

# Shell morphology and genetic characteristics of *Corbula (Potamocorbula)* spp. (Bivalvia: Corbulidae) from the waters adjacent to Japan, Korea and San Francisco Bay

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**ABSTRACT:** *Corbula (Potamocorbula)* spp. are natively distributed in the waters adjacent to Japan and Korea. These populations are distinguished by 6 morphological characteristics. However, they have high geographic variation, and it is quite difficult to distinguish among them. In the present study, we investigated the geographic distribution of the morphological and genetic characteristics of *Corbula (Potamocorbula)* spp. collected from the waters adjacent to Japan, Korea and San Francisco Bay, USA. Shell morphology was inferred through canonical discriminant analysis (CDA) of 8 shell characteristics, and genetic characteristics were analysed using the DNA sequences of mitochondrial cytochrome *c* oxidase subunit 1 (CO1) and nuclear internal transcribed spacer 2 (ITS2) regions. The results of CDA showed that the samples from Ganghwa-do in western Korea could be clearly separated from those of all the other localities. The molecular phylogenetic tree and haplotype network that were constructed from the CO1 and ITS2 regions, respectively, did not show clear groupings among the collected samples, except for 1 group composed of samples from Ganghwa-do. In addition, no differences in CO1 amino acid sequences could be found among any of the samples examined, including the Ganghwa-do samples. These facts suggest that *Corbula (Potamocorbula)* spp. distributed in the waters adjacent to Japan and Korea were separated into 2 groups. However, these 2 groups were genetically closely related, and it seems appropriate to treat them as 2 distinct species. The classification of the subgenus *Potamocorbula* should be re-examined at the species level.

**KEY WORDS:** Mollusca · Bivalvia · Corbulidae · *Potamocorbula* · Phylogeography · Classification · Mitochondrial cytochrome *c* oxidase subunit 1 · Nuclear internal transcribed spacer 2

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## INTRODUCTION

Populations of the Asian (overbite) clam, *Corbula (Potamocorbula) amurensis* Schrenck, 1861, in San Francisco Bay, USA, have markedly increased since 1986. They were introduced to the region through ballast waters from a cargo ship that had stopped in

East Asia (Carlton et al. 1990, Nichols et al. 1990). It is known that the waters adjacent to Japan and Korea in the Northwest Pacific are inhabited by at least 4 species of the subgenus *Potamocorbula* Habe, 1955. These species are distinguishable from each other by 6 morphological characteristics: shell size, shell outline, shell inflation, shell thickness, posterior

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margin and position of the umbo (Zhuang & Cai 1983, Habe 1994, Horikoshi & Okamoto 1994, Tsuchida & Okamura 1997). However, Sato (2002, 2005) and Sato & Kanazawa (2004) reported that there were high geographic variations in these morphological characteristics, making it challenging to distinguish among these species.

*Corbula (Potamocorbula) amurensis* is regarded as an invasive species in San Francisco Bay and may have a significant impact on the estuarine ecosystem of the bay (Carlton 1999, Coan 2002, Kimmerer 2002, Greene et al. 2011). In addition, fossil specimens of this species have been used as indicators of a cool climate or brackish water during reconstructions of the paleoenvironments of the Late Pleistocene and Holocene in Japan (Matsushima 1984, Habe 1994). However, these may not be accurate until the relationships between the geographic distribution and morphological characteristics of *Corbula (Potamocorbula)* spp. are fully understood at both population and species levels. To clarify the relationships among members of *Corbula (Potamocorbula)* spp., it will be necessary to investigate the morphological and genetic characteristics of populations from various localities. There have been several discussions of the genetic characteristics of *Corbula (Potamocorbula)* spp. For example, Duda (1994) reported that *C. (P.) amurensis* from San Francisco Bay, California, shows high genetic variation. Further, Smith et al. (2012) indicated that *C. (P.) amurensis* from San Francisco Bay and the Ariake Sea, western Japan, can be identified using the same genetic marker, 18S ribosomal

DNA. However, they did not simultaneously discuss the relationships between the morphological and genetic characteristics. In the present study, we investigated the geographic distribution of the morphological and genetic characteristics of the populations from the waters adjacent to Japan, Korea and San Francisco Bay through canonical discriminant analysis (CDA) of 8 shell characteristics and through the DNA sequences of mitochondrial cytochrome *c* oxidase subunit 1 (CO1) and nuclear internal transcribed spacer 2 (ITS2) regions.

## MATERIALS AND METHODS

### Sampling

*Corbula (Potamocorbula)* spp. were collected from 8 localities shown in Fig. 1: Rokkaku River Estuary, Saga Prefecture, Ariake Sea, western Japan (N = 25 individuals collected on 12 July 1998 and 23 November 2008); Dong River Estuary, Suncheon-si, southern Korea (N = 30, 20 August 2011); Mangyeong River Estuary, Gunsan-si, western Korea (N = 30, 3 May 2000 and 11 July 2008); Ganghwa-do, intertidal muddy sand flat, western Korea (N = 30, 8 May 1998 and 18 August 2011); Maeho Lake, Yangyang-gun, eastern Korea (N = 30, 16 July 2008); Hinuma Lake, Ibaraki Prefecture, central Japan (N = 30, 31 May 1998 and 30 July 2011); Obuchi Swamp, Aomori Prefecture, northern Japan (N = 46, 3 September 1998 and 27 July 2009); and San Francisco Bay, California,

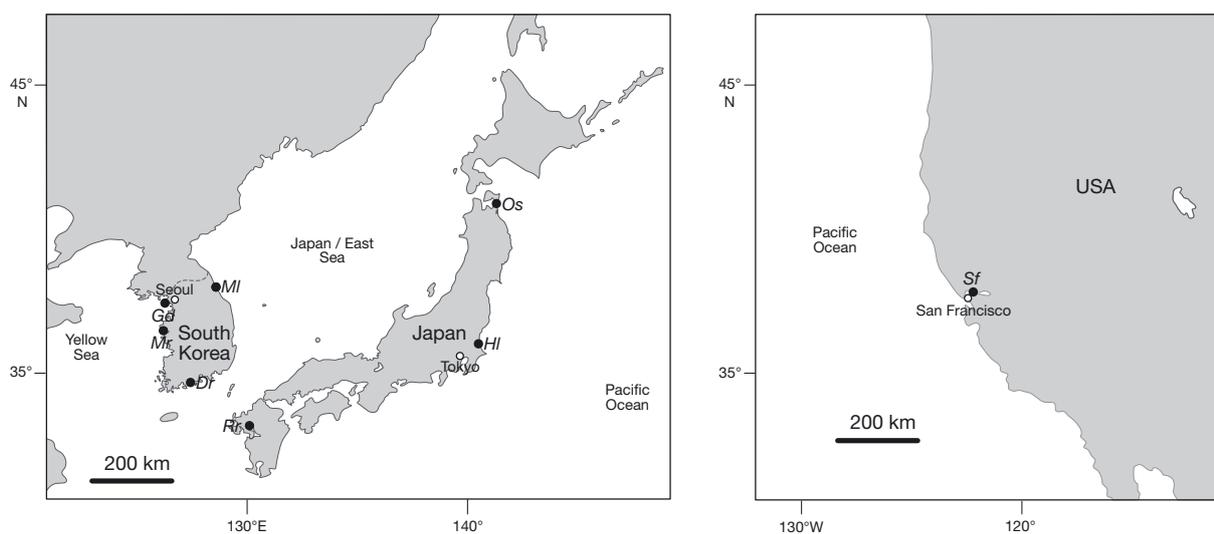


Fig. 1. Localities where the samples were collected. Rr: Rokkaku River Estuary (33°11' N, 130°12' E); Dr: Dong River Estuary (34°51' N, 127°30' E); Mr: Mangyeong River Estuary (35°51' N, 126°40' E); Gd: Ganghwa-do (37°36' N, 126°22' E); MI: Maeho Lake (37°56' N, 128°46' E); HI: Hinuma Lake (36°16' N, 140°30' E); Os: Obuchi Swamp (40°57' N, 141°22' E); Sf: San Francisco Bay (37°27' N, 122°1' W)

USA (N =30, 9 February 2000 and 9 November 2010). In addition, *C. (P.) fasciata* and *Solidicorbula erythrodon*, which were used as outgroups in the analysis of genetic variation, were collected from Bohol Island in the Philippines (9°44'N, 123°53' E) on 8 June 2007 and from Sagami Bay in Japan (35°17' N, 139°20' E) on 7 September 2011, respectively. The soft tissue of living animals was preserved in 100% ethanol, and DNA was extracted from the foot or adductor muscle using the DNeasy Blood & Tissue Kit (Qiagen). The shell and soft tissue were numbered in each individual. All voucher samples were deposited in the Tohoku University Museum (TUMC Nos. 111200 to 111207).

### Morphological analysis

Eight shell characteristics (in mm): shell length (*L*), shell breadth (*B*), shell height of left valve (*HL*), shell height of right valve (*HR*), thickness of shell margin (*T*), height of umbo (*U*), posterior shell length (*PL*) and upper shell height (*UH*) were measured on each individual (Fig. 2). For measurement of *L*, *HR*, *U*, *PL* and *UH*, the outside of the right shell valve was photographed with a digital camera, and then each characteristic was measured using image analysis software, ImageJ ver. 1.45s. Further, *L*, *B*, *HL*, *HR* and *T* were measured using a digital slide caliper (accuracy  $\pm 0.01$  mm). *L* and *HR* were measured using both methods, but there were no significant differences ( $p > 0.05$ ) between them. Then, to standardize the variability for size, all characteristics were log-transformed (using base 10 logs), and the 7 characteristics excluding *L* were divided by the log *L*. CDA was tested with the standardized 7 characteristics using SPSS (Ver. 16.0).

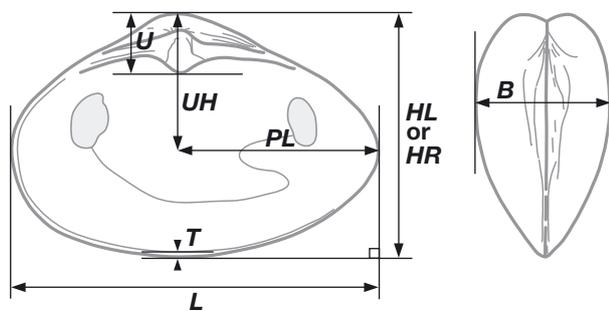


Fig. 2. *Corbula* (*Potamocorbula*) spp. Eight characteristics of left and right shell valves and shell breadths measured on shells. *L*: shell length; *B*: shell breadth; *HL*: shell height of left valve; *HR*: shell height of right valve; *T*: thickness of shell margin; *U*: height of umbo; *PL*: posterior shell length; *UH*: upper shell height

### Polymerase chain reaction (PCR)

CO1 and ITS2 regions were amplified using Pre-mix Taq (Takara) and a PCR Thermal Cycler PERSONAL (Takara). The following primers were newly designed for the present study: 5'-TCW ACA AAT CAY AAA GAT ATT GG-3' (forward) and 5'-ACY TCM GGR TGM CCA AAA AAT CA-3' (reverse) for the CO1 region, and 5'-ATT GCA GGA CAC ATT GAA CAT CG-3' (forward) and 5'-GTT ACT GAG GGA ATC CTT GTT A-3' (reverse) for the ITS2 region. The conditions for PCR amplification were as follows: denaturation at 94°C for 30 s, annealing at 54°C for 30 s (CO1) and 55°C for 30 s (ITS2) and extension at 72°C for 60 s. These steps were repeated 30 times. The PCR products were purified using ExoSAP-IT (USB), and cycle-sequencing reactions were performed using BigDye Terminator Version 3.1 Cycle Sequencing Kit (ABI PRISM). The sequences were compiled using a 3130 Genetic Analyzer (ABI PRISM) from both the 5' and 3' directions. These sequences were registered in the DNA Data Bank of Japan (CO1: AB740093 to AB740137 and AB827932 to AB827935, ITS2: AB740177 to AB740218 and AB827936 to AB827938).

### Genetic analysis

In the analysis of genetic variation in the CO1 region, the sequences of *Varicorbula dissimilis* (AF 120669), *Solidicorbula erythrodon* (AB740137), and *Corbula* (*Potamocorbula*) *fasciata* (AB740136) were included as outgroups. After alignment using Clustal W Version 1.8 (Thompson et al. 1997), sites that contained gaps or those that could not be confirmed by homology were removed from the analysis. A molecular phylogenetic tree was reconstructed using maximum-likelihood (ML) and Bayesian methods, respectively. The ML method was performed by PAUP Version 4.0\*b10 (Swofford 2002). The tree search algorithm was a likelihood ratchet algorithm (2000 times) (Vos 2003), and the model for the ML method was selected using Modeltest Version 3.7 (Posada & Crandall 1998). A bootstrap test was conducted 1000 times. The Bayesian method was performed by MrBayes Version 3.1.2 (Ronquist & Huelsenbeck 2003). This program was run for 5 000 000 generations, with sampling every 1000th generation. The model for the Bayesian method was determined using Mrmodeltest Version 2.3 (Posada & Buckley 2004).

In the analysis of genetic variation in the ITS2 region, the sequences were aligned using Clustal W

Version 1.8 (Thompson et al. 1997). Sites that contained gaps and those that could not be confirmed by homology were removed from the analysis. Further, a haplotype network was built using TCS Version 1.21 (Clement et al. 2000).

## RESULTS

### Canonical discriminant analysis

Shells of representative individuals collected from each locality are shown in Fig. 3. According to the results of CDA, individuals from Ganghwa-do can be clearly separated from those of all the other localities by the 7 standard morphological characteristics (Fig. 4). The proportions of Canonical Variates 1 and 2 were 84.6 and 8.3%, respectively (Table 1). The centroid of Canonical Variate 1 in individuals from Ganghwa-do was less than  $-6$ , but those from the other 7 localities were all more than  $-2$ . The shell characteristics that

largely affect Canonical Variate 1 were shell height and height of umbo (Table 2). Namely, individuals from Ganghwa-do have smaller shell heights and umbos rather than those from the other 7 localities (Fig. 3).

The other 7 samples beside Ganghwa-do cannot be clearly separated from each other (Fig. 4). However, individuals from Maeho Lake and Hinuma Lake have similar shell morphology, and those from Rokkaku River Estuary, Mangyeong River Estuary and San Francisco Bay were also of similar shell shape (Fig. 4). The samples from Dong River Estuary and Obuchi Swamp were slightly different from the other 5 samples, but some individuals overlapped morphologically (Fig. 4). In conclusion, though these 7 samples have a characteristic shell morphology, they cannot be distinguished because of the high morphological variability.

Among the samples from each locality, the percent of correct cases in multivariate discriminant analysis ranged between 100 and 60.0% (Table 3). All indi-

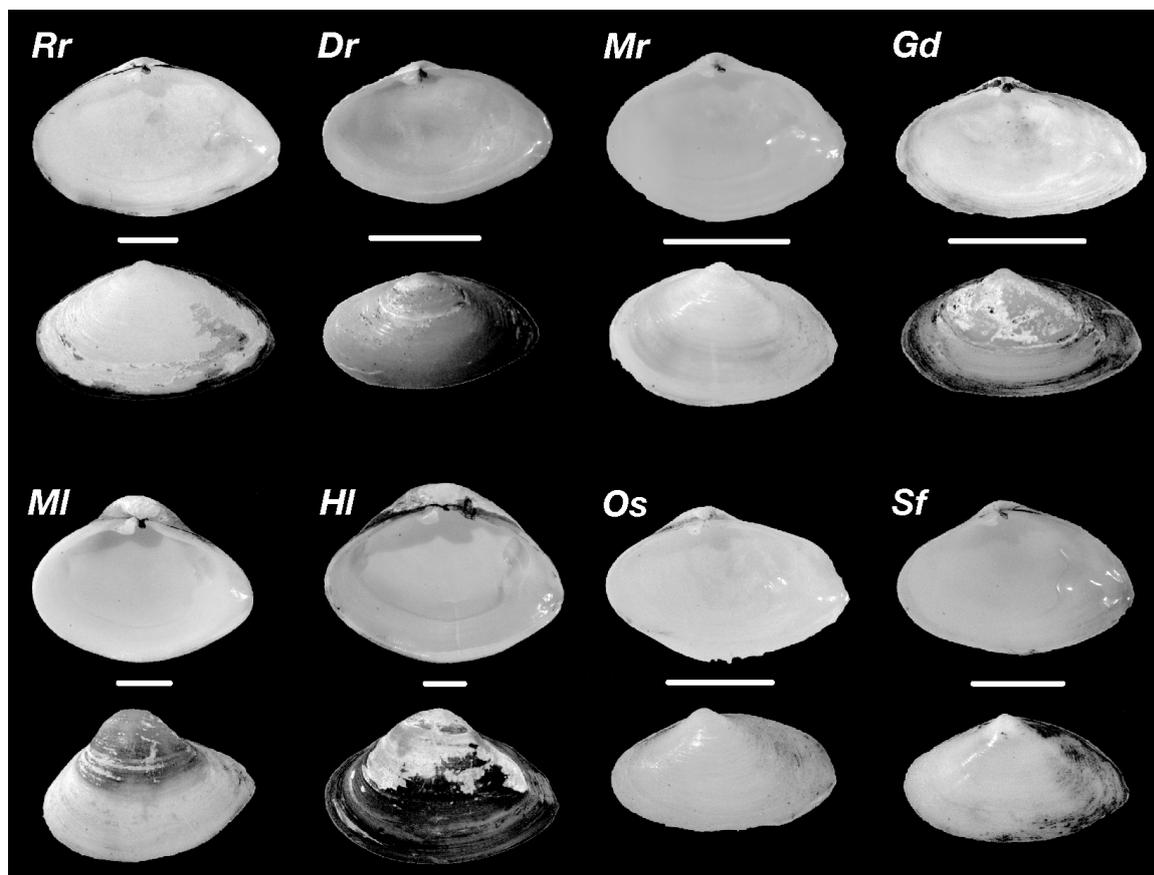


Fig. 3. *Corbula* (*Potamocorbula*) spp. Representative individuals collected from each locality. For each locality the upper 2 rows display the right valve and the lower 2 rows display the left valve. Scale bars: 5 mm. Rr: Rokkaku River Estuary; Dr: Dong River Estuary; Mr: Mangyeong River Estuary; Gd: Ganghwa-do; MI: Maeho Lake; HI: Hinuma Lake; Os: Obuchi Swamp; Sf: San Francisco Bay

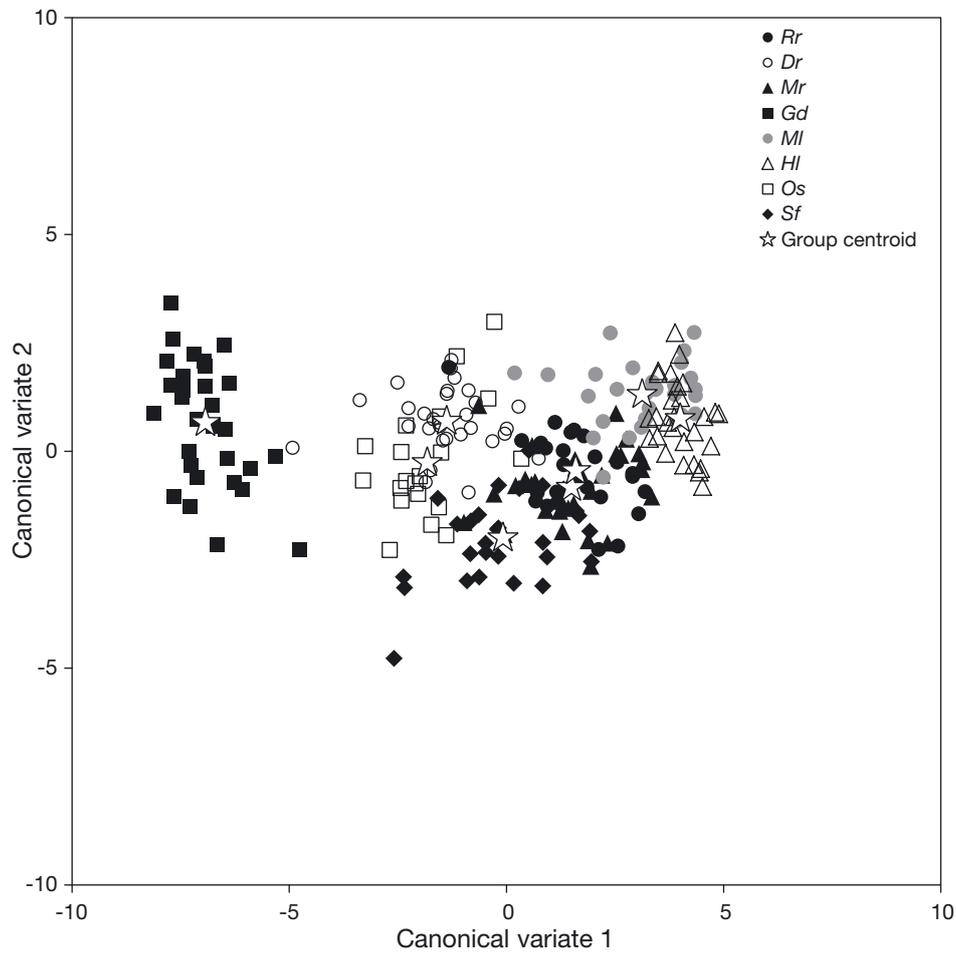


Fig. 4. *Corbula (Potamocorbula)* spp. Two-dimensional scattergrams of *Corbula (Potamocorbula)* spp. from 8 localities obtained with canonical discriminant analysis. The values of Canonical Variates 1 and 2 for each individual and the centroids for individuals from each locality are shown. *Rr*: Rokkaku River Estuary; *Dr*: Dong River Estuary; *Mr*: Mangyeong River Estuary; *Gd*: Ganghwa-do; *MI*: Maeho Lake; *HI*: Hinuma Lake; *Os*: Obuchi Swamp; *Sf*: San Francisco Bay

Table 1. *Corbula (Potamocorbula)* spp. Standardized coefficients of 7 characteristics, eigenvalue and proportion of Canonical Variates 1 and 2 for 8 groups. *L*: shell length; *B*: shell breadth; *HL*: shell height of left valve; *HR*: shell height of right valve; *T*: thickness of shell margin; *U*: height of umbo; *PL*: posterior shell length; *UH*: upper shell height

Characteristics	Standardized coefficients of Canonical Variates:	
	1	2
log <i>B</i> /log <i>L</i>	-0.248	1.115
log <i>HL</i> /log <i>L</i>	0.385	0.255
log <i>HR</i> /log <i>L</i>	0.361	-0.475
log <i>T</i> /log <i>L</i>	0.357	-0.199
log <i>U</i> /log <i>L</i>	0.451	0.226
log <i>PL</i> /log <i>L</i>	-0.200	0.227
log <i>UH</i> /log <i>L</i>	0.095	-0.853
Eigenvalue	11.314	1.108
Proportion	84.6	8.3

Table 2. *Corbula (Potamocorbula)* spp. Centroids of Canonical Variates 1 and 2 for individuals from 8 localities. *Rr*: Rokkaku River Estuary; *Dr*: Dong River Estuary; *Mr*: Mangyeong River Estuary; *Gd*: Ganghwa-do; *MI*: Maeho Lake; *HI*: Hinuma Lake; *Os*: Obuchi Swamp; *Sf*: San Francisco Bay

Locality	Centroid of Canonical Variate:	
	1	2
<i>Rr</i>	1.589	-0.440
<i>Dr</i>	-1.377	0.702
<i>Mr</i>	1.496	-0.809
<i>Gd</i>	-6.914	0.651
<i>MI</i>	3.127	1.317
<i>HI</i>	4.008	0.752
<i>Os</i>	-1.822	-0.258
<i>Sf</i>	-0.077	-1.980

Table 3. *Corbula (Potamocorbula)* spp. Discrimination scores using multivariate discriminant analysis among individuals from 8 localities. *Rr*: Rokkaku River Estuary; *Dr*: Dong River Estuary; *Mr*: Mangyeong River Estuary; *Gd*: Ganghwa-do; *MI*: Maeho Lake; *HI*: Hinuma Lake; *Os*: Obuchi Swamp; *Sf*: San Francisco Bay

	No. of total cases	No. of correct cases	Percent of correct cases	Number of classified cases								
				<i>Rr</i>	<i>Dr</i>	<i>Mr</i>	<i>Gd</i>	<i>MI</i>	<i>HI</i>	<i>Os</i>	<i>Sf</i>	
<i>Rr</i>	25	19	76.0	19	1	5	0	0	0	0	0	0
<i>Dr</i>	30	27	90.0	0	27	2	1	0	0	0	0	0
<i>Mr</i>	30	18	60.0	6	1	18	0	1	1	0	0	3
<i>Gd</i>	30	30	100.0	0	0	0	30	0	0	0	0	0
<i>MI</i>	29	22	75.9	1	1	1	0	22	4	0	0	0
<i>HI</i>	28	24	85.7	0	0	0	0	4	24	0	0	0
<i>Os</i>	20	19	95.0	0	1	0	0	0	0	19	0	0
<i>Sf</i>	30	20	66.7	1	0	8	0	0	0	1	20	0
Total	222	179	80.6	27	31	34	31	27	29	20	23	0

viduals from Ganghwa-do were classified correctly, but 25.0% of the individuals from the other 7 localities were classified incorrectly (43 out of 172 ind.) (Table 3).

#### Genetic variation of the CO1 region

DNA sequences of 606 bp in length were compiled for 47 individuals. They were 5 sequences from Rokkaku River Estuary, 4 from Dong River Estuary, 8 from Mangyeong River Estuary, 8 from Ganghwa-do, 3 from Maeho Lake, 4 from Hinuma Lake, 8 from Obuchi Swamp and 7 from San Francisco Bay. After the alignment, a 594 bp sequence was used for the genetic analysis. Genetic variation among the sequences was found at 64 nucleotide sites (11%); however, the sequences of some individuals were identical. The sequences that were translated to amino acids were the same in all the individuals from the 8 localities. There were 25 amino acid substitutions between the ingroup and the outgroup *Corbula (Potamocorbula) fasciata*.

In the ML method, the K81uf+G model was selected by Modeltest Version 3.7 and the likelihood index ( $-\ln L$ ) was 2593.56183. In the Bayesian method, the GTR+G model was selected by Mrmodeltest. A strict consensus tree was computed from these 2 trees (Fig. 5). In the strict consensus tree, all individuals from the 8 localities formed a monophyletic group and *Corbula (Potamocorbula) fasciata* was identified as a sister group of the monophyletic group. The individuals from

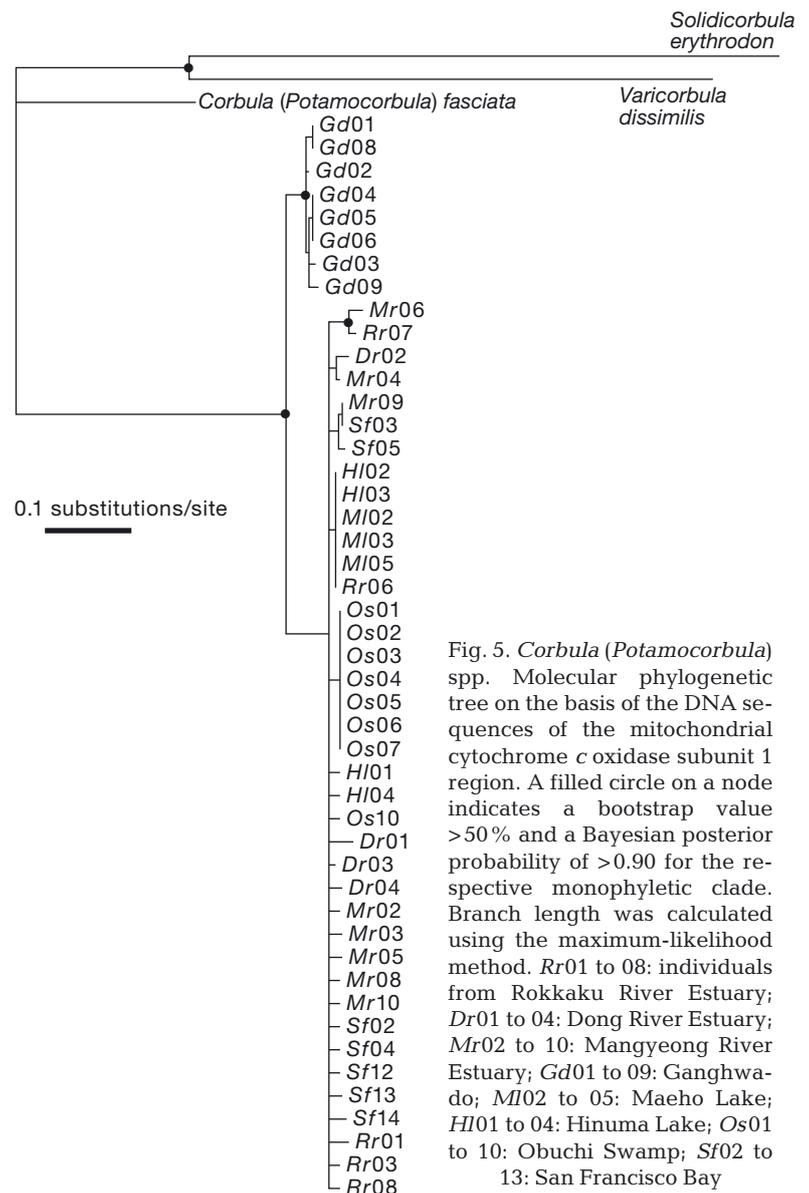


Fig. 5. *Corbula (Potamocorbula)* spp. Molecular phylogenetic tree on the basis of the DNA sequences of the mitochondrial cytochrome c oxidase subunit 1 region. A filled circle on a node indicates a bootstrap value >50% and a Bayesian posterior probability of >0.90 for the respective monophyletic clade. Branch length was calculated using the maximum-likelihood method. *Rr*01 to 08: individuals from Rokkaku River Estuary; *Dr*01 to 04: Dong River Estuary; *Mr*02 to 10: Mangyeong River Estuary; *Gd*01 to 09: Ganghwa-do; *MI*02 to 05: Maeho Lake; *HI*01 to 04: Hinuma Lake; *Os*01 to 10: Obuchi Swamp; *Sf*02 to 13: San Francisco Bay

Ganghwa-do formed a monophyletic group, and it was a sister group of the other 7 localities. Some individuals from Rokkaku River Estuary, Mangyeong River Estuary and San Francisco Bay formed monophyletic groups (Fig. 5).

### Genetic variation of the ITS2 region

Sequences of 384 to 393 bp in length were compiled for 45 individuals. They were 5 sequences from Rokkaku River Estuary, 3 from Dong River Estuary, 8 from Mangyeong River Estuary, 8 from Ganghwa-do, 4 from Maeho Lake, 4 from Hinuma Lake, 6 from Obuchi Swamp and 7 from San Francisco Bay. After the alignment, a 377 bp sequence was used for genetic analysis. Genetic variation among the sequences was found at 16 sites (4%).

The haplotype network is shown in Fig. 6, and it revealed 10 unique haplotypes forming 2 groups. The highest divergence among them was 11 bp. One group included only individuals from Ganghwa-do, and the other group was composed of the remaining

individuals. In the Ganghwa-do group, 3 haplotypes were identified, and the highest divergence was 3 bp. In the group containing all the other individuals, 7 haplotypes were identified, and the highest divergence was 4 bp. One haplotype was found in the samples from 6 investigated localities, except for those from Dong River Estuary and Ganghwa-do (Fig. 6).

### DISCUSSION

According to the criteria of Zhuang & Cai (1983), the samples from Rokkaku River Estuary, Mangyeong River Estuary and San Francisco Bay were identified as *Corbula (Potamocorbula) amurensis*, those from Ganghwa-do as a morphological type of *C. (P.) laevis* Hinds, 1843, those from Maeho Lake and Hinuma Lake as *C. (P.) ustulata* (Reeve 1844) and those from Dong River Estuary and Obuchi Swamp as *C. (P.) rubromuscula* (Zhuang & Cai 1983). However, Horikoshi & Okamoto (1994) identified the samples from Rokkaku River Estuary as *C. (P.) cf. laevis*. Tsuchida & Okamura (1997) identified the

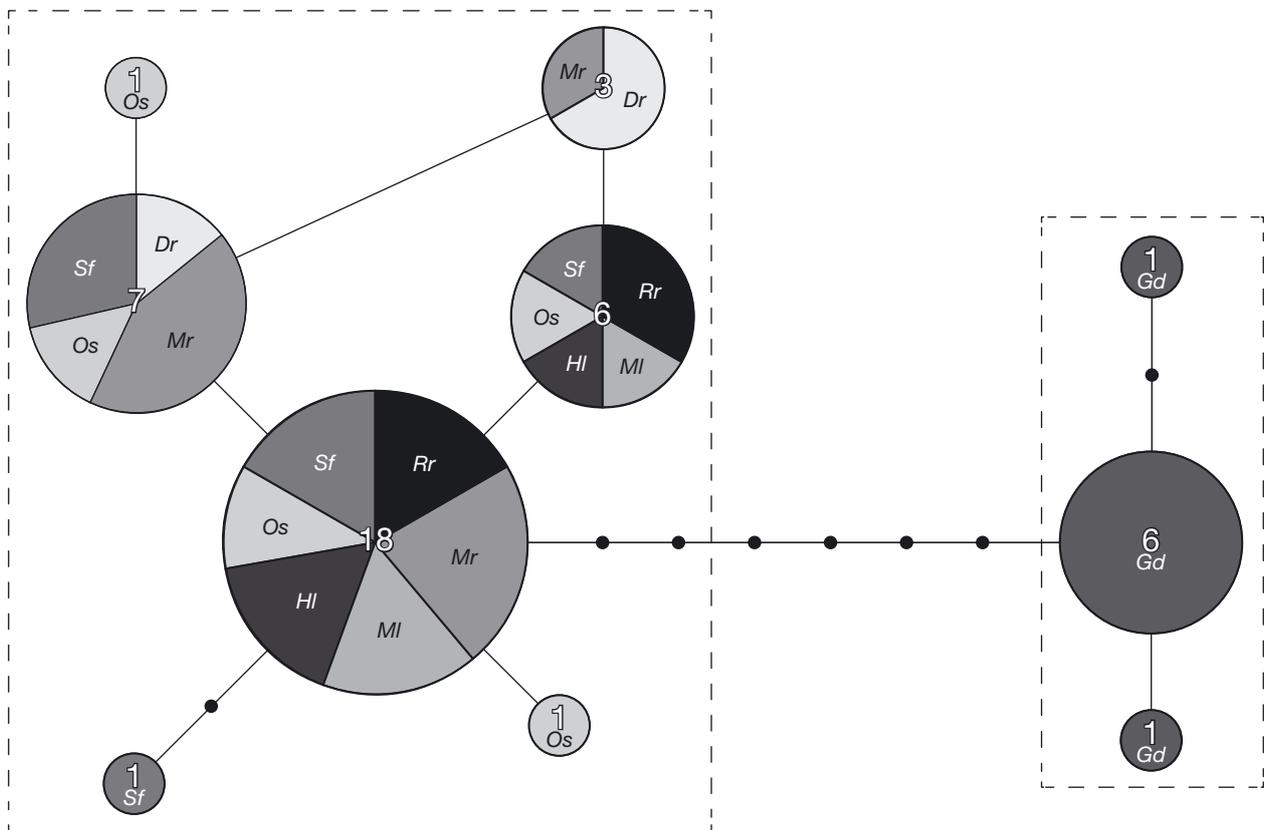


Fig. 6. *Corbula (Potamocorbula)* spp. Haplotype network on the basis of the DNA sequences of the nuclear internal transcribed spacer 2 region. Numerals indicate the number of individuals in the samples. Rr: Rokkaku River Estuary; Dr: Dong River Estuary; Mr: Mangyeong River Estuary; Gd: Ganghwa-do; MI: Maeho Lake; HI: Hinuma Lake; Os: Obuchi Swamp; Sf: San Francisco Bay

samples from Hinuma Lake as *C. (P.) ustulata*, in agreement with Zhuang & Cai (1983), though Huber (2010) defined this species as a junior synonym of *C. (P.) nimbosa* Hanley, 1843.

On the basis of their classification, *Corbula (Potamocorbula)* spp. distributed in the waters adjacent to Japan and Korea are divided into at least 4 species. If the classification is accurate, then these groups could be indicated through the analysis of morphological and molecular characteristics. However, the investigated samples did not form any species groups, except for the group composed of samples from Ganghwa-do. In the CDA results, samples from Ganghwa-do were clearly separated from those of the other 7 localities. Among the CO1 and ITS2 sequences, which were used to analyze intra-species relationships, the greatest difference was found between the groups composed of samples from Ganghwa-do and those composed of the remaining samples. This difference was larger than the largest difference among the samples from the other 7 localities. Therefore, the samples from Ganghwa-do can be distinguished from those of the other localities on the basis of both morphological and genetic characteristics. Furthermore, the samples from Ganghwa-do inhabited the intertidal zone of a muddy sand flat at an extremely high population density (maximum density >150 000 ind. m<sup>-2</sup>). This habitat is quite different from those of the other samples, which mainly inhabited the subtidal zone of mud flats or sand coasts. Therefore, reproductive isolation may have occurred between the Ganghwa-do population and the populations from the other localities. On the other hand, no differences in CO1 amino acid sequences, which has been frequently applied as a criterion for differences between species, were identified among any of the investigated samples. This suggests that the reproductive isolation occurred relatively recently.

Although the populations of Dong River Estuary, Mangyeong River Estuary, Maeho Lake and Obuchi Swamp are native to these places, we found no population-specific substitutions in CO1 and ITS2 regions. Further, the geographical distance between these localities would make it difficult for the populations inhabiting them to exchange genes. Thus, it may be concluded that these populations originated from a common, historically large population. In contrast, it is known that the populations of Rokkaku River Estuary, Hinuma Lake and San Francisco Bay were artificially introduced from other localities (Carlton et al. 1990, Nichols et al. 1990, Horikoshi & Okamoto 1994, Tsutsumi et al. 1997). Some indi-

viduals from Mangyeong River Estuary and Rokkaku River Estuary formed a clade with those from San Francisco Bay in the phylogenetic tree, and the samples from all the investigated localities, except for those from Dong River Estuary and Ganghwa-do, contained the same haplotype in the haplotype network. These facts suggest that the artificially introduced populations also belonged to the same historically large population. Therefore, it may be that the historical population was naturally or artificially separated and that the populations of the 7 localities were recently formed. Further, the population of Ganghwa-do may have separated from the historical population earlier than those of the other localities.

Nichols et al. (1990) and Nicolini & Penry (2000) reported that *Corbula (Potamocorbula) amurensis* is euryhaline and inhabits seawater with salinity ranging from 5 to 25‰. In addition, the populations of Rokkaku River Estuary, Mangyeong River Estuary and San Francisco Bay have been commonly shown to increase quickly after sudden environmental changes such as reclamation (Takayasu et al. 2000, Sato et al. 2001, Sato 2002, 2005, Sato & Azuma 2002, Sato & Kanazawa 2004). These facts could cause high variations in morphological characteristics. Although *Corbula (Potamocorbula)* spp. have been treated as indicators of a cool climate or brackish water in the past, the results of the present study do not support this. The smaller shells of the Ganghwa-do population might indicate a high population density, but it is not clear whether genetic or environmental factors caused the shell reduction. Therefore, *Corbula (Potamocorbula)* spp. may not be used as a paleoenvironmental indicator.

The present study showed that *Corbula (Potamocorbula)* spp. distributed in the waters adjacent to Japan and Korea are divided into 2 groups on the basis of morphological and genetic characteristics. The 2 groups are closely related genetically; however, reproductive isolation between them is possible. Hence, currently, it seems appropriate to treat them as at least 2 distinct species. The classification of the subgenus *Potamocorbula* should be re-examined at the species level.

Hallan & Willan (2010) described 2 species of genus *Lentidium* from northern Australia. *Lentidium* conspicuously resembles the subgenus *Potamocorbula* in shell morphology. Thus, *Lentidium* and *Potamocorbula* could be closely related, but further information on both morphological and genetic characteristics is needed to investigate the relationship between the 2 genera (cf. Hallan et al. 2013).

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