Communication

# A Single Mitochondrial Lineage Is Shared by Morphologically and Allozymatically Distinct Freshwater *Corbicula* Clones

Joong-Ki Park, Jun-Sang Lee<sup>1</sup>, and Won Kim\*

School of Biological Sciences, Seoul National University, Seoul 151-742, Korea; <sup>1</sup>Department of Biology, Kangwon National University, Chunchon 200-701, Korea.

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Despite that the exotic invasion and rapid range expansion of Asian freshwater Corbicula into new environments have been of intensive research topic in freshwater ecology, the genetic structures of freshwater Corbicula in its native range remain poorly understood. In this study, the genetic structures of two Korean freshwater Corbicula clonal lineages were characterized by cross-referencing the nuclear genomic structures with mtDNA sequence analysis. In spite of substantial genetic differences (Nei's D = 0.363 - 0.372) and a pronounced level of fixed allelic distinctions (in six of 20 allozyme loci) between Corbicula lineages, no lineage-specific mtDNA differentiation was observed. The evident disjunction between mtDNA sequences and nuclear genomes is a compelling evidence for the existence of interspecific nuclear hybrid genome structures, comprising different combinations of paternal and maternal contributions. This unusual novel finding is the first case demonstrating that morphologically and allozymatically distinct, yet mitochondrially identical clonal lineages exist in the genus Corbicula. However, we could not find the ancestral species for these two clonal lineages in the present study, and the answer for this question must wait until the genetic structure of Asian Corbicula taxa is fully characterized.

Keywords: Allozymes; Corbicula; MtDNA COI.

### Introduction

The freshwater *Corbicula* is one of the most well-known 'biofouling' pests that cause nuisance problems in fresh-

Tel: 82-2-887-0752; Fax: 82-2-872-1993

E-mail: wonkim@plaza.snu.ac.kr

World (Araujo et al., 1993; Ituarte, 1994; Kijviriya et al., 1991; McMahon, 1991; Morton, 1986; 1997). Despite that the invasion and its rapid expansion into new environments have been of intensive research topic in freshwater ecology, the genetic structures of freshwater Corbicula in its native range remain poorly understood. Over the last few decades, the existence of two morphotypes (white and purple morphs) in freshwater Corbicula has been reported from a number of regional populations [North America (Hillis and Patton, 1982); Thailand (Kijviriya et al., 1991); Hong Kong (Tsoi et al., 1991); Taiwan (Komaru and Konishi, 1999)]. A major distinction between the two morphs was made on the basis of color patterns of the inner shell surface and shell sculptures. Although morphological dichotomy of freshwater Corbicula forms is well documented by separate research groups, the conclusions on the occurrence of a single (Kijviriya et al., 1991) or two distinct lineages (Hillis and Patton, 1982; Siripattrawan et al., 2000) appear far from being generalized, and cannot be applied to other unstudied populations because these studies showed inconsistent results depending on the regional populations used in allozyme and mitochondrial analyses.

Similar to other regional taxa, most workers have undoubtedly agreed that two Korean morphotypes should be relegated to two separate species: *C. fluminea* (Müller, 1774) (white morph) and *C. leana* Prime, 1864 (purple morph) (Choe *et al.*, 1999; Kwon, 1990; Kwon *et al.*, 2001; Martens, 1905; Oyama, 1943). In contrast, Morton (1986) has proposed a single-species model for global freshwater members of this genus, including the Korean freshwater forms. However, Morton's single-species hypothesis, based primarily on morphological perspectives, has recently been challenged by genetic evidence. In an earlier allozyme study, Lee and Kim (1997) uncovered fixed allelic distinctions in a total of six loci, correlating with conchological distinction (white and purple morphs), which was interpreted as a reliable evidence for the exis-

<sup>\*</sup> To whom correspondence should be addressed.

water systems in many countries of Europe and the New

tence of two independent lineages in Korea, namely C. *fluminea* and C. *leana*.

A molecular survey of mitochondrial DNA has recently provided valuable insights into the long-standing debate on morphologically complicated species-level taxonomy, including that of *Corbicula* (Renard *et al.*, 2000; Siripattrawan *et al.*, 2000). In the present study, we re-visited this issue using an independent genetic dataset, mtDNA COI (mitochondrial DNA cytochrome c oxidase I) gene sequences. The COI sequences obtained from the two Korean freshwater *Corbicula* clonal lineages displayed a complete identity, demonstrating that morphologically and allozymatically distinct, but mitochondrially identical clonal lineages exist in the genus *Corbicula*. We further suggest that the two Korean freshwater *Corbicula* lineages are of inter-specific hybrid origin with different paternal ancestry.

#### Materials and Methods

**Sampling** Materials for mtDNA typing in this study were the *partial* fractions of the individuals used by Lee and Kim (1997) in their allozyme study. Samples were taken from seven freshwater populations of *Corbicula*, covering a broad range of Korean freshwater forms (Table 1). In addition, a population (Lake Songji, Kangwon-do) of Korean estuarine sexual species *C. japonica* Prime, 1864 was included for genetic comparison with the Japanese congener.

DNA sequencing and analysis Twenty individuals from the North Han River and five from each of the other seven populations, including the brackish species C. japonica Prime, 1864 were genetically characterized by amplifying and directly sequencing a homologous 614 nt fragment of the mitochondrial cytochrome oxidase I (COI) gene (corresponds to positions 1,539-2,152 of Drosophila yakuba mtDNA sequence; Clary and Wolstenholme, 1985). PCR amplification and sequencing reaction were performed using Folmer et al. (1994) primer set LCOI490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). Details for the molecular techniques were referred to Park and O'Foighil (2000) and Whang et al. (2002). The COI sequences of Japanese freshwater triploid androgenetic C. leana and estuarine diploid sexual species C. japonica published in a previous study (Siripattrawan et al., 2000) were incorporated into the dataset for comparison with Korean populations.

**Allozyme typing** To illustrate allelic distinction between two morphotypes (white and purple morphs), we replicated allozyme typing for esterase (E.C. No.: 3.1.1.1) and 6-phosphogluconate dehydrogenase (E.C. No.: 1.1.1.43).

**Sperm morphology** Clams were collected alive from the North **Table 1.** The sampling sites and number of individuals se-

quenced for mtDNA COI gene fragment.

Locality	No. of inds. sequenced	Occurrence
North Han River, Kyunggi-do Province	20	
Lake Uiam, Kangwon-do Province	5	õ
Musim Stream, Chungcheongbook-do Province	5	•
Tamjin River, Jeonranam-do Province	5	•
Taehwa River, Kyungsangnam-do Province	10	$\bullet$
Lake Songji, Kangwon-do Province	5	
Total	50	

Collection localities are represented by four different symbols:  $\bigcirc$  (white morph, *C. fluminea*), (purple morph, *C. leana*), (sympatric occurrences of *C. fluminea* and *C. leana*), and  $\blacksquare$  (estuarine form *C. japonica*).



Fig. 1. A. External and internal views of the shell types of freshwater *Corbicula* [Left, *C. fluminea* (white morph, Korea); Middle, *C. leana* (purple morph, Korea); Right, *C. leana* (Japan)]. B. The zymograms [*Est-3*, *Est-4*] showing the allelic distinctions and lineage-specific fixed heterozygosity between 2 Korean *Corbicula* lineages [left 5 lanes (*C. fluminea*); right 5 lanes (*C. leana*)].

Han River in September of 2001 and dissected for observation of sperm morphology. Sperms were extracted from the gonad of adult individuals. The pictures of biflagellate sperm of two morphotypes were taken using the DIC (differential interference contrast) microscope.

## **Results and Discussion**

Within the Korean freshwater *Corbicula* population, we recognized two morphologically distinct forms, coupled

with the patterns in their inner shell coloration (Fig. 1A). Besides the color of the inner nacre surface, the two morphotypes differed from each other in the number of annuli of the outer surface and shell shape. While the white morph is somewhat laterally corpulent with sparsely ornamented annuli in outer surface, the purple morph with its densely ornamented annuli is laterally compressed, and widens toward the anterior-posterior ends of the shell body. In this study, the two Korean freshwater morphotypes of *Corbicula* were denoted *C. fluminea* (white morph) and *C. leana* (purple morph) respectively, in accordance with an earlier allozyme study (Lee and Kim, 1997).

In our dataset of COI sequences, the genetic divergence level between haplotypes of the Korean and Japanese estuarine diploid sexual species C. japonica was very low, with a maximum of four base substitutions (p = 0.005-0.007%) among pairwise comparisons. In contrast to the variations encountered among estuarine forms of C. japonica, only a single haplotype, and no intra- or interpopulation differences were observed from 45 individuals of seven freshwater local populations representing two Korean Corbicula lineages (C. fluminea and C. leana). This result was greatly unexpected, and differed from that of a previous allozyme study which showed pronounced allelic distinctions between the two lineages (Lee and Kim, 1997; see also Fig. 1B). Although a significant mtDNA difference was expected between these two lineages, our COI sequences displayed a complete identity, i.e., no lineage-specific mtDNA differentiation was discovered. This disparity between mtDNA sequence uniformity and nuclear genetic differentiation was surprising, since the specimens for mtDNA typing in this study were the partial fractions of individuals used for the allozyme study which uncovered a pronounced level of fixed allelic distinctions at six of 20 allozyme loci (Lee and Kim, 1997). Indeed, a parallel divergence of mtDNA sequences and nuclear genomes between the two morphologically distinct lineages has been recorded in North American Corbicula populations: allozyme studies revealed the fixed inter-morph differences in a total of six loci between two distinct North American forms (Hillis and Patton, 1982; McLeod, 1986). Consistently, morphotype-specific mtDNA genotypes were disclosed in North America that differed by 2.85% in sequence, and were respectively identical to haplotypes discovered in Japanese C. leana and Korean C. fluminea (Siripattrawan et al., 2000; see Fig. 3). Therefore, our a priori expectation was that interlineage mtDNA sequence differences between the two Korean morphotypes were nearly as divergent as those recorded between the two North American lineages.

The salient disjunction between the mtDNA/nuclear genomes was repetitively reproduced by our complementary re-performance of the mtDNA and allozyme typings



**Fig. 2.** The biflagellate spermatozoa of two Korean freshwater *Corbicula* lineages [*C. fluminea* (A), *C. leana* (B)]. Scale bars, 10 µm.

for two lineages (see Fig. 1B), indicating that it is real, not of experimental artifact.

Cross-referencing the nuclear genomic structures with mtDNA uniformity encountered from two Korean lineages has a significant implication in their respective genetic structures. In general, species-specific life history patterns and other biological attributes, such as dispersal modes, developmental and reproductive traits shown in many animal taxa, play important roles in shaping the genetic structures of contemporary populations (Avise, 2000). In contrast to the results of the allozyme study, which uncovered fixed allelic differences in six of 20 allozyme loci and substantial genetic differences (Nei's D = 0.363-0.372) between two Korean freshwater Corbicula lineages (Lee and Kim, 1997), our COI dataset revealed a complete mtDNA sequence identity. This apparent disparity between mtDNA sequence uniformity and nuclear genetic differentiation is not always implausible, when interpreted in conjunction with the reproductive traits and genetics of freshwater Corbicula lineages. Both Japanese C. leana and Korean C. fluminea are triploids, with a chromosomal complement of 54 (Okamoto and Arimoto, 1986; Park et al., 2000), and the reproduction mode of Japanese C. leana is characterized as androgenetic (Komaru et al., 1998). Like many other regional freshwater populations [North American C. fluminea (Kramer, 1983); Japanese C. leana (Komaru and Konishi, 1996), C. fluminea (Konishi et al., 1998), Taiwanese Corbicula (Komaru and Konishi, 1999); Australian C. australis (Byrne et al., 2000)], the triploid hermaphroditic two Korean freshwater Corbicula lineages produce a biflagellate sperm (Fig. 2), indicating that they maintain the clonal reproduction (Byrne et al., 2000; Siripattrawan et al., 2000). In most animal taxa, the acquisition of clonal reproduction may be a consequence of hybridization be-

C. fluminea (KR)*	TTGATCTGGTTTAATAGGAACTGCTTTTAGGGTAATTATTCGAATGGAGC
C. leana C. ianonica (KP1)	
C. japonica (KR1)	А
C japonica (IP)	G A T
c.juponicu (si )	
	100
C. fluminea (KR)*	TTGCTATTCCAGGGACTTTACTAGATGATGGGCAGTTGTATAATACTATT
C. leana	C
C. japonica (KR1)	.A
C. japonica (KR2)	.ATAA
C. japonica (JP)	.A
	150
C. fluminea (KR)*	GTTACTGCTCATGCTTTAGTAATAATTTTTTTTTTTAGTAATGCCAATAAT
C. leana	G
C. japonica (KR1)	CTG
C. japonica (KR2)	CTG
C. japonica (JP)	CTG
	200
C. fluminea (KR)*	GATGGGTGGTTTTGGAAATTGACTTGTTCCATTAATGTTAAGGGCTCCTG
C. leana	A
C. japonica (KR1)	AC
C. japonica (KR2)	AC
C. japonica (JP)	AC
	250
C. fluminea (KR)*	200 ATTATTACCTTTTTTACCACCACTTAAATAATTAACACTTTTTCCCCTTTTTCCCCACCA
C. leana	
C. japonica (KR1)	GTCGAG
C. japonica (KR2)	G
C. japonica (JP)	GT
	200
C fluminga (KP)*	300 ൙൲ൔ൬൙൙൲൲൱൙൲൛ൔ൙൲൛ൔൔ൱൙൙൙൛ൔ൱൙൙൲ൔ൱൙൲൙ൔ൙ൔ൙൱൙൙൙൙൙
C leana	G A
C. janonica (KR1)	
C. japonica (KR2)	AAAAAA
C. japonica (JP)	A
	250
C fumines (VD)*	U35 macanaca anonna machannanana a a mannacha machanaca
C leana	C
C. janonica (KR1)	Т. АТ.
C. japonica (KR2)	TA
C. japonica (JP)	TA
C Aumin on (VD)*	
C. Juminea (KK)*	CGICAGIAGAIIIAGCIAIIIIIICICICIICAIIIAGGGGGIAIIICIICA
C ianonica (KR1)	т с ст а т
C. japonica (KR2)	.TGT
C. japonica (JP)	.TGGT
•••	
C Aumin on (VD)*	
C. Juminea (KK)*	ATTTAGCTICAATTAATTTIGTIGTCACTAGATTTIGTATGCGTCCTGG
C ianonica (KR1)	с. т
C. japonica (KR2)	G
C. japonica (JP)	G
C fluming (VD)*	
C. Juminea (KK)*	AGOGCAAAAGCTAATTUGGACTACAATGTTTATTIGATGTATTGTTGTAA
C. ianonica (KR1)	
C. japonica (KR2)	
C. japonica (JP)	AAT
C Aumin on (VD)*	
C. Jununea (KK)	CIGGAATTIGTIGATTATIGCAATGCCIGIGTIAGCIGGGGCICTIACT
C ianonica (KR1)	Δ Δ
C. japonica (KR2)	
C. japonica (JP)	AA
C fuminas (ZD)*	
C. juminea (KK)* C. leana	AIGIIGITAACIGATUGTAATITTAACACITCATTTTTTGATCCGGTAGG
C. ianonica (KR1)	Α
C. japonica (KR2)	A
C. japonica (JP)	A
C fuminas (ZD)*	614
C. juminea (KK)* C. leana	LI IAGGGATUUTT
C ianonica (KP1)	Δ
C japonica (KR2)	Δ
C. japonica (JP)	A

Fig. 3. Alignment of the 614 nucleotide mt COI gene fragment sequenced for Korean *Corbicula* samples. Sequence comparisons between Korean *C. fluminea* and Japanese *C. leana* haplotypes, and between Korean *C. japonica* and Japanese *C. japonica* haplotypes are shown. The COI sequence obtained from two Korean morphotypes (white and purple morphs) is identical to the sequence indicated by *C. fluminea* (KR)\*. Dots depicted in a sequence represent the same character state as that in the first sequence. KR, Korea; JP, Japan. The GenBank accession numbers: Korean *C. fluminea* (KR, AF196269), Japanese *C. leana* (JP, AF196268), Japanese *C. japonica* (JP, AF196271), Korean *C. japonica* [(KR1, AF367440); (KR2, AF367441)].

tween sexual species (Dawley, 1989; Vrijenhoek, 1994; Wilson and Hebert, 1992). Therefore, most clonal lineages carry a mix of the nuclear genomes of two or more sexual species (Avise *et al.*, 1992). Although direct empirical evidence that allows us to identify plausible paternal ancestry is not yet available, the lineage-specific fixed heterozygosity observed in some loci [*Mdh* (Lee and Kim, 1997); *6-pgd* (not shown), *Est-3*, *Est-4* (Fig. 1B)] is consistent with our assumption that the two Korean freshwater *Corbicula* clonal lineages are of interspecific hybrid origin.

A number of studies, in general, show agreement between mtDNA and allozyme analyses but this is not always the case for asexually reproducing polyploid taxa with interspecific hybrid origin. If maternal transmission of mtDNA genome is the case for Corbicula, like almost the other animal taxa (Avise, 1986; Hayashi et al., 1978), the complete mtDNA uniformity found between the two Korean lineages likely reflects the evolutionary recency of common maternal origin. In contrast, the pronounced level of distinct allelic differences and substantial genetic differentiation in the nuclear genome structure discovered between two clonal lineages may result from different paternal ancestry. The evident discrepancy between mtDNA sequences and nuclear genomes revealed in this study provides a compelling evidence for the existence of interspecific nuclear hybrid genome structures, with different combinations of paternal and maternal contributions. This finding is the first atypical case demonstrating that morphologically and allozymatically distinct, but mitochondrially identical clonal lineages exist in the genus Corbicula. Our data further suggest that the two Korean freshwater Corbicula clonal lineages are of interspecific hybrid origin with different paternal ancestry. However, we could not find the ancestral species for these two clonal lineages in the present study, and the answer for this question must wait until the genetic structure of Asian Corbicula taxa is fully characterized.

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#### References

Araujo, R., Moreno, D., and Ramos, M. A. (1993) The Asiatic clam, *Corbicula fluminea* (Müller, 1774) (Bivalvia: Corbiculidae) in Europe. Am. Malacol. Bull. 10, 39-49.

- Avise, J. C. (1986) Mitochondrial DNA and the evolutionary genetics of higher animals. *Phil. Trans. R. Soc. London B.* 312, 325–342.
- Avise, J. C. (2000) Phylogeography. The history and formation of species. Harvard University Press, London.
- Avise, J. C., Quattro, J. M., and Vrijenhoek, R. C. (1992) Molecular clones within organismal clones: mitochondrial DNA phylogenies and the evolutionary histories of unisexual vertebrates. *Evol. Biol.* 26, 225–246.
- Byrne, M., Phelps, H., Church, T., Adair, V., Selvakumaraswamy, P., and Potts, J. (2000) Reproduction and development of the freshwater clam *Corbicula australis* in southeast Australia. *Hydrobiologia* **418**, 185–197.
- Choe, B. L., Park, M. S., Jeon, L. G., Park, S. R., and Kim, H. T. (1999) Commercial molluscs from the freshwater and continental shelf in Korea. National Fisheries Research and Development Institute. Kuduk Pub. Co., Pusan.
- Clary, D. O. and Wolstenholme, D. R. (1985) The mitochondrial DNA molecular of *Drosophila yakuba*: nucleotide sequence, gene organization, and genetic code. J. Mol. Evol. 22, 252–271.
- Dawley, R. M. (1989) An introduction to unisexual vertebrates; in *Evolution and Ecology of Unisexual Vertebrates*, Dawley, R. M. and Bogart, J. P. (eds.), pp. 1–18, New York State Museum, Albany, New York.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* **3**, 294–299.
- Hayashi, J. I., Yonekawa, H., Gotho, O., Watanabe, J., and Tagashira, Y. (1978) Strictly maternal inheritance of rat mitochondrial DNA. *Biochem. Biophys. Res. Commun.* 83, 1032– 1038.
- Hillis, D. M. and Patton, J. C. (1982) Morphological and electrophoretic evidence for two species of *Corbicula* (Bivalvia: Corbiculidae) in North America. *Am. Midl. Nat.* **108**, 74–80.
- Ituarte, C. F. (1994) Corbicula and Neocorbicula (Bivalvia: Corbiculidae) in the Paraná Uruguay, and Río de La Plata basins. Nautilus 107, 129–135.
- Kijviriya, V., Upatham, E. S., and Woodruff, D. S. (1991) Genetic studies of Asian clams, *Corbicula*, in Thailand: allozymes of 21 nominal species are identical. *Am. Malacol. Bull.* 8, 97–106.
- Komaru, A. and Konishi, K. (1996) Ultrastructure of biflagellate spermatozoa in the freshwater clam, *Corbicula leana* (Prime). *Invert. Reprod. Dev.* 29, 193–197.
- Komaru, A. and Konishi, K. (1999) Non-reductional spermatozoa in three shell color types of the freshwater clam *Corbicula fluminea* in Taiwan. *Zool. Sci.* 16, 105–108.
- Komaru, A., Kawagishi, T., and Konishi, K. (1998) Cytological evidence of spontaneous androgenesis in the freshwater clam *Corbicula leana* Prime. *Dev. Genet. Evol.* 208, 46–50.
- Konishi, K., Kawamura, K., Furuita, H., and Komaru, A. (1998) Spermatogenesis of the freshwater clam *Corbicula aff. fluminea* Müller (Bivalvia: Corbiculidae). J. Shellfish Res. 17, 185–189.
- Kraemer, L. R. (1983) Ontogenetic aspects of biflagellate sperm

in Corbicula fluminea (Müller) (Bivalvia: Sphaeriacea). Trans. Am. Microsci. Soc. 102, 88.

- Kwon, O. K. (1990) Illustrated encyclopedia of fauna and flora of Korea. Vol. 32. Mollusca (I), Ministry of Education, Seoul.
- Kwon, O. K., Min, D. K., Lee, J. R., Lee, J. S., Je, J. G., and Choe, B. L. (2001) Korean mollusks with color illustration. Hanguel Pub., Co., Pusan.
- Lee, J. S. and Kim, J. B. (1997) Systematic study on the genus *Corbicula* (Bivalvia: Corbiculidae) in Korea. *Korean J. Syst. Zool.* 13, 233–246. (in Korean with an English abstract).
- Martens, E. (1905) Koreanische Süsswasser-Mollusken. Zoologischen Jahrbüchern Suppl. 8, 23–70, Pls. 1–3.
- McLeod, M. J. (1986) Electrophoretic variation in North American Corbicula. Am. Malacol. Bull. Special Ed. 2, 125–132.
- McMahon, R. F. (1991) Mollusca: Bivalvia; in *Ecology and Classification of North American Freshwater Invertebrates*, Thorp, J. H. and Covich, A. P. (eds.), pp. 315–399, Academic press, New York.
- Morton, B. (1986) *Corbicula* in Asia-an updated synthesis. *Am. Malacol. Bull. Special Ed.* **2**, 113–124.
- Morton, B. (1997) The aquatic nuisance species problem: a global perspective and review; in *Zebra Mussels and Aquatic Nuisance Species*, D'Itri, F. M. (ed.), pp. 1–54, Ann Arbor Press, Ann Arbor, MI.
- Okamoto, A. and Arimoto, B. (1986) Chromosomes of Corbicula japonica, C. sandai and C. (Corbiculina) leana (Bivalvia: Corbiculidae). Venus 45, 194–202.
- Oyama, K. (1943) Study on East Asian Corbicula family. 2. On Korean Corbicula. Jpn. J. Malacol. (Venus) 12, 150–158.
- Park, G. M., Yong, T. S., Im, K. I., and Chung, E. Y. (2000) Karyotypes of three species of *Corbicula* (Bivalvia: Veneroida) in Korea. J. Shellfish Res. 19, 979–982.
- Park, J. K. and O'Foighil, D. (2000) Genetic diversity of Oceanic island *Lasaea* (Mollusca: Bivalvia) lineages exceeds that of continental populations in the Northwestern Atlantic. *Biol. Bull.* **198**, 396–403.
- Renard, E., Bachmann, V., Cariou, M. L., and Moreteau, J. C. (2000) Morphological and molecular differentiation of invasive freshwater species of the genus *Corbicula* (Bivalvia, Corbiculidea) suggest the presence of three taxa in French rivers. *Mol. Ecol.* 9, 2009–2016.
- Siripattrawan, S., Park, J. K., and O'Foighil, D. (2000) Two lineages of the introduced Asian freshwater clam *Corbicula* occur in North America. J. Moll. Stud. 66, 423–429.
- Tsoi, S. C. M., Lee, S. C., Wu, W. L., and Morton, B. (1991) Genetic variation in *Corbicula fluminea* (Bivalvia: Corbiculoidea) from Hong Kong. *Malac. Rev.* 24, 25–34.
- Vrijenhoek, R. C. (1994) Unisexual fish: model systems for studying ecology and evolution. *Annu. Rev. Ecol. Syst.* 25, 71–96.
- Whang, I.-J., Jung, J., Park, J.-K., Min, G. S., and Kim, W. (2002) Intragenomic length variation of the ribosomal DNA intergenic spacer in malaria vector, *Anopheles sinensis*. *Mol. Cells* 14, 158–162.
- Wilson, C. C. and Hebert, P. D. N. (1992) The maintenance of taxon diversity in an asexual assemblage: an experimental analysis. *Ecology* 73, 1462–1472.