Sains Malaysiana 46(12)(2017): 2251–2261
http://dx.doi.org/10.17576/jsm-2017-4612-01

Population Genetic Analysis of Oceanic Paddle Crab (*Varuna litterata*) in Thailand

(Analisis Populasi Genetik Ketam Meranduk Laut (*Varuna litterata*) di Thailand)

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ABSTRACT

Population genetic structure of Varuna litterata *living along the coast of Thailand were examined in this study. The samples were collected from 3 coastal regions: The Andaman sea (Satun, Trang, Phang Nga), the lower Gulf of Thailand (Pattani, Songkhla, Nakhon Si Thammarat) and the upper Gulf of Thailand (Petchburi, Samut Songkram, Rayong, Trat). Intraspecific variation was determined based on partial sequences of the cytochrome oxidase subunits I gene. A total of 182 samples were collected but only 32 haplotypes were obtained from these samples. An excess of rare haplotypes indicated that the female effective population size of* V. litterata *living along the coast of Thailand is large. Estimated values of haplotype diversity and nucleotide diversity were 0.790 and 0.003, respectively. The AMOVA (analysis of molecular variance) and phylogenetic analysis results showed that based on genetic variation, the population of this organism was found to have 2 genetically different populations: The Andaman sea population and the Gulf of Thailand population. Genetic exchange of* V. litterata *among populations inhabiting along the coast of Thailand could be described by the stepping stone model. The results of neutrality tests, both Tajima's D and Fu's Fs statistics, yielded negative values (-1.992 and -26.877, respectively) and statistically significant deviation from the neutrality, indicating that the* V. litterata *living along the Thailand coast had experienced population expansion. Mismatch distribution analysis indicated that a possible expansion occurred 211,428 years ago during the Pleistocene glaciations period.*

Keywords: COI gene; genetic diversity; grapsid crab; mitochondrial DNA

ABSTRAK

Struktur populasi genetik Varuna litterata *yang hidup di sepanjang pesisir pantai di Thailand telah dikaji. Sampel dikumpul daripada tiga rantau pesisir pantai: Laut Andaman (Satun, Trang, Phang Nga), bahagian bawah Teluk Thailand (Pattani, Songkhla, Nakhon Si Thammarat) dan di bahagian atas Teluk Thailand (Petchburi, Samut Songkram, Rayong, Trat). Variasi intraspesies ditentukan berdasarkan urutan separa subunit sitokrom oksidase gen I. Sejumlah 182 sampel telah diambil tetapi hanya 32 haplotip telah diperoleh daripada sampel tersebut. Lebihan haplotip langka menunjukkan saiz populasi betina* V. litterata *yang berkesan hidup di sepanjang Pantai di Thailand adalah besar. Anggaran nilai kepelbagaian haplotip dan kepelbagaian nukleotida masing-masing adalah 0.790 dan 0.003. Keputusan AMOVA (analisis molekul varians) dan analisis filogeni menunjukkan bahawa berdasarkan variasi genetik, populasi organisma ini didapati mempunyai dua populasi genetik berbeza: Populasi Laut Andaman dan Teluk Thailand. Pertukaran genetik* V. litterata *antara populasi yang mendiami di sepanjang Pantai Thailand dapat diterangkan dengan model batu loncatan. Ujian keputusan berkecuali, kedua-dua statistik D Tajima dan Fs Fu masing-masing menghasilkan nilai negatif (-1.992 dan -26.877) dan signifikan secara statistik sisihan daripada yang berkecuali, menunjukkan bahawa* V. litterata *yang hidup di sepanjang Pantai Thailand telah mengalami pengembangan populasi. Analisis pengagihan tak sepadan menunjukkan potensi pengembangan berlaku di 211,428 tahun yang lalu semasa tempoh Pleistocene glaciations.*

Kata kunci: DNA mitokondrium; gen COI; kepelbagaian genetik; ketam grapsid

INTRODUCTION

Varuna litterata is a grapsid crab belonging to the family Varunidae. *V. litterata* is an important fishery product for people living in Southeast Asia (Carpenter & Niem 1998). In Thailand, *V. litterata* is also collected for their tasty ovaries and they are especially common in markets during their breeding period. *V. litterata* is generally preserved with fish sauce. This preserved form is an ingredient found in many Thai dishes, including Yum Poo Pan and pickled crab. In Thailand, a large number of *V. litterata*, approximately 18,000 tons per year, has been consumed; however, only approximately 12,000 tons can be domestically produced per year. Therefore, at least 6,000 tons of *V. litterata* have to be annually imported from Myanmar and Cambodia (Tiensongrassamee 2009). *V. litterata* have a crucial role as decomposers in esturine ecosystems by degrading organic matter. Furthermore, its fecal material potentially contributes to secondary production via a coprophagous food chain (Gillikin & Schubart 2004). *V. litterata* is mainly found

in estuarine swamps up to 20 km inland in completely freshwater located along the Thailand coast with a coastline of approximately 3,000 km (Naiyanetr 2007). These regions are major habitats for this species and main fishery areas for local people. Thailand's coastal habitats are biologically complex due to the variability of their taxonomic composition and overall community structure. Additionally, the Andaman sea coast and the Gulf of Thailand, separated by a geographic barrier known as the Thai-Malay peninsula, were reported to have different topographic and oceanographic variations (Nakthon 1992). These factors may affect genetic variations of *V. litterata*; however, its genetic features have not yet been reported. Due to dramatic decreases in *V. litterata* populations, caused by overexploitation for commercial purposes (Tiensongrasamee 2009), an effective sustainable management strategy is needed. This plan needs to be based on detailed information of genetic features and the historical demography of this species.

In this study, we hypothesized that the geographic barrier observed along the Thailand coast generates genetic variations of *V. litterata* living in this area. In order to obtain information about genetic features of *V. litterata* within and among habitats in this area, both the population genetic structure and historical demography were studied. The mitochondrial DNA is exclusively maternally inheritance with a relatively fast evolutionary rate and lack of recombination (Avise 2000). Genetic variations within this species were identified by examining the partial sequence of the mitochondrial genome in cytochrome oxidase subunit I gene (*COI*). The results of this study would certainly help us gain insight into the population genetic structure and the demographic history of these species living along the coast of Thailand. This genetic information would be helpful for designing an appropriate management and sustainable exploitation of *V. litterata* in Thailand.

MATERIALS AND METHODS

SAMPLE COLLECTION

A total of 182 individuals of *V. litterata* were collected from ten localities along the Thailand coast including Satun (ST), Trang (TG), Phang Nga (PN), Pattani (PT), Songkhla (SK), Nakhon Si Thammarat (NS), Petchburi (PB), Samut Songkram (SM), Rayong (RY) and Trat (TR) (Table 1, Figure 1). The samples were immediately stored on ice, transferred to a laboratory and stored at -20ºC for further analysis.

DNA EXTRACTION, AMPLICATION AND SEQUENCING

Total genomic DNA was extracted from *V. litterata* muscle tissue of the first or second walking legs using Tissue Genomic DNA Extraction Mini Kit (FAVORGEN, BIOTECH CORP.) according to the manufacturer's protocol. Partial nucleotide sequence of *COI* gene from each crab specimen was amplified. The primer pair, PMT1 5' GGT CAA CAA ATC ATA AGA TAT TGG 3' and PMT2 5' TAA ACT TCA GGG TGA CCA AAA AAT CA 3' (Tang et al. 2003) was used to amplify the fragment of the *COI* gene. PCR reaction was conducted in total volume of 50 μL containing 10X *Taq* buffer 5 μL, 25 mM $MgCl_2$ 7.5 μL, 2 mM dNTPs mix 4 μL, 10 μM forward and reverse primers 2μL each, 2.5 unit *Taq* DNA polymerase (RBCbiosciences, USA) 0.5 μL, total DNA 2.5 μL (50-100 ng) and ultrapure water 26.5 μL. The PCR was performed using the following conditions in a thermocycler; step 1-initialization at 94ºC for 4 min; step 2-35 cycles of 94ºC for 40 s, 51ºC for 1 min , 72ºC for 1 min; step 3-final extension at 72ºC for 10 min. The PCR products were purified using Gel/PCR Purification Mini Kit (FAVORGEN, BIOTECH CORP.) and sequenced (1st Base Laboratory, Malaysia).

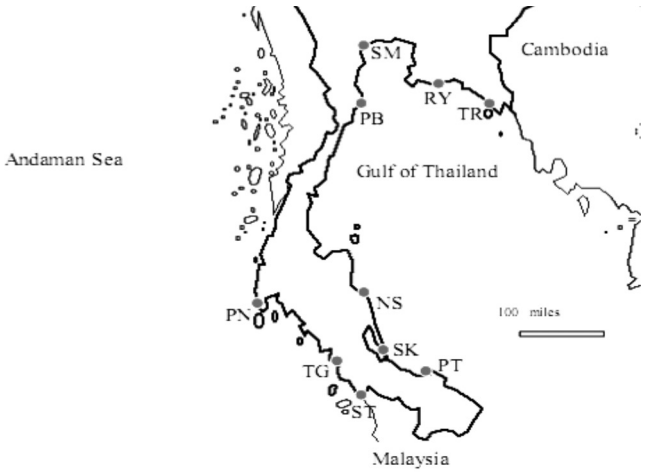

FIGURE 1. The collecting localities for *V. litterata* along the coast of Thailand and the localities abbreviated in the parentheses: Satun (ST); Trang (TG); Phang Nga (PN); Pattani (PT); Songkhla (SK); Nakhon Si Thammarat (NS); Petchburi (PB); Samut Songkram (SM); Rayong (RY); Trat (TR)

DATA ANALYSIS AND GENETIC VARIATION STUDY

Sequences obtained from each specimen were assembled using CAP3 (Huang & Madan 1999) under default parameters to construct a partial sequence of *COI* gene. Multiple sequence alignments were performed using ClustalW version 1.83 (Thompson et al. 1994). Ambiguous positions of the aligned sequences were adjusted manually. Standard indices of genetic diversity including the nucleotide diversity (π) (Nei & Tajima 1981), haplotype diversity (h) (Nei 1987) and mean number of nucleotide differences among all haplotypes were estimated using DnaSP version 5.00 (Librado & Rozas 2009).

POPULATION GENETIC STRUCTURE AND HISTORICAL DEMOGRAPHIC ANALYSES

Population genetic structure of *V. litterata* was determined under seven putative geographic-base structures. In the first structure, the *V. litterata* specimens were separated into ten populations according to the sampling provinces (ST, TG, PN, PT, SK, NS, PB, SM, RY and TR). The second putative structure was determined according to the two geographic regions: the Andaman sea coast (ST, TG and PN) and the coast of the Gulf of Thailand (PN, SK, NS, PB, SM, RY and TR). The third putative structure was determined according to the two geographic regions: the southern coast of Thailand (ST, TG, PN, PT, SK and NS) and the northern coast of Thailand (PB, SM, RY and TR). The fourth putative structure was determined according to the three geographic regions: The Andaman sea coast (ST, TG and PN), the coast of the lower Gulf of Thailand (PT, SK and NS) and the coast of the upper Gulf of Thailand (PB, SM, RY and TR). The fifth putative structure was determined according to the two geographic regions: The Andaman sea coast (ST, TG and PN) and the coast of the lower Gulf of Thailand (PT, SK and NS). The sixth putative structure was determined according to the two geographic regions: the Andaman sea coast (ST, TG and PN) and the coast of the upper Gulf of Thailand (PB, SM, RY and TR). The seventh putative structure was determined according to the two geographic regions: the coast of the lower Gulf of Thailand (PT, SK and NS) and the coast of the upper Gulf of Thailand (PB, SM, RY and TR). Hierarchical analysis of molecular variance (AMOVA) was performed to compare levels of genetic diversity within and among putative populations using ARLEQUIN v. 3.5 (Excoffier & Lischer 2010). The associated F-statistic analogs including Φ_{CT}, Φ_{SC} and Φ_{ST} were estimated at the different hierarchical levels. The significance of Φ-statistic was tested by 10,000 permutations (p<0.05). In addition, genetic distances between all possible combinations of populations (pairwise F_{ST}) were estimated. The significance of the pairwise was tested with 10,000 permutations. Phylogenetic analysis using Neighbor-joining (NJ) method (Saitou & Nei 1987) based on the matrix of Kimura 2-parameter distances implemented in MEGA version 4 (Tamura et al. 2007) was used to examine the relationships among individuals of *V. litterata*. Relative support for tree topology was obtained by bootstrapping (Felsenstein 1985) using 1,000 replicates under Kimura 2-parameter model.

Demographic history of *V. litterata* from all localities across the Thailand coast was examined using three independent approaches. In the first approach, selective neutrality for each sampling localities and for the whole populations were tested using the Tajima's D (Tajima 1989) and the Fu's Fs (Fu 1997) statistics based on 10,000 replicates. Secondly, the population expansion was tested by analyzing the mismatch distributions of the pairwise different between individuals (Rogers & Harpending 1992). The smoothness of the mismatch distribution was measured by Harpending's Raggedness (Harpending 1994). The parameter τ, θ_0 and θ_1 were estimated from the data. Thirdly, the time since population expansion (T) was calculated as $T = \tau/2u$ (Rogers & Harpending 1992) where τ is expansion time and $2u = \mu \times$ generation time $\times$ number of bases sequence where μ is the mutation rate.

RESULTS

GENETIC VARIATION

Sizes of the partial nucleotide sequences amplified from each *V. litterata* individual were 525 bp. The alignment results showed that out of 525 aligned sites, 492 were monomorphic and 33 were polymorphic. Among the 33 polymorphic sites, 13 were singleton and 20 sites were parsimoniously informative sites. The number of haplotypes, the number of polymorphic sites, nucleotide diversity value (π) and haplotype diversity value (h) of ten crab populations were estimated (Table 1). The haplotype diversity and nucleotide diversity value of each population were in the range of 0.614 - 0.924 and 0.001 - 0.006, respectively. The haplotype diversity and the nucleotide diversity value of the overall populations were 0.790±0.025 and 0.003±0.000, respectively (Table 1). In total, 32 haplotypes were identified, consisting of 10 shared (H02, H03, H04, H06, H07, H09, H10, H12, H13 and H24), 2 population-specific (H15 and H19) and 20 rare haplotypes (Table 2). Each population except TG, SM and TR had its own unique haplotypes called 'private alleles'. A total private allele of *V. litterata* was 22. RY population possessed the highest number of private alleles of 8. PB and ST had 5 and 3 private alleles, respectively. SK and NS had 2 private alleles. Both PN and PT had 1 private allele.

POPULATION GENETIC STRUCTURE

The AMOVA analysis showed that the genetic variations (88.18 - 93.30 %) of the *V. litterata* were within-population variations in each putative structure. The F-statistic of the first putative structure, *V. litterata* population along the coast of Thailand, was statistically significant (Φ_{ST}=0.073, p=0.001). The F-statistic of the second putative structure, Andaman sea, was significantly different from the Gulf of Thailand (Φ_{CT}=0.089, p=0.008). The F-statistic of the third putative structure, the southern coast of Thailand

TABLE 1. Collecting localities, code of collecting site, number of individuals per sampling site (*N*) and summary statistics of genetic variability for *V. litterata* along the Thailand coast

Locality	Code	*N*	No. haplotypes	No. polymorphic sites	Haplotype diversity (*h*) (mean ± SD)	Nucleotide diversity (π) (mean ± SD)
Satun	ST	17	8	13	0.816±0.082	0.003±0.001
Trang	TG	18	5	11	0.660±0.102	0.005±0.001
Phang Nga	PN	17	5	12	0.699±0.102	0.006±0.002
Pattani	PT	18	6	5	0.784±0.065	0.002±0.000
Songkhla	SK	18	5	4	0.614±0.117	0.001±0.000
Nakhon Si Thammarat	NS	20	6	6	0.763±0.066	0.002±0.000
Petchburi	PB	18	8	7	0.843±0.060	0.002±0.000
Samut Songkram	SM	20	6	5	0.621±0.109	0.001±0.000
Rayong	RY	19	12	12	0.924±0.042	0.004±0.000
Trat	TR	17	4	3	0.750±0.063	0.002±0.000
Total		182	32	33	0.790±0.025	0.003±0.000

TABLE 2. Haplotype distributions of *V. litterata* from 10 localities along the Thailand coast

Haplotype	ST	TG	PN	PT	SK	NS	PB	SM	RY	TR	Total
H01	1	-	-	-	-	-	-	-	-	-	1
H02	7	10	9	7	11	6	4	12	4	4	74
H03	2	-	-	4	3	2	6	1	4	7	29
H04	3	4	2	1	1	8	-	4	2	-	25
H05	1	-	-	-	-	-	-	-	-	-	1
H06	1	-	-	-	-	2	-	-	-	-	3
H07	1	2	-	-	-	-	-	-	-	-	3
H08	1	-	-	-	-	-	-	-	-	-	1
H09	1	-	2	-	-	-	1	1	-	2	7
H10	-	1	3	-	-	-	-	-	-	-	4
H11	-	-	1	-	-	-	-	-	-	-	1
H12	-	-	-	4	-	-	-	1	-	-	5
H13	-	-	-	1	-	-	-	-	-	4	5
H14	-	-	-	1	-	-	-	-	-	-	1
H15	-	-	-	-	2	-	-	-	-	-	2
H16	-	-	-	-	1	-	-	-	-	-	1
H17	-	-	-	-	-	1	-	-	-	-	1
H18	-	-	-	-	-	1	-	-	-	-	1
H19	-	-	-	-	-	-	3	-	-	-	3
H20	-	-	-	-	-	-	1	-	-	-	1
H21	-	-	-	-	-	-	1	-	-	-	1
H22	-	-	-	-	-	-	1	-	-	-	1
H23	-	-	-	-	-	-	1	-	-	-	1
H24	-	-	-	-	-	-	-	1	1	-	2
H25	-	-	-	-	-	-	-	-	1	-	1
H26	-	-	-	-	-	-	-	-	1	-	1
H27	-	-	-	-	-	-	-	-	1	-	1
H28	-	-	-	-	-	-	-	-	1	-	1
H29	-	-	-	-	-	-	-	-	1	-	1
H30	-	-	-	-	-	-	-	-	1	-	1
H31	-	-	-	-	-	-	-	-	1	-	1
H32	-	-	-	-	-	-	-	-	1	-	1
Total	18	17	17	18	18	20	18	20	19	17	182

showed no significant difference from the northern coast of Thailand (Φ_{CT}=0.027, p=0.080). The F-statistic of the fourth putative structure, *V. litterata* population between the Andaman sea and the lower and upper Gulf of Thailand, showed a statistically significant finding (Φ_{CT}=0.056, p=0.010). The F-statistic of the fifth putative structure showed no statistically significant difference between the Andaman sea and the lower Gulf of Thailand (Φ_{CT}=0.066, p=0.099). The F-statistic of the sixth putative structure, Andaman sea showed a statistically significant difference from the upper Gulf of Thailand (Φ_{CT}=0.089, p=0.028). The F-statistic of the seventh putative structure showed no statistically significant difference between the lower and upper Gulf of Thailand (Φ_{CT}=-0.004, p=0.517) (Table 3). Every pairwise F_{ST} of the geographic-based populations, as shown in Table 4, showed significant differences between populations, except for the comparison between ST and the group of PT, PB and TR; between TG and the group of PT, SK, PB, RY and TR; between PN and the group of PT, SK, NS, PB, RY and TR; between PT and NS ; between SK and the group of NS and TR; between NS and the group of PB, RY and TR; between PB and SM; and between SM and the group of RY and TR. The neighbor-joining tree showed two distinct clade (clade A and B), which were supported by bootstrap values (56%). Clade A was consisted of individuals from Andaman Sea in a large proportion (76.19%), while clade

TABLE 3. Hierarchical analysis of molecular variance (AMOVA) of *V. litterata*

Source of variation	df	Sum of squares	Variance components	Percentage of variation	p-value
Single region					
Among populations	9	18.074	0.065 Va	7.30	Φ_{ST} = 0.073*(p=0.001)
Within populations	172	141.932	0.825 Vb	92.70	
Total	181	160.005	0.890		
Andaman Sea and Gulf of Thailand					
Among groups	1	7.502	0.083 Va	8.91	Φ_{CT}= 0.089*(p=0.008)
Among populations within groups	8	10.572	0.027 Vb	2.91	Φ_{SC}= 0.031 (p=0.051)
Within populations	172	141.932	0.825 Vc	88.18	Φ_{ST}= 0.118*(p=0.020)
Total	181	160.005	0.935		
Southern and northern coast of Thailand					
Among groups	1	3.967	0.024Va	2.77	Φ_{CT}= 0.027(p=0.080)
Among populations within groups	8	14.106	0.051Vb	5.72	Φ_{SC}= 0.058* (p=0.001)
Within populations	172	141.932	0.825Vc	91.51	Φ_{ST}= 0.084*(p=0.000)
Total	181	160.005	0.901		
Andaman Sea, lower Gulf of Thailand and upper Gulf of Thailand					
Among groups	2	8.765	0.050 Va	5.64	Φ_{CT}= 0.056*(p=0.010)
Among populations within groups	7	9.309	0.027 Vb	3.07	Φ_{SC}= 0.032 (p=0.069)
Within populations	172	141.932	0.825 Vc	91.29	Φ_{ST}= 0.087*(p=0.030)
Total	181	160.005	0.903		
Andaman Sea and lower Gulf of Thailand					
Among groups	1	4.797	0.066 Va	6.61	Φ_{CT}= 0.066 (p=0.099)
Among populations within groups	4	4.795	0.015 Vb	1.49	Φ_{SC}= 0.015(p=0.230)
Within populations	102	94.649	0.927 Vc	91.90	Φ_{ST}= 0.080*(p=0.009)
Total	107	104.241	1.009		
Andaman Sea and upper Gulf of Thailand					
Among groups	1	7.245	0.097 Va	8.95	Φ_{CT}= 0.089*(p=0.028)
Among populations within groups	5	6.549	0.018 Vb	1.73	Φ_{SC}= 0.019 (p=0.180)
Within populations	119	115.460	0.970 Vc	89.32	Φ_{ST}= 0.106*(p=0.004)
Total	125	129.254	1.086		
lower and upper Gulf of Thailand					
Among groups	1	1.263	-0.003 Va	-0.48	Φ_{CT}=- 0.004 (p=0.517)
Among populations within groups	5	7.274	0.046 Vb	7.18	Φ_{SC}= 0.071* (p=0.003)
Within populations	123	73.756	0.599 Vc	93.30	Φ_{ST}= 0.066 * (p=0.002)
Total	129	82.292	0.642		

*significant differentiation (p<0.05)

TABLE 4. Population pairwise F_{ST} values of *V. litterata*

	Andaman sea			lower Gulf of Thailand			upper Gulf of Thailand			
	ST	TG	PN	PT	SK	NS	PB	SM	RY	TR
ST	-									
TG	-0.009 (0.550)	-								
PN	0.016 (0.196)	-0.048 (0.802)	-							
PT	0.051* (0.038)	0.108* (0.002)	0.108* (0.006)	-						
SK	0.031 (0.099)	0.097* (0.020)	0.103* (0.010)	0.026 (0.024)	-					
NS	-0.016 (0.722)	0.075 (0.059)	0.110* (0.008)	0.115* (0.005)	0.097* (0.019)	-				
PB	0.080* (0.008)	0.132* (0.000)	0.128* (0.000)	0.049 (0.078)	0.048 (0.085)	0.158* (0.000)	-			
SM	-0.002 (0.408)	0.072 (0.119)	0.093 (0.056)	0.047 (0.099)	0.025 (0.160)	0.019 (0.028)	0.124* (0.002)	-		
RY	0.031 (0.080)	0.100* (0.001)	0.110* (0.000)	0.002 (0.364)	0.029 (0.110)	0.061* (0.032)	0.016 (0.179)	0.051* (0.040)	-	
TR	0.110* (0.004)	0.148* (0.000)	0.121* (0.014)	0.064 (0.069)	0.094* (0.033)	0.195* (0.000)	0.002 (0.194)	0.169* (0.000)	0.034 (0.097)	-

*significant differentiation ($p< 0.05$)
p values in parentheses

B was consisted of individuals from Gulf of Thailand in a large proportion (85.74%) (Figure 2). The result suggested that dominate population of *V. litterata* from Andaman sea and Gulf of Thailand have evolved separately as at least two major evolutionary lineages. Probably, barrier that caused the reproductive isolation of marine species in this geographic area may be the landmass of Malay Peninsula. Role of Malay Peninsula on genetic variation have been reported in Asian horseshoe crab (*Carcinoscorpius rotundicauda*) (Tan et al. 2015) and Asian moon scallop (*Amusium pleuronectes*) (Mahidol et al. 2007).

DEMOGRAPHIC HISTORY

Two methods of neutrality tests, Tajima's *D* and Fu's *Fs* statistics, were applied to examine the historical demography of this species for each population and for all populations pooled together. The results of these analyses are shown in Table 5. Tajima's *D* statistics of ST, TG, PN, PT, SK, NS, PB, SM, RY and TR populations were negative. When all populations were pooled together, the *D* statistic was statistically significant (D=-1.992, p=0.001). Fu's *Fs* statistics of ST, TG, PN, PT, SK, NS, PB, SM, RY and TR populations were negative except for TG and PN. The *Fs* statistic of the pooled population was a statistically significant negative value (Fs=-26.877, 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=0.012, p=0.008), although those of each population could not reject the model. The Harpending raggedness indices were significant values (rg=0.112, p=0.000) (Table 5). The estimated θ_1 was higher than θ_0 for every sampling location, indicating population expanded from a very small to a very large size (Table 5). The initiation time of this species and population expansion across the Thailand sea coast was approximately 211,428 years ago. This initiation time was estimated from the mutation rate of 1.2% per million years with a generation time of 1 year. Due to a lack of fossil records of grapsid crabs, this mutation rate of the *COI* gene was estimated from fossil records of a crustacean (Vanschoenwinkel et al. 2012).

DISCUSSION

GENETIC VARIATION

A total of 32 mtDNA haplotypes were detected in the *COI* gene segment. These haplotypes consisted of 10 shared, 2 population-specific and 20 rare haplotypes. The presence of rare haplotypes in the many populations included in this study indicated the existence of a large female effective population size of the *V. litterata* along the coast of Thailand (Lewontin 1974). This skewed haplotype frequency is thought to reflect a large effective population size that allowed for the retention of numerous unique haplotypes in female. In addition, the presence of many

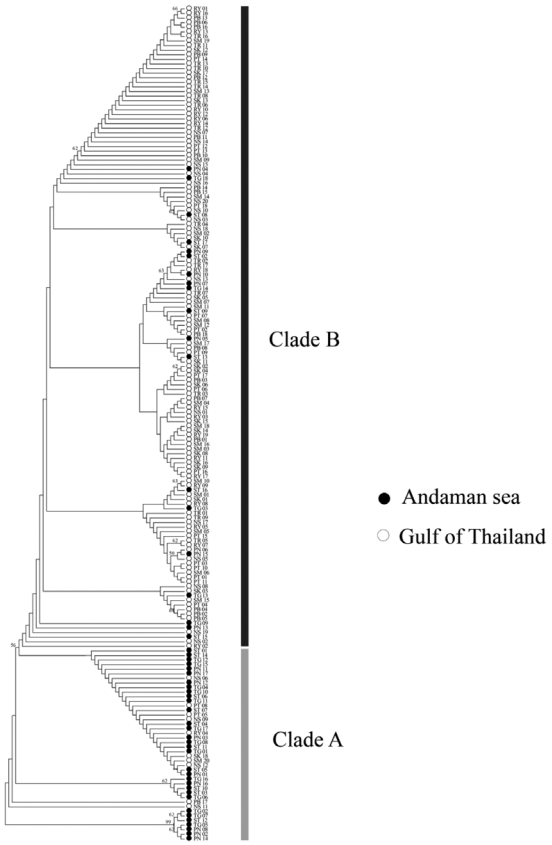

FIGURE 2. Neighbor-joining phylogenetic tree based on cytochrome oxidase subunits I gene of ten populations of *V. litterata*. The node support ration at each branch is evaluated by performing bootstrapping with 1,000 replicates under Kimura 2-parameter model and hidden lower than 50%. Name of localities are abbreviated: Satun (ST); Trang (TG); Phang Nga (PN); Pattani (PT); Songkhla (SK); Nakhon Si Thammarat (NS); Petchburi (PB); Samut Songkram (SM); Rayong (RY); Trat (TR)

rare haplotypes gives an indication of a historic period of sudden population expansion in *V. litterata*. A sudden population expansion may result in an excess of rare haplotypes, as more haplotype are produced by mutation than are removed by genetic drift. Alternatively, the same patterns could have resulted from a recovery of variation following selective sweep for a favorable haplotype, with new haplotypes being generated by mutation (Croos & Pálsson 2010). In this study, there were 22 private alleles observed in the specific populations of *V. litterata*. The haplotype distribution pattern suggested that those 'private alleles' could be used as indicators of stock identification (Xu et al. 2009). A pattern of genetic diversity in the population of *V. litterata* living along the Thailand coast presented high haplotype diversity but a low nucleotide diversity. This pattern was reported as a typical character of genetic variations of marine species (Chu et al. 2012; Kong et al. 2010). Regarding the population genetic theory, this pattern can be generated by an accumulation of new mutations in a rapidly expanding population (Watterson 1984). This pattern of genetic variation has been observed in various grapsid crab species, such as the Violet vinegar crab (*Episesarma versicolor*) (Supmee et al. 2012a), the Thai vinegar crab (*E. mederi*) (Supmee et al. 2012b) and the *Neosarmatium meinerti* (Ragionieri et al. 2010).

POPULATION GENETIC STRUCTURE

In this study, the *V. litterata* population in Thailand was found to have 2 genetically different populations: The Andaman sea population and the Gulf of Thailand population. This genetic separation could be the result of the gene flow disruption between the populations of the Andaman Sea and the Gulf of Thailand that is caused by the geographical barrier on the Malay Peninsula. Genetic divergence between populations of marine organisms in the Andaman sea and the Gulf of Thailand has been reported for several other species including the surf calm (*Paphia undulata*) (Donrung et al. 2011), the orange spotted grouper (*Epinephelus coioides*) (Antoro et al. 2006) and the black tiger shrimp (*Penaeus monodon*) (Supungul et al. 2000). The results of this study showed significant genetic differences of *V. litterata* between the Andaman Sea and the upper Gulf of Thailand. In marine environments, most species spend part of their life cycle (free-moving gametes, larvae or adults) in open waters. It could be expected that species with high dispersal capabilities have high gene flow and little genetic structure (Russo et al. 1994; Uthicke & Benzie 2003). Genetic homogeneity between long-distance populations of many marine crabs is maintained by high larva dispersal. For example, genetic homogeneity of *N. meinerti* (Ragionieri et al. 2010) and *Uca annulipes* (Silva

TABLE 5. Parameter indices of mismatch distribution analysis and neutrality test of *V. litterata*

Locality	Tajima's *D*	Fu' s *Fs*	τ^a	θ_0^b	θ_1^c	SSD^d	Rag	Expansion time (year ago)
ST	-1.760* (0.026)	-2.557* (0.045)	1.412	0.000	99999.000	0.018 (0.240)	0.117 (0.263)	224,126
TG	-0.293 (0.427)	1.627 (0.807)	0.845	0.031	99999.000	0.061 (0.075)	0.126 (0.441)	134,126
PN	-0.223 (0.457)	1.901 (0.838)	1.217	0.003	99999.000	0.845 (0.299)	0.137 (0.541)	193,174
PT	-0.845 (0.220)	-2.254* (0.031)	1.492	0.000	99999.000	0.027 (0.072)	0.211 (0.041)	236,825
SK	-1.128 (0.148)	-2.095* (0.015)	0.952	0.000	99999.000	0.022 (0.116)	0.179 (0.144)	151,111
NS	-0.955 (0.186)	-1.753 (0.093)	1.408	0.000	99999.000	0.007 (0.393)	0.097 (0.341)	223,492
PB	-1.137 (0.127)	-4.013* (0.003)	1.685	0.000	99999.000	0.039 (0.063)	0.191 (0.041)	267,460
SM	-1.460 (0.059)	-3.241* (0.002)	0.953	0.001	99999.000	0.022 (0.183)	0.181 (0.112)	151,269
RY	-1.302 (0.091)	-7.490* (0.000)	2.314	0.000	99999.000	0.002 (0.755)	0.054 (0.475)	367,301
TR	-0.734 (0.790)	-0.062 (0.493)	1.526	0.000	99999.000	0.016 (0.182)	0.152 (0.192)	242,222
TOTAL	-1.992* (0.001)	-26.877* (0.000)	1.332	0.000	99999.000	0.012 (0.008)	0.112 (0.000)	211,428

*significant differentiation ($p<0.05$), [a]time in number of generation, [b]pre-expansion population size ($\theta_0=2N_0\mu$), [c]postexpansion population size ($\theta_1=2N_1\mu$), [d]sum of squared deviations and *p* values in parentheses

et al. 2010) are maintained by a high level of gene flow along 3,000 km. Although several stages of a marine species life cycle are free-moving, have high dispersal capabilities and promote gene flow between populations, many factors including larval behavior, geographic barrier and geographic distance can limit gene flow between populations (Riginos & Nachman 2001; Schmidt & Rand 1999). In this study, *V. litterata* could not maintain gene flow between the Andaman Sea and the upper Gulf of Thailand, whereas the distance between these regions is approximately 3,000 km. This result could be caused by the behavior of *V. litterata* in the last larval stage where this species generally returned to the parental habitats (Ryan & Choy 1990). This larva behavior may disrupt the gene flow of the *V. litterata* population since there is a long geographic distance between the Andaman sea and the upper Gulf of Thailand. In addition, the geographic barrier (Malaysian Peninsula) could be limiting the gene flow of the *V. litterata* population between the Andaman sea and the upper Gulf of Thailand. The *V. litterata* living in the Gulf of Thailand is a single population. Gene flow between the lower and upper Gulf of Thailand was plausibly maintained by many factors; for example, a high dispersal ability in the larval phase (Ryan & Choy 1990), a short geographic distance and a current circulation in the Gulf of Thailand, which is a clockwise gyre during the Southwest monsoon and a counter clockwise gyre in the Northeast monsoon period (Nakthon 1992).

Surprisingly, genetic differentiation between the populations of *V. litterata* inhabiting along the coast of the Andaman Sea and the lower Gulf of Thailand was not found even though these two regions are separated by a geographic barrier. The present geographic boundary (part of the southern continent from Thailand to Malaysia peninsula) seems not to effectively prevent gene flow between the two populations. Genetic similarity of *V. litterata* between the Andaman Sea and the lower Gulf of Thailand population could be explained by a sea surface current. Wind direction generated by the northeast monsoon forced the sea surface current from the China Sea southward to the lower Gulf of Thailand, then westward passing the Malacca strait and into the Andaman Sea (Snidvong & Sojisuporn 1999). Thus, this wind direction facilitated dispersal of larvae from the lower Gulf of Thailand to the Andaman Sea. The larva can migrate via the Malacca strait by stepping stone mechanisms along feeding grounds (Thia-Eng et al. 2000). In Southern Thailand, intensive shrimp farming use sea water from the Andaman Sea for aquaculture. The transportation of sea water from the Andaman Sea to the lower Gulf of Thailand for shrimp culture mixed with planktonic larva and interbreeding with native populations was responsible for such genetic similarity between the Andaman Sea and the lower Gulf of Thailand. A similar result was obtained in black tiger shrimp (*P. monodon*) (Supungul et al. 2000; Tassanakajon

et al. 1998) whereby a population from the Andaman sea did not show significant differentiation from the lower Gulf of Thailand population. In summary, genetic exchange of *V. litterata* among populations inhabiting along the coast of Thailand can be described by the stepping stone model (Kimura & Weiss 1964). Under this model, the genetic exchange of *V. litterata* happens exclusively between adjacent demes (upper Gulf of Thailand to lower Gulf of Thailand to Andaman sea). Thus the connection between populations is proportional to the geographic distance between them (Palumbi 2003).

DEMOGRAPHIC HISTORY

Both Tajima's *D* and Fu's *F*s statistics of the pooled population were negative and statistically significant deviation from a neutral state. In the case of Tajima's *D* test, the statistically significant negative value means that either purifying selection or population expansion determines the genetic variation of the population of interest (Yang 2006). The statistically significant negative value of Fu's *Fs* statistics suggested population expansion because this test is a powerful statistical test for detecting demographic expansion, especially from non-recombinant genetic data (Ramirez-Soriano et al. 2008). In addition, the estimated value of θ_1 was higher than θ_0 in every population supporting the demographic expansion. However, the results of the mismatch distribution were not supported by the goodness-of-fit test showing that the sudden expansion model could not well fit to the demographic history of the *V. litterata* population.

In this study, we estimated that the population expansion of *V. litterata* occurred around 211,428 years ago. The estimated expansion spans the Pleistocene glaciations period, coincides with the drastic climate changes occurred in the same period. During the Pleistocene period (2,588,000 to 11,700 years ago), glacial buildup on land resulted in significant decreases in sea levels that profoundly altered coastal environments (Gradstein et al. 2004). Sea levels fell from near modern levels to 130 m below present as a result of the buildup of glaciers on land during the Pleistocene (Voris 2000). These changes in sea levels resulted in a progradation of *V. litterata* habitat across the bays (Rhodes et al. 2011; Tanabe et al. 2003). Therefore, populations of *V. litterata* possibly expanded concomitantly with habitat expansion. The estimated time of the population expansion obtained in this study supported the theory that diversification of marine species in Southeast Asia had been generated by environmental factors 2.4 million to 10,000 years ago (McMillan & Palumbi 1995; Panithanarak et al. 2010). Changes in sea levels during the Pleistocene and Miocene periods, after approximately 1.81 mya (Gradstein et al. 2004), affected the diversification of *V. litterata* on the Thailand coast and also diversification of other species: for example, other marine species and freshwater decapod species living along the Atlantic-Mediterranean transition area (Garcia-Merchan et al. 2012), Mud crab, *S. paramamosain*, living along the Chinese coasts of the South China and East China Seas (He et al. 2010).

IMPLICATIONS FOR MANAGEMENT OF *V. litterata*

The obtained genetic variability and population genetics structures of *V. litterata* obtained in this study might facilitate fishery management, development of captive breeding and the restocking programs for aquaculture and conservation. In this study, the results showed that population differentiations occurred between Andaman Sea and the Gulf of Thailand. Thus *V. litterata* population along the Thailand coast should not be treated as a single management unit. Because different populations carrying different genetic structure should be managed as distinct units, the natural resource management of the *V. litterata* population living in the Andaman sea should be separated from that of the population inhabiting along the coast of Gulf of Thailand. The comparison results showed the crab samples collected from Phang Nga, Pattani and Rayong carried the highest level of genetic variations among the sites located in the Andaman sea coast, lower and upper Gulf of Thailand coast, respectively; therefore, the populations of these sites should be preserved as the base-line stocks for natural resource management. A genetic-based stock enhancement program should be implemented to resolve problems of overexploitation, as illustrated by an increasing proportion of small sizes of captured *V. litterata* and to maintain the genetic diversity of *V. litterata*.

CONCLUSION

In this study, 182 nucleotide sequences of the mtDNA in cytochrome oxidase subunits I gene with a size of 525 bp were analyzed to determine genetic variation and historical demography of *V. litterata* living along the coast of Thailand. An abundance of rare haplotypes indicated that the female effective population size of *V. litterata* living along the coast of Thailand is large. The results of the demographic history analysis indicated that the *V. litterata* population had experienced population expansion. The AMOVA analysis result presented the genetic structure of *V. litterata* found in 2 populations: The Andaman Sea population and the Gulf of Thailand population. This genetic structure corresponded well to the geographic barrier. The pattern of genetic structure of *V. litterata* could be well explained by a stepping stone model. These results should provide the necessary information for constructing effective sustainable management strategies that will help recolonize this species across the Thailand coast and reduce overexploitation of this species in this area.

ACKNOWLEDGEMENTS

The authors wish to thank Rajamangala University of Technology Srivijaya, Nakhon Si Thammarat, Thailand for their financial support and Christopher J. Forti for proof reading and editing this paper.

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Received: 7 August 2016
Accepted: 22 May 2017