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Genetic relationship of asiatic hard clam populations collected in northern coastal provinces in Vietnam based on mtDNA sequence analysis

Abstract

The genetic relationship of some Asiatic hard clam (*Meretrix meretrix*) based on mtDNA COI sequence analysis was investigated for populations collected in Thai Binh, Nam Dinh, Nghe An provinces in Vietnam. In addition, this research also targets at species identification based on COI sequences. In total of 59 sequences analyzed, 19 sequences belonged to *Meretrix meretrix* species with Gen Bank accession number DQ399399.1. 17 sequences of *M. meretrix* were used for genetic relationship analysis among 3 populations. In which, 6 polymorphic sites, 3 parsimony informative sites and 4 haplotypes observed for the COI gene. Moderately genetic population diversity was observed, overall haplotype and nucleotide diversity were 0.476±0.233 and 0.00151±0.00069, respectively. Generally, genetic differentiation (F_{ST}) (F_{ST} < 0.15) was moderate. The genetic distance was rather low, which ranged from 0.001 (Thai Binh–NgheAn, Thai Binh–Nam Dinh populations) to 0.002 (Nam Dinh – Nghe An populations). The result of haplotype network constructing indicated that populations shared common haplotype and there was no specific isolation of the haplotypes of the populations. Hence, it showed *M. meretrix* populations had intimate genetic relationship. The result of phylogenic tree indicated that three *M. meretrix* populations (Thai Binh, Nam Dinh, Nghe An) had a very small or no genetic variation among populations.

Keywords: Population, genetic diversity, genetic relationship, meretrixmertrix, phylogenetic analysis

Research Article

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Introduction

Asiatic hard clams (*Meretrix meretrix*), genus *Meretrix* (Veneridae), are commercially important species in coastal areas of South and Southeast Asia.[1] In Vietnam, the northern coastal provinces is the main distributor for the total production of this species.[2] These clams were considered to be one of the indigenous mollusks in this region. However, recently, in the coastal areas of some Northern provinces, White clams (*Meretrix lyrata*) or Ben Tre clams were entered from Southern provinces and produced artificially. As the consequences of rapid development, White clams dominate in number compared to indigenous clams with 85-90% of the mollusk yield. This has led to the changes in the structure of coastal organism communities in general and decrease rapidly the resource of *M. meretrix* in particular.[3]

There was a considerable number of studies about genetic of *M. meretrix* in Asia. Chen et al.[4] present phylogenetic relationships of the genus *Meretrix* by using COI gene sequences. Thereafter, Chen et al.[5] built phylogenetic tree for 106 individuals belonging to Veneridae family including *M. meretrix*. In 2011, He et al.[6] used clam specimens collected from the coast of Panjin, Liaoning province, China for complete mitochondrial genome sequencing. The results showed that mitochondrial genome sequence of *M. meretrix* is 19,826 bp in length, containing 37 genes, in which 12 protein-coding genes, 2 ribosomal RNAs, and 23 tRNAs.[6] In general, the genetic studies of *M. Meretrix* in Asia focus primarily on analyzing the genetic relationship of them to closely related species. However, in Vietnam, studies have focused on resource assessment and reproductive biology, meanwhile, research on genetic of Asiatic hard clam has not paid much attention. The understanding of genetic structure and information about *M. meretrix* genetic diversity is necessary for the conservation, restoration, and development of this clam resource in Northern part of Vietnam.

Mitochondrial DNA (mtDNA) has been widely studied in almost marine and freshwater fish species, mainly for taxonomic and phylogenetic purposes. The advantages of using mtDNA include its simple maternal inheritance, absence of recombination, and high substitution rates.[7] The mitochondrial COI gene is often used to distinguish species in animals because of faciliating in amplification by using PCR method and universal primers.[8] This sequence of genes is always conserved among individuals in the same species and the rate of mutation is fast enough to distinguish between species with close genetic relationships.[9] In this study, the mitochondrial COI gene sequence was used to identify species and genetic relationship analysis in *Meretrix* genus that were collected in some Northern coastal provinces in Vietnam.

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Materials and methods

Samples collection

In total, 60 samples were collected in six locations, including HaiPhong (HP), Thai Binh (TB), Nam Dinh (ND), ThanhHoa (TH), NgheAn (NA) and Ha Tinh (HT) with 10 samples per province (Table 1) (Figure 1). The name Asiatic hard clam is called according to the local community (with the Latin name is *Meretrix meretrix*). Based on morphological characteristics, collected samples were preliminarily identified as belonging to the *M. meretrix*. They are large clams with thick shell covered by thin, delicate, straw-coloured or grey periostracum, and a greyish-blue or bluish-brown band on its postero-dorsal margin. The length is greater than the height. The muscle tissue 1-2g/sample was cut and preserved in 96% alcohol at 4°C.

Table 1 Collection details for Asiatic hard clam samples

Geographic populations (abbreviation used)	Sample location (longitude and latitude)	Collection time
HP	HaiPhong (20°51′59″N, 106°40′57″E)	August, 2017
TB	Thai Binh (20°32′20″N, 106°23′40″E)	August, 2017
ND	Nam Dinh (20°25′13″N, 106°10′05″E)	September, 2017
TH	ThanhHoa (20°08′28″N, 105°18′34″E)	June, 2017
NA	NgheAn (19°10′35″N, 104°58′38″E)	May, 2017
HT	Ha Tinh (18°20′28″N, 105°54′26″E)	June, 2017

Figure 1 Map showing sample locations.

DNA extraction, PCR amplification and sequencing

Total DNA of 60 clam samples was extracted according to the alcohol precipitation method [10]. DNA quality was checked by 0.8% agarose gel electrophoresis and the absorbance at 260nm was measured using Nanodrop and cuvette spectrophotometer (NanoDrop™ 2000C) to determine DNA concentration.

The fragments of COI gene of 60 samples were amplified by PCR reaction with primers according to Folmer et al.[8] The primer sequence is as follows: Fw – 5'GGTCAACAAATCATAAAGATATTGG3' and Rw – 5'TAAACTTCAGGGTGACCAAAAAATCA3'. PCR was carried out in a 37µl volume containing 1U/µlTaq DNA polymerase, 100ng/µl template DNA, 10µM each primer (1µl), 5mM (0.5µl) of each dNTPs, 100mM TrisHCl (pH 8.3), 25mM $MgCl_2$ (2.5µl), 500mM KCl (pH 8.3). The PCR was employed with initial denaturation of 2 min at 94°C followed by 30 cycles of denaturation for 30s at 94°C, annealing at 45°C for 45s and an extension of 72°C for 50s. After the completion of 30 cycles, a final extension step of 10 min at 72°C was performed. The PCR product was then kept at 4°C until removed from the machine. The amplified product was tested in 1.5% agarose gel and visualized using the Uvitec system. The appropriate PCR products then were purified and sequenced.

Data analysis

Sequences of COIwaschecked by Finch TV 1.4.0sofware.[11] Then, they were aligned and cut into the same length with BioEdit 7.2.5[12] using Clustal W under default settings. The BioEdit software was also used to check and determine the similarity degree of sequences and to create the consensus sequence of each population. The program DnaSP 5.0[13] was used to analyze molecular diversity indices including haplotype diversity (Hd), nucleotide diversity (π). Hierarchical analyses of molecular variance (AMOVA) were performed using Arlequin 3.5[14] to evaluate population structure. Haplotype network was constructed by using Network 4.6.1.[15]

Analysis of genetic distance between populations was used MEGA 6.0.[16] The evolutionary history was inferred using the Neighbor-Joining method.[17] The evolutionary distances were computed using the Kimura 2-parameter method[18] and are in the units of the number of base substitutions per site. *Venerupis/Ruditapes philippinarum* (EU266378.1) and *Meretrix petachialis* (KY318134.1) were used as out group.

Results and Discussion

M. meretrix identification based on COI region

The Blast results from National Center for Biotechnology Information (NCBI) showed that in total of 59 samples, there are 19 samples (32.2%) of *M. meretrix* with 99-100% identity (Table 2).

These results illustrated that species identification by morphology and molecular biology produced different results. By morphology method, 100% of the samples were classified as *M. meretrix*. However, by molecular biology method, only 32.2% of samples were identified as *M. meretrix* based on COI sequences. The remaining (67.8%) were identified as *M. petechialis*. According to Prashad,[19] *M. meretrix* is a species which experienced the greatest variation in the group of bivalves. Because of shades of shells and shell colors, it was wrongly identified with other species.[19] The results obtained in this study do not support the present taxonomic status of *M. meretrix* and *M. petechialis*, our goal is to analyze the genetic relationship of *M.meretrix* in different geographic regions of Vietnam. Specially, we focused to analyze genetic relationship of 3 *M. meretrix* populations in Thai Binh, Nam Dinh, Nghe An provinces.

Table 2 Number of samples belonging *M. meretrix* species in investigated populations

Population	No. of samples	No. of analyzed sequences	No. of *M.meretrix* species	Rate (%)
Hai Phong	10	10	0	0
Thai Binh	10	10	6	60
Nam Dinh	10	10	3	30
Thanh Hoa	10	10	0	0
Nghe An	10	10	8	80
Ha Tinh	10	9	2	22

Genetic relationship between *M. meretrix* populations

Mitochondrial genetic diversity

The fragments of COI sequences (650 bp) were obtained from 17 clams from the 3 of 6 populations studied (Thai Binh, Nam Dinh, NgheAn) which had more than 2 clams identified as *M. meretrix* (with GenBank accession numbers is DQ399399.1). In which, 6 polymorphic sites, 3 parsimony informative sites and 4 haplotypes were observed for the COI gene. The results of *M. meretrix* populations genetic diversity were shown in Figure 2 & Table 3.

Table 3 Mitochondrial genetic diversity of studied *M. Meretrix* populations

Sample site	No. of sequences	No. of haplotype (h)	Haplotype diversity (Hd ± SD)	Nucleotide diversity (π± SD)
Thai Binh	6	2	0.333 ± 0.215	0.00051 ± 0.00033
Nam Dinh	3	2	0.667 ± 0.314	0.00204 ±0.00096
Nghe An	8	2	0.429 ± 0.619	0.00197 ± 0.00078
Total	17	4	0.476 ± 0.233	0.00151 ± 0.00069

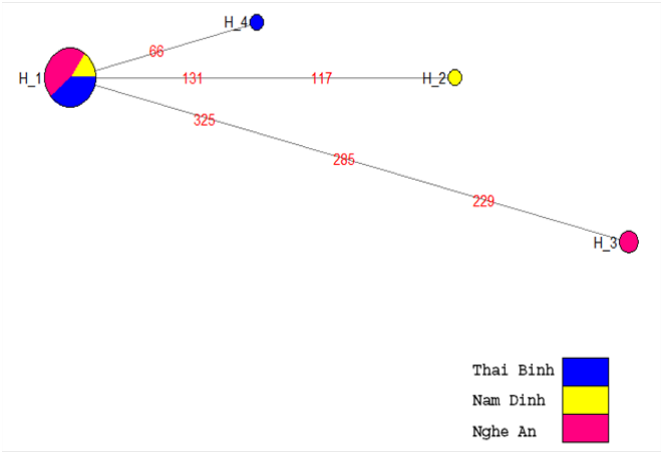

Figure 2 Network of studied *M. meretrix* populations.

The COI network was radial-like with a number of unique haplotypes closely related to central haplotype. Figure 2 indicated that the haplotype H_1, accounted for 76.47% (13/17), of all 17 individuals; occupied the central position of the network, one step away with other 3 haplotypes. This also suggested that H_1 was the ancestral haplotype and *M. meretrix* populations had an intimate genetic relationship. Besides, each population had its own haplotype which specific to the population. Hence, there was no population structure or population structure is not clearly established among studied populations the overall haplotype diversity and nucleotide diversitywere 0.476±0.233 and 0.00151±0.00069, respectively. The Hd ranged from 0.333±0.215 (Thai Binh population) to 0.667±0.314 (Nam Dinh population). Nucleotide diversity was highest (0.00204±0.00096) in Nam Dinh population and lowest (0.00051±0.00033) in Thai Binh population. Hd and π values in this study were lower than previous study on *Meretrix petechialis* and *Ruditapes philippinarum* using the same method. In fact, these values were 0.9483±0.0054 and 0.03364±0.01638, respectively

in *Meretrix petechialis* collected in the Northwestern Pacific.[20] In Manila clam (*Ruditapes philippinarum*), Hd values were ranged from 0.80 to1.00 while π values fluctuated 0.17–1.08 in populations collected in Asia.[21] Grant and Bowen, 1998 pointed out that marine species which experienced rapid expansion following a period of low effective population size often display high haplotype but medium to low nucleotide diversities.[22] Genetic diversity can be influenced by a range of factors including sample size, natural selection, mutation rates, gene flow among populations and human factors.[23] Previous studies revealed high genetic diversity in marine species including the miiuy croaker (*Miichthys miiuy*),[24] the fat greenling (*Hexagrammos otakii*),[25] and the clam (*Macridiscus multifarius*).[26]

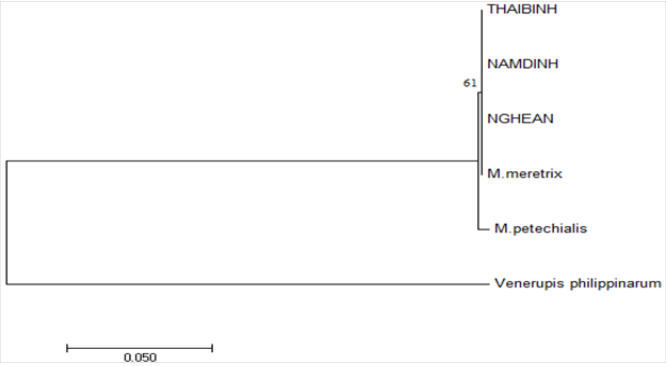

Figure 3 Dendrogram (NJ tree) based on Nei (1978) genetic distance between 3 *M. meretrix* populations.[28]

Genetic differentiation and genetic distance

F_{ST} values (Table 4) indicated the levels of pair wise genetic differentiation between the 3 populations. The F_{ST} values between populations were moderate ($F_{ST} < 0.15$), in which the lowest F_{ST} value was observed between the Nam Dinh and NgheAn populations ($F_{ST} = 0.07996$), while the highest F_{ST} value was observed between the Nam Dinh and Thai Binh populations ($F_{ST} = 0.13333$).

Table 4 Genetic differentiation (above) and genetic distance (below) of 3 *M. meretrix* populations

	Nam Dinh	Thai Binh	Nghe An
Nam Dinh	☐	0.13333*	0.07996*
Thai Binh	0.001	☐	0.08789*
Nghe An	0.002	0.001	☐

(*: *P value < 0.05*)

The genetic differentiation (F_{ST}) increased as geographic distance increased was correct for the case of the relationship between Nghe An population and the other, but it was not correct for other relationships. Genetic differentiation is influenced by many factors including habitat differences, historical events and human activities.[27]

The genetic distance values ranged from 0.001 (Thai Binh – NgheAn, Thai Binh – Nam Dinh) to 0.002 (Nam Dinh – Nghe An). The variation in genetic distance was not correlated with geographic distance. In this study, the geographic distance between NgheAn – Thai Binh was highest, but the genetic differentiation between Nam Dinh – NgheAn were largest.

Hierarchical analysis of AMOVA (Table 5) showed that majority of the molecular variation was distributed within populations (91%) rather than among populations (9%), indicating that the total genetic variation was intrapopulation variation. Therefore, it can be found that the population structure was not clearly established and the genetic diversity was low among studied populations. The F_{ST} value was 0.08999 which means there were moderate significant genetic variations among the three *M. meretrix* populations. Therefore, the use of only COI marker for the mtDNA region had not been polymorphic in this study.

Table 5 Results from analysis of molecular variance (AMOVA) of populations

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation Index F_{ST}
Among populations	2	1.451	0.04709	9	0.08999
Within populations	14	6.667	0.47619	91	
Total	16	8.118	0.52328		

Phylogenic analysis

Phylogenetic relationships were showed among *M. meretrix* species and outgroup *Meretrix petechialis* and *Venerupis philippinarum*. Because of the limitation in number of *M. meretrix* samples (3–8 samples per population) and the mixing population among three provines, there was no or less significant differences between populations consensus sequences. Tree topologies indicated that three *M. meretrix* populations and *M. meretrix* COI gene sequence (Accession number: DQ399399.1) formed a monophyletic group with very small or no genetic variation among populations.

M. meretrix and *M. petechialis* have been known as closely related species and there were some suggestions that they should be considered as synonyms[4,6]. However, as of now, the classification of *M. meretrix* and *M. petechialis* are still debated and unanimously agreed on the morphological and molecular biology identification methods. According to previous studies, *M. meretrix* was only distributed in the South China Sea, while *M. petechialis* was widely distributed throughout the coasts of China[29] and they were often misidentified.[20] In another similar case, Chen et al.,[4] supposed that *M. petechialis* and *M. lusoria* should be treated as a junior synonym of *M. meretrix* but as the reported by Torii et al.,[30] *M. petechialis* and *M. lusoria* are the two different species. Moreover, these authors established a method to identify *M. lusoria* and *M. petechialis* from shell morphology which can identify with 98.89% correct percentage.

Conclusions

Nineteen out of 59 samples were identified as *M. meretrix*. The *M. meretrix* populations had moderate genetic diversity that revealed by values of haplotype diversity and nucleotide diversity. The genetic differentiation (F_{ST}) was relatively high, however, the genetic distance (DA) was low and not related with geographic distance. Total genetic variation was intrapopulation variations. There was no clear population structure established among studied populations. The obtained results of this study have contributed scientific basis about genetic data of Asiatic hard clam in some regions in Vietnam. This is the basis for scientists, managers and people to build timely measures to research, preserve and develop clam genetic resources in the future.

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Conflict of interest

None.

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