Population Genetic Structure of the Wedge Clam (*Donax scortum*) along the Andaman Sea Coast of Thailand

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ABSTRACT

The wedge clam (*Donax scortum*) is an important commercial species in Thailand. In our study, we performed an analysis of the genetic structure of the wedge clam population in the Andaman Sea along the coast of Thailand to provide information for developing a management plan. Partial sequences of the mitochondrial DNA cytochrome oxidase subunit I gene (mtDNA *COI*) with a size of 426 base pairs were investigated in 115 individuals, collected from four sampling sites within fishing grounds along the Andaman Sea coast of Thailand. Thirty-two haplotypes were observed. Haplotype diversity and nucleotide diversity were 0.887 and 0.00541, respectively. The population genetic analysis shows a lack of genetic structure, possibly caused by a high level of gene flow due to the high dispersal ability of wedge clam larvae. Demographic history tests reveal that the wedge clam population experienced expansion approximately 1,000 years ago. The results of our study can be used to guide the management of the wedge clam in this part of the Andaman Sea to maintain genetic diversity.

Keywords: *Donax scortum*, Genetic diversity, Mitochondrial DNA, Thailand

INTRODUCTION

The wedge clam (*Donax scortum*) is a marine bivalve found along coastal areas in shallow subtidal waters. It lives in habitats that are characterized by compacted fine sand mixed with mud (Singh *et al*., 2012; Singh, 2017) and is widely distributed in the Indo-West Pacific region (Poutiers, 1998). It is popularly consumed in many countries in Southeast Asia (Singh, 2017). In Thailand, it is one of the economically important clams harvested along the Andaman Sea coast (Tanyaros, 2010). In the past several years, the number of wedge clams in natural populations has decreased, due mainly to overfishing, so implementation of an effective management strategy is necessary (Pengsakun *et al*., 2017).

Population genetic structure is the study of patterns of genetic diversity among subpopulations (Reed and Frankham, 2003). Genetic diversity is maintained by gene flow. Measurement of genetic diversity is necessary for planning the conservation of living organisms because it indicates the fitness of the population (Garner *et al*., 2005). In Thailand, the wedge clam is found along the coast of the Andaman Sea from Satun Province to Krabi Province, a distance of 500 km (Jitpukdee *et al*., 2015).

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This area is the main fishing ground for local fishermen. The areas with appropriate habitat for the wedge clam are separated by some distance (Aungtonya *et al*., 2000), which may generate genetic structure within the wedge clam population; however, genetic information on this species has not been previously reported. The genetic diversity of other marine animals in this area, such as violet vinegar crab (*Episesarma versicolor*) (Supmee *et al*., 2012), cobia (*Rachycentron canadum*) (Phinchongsakuldit *et al*., 2013), and oceanic paddle crab (*Varuna litterata*) (Suppapan *et al*., 2017) has been reported. In our study, we hypothesized that the distance separating individual fishing grounds, i.e., wedge clam habitat, along the Andaman Sea coast of Thailand generates genetically distinct subpopulations within the wedge clam population. We assess both population genetic structure and demographic history of the wedge clam in the Andaman Sea.

Mitochondrial DNA (mtDNA) has been extensively used to study the population genetic structure of various metazoan species (Guo *et al*., 2012) due to its rapid evolutionary rate, lack of recombination, and the fact that it is maternally inherited (Avise, 2000). In the past decade, the nucleotide sequence of mtDNA in the cytochrome oxidase subunit I gene (mtDNA *COI*) has been one of the most frequently used to examine population genetic structure (Li *et al*., 2016). Due to the mtDNA *COI* sequence being highly variable, easily retrieved from the genetic database, and suitable for systematic analysis, it is appropriate for the study of population genetic structure in animals (Feng *et al*., 2011). The nucleotide sequence from mtDNA *COI* has been used to study the genetic variation in other mollusks such as slipper limpet (*Crepipatella dilatata*) (Brante *et al*., 2012), pen shell (*Atrina pectinata*) (Xue *et al*., 2014), and Chinese freshwater snail (*Bellamya aeruginosa*) (Gu *et al*., 2015). In our study, genetic variation of this species was observed in a partial sequence of the mtDNA *COI* gene. The results of this study provide information to guide the management of wedge clams in the Andaman Sea to maintain their genetic diversity.

MATERIALS AND METHODS

Sample collection

One hundred fifteen (115) wedge clams were collected from four localities within fishing grounds in Thai waters along the Andaman coast, including Bo Jed Luk in Satun Province, Had Samran in Trang, Pakmeng in Trang, and Khao Thong in Krabi (Figure 1). Fresh samples were stored on ice immediately, transferred to the laboratory, and preserved at -20 °C for subsequent DNA extraction.

DNA extraction, PCR amplification and nucleotide sequencing

Total genomic DNA was extracted from muscle tissue using the Genomic DNA Extraction Kit from Tiangen Biotech (China) following the manufacturer's protocol. The primer pair, DS_COI_H1: GTA ACG TCT CAC GGG TTG TT 3' and DS_COI_L1: 5' CCA CCT CCA ACA GGA TCA A 3', was designed using the Primer 3 program (Untergasser *et al*., 2012) and used to amplify a fragment of the nucleotide sequence from the mtDNA *COI* gene. Polymerase chain reaction (PCR) was conducted in a 50 µL reaction mixture containing 5 µL of 10X *Taq* buffer, 5 µL of 25 mM $MgCl_2$, 4 µL of 2 mM dNTPs mix, 2 µL of 10 mM each primer, 0.5 µL of 2.5 unit *Taq* DNA polymerase (Thermo Scientific, USA), 26.5 µL of ddH_2O and 5 µL of total genomic DNA (50-100 ng). PCR was performed in a thermocycler (Major Cycler, Cycler, Taiwan) under the following conditions: initialization at 94 °C for 4 min, 35 cycles of; denaturation at 94 °C for 40 sec, annealing at 55 °C for 1 min, extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. The correct size of PCR products was checked on 1% agarose gel (1×TAE) for 40 min at 100 V, stained with ethidium bromide, and visualized under UV light. The amplification product was purified using a DNA product purification kit (Tiangen Biotech, China) and sequenced by 1ST Base Laboratory (Selangor, Malaysia).

Data analysis and genetic diversity

DNA sequences were checked against entries archived in the National Center for Biotechnology Information database and then edited. Multiple sequences were aligned using ClustalW ver. 1.83 (Thompson *et al*., 1994). Standard indices of genetic diversity, including polymorphic sites, nucleotide diversity (π) (Nei and Tajima, 1981), and haplotype diversity (h) (Nei, 1987), were estimated using DnaSP version 6.00 (Rozas *et al*., 2017). Since mtDNA is maternally inherited, the female effective population size (N_{ef}) was calculated according to the equation: $N_{ef} = \theta/2\mu m$ (Zhang *et al*., 2003), where the estimator of the mutation parameter theta (θ) is obtained from the number of polymorphic sites as implemented in ARLEQUIN ver. 3.5 (Excoffier and Lischer, 2010), μ is the mutation rate per nucleotide sequence per generation (Tajima, 1996), and m is the DNA sequence length. In our study, we assumed a widely accepted mutation rate of 1 % per site per million years for the mtDNA *COI* (Weigelt *et al*., 2017).

Population genetic structure

The population genetic structure of the wedge clam was analyzed. The analysis of molecular variance (AMOVA) was performed with ARLEQUIN ver. 3.5 (Excoffier and Lischer, 2010). The significance of the Φ-statistic was tested using 10,000 permutations. Genetic distance between pairs of populations (pairwise F_{ST}) was estimated. The significance of the pairwise differentiation metrics was tested with 10,000 permutations. Phylogenetic analysis was conducted to examine the relationships among haplotypes of the wedge clam. The neighbor-joining (NJ) method (Saitou and Nei, 1987) is based on the matrix of Kimura 2-parameter distances as implemented in MEGA version 7.0 (Kumar *et al*., 2016) using 1,000 bootstrapping replicates to reconstruct phylogenetic relationships among haplotypes.

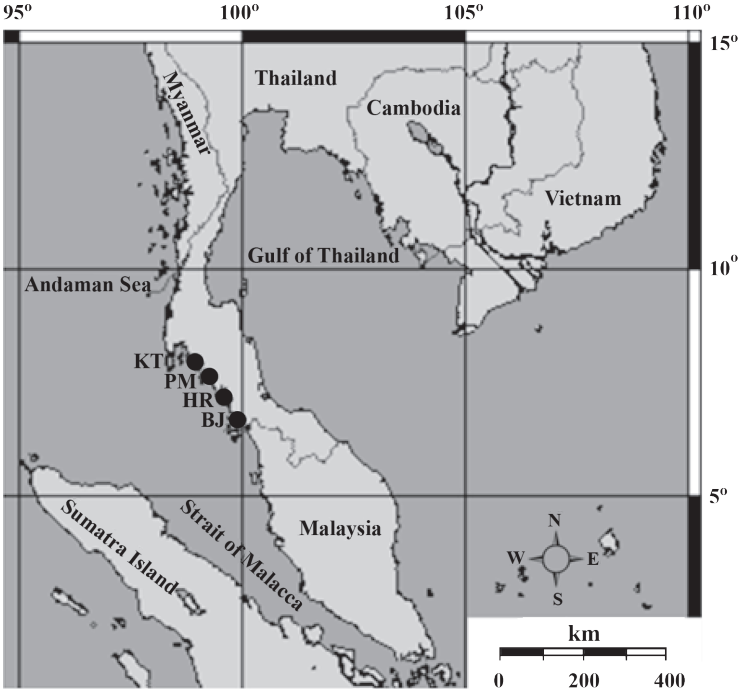

Figure 1. Sample collection sites for the wedge clam along the Andaman Sea coast of Thailand. (BJ = Bo Jed Luk, HR = Had Samran, PM = Pakmeng, KT = Khao Thong)

Demographic history analysis

Four different analyses were used to assess the historical demography of the wedge clam. First, selective neutrality was tested using Tajima's *D* (Tajima, 1989) and Fu's *Fs* (Fu, 1997) statistics based on 10,000 replicates. Second, mismatch distributions under the sudden expansion model were examined. The fit between the observed and expected mismatch distributions was tested with the raggedness index (Harpending, 1994) and the sum of squared deviations (*SSD*) of the goodness-of-fit test (Schneider and Excoffier, 1999), implemented in ARLEQUIN v. 3.5 (Excoffier and Lischer, 2010) using 10,000 bootstrap replicates. The mutation parameters, including the population size before expansion ($\theta_0 = 2N_0\mu$) and the population size after expansion ($\theta_1 = 2N_1\mu$) (Rogers, 1995) of the mismatch distribution, were calculated (Rogers and Harpending, 1992). Third, a minimum spanning network (MSN) based on the mean number of pairwise differences among haplotypes of the mtDNA *COI* region was constructed using ARLEQUIN v. 3.5 (Excoffier and Lischer, 2010). Finally, the change of the effective population size (*Ne*) over time was evaluated by Bayesian skyline analysis using BEAST/BEAUTi ver. 1.7.2 (Drummond *et al*., 2012). Based on the evolutionary rate of mtDNA *COI* in bivalves, the mutation rate was adjusted to 1.0 % per million years (Weigelt *et al*., 2017). The analysis was implemented with 10 million steps in a Markov chain Monte Carlo (MCMC) simulation with a relaxed molecular clock model. The result was generated by Tracer ver. 1.6 (Rambaut *et al*., 2014).

RESULTS

Genetic diversity

The alignment of the mtDNA COI partial sequence results showed 426 aligned sites; 29 sites were polymorphic, defining 32 haplotypes. Of the 29 polymorphic sites, 12 were singleton sites, and 17 were parsimonious informative sites. The nucleotide sequences of 32 haplotypes from this study were deposited in GenBank with accession numbers MW177867- MW177873, MW177876, MW177879, MW177880-MW177885, MW177887-MW177895, MW177936, MW177938-MW177943, and MW177945. Haplotype diversity and nucleotide diversity of the total population were 0.887±0.017 and 0.00541±0.00032, respectively (Table 1). The number of polymorphic sites, number of haplotypes, haplotype diversity (*h*), and nucleotide diversity (π) in each of the four samples (i.e., four collection sites) are presented in Table 1. Thirty-two haplotypes were identified. Eleven haplotypes (H01, H02, H03, H04, H05, H06, H07, H08, H09, H12, H19) were shared among samples, two haplotypes (H22, H27) were shared among individuals within one sample, and 19 haplotypes (H10, H11, H13, H14, H15, H16, H17, H18, H20, H21, H23, H24, H25, H26, H28, H29, H30, H31, H32) were unique haplotypes (Table 2). Twenty-one haplotypes were found only in a single sample, i.e., "private" haplotypes (Table 2). All samples had private haplotypes. The KT sample possessed eight private haplotypes, followed by the BJ, HR, and PM samples, with five, four, and two private haplotypes, respectively (Table 2). The female effective population size was 640,023.

Table 1. Sample collection sites, sample code, number of individuals per sample (*N*), and genetic diversity indices for wedge clams estimated using mtDNA *COI* sequences.

Collecting site	Code	*N*	No. polymorphic sites	No. haplotypes	Haplotype diversity (*h*) (mean±SD)	Nucleotide diversity (π) (mean±SD)
Bo Jed Luk	BJ	31	15	15	0.901±0.035	0.00553±0.00881
Had Samran	HR	28	9	10	0.810±0.059	0.00401±0.00051
Pakmeng	PM	24	15	12	0.895±0.045	0.00612±0.00067
Khao Thong	KT	32	20	18	0.921±0.034	0.00600±0.00066
Total		**115**	**29**	**32**	**0.887±0.017**	**0.00541±0.00032**

Table 2. Mitochondrial *COI* haplotype distributions for wedge clams from four localities along the Andaman Sea coast of Thailand.

Haplotype	BJ	HR	PM	KT	Total
H01	2	-	1	-	3
H02	1	1	-	-	2
H03	1	-	1	1	3
H04	8	5	3	8	24
H05	1	-	-	1	2
H06	5	11	7	3	26
H07	1	1	1	1	4
H08	4	4	2	4	14
H09	2	-	3	2	7
H10	1	-	-	-	1
H11	1	-	-	-	1
H12	1	-	1	1	3
H13	1	-	-	-	1
H14	1	-	-	-	1
H15	1	-	-	-	1
H16	-	1	-	-	1
H17	-	1	-	-	1
H18	-	1	-	-	1
H19	-	2	1	1	4
H20	-	1	-	-	1
H21	-	-	1	-	1
H22	-	-	2	-	2
H23	-	-	1	-	1
H24	-	-	-	1	1
H25	-	-	-	1	1
H26	-	-	-	1	1
H27	-	-	-	2	2
H28	-	-	-	1	1
H29	-	-	-	1	1
H30	-	-	-	1	1
H31	-	-	-	1	1
H32	-	-	-	1	1
Total	**31**	**28**	**24**	**32**	**115**

Note: BJ = Bo Jed Luk, HR = Had Samran, PM = Pakmeng, KT = Khao Thong

Population genetic structure

The population genetic structure of wedge clams collected from the Andaman Sea coast of Thailand was determined. Based on the analysis of molecular variance (Table 3), the *F*-statistic was not statistically significant (Φ_{ST} = -0.00009, p = 0.426), indicating a lack of genetically distinct subpopulations in the study area. Further, pairwise F_{ST} values (Table 4) showed no significant differences for any sample combination, confirming a lack of genetic structure. Also, the neighbor-joining tree showed no distinct lineages of haplotypes, supporting the inference that the wedge clam population along the Andaman Sea coast of Thailand is not genetically structured (Figure 2).

Table 3. Analysis of molecular variance (AMOVA) for wedge clams sampled at four sites along the Andaman Sea coast of Thailand based on mtDNA *COI* sequences.

Source of variation	df	Sum of squares	Variance components	Percentage of variation	p-value
Among samples	3	3.451	-0.0001Va	-0.01	Φ_{ST} = -0.00009 (p = 0.426)
Within samples	111	128.010	1.15324Vb	100.01	
Total	114	131.461	1.15314		

Note: p values in parentheses

Table 4. Population pairwise F_{ST} values between wedge clam samples from four sites along the Andaman Sea coast of Thailand based on mtDNA *COI* sequences.

	BJ	HR	PM	KT
BJ	-			
HR	0.01132 (0.21186)	-		
PM	-0.02573 (0.97565)	0.01268 (0.20661)	-	
KT	-0.01632 (0.90743)	0.03840 (0.05099)	-0.01634 (0.84566)	-

Note: p values in parentheses; BJ = Bo Jed Luk, HR = Had Samran, PM = Pakmeng, KT = Khao Thong

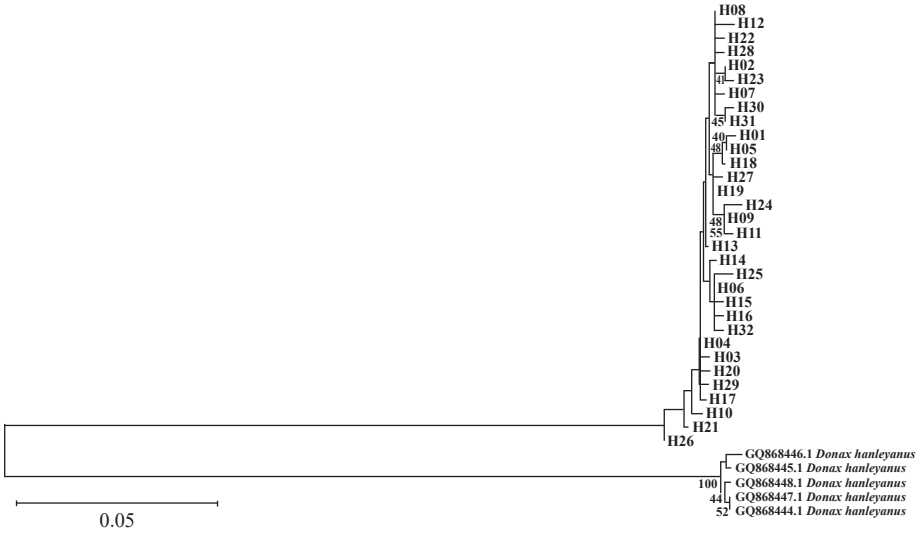

Figure 2. Neighbor-joining phylogenetic tree constructed under the Kimura 2-parameter model based on 32 mtDNA *COI* haplotypes of wedge clam, with *Donax hanleyanus* as the outgroup. Bootstrap values are shown to the left of nodes.

Demographic history

The historical demography analysis of the wedge clam population from the Andaman Sea coast of Thailand was examined. In the results of neutrality tests (Table 5), Tajima's *D* statistic was significantly negative for the total population (*D* = -1.72180, p = 0.01070) and Fu's *Fs* statistic showed a significant negative value (*Fs* = -25.76639, p = 0.00000). According to the goodness-of-fit test, the mismatch distribution observed for the total population best matched a sudden expansion model (*SSD* = 0.00302, p = 0.28640), and the raggedness indices were not significant (*rg* = 0.03081, p = 0.58070) (Table 5). The mismatch distribution (Figure 3) was unimodal and fit well with a population expansion model. The estimated θ_1 (31.64000) was higher than θ_0 (0.02109) for the total population, indicating that the wedge clam population had expanded (Table 5). The topology of the mitochondrial haplotype network (Figure 4) showed a star-like configuration and did not

Table 5. Parameter indices for the neutrality test and mismatch distribution analysis of the wedge clam based on mtDNA *COI* sequences.

Collecting site	Tajima's *D*	Fu's *Fs*	*SSD*	Rag	θ_0	θ_1
BJ	-1.24556	-8.43751	0.00464	0.03730	0.00000	325.00000
	(0.09710)	(0.00000)	(0.32380)	(0.55800)		
HR	-0.82426	-4.12776	0.00146	0.03043	0.00352	12.50547
	(0.22600)	(0.00730)	(0.83480)	(0.87960)		
PM	-1.24191	-4.97384	0.01349	0.04756	0.00000	33.08594
	(0.09300)	(0.00450)	(0.19160)	(0.36330)		
KT	-1.67009	-12.52458	0.00443	0.04698	0.00000	99999.00000
	(0.02480)	(0.00000)	(0.34230)	(0.32050)		
Total	**-1.72180**	**-25.76639**	**0.00302**	**0.03081**	**0.02109**	**31.64000**
	(0.01070)	**(0.00000)**	**(0.28640)**	**(0.58070)**		

Note: p values in parentheses, *SSD* = sum of squared deviations; Rag = raggedness index; θ_0 = population size before expansion ($\theta_0 = 2N_0\mu$); θ_1 = population size after expansion ($\theta_1 = 2N_1\mu$); BJ = Bo Jed Luk; HR = Had Samran; PM = Pakmeng; KT = Khao Thong

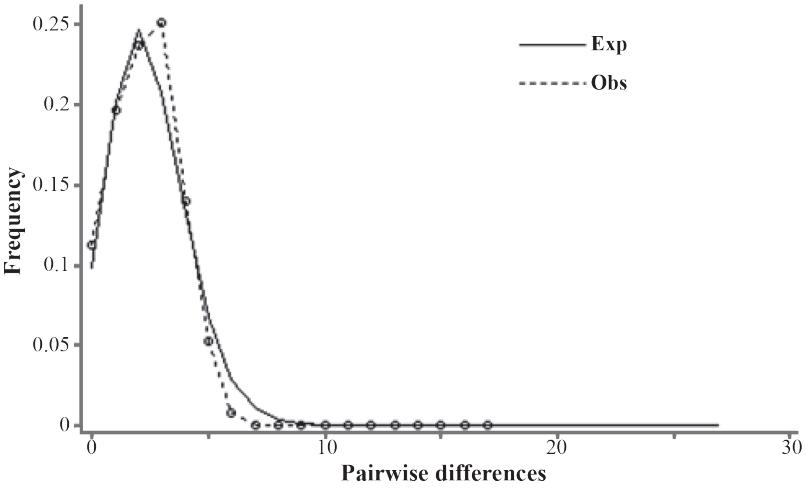

Figure 3. DNA sequence mismatch distribution under a sudden population expansion model for the wedge clam. The solid line represents the expected mismatch distribution, and the dotted line represents the observed pairwise differences.

indicate any distinct pattern of phylogeographic structure. The most common haplotype of the network was H04, which was observed in all populations and was connected to other haplotypes by relatively few mutation steps. The Bayesian skyline analysis (Figure 5) revealed that the expansion of the wedge clam population occurred around 1,000 years ago.

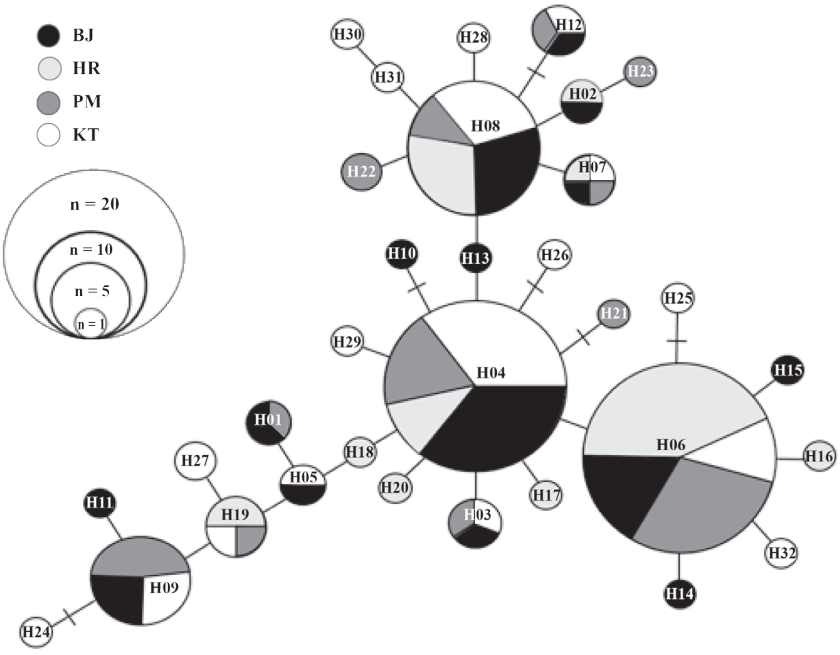

Figure 4. Minimum spanning network based on 32 mtDNA *COI* haplotypes of wedge clam. Circles represent haplotypes, and the proportion size is the observed frequency. Colors within circles represent the four collecting sites. Single lines directly connecting haplotypes indicates separation by one mutation step. A bar crossing a connecting line indicates one additional mutation step.

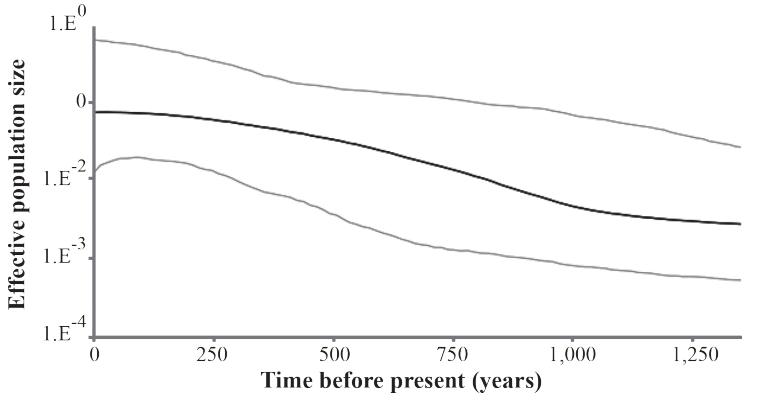

Figure 5. Bayesian skyline plot showing the inferred historical demography of the wedge clam along the Andaman Sea coast of Thailand. The black line is the median estimation, and the grey lines indicate the 95 % confidence interval.

DISCUSSION

Genetic diversity

Among 115 wedge clam individuals collected from four sites in the present study, we found numerous private mitochondrial *COI* sequence haplotypes. The observation of many private haplotypes in this study suggests the existence of a large female effective population size (William and Allendorf, 2007) for the wedge clam in the study area. The large effective female population size indicates that good potential exists for management of the wedge clam along the Andaman Sea coast in the future. Haplotype diversity was relatively high, whereas nucleotide diversity was relatively low in all samples. This pattern indicates that the wedge clam has undergone a relatively recent population expansion. This genetic character can be generated by an accumulation of new mutations, maintaining high haplotype diversity in a rapidly expanding population (Watterson, 1984; Ma *et al*., 2010). Molecular genomic characterization of mtDNA in our study is consistent with other reports for several marine invertebrate species in the Andaman Sea, such as violet vinegar crab (*Episesarma versicolor*) (Supmee *et al*., 2012), oceanic paddle crab (*Varuna litterata*) (Suppapan *et al*., 2017) and Asiatic hard clam (*Meretrix meretrix*) (Supmee *et al*., 2020). Other results from our study such as the star-shaped haplotype networks support a rapid population expansion of the wedge clam population along the Andaman Sea, and the skyline plot shows that the expansion occurred over the past 1,000 years.

Population genetic structure

Our genetic structure analysis reveals that wedge clam samples from the four sites do not represent distinct subpopulations. There are many factors that can maintain genetic homogeneity between populations, such as high larval dispersal ability, hydrographic variation, and a lack of geographic barriers. Many marine animals release gametes or planktonic larvae into open waters, which migrate with the current, promoting gene flow among populations (Russo *et al*., 1994; Uthicke and Benzie, 2003). In some mollusks, dispersal distance of planktonic larva can be more than 1,000 km, such as in *Strombus* species (Davis *et al*., 1993), and the black ark (*Anadara tuberculosa*) (Diringer *et al*., 2019); the wedge clam habitat along the Andaman Sea coast of Thailand spans 500 km. Further, the Andaman coast does not have any geographic barriers, and water circulation flows northward in the spawning season of the wedge clam (Aungtonya *et al*., 2000; Singh, 2017). These factors promote gene flow and maintain genetic homogeneity among populations in the study area. A lack of population genetic structure caused by a high level of gene flow has been reported for other marine animals along the Andaman coast of Thailand, such as violet vinegar crab (*Episesarma versicolor*) (Supmee *et al*., 2012), cobia (*Rachycentron canadum*) (Phinchongsakuldit *et al*., 2013), Indian mackerel (*Rastrelliger kanagurta*) (Munpholsri *et al*., 2013), and oceanic paddle crab (*Varuna litterata*) (Suppapan *et al*., 2017). Based on the results of our study, we suggest that the wedge clam population in the Andaman Sea of Thailand should be managed as a single fishery unit, with stock assessment and any harvest limits set for the entire area.

Demographic history

All of our independent analyses of demographic history reveal that the wedge clam population in the study area experienced a rapid population expansion during the past 1,000 years. First, from the neutrality test, Tajima's *D* and Fu's *Fs* were negative and significantly deviating from the neutral state, indicating that the wedge clam might have undergone purifying selection or a population expansion (Yang, 2006). Also, Fu's *Fs* test, the test statistic for detecting population expansion in haplotype data was negative, showing a population expansion (Ramirez-Soriano *et al*., 2008). Second, DNA sequence mismatch analysis was consistent with a sudden expansion model, and a goodness-of fit-test fit the expected mismatch distribution well, indicating that the wedge clam has undergone population expansion. Furthermore, the population size after expansion (θ_1) was higher than the population size before expansion (θ_0), indicating a demographic expansion. Third, the topology of the mitochondrial network was star-like and did not show any distinct pattern of

phylogeographic structure, concordant with the phylogenetic tree results showing no distinct lineages. In the haplotype network, the common haplotype was connected to other haplotypes directly in a few mutational steps, indicating that the wedge clam has a short demographic history. Finally, the expansion time was confirmed with the Bayesian skyline plot analysis, whose results indicate that the wedge clam population expansion along the Andaman Sea coast of Thailand occurred around 1,000 years ago during the Holocene epoch. Coastal evolution in the Thai-Malay peninsula has been attributed to sea-level change (Culver *et al*., 2015). The high point of sea level occurred around 6,000 years ago and fell continuously until it became stable around 1,000 years ago, leading to the formation of the current coastal marine habitat (Rhodes *et al*., 2011; Surakiatchai *et al*., 2018). Thus, the wedge clam populations possibly expanded with habitat formation from changes in sea level during the Holocene epoch, which limited the time for diversification of the wedge clam living in the Andaman Sea. The effect of the sea-level fall in the Holocene epoch has been reported in many marine species, such as violet vinegar crab (*Episesarma versicolor*) in the Andaman Sea coast of Thailand (Supmee *et al*., 2012), a crustacean in the family Kalliapseudidae (*Mesokalliapseudes macsweenyi*) in the northwestern Atlantic Ocean and Gulf of Mexico (Drumm and Kreiser, 2012), and the black ark (*Anadara tuberculosa*) in the East Pacific (Diringer *et al*., 2019).

CONCLUSION

In our study, 115 mtDNA *COI* nucleotide sequences with a size of 426 base pairs were analyzed to determine the genetic structure and demographic history of the wedge clam in the Andaman Sea along the coast of Thailand. Results of multiple genetic structure analyses indicate that there is a single wedge clam population in this area. Results of demographic history tests show that the wedge clam population experienced expansion around 1,000 years ago during the Holocene epoch. This study provides essential information for developing sustainable management strategies to maintain the genetic diversity of the wedge clam population in this part of Thailand. To gain more robust information and additional insights on fine-scale genetic differentiation, we suggest that nuclear DNA markers such as microsatellites or SNPs should be used in further analyses.

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