Population genetic structure of Asiatic Hard Clam (*Meretrix meretrix*) in Thailand based on Cytochrome Oxidase subunit I gene sequence

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Abstract. Supmee V, Sangthong P, Songrak A, Suppapan J. 2020. Population genetic structure of Asiatic Hard Clam (*Meretrix meretrix*) in Thailand based on Cytochrome Oxidase subunit I gene sequence. *Biodiversitas* 21: 2702-2709. The Asiatic hard clam (*Meretrix meretrix*) is an important commercial clam in Thailand. Last decade, this species had a dramatic decrease. Thus, to conserve this species, genetic information is essential. In our study, 135 samples of *M. meretrix* were collected from 7 sampling sites along the Thailand coast. The genetic structure was examined based on the variation of the nucleotide sequence in cytochrome oxidase subunit I gene. Twenty haplotypes were identified. Numerous rare haplotypes were revealed indicating the existence of a large female effective population size. In the northern Gulf of Thailand population, the genetic diversity was low. The neutrality test and minimum spanning network showed experienced expansion events of *M. meretrix* in Thailand. The genetic structure showed that the *M. meretrix* population was separated into the Gulf of Thailand and the Andaman population. This finding was possibly caused by a disruption of gene flow from the Thailand-Malay Peninsula and larval behavior. Our finding suggested that the construction management program to maintain the genetic diversity of this species could be separated into 2 conservation units.

Keywords: Mitochondrial DNA, genetic diversity, cytochrome oxidase subunit I, Asiatic hard clam, Thailand

INTRODUCTION

The Asiatic hard clam (*Meretrix meretrix*) is bivalve and widely distributed in the Indo-West Pacific. It is found in open coasts with more sand and less muddy bottom (Sienes et al. 2018). It is an economic bivalve in both the Gulf of Thailand and the Andaman Sea coast of Thailand (Fishery Statistics Analysis and Research Group 2017). Nowadays, the amount of *M. meretrix* in Thailand is decreasing because it is easy to catch in nature and fishermen fished it in various sizes without considering its sustainability. Further, the demand for *M. meretrix* consumption in Thailand is increasing, tends to overexploitation, and affects natural resource sustainability (Fishery Statistics Analysis and Research Group 2017).

The population genetic structure describes the genetic diversity pattern in the subpopulation. Usually, the marine species in subpopulation will breed within the group, and there may be some crossbreeding among subpopulations. Mating across populations creates opportunities for gene flow between sub-populations. If there is a large gene flow, the population will be slightly different and a low gene flow the population will be more different. In the marine species, the genetic structure is shaped by many factors, including geographical barriers, gene flow, population size, life history, and bottlenecks effect (Gilleard and Redman 2016). Typically, mitochondrial DNA is exclusively inherited through the maternal line, high copy number, and lack of recombination (Li et al. 2016). In our study, the information of the mitochondrial DNA sequencing on cytochrome oxidase subunit I gene (mtDNA COI) was used to discuss the population genetic structure of the *M. meretrix* in Thailand. The nucleotide sequencing of mtDNA COI has been used for studies population genetic structure of various clams such as *Tridacna crocea* and *T. squamosa* (Neo and Todd 2012), *Atrina pectinata* (Xue et al. 2014), and *M. petechialis* (Zheng et al. 2019). Several population genetic structure studies on *M. meretrix* have been revealed in Vietnam (Trang et al. 2018) and China (Gu et al. 2015) but no studies have been reported from Thailand. The genetic information of the genetic structure in marine species is essential to the management of marine resources, especially when sharing information with biological studies, such as reproduction, behavior, and spawning time. Thus, the genetic information of *M. meretrix* in our study may be used for consideration in maintaining the genetic diversity of the natural stock to achieve the sustainable exploitation of *M. meretrix* in Thailand.
MATERIALS AND METHODS

Sampling sites and DNA extraction

A total of 135 Asiatic hard clams (M. meretrix) were caught from 7 sampling sites along the Thailand coast including Chonburi (CB), Samut Songkhram (SM), Chumphon (CP), Surat Thani (SR), Nakhon Si Thammarat (NS), Songkhla (SK), Satun (ST), and Trang (TG) (Table 1). Fresh samples were preserved on the ice and transported to a laboratory for DNA extraction. Total genomic DNA was isolated from the adductor muscle tissue followed by the manufacturer protocol from the Genomic DNA Extraction Kit (Tiangen BioTech, China).

PCR amplification and nucleotide sequencing

A region of the mtDNA COI gene was amplified by Polymerase chain reaction (PCR). Primer COI_MM_F: 5’ GCA GTA TTA AAA TTT CGA TCT 3’ and COI_MM_F: 5’ GTG GTT TTG GAA ATT GGT TG 3’ were designed for amplifying target DNA. Polymerase chain reaction was conducted in total volume 50 μl consisting 10X Taq buffer 5 μl, 25 mM MgCl₂ 5 μl, 2 mM dNTPs mix 4 μl, 10 μM forward and reverse primers 2 μl each, 2.5 unit Taq DNA polymerase (Thermo SCIENTIFIC, USA) 0.5 μl, DNA template 5 μl (50-100 ng) and ultrapure water 26.5 μl. The polymerase chain reaction was performed in a thermocycler (Major Cycler, CYCLER, TAIWAN). The PCR conditions was followed; step 1-first denaturation at 94 ºC for 4 min; step 2-35 cycles of 94ºC for 40 sec, 51 ºC for 1 min, 72 ºC for 1 min; step 3-final extension at 72 ºC for 10 min. The PCR products were purified using Gel/PCR Purification Mini Kit (Tiangen BioTech, China) and sent to 1ST Base Laboratory, Malaysia for direct sequencing.

Population genetic structure

The population genetic structure of M. meretrix was examined under six assumptions according to the geographic-based structures. Firstly, samples were separated into seven populations regarding the sampling sites (CB, SM, CP, SR, NS, ST, and TG). Secondly, samples were determined according to the geographic region: the Gulf of Thailand (CB, SM, CP, SR, and NS) and the Andaman Sea (ST, and TG). Thirdly, samples were divided according to the three geographic regions: the northern Gulf of Thailand (CB and SM), the southern Gulf of Thailand (CP, SR, and NS) and the Andaman Sea (ST and TG). Fourthly, samples were determined according to the two geographic regions: the northern Gulf of Thailand (CB and SM) and the Andaman Sea (ST and TG). Fifthly, samples were separated according to the two geographic regions: the southern Gulf of Thailand (CP, SR, and NS) and the Andaman Sea (ST and TG). Sixthly, samples were divided according to the two geographic regions: the southern Gulf of Thailand (CP, SR, and NS) and the northern Gulf of Thailand (CB and SM).

An analysis of molecular variance (AMOVA) was used to examine the genetic differentiation within and among putative populations using ARLEQUIN v. 3.5 (Excoffier and Lischer 2010). The Φ-statistic analogs were estimated the different levels (ΦCT; among groups, ΦSC; among populations within groups and ΦST; within populations) and tested by 10,000 permutations (p<0.05). Pairwise FST were estimated genetic distances between all combinations of populations. The significance of the pairwise was determined with 10,000 permutations (p<0.05). The neighbor-joining (NJ) phylogenetic tree the under Kimura 2-parameter model was constructed using MEGA 7.0 (Kumar et al. 2016) to depict the relationships among haplotypes. The statistical of the tree topology robustness was obtained by bootstrapping using 1,000 replicates. A minimum spanning network based on the mean number of pairwise differences among haplotypes of the mtDNA COI was constructed using ARLEQUIN v. 3.5 (Excoffier and Lischer, 2010) and drawn by hand.

RESULTS AND DISCUSSION

Genetic diversity

The length of the mtDNA COI fragment was 446 base pairs and 20 haplotypes were determined. Twenty-one
polymorphic sites were observed, including 13 singleton variable sites and 8 parsimony informative sites. The average nucleotide composition of A, T, G, and C were 43.7%, 20.4%, 14.3%, and 21.6%, respectively. The A/T base content was 64.1% and G/C was 35.9%. The variable sites among 20 mtDNA COI haplotypes were shown in Table 1.

Haplotype diversity was ranged from 0.195±0.115 at SM to 0.693±0.114 at SR. Haplotype diversity (h) of the northern Gulf of Thailand, the southern Gulf of Thailand, the Andaman Sea and the total of Thailand was 0.249±0.093, 0.587±0.075, 0.549±0.066 and 0.507±0.052, respectively (Table 2). Meanwhile, the nucleotide diversity (π) of the northern Gulf of Thailand, the southern Gulf of Thailand, the Andaman Sea and the total of Thailand was 0.00059±0.00023, 0.00318±0.00072, 0.00149±0.00028 and 0.00210±0.00038, respectively. The range of nucleotide diversity was 0.00045±0.00027 at SM to 0.00551±0.00132 at CP (Table 2). The number of samples, number of polymorphic sites, number of haplotypes, haplotype diversity, and nucleotide diversity within either population were shown in Table 2.

Table 1. Variation nucleotide position among 20 mtDNA COI haplotypes of the M. meretrix from the 7 sampling sites along the Thailand coast. All haplotypes are compared with haplotype MM 01. The dot indicates identical nucleotides.

| Haplotype | 1 | 2 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 4 |
|-----------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MM 01     | T | A | C | G | A | A | G | T | G | C | A | A | C | G | G | G | A | G | T | G |   |
| MM 02     | C | T | A | G | C | A |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| MM 03     |   |   | A | G |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| MM 04     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| MM 05     | C | T | A | G | C | A |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| MM 06     | C | G | A | A | G | C | A | A |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| MM 07     | C | G | T | A | G | C | A | A | A | T | T | A | A | A | A | G | A | A | G | C |   |
| MM 08     | C | G | T | A | G | C | A | A | A | T | T | A | A | A | A | G | A | C |   |
| MM 09     | C | G | T | A | G | C | C | A | A | C | T | T | A | A | A | G | A | A |   |
| MM 10     | C | G | T | A | G | C | A | A | A | T | T | A | A | A | A | G | A | C |   |
| MM 11     | C | G | T | A | G | G | A | A | A | T | T | A | A | A | A | G | A | C |   |
| MM 12     | C | G | T | A | G | A | A | T | T | A | A | A | A | A | A | G | A | C |   |
| MM 13     | C | G | T | A | G | C | A | A | T | T | A | A | A | A | A | G | A | C |   |
| MM 14     | C | G | T | A | G | C | A | A | T | T | A | A | A | A | A | G | A | C |   |
| MM 15     | C | G | T | A | G | C | A | A | T | T | A | A | A | A | A | G | A | C |   |
| MM 16     | C | G | T | A | G | C | A | A | T | T | A | A | A | A | A | G | A | C |   |
| MM 17     | C | G | T | A | G | C | A | A | T | T | A | A | A | A | A | G | A | C |   |
| MM 18     | C | G | T | A | G | C | A | A | T | T | A | A | A | A | A | G | A | C |   |
| MM 19     | C | G | T | A | G | C | A | A | T | T | A | A | A | A | A | G | A | C |   |
| MM 20     | C | G | T | A | G | C | A | A | T | T | A | A | A | A | A | G | A | C |   |

Table 2. Collecting localities, number of individuals per sampling site (N), number of polymorphic sites, haplotype diversity (h), nucleotide diversity (π) and neutrality test parameter of M. meretrix along the Thailand coast.

| Collecting localities       | N  | No. polymorphic sites | No. haplotypes | Haplotype diversity (h) (mean±SD) | Nucleotide diversity (π) (mean±SD) | Tajima’s D | Fu’s Fs |
|-----------------------------|----|-----------------------|----------------|-----------------------------------|-----------------------------------|------------|--------|
| Chonburi (CB)               | 18 | 3                     | 4              | 0.314±0.138                       | 0.00075±0.00035                   | -1.71304   | -2.60267|
| Samut Sakhon (SM)           | 20 | 2                     | 3              | 0.195±0.115                       | 0.00045±0.00027                   | -1.51284   | -1.86305|
| Chumphon (CP)               | 19 | 9                     | 4              | 0.684±0.092                       | 0.00551±0.00132                   | 0.24989*   | 1.18703*|
| SuratThani (SR)             | 18 | 7                     | 6              | 0.693±0.114                       | 0.00259±0.00064                   | -1.46445*  | -3.25454*|
| Nakhon Si Thammarat (NS)    | 21 | 5                     | 5              | 0.352±0.131                       | 0.00107±0.00047                   | -1.98137*  | -3.06773*|
| Satun (ST)                  | 20 | 4                     | 4              | 0.595±0.098                       | 0.00176±0.00046                   | -0.088138  | -0.56034|
| Trang (TG)                  | 19 | 3                     | 4              | 0.526±0.089                       | 0.00126±0.00026                   | -0.04521   | -0.03167|
| Northern Gulf of Thailand   | 38 | 5                     | 6              | 0.249±0.093                       | 0.00059±0.00023                   | -0.98642   | -2.14589|
| Southern Gulf of Thailand   | 58 | 15                    | 14             | 0.587±0.075                       | 0.00318±0.00072                   | -1.57892*  | -1.94538*|
| Andaman Sea                 | 39 | 4                     | 4              | 0.549±0.066                       | 0.00149±0.00028                   | -0.56984   | -4.23568|
| Total                       | 135| 21                    | 20             | 0.507±0.052                       | 0.00210±0.00038                   | -1.04977*  | -1.45614*|

Note: *significant differentiation (p<0.05)
Twenty haplotypes were identified. In total, 7 haplotypes were shared; 2 haplotypes were shared within the population (MM 09 and MM 14) and 5 haplotypes were shared among the population (MM 01, MM 02, MM 03, MM 04, and MM 16). Haplotype MM 02 was shared by all populations, while haplotype MM 01, MM 03, and MM 04 were shared only in the Andaman Sea populations. The remaining 13 haplotypes were rare haplotype (MM 05, MM 06, MM 07, MM 08, MM 10, MM 11, MM 12, MM 13, MM 15, MM 17, MM 18, MM 19, MM 20) (Table 3). SR and NS population possessed 4 rare haplotypes, CB and SM population had 2 rare haplotypes, and the CP population possessed 1 rare haplotype (Table 3). Tajima’s D statistic of the total population and within-population in the southern Gulf of Thailand was -1.04977 and -1.57892, and statistically significant. Meanwhile, the population of the northern Gulf of Thailand and the Andaman Sea, the D statistic was -0.98642 and -0.56984, and non-statistically significant (Table 2). Tajima’s D statistic of CP, SR, and NS populations, were statistically significantly negative, while this statistic for the CB, SM, ST, and TG populations was statistically non-significantly negative (Table 2). Fu’s Fs statistics of the total population and within-population in the southern Gulf of Thailand was statistically significant (-1.45614 and -1.94538). In the meantime, the Fs statistic of the population in the northern Gulf of Thailand and the Andaman Sea was -2.14589 and -4.23568, and non-statistically significant (Table 1). Fu’s Fs statistic was statistically significantly negative in CP, SR, and NS populations, and this statistic was statistically non-significantly negative for the CB, SM, ST, and TG populations (Table 2).

Population genetic structure

The AMOVA analysis revealed the genetic structure in 5 putative genetic structure. Firstly, the M. meretrix population along the Thailand coast showed a different statistically significant ($\Phi_{CT}=0.109, p=0.000$). Secondly, the Gulf of Thailand population was significantly different from the Andaman Sea ($\Phi_{CT}=0.058, p=0.036$). Thirdly, the northern Gulf of Thailand, the southern Gulf of Thailand and the Andaman sea showed a different statistically significant ($\Phi_{CT}=0.059, p=0.041$). Fourthly, the northern Gulf of Thailand showed significant differences with the Andaman Sea ($\Phi_{CT}=0.153, p=0.038$). Fifthly, the southern Gulf of Thailand revealed significant differences from the Andaman Sea ($\Phi_{CT}=0.065, p=0.045$). Meanwhile, the $\Phi$-statistic of the putative structure showed no different significant between the northern and southern Gulf of Thailand ($\Phi_{CT}=0.020, p=0.200$) (Table 4). The pairwise $F_{ST}$ showed significant differences among the population between the Gulf of Thailand population and the Andaman Sea population. Every pairwise $F_{ST}$ among sampling populations was shown in Table 5. The neighbor-joining phylogenetic tree showed that the haplotype MM 01, MM 03, and MM 04 was divided into the Andaman Sea population and another haplotype was grouped into the Gulf of Thailand population (Figure 2). The topology of the minimum spanning network showed a star-like network. Haplotype MM 02 was a common haplotype and connected directly with other haplotypes by 1-5 mutation steps. The minimum spanning network was divided into two groups from a distinct pattern of geographic structure among 20 haplotypes. The Gulf of Thailand group consisted of haplotype MM 02, MM 05, MM 06, MM 07, MM 08 MM 09, MM 10, MM 11, MM 12, MM 13, MM 14, MM 15, MM 16, MM 17, MM 18, MM 19, and MM 20. The Andaman Sea group consisted of haplotype MM 01, MM 03, and MM 04. The haplotype of the Gulf of Thailand group and the Andaman Sea group was separated by 5 mutation steps. (Figure 3).

Discussion

Genetic diversity

The genetic information of 20 mtDNA COI haplotypes of M. meretrix in Thailand using the nucleotide sequence of mtDNA COI was analyzed. In our study, the A/T base content was higher than G/C base content, concordant with other reports that mtDNA COI sequence of Meretrix species is an A/T rich sequence such as M. meretrix (Wang et al. 2016), and M. petechialis (Zheng et al. 2019). The A/T rich base composition is a common feature of the mitochondrial genome in animals (Fourdrilis et al. 2018).

Many rare haplotypes were presented indicated that the large female effective population size of the M. meretrix was existence (Croos and Pålsson 2010). Numerous rare haplotypes are the retention of a new mutation and reflect a large female effective population size of the population (Zheng et al. 2019). The genetic information of 20 mtDNA COI haplotypes of M. meretrix in Thailand using the nucleotide sequence of mtDNA COI was analyzed. In our study, the A/T base content was higher than G/C base content, concordant with other reports that mtDNA COI sequence of Meretrix species is an A/T rich sequence such as M. meretrix (Wang et al. 2016), and M. petechialis (Zheng et al. 2019). The A/T rich base composition is a common feature of the mitochondrial genome in animals (Fourdrilis et al. 2018).

Many rare haplotypes were presented indicated that the large female effective population size of the M. meretrix was existence (Croos and Pålsson 2010). Numerous rare haplotypes are the retention of a new mutation and reflect a large female effective population size. High haplotype diversity and low nucleotide diversity were presented in all sampling populations and all geographic regions. This pattern is generated by new mutations accumulation in a sudden population expansion (Chu et al. 2012). This genetic diversity pattern was reported in various clam species including Donax viattus (Fernández-Pérez et al. 2017), M. petechialis (Wang et al. 2017a), and M. meretrix (Trang et al. 2018).

Table 3. Haplotype distributions of M. meretrix from 7 localities along the Thailand coast. Stations codes are given in Table 2.
In Thailand, the lowest nucleotide diversity value was found in the northern Gulf of Thailand population indicating a higher genetic diversity in the population (Ma et al. 2010). The larger nucleotide diversity value indicates a higher genetic diversity in the population (Wang et al. 2017b). Nucleotide diversity can be influenced by many factors including population size, life history, and environmental change (Ma et al. 2010). In this study, the lowest nucleotide diversity value was found in the northern Gulf of Thailand population indicating a low genetic diversity in this region. Last decade, the harvesting of *M. meretrix* in the northern Gulf of Thailand was changed to the machine harvesting, it fished *M. meretrix* in various sizes without considering its sustainability. As a result, the population size of *M. meretrix* in the northern Gulf of Thailand decreased dramatically. Therefore, the overexploitation of the *M. meretrix* in natural resources in the northern Gulf of Thailand population may result in genetic bottleneck.

In the Thailand population, the neutrality test revealed negative and deviated significantly from the neutral population. The statistically significant negative value in Tajima’s *D* test might have been caused by a population expansion (Omori and Wu 2017). Further, Fu’s *F*<sub>S</sub> values of population differentiation are presented in Table 4.

| Source of variation | df | Sum of squares | Variance components | Percentage of variation | *p*-value |
|---------------------|----|----------------|----------------------|-------------------------|-----------|
| **Single region**   |    |                |                      |                         |           |
| Among populations   | 6  | 8,545          | 0.050V<sub>a</sub>   | 10.99                   | Φ<sub>ST</sub> = 0.109*(p=0.000) |
| Within populations  | 128| 53,936         | 0.421V<sub>c</sub>   | 89.01                   |           |
| Total               | 134| 62,481         | 0.473                |                         |           |
| **Gulf of Thailand and Andaman Sea** |    |                |                      |                         |           |
| Among groups        | 1  | 2,752          | 0.028V<sub>a</sub>   | 5.85                    | Φ<sub>CT</sub>=0.058*(p=0.036) |
| Among populations within groups | 5 | 5,794         | 0.038V<sub>b</sub>   | 7.85                    | Φ<sub>SC</sub>=0.083*(p=0.001) |
| Within populations  | 128| 53,936         | 0.421V<sub>c</sub>   | 86.30                   | Φ<sub>ST</sub>=0.137*(p=0.000) |
| Total               | 134| 62,481         | 0.488                |                         |           |
| **Northern Gulf of Thailand, southern Gulf of Thailand, and Andaman Sea** |    |                |                      |                         |           |
| Among groups        | 2  | 4,532          | 0.028V<sub>a</sub>   | 5.96                    | Φ<sub>CT</sub>=0.059*(p=0.041) |
| Among populations within groups | 4 | 4,013         | 0.030V<sub>b</sub>   | 6.29                    | Φ<sub>SC</sub>=0.066*(p=0.020) |
| Within populations  | 128| 53,936         | 0.421V<sub>c</sub>   | 87.75                   | Φ<sub>ST</sub>=0.122*(p=0.000) |
| Total               | 134| 62,481         | 0.480                |                         |           |
| **Southern Gulf of Thailand and Andaman Sea** |    |                |                      |                         |           |
| Among groups        | 1  | 1,737          | 0.041V<sub>a</sub>   | 15.40                   | Φ<sub>CT</sub>=0.153*(p=0.038) |
| Among populations within groups | 2 | 0,248         | -0.005V<sub>b</sub>  | -2.14                   | Φ<sub>SC</sub>=0.025(p=0.723) |
| Within populations  | 73 | 17,236         | 0.236V<sub>c</sub>   | 86.75                   | Φ<sub>ST</sub>=0.132'(p=0.003) |
| Total               | 76 | 19,221         | 0.272                |                         |           |
| **Northern and southern of Gulf of Thailand** |    |                |                      |                         |           |
| Among groups        | 1  | 1,781          | 0.010V<sub>a</sub>   | 2.05                    | Φ<sub>CT</sub>=0.020(p=0.200) |
| Among populations within groups | 3 | 3,900         | 0.044V<sub>b</sub>   | 8.64                    | Φ<sub>SC</sub>=0.088*(p=0.015) |
| Within populations  | 91 | 41,434         | 0.455V<sub>c</sub>   | 89.31                   | Φ<sub>ST</sub>=0.106*(p=0.001) |
| Total               | 95 | 47,115         | 0.509                |                         |           |

Note: *significant differentiation (p<0.05)

| Population | Northern Thailand | Gulf of Thailand | Southern Gulf of Thailand | Andaman Sea |
|------------|-------------------|------------------|---------------------------|-------------|
|            | CB                | SM               | CP                        | SR          | NS           | ST | TG |
| Northern Gulf of Thailand |       |                  |                           |             |              |    |    |
| CB         | -                 | -                |                           |             |              |    |    |
| SM         | 0.012             | -                |                           |             |              |    |    |
| Southern Gulf of Thailand |       |                  |                           |             |              |    |    |
| CP         | 0.162*            | 0.188            |                           |             |              |    |    |
| SR         | 0.034             | 0.051*           | 0.058                     |             |              |    |    |
| NS         | -0.007            | -0.001           | 0.149*                    | 0.011       | -            |    |    |
| Andaman Sea |       |                  |                           |             |              |    |    |
| ST         | 0.090*            | 0.105*           | 0.151*                    | 0.057*      | 0.072*       |    |    |
| TG         | 0.162*            | 0.190*           | 0.187*                    | 0.108*      | 0.138*       | -0.035 |    |

Note: *significant differentiation (p<0.05)
statistics, a strong statistical test for detecting demographic expansion from mtDNA sequence data (Ramirez-Soriano et al. 2008) showed the statistically significant negative value, indicated a population expansion. Thus, these results suggested that the *M. meretrix* population in Thailand might have experienced expansion events. Further, Tajima’s *D* and Fu’s *Fs* results in the southern Gulf of Thailand population was statistically significant negative value indicated a population expansion had undergone. For the northern Gulf of Thailand and the Andaman Sea population, Tajima’s *D* and Fu’s *Fs* were negative but no significant deviation from the neutral state. These results support for slight population expansion and the non-significant negative value may be caused by the expansion that has been restricted to sampling sites (Rosly et al. 2013). Besides, a minimum spanning network showed a star-like topology indicated a signature of sudden population expansion (Slatkin and Hudson, 1991).

**Population genetic structure**

Population genetic structure in several marine animals caused by gene flow disruption. The geographic barrier, dispersive ability, and larva behavior are the main factor to prevent or promote gene flow among the population (Wang et al. 2013). In our study, the AMOVA, pairwise *F*<sub>ST</sub>, the neighbor-joining phylogenetic tree, and minimum spanning network could be genetically separated *M. meretrix* population into the Gulf of Thailand population and the Andaman Sea population. The genetic differentiation of the *M. meretrix* population between these two seas may cause by two factors. Firstly, Thailand's coast is located between the Pacific Oceans (Gulf of Thailand) and the Indian Oceans (Andaman Sea), separated by the Thai-Malaysian peninsula. Thus, this geographic barrier may prevent the gene flow of *M. meretrix* living in the Gulf of Thailand and the Andaman Sea. Secondly, the first larva stage of many marine animals is a planktonic larva. It is a free-moving larva and migrates to the open sea by the current. So, the water circulation is to promote a dispersal ability of many marine animals and maintain gene flow between populations (Johannesson et al. 2018). The planktonic larva stage of *M. meretrix* was mostly complete and settlement on 12 days (Sienes et al. 2018; Hamli et al. 2019). As the short of planktonic larva stage and the rapid settlements of *M. meretrix* may reduce a dispersion ability. Thus, the larva behavior of *M. meretrix* may decrease gene flow for a long geographic distance between the Gulf of Thailand and the Andaman Sea population. The genetic structure between these two regions has been reported in various marine species, such as *Hippocampus kuda* (Panithanarak et al. 2010), *Paphia undulata* (Donrung et al. 2011), and *Varuna litterata* (Suppapan et al. 2017).

**Figure 2.** Neighbor-joining phylogenetic tree based on the Kimura 2-parameter model and *Cyclina sinensis* as the outgroup. The number on the branches is bootstrap values.
Our finding showed that *M. meretrix* living along the Gulf of Thailand was only 1 population. Genetic homogeneity of the *M. meretrix* population in the Gulf of Thailand was plausibly maintained by two factors. Firstly, a lack of geographic barriers and a short geographic distance in the Gulf of Thailand maybe not prevented the gene flow of the *M. meretrix* population between the northern and the southern Gulf of Thailand. Secondly, the sea current in the Gulf of Thailand is a clockwise direction during the southwestern monsoon and a counter-clockwise direction in the northeastern monsoon (Saramul 2017). Further, the reproductive cycle of *M. meretrix* is biannual (Hamli et al. 2019) concordant with the monsoon period. Therefore, the homogenizing of the planktonic larva by sea circulation in the reproductive time may be maintained the gene flow of the *M. meretrix* in the Gulf of Thailand. The lack of genetic structure in the Gulf of Thailand was also reported in populations of other marine species; for example, *H. kuda* (Panithanarak et al. 2010), *Perna viridis* (Prakoon et al. 2010), and *Rachycentron canadum* (Phinchongsakuldit et al. 2013).

**Implications for management of *M. meretrix***

The genetic information of *M. meretrix* obtained in our study might facilitate fishery management. Our finding showed that the genetic structure has occurred between the Gulf of Thailand and the Andaman Sea. Thus, the management program to maintain genetic diversity should be treated as 2 separate conservation units because each population carries a unique genetic structure. For example, the increase of the abundance of *M. meretrix* population in the Gulf of Thailand via restocking should not be collected individuals from the Andaman Sea because it could lead to genetic contamination and outbreeding depression of natural populations. Interestingly, the *M. meretrix* population in the northern Gulf Thailand had a low genetic diversity, thus the management strategy in this region should be the focus. For instance, to enhance the genetic diversity of *M. meretrix* populations in the northern Gulf Thailand, the regulatory enforcement to capture and trade must be used to prevent population declines.

To conclude, in the present study, a total of 20 haplotypes from partial nucleotide sequences in the cytochrome oxidase subunit I gene were analyzed. A numerous of rare haplotypes indicating a large female effective population size of *M. meretrix* in Thailand. All populations, the haplotype diversity was high and the nucleotide diversity was low indicating that *M. meretrix* population expansion had undergone. The neutrality tests (Tajima's *D* and Fu’s *F**) were negative values, indicating that the *M. meretrix* population had experienced population expansion. Besides that, a minimum spanning network interpreted a signature of sudden population expansion. The population genetic structure analysis could be separated *M. meretrix* into the Andaman Sea population and Gulf of Thailand population. The genetic structure of *M. meretrix* might be caused by gene flow disruption by the geographic barrier from Thai-Malaysian peninsular and larval behavior. Genetic information in our study is a proper way to construct effective sustainable management.
strategies to maintain and increase the genetic diversity of *M. meretrix* in Thailand.

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