Genetic Diversity of Blue Swimming Crab (*Portunus pelagicus*) from a Crab Bank Project and Wild Crabs in Trang and Krabi Province, Thailand Using mtDNA Control Region Sequences

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ABSTRACT

The blue swimming crab (*Portunus pelagicus*) is an economically important crustacean with increasing consumption demands, while wild stock is declining. Therefore, a crab bank project has been initiated to restore crab resources. The crab bank, which has been operating for at least 10 years, collects berried females, hatches their eggs to the zoea stage, and release them into the sea. However, the project's success has not been evaluated. This study investigates the genetic diversity of berried females from the crab bank project and wild crabs in Trang and Krabi provinces. The nucleotide sequences within the mitochondrial DNA control region of *P. pelagicus* were analyzed. We found that 65.72% of wild crabs in Trang and 39.72% in Krabi shared haplotypes with berried females from the crab bank project. This indicates a division within the *P. pelagicus* population, forming two distinct groups corresponding to Trang and Krabi. The demographic history analysis suggests a period of population expansion. Based on these genetic diversity findings, we propose management strategies for the crab bank projects in both areas. However, this study is preliminary, and further research incorporating additional genetic markers from the nuclear genome and more samples from areas beyond Trang and Krabi is recommended.

Keywords: Crab bank, Mitochondrial DNA, Restocking, Thailand

INTRODUCTION

The blue swimming crab (*Portunus pelagicus*) holds significant importance as a marine crab species in Thailand (Fishery Statistics Analysis and Research Group, 2022). These crabs are typically found in coastal regions, particularly near seagrass ecosystems with sandy ground (Asphama *et al*., 2015). In Thailand, *P. pelagicus* is distributed along the shores of the Andaman Sea and the Gulf of Thailand. Most *P. pelagicus* harvests come from wild catches because commercial growing attempts have proven to be costly and ineffective. From 2019 to 2022, *P. pelagicus* was the most heavily caught crab species in Thailand (Fishery Statistics Analysis and Research Group, 2022). As a result, initiatives have been undertaken to replenish Thailand's coastal waters with sufficient resources to sustain fisheries and preserve the crab's native environment.

Several initiatives aimed at increasing stocks, such as releasing hatchery-reared offspring into natural habitats, have been implemented for multiple species (Cai *et al*., 2020). An efficient approach to minimizing resource depletion and

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swiftly enhancing population figures involves introducing larvae into natural aquatic environments. Over the last decade, the crab bank project has served as a prime example of this approach in Thailand. The typical practice in crab hatcheries involves gathering eggs from female crabs with berries, which are then hatched into the zoea stage, the initial larval stage, and subsequently released into the seawater (Thiammueang *et al*., 2012). Surprisingly, studies on the genetic information of *P. pelagicus* in the context of crab bank project management have not been extensively reported before. To efficiently plan the management of the crab bank project, it is essential to study the genetic information of berried females and wild crabs simultaneously.

Genetic diversity refers to the presence of diverse genetic traits within a specific species, a population. This diversity is critical for the survival of species because it enables living organisms to adapt to changing environments and has the potential for further evolution (Frankham *et al*., 2002). Genetic structure is characterized by systematic variations in allele frequencies among subpopulations. Alterations in genetic composition arise from factors that impact gene frequencies or the patterns of genetic diversity. Comprehending the genetic structure is essential in developing efficient management strategies and guaranteeing the sustainable exploitation of marine animals (Wellmann and Bennewitz, 2019). Thus, information on genetic diversity should ideally inform and support management strategies for berried females and wild blue-swimming crabs.

The examination of nucleotide sequences derived from mitochondrial DNA (mtDNA) is employed to explore genetic diversity and patterns of genetic structure in various animal species. This approach focuses on the nucleotide sequence found in the control region of mitochondrial DNA (mtDNA CR) (Tokuyama *et al*., 2020; Wang *et al*., 2020). This specific region was chosen due to its higher mutation rate compared to other mtDNA regions, making it well-suited for assessing genetic diversity. Additionally, because of its haploid maternal inheritance, this genetic marker is suitable for tracing genetic transmission through maternal lines (Boore, 1999; Avise, 2000). Given these reasons, studying the nucleotide sequence in mitochondrial DNA is suitable for confirming maternal relationships with offspring. Therefore, in this study, we used mtDNA nucleotide sequences to track the offspring born from the berried female crabs in the crab bank project to assess the success of releasing offspring into the sea.

In this study, we selected crab banks located in Trang and Krabi to conduct a preliminarily investigation of the genetic information of *P. pelagicus*. Genetic diversity, genetic structure, and demographic history of *P. pelagicus* were studied to provide genetic information for managing crab banks in the area. Furthermore, this study analyzes the similarity of haplotypes between the berried females from the crab bank project and the wild crab populations after releasing offspring to assess the success of crab releases following a period of operation. The findings of this research offer insights to assist in the conservation of genetic diversity in *P. pelagicus* populations in Trang and Krabi.

MATERIALS AND METHODS

Ethics statement

All procedures in this study were conducted by the approved protocols of the Institutional Animal Care and Use Committee of Rajamangala University of Technology Srivijaya, authorized under approval number IAC 02-01-2023.

Sample collection and DNA extraction

Samples of berried female *Portunus pelagicus* were collected by gill net within a 9 km^2 area surrounding the crab bank locations in Trang (Crab Bank, Ban Pak Klong, Sikao District, Trang Province) and Krabi (Crab Bank Learning Center, Tha Khlong, Ko Lanta District, Krabi Province) in January 2023 (Figure 1). A total of 418 berried females were placed in water tank containers to facilitate egg-laying. The crab larvae were temporarily raised to the zoea stage before being released into the sea or estuaries within a 9 km^2 area surrounding the crab bank locations. The release process occurred from the end of January to early February (Table 1).

Approximately 5 months later (from 22 June – 20 July 2023), when the released crabs were presumably reaching adulthood, six surveys were conducted to capture wild crabs in the coastal waters within a 9 km^2 area surrounding the crab bank locations in Trang and Krabi (Figure 1, Table 2). This effort resulted in the retrieval of 424 crabs, with carapace widths ranging from 8.7 to 16.2 cm. These crabs primarily inhabit limited regions and do not exhibit significant long-distance migration. Additionally, the two crab banks in these areas are separated by Lanta Island, suggesting a disrupted genetic exchange between the crab populations in Trang and Krabi. Therefore, we divided the samples into Trang and Krabi segments for further analysis.

A single pereiopod from both the berried females and wild crabs was gathered and stored in 95% ethanol at room temperature. Samples were transferred to the laboratory and preserved at -20 °C for DNA extraction. Genomic DNA was obtained from the pereiopod muscles using a tissue genomic DNA extraction mini-kit (TIANGEN, Taiwan), following the guidelines provided by the manufacturer.

PCR and nucleotide sequencing

A specific DNA segment in the control region of mitochondrial DNA (mtDNA CR) was selected for amplification using the standard polymerase chain reaction (PCR). The primers used were PPCR_H1 (5'-TTG AGG GAA ACC AGA A AG ATT 3') and PPCR_L1 (5'-CCA TGC GTT AA A ATA CAA ATT C 3') (Supmee *et al*., 2020). The optimized PCR protocol included an initial denaturation step of 4 min at 94 °C, followed by 35 cycles comprising 40 s at 94 °C, 1 min at 52.5 °C, and 1 min at 72 °C, with a final incubation step of 10 min at 72 °C. The reaction mixture had a final volume of 50 µL and contained ultrapure water (24 µL), 25 mM $MgCl_2$ (7.5 µL), 2 mM dNTPs mix (4 µL), each 10 µM forward primer and reverse primer (2 µL), Taq DNA polymerase (0.5 µL), 10X Taq buffer (5 µL), and DNA template (50–100 ng) (5 µL). The resulting PCR products were separated through a 1% agarose gel, stained with ethidium bromide, and detected via UV transillumination. The accurate size of the PCR product was purified and subsequently submitted for nucleotide sequencing by the ABI® BigDye Terminator Cycle Sequencing Kit v3.1. (ATGC Co., Ltd., Thailand).

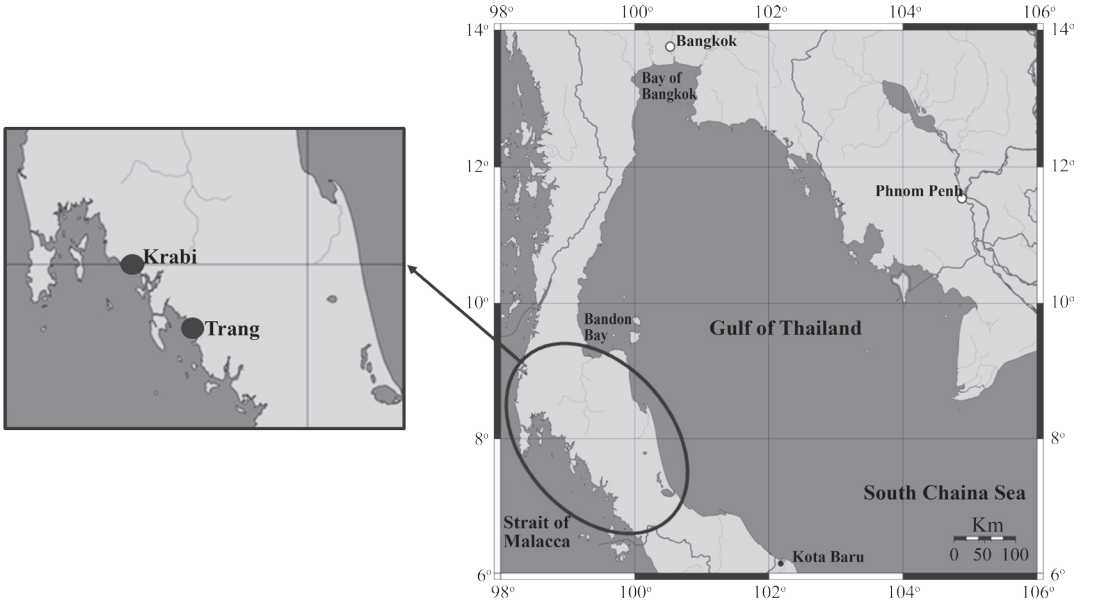

Figure 1. Sample collection sites for the *Portunus pelagicus* from the crab bank project in Trang and Krabi. (Source: https://upload.wikimedia.org/wikipedia/commons/b/b7/Gulf_of_Thailand.svg)

Table 1. Number of berried female crabs, released zoea larvae, and release date.

Location	Number of berried female crabs	Number of zoea (x10^6)	Release date
Trang	45	8	20 January 2023
	57	9	25 January 2023
	61	11	7 February 2023
	41	7	14 February 2023
Total	**204**	**35**	
Krabi	36	6	18 January 2023
	65	11	26 January 2023
	59	11	6 February 2023
	54	10	13 February 2023
Total	**214**	**42**	

Table 2. Number, sizes (carapace width and weight) and capture dates of the wild crabs used in this study.

Location	Number of wild crabs	Carapace widths (cm)	Weight (g)	Capturing time
Trang	38	9.7–14.3	100.21–189.27	22 June 2023
	37	8.9–15.2	98.54–211.36	27 June 2023
	35	9.3–14.7	99.47–190.14	4 July 2023
	34	10.1–15.8	105.85–212.73	12 July 2023
	36	8.7–14.1	94.26–178.52	16 July 2023
	30	9.1–15.6	105.37–245.41	19 July 2023
Total	**210**	**8.7–15.8**	**98.54–245.41**	
Krabi	35	9.4–15.6	101.56–223.58	23 June 2023
	36	8.8–15.2	95.25–218.47	26 June 2023
	37	9.3–16.2	99.78–238.22	3 July 2023
	33	8.7–15.3	93.41–201.25	10 July 2023
	34	8.9–15.9	98.11–237.58	17 July 2023
	39	9.1–16.1	104.45–246.42	20 July 2023
Total	**214**	**8.7–16.2**	**95.25–246.22**	

Genetic diversity and haplotype classification

The nucleotide sequences obtained from the service unit underwent validation. These validated sequences were then aligned using the ClustalW ver. 1.83 program (Thompson *et al*., 1994) and subsequently edited. Genetic diversity parameters, including the count of polymorphic sites, haplotype numbers, nucleotide diversity (π; Nei, 1987), and haplotype diversity (h; Nei, 1987), were evaluated using DnaSP version 6.00 (Rozas *et al*., 2017). Shared haplotypes and unique haplotypes between berried female crabs and wild crabs were investigated.

Genetic structure analysis

Analysis of molecular variance (AMOVA) was used to assess genetic structure. This analysis was conducted using ARLEQUIN version 3.5.1.2 software (Excoffier and Lischer, 2010) with 10,000 permutations for statistical significance. The genetic differentiation between every conceivable pair of populations was assessed using pairwise F_{ST}, employing 10,000 permutations within ARLEQUIN version 3.5.1.2. Subsequently, the Gamma ST (γ_{ST}) parameter was calculated using DnaSP version 6.00 to assess genetic distances (D) among populations (Nei, 1982; Rozas *et al*., 2017). The estimation of gene flow [$Nm = (1-F_{ST})/2F_{ST}$] between localities was derived from the pairwise F_{ST} values (Slatkin, 1987). An individual crab phylogenetic tree was constructed using the neighbor-joining (NJ) method in MEGA version 7.0 (Kumar *et al*., 2016) based on the Kimura 2-parameter distance matrix. The statistical strength of the tree was assessed by conducting 1,000 replicates using the bootstrapping method. A minimum spanning network (MSN) was created with ARLEQUIN version 3.5.1.2 software (Excoffier and Lischer, 2010), relying on the average count of differences between every pair of mtDNA *CR* haplotypes, and then manually sketched.

Demographic history analysis

Three distinct analyses were employed to investigate the demographic history of *P. pelagicus*. First, Tajima's D (Tajima, 1989) and Fu's Fs (Fu, 1997) tests were performed to evaluate population neutrality. Second, the rapid expansion model was assessed through mismatch distribution analysis. Finally, the population size was estimated using the parameters θ_0 (population size before growth) and θ_1 (population size after expansion). All demographic history analyses were conducted using ARLEQUIN version 3.5.1.2 software (Excoffier and Lischer, 2010) with a statistical significance threshold of 10,000 permutations.

RESULTS

Genetic diversity and haplotype classification

The mitochondrial DNA control region (mtDNA *CR*) sequence of *Portunus pelagicus* consisted of 508–512 base pairs. As per the alignment of berried female crabs, 592 aligned sites were identified, including 54 gaps or missing data, 378 monomorphic sites, and 160 polymorphic sites, which included 36 singleton sites and 124 parsimonious informative sites, leading to the definition of 199 haplotypes. Among these, 43 haplotypes were shared, with 12 being shared between populations and 31 within a population, while 156 were unique (Figure 3a). Haplotype diversity values varied between 0.924 and 0.983, and nucleotide diversity ranged from 0.032 to 0.036. The overall haplotype diversity across the entire population was calculated as 0.968, and the nucleotide diversity for the total population was 0.033 (Table 3). In the alignment of wild crabs, 589 aligned sites were observed, consisting of 83 gaps or missing data, 432 monomorphic sites, and 174 polymorphic sites, encompassing 49 singleton sites and 125 parsimonious informative sites,

resulting in 204 haplotypes. Among these, 38 haplotypes were shared, with 14 being shared between populations and 24 within a population, while 166 were unique (Figure 3b). Haplotype diversity values ranged from 0.917 to 0.924, and nucleotide diversity ranged from 0.020 to 0.023. The overall haplotype diversity across the entire population was calculated as 0.948, and the nucleotide diversity for the total population was 0.021 (Table 3). Genetic diversity parameters for the berried female and wild crab populations in Trang and Krabi are detailed in Table 3.

Within the Trang population, we identified 211 different haplotypes among 204 berried female crabs and 210 wild crabs. Of these, 147 unique haplotypes observed in either the berried female group (80 individuals) or the wild crab group (67 individuals) were removed. The remaining set, consisting of 114 berried female crabs and 138 wild crabs, shared 64 haplotypes. In Krabi, 214 berried female crabs and 214 wild crabs resulted in 194 distinct haplotypes. Of these, 134 unique haplotypes found in 66 females or 68 wild individuals were excluded. The remaining crabs (136 berried female crabs and 129 wild crabs) shared 60 haplotypes (Table 4). In summary, 48.55% of the wild individuals were excluded from the analysis (Table 4).

Genetic structure

The genetic structure of berried female and wild *P. pelagicus* from Trang and Krabi was examined. The analysis of molecular variance revealed significant differences in the Φ_{ST} statistics when comparing the population of berried female crabs between the two locations (Φ_{ST} = 0.907, p = 0.00001), suggesting a distinct population separation among the berried female crabs in Trang and Krabi. Likewise, the Φ_{ST} statistic revealed noteworthy distinctions (Φ_{ST} = 0.906, p = 0.00001) when assessing wild crab populations in Trang and Krabi. This indicates the presence of a genetic structure among the wild crab populations in both regions (Table 5). The analysis of the population genetic structure between berried females and wild crabs in Trang revealed no significant genetic differences (Φ_{ST} = 0.062, p = 0.10002) (Table 5), similar to the analysis of the population genetic structure between berried females and wild crabs in Krabi, which also showed no significant genetic differences (Φ_{ST} = 0.059, p = 0.11130) (Table 5).

The pairwise F_{ST} revealed significant distinctions when comparing the berried female populations in Trang and Krabi, confirming the population structure among berried female crabs in both areas (F_{ST} = 0.872, p = 0.00003) (Table 6).

Table 3. Genetic diversity parameters of berried females (used for the restocking program), and wild *Portunus pelagicus* populations collected 5 months after zoea release, in Trang and Krabi Provinces.

Population	No. of individual	No. haplotype	No. polymorphic sites	Haplotype diversity (h) Mean±SD	Nucleotide diversity (π) Mean±SD
Berried female					
Trang	204	103	157	0.983±0.003	0.036±0.002
Krabi	214	98	169	0.924±0.003	0.032±0.003
Total	**418**	**199**	**160**	**0.968±0.002**	**0.033± 0.000**
Wild crab					
Trang	210	106	154	0.917±0.005	0.020±0.004
Krabi	214	112	172	0.924±0.006	0.023±0.003
Total	**424**	**204**	**174**	**0.948±0.002**	**0.021±0.000**

Table 4. Classification of haplotypes in the mtDNA control region of berried female and wild *Portunus pelagicus* collected 5 months after zoea release, and the number of wild crab mtDNA excluded.

Crab bank	No. of berried female	No. of wild crab	Unique haplotype (berried female crabs)	Share haplotype	Unique haplotype (wild crabs)	Exclusion of mtDNA
Trang	204	210	80 (90b)	64 (114b+138w)	167 (72w)	72 (34.28%)
Krabi	214	214	66 (78b)	60 (136b+129w)	68 (85w)	129 (60.28%)
Total	**418**	**428**				201 (48.55%)

Note: *Specific number of berried female and wild crabs is in brackets; b = berried female crabs; w = wild crabs

Table 5. Genetic structure analysis of berried female and wild *Portunus pelagicus* population based on analysis of molecular variance.

Source of variation	df	Sum of squares	Variance components	Percentage of variation	Φ-statistic
Berried female crabs (Trang & Krabi)					
Among populations	1	404.599	1.855 Va	9.79	Φ_{ST} = 0.907 (p = 0.00001)
Within populations	416	7111.708	17.095 Vb	90.21	
Total	417	7516.307	18.950		
Wild crabs (Trang & Krabi)					
Among populations	1	435.149	1.966 Va	9.71	Φ_{ST} = 0.906 (p = 0.00001)
Within populations	422	7720.219	18.294 Vb	90.29	
Total	423	8155.368	20.260		
Berried female crabs and wild crabs in Trang					
Among populations	1	414.218	1.962 Va	8.72	Φ_{ST} = 0.062 (p = 0.11002)
Within populations	412	7174.823	18.103 Vb	91.28	
Total	413	7589.041	20.065		
Berried female crabs and wild crabs in Krabi					
Among populations	1	446.498	1.891 Va	9.01	Φ_{ST} = 0.059 (p = 0.11130)
Within populations	426	7744.252	18.545 Vb	90.99	
Total	427	8190.750	20.436		

Similarly, the analysis of pairwise F_{ST} indicated significant variations when comparing wild crab populations in Trang and Krabi, confirming the existence of a genetic structure among wild crabs in these regions (F_{ST} = 0.714, p = 0.00002) (Table 6). The results of the genetic difference study using pairwise F_{ST} analysis between berried females and wild crabs in Trang revealed no significant genetic differences (F_{ST} = 0.245, p = 0.09714). Similarly, the genetic difference analysis between berried females and wild crabs in Krabi showed no significant genetic differences (F_{ST} = 0.301, p = 0.11812).

The genetic distances of berried female crabs between Trang and Krabi populations were 0.407, and for the wild crab populations in Trang and Krabi, it was 0.401 (Table 6). The obtained genetic distance value is considered to be close to the maximum genetic divergence (D =1) (Nei, 1972), indicating a genetic difference within the *P. pelagicus* population between these areas. The gene flow (Nm) between the Trang and Krabi populations of berried female crabs was 0.073, and for wild crabs, it was 0.200 (Table 6). In theory, if the value of Nm is less than 1, it indicates low migration among populations (Slatkin, 1987). Therefore, it suggests that the crab populations in Trang and Krabi experience a restriction of gene flow.

The phylogenetic tree revealed distinct lineages among berried female crabs between Trang and Krabi (Figure 2a). Furthermore, the phylogenetic tree indicated that the population of wild crabs between Trang and Krabi was divided into two groups (Figure 2b). The haplotype network of berried female crabs was categorized into two primary haplogroups. In the first haplogroup, there were 93 haplotypes, comprising 6 shared haplotypes between populations, 86 haplotypes from Trang, and 1 from Krabi, with haplotype B68 being the common haplotype. The second haplogroup consisted of 106 haplotypes, and also featured 6 shared haplotypes between populations, consisting of 2 haplotypes from Trang and 98 from Krabi, with haplotype B82 being a common haplotype. The most common haplotype in the network was B82. Although haplogroup I and haplogroup II were linked, they were separated by a distance of 45 mutation steps (Figure 3a). The haplotype network of wild crabs was similarly divided into two haplogroups. Haplogroup I comprised of 101 haplotypes (7 shared haplotypes between populations, 87 haplotypes from Trang, and 7 from Krabi), with haplotype W20 being the common haplotype. Haplogroup II consisted of 103 haplotypes (7 shared haplotypes between populations, with 5 haplotypes from Trang and 91 from Krabi), and haplotype W11 being the common haplotype. The most common haplotype in the minimum spanning network was W20. Haplogroup I and haplogroup II were connected but were separated by 51 mutation steps (Figure 3b).

Demographic history

The demographic history of female crabs with berries in Trang and Krabi was investigated using three separate tests. First, Tajima's D yielded a result of -1.241, and Fu's Fs was -23.472. Both parameters indicated statistically significant differences (Table 7). Second, the mismatch distribution was fitted with a rapid expansion model (Figure 4a). The SSD and Rag index, with values of 0.001 and 0.004, respectively, showed no statistical significance, supporting a rapid expansion model (Table 7). Finally, θ_1 surpassed θ_0 across all sampling sites, with values of 9.346 for θ_0 and 302.087 for θ_1, as shown in Table 7.

Similarly, the demographic trajectory of *P. pelagicus* individuals caught in Trang and Krabi was studied using three different techniques. First, Tajima's D yielded a score of -1.230, and Fu's Fs recorded -23.484, indicating statistical significance (Table 7). Second, the mismatch distribution was adjusted using a rapid expansion model (Figure 4b). The SSD and Rag index, with values of 0.001 and 0.001, respectively, showed no statistical significance, thus supporting a rapid expansion model (Table 7). Finally, θ_1 exceeded θ_0 at all sampling locations, with values of 4.679 for θ_0 and 219.549 for θ_1, as indicated in Table 7.

Table 6. Pairwise F_{ST} (F_{ST}), genetic distance (D), and gene flow (Nm) value of *Portunus pelagicus* populations used in this study.

population	Berried female in Trang	Berried female in Krabi
Berried female in Trang		
Berried female in Krabi	F_{ST} = 0.872 (p = 0.00003)	-
	D = 0.407	
	Nm = 0.073	
population	**Wild crabs in Trang**	**Wild crabs in Krabi**
Wild crabs in Trang	-	
Wild crabs in Krabi	F_{ST} = 0.714 (p = 0.00002)	-
	D = 0.401	
	Nm = 0.200	

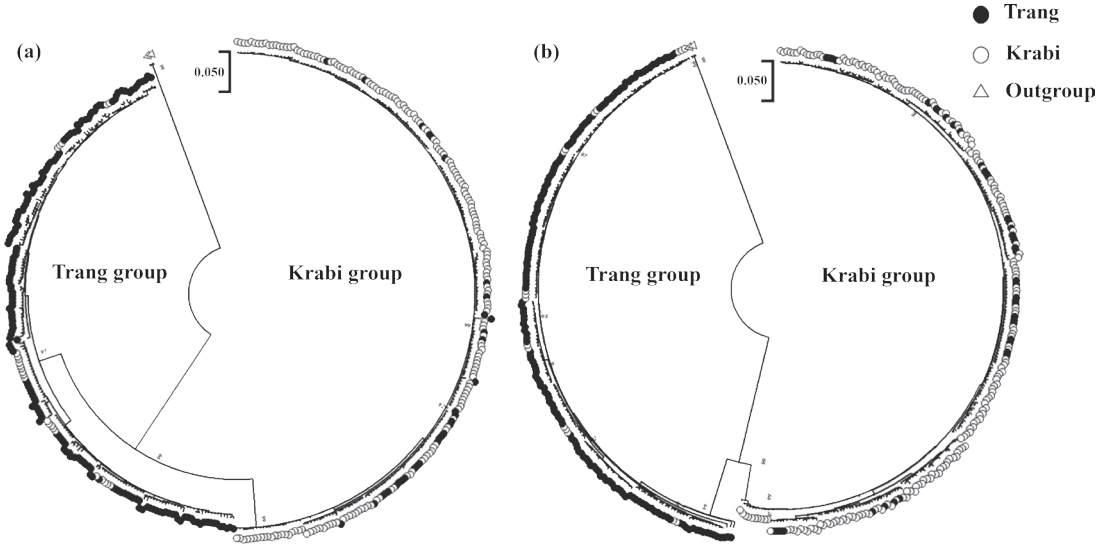

Figure 2. A phylogenetic tree constructed for the individuals of *Portunus pelagicus* from (a) Berried female crabs (Trang & Krabi) and (b) Wild crabs (Trang & Krabi), with *Squilla mantis* serving as the outgroup (accession number: NC_006081.1:15133-15994). The robustness of the tree's statistical validity was evaluated through 1,000 iterations employing the bootstrapping technique.

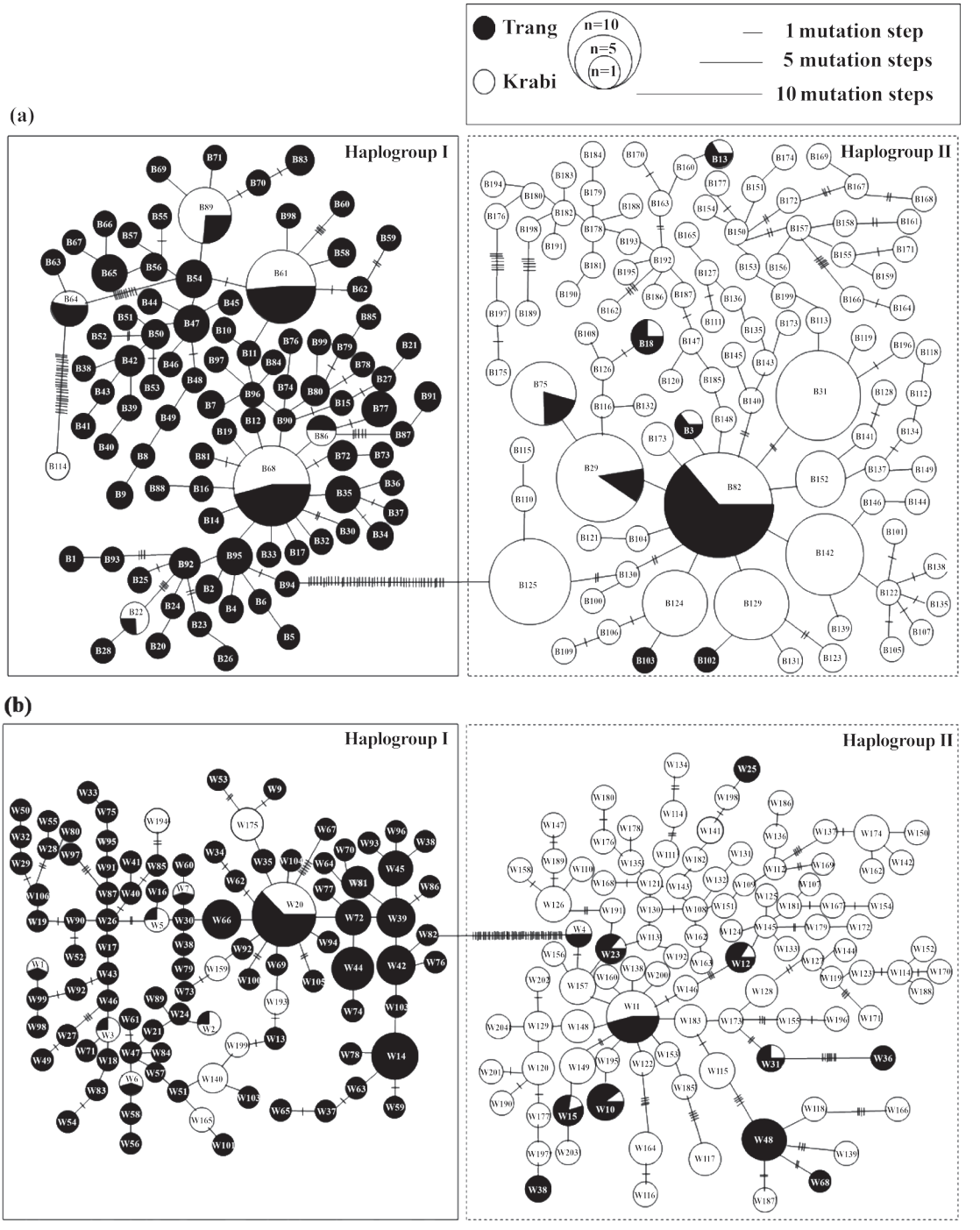

Figure 3. The minimum spanning network of mtDNA *CR* haplotypes of *Portunus pelagicus* (a) Berried female crabs (Trang & Krabi) and (b) Wild crabs (Trang & Krabi); Haplotypes are depicted as circles, with their size corresponding to their observed frequency. The color within each circle indicates the collection site. A single line connecting two haplotypes represents one mutation step. The number of vertical bars on the connecting lines indicates an increasing number of mutation steps.

Table 7. Parameter indices for the demographic history test of berried female and wild *Portunus pelagicus*.

Collecting localities	Tajima's D	Fu' s Fs	SSD[a]	Rag[b]	θ_0[c]	θ_1[d]
Berried female						
Trang	-1.065*	-23.733*	0.001	0.001	9.093	309.90.
Krabi	-0.970*	-23.672*	0.001	0.001	9.124	252.771
Total	**-1.241***	**-23.472***	**0.001**	**0.004**	**9.346**	**302.087**
Wild crabs						
Trang	-0.921*	-23.758*	0.001	0.001	5.909	204.196
Krabi	-1.079*	-23.676*	0.001	0.001	0.005	147.996
Total	**-1.230***	**-23.484***	**0.001**	**0.001**	**4.679**	**219.549**

Note: *Statistically significant ($p<0.05$); [a]SSD = sum of squared deviations; [b]Rag = raggedness index; [c]θ_0 = population size before expansion; [d]θ_1 = population size after expansion

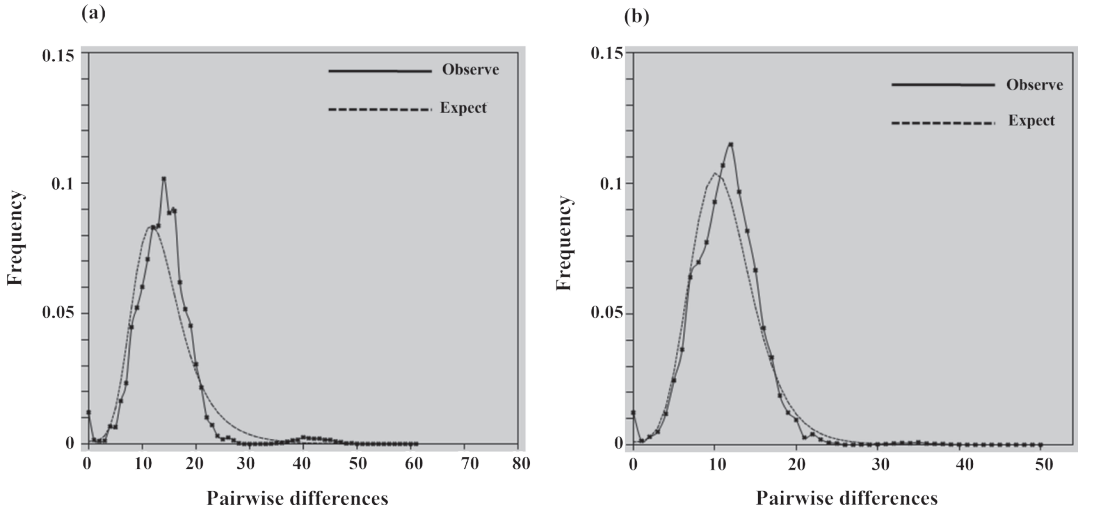

Figure 4. The mismatch distribution under a rapid population expansion paradigm of (a) Berried female crabs (Trang & Krabi) and (b) Wild crabs (Trang & Krabi). The expected mismatch distribution is represented by the dotted line, and the observed pairwise discrepancies are represented by the solid line.

DISCUSSION

Genetic diversity

Our study identified numerous unique mtDNA *CR* haplotypes in berried females and wild crabs. The presence of multiple exclusive haplotypes underscores the considerable effective female population size of *Portunus pelagicus* in Trang and Krabi (William and Allendorf, 2007). An effective female population size serves as evidence of successful female reproduction. The considerable number of effective female *P. pelagicus* individuals minimizes the likelihood of inbreeding, potentially expediting the recovery of the population. Consequently, ongoing conservation efforts remain justified. The effectiveness of the artificial propagation program for *P. pelagicus* relies on the proactive preservation of genetic diversity. Hence, the significant effective female population size indicates a promising outlook for the future recovery of the *P. pelagicus* population along the coasts of Trang and Krabi.

In samples collected from berried females and wild crabs, there was a high variation in haplotypes, while nucleotide diversity remained relatively low. This observed pattern indicates a recent expansion in the population of the *P. pelagicus*. In a population that has experienced rapid expansion, the genetic characterization is shaped by the accumulation of new mutations and the retention of elevated haplotype diversity (Watterson, 1984). Earlier studies have also observed elevated haplotype diversity and diminished nucleotide diversity in marine populations, including those of brown croaker (*Miichthys miiuy*) (Gwak and Roy, 2023), so-iny mullet (*Planiliza haematocheilus*) (Supmee *et al*., 2023), Chinese pomfret (*Pampus chinensis*) (Sun *et al*., 2021), and blue-spotted mudskipper (*Boleophthalmus boddarti*) (Theeranukul *et al*., 2021).

Nucleotide diversity (Nei and Li, 1979), the measure of polymorphism within a population, was 0.033 and 0.021 in berried females and wild *P. pelagicus*, respectively. These values indicate that *P. pelagicus* in Trang and Krabi have lower nucleotide diversity compared to *P. pelagicus* in the Andaman Sea (0.042) (Suppapan *et al*., 2023). Nevertheless, we found that the nucleotide diversity values of *P. pelagicus* in Trang and Krabi are similar to those of other crustacean species. For instance, the nucleotide diversities of the swimming crabs (*P. sanguinolentus*), the Chinese mitten crab (*Eriocheir sinensis*), and the swimming crab (*P. trituberculatus*) were recorded as 0.011 (Lu *et al*., 2022), 0.010 (Wang *et al*., 2020), and 0.020 (Cho *et al*., 2009), respectively. Additionally, we observed that the nucleotide diversity of the *P. pelagicus* in Trang exceeded that in Krabi. Consequently, our findings demonstrate that the *P. pelagicus* in Trang exhibits greater genetic diversity compared to its counterpart in Krabi.

Although mtDNA *CR* exhibits exceptionally high nucleotide variation in genetic analyses (Avise *et al*., 1987), its application in parentage assignment for stock enhancement is infrequent. Only a few studies have employed it as a supplementary tool to provide additional valuable insights, such as in the swimming crab (*P. trituberculatus*) (Cai *et al*., 2020), the black rockfish (*Sebastes inermis*) (Murakami *et al*., 2006), and the Japanese flounder (*Paralichthys olivaceus*) (Sekino *et al*., 2003). Given the significant nucleotide variation in mtDNA *CR* fragments, exceptional performance can be anticipated, particularly in species with high haplotype diversity (Cai *et al*., 2020). An initial screening was carried out utilizing fragments from the mitochondrial DNA control region in this study. Considering cost-effectiveness and efficiency, utilizing mitochondrial DNA control region fragments may be regarded as a highly effective approach for the initial screening phase in future evaluations of enhancement programs.

The most common haplotypes identified in this research are present in exceedingly low proportions (5.02% for berried female crabs and 4.48% for wild crabs) among the specimens under examination. Furthermore, a significant number of unique haplotypes was identified. As a result, the potential influence of unreleased crabs on haplotype distribution frequencies is deemed insignificant in this study. Based on the similarity of haplotypes between the berried females from the crab bank project and the wild crabs, our study found that 65.72% and 39.72% of the wild crabs shared haplotypes with berried female crabs from the crab bank project in Trang and Krabi, respectively. The higher occurrence of a shared haplotype between berried females and wild crabs in Trang compared to Krabi may be attributed to the topographical features of the study area. Trang features a semi-enclosed bay, facilitating crab movement within a confined space, while Krabi features a coastal landscape with an open sea environment, allowing crabs to move greater distances. Consequently, the likelihood of discovering shared haplotypes is higher in Trang compared to Krabi.

Our study found that implementing a crab bank project in bay areas is more effective for recolonization compared to open sea locations. The offspring released in bay areas are more likely to successfully reestablish themselves in the local population. The results of this study demonstrate that releasing crab offspring into the wild leads to the return of adult crabs, potentially increasing the wild crab population to a level sufficient for sustainable fishing. However, since this study is

preliminary, further investigation is necessary to confirm these findings by collecting more crabs from nearby areas other than Trang and Krabi. Additionally, studying mitochondrial DNA control region sequences, as done in this study, could provide more insights. Furthermore, it is advisable to conduct additional studies that include other genetic markers within the nuclear genome.

Genetic structure

The study on the genetic structure of berried crabs in Trang and Krabi provinces, utilizing six testing methods, revealed genetic differences between the berried crab populations in the two crab banks. Additionally, there was a population structure difference among wild crabs in Trang and Krabi crab banks. The *P. pelagicus* populations exhibited genetic isolation when located more than 60 km apart (Madduppa *et al*., 2021), indicating notable genetic divergence at a micro-scale level among the conspecific samples (Khamnamtong *et al*., 2021). The crab bank, serving as the sample repository for this study, is located approximately 150 km away. Our research identified a genetic structure among *P. pelagicus* in closely situated areas, suggesting significant genetic variation at a micro-scale level. Genetic differentiation patterns at this level correspond with numerous patterns observed in previous genetic markers, such as those documented by Klinbunga *et al*. (2007) (AFLP), Klinbunga *et al*. (2010) (RAPD), and Khamnamtong *et al*. (2021) (SSCP).

Portunus pelagicus is characterized by considerable mobility, with adults capable of covering about 20 km daily (Kangas, 2000). Adults and juveniles live in sheltered benthic coastal settings, with females moving into the open ocean for spawning and then returning to estuaries. Migration from estuaries by males and females is triggered by reduced salinity (Meagher, 1971). These migratory habits likely influence the observed levels of genetic differentiation in this species. This corresponds to findings of the genetic structure influenced by the migratory behavior of various marine species, such as the so-iny mullet (Supmee *et al*., 2023), the greenback mullet (*Liza subviridis*) (Supmee *et al*., 2017), mullet (*Mugil cephalus*) (Liu *et al*., 2009), and the Pacific blue shrimp (*Penaeus stylirostris*) (Aubert and Lighter, 2000).

Another factor contributing to genetic structure is the presence of geographical barriers that hinder gene flow between populations (Pearman *et al*., 2020). Marine animals that inhabit areas between geographically isolated regions, such as islands or peninsulas, experience reproductive isolation mechanisms. This leads to variations in genetic diversity patterns, resulting in the population dividing into separate population structures. An example of the formation of population structures in marine animals due to geographical barriers in Thailand is the Malay Peninsula, which divides marine animal populations between the Andaman Sea and the Gulf of Thailand. This barrier affects populations such as the hard clam (*Meretrix lyrata*) (Suppapan *et al*., 2021), the ornate threadfin bream (*Nemipterus hexodon*) (Supmee *et al*., 2021a), and the oceanic paddle crab (*Varuna literata*) (Suppapan *et al*., 2017). In this investigation, the crab banks in both regions are isolated by the Lanta Islands. This geographical separation could serve as an additional obstacle to gene exchange among *P. pelagicus* populations in the respective areas, potentially contributing to the emergence of distinct genetic structures. Our genetic structure study found genetic differences among the *P. pelagicus* populations between Trang and Krabi, but no genetic differences between berried females and wild crabs within the same region. This suggests that the restocking program has been successful, as there is no evidence of genetic contamination within the population.

Demographic history

Our independent demographic history analyses demonstrate a rapid expansion of populations for berried females and wild *P. pelagicus* in the study area. Initially, the neutrality assessment using Tajima's D and Fu's Fs showed negative values, indicating a significant departure from the neutral population. This implies that P. pelagicus may have experienced population growth or purifying selection (Yang, 2006). Moreover, the negative value of Fu's Fs test, which detects population expansion in haplotype data, serves as supplementary evidence

supporting the notion of population expansion (Ramirez-Soriano *et al*., 2008). Secondly, the mismatch distribution endorsed a sudden expansion model, and the goodness-of-fit test confirmed a well-fitted rapid expansion pattern. This supports the concept that berried females and the wild *P. pelagicus* population have experienced expansion. Finally, θ_1 was higher than θ_0, providing additional evidence in favor of demographic expansion. Recent reports have indicated population expansion among marine species in the study area, including the blue swimming crab (Suppapan *et al*., 2023), the sandfish (*Holothuria scabra*) (Ninwichian and Klinbunga, 2020), and the wedge clam (*Donax scortum*) (Supmee *et al*., 2021b).

Guidelines for management

The crab bank project aims to bolster the crab population in the wild to offset the impacts of intensive crab fishing. The offspring from the project grow and mature into breeding adults or become suitable targets for continued fishing. According to fisheries statistics, there has been an increase in *P. pelagicus* catches since 2019, following the Thai government's promotion of the crab bank project (Fishery Statistics Analysis and Research Group, 2022). However, no published study has evaluated the stock enhancement of the crab bank in Thailand.

The evaluation report on the stock enhancement of *P. pelagicus* has been documented in Australia, using anchor tags attached to mature crabs with the mark-recapture method for examination. It was found that the recapture rate was significantly low (Williams, 1986). Another study conducted in Japan involved clipping the dactylus of the swimming leg from the central tip of crab offspring and employing the mark-recapture method. Approximately 4% of the crab offspring were recaptured (Obata, 2016). From both studies, it can be observed that the mark-recapture method in *P. pelagicus*, using tags attached to the crabs' bodies, yields low recapture rates due to the crabs' molting process, which causes the tags to become detached and lost. As for the method of marking by clipping the dactylus, it may affect the behavior of the crabs when in their natural habitat, leading to lower survival rates. Additionally, the number of released crabs in both studies was much lower (approximately 10,000 individuals) than in our study (> 1,000,000 individuals), resulting in lower recapture rates. Therefore, our study utilizes genetic markers from the mitochondrial DNA control region (mtDNA *CR*), specifically from the nucleotide sequence, which revealed a higher success rate in wild crabs. This could be because using DNA markers for marking, sourced from a single-berried female crab, can potentially track millions of crab offspring. Additionally, it allows for the release of a large number of crab offspring into the sea, increasing the likelihood of detecting genetic markers from crabs in the wild. Evaluating stock enhancement using genetic markers is an effective and suitable method for monitoring crab populations in the wild. Nevertheless, it is advisable to complement tracking efforts with the use of additional genetic markers, which should be confirmed by collecting more crabs from nearby areas, excluding Trang and Krabi, or from other regions.

Our study, analyzing nucleotide sequences in the mitochondrial DNA control region, uncovered high genetic diversity within each crab bank, suggesting robust population fitness of *P. pelagicus* in this region. Furthermore, the issue of inbreeding does not pose a concern for this crab. Genetic structure results indicate that the genetic structure of *P. pelagicus* differs between the Trang and Krabi areas. Therefore, management strategies should be separate to prevent genetic contamination. Examples of management include not releasing crab offspring across different regions. Controlling coastal pollution is one of the prompt activities that can be taken to improve the spread of larvae and increase the number of breeding individuals. Additionally, effective population management might include the regulation of gear, habitat monitoring, and the initiation of restoration endeavors. Periodic surveys on genetic diversity and seascape studies are required to provide a thorough picture of the crab population's temporal and spatial dynamics.

In a program aimed at bolstering stock, the key goals include ecological, economic, and social benefits, emphasizing the need for a well-balanced assessment of release strategies and their environmental impact (Secor *et al*., 2002). Hence, the success of stock enhancement relies not solely on the performance of the released recruits but also on effective fishery management and environmental enhancements.

CONCLUSIONS

In our research, we analyzed 508–512 base pair mtDNA *CR* nucleotide sequences to preliminary evaluate the genetic diversity of berried and wild *Portunus pelagicus* sourced from crab banks in Trang and Krabi, Thailand. The findings of our study revealed that *P. pelagicus* from the crab bank initiative exhibits high genetic diversity, indicating heightened population fitness. Our study found that 65.72% and 39.72% of the wild crabs shared haplotypes with berried female crabs from the crab bank project in Trang and Krabi, respectively. Our findings indicate that releasing crab offspring into their natural habitat leads to the eventual presence of adult crabs, potentially boosting the wild crab population to a sustainable level for fishing in the region. The exploration of the genetic structure uncovered a distinct division into two separate populations: Trang and Krabi. Demographic history tests suggested a recent population expansion within the *P. pelagicus* population. This research provides information for creating management measures aimed at preserving the genetic variation of the *P. pelagicus* population in this location. We suggest using nuclear DNA markers in upcoming studies to improve the breadth of data and provide new insights into genetic information.

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