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Two *Corbicula* (Corbiculidae: Bivalvia) mitochondrial lineages are widely distributed in Asian freshwater environment

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Abstract

We investigated the biogeography of Asian *Corbicula* using mitochondrial gene sequence variation for *Corbicula* members sampled from 24 localities of eight Asian regions. A total of 210 individuals were genetically characterized by examining sequence variations of a 614 bp fragment of the mitochondrial cytochrome oxidase I (COI) gene. Phylogenetic analyses of the COI dataset revealed that *Corbicula* members are subdivided into two well-supported clades: estuarine and freshwater. A robust dichotomy between the Japanese/Korean (*Corbicula japonica*) and Chinese (*Corbicula fluminalis*) estuarine forms was evident, suggesting that these two regional populations represent a deep phylogeographic split. Our mitochondrial gene tree showed that among the freshwater members, two *Corbicula* mitochondrial lineages are the most common, having attained extensive geographic distribution in the Asian freshwater environment. While the present study provides significant biogeographic information on Asian *Corbicula*, a comprehensive phylogenetic study by cross-referencing the mitochondrial-based *Corbicula* phylogeny with nuclear gene data is required to fully understand the evolutionary origin(s) of triploidy/clonality in this genus. © 2003 Elsevier Science (USA). All rights reserved.

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1. Introduction

The introduction of exotic species has often caused many problems to native ecosystems (Avise, 1992; Garton and Johnson, 2000; Morton, 1997; Stepien et al., 1999; Williamson, 1996) and the rapid spread and subsequent ecological success of invader species are often closely linked with species-specific biological attributes, such as physiology, habitat preference, life history, and reproductive mode (di Castri, 1990; Wilson and Naish, 2001). Therefore, well-defined species-level taxonomy, genetic diversity, and present-day biogeographic information may help to provide more effective management guidelines for exotic biofouling species as well as being of intense interest to evolutionary biology (Avise, 1992; Hedrick et al., 2001; Mulvey et al., 1998; Sasaki et al., 2001).

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The genus Corbicula is a morphologically variable and taxonomically complicated group of bivalves that are most commonly found in lakes, rivers and estuarine systems of Asia (McMahon, 1983). The members of this genus are reproductively and developmentally heterogeneous. While estuarine Corbicula fluminalis sensu (Morton, 1986) and Japanese Lake Biwa endemic Corbicula sandai are both diploid sexual species and retain gonochoristic, non-brooding, and indirect developmental modes (Fukurawa and Mizumoto, 1953; Komaru et al., 1997; Okamoto and Arimoto, 1986; Sakai et al., 1994), most other freshwater members are known to be hermaphroditic, brooding, triploid, and ameiotic clonal lineages (Byrne et al., 2000; Komaru et al., 1997; Komaru et al., 1998; Miyazaki, 1936; Okamoto and Arimoto, 1986; Park et al., 2000).

Over the last few decades, freshwater *Corbicula* have invaded the drainage systems of North America, South America, and many European countries (Araujo et al., 1993; Bij de Vaate and Greijdanus-Klaas, 1990; Howlett

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and Baker, 1999; Ituarte, 1994; McMahon, 1983; Morton, 1986). Their rapid spread has had a serious ecological impact on the native freshwater fauna (Britton and Morton, 1982; MacIsaac, 1996; Mills et al., 1996; Morton, 1997). In spite of its ecological significance, *Corbicula* systematics and biogeography are poorly understood to date, primarily due to the extraordinary range of morphological variation (Kijviriya et al., 1991; Tsoi et al., 1991). Traditionally, many hundreds of species names have been assigned to Asian *Corbicula* alone based on only a few conchological characters (Britton and Morton, 1982), but only a small fraction of these is considered taxonomically valid (Araujo et al., 1993; Kijviriya et al., 1991; Morton, 1986).

In a review on Asian Corbicula, Morton (1986) proposed a dual-species model for global members of this genus largely based on a suite of morphological and life history characteristics: the freshwater form, Corbicula fluminea (Müller, 1774) and estuarine form, C. fluminalis (Müller, 1774). Recently, however, a growing body of independent genetic evidence has challenged Morton's dual-species model. Molecular surveys of different regional taxa (Japan, Korea, North America, Europe, etc.) based on either allozyme (Hatsumi et al., 1995; Hillis and Patton, 1982; Lee and Kim, 1997) or mtDNA genetic markers (Renard et al., 2000; Siripattrawan et al., 2000) suggested the existence of genetically divergent multiple Corbicula lineages, which is incongruent with Morton's proposal. However, it must be noted that these works were primarily centered on the genetic structures of limited regional taxa. For example, Renard et al. (2000) disclosed important data on the distribution of European freshwater Corbicula, but this study was restricted entirely to non-indigenous freshwater taxa lacking native Asian populations. Siripattrawan et al. (2000) performed the first comparative mtDNA survey to test the competing hypothesis on whether two North American freshwater forms represent two independent lineages or a morphologically variable single lineage. They found morphotype-specific North American mtDNA genotypes that were respectively identical to haplotypes encountered in Japanese and Korean triploid *Corbicula*, and concluded that two freshwater lineages were introduced into North America. Although additional information on the phylogeny among a limited selection of Asian Corbicula taxa was revealed in the survey, a large fraction of the genetic diversity and biogeography of native Asian Corbicula still remains unknown.

In the present study, we utilized mitochondrial gene sequence variation to investigate the biogeography of Asian *Corbicula*. We genetically characterized Asian *Corbicula* populations based on mitochondrial COI sequence data. Additionally, we combined Asian mtDNA genotype variations with pre-existing COI datasets (Baldwin et al., 1996; Renard et al., 2000; Siripattrawan et al., 2000) to elucidate the present-day biogeography of globally occurring freshwater members, including both native and non-indigenous exotic populations.

2. Materials and methods

2.1. Sampling and DNA Sequencing

The Corbicula specimens in this study were sampled from a wide range of Asian freshwater and estuarine systems. Table 1 displays details of sampling localities and the number of Corbicula specimens sequenced in this study. A total of 210 individuals sampled from 24 collection localities of eight Asian regions: Japan, Korea, China, Taiwan, Malaysia, Indonesia, Thailand, and Vietnam. Five additional specimens sampled from an exotic region (Spain) were sequenced and incorporated into the dataset. DNA templates were extracted from the mantle tissues of individual specimens using a DNeasy tissue kit (Qiagen). The target gene fragment was amplified by polymerase chain reaction (PCR) using the universal COI primer sets designed by Folmer et al. (1994): LCO1490 (5'-GGTCAACAAATCATAAAGA-TATTGG-3'), and LCO2198 5'-TAAACTTCAGGGT GACCAAAAAATCA-3'). A negative control (no template) was included in each run of 35 cycles of amplification (1 min 94 °C denaturing; 30 s 45 °C annealing; 1 min 72 °C extension). Double-stranded products were isolated on 1% agarose gels, excised under long-wavelength UV light and extracted using a GeneClean (Bio 101) NaI/glass powder kit. Both strands of target fragments were cycle-sequenced using a Big Dye DNA sequencing kit (PE Applied Biosystems) and reaction products were electrophoresed on an ABI 310 automated DNA sequencer. Although an approximately 710 bp region of the mitochondrial COI gene was amplified and directly sequenced, only the 614 bp fragment was selected as the dataset for phylogenetic analyses to avoid erroneous sequence determination at the 5'- and 3'-end regions.

2.2. Data analyses

The novel COI genotype sequences identified were combined with those previously published (Baldwin et al., 1996; Renard et al., 2000; Siripattrawan et al., 2000). Sequence alignments were initially performed using the CLUSTAL option of Sequence Navigator 1.0.1 (Kececioglu and Myers, 1994) and optimized manually. Phylogenetic analyses were conducted by neighbor-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) methods using the 4.0b8 version of PAUP (Swofford, 2001) with two veneroid species [*Mercenaria mercenaria* (GenBank Accession No.: U47648) and *Rangia cuneata* (GenBank Accession

Table 1

Geographic origin and the number of Corbicula samples used in this study

Locality	Abbreviation of locality	No. of individuals sequenced
Freshwater		
Lake Uiam, Korea	KR1	$10(8+2^{a})$
Taehwa River, Korea	KR2	10
North Han River, Korea	KR3	10
Somjin River, Korea	KR4	10
Toyo River, Aichi Prefecture, Japan	JP1	$11(8+3^{a})$
Itoshima-gun, Fukuoka Prefecture, Japan	JP2	10
Lake Biwa, Shiga Prefecture, Japan	JP3	2ª
Motohama, Motooka, Nishi-ku, Fukuoka, Japan	JP4	6
Lake Tong-Ting, China	CN1	16
Lake Qingshan, China	CN2	15
Lake Poyang, China	CN3	14
Ho-Long River, Taiwan	TW1	12
Drainage of Rice farm, I-Lan, Taiwan	TW2	8
Yan-Ming, Taiwan	TW3	8
Langat River, Malaysia	MY	10
Taman Sari, Ciapus, Ciomas, Bogor, Indonesia	ID1	17
Muara, Cikaret, Bogor, Indonesia	ID2	8
Harapan Jaya village, Cibinong, Indonesia	ID3	10
Tugu Jaya village, Cigombong, Bogor, Indonesia	ID4	8
Lampao Dam, Kalasin Province, Thailand	TH	$8(6+2^{a})$
Long Xuyên, Vietnam	VT	7
Miňo River at Goian, Spain	SP	5
Estuarine		
Lake Pomae (saline). Korea	KR5	4
Estuary of Toyo River, Aichi Prefecture, Japan	JP5	2^{a}
Estuary of Yangtz River, China	CN3	5

^a Number of individuals sequenced in the previous study (Siripattrawan et al., 2000).

No.: U47652)] designated as outgroups. In order to test the monophyly of Corbicula, the COI sequences of two New World corbiculids [Polymesoda caroliniana (Gen-Bank Accession No.: AF196276), Neocorbicula limosa (GenBank Accession No.: AF196277)] were included in the phylogenetic analysis. For NJ and ML analyses, the general time reversible model with among-site rate heterogeneity (GTR+ Γ) was selected as the best-fit DNA substitution model for our dataset using Modeltest 3.06 (Posada and Crandall, 1998). The parameters estimated were a substitution rate matrix ([A-C]=0.4604, [A-G = 11.9805, [A-T] = 2.1328, [C-G] = 1.7651, [C-T = 3.9751, and [G-T] = 1.0000) with unequal base frequencies (A = 0.2222, C = 0.1255, G = 0.2146, and T = 0.4377) and Γ distributed among-site rate heterogeneity ($\Gamma = 0.3983$). The pairwise genetic distance matrix among taxa was estimated using the selected substitution model (GTR+ Γ). MP and ML analyses were performed with the heuristic search option using random stepwise addition with 10 replications. Branch support levels of NJ and MP trees were estimated with bootstrapping (1000 heuristic iterations using random stepwise addition with 10 replications). The sequence length of two European haplotypes (II and IV) reported exclusively by Renard et al. (2000) is 52 bp shorter than that of our dataset. For this reason, we excluded these

two haplotype sequences from the dataset for phylogenetic analyses.

3. Results

3.1. Geographic distribution of Asian mtDNA genotypes

In this study, 16 novel mtDNA COI genotypes were detected from a total of 210 Corbicula individuals sampled from 24 sites of eight Asian regions (Japan, Korea, China, Taiwan, Malaysia, Indonesia, Thailand, and Vietnam). No novel haplotype was identified from the exotic population analyzed (Spain) and its mitotype sequence was identical to the Asian FW5 haplotype. A combination of our novel haplotypes with the pre-existing COI dataset obtained from previous studies (Renard et al., 2000; Siripattrawan et al., 2000) yielded a total of 25 mtDNA genotypes, of which 19 were from freshwater members and the remaining six from estuarine forms (see Table 2). Among the 19 freshwater haplotypes, three (FW1, FW4, and FW5) were encountered in both native and introduced regions, while each remaining haplotype was restricted to a single location. Note that these three mtDNA genotypes were by far the most common in both Asian (158 of 210

Native range Haplotype: KR1 KR2 KR3 KR4 KR5 JP1 JP2 Freshwater 8+2 ^a 10 10 6 FW2 FW2 FW3 FW4 FW3 FW4 FW3 FW4 FW4														
Haplotype: KR1 KR2 KR3 KR4 KR5 JP1 JP2 Freshnater FW1 8+2 ^a 10 10 6 FW2 FW3							In	troduced rai	nge				Nos.	
Haplotype: KR1 KR2 KR3 KR4 KR5 JP1 JP2 Preshnater FW1 8+2 ^a 10 10 6 FW2 FW3 FW3							Ē	adouri			North America			
<i>Freshwater</i> FW1 8+2 ^u 10 10 6 FW2 FW3 FW4	P2 JP3 JP4 J	P5 CNI CN2 CI	43 CN4 TW1	TW2 TW3	MY IDI IE	12 ID3 ID4 TH VT	AU MD F.	R1 FR2 FR	3 FR4 NE	I NE2 SP	UT NM MI NC I	īz		
FWI 8+2 ⁴ 10 10 6 FW2 FW3 FW4														
FW2 FW3 FW4	4	10 4									3a 2a	52+7 ^a	AF196269	P, 2
FW3 FW4		9										б	AF457989	Р
FW4		3										б	AF457990	Ь
		5			13 8	10 8	3ª		8 ^a	6^{a}		44+17	 AF269096- 	8 P, 3
FW5 8 + 3 ^a 10	0 2	, S	4	8	10		⁸ 0	6 ^a 4 ^a	5 ^a	5	3 ^a 3 ^a	62+33	¹ AF196268	P, 2, 3
FW6		4									1	1a 1a	1147647	-
FW7								1a			•	19	AF269094	
FW8			×									~ ~	AF457991	. <u>-</u>
EW9			2		4) 1	AF457993	
FW10 4												4	AF457992	Ч
FWII												2	AF457994	Р
		2												
FW12												7	AF457995	Ь
		7												
FW13												4	AF457999	Ъ
		4												•
FW14						$6 + 2^{-4}$						-7+0I	AF1962/0	г, г
FW15						-						_	AF468017	<u>م</u>
FW16						2						7	AF468018	Ч.
FW17									5ª			Sa	AF269095	ŝ
C. sandai A	la]a	AF196272	2
C. snadai B	la											la	AF196273	2
AUST							la					la	AF196274	2
MADA							Ia					la	AF196275	7
Economico Actorizados														
C. ja-		2^{a}										С ^а	AF196271	7
ponica A														
C. japonica B 2												7	AF367440	Ч
C. japonica C												2	AF367441	Ч
C. fluminalis A			2									2	AF457996	Ч
C. fluminalis B			2									7	AF457997	Ч
C. fluminalis C			-									Г	AF457998	Ч

Table 2 Haplotype frequencies and distribution patterns of the 27 mtDNA COI genotypes [including two European haplotypes II (=FW7) and IV (=FW17] of Corbicula

532

individuals) and exotic freshwater samples (57 of 64 individuals; see Table 2 for details).

3.2. mtDNA variation and phylogeny of mtDNA genotypes

A total of 300 of the nucleotide positions were variable, 167 of which were phylogenetically informative under conditions of parsimony. No indels were observed and sequence differences in the dataset were unevenly distributed across the three codon positions, a typical pattern of mitochondrial protein-coding genes in most metazoan animal groups (Brown, 1985). Within the Corbicula members, most sequence variation was observed at the third position (86.0%), while the first and second positions were responsible for 12.0 and 2.0% of the remaining variable sites, respectively. The corrected pairwise sequence differences across the three codon positions were moderate, ranging from 0.2 to 13.0% among Corbicula members. However, these were remarkably increased (ranging from 0.5 to 64.4%) when estimated at the third codon position only. An alignment of the 25 COI haplotypes detected in the present study and the corrected pairwise distance matrix are available from the authors on request.

Maximum parsimony analyses of the COI dataset using two veneroid species (*M. mercenaria* and *R. cuneata*) as outgroup taxa resulted in four most equally parsimonious trees (tree length = 563; CI = 0.725; RI = 0.770). The semi-strict consensus of the four most parsimonious trees is shown in Fig. 1A. NJ trees under the general time reversible model with among-site rate heterogeneity (GTR+ Γ) are depicted in Fig. 1B. Tree topologies from MP, NJ, and ML (not shown) analyses were consistent with each other in that Corbicula members formed a monophyletic group relative to other members (N. limosa and P. caroliniana) of the family Corbiculidae and estuarine members formed a robust dichotomy. However, within the freshwater Corbicula clade, three phylogenetic methods yielded tree topologies incongruent with each other in terms of branching order, making it difficult to infer reliable interrelationships among shallow branches (Figs. 1A and B). We consider our dataset contains a relatively weak phylogenetic signal insufficient to resolve interrelationships among them. It becomes more evident that most of these shallow branches in the freshwater clade were collapsed when the bootstrap consensus tree topologies were inferred from MP and NJ analyses (see Fig. 2). Nevertheless, bootstrap consensus trees obtained with these methods yielded similar results, specifically that the freshwater clade is composed of seven major subclades or haplotype groupings (A-G in Fig. 2). Note that the application of the NJ criterion in phylogenetic analyses yielded much higher bootstrap values for most nodes. This may be partly due to the relatively few



Fig. 1. (A) Semi-strict consensus tree of the four equally most parsimonious trees (tree length = 563; CI = 0.725; RI = 0.770). (B) NJ tree based on the corrected distance matirix (GTR+ Γ) for our COI dataset. The numbers above or below the branches indicate bootstrap support values (\geq 50%).



Fig. 2. Bootstrap majority-rule consensus tree obtained from NJ and MP methods. The numbers above (NJ) and below (MP) the branches indicate bootstrap support values ($\ge 50\%$). Application of MP criterion yielded the same results with the NJ tree, with minor changes in terminal branches.

synapomorphic character states shared among terminal haplotypes. For example, although the FW1, FW2, FW3, and FW4 haplotypes differed from each other by a maximum of two base substitutions (average sequence divergence of 0.24%), the nodes for ((FW1, FW2, FW4) FW3) were collapsed in the strict consensus of four most parsimonious trees and received a bootstrap value of only 54% when the MP criterion was employed. However, bootstrap support for this node was remarkably increased to 82% in NJ analyses.

In our phylogenetic analyses, the monophyly of the genus *Corbicula* was robustly supported (a bootstrap value of 100%) and all members of this genus were subdivided into two well-supported clades: one representing estuarine forms (bootstrap values of 100%, both in NJ and MP) and the other including assemblages of globally distributed freshwater forms (bootstrap values of 96 and 76% from NJ and MP analyses, respectively). Among *Corbicula* members, the estuarine clade occupied a well-supported basal position (bootstrap values of 100% both from NJ and MP analyses, respectively) relative to the freshwater clade in which Japanese/Korean *C. japonica* formed a subclade sister to the Chinese estuarine species, *C. fluminalis* sensu (Morton, 1986). Within the freshwater clade, the African freshwater lineage (MADA) occupied a basal position with a high bootstrap value (100 and 96%) in NJ and MP analyses, respectively). A terminal polytomy was subsequently formed by the remaining multiple assemblages of mostly Asian/Australian freshwater forms. Despite the lack of phylogenetic information on the reliable branching order among freshwater components displaying polytomy, seven major subclades or haplotype branches (A-G) within the freshwater clade were evident from the bootstrap consensus tree topologies obtained with NJ and MP methods (Fig. 2). These subclades were distinguished from each other by a minimum of four nucleotide differences (subclade A/C). Among the 19 freshwater mtDNA genotypes detected, some formed well-defined haplotypic groupings (subclades A, B, D, and E), although subclade C received a relatively weak bootstrap support value (56 and 55% with NJ and MP analyses respectively). Of these, subclades A and B included multiple haplotypes encountered from widespread Asian populations, whereas the remaining three were restricted to a single location (E) or narrow geographic distributions (C and D). Notably, many Asian regional populations (Japan, Korea, China, and Indonesia) encompassed more than two divergent haplotypes that were resolved into the separate subclades A and B. Our Japanese freshwater samples, except Lake Biwa endemic C. sandai, comprised two divergent haplotypes (FW1 and FW5 with 2.85% sequence divergence) that were nested within the well- defined subclades A and B, respectively. The Korean freshwater populations were predominated by the haplotype FW1 (36/40 samples), whereas the rare haplotype FW10 (4/40 Korean freshwater samples) was basal to the rest of subclade B. The Indonesian samples contained two divergent haplotypes (FW4 and FW9) that were nested within the subclades A and B, respectively. Each of our Chinese specimens sampled from two localities (Lakes Qingshan and Poyang) comprised a mixture of two genetically divergent lineages. With the exception of two Lake Qingshan individuals displaying sequences identical to FW1, the remaining 13 individuals formed a separate subclade C. The Lake Poyang population comprised three haplotypes identical to FW1, FW4, and FW5, respectively. In subclade D, the Thailand samples were monomorphic to FW14 and the Vietnamese haplotypes were either identical to or closely related with that of the Thai population.

4. Discussion

4.1. Phylogenetic relationships and biogeography of Asian Corbicula

Phylogenetic analyses of the COI dataset yielded two major distinct clades within the genus *Corbicula*: estuarine (composed exclusively of brackish members) and freshwater (exclusively comprising freshwater members). Our analyses strongly support the sister status of the estuarine clade to all its freshwater congeners, consistent with previous results from karyological (Okamoto and Arimoto, 1986), allozymatic (Hatsumi et al., 1995) and mtDNA studies on *Corbicula* (Siripattrawan et al., 2000). In addition, the COI gene trees obtained from the present study have novel implications for the existence of two genetically differentiated brackish *Corbicula* lineages. A robust dichotomy between the Japanese/Korean (*C. japonica*) and Chinese (*C. fluminalis*) estuarine

 Table 3

 Summary of reproduction modes found in *Corbicula*

forms was consistently supported, suggesting that these two regional populations may represent a deep phylogeographic split (average sequence divergence of 2.04% between the clades). This result is in contrast with Morton's (1986) proposal that the Chinese form (*C. fluminalis*) is the only taxonomically valid estuarine species representing all global brackish members of this genus. To resolve the question of whether these represent a genetically differentiated single species or two well-established species, more comprehensive evidence is required. Genetic characterization based on more extensive sampling from East Asian estuarine regions is currently in progress for the detailed elucidation of this issue.

Apart from the best NJ tree, the multiple haplotype assemblages representing the Asian/Australian freshwater forms displayed a terminal polytomy in bootstrapped NJ and MP consensus trees (Fig. 2). Interestingly, the freshwater clade is composed of a mixture of genetically divergent and reproductively heterogeneous freshwater lineages. Multiple empirical evidence published so far, including cytology, spermatomorphology and allozymes, strongly suggests that the majority of freshwater Corbicula members maintain triploidy/clonal entity in this group (Table 3). Most of the Japanese and Korean freshwater Corbicula (represented here by mtDNA genotypes of FW5 and FW1, respectively) are triploid [3n = 54 (*Corbicula leana* sensu Okamoto and Arimoto, 1986; C. fluminea sensu Park et al., 2000)] and the reproductive mode of Japanese C. leana (sensu Komaru et al., 1998) is androgenetic (i.e., only chromosomes derived from unreduced spermatozoa contribute to zygote development). A number of independent lines of spermatogenic evidence consistently demonstrate that many other regional freshwater Corbicula [North America (Kraemer, 1983); Japan (Komaru and Konishi, 1996; Konishi et al., 1998), Taiwan (Komaru and Konishi, 1999); Australia (C. australis sensu Byrne et al., 2000); Korea (Park et al., 2002)] produce biflagellate sperm, which is a well-known diagnostic marker for clonality in this genus (Byrne et al., 2000; Komaru et al., 1997; Siripattrawan et al., 2000). An allozyme study revealed a close resemblance

Taxa	Geographic origin	Reproduction mode	Habitat	Reference
C. japonica	Japan	Sexual	Estuarine	Asahina (1941)
C. sandai	Japan	Sexual	Freshwater	Komaru et al. (1997)
C. leana	Japan	Clonal	Freshwater	Komaru et al. (1998)
C. fluminea	Japan	Clonal	Freshwater	Komaru et al. (1997)
	Taiwan	Clonal	Freshwater	Komaru and Konishi (1999)
	Korea	Clonal ^a	Freshwater	Park et al. (2002)
	North America	Clonal ^a	Freshwater	Siripattrawan et al. (2000)
C. australis	Australia	Clonal ^a	Freshwater	Byrne et al. (2000)

^a Evidenced by biflagellate sperm morphology.

of the genetic structure of Thai Corbicula populations to the Japanese ameiotic congener (Kijviriya et al., 1991). Unlike most other members in the freshwater clade, the Japanese Lake Biwa endemic C. sandai Reinhardt, 1878 produces uniflagellate sperm (Komaru and Konishi, 1996) and is the only known non-brooding meiotic diploid freshwater species (Fukurawa and Mizumoto, 1953; Hachiri and Higashi, 1970; Komaru et al., 1997; Okamoto and Arimoto, 1986). Therefore, it was considered as a parental species for the Japanese triploid Corbicula (Okamoto and Arimoto, 1986). Although the MP analyseis of our COI dataset placed C. sandai basal to the remaining Asian/Australian freshwater taxa (Fig. 1A), this positioning is far from robust (bootstrapping value of <50%). Our mtDNA gene tree does not contain a convincing phylogenetic signal either supporting or rejecting the proposition that C. sandai represents an ancestral species for members of the Asian/Australian freshwater clade.

Despite the lack of phylogenetic resolution among the freshwater mtDNA lineages, our gene tree suggests that the freshwater Corbicula is composed of genetically divergent multiple lineages (subclades A-G). Although clonally reproducing asexual lineages are not biological species in the usual sense, our result indicates that there are genetically divergent and reproductively heterogeneous multiple evolutionary lineages in freshwater Corbicula. Significantly, the distribution pattern of mtDNA haplotypes observed in this study provides valuable information on the biogeography of Asian freshwater Corbicula. Our COI dataset reveals that most of the Asian regional populations (Japan, Korea, China, and Indonesia) include more than two divergent haplotypes grouped into two subclades (A and B). It is worth noting that within this Asian mtDNA genotypic diversity, two haplotypes (FW1 and FW5) are the most commonly found across the Asian range. Of seven freshwater lineages in Fig. 2, two Corbicula mitochondrial lineages [subclades A (FW1-4) and B (FW5, 6, 8-10)] in particular, represented by an assemblage of multiple mtDNA genotypes, account for the majority of freshwater individuals (180 of 210; 85.7%) sampled from multiple Asian populations [subclade A (Korea, Japan, China, and Indonesia) and subclade B (Japan, Taiwan, Malaysia, China, and Indonesia); see Table 2 and Fig. 2]. Our results strongly suggest that these two freshwater Corbicula mitochondrial lineages are the most common, having attained extensive geographic distribution in Asian freshwater environments.

4.2. Mitochondrial genotype distribution and biogeography of exotic freshwater Corbicula

The present study provides subsidiary, but significant information on the present-day distribution of exotic freshwater *Corbicula*. When compared with results previously recorded from exotic regions (e.g., Europe and North America), it becomes clear that a small fraction of Asian genotypic diversity has invaded exotic freshwater environments. Indeed, the haplotypic identity between native and exotic members was evident: among three North American haplotypes, two were identical to the Asian haplotypes, FW5 and FW1, respectively (Siripattrawan et al., 2000). Renard et al. (2000) uncovered three European freshwater lineages comprising four mtDNA COI genotypes (European haploytpes I, II, IV, and V), two of which were shared by Asian populations: The European haplotype I was identical to FW5, one of the most common Asian freshwater system members, while European haplotype V was identical to FW4 natively found in Indonesia and Lake Poyang of China. Despite our sampling efforts from the native range, we identified no Asian haplotypes identical to other exotic lineages and/or haplotypes [FW6, FW7 (=European II), and FW17 (=European IV)]. Moreover, the two European haplotypes (II, IV) reported by Renard et al. (2000) are 52 bp shorter in length than those found in our dataset. For this reason, the extra section of our original dataset was excised and additional phylogenetic analyses based on the truncated COI dataset were also performed. This analysis yielded tree topologies nearly identical to those in Fig. 2 (data not shown), but some shallow branches in the freshwater clade were collapsed or received relatively weak supporting values. We consider this is due in part to loss of informative sites that are contained in the excised regions of our original dataset.

Of 19 freshwater mtDNA genotypes in the combined global COI dataset, three (FW1, FW4, and FW5) representing most of the two predominant Asian mitochondrial lineages account for the majority of exotic samples (57 of 64; 89.1%) and are widespread in the introduced range, specifically, Europe [France, the Netherlands (Renard et al., 2000); Spain (this study)], North America (Siripattrawan et al., 2000), and South America (D.O' Foighil, personal communication). Although confirmative evidence for the clonality of samples possessing FW4 (which differs from FW5 by a single base substitution) is not yet available, data from empirical studies on karyology (Okamoto and Arimoto, 1986; Park et al., 2000) and spermato-morphology (Komaru and Konishi, 1996; Komaru and Konishi, 1999; Konishi et al., 1998; Park et al., 2002) strongly suggest that our samples, represented by the FW1 and FW5 haplotypes, respectively, maintain a triploidy/ clonal entity. This indicates that the two predominant Asian freshwater clonal lineages have successfully undergone wide-range expansion in exotic freshwater fauna. This interpretation is consistent with the results of previous mtDNA surveys [North America (Siripattrawan et al., 2000); Europe (Renard et al., 2000)] and additionally corroborated by the most recent mtDNA

The invasion of exotic mollusks, including Corbicula, is directly or indirectly linked with human activities (McMahon, 1991; Morton, 1997). Moreover, the ecological success of invader species is often closely associated with species-specific biological attributes, such as physiology, habitat preference, life history, and reproductive mode (di Castri, 1990; Wilson and Naish, 2001). As a short-term evolutionary benefit, the hermaphroditic asexual lineages would appear to have a colonization advantage over outcrossing sexual congener because they may be able to establish themselves in a new habitat with a relatively smaller propagule size (Beukeboom and Vrijenhoek, 1998; Sasaki et al., 2001; Wetherington et al., 1987). Indeed, *Corbicula* are unique among studied freshwater bivalves in that it includes both gonochoristic, diploid, meiotic (Fukurawa and Mizumoto, 1953; Hachiri and Higashi, 1970; Komaru et al., 1997; Okamoto and Arimoto, 1986) and self-fertilizing hermaphroditic, triploid, ameiotic lineages [C. leana (sensu Miyazaki, 1936; Okamoto and Arimoto, 1986; Komaru et al., 1997, 1998) and C. fluminea (sensu Morton, 1977; Morton, 1982; Kraemer, 1983; Park et al., 2000)]. In contrast to the geographic distribution of the diploid sexual species, C. sandai, which is limited to the Japanese Lake Biwa, it is clear that two major Asian self-fertilizing hermaphroditic, triploid, clonal lineages have attained extensive global geographic distribution. The ecological success and rapid-range expansion of these two prevalent Asian freshwater Corbicula lineages in exotic environments may have been facilitated by their unusual developmental and reproductive traits, such as high fecundity, self-fertilizing hermaphroditism and clonality (Britton and Morton, 1982; Byrne et al., 2000; McMahon, 1983), which moderate the requirements for successful colonization.

Despite the biogeographic insights presented in this study, our COI gene tree provides limited information on the phylogenetic relationships of Asian freshwater Corbicula. In most animal taxa, the acquisition of clonal reproduction may be a consequence of hybridization between sexual species (Dawley, 1989; Vrijenhoek, 1994; Wilson and Hebert, 1992). Most clonal lineages therefore contain a mixture of the nuclear genomes of two or more sexual species (Avise et al., 1992). As with most cases of other clonally reproducing animal taxa with interspecific hybrid origin, our mitochondrial COI gene tree merely represents matrilineal genealogy. Based on our preliminary surveys on mtDNA and allozyme characterization, the discrepancy between mtDNA and nuclear genomic structures is real for Korean populations (Park et al., 2002), indicating that mtDNA identity does not necessarily correlate with the identity of nuclear genomic

structure. In this case, the mtDNA gene tree is a poor representation of phylogenetic relationships among freshwater *Corbicula* lineages. A comprehensive phylogenetic study by cross-referencing the mitochondrialbased *Corbicula* phylogeny with nuclear gene data is required to elucidate the evolutionary origin(s) of triploidy/clonality in this genus. Empirical evidence from Asian populations that have not been characterized for ploidy level and reproduction mode is also critical for resolving taxonomic complexities of this genus.

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