue swimming crab estimated using mtDNA *CR* sequences

Collecting sites	Date of collection	Geographic coordinates	N	No. polymorphic sites	No. haplotypes	Haplotype diversity (h) (Mean \pm SD)	Nucleotide diversity (π) (Mean \pm SD)
Satun	5 December 2021	6° 40' 37" N, 99° 50' 47" E	30	58	16	0.857 \pm 0.058	0.032 \pm 0.003
Trang	7 December 2021	7° 20' 09" N, 99° 22' 46" E	30	57	17	0.936 \pm 0.026	0.039 \pm 0.002
Krabi	10 December 2021	8° 02' 28" N, 98° 48' 45" E	30	67	18	0.947 \pm 0.023	0.043 \pm 0.002
Phang Nga	13 December 2021	8° 26' 23" N, 98° 14' 04" E	30	61	15	0.908 \pm 0.032	0.048 \pm 0.003
Ranong	17 December 2021	9° 52' 27" N, 98° 31' 54" E	30	59	17	0.920 \pm 0.030	0.036 \pm 0.002
Total			150	127	71	0.927 \pm 0.014	0.042 \pm 0.001

TABLE 2. The mtDNA *CR* haplotype distributions for the blue swimming crab from 5 localities along the Andaman Sea coast of Thailand

Haplotype	ST	TR	KB	PN	RN	Total	Haplotype	ST	TR	KB	PN	RN	Total
H01	11	5	4	7	5	32	H37	-	-	1	-	-	1
H02	1	-	-	-	-	1	H38	-	-	1	-	1	2
H03	1	-	-	-	-	1	H39	-	-	3	-	-	3
H04	1	-	-	-	-	1	H40	-	-	1	-	-	1
H05	1	-	-	-	-	1	H41	-	-	1	-	-	1
H06	1	-	-	-	-	1	H42	-	-	1	-	-	1
H07	1	-	-	-	-	1	H43	-	-	2	-	-	2
H08	1	-	-	-	-	1	H44	-	-	1	-	-	1
H09	1	-	-	-	-	1	H45	-	-	1	-	-	1
H10	1	-	-	-	-	1	H46	-	-	1	-	-	1
H11	2	5	5	5	6	23	H47	-	-	-	4	-	4
H12	1	-	-	-	-	1	H48	-	-	-	1	-	1
H13	4	-	-	-	-	4	H49	-	-	-	1	-	1
H14	1	-	-	-	-	1	H50	-	-	-	1	-	1
H15	1	-	-	-	-	1	H51	-	-	-	1	-	1
H16	1	-	-	-	-	1	H52	-	-	-	1	-	1

H17	-	2	-	-	-	2	H53	-	-	-	1	-	1
H18	-	1	-	-	-	1	H54	-	-	-	1	-	1
H19	-	1	-	-	-	1	H55	-	-	-	1	-	1
H20	-	1	-	-	-	1	H56	-	-	-	1	-	1
H21	-	1	-	-	-	1	H57	-	-	-	1	-	1
H22	-	4	1	3	1	9	H58	-	-	-	1	-	1
H23	-	1	-	-	-	1	H59	-	-	-	-	1	1
H24	-	1	-	-	-	1	H60	-	-	-	-	1	1
H25	-	1	-	-	-	1	H61	-	-	-	-	1	1
H26	-	1	-	-	-	1	H62	-	-	-	-	1	1
H27	-	1	-	-	-	1	H63	-	-	-	-	5	5
H28	-	1	-	-	-	1	H64	-	-	-	-	1	1
H29	-	1	-	-	-	1	H65	-	-	-	-	1	1
H30	-	2	-	-	-	2	H66	-	-	-	-	1	1
H31	-	1	-	-	-	1	H67	-	-	-	-	1	1
H32	-	-	1	-	-	1	H68	-	-	-	-	1	1
H33	-	-	1	-	-	1	H69	-	-	-	-	1	1
H34	-	-	1	-	-	1	H70	-	-	-	-	1	1
H35	-	-	1	-	-	1	H71	-	-	-	-	1	1
H36	-	-	3	-	-	3	Total	30	30	30	30	30	150

ST= Satun, TR= Trang, KB= Krabi, PN= Phang Nga, RN=Ranong

TABLE 3. Analysis of molecular variance (AMOVA) for the blue swimming crab populations along the Andaman Sea coast of Thailand based on mtDNA *CR* sequences

Source of variation	df	Sum of squares	Variance components	Percentage of variation	Φ -statistic
1) Single region					
Among populations	4	99.073	0.579Va	7.26	$\Phi_{ST} = 0.012$ ($p=0.081$)
Within populations	145	1072.033	7.393Vb	92.74	
Total	149	1171.107	7.972		
2) Lower and upper Andaman Sea					
Among groups	1	14.218	-0.185Va	-2.47	$\Phi_{CT} = -0.024$ ($p=0.062$)
Among populations within groups	3	84.856	0.696Vb	8.82	$\Phi_{SC} = 0.086$ ($p=0.074$)
Among populations	145	1072.033	7.393Vc	93.65	$\Phi_{ST} = 0.063$ ($p=0.0853$)
Total	149	1171.107	7.894		

TABLE 4. Population pairwise F_{ST} values between the blue swimming crab populations along the Andaman Sea coast of Thailand based on mtDNA *CR* sequences

	Lower Andaman Sea			Upper Andaman Sea		
	Population	Satun	Trang	Krabi	Phang Nga	Ranong
Lower Andaman Sea	Satun	-				
	Trang	0.130 (0.060)	-			
	Krabi	0.099 (0.061)	0.104 (0.091)	-		
Upper Andaman Sea	Phang Nga	0.071 (0.073)	0.058 (0.068)	0.061 (0.073)	-	
	Ranong	0.047 (0.061)	0.072 (0.093)	0.039 (0.070)	0.038 (0.058)	-

p values in parentheses

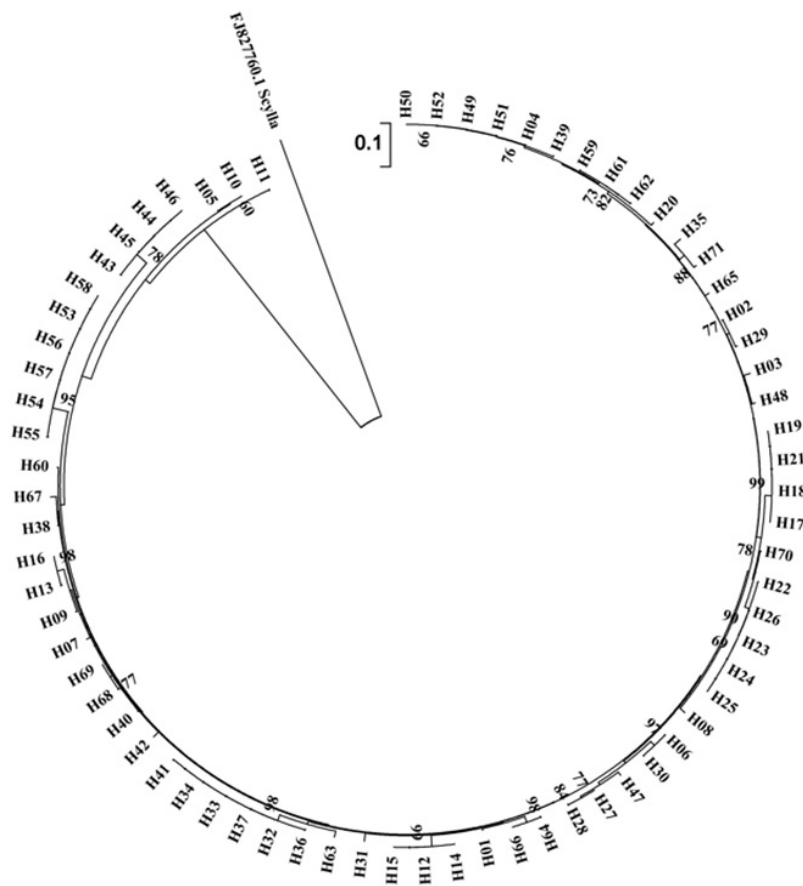


FIGURE 2. A neighbor-joining phylogenetic tree based on mtDNA *CR* of the blue swimming crab constructed under the Kimura 2-parameter model with a bootstrap value of 1,000 replicates and *Scylla olivacea* (accession number: FJ827760.1) as an outgroup

DEMOGRAPHIC HISTORY

The demographic history of the blue swimming crab from the Andaman Sea was analyzed using three independent tests. First, the selective neutrality test (Tajima's D and Fu's F_s tests) was negative and statistically significant. Tajima's D and Fu's F_s values were negative in all sampling sites and were statistically significant. In the total population, Tajima's D and

Fu's F_s were -1.987 and -23.003 and were statistically significant (Table 5). Second, mismatch distribution tests showed that the SSD value and the Harpending Raggedness index were statistically insignificant at 0.008 and 0.015, and accepted a sudden expansion model (Table 5). Third, the parameter values θ_0 and θ_1 of the total population were 3.171 and 10,061.638, respectively, with all sampling sites θ_1 greater than θ_0 (Table 5).

TABLE 5. Parameter indices for the neutrality test and mismatch distribution analysis of the blue swimming crab based on mtDNA CR sequences

Collecting localities	Tajima's D	Fu's F_s	SSD^a	Rag ^b	θ_0^c	θ_1^d
Satun	-1.692* (0.034)	-4.096* (0.041)	0.039 (0.073)	0.077 (0.325)	3.946	4,430.859
Trang	-2.048* (0.025)	-7.086* (0.023)	0.029 (0.089)	0.044 (0.236)	1.868	4,354.716
Krabi	-2.154* (0.040)	-5.110* (0.037)	0.020 (0.100)	0.028 (0.137)	0.003	7,659.531
Phang Nga	-1.580* (0.028)	-8.233* (0.030)	0.029 (0.102)	0.063 (0.145)	7.781	9,661.171
Ranong	-1.365* (0.016)	-6.242* (0.027)	0.022 (0.076)	0.042 (0.123)	4.162	8,755.156
Total	-1.987* (0.013)	-23.003* (0.024)	0.008 (0.098)	0.015 (0.100)	3.171	10,061.638

p values in parentheses, *significant differentiation ($p < 0.05$), ^asum of squared deviations, ^bRaggedness index, ^cpopulation size before expansion, ^dpopulation size after expansion

DISCUSSION

GENETIC DIVERSITY

Our study examined 150 blue swimming crab individuals from five different sampling sites. We found many distinct private mtDNA CR sequencing haplotypes. The presence of several private haplotypes in this study shows that the blue swimming crab's effective female population size in the studied area is large (William & Allendorf 2007). An effective female population indicates the breeding of females or female reproductive success. The blue swimming crab's highly effective female population reduces the likelihood of inbreeding, potentially allowing the population to

recover quickly. As a result, ongoing conservation efforts are still worthwhile. The blue swimming crab artificial propagation program would benefit from actively ensuring that such genetic variability is maintained. Thus, the large effective female population size suggests that the blue swimming crab along the Andaman Sea coast has a good chance of recovering the population in the future.

In all samples, haplotype diversity was relatively high, whereas nucleotide diversity was relatively low. This pattern suggests that the blue swimming crab has experienced a recent population expansion. In a rapidly expanding population, genetic characterization can be generated by the accumulation of new mutations and by maintaining high haplotype diversity (Ma et al. 2010;

Watterson 1984). Studies have previously reported that marine populations in the Andaman Sea have high haplotype diversity and low nucleotide diversity values, for example, the violet vinegar crab (*Episesarma versicolor*) (Supmee et al. 2012a), the oceanic paddle crab (*Varuna literate*) (Suppapan et al. 2017), the Asiatic hard clam (*Meretrix meretrix*) (Supmee et al. 2020b), and the wedge clam (*Donax scortum*) (Supmee et al. 2021).

Nucleotide diversity is a concept used in molecular genetics to quantify the degree of polymorphism within a population (Nei & Li 1979). In our study, the nucleotide diversity in the mtDNA *CR* of the blue swimming crab in the Andaman Sea was high (0.042) when compared to that of other crabs. For instance, the nucleotide diversities of the Thai vinegar crab (*E. mederi*), the blue land crab (*Cardisoma guanhumi*), and the swimming crab (*P. trituberculatus*) were 0.023 (Supmee et al. 2012b), 0.027 (Amaral et al. 2015), and 0.025 (Hui et al. 2019), respectively. Besides, we found that the nucleotide diversity of the blue swimming crab in the Andaman Sea was higher than in the Gulf of Thailand, which was 0.008 (Supmee et al. 2020a). Therefore, it is shown that the blue swimming crab in the Andaman Sea has higher genetic diversity than the blue swimming crab in the Gulf of Thailand.

POPULATION GENETIC STRUCTURE

According to our genetic structure analysis, the blue swimming crab samples from the five sites did not represent distinct subpopulations. Many factors, such as high larval dispersal ability, hydrographic variation, and a lack of geographic barriers, can help to maintain genetic homogeneity between populations. Many marine animals release gametes or planktonic larvae into open waters, where they migrate with the current and promote gene flow between populations (Uthicke & Benzie 2003). Marine species with long larval stages, in particular, are thought to have high levels of genetic variation within populations (Russo, Sole-Cava & Thorpe 1994). Furthermore, the absence of genetic differentiation in several species indicates that a long-duration planktonic larval stage influences the opportunity for a high degree of gene flow. For example, the larval stages of the amphidromous gastropods (*Neritina canalis*), the mangrove crab (*Neosarmatium meinerti*), and the fiddler crab (*Uca annulipes*) were 46 days (Crandall, Taffel & Barber 2010), 43 days (Ragionieri et al. 2010), and 28 days (Silva, Mesquita & Paula 2010), respectively. It was found that the blue swimming crab has a larval period of 30 days (Josileen & Menon 2005). Besides, the

connection of the water circulation between the lower and upper Andaman Sea in Phang Nga province may enhance the mixing of planktonic larvae. As a result, it was possible to promote gene flow that was extensive in these two areas. Furthermore, the absence of geographic barriers in the Andaman Sea may not disrupt the gene flow of the blue swimming crab between the upper and lower Andaman Sea populations. The lack of genetic structure of the blue swimming crab along the Andaman Sea coast of Thailand was previously reported by Klinbunga et al. (2010, 2007) and Khamnamtong et al. (2021). Moreover, we found that a previous study of the population genetic structure of the blue swimming crabs in Malaysia, collecting samples along the Strait of Malacca, the area adjacent to our experiment, showed no genetic differences (Chai et al. 2017; Sukimin, Esa & Amin 2017). Several previous studies have shown that the blue swimming crab has an extended planktonic larval stage with potentially high larval dispersal. As a result, there is a low level of genetic differentiation among the blue swimming crab populations. In Vietnam, there were no genetic differences between the blue swimming crabs found between northern Vietnam and central Vietnam over a distance of about 1,500 kilometers (Dang et al. 2019). In Indonesia, the blue swimming crab population habits along the Java Sea coast were those of a single population (Madduppa et al. 2021). In China, in the swimming crab distribution areas in the East and South China Seas, there was no population genetic structure (Ren et al. 2018). In Southeast Asia, the blue swimming crab populations in China, Vietnam, and Singapore have no genetic differences (Lu et al. 2022). Thus, we concluded that the high ability of larval dispersal maintains genetic homogeneity among the blue swimming crab populations.

Based on our findings, we propose that the blue swimming crab population in the Andaman Sea coast of Thailand be managed as a single fishery unit, with stock assessments and harvest limits set for the entire area. The management of this species should take precautions to prevent overfishing and habitat destruction from endangering the survival of existing populations. Immediate actions include not catching female crabs with eggs located outside of their shell to increase successful spawning, establishing marine reserves to reduce genetic losses, and controlling coastal pollution to increase the number of breeding individuals and larval dispersal. Furthermore, gear regulation, habitat monitoring, and restoration may be among the most effective methods of managing healthy populations. To provide an overall temporal and spatial view of crab populations, periodic

surveys on genetic diversity and seascape research should be conducted.

DEMOGRAPHIC HISTORY

All of our independent demographic history analyses show that the blue swimming crab population in the study area has expanded rapidly. First, Tajima's D and Fu's F_s were negative and significantly deviated from neutrality, indicating that the blue swimming crab may have undergone purifying selection or population expansion (Yang 2006). Fu's F_s test, which detected population expansion in haplotype data, was also negative, indicating population expansion (Ramirez-Soriano et al. 2008). Second, DNA sequence mismatch analysis supported a sudden expansion model, and a goodness-of-fit-test fitted the expected mismatch distribution well, indicating that the blue swimming crab population has expanded. Third, the population size after expansion (θ_1) was greater than the population size before expansion (θ_0), indicating that there was a demographic expansion. The recent population expansion of marine species in the Andaman Sea has been previously reported, such as the violet vinegar crab (*Episesarma versicolor*) (Supmee et al. 2012a), the wedge clam (*Donax scortum*) (Supmee et al. 2021), the greenback mullet (*Liza subviridis*) (Supmee et al. 2017), and the sandfish (*Holothuria scabra*) (Ninwichian & Klinbunga 2020).

CONCLUSIONS

In our study, we examined 150 mtDNA *CR* nucleotide sequences ranging in size from 369 to 371 base pairs to determine the genetic structure and demographic history of the blue swimming crab on the Andaman Sea coast of Thailand. Multiple genetic structure analyses show that there is only one blue swimming crab population in this area. The results of demographic history tests showed that the blue swimming crab population had expanded. This study provides critical information for developing long-term management strategies to preserve the genetic diversity of Thailand's blue swimming crab population. We propose that nuclear DNA markers such as microsatellites or SNPs be used in future analyses to obtain more robust information and additional insights on fine-scale genetic differentiation.

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*Corresponding author; email: verakiat.s@rmutsv.ac.th