The comparison of shell morphology and genetic relationship between *Meretrix lusoria* and *M. petechialis* in Japan and Korea

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**Abstract:** Morphological and genetic traits of *Meretrix lusoria* and *M. petechialis* were compared among individuals from Japan and Korea. Multivariate analysis of shell morphology revealed that *M. lusoria* from all localities of Japan (Aomori to Kyushu) and from the southern and southwestern coasts of Korea (Sacheon Bay and Gangjin Bay) have some common characters, namely more linear shape in posterior-dorsal margin, smaller width of socket and larger shell breadth rather than *M. petechialis* from the western coasts of Korea (Baeksu and Saemangeum). Among *M. lusoria*, individuals from the Japan/East Sea coasts (Yuya Bay, Aso Sea and Mutsu Bay) have more linear shape in posterior-dorsal margin than those from other localities. The distribution border between *M. lusoria* and *M. petechialis* is located around the southwestern coasts of Korea (from Gangjin Bay to Baeksu). Analyses of mitochondrial COI and nucleus ITS also revealed that individuals from Japan and the southern coasts of Korea (Sacheon Bay) were classified as *M. lusoria*, and those from the western coasts of Korea (Baeksu and Saemangeum) as *M. petechialis*. However, all individuals from Gangjin Bay were classified as *M. petechialis* based on the analysis of mitochondrial COI, although most individuals were classified as *M. lusoria* by the analysis of nucleus ITS. These results suggest that hybridization between *M. lusoria* and *M. petechialis* occurs around Gangjin Bay. Further, we established a method to identify *M. lusoria* and *M. petechialis* from shell morphology. The modified discriminant score using the 5 selected characters, i.e. shell length (L), shell breadth (B), width of socket (SW), length of posterior-dorsal margin (LPM) and height of posterior-dorsal margin (HPM), is \( D = 110.26 - 61.61(\log B/\log L) + 10.90(\log SW/\log L) - 81.72(\log LPM/\log L) + 27.27(\log HPM/\log L) \). Using this discriminant score, we can identify *M. petechialis* and *M. lusoria* with 98.89% correct percentage.

**Key words:** Japan, Korea, *Meretrix lusoria*, *Meretrix petechialis*, mitochondrial COI gene, shell morphology

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**Introduction**

*Meretrix lusoria* (Röding) is one of the most popular clams around the Japanese coasts. However, after the 1970’s, most habitats and populations of this species decreased drastically due to reclamation and ocean pollution (Yamashita et al. 2004, Henmi 2009). In Japan, more than 800 km² of tidal flats formerly existed, but more than 40% of them have been lost during the past 50 years (Tsutsumi et al. 2000). Then, many benthic animals such as *M. lusoria* are drastically decreasing (Wada et al. 1996, Tsutsumi 2006). In Korea, the Saemangeum area, which is the most productive site of *Meretrix petechialis* Lamarck in that country, has also been isolated by the reclamation dike of 33 km in length (the longest in the world) since April 2006 (Sato 2006, Hong et al. 2007, Sato et al. 2007).

Furthermore, *M. petechialis* is now artificially introduced from China and Korea to Japan, and it has been pointed out that the hybrid between *M. lusoria* and *M. petechialis* may have occurred in Japan (e.g. Kosuge 1995, Wada et al. 2000).
However, there are few studies that make clear the relationship between these two species using morphological and genetic analysis, and a method to identify *M. lusoria* and *M. petechialis* has not been established (Yamakawa et al. 2008). Thus, the objectives of the present study are 1) to distinguish clearly between *M. lusoria* and *M. petechialis* based on morphological and genetic analyses, 2) to consider the phylogenetic relationship between these species, and 3) to discuss possibility of hybridization and fear an artificial mating between them. Using these results, we established the method to identify *M. lusoria* and *M. petechialis* from shell morphology.

**Materials and Methods**

Individuals of *M. lusoria* and *M. petechialis* were collected from 11 localities: i.e. Mutsu Bay (MT), Sendai Bay (SN), Aso Sea (AS), Ise Bay (IS), Yuya Bay (YY), Kafuri Bay (KF) and Ariake Bay (AR) in Japan, and Sacheon Bay (SC), Gangjin Bay (GJ), Baeksu (BK) and Saemangeum (SM) in Korea (Fig. 1). All individuals are stored at the Tohoku University Museum (TUMC111000-111010, Table 1).

Living animals from the intertidal zone were dug at low tide, and those from the subtidal zone were collected from commercial port landings. In order to examine the genetic traits, the soft tissue of living animal was removed from its shell and preserved in 99.5% ethanol. The soft tissue and shell were numbered in each individual, so that genetic and morphological data could be collated and related.

**Morphological analysis**

10 shell characters (in mm): shell length (L), shell height (H), shell breadth (B), pallial sinus length (PL), ligament

![Fig. 1. Sampling localities of *Meretrix lusoria* and *M. petechialis* in Japan and Korea. MT: Mutsu Bay, SN: Sendai Bay, AS: Aso Sea, IS: Ise Bay, YY: Yuya Bay, KF: Kafuri Bay, AR: Ariake Bay, SC: Sacheon Bay, GJ: Gangjin Bay, BK: Baeksu, SM: Saemangeum.](image)

**Table 1.** Information of locality, number of individuals, sample date and catalogue number at the Tohoku Univesity Museum (TUMC) for *Meretrix lusoria* and *M. petechialis* from Japan and Korea.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Sample point</th>
<th>Latitude and Longitude</th>
<th>No. of indiv.</th>
<th>Sample date</th>
<th>Catalogue No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutsu Bay (MT)</td>
<td>The mouth of Sin-Tanabe River, Mutsu City, Aomori Pref., Japan</td>
<td>41°16'21&quot;N, 141°11'46&quot;E</td>
<td>44</td>
<td>May 10, 2005</td>
<td>TUMC111000</td>
</tr>
<tr>
<td>Sendai Bay (SN)</td>
<td>The mouth of Nanakita River, Sendai City, Miyagi Pref., Japan</td>
<td>38°15'13&quot;N, 141°00'37&quot;E</td>
<td>83</td>
<td>Feb.–Sep. 2007</td>
<td>TUMC111001</td>
</tr>
<tr>
<td>Aso Sea (AS)</td>
<td>The mouth of Noda River, Yosano Town, Kyoto Pref., Japan</td>
<td>35°33'39&quot;N, 135°09'30&quot;E</td>
<td>34</td>
<td>June 8, 2008</td>
<td>TUMC111002</td>
</tr>
<tr>
<td>Ise Bay (IS)</td>
<td>The mouth of Sakauchi River, Matsuoka City, Mie Pref., Japan</td>
<td>34°36'30&quot;N, 136°32'54&quot;E</td>
<td>28</td>
<td>Apr. 23, 2005</td>
<td>TUMC111003</td>
</tr>
<tr>
<td>Yuya Bay (YY)</td>
<td>Yuya-igami, Nagato City, Yamaguchi Pref., Japan</td>
<td>34°22'17&quot;N, 131°01'42&quot;E</td>
<td>30</td>
<td>June 28, 2004</td>
<td>TUMC111004</td>
</tr>
<tr>
<td>Kafuri Bay (KF)</td>
<td>The mouth of Izumi River, Itoshima City, Fukuoka Pref., Japan</td>
<td>33°33'10&quot;N, 130°09'40&quot;E</td>
<td>72</td>
<td>Apr. 2005–Aug. 2006</td>
<td>TUMC111005</td>
</tr>
<tr>
<td>Sacheon Bay (SC)</td>
<td>Seonjin-ri, Sacheon City, Gyeongsangnam-do, Korea</td>
<td>35°02'26&quot;N, 128°02'16&quot;E</td>
<td>31</td>
<td>Apr. 26, 2003</td>
<td>TUMC111007</td>
</tr>
<tr>
<td>Baeksu (BK)</td>
<td>Baekpawi, Duu-ri, Yeonggwang-gun, Jeonnanam-do, Korea</td>
<td>35°14'23&quot;N, 126°18'30&quot;E</td>
<td>60</td>
<td>Apr. 9, 2008, May 7, 2003</td>
<td>TUMC111009</td>
</tr>
<tr>
<td>Saemangeum (SM)</td>
<td>Sura, Okbong-ri, Gunsan City, Jeonrabuk-do, Korea</td>
<td>35°55'43&quot;N, 126°36'05&quot;E</td>
<td>49</td>
<td>Aug. 25, 2007</td>
<td>TUMC111010</td>
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</table>
length (LL), socket width (SW), anterior shell length (AL), upper shell height (UH), length of posterior-dorsal margin (LPM) and height of posterior-dorsal margin (HPM) were measured on each individual (Fig. 2). For measurement of L, H, AL, UH, LPM and HPM, the outside of the right shell valve was photographed with a digital camera, and then each character was measured using image analysis software (Scion Image ver. 1.63). Further, L, H, B, PL, LL and SW were measured using a digital slide caliper (accuracy ±0.01 mm). L and H were measured using both methods, but there were no significant differences (p > 0.05) between them. Measured characters were analyzed with reduced major axis regression (RMA) against shell length and we compared their slopes using the method of significance test at 95% confidence level (Hayami & Matsukuma 1971). Then, to standardize the variability for size, all characters were log-transformed (using base 10 logs), and the 9 characters excluding shell length were divided by the log-transformed shell length (Table 2). Canonical discriminant analysis (CDA) was tested with the standardized 9 characters using SPSS (ver. 16.0). The method of multivariate analysis was partly borrowed from Takada (1992), Matsumasa et al. (1999) and Sato & Matsushima (2000).

**Genetic analysis**

Genomic DNA was isolated from adductor muscle of the clam by the DNeasy Blood & Tissue kit (Qiagen) following

<table>
<thead>
<tr>
<th>Characters</th>
<th>Locality (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MT (44)</td>
</tr>
<tr>
<td>log H/log L</td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>0.946</td>
</tr>
<tr>
<td>SD</td>
<td>0.005</td>
</tr>
<tr>
<td>log B/log L</td>
<td></td>
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<tr>
<td>mean</td>
<td>0.811</td>
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<tr>
<td>SD</td>
<td>0.011</td>
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<tr>
<td>log PL/log L</td>
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<td>mean</td>
<td>0.717</td>
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<td>SD</td>
<td>0.021</td>
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<td>log LL/log L</td>
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<td>mean</td>
<td>0.650</td>
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<td>SD</td>
<td>0.034</td>
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<td>log SW/log L</td>
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<td>mean</td>
<td>0.315</td>
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<tr>
<td>SD</td>
<td>0.042</td>
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<td>log AL/log L</td>
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<td>mean</td>
<td>0.395</td>
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<td>SD</td>
<td>0.103</td>
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<tr>
<td>log UH/log L</td>
<td></td>
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<tr>
<td>mean</td>
<td>0.519</td>
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<td>SD</td>
<td>0.068</td>
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<td>log LPM/log L</td>
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<td>mean</td>
<td>0.936</td>
</tr>
<tr>
<td>SD</td>
<td>0.007</td>
</tr>
<tr>
<td>log HPM/log L</td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>0.401</td>
</tr>
<tr>
<td>SD</td>
<td>0.034</td>
</tr>
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</table>
the manufacture’s protocol.

The mitochondrial cytochrome C oxidase subunit 1 (COI) was amplified from the genomic DNA using the primers reported by Folmer et al. (1994). Internally transcribed spacer-1 (ITS-1) gene was amplified using our original primers, PEF-10 (5’-TAG AGG AAG GAG AAG TCG TAA CAA GG) and 5.8R (5’-CAA KRT GCG TTC RAR RTG TCG ATG WTC A). PCR was performed in a total volume of 25 μL containing 1.25 U of Ex-Taq polymerase (Takara), 2.5 μL of 10× Ex-Taq buffer, 2 mM MgCl₂, 200 μM each of dNTPs, 50 pmol each of oligonucleotide primers, and 10 to 50 ng of the genomic DNA. The mixture was subjected to 35 cycles of amplification in a thermal cycler (Bioread). The first cycle was preceded by an initial denaturation for 1 min at 94°C. Each cycle consisted of denaturation for 30 sec at 94°C, annealing for 30 sec at 40°C, and extension for 60 sec at 72°C. The last cycle was followed by a final extension step for 10 min at 72°C.

PCR products of COI were directly sequenced by their PCR primers. Each PCR products was treated with ExoSAP-IT (Amersham Bioscience) for inactivation of residual primers and dNTPs. Nucleotide sequences were determined by ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) using a DYEnamic ET Terminator Cycle Sequencing Kit and DYEnamic Terminator Dilution Buffer (Amersham Bioscience).

For cloning, the PCR product of ITS genes was loaded onto an Agarose S agarose gel (Nippon gene) and electrophoresed in TBE (89 mM Tris-borate, 89 mM boric acid, 2 mM EDTA) buffer. The gel portion containing the amplified DNA fragment was removed, and the DNA was extracted with Quiagift spin kit (Qiagen). The extracted DNA was sequenced by the dideoxy-chain termination method of Sanger et al. (1977), using an automatic DNA sequencer, model 3100 (Applied Biosystems).

Homology searches of the nucleotide sequences were performed by BLAST and FASTA program supplied by GenBank for identification of the M. lusoria and M. petecialis.

Results

Morphological comparison among individuals from each locality

Among the 9 shell characters, the correlation coefficients (r²) of RMA in H, B, PL, LL, AL, UH and LPM against L were usually >0.9, but those in SW and HPM against L were relatively low in some localities although all regressions were significant at 95% confidence level (Table 3). The slopes of RMA (coefficient α) in the 9 characters ranged between 0.7 and 1.4 (Table 3), and there were significant differences in some characters among individuals from each locality (Table 4).

In individuals from Ariake Bay and Saemangeum, the slopes in H-L and UH-L were significantly larger (K>1.96, p<0.05) than those from the other localities (Table 4A and 4G, Fig. 3A and 3G). Shell morphology of these individuals looks more isosceles triangular than individuals from other localities. Further, the slopes in B-L were significantly larger in individuals from Ariake Bay and Saemangeum (Table 4B), and the shell breadth especially becomes larger with increase of the shell size in individuals from Ariake Bay (Fig. 3B).

The slopes in HPM-L were significantly smaller in individuals from Mutsu Bay and Yuya Bay but significantly larger in those from Aso Sea and Saemangeum than the other locality individuals (Table 4I). However, the coefficient β of RMA in HPM-L was much smaller in individuals from Aso Sea (Table 3), therefore the shape of posterior-dorsal margin is usually straighter than those from Saemangeum and Baeksu through ontogeny (Fig. 4I). As a result, individuals from Mutsu Bay, Yuya Bay and Aso Sea, which are located around or near the Japan/East Sea coasts, have more linear shape of posterior-dorsal margin, and those from Baeksu and Saemangeum, located around the Yellow Sea coasts, have more rounded shape of posterior-dorsal margin compared to those from other localities. In the same manner, the slopes in PL-L were also significantly larger in individuals from Yuya Bay but smaller in those from Baeksu and Saemangeum (Table 4C, Fig. 3C).

The slopes in LL-L were significantly larger in the Saemangeum but smaller in the Sacheon Bay individuals, and those in SW-L were significantly larger in the Ariake Bay but smaller in the Sendai Bay individuals than those from the other localities (Table 4D, 4E, Fig. 3D, 3E). In AL-L and LPM-L, there were no or few significant differences among all the individuals (Table 4F, 4H, Fig. 3F, 3H).

Multivariate analysis among the individuals of Meretrix spp.

According to the results of CDA, individuals from Baeksu and Saemangeum, western coasts of Korea, can be divided clearly from those from all the other localities, i.e. southwestern and southern coasts of Korea and Japan, by the standardized 9 morphological characters (Fig. 4).

The proportions of canonical variate 1 and 2 were 60.9% and 13.0% respectively (Table 5). The centroid of canonical variate 1 in individuals from Baeksu and Saemangeum were both more than 3.5, but those from the other 9 localities were all less than 0 (Table 6). The shell characters that largely affect canonical variate 1 were length and height of posterior-dorsal margin (log LPM/log L: −1.372, log HPM/log L: 1.137), socket width (log SW/log L: −1.070), and shell breadth (log B/log L: −0.974) against shell length (Table 5). Namely, individuals from Baeksu and Saemangeum have 1) more rounded shape in posterior-dorsal margin, 2) larger width of socket and 3) smaller shell breadth rather than those from the other 9 localities.
Among individuals from each locality, percent of correct cases in multivariate discriminant analysis ranged between 97.1% and 48.2% (Table 7). However, most cases of misclassification were found among individuals from all localities in Japan and southern and southwestern coasts in Korea (MT-GJ in Table 7) or between those from the western coasts of Korea (BK & SM in Table 7). Most individuals from Baeksu and Saemangeum (81 indiv./82 indiv.; 98.8%) were classified into those from the 2 localities (BK & SM), and almost all individuals from the other 9 localities (447 indiv./448 indiv.; 99.8%) were also classified into these individuals (MT-GJ) (Table 7).

GENETIC ANALYSIS OF MERETRIX SPP.

The 710 bp of the COI and 823–837 bp of ITS-1 nucleotide sequences were determined from each population collected in the present study. The matching scores between *M. lusoria* and *M. petechialis* were 93% per 710 bp of COI nucleotide sequences and 97% per 823–837 bp of ITS-1 nucleotide sequences, respectively. Insertions or deletions of some nucleotides were observed in ITS-1 genes compared from *M. lusoria* and *M. petechialis*. The nucleotide sequences of ITS-1 of *M. lusoria* and *M. petechialis* are deposited in DDBJ/EMBL/Gen Bank (accession numbers AB499129 and AB499729, respectively). Individuals from the Japanese coasts, e.g. Mutsu Bay, Sendai Bay, Aso Sea,
### Table 4

Results of significance test of *Meretrix lusoria* and *M. petechialis* for the slopes of reduced major axis regression (RMA) in the 9 characters against shell length among each pair of individuals from 2 localities. — : There are no significant differences ($p>0.05$) in slope of RMA between the pair of individuals from localities 1 and 2. $+$: The slope of RMA in individuals from locality 1 is significantly larger ($p<0.05$) than that from locality 2. $-$: The slope of RMA in individuals from locality 1 is significantly smaller ($p<0.05$) than that from locality 2. MT: Mutsu Bay, SN: Sendai Bay, AS: Aso Sea, IS: Ise Bay, YY: Yuya Bay, BF: Kafuri Bay, AR: Ariake Bay, SC: Sacheon Bay, GJ: Gangjin Bay, BK: Baeksu, SM: Sae-mangaeum.

<table>
<thead>
<tr>
<th>Character</th>
<th>Locality 1</th>
<th>Locality 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. shell height (H)—shell length (L)</strong></td>
<td>SN</td>
<td>AS</td>
</tr>
<tr>
<td></td>
<td>IS</td>
<td>YY</td>
</tr>
<tr>
<td></td>
<td>SC</td>
<td>BK</td>
</tr>
<tr>
<td><strong>B. shell breadth (B)—L</strong></td>
<td>SN</td>
<td>AS</td>
</tr>
<tr>
<td></td>
<td>YY</td>
<td>AL</td>
</tr>
<tr>
<td></td>
<td>SC</td>
<td>BK</td>
</tr>
<tr>
<td><strong>C. pallial sinus length (PL)—L</strong></td>
<td>SN</td>
<td>AS</td>
</tr>
<tr>
<td></td>
<td>YY</td>
<td>AL</td>
</tr>
<tr>
<td></td>
<td>SC</td>
<td>BK</td>
</tr>
<tr>
<td><strong>D. ligament length (LL)—L</strong></td>
<td>SN</td>
<td>AS</td>
</tr>
<tr>
<td></td>
<td>YY</td>
<td>AL</td>
</tr>
<tr>
<td></td>
<td>SC</td>
<td>BK</td>
</tr>
<tr>
<td><strong>E. socket width (SW)—L</strong></td>
<td>SN</td>
<td>AS</td>
</tr>
<tr>
<td></td>
<td>YY</td>
<td>AL</td>
</tr>
<tr>
<td></td>
<td>SC</td>
<td>BK</td>
</tr>
<tr>
<td><strong>F. anterior shell length (AL)—L</strong></td>
<td>SN</td>
<td>AS</td>
</tr>
<tr>
<td></td>
<td>YY</td>
<td>AL</td>
</tr>
<tr>
<td></td>
<td>SC</td>
<td>BK</td>
</tr>
<tr>
<td><strong>G. upper shell height (UH)—L</strong></td>
<td>SN</td>
<td>AS</td>
</tr>
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<td>AL</td>
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<td>SC</td>
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<tr>
<td><strong>H. length of posterior-dorsal margin (LPM)—L</strong></td>
<td>SN</td>
<td>AS</td>
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<td></td>
<td>YY</td>
<td>AL</td>
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<td><strong>I. height of posterior-dorsal margin (HPM)—L</strong></td>
<td>SN</td>
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<td>AL</td>
</tr>
<tr>
<td></td>
<td>SC</td>
<td>BK</td>
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</table>
Fig. 3. 2 dimension scattergrams of log-transformed 9 shell characters against log-transformed shell length (L: mm) with reduced major axis regression (RMA) for 11 groups of *Meretrix lusoria* and *M. petechialis*. The regression lines of groups have the smallest and largest slopes (coefficient a) in RMA were shown with their locality symbols. MT: Mutsu Bay, SN: Sendai Bay, AS: Aso Sea, IS: Ise Bay, YY: Yuya Bay, KF: Kafuri Bay, AR: Ariake Bay, SC: Sacheon Bay, GJ: Gangjin Bay, BK: Baeksu, SM: Sae-mangeum.

Fig. 4. 2 dimension scattergrams of *Meretrix lusoria* and *M. petechialis* from 11 localities obtained with canonical discriminant analysis (CDA). The values of canonical variate 1 and 2 for each individual and centroid for individuals from each locality were shown.
Ise Bay, Yuya Bay, Kafuri Bay and Ariake Bay, were mostly classified as *M. lusoria* by COI and ITS-1 genes, except for 3 individuals from Ariake Bay (3.1% among 96 individuals examined) have ITS-1 of *M. petechialis* genes (Fig. 5). Further, all individuals from Sacheon Bay, southern coast of Korea, were classified as *M. lusoria* by both COI and ITS-1 genes. By contrast, the analyses of mitochondrial COI and nucleus ITS revealed that individuals from the western coasts of Korea, Baeksu and Saemangeum, were almost all classified as *M. petechialis*, except that 1 individual from Baeksu (10.0% among 10 individuals examined) has ITS-1 of both *M. lusoria* and *M. petechialis* genes. All individuals from Gangjin Bay, southwestern coast of Korea, were classified as *M. petechialis* by COI genes (Fig. 5). However, in the analysis of nucleus ITS-1, 40 individuals among them were classified as *M. lusoria*, 2 individuals as *M. petechialis*, and 1 individual has both *M. lusoria* and *M. petechialis* genes.

### Discussion

**Discrimination of *M. lusoria* and *M. petechialis* based on morphological and genetic analyses**

Multivariate analysis of shell morphology revealed that individuals from all localities in Japan (Mutsu Bay to Ariake Bay) and from the southern and southwestern coasts in Korea (Sacheon Bay and Gangjin Bay) have some common characters, namely more linear shape in posterior-dorsal margin, smaller width of socket and larger shell breadth than those from the western coasts of Korea (Baeksu and Saemangeum) (Fig. 4, Table 5). Only 2 individuals from Sendai Bay and Saemangeum were misclassified into the...
individuals have similar shell morphology to those of *M. petechialis* and ITS-1 genes of this species. These results may be concerned with their faunal similarities between Ariake Bay and the Yellow Sea (Sato & Takita 2000).

The distribution border between *M. lusoria* and *M. petechialis* is located around the southwestern coasts of Korea (from Gangjin Bay to Baeksu), as Yamashita et al. (2004) reported. According to mitochondrial COI, all individuals from Gangjin Bay were classified as *M. petechialis*, but in nucleus ITS-1, most individuals were classified as *M. lusoria* (Fig. 5) and 1 individual has ITS-1 genes both *M. lusoria* and *M. petechialis*. However, the result of CDA discriminated all individuals from Gangjin Bay as *M. lusoria* (Fig. 4), and the slopes of RMA in most shell characters were significantly different between individuals from Gangjin Bay and those from Saemangeum (Table 4). Therefore, most individuals from Gangjin Bay can be identified as *M. lusoria*, and hybridization between *M. lusoria* and *M. petechialis* may occur around Gangjin Bay.

If individuals from Gangjin Bay are assumed as native ones without the artificial introduction, *M. lusoria* and *M. petechialis* are considered as a subspecies producing weak geographical isolation. In Japan, many individuals of *M. petechialis* are artificially introduced from China and Korea, because native individuals of *M. lusoria* decrease drastically. In such cases, there is fear that an artificial mating may occur among *M. lusoria* and *M. petechialis* (Kosuge 1995, Wada et al. 1996, Yamashita et al. 2004). The present study found that 3 individuals from Ariake Bay have ITS-1 genes of *M. petechialis* (Fig. 5), and the results of AFLP analysis also showed that individuals from Ariake Bay are genetically similar to those of *M. petechialis* (Hamaguchi et al. unpublished data). These facts suggest that individuals from Ariake Bay might have been influenced by the recent artificial hybrid in addition to the ancient relationship with *M. petechialis* from the Yellow Sea. In any case, the artificial introduction of *M. petechialis* to the Japanese coasts should be stopped.

**Method to identify *M. lusoria* and *M. petechialis* from shell morphology**

Genus *Meretrix* comprises 9 recognized species at the present day; however, systematic descriptions of these species are often confusing because of their morphological similarities (Yamakawa et al. 2008). *M. lusoria* and *M. petechialis* are especially similar in their shell morphology, and there are many erroneous identifications and notations in many books, reports, and articles (Yamakawa et al. 2008). Pan et al. (2006) analyzed 16S rRNA and ITS-1 sequences of *Meretrix* spp. including *M. lusoria* and *M. petechialis*, and Chen et al. (2009) also studied COI of these species. However, they did not explain how to identify the two species from shell morphology. The present study succeeded in discriminating clearly between the two species using analyses of shell morphology and genetics. Then, we establish the method to identify *M. lusoria* and *M. pe-
In the past studies, shell color patterns, shell height, shell breadth, position of umbo (represented by AL and UH) and the shape of posterior-dorsal margin have been usually used as remarkable characters to identify *M. petechialis* and *M. lusoria* (e.g. Habe 1983, Matsukuma 2000, Min et al. 2004). In the present study, the results of CDA revealed that the shape of posterior-dorsal margin, shell breadth and width of socket are more important to discriminate the two species than shell height and the other characters (Table 5). It was also impossible to discriminate the two species based on only shell color patterns (Torii, unpublished data).

Then, in order to simplify the method to identify *M. petechialis* and *M. lusoria* from shell morphology, we modify the discriminant score (*D*) using the 5 selected characters as follows (Table 8A).

\[
D = 110.26 - 61.61(\log B/\log L) + 10.90(\log SW/\log L) - 81.72(\log LPM/\log L) + 27.27(\log HPM/\log L) .
\]

This discriminant score can indicate that when *D* value >0 that individual is identified as *M. petechialis*, and when *D* <0 that individual is identified as *M. lusoria*. The *D* values for almost all individuals of *M. lusoria* were less than 0 (456 indiv./458 indiv., 99.6%), and those of *M. petechialis* were more than 0 (78 indiv./82 indiv., 95.1%) among the all individuals studied in the present study (Fig. 6). The percentage of correct cases in this discriminant score is 98.89% (Table 8B). Using this discriminant score, we can identify *M. lusoria* and *M. petechialis* clearly based on the 5 shell characters, i.e. shell length (L), shell breadth (B), width of socket (SW), length of posterior-dorsal margin (LPM) and height of posterior-dorsal margin (HPM) (Fig. 6).

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