Genetic and morphological variation in three populations of *Donax* spp. in the gulf of Thailand

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ABSTRACT

The aim of this research was to evaluate the genetic and morphological variation among the three populations of *Donax* spp. along the Gulf of Thailand. Samples were collected from Bangsean, Cha-Am and Suanson sandy beaches, and analyzed to reveal genetic and morphological variation by using 4 loci based on Inter-Simple Sequence Repeatsmarkers and 5 morphological variables. Thediscriminant function analysis of morphology presented a clear separation among three populations. Polymorphisms were detected at 4 loci across all three populations. The mean number of alleles ranged from 1.65±0.48 to 1.90±0.30 and the effective number of alleles ranged from 1.18±0.27 to 1.29±0.30. *Donax* spp. from Suanson beach showed the lowest level of genetic variation at which Nei's (1973) gene diversity was 0.16±0.17, whereas Nei's (1973) gene diversity of *Donax* spp. from Bangsean and Cha-Am beaches were 0.18±0.15 and 0.19±0.16, respectively. Percentage of polymorphic loci ranged from 66.22 to 89.86%. Overall, the results from this research based on genetic variation were in the same range of most other marine bivalves, which allows for potential adaptation to environmentalchanges. Furthermore, the results indicated that the population of *Donax* spp. from Suanson beach should be treated as a separate unit for conservation management.

Keywords*:* morphometric, variation, ISSR marker, *Donax* spp.

INTRODUCTION

Donax spp. are widely distributed along the sandy beaches of Thailand. People usually collect *Donax* spp. for consuming or selling them as fresh or preserved food. *Donax* spp. are in the group of bivalve mollusks that can be used as biological indicators of heavy metal pollution in the sea (Fishelson *et al*., 1999; Valle *et al*., 2011). The concentrations of heavy metals found in tissue of *Donax* spp. in

the sandy beaches of Rayong province, Thailand have been reported (Dungchangwat *et al*., 2011; Thairit *et al*., 2011). The results showed that most of the concentrations of heavy metals including Cd, Cu, Pb and Zn still have not exceeded the acceptable values for human consumption designated by the European Communities.

Nowadays, the population of *Donax* spp. in Thailand has been decreasing due to many causes. The major causes were the growth of tourism and the industrial development that disturb the natural habitats of *Donax* spp. The population management of *Donax* spp. should be established for conservation of *Donax* spp. in nature. However, the population structure of *Donax* spp. based on morphological or genetic data from different locations along the Gulf of Thailand is currently unknown.

Inter-Simple Sequence Repeats (ISSR) marker is a technique that can amplify inter-microsatellite sequences at multiple loci throughout the genome with oligonucleotide primers based on simple sequence repeats anchored at either the 3´ or 5´ end (Hou *et al*., 2006). The advantage of this technique is that it can detect polymorphisms in microsatellites loci without previous knowledge of DNA sequences. ISSR marker has been widely used to investigate genetic diversity and population genetic structure in bivalves (Laudien *et al*., 2003; Hou *et al*., 2006; Kong *et al*., 2007). However, the degree of morphological and genetic variation of *Donax* spp. in Thailand has not yet been estimated.

Therefore, the purpose of this study was to examine the morphological and genetic variation assessed by ISSR markers among three natural populations of *Donax* spp. The results from genetic data can be used to explain the population structure that is crucial for conservation management of Thai *Donax* spp. in the future.

MATERIALS AND METHODS

Sample collection

Samples of *Donax* spp. were collected between November 2010 and January 2011 from three beaches of Thailand (Fig. 1): Bangsaen beach (BB), Chonburi province; Cha-Am beach (CB), Phetchaburi province and Suanson beach (SB), Prachuapkhirikhan province. *Meretrix meretrix* (MM) was collected as a reference species. A hundred samples of each population were collected and measured to study morphological variation. Then, thirty individuals randomly taken from each population were used for genetic testing.

Morphometric analyses

Five morphological variables were measured according to Carstensen *et al*. (2006) and Zhao *et al*. (2009). Shells were measured with vernier caliper (±0.02 mm) for total shell length (L), maximum shell height (H) and total width of two valves (W) (Fig. 2), then H/L and W/H relations were calculated. Discriminant

function analysis (DFA) was used to isolate 5 morphometric characters accounting for the most variation among a priori defined groups (population in this case). The classification functions within DFA were then employed to assess how accurately individual shells had been assigned to the different populations.

Figure 1 Location of sampling area.

Genetic analysis by ISSR-PCR markers

Genomic DNA was isolated from foot muscle using DNA extraction kit from DNA technology laboratory, Kasetsart University, Thailand. The quality and quantity of DNA were checked by 1% agarose gel electrophoresis under ultraviolet transilluminator. The final DNA concentration of each sample was adjusted to 20ng/µl. Four primers that showed high polymorphisms in bivalves (Hou *et al*., 2006) were chosen for this analysis. The sequences of 4 ISSR primers were 5´- $(AC)_8$T-3´ for ISSR-8, 5´- $(AC)_8$G-3´ for ISSR-9, 5´- $(TG)_8$GT-3´ for ISSR-10 and 5´- $(AG)_8$TG-3´ for ISSR-11. PCR was carried out in a final volume of 10 µl containing 20 ng DNA template, 0.5 pmol primer, 1.6 mM $MgCl_2$, 200 µM dNTPs and 1 unit of *Taq* polymerase. DNA amplifications were performed using GeneAmp^R PCR System 9700 (Applied Biosystem) programmed for an initial cycle at 94 ^{O}C, 5 min; 35 cycles of 94^{O}C, 1 min, 46-52 ^{O}C, 1 min and 72 ^{O}C, 2 min; followed by 72 ^{O}C, 7 min. Amplification products were electrophoresed on 2.0% agarose gels at 100 V in 0.5X TBE buffer, visualized by staining with 1 ug/ml ethidium bromide, and photographed under ultraviolet light.

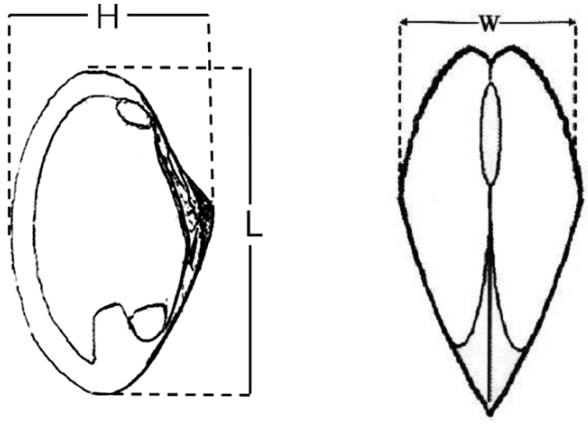

Figure 2 Three shell characters measured for each individual (*Donax* spp. and *M. meretrix*): total shell length (L), maximum shell height (H) and total width of two valves (W).

The ISSR-PCR fragments were scored as dominant binary markers, by presence (1) or

absence (0), for all 4 loci. The following parameters were generated using PopGene version 1.32 to describe genetic variation: the observed number of alleles (Na), the effected number of alleles (Ne), Nei's gene diversity (H), Shannon's information index (SI) and the percentage of polymorphic loci. Jaccard's similarity coefficients and chord distance from allele frequency estimates based on the Bayesian method were generated to determine the genetic distances among individuals and populations, respectively by using FAMD program as described by Schlüter and Harris (2006). Radial tree of 120 individuals and population tree were constructed using the neighbour-joining analysis by PhyloDraw program (Choi *et al*., 2000). STRUCTURE was applied to estimate the number of genetic subpopulations or clusters (K). Ten independent runs of K=1-5 with 100,000 Markov chain Monte Carlo (MCMC) iterations and burn-in period of 100,000 were performed, using admixture model with correlated allele frequencies and no prior information about individual membership. The program DISTRUCT written by Rosenberg (2004) was used to visualize these estimated membership coefficients. Subpopulations were presented as colors, and individuals were depicted as bars partitioned into colored segments that correspond to membership coefficients in the subgroups.

RESULTS AND DISCUSSION

Morphological variation

The biometric measurements were shown in Table 1. The results showed that the *Donax* spp. from SB were the shortest in shell length (1.32±0.26 cm), the longest in shell height (1.10±0.19 cm) and the thinnest in total width of two valves (0.59±0.10 cm) compared to those from BB (1.81±0.19 cm, 1.17±0.10 cm and 0.66±0.08 cm, respectively) and from CB (2.18±0.25 cm, 1.42±0.19 cm and 0.71±0.10 cm, respectively). The *Donax* spp. from SB showed the highest H/L ratio (0.86±0.30) whereas those from BB and CB showed similar values which were 0.65±0.12 and 0.65±0.03, respectively. The W/H ratio of *Donax* spp. ranged from 0.50 (CB) to 0.57 (BB). Based on the results of biometric measurements, the *Donax* spp. form SB exhibited thinner and rounder valves than the ones from BB and CB. In addition, MM showed the highest values in all 5 characters which were shell length (4.22±0.43 cm), maximum shell height (3.37±0.40 cm), the total width of two valves (2.25±0.32 cm), H/L ratio (0.80±0.03) and W/H ratio (0.67±0.03).

The DFA gave a number of combinations of the morphometric variables for the three populations of *Donax* spp. and one population of *M. meretrix*. The first function gave the best overall discrimination among the populations (Wilks' Lambda = 0.013; approximate Chi-square = 3,450; $P<0.001$). In the DFA of all

shell morphometric values, 82% of the variation was explained by Function I, 15.8% by Function II and 2.2% by Function III. The total width of two valves was the first of the five variables that contributed to the separation of populations along Function I, whereas shell length and maximum shell height contributed to the separation of populations along Function II.

Plots of individuals along the first two canonical variables (Fig. 3) showed a clear separation between the *Donax* spp. from SB and the other two populations (BB and CB), with MM as an outgroup. The classification function of DFA indicated that 94% of BB

Table 1 Biometric measurements (means±SD, minimum and maximum) of one hundred individuals of *Donax* spp. from three beaches and *M. meretrix*.

	Shell length (L) (cm)	Maximum shell height (H) (cm)	Total width of two valves (W) (cm)	H/L ratio	W/H ratio
Bangsean					
means±SD	1.81±0.19	1.17±0.10	0.66±0.08	0.65±0.12	0.57±0.06
min-max	0.54-2.29	0.96-1.50	0.50-1.14	0.59-1.83	0.46-1.03
Cha-Am					
means±SD	2.18±0.25	1.42±0.19	0.71±0.10	0.65±0.03	0.50±0.02
min-max	1.65-2.76	1.04-1.89	0.54-0.96	0.57-0.72	0.43-0.56
Suanson					
means±SD	1.32±0.26	1.10±0.19	0.59±0.10	0.86±0.30	0.53±0.06
min-max	0.24-2.12	0.72-1.48	0.38-0.83	0.61-3.79	0.43-1.08
M. meretrix					
means±SD	4.22±0.43	3.37±0.40	2.25±0.32	0.80±0.03	0.67±0.03
min-max	3.57-5.77	2.81-4.92	1.82-3.34	0.63-0.85	0.61-0.77

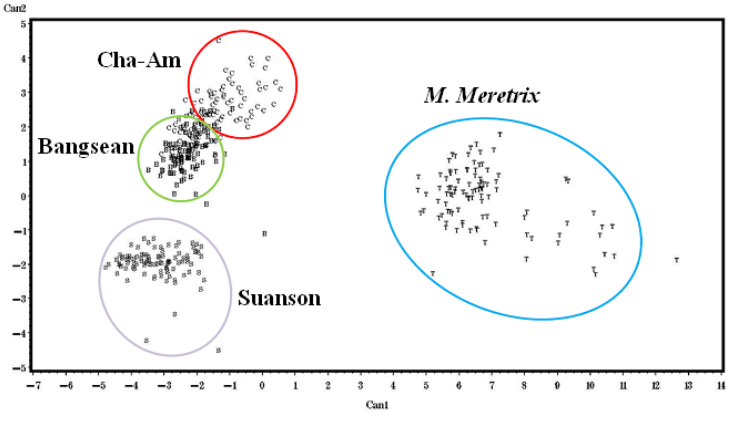

Figure 3 Relationships between scores on Function 1 and Function 2 for discriminant function analysis of morphometric variation of *Donax* spp from three beaches. (B = Bangsean, C = Cha-Am and S = Suanson) and T = *M. meretrix*.

population, 86% of CB population, 97% of SB population and 100%of MM population, individuals could be correctly reassigned by morphology. Individuals from CB population (14%) were incorrectly reassigned to BB population (Table 2).

Genetic variation

The results from ISSR-PCR markers were shown in Table 3. Polymorphisms were detected at 4 loci across all three populations. The mean number of alleles ranged from 1.65±0.48 to 1.90±0.30 and the effective number of alleles ranged from 1.18±0.27 to 1.29±0.30. The *Donax* spp. from SB showed the lowest level of genetic variation at which the Nei's (1973) gene diversity was 0.16±0.17. Concurrently, the Nei's (1973) gene diversity of *Donax* spp. from BB and CB were 0.18±0.15 and 0.19±0.16, respectively. The percentage of polymorphic loci ranged from 66.22 to 89.86%.

Genetic differentiation among populations and individuals were recognized by neighbor-joining tree and radial tree, respectively (Fig. 4 and Fig. 5). The result from population tree showed the closest relationship between the *Donax* spp. from CB and BB, with MM as an outgroup. The result from radial tree showed that individuals were split into 4 clusters corresponding to the 4 populations with some individuals misclassified.

Table 2 Percents of individuals that classified into populations based on morphological data.

	Percents of individuals that classified into populations			
	Bangsean	Cha-Am	Suanson	*M. meretrix*
Bangsean	94	5	1	0
Cha-Am	14	86	0	0
Suanson	1	2	97	0
M. meretrix	0	0	0	100

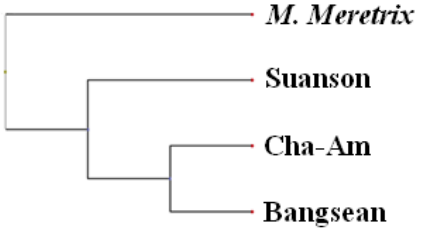

Figure 4 Neighbor-joining tree based on chord distance from allele frequency.

Clustering results of *Donax* spp. populations obtained by STRUCTURE applying K=1 to K=5 are shown in Fig. 6. At K=2, the four populations split into 2 groups, one containing the *Donax* spp. from BB and CB when the *Donax* spp. from SB was in the same group with MM. However, at K=3, the *Donax* spp. from SB split from MM. At K=4, the four

Table 3 Genetic variation in three populations of *Donax* spp. and *M. meretrix.*

	N_a[1]	N_e[2]	H[3]	SI[4]	Number of polymorphic loci (%)
Bangsean	1.90±0.30	1.26±0.28	0.18±0.15	0.29±0.21	62 (89.86)
Cha-Am	1.84±0.37	1.29±0.30	0.19±0.16	0.31±0.22	58 (84.06)
Suanson	1.65±0.48	1.25±0.31	0.16±0.17	0.26±0.25	45 (65.22)
M. meretrix	1.74±0.44	1.18±0.27	0.12±0.15	0.21±0.21	51 (73.91)
Means	2.00±0.00	1.29±0.24	0.20±0.13	0.34±0.18	69 (100.00)

[1] N_a = Mean number of alleles, [2] N_e= Effective number of alleles (Kimura and Crow, 1964), [3] H=Nei's (1973) gene diversity, [4] SI=Shannon's information index (Lewontin, 1972)

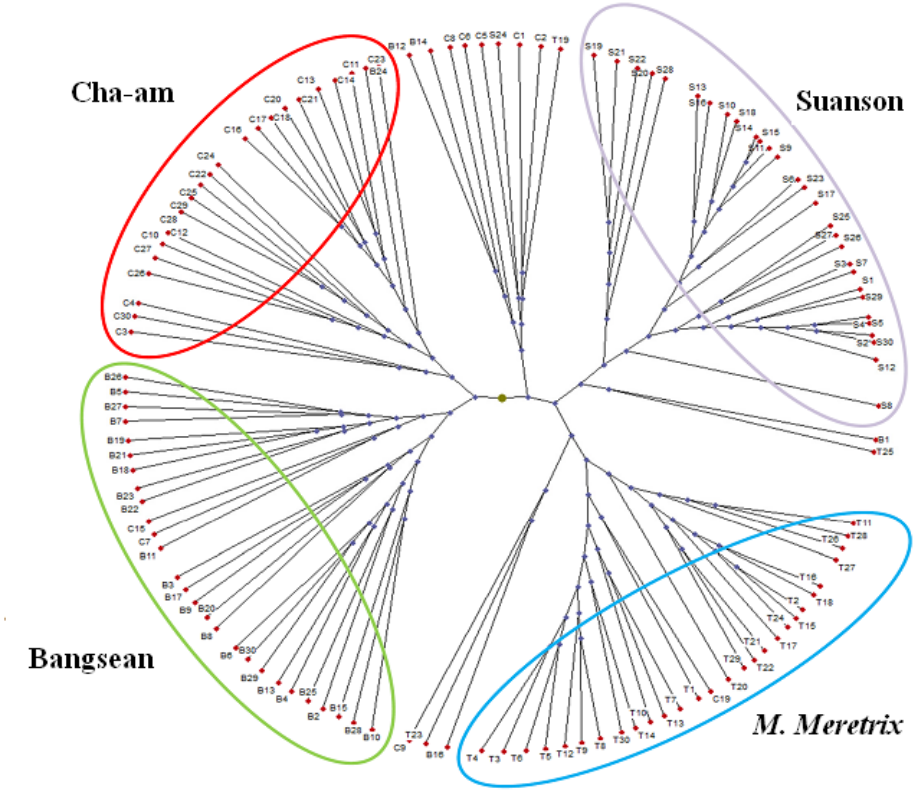

Figure 5 Radial tree for 120 individuals based on Jaccard's similarity coefficient, Neighbor joining method.

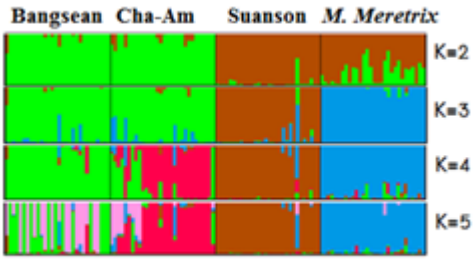

Figure 6 Graphical presentation of the population structure. Each population was separated by vertical lines, which was sectioned into K colored segments that represent the proportion of membership of each pre-defined population in K clusters. The populations were labeled above the figure. See color figure on the website.

populations were separated into 4 groups with the proportional of membership of each pre-defined population of 89%, 64%, 92% and 92% for BB, CB, SB and MM, respectively (Table 4). Misclassified individuals were distributed among all populations. Misclassification proportions varied from 8% to 36%. The *Donax* spp. from SB was the most correctly classified population, whereas the population of *Donax* spp. from CB was the most misclassified. The most misclassified individuals were classified into BB population (24%). However, the genetic structure of SB population seemed to be highly uniform and never separated at any K up to K=5. The highest value of Ln Pr(G|K) with the lowest variation between runs was obtained for K=4 as shown in Fig. 7.

The result based on morphology and genetic data suggested a clear separation of *Donax* spp. between SB and BB/CB. The *Donax* spp. from SB differed from others populations; this might be associated with habitat differences and directional selection with microevolutionary changes maintained by geographical isolation. The variations of morphological characteristics of many species are believed to be determined by changes in the physical (Soares *et al*., 1999) and/or biological environment (Levitan, 1988). These results, in particular the splitting of the SB population, may be of general interest to conservationists dealing with unique threatened populations.

Table 4 Percents classified into populations at K=4 (the results from STRUCTURE software).

	Inferred clusters			
	Bangsean	Cha-Am	Suanson	*M. meretrix*
Bangsean	89	4	3	3
Cha-Am	24	64	3	9
Suanson	2	1	92	5
M. meretrix	4	2	2	92

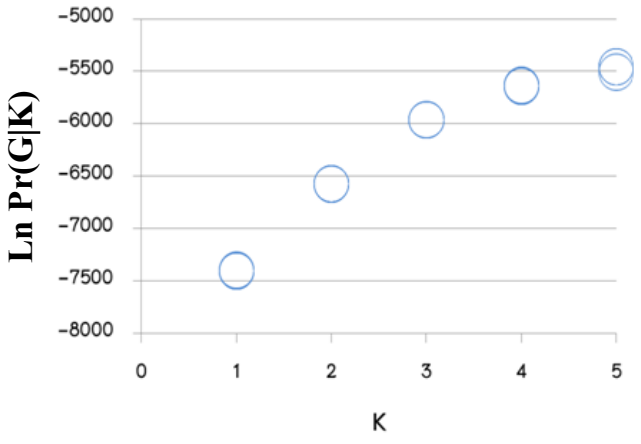

Figure 7 Ln Pr(G|K) values presented as a function of the number of clusters (each K = 10 runs), burn-in period and collected data for 100,000 iterations.

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