

Invasion of the clonal clams: *Corbicula* lineages in the New World*

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Abstract: Unknown in the New World prior to 1924, members of the exotic bivalve genus *Corbicula* now ranges from Michigan to Patagonia and are among the most common freshwater molluscs in the Americas. Michigan populations are triploid and share a distinct shell morphotype (Form A), mitochondrial (mt) haplotype, and nuclear ribosomal genotype with New World populations spanning temperate, subtropical, and tropical watersheds over 85° of latitude. Two additional mt and nuclear ribosomal lineages were detected in a minority of New World populations; all three produced biflagellate sperm, a morphological marker for clonality in this clam taxon. Two of 12 New World samples exhibited qualitatively distinct nuclear/organelle genetic disjunctions. One of these (Iguazú Falls) appears to have resulted from hybridization, but the other (Rio Grande Form A) appears inconsistent with hybridization, and we propose that it may have resulted from *in situ* androgenetic clonal capture. These clones of *Corbicula* are but several of hundreds of recent New World freshwater exotics. Their extraordinarily rapid bicontinental spread may well be a portent of things to come. We anticipate that over the next decades many other New World freshwater exotics will gradually attain extensive bicontinental distributions.

Keywords: alien clam, freshwater, androgenesis, phylogeny, genetic disjunction

Continental ecosystems are being profoundly reshaped by ongoing human-mediated transoceanic biotic exchange (Vermeij 1996). Freshwater biotas that were previously insulated by oceanic barriers to dispersal are now vulnerable to inter-continental alien introductions, and a large variety of such invasive taxa have recently become established in New World watersheds (Benson 1999). In North America, much research attention is focused on the Eurasian “zebra mussel,” *Dreissena polymorpha* (Pallas, 1771) (Nalepa and Schoesser 1993). The geographic scale of its spread in the New World, however, is dwarfed by that of the “Asian clam” *Corbicula* Mühlfeld, 1811, another freshwater alien.

Individuals of the genus *Corbicula* were first recorded in the New World in western North America almost 80 years ago, and, after breaching the Continental Divide in the late 1950s, members of the genus rapidly extended across the continental USA and into Mexico (McMahon 1999). In South America, the genus has spread extensively in the quarter century since its first detection in southern Brazil and Argentina (Ituarte 1994), becoming established in Venezuela in the 1980s (McMahon 1999) and subsequently in the Pannatal (Callil and Mansur 2002), as well as the lower (Beasley and Tagliaro 2001) and upper (this study) sections of the

Amazonian drainage system. At present, New World populations of *Corbicula* range from Lake Superior (Ward and Hodgson 1997) to Patagonia (Cazzaniga 1997), dominate the benthic macrofauna of many warm temperate-to-tropical New World watersheds, and frequently impede domestic and industrial water supply infrastructure (Ituarte 1994, McMahon 1999). Our aim in this study was to perform a phylogenetic analysis of this hyper-invasive alien focusing on its current New World distribution, a task complicated by the unusual genetic structure and obscure sister relationships of many freshwater lineages of *Corbicula*.

Background on Old World Species of *Corbicula*

Prior to the global transoceanic spread of freshwater lineages in the 20th century, the extant range of the clam genus *Corbicula* was restricted to Eurasia (excluding Western Europe), Africa, Madagascar, and Australia (Pilsbry and Bequaert 1927). The most contentious issue in the taxonomy of *Corbicula* has traditionally involved the status of hermaphroditic Asian freshwater lineages. They exhibit considerable shell phenotypic variation, at least some of which is ecophenotypic (Kijviriya *et al.* 1991), and Morton (1986) proposed that there exists but a single species of freshwater

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Corbicula. Subsequent research has shown this to be an oversimplification. In addition to distinct gonochoric freshwater species in Japan (Lake Biwa; Hurukawa and Mizumoto 1953), Indonesia (Glaubrecht and Korniusshin 2003), and, as an exotic, Western Europe (Rajagopal *et al.* 2000), a variety of hermaphroditic clonal lineages have been uncovered in Asia. These latter lineages lack meiosis, generate unreduced biflagellate sperm cells, and come in diploid (Komaru *et al.* 1997, Komaru and Konishi 1999, Park *et al.* 2000), triploid (Okamoto and Arimoto 1986, Komaru *et al.* 1997, Komaru and Konishi 1999, Park *et al.* 2000, Qiu *et al.* 2001), and tetraploid (Qiu *et al.* 2001) genomic iterations. Remarkably, at least some of these clonal lineages, including diploid and triploid clones, are androgenetic, an unusual method of reproduction in which the embryonic nuclear genome is inherited paternally (derived from an unreduced sperm nucleus) and the maternal nuclear genome is ejected via the polar bodies (Komaru *et al.* 1998, 2000, Ishibashi *et al.* 2002, 2003, McKone and Halpern 2003). Pending identification of convincing sexual parental species, the evolutionary relationships and taxonomic nomenclature of these diverse clones remain obscure (Siripattrawan *et al.* 2000). Identifying parental taxa may be relatively complicated as emerging data suggest that nuclear/mitochondrial disjunctions and introgressive events may be quite common in freshwater lineages of *Corbicula* (Park *et al.* 2002, Pfenninger *et al.* 2002).

Background on Alien New World *Corbicula*

The systematic uncertainty concerning hermaphroditic Asian freshwater *Corbicula* taxa has inevitably complicated the study of exotic New World lineages. Three distinct shell morphotypes have been distinguished. Two morphotypes occur in North America (Hillis and Patton 1982), a widespread "Form A" and a southwestern "Form B" (Britton and Morton 1986) (Fig. 1). A third morphotype, "Form C" (Fig. 1), previously identified as *Corbicula largillerti* (Philippi, 1844), has been described in La Plata, Argentina (Ituarte 1994). All three New World morphotypes are hermaphroditic (McMahon 1991, Ituarte 1994). Attempts to genetically characterize New World populations of *Corbicula* have been restricted to North American temperate populations where the Form A and Form B morphotypes represent two distinct allozymic (Hillis and Patton 1982, McLeod 1986) and mitochondrial (Siripattrawan *et al.* 2000) lineages. Preliminary karyological characterization has been performed on samples of Form A and Form B morphs and both have been found to be triploid ($3n = 54$), to lack meiotic metaphases, and to produce unreduced sperm (Burch *et al.* 1998, J. B. Burch, pers. comm.).

The rapid spread of multiple alien morphotypes and lineages of *Corbicula* across bicontinental New World temperate, subtropical, and tropical watersheds raises obvious

questions regarding the genetic diversity associated with this enormous range extension. Our study aimed to provide an initial estimate of genetic structuring in these exotic populations, using both mitochondrial and nuclear markers.

MATERIALS AND METHODS

Sampling

Live animals were sampled at each of the 12 study locations, which collectively spanned the bicontinental New World range. All specimens sampled outside of Michigan were preserved in 95% ethanol prior to shipment. Voucher specimens for each location have been deposited in the University of Michigan's Museum of Zoology Mollusk Division. Details of the sampling site locations, date sampled, collector, UMMZ catalog number, and GenBank Accession numbers are given in Table 1.

Morphotype Designation

Freshwater morphs of *Corbicula* exhibit a significant degree of ecophenotypic variation in shell phenotype, especially in shell outline, shape, sculpture, and pigmentation (Prezant *et al.* 1988, Kijviriya *et al.* 1991, Tsoi *et al.* 1991, Ituarte 1994). Although the two North American morphs (Form A and Form B) have been previously dismissed as ecophenotypes (Britton and Morton 1986), this interpretation is inconsistent with the maintenance of pronounced morphological distinctiveness in microsympatric populations (McLeod 1986, present study) and with the presence of fixed multi-locus differences among the morphs (Hillis and Patton 1982, McLeod 1986, present study). North American Form A is easily distinguished (especially in 3 dimensions) from Form B by its more trigonal shape, taller and more inflated umbos, coarser and more widely spaced external commarginal ribs, and lighter colored inner shell surface (McMahon 1991; Fig. 1). These distinctions are most obvious in juvenile specimens (Hillis and Patton 1982), and they were unambiguously evident in our microsympatric specimens of Form A and Form B from the Rio Grande study site. The Form C morphotype has by far the finest external surface sculpture and the thinnest and least inflated shell of all three New World morphotypes. It is further distinguished from the co-occurring Form A morphotype by the latter's prominent umbo, posterior rostrum, and lighter coloration (Ituarte 1994; Fig. 1).

Cytological Methods

Cytological investigation of clams of *Corbicula* Form A from Michigan was performed using an acetic orcein squash technique (Lee 1999). Freshly sampled clams were injected in their visceral masses with 0.2 ml of 0.05% colchicine. After a 2 hour incubation, the gonads were dissected, hypo-

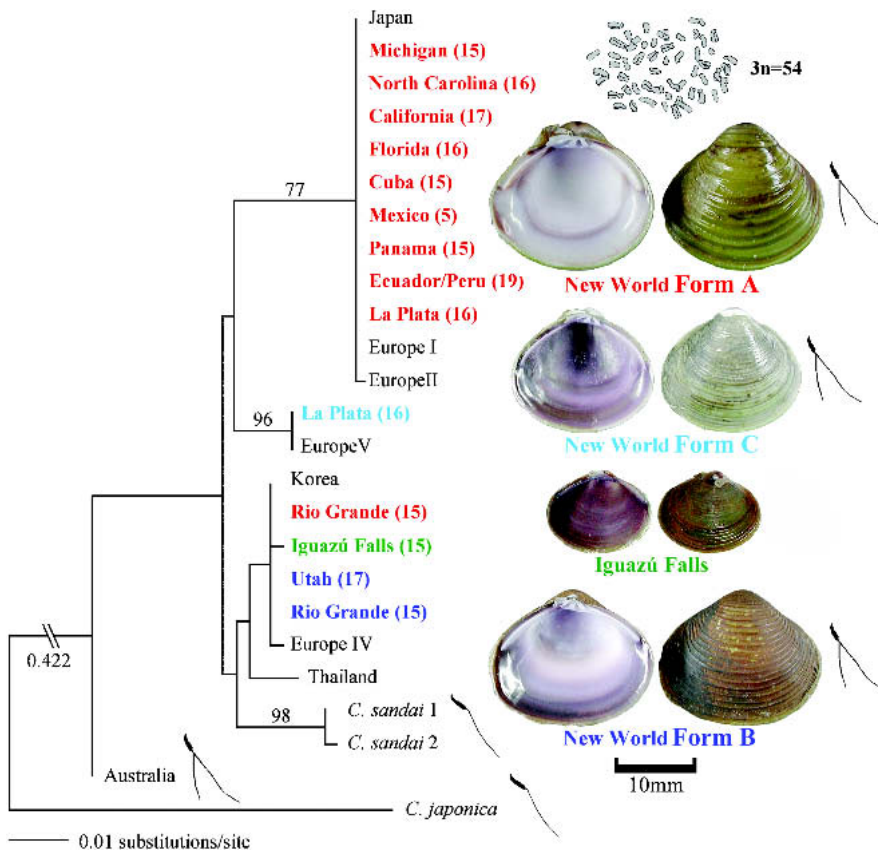


Figure 1. Maximum-likelihood phylogram ($\ln L = -1171.63$) of COI DNA sequences of freshwater individuals of *Corbicula*. The ML tree was recovered with PAUP using the HKY+I+ Γ model of nucleotide substitution and rooted with the Asian estuarine sexual species *Corbicula japonica*. The ingroup contained a second Asian diploid sexual species (the Lake Biwa endemic *Corbicula sandai* Reinhardt, 1878). The known sperm morphology of the species/morphotypes of *Corbicula* is indicated by monoflagellate (sexual) and biflagellate (clonal) sperm icons (unknown for the Iguazú Falls morphotype). Bracketed numbers after each New World sampling location indicate the number of individuals bearing that haplotype. Upper numbers on ingroup branches indicate Bayesian posterior probability values > 50. Exemplars of the three primary New World shell morphotypes A, B, and C, and of the Iguazú Falls morphotype are depicted showing internal views of the left valves and external views of the corresponding right valves. New World mt haplotypes recovered from each study population are color-coded to indicate which shell morphotype(s) they were associated with. A triploid ($3n = 54$) mitotic metaphase chromosome spread is depicted from our Michigan sample of New World Form A.

tonically treated, fixed with Carnoy's solution, stained with 1% acetic-orcein, macerated, squashed on a microscope slide, and viewed with a compound microscope. Metaphase spreads were drawn with the aid of a camera lucida.

Molecular Methods

DNA templates were extracted from mantle edge tissue using a DNeasy Tissue Kit (QIAGEN) according to the manufacturer's protocols. Two target gene fragments were

PCR-amplified: a 656 nucleotide (nt) portion of the mt gene *Cytochrome oxidase I* (COI) using primers LCO1490/HCO2198 (Folmer *et al.* 1994) and a 412 nt Domain 2 28S nuclear ribosomal DNA fragment using primers D23F and D4RB (Park and Ó Foighil 2000). The target fragments were amplified with 4 units of *Taq* DNA Polymerase (Promega, storage buffer A) and a negative control (no template) was included in each PCR run. For all PCR reactions, a touchdown protocol (Palumbi 1996) was utilized. After a 3 min 94°C denaturation, an initial annealing temperature of 65°C was decreased by 2°C/cycle (40 s 94°C denaturing, 40 s annealing and 1 min 72°C extension) until the final annealing temperature (45°C for COI and 55°C for 28S) was reached and subsequently maintained for an additional 30 cycles. The resulting double-stranded PCR products were isolated on 1% agarose gels, excised under long wavelength UV light, and purified using a QIAEX II Gel Extraction Kit (QIAGEN). Both strands of the amplified fragments were directly cycle-sequenced using the original amplification primers (annealing temperature 50°C) and Big-Dye Terminator Cycle Sequencing Ready Reaction (Perkin Elmer Applied Biosystems). The sequencing reactions were cleaned using Centri-Sep spin columns (Princeton Separations) loaded with G-50 Sephadex (Sigma) and then electrophoresed on an ABI 377 (Applied Biosystems) automated DNA sequencer.

Phylogenetic methods

Our COI phylogenetic data set for morphs of *Corbicula* incorporated sequences from earlier studies: Siripatrawan *et al.* 2000 (GenBank AF196268–74; AF196278; AF196278; AF196281) and Renard *et al.* 2000 (GenBank AF269090; AF269094–96), in addition to novel New World sequences obtained in this study (GenBank AF519495–519512). In all analyses, the estuarine, diploid, meiotic, dioecious, non-brooding, indirect-developer *Corbicula japonica* Prime, 1864 was utilized as an outgroup (Siripatrawan *et al.* 2000). Phylogenetic analyses were carried out under maximum parsimony (MP) and maximum likelihood (ML) op-

timality criteria using PAUP*4.0b10 (Swofford 2002). ML searches (heuristic, 5 random sequence additions) were carried out under the HKY model (Hasegawa *et al.* 1985) with rate heterogeneity. The transition/transversion ratio, proportion of invariable sites, and gamma shape parameter were estimated by maximum likelihood. To estimate the phylogenetic uncertainty of the tree topology obtained, we utilized a Bayesian statistical method with Markov chain Monte Carlo sampling (Huelsenbeck *et al.* 2000) using MrBayes 3.0 (Ronquist and Huelsenbeck 2003). We sampled 40,000 trees and discarded the first 8,000 found to ensure the Markov chains had converged on a stationary posterior probability distribution. A 50% majority-rule consensus was obtained from the remaining trees and the proportion of trees containing a specific branching order represented its posterior probability given the data and model of evolution. Unweighted MP analyses yielded 5 most parsimonious trees (92 steps, CI = 0.8152, RI = 0.9000) in which the Australian and Thai mt lineages had alternate topological placements within the ingroup crown clade.

RESULTS

Morphotype distribution

Eleven of the 12 sampling locations yielded clams that could be readily assigned to one of the three New World morphotypes of *Corbicula* (Table 1; Fig. 1). Form A was by far the most common and was encountered in 10/12 bicontinental locations spanning the New World range of the genus *Corbicula* (Table 1; Fig. 2). Typically, Form A was the only morphotype present at these locations. However, in two cases it occurred in microsympatry: in the Rio Grande with Form B and in La Plata with Form C (Table 1). In addition to its presence in the Rio Grande, the Form B morphotype was also encountered (on its own) in a Utah watershed (Table 1). The Iguazú Falls sample was an exception to the three morphotype system. Clams from Iguazú Falls, although most closely resembling Form C, were noticeably less trigonal and had slightly coarser and more widely-spaced external commarginal ribs (Fig. 1).

Mitochondrial Haplotype Distribution

A total of 212 individuals, retrieved from 12 sampling locations spanning the bicontinental New World range (Table 1), were sequenced for the target COI gene fragment. Eleven of the 12 study populations were monotypic, *i.e.*, only one mt genotype was encountered within each population. A total of four New World haplotypes were recovered comprising three phylogenetically distinct mitochondrial lineages, all of which nested firmly within a shallow crown clade of predominantly clonal Austral/Asian freshwater congeners (Fig. 1).

Our New World samples were dominated (150/212 individuals) by a single mt haplotype which was present in nine temperate, subtropical, and tropical sampling locations and was exclusive to the Form A morphotype (Fig. 1). Remarkably, its distribution encompassed the New World latitudinal range of the genus *Corbicula* (Fig. 2). Outside of the New World, the Form A mt haplotype occurs widely in Asia (Park and Kim 2003) and has been recovered from an androgenetic Japanese triploid ($3n=54$) clone (Komaru *et al.* 1998, Siripattawan *et al.* 2000; Fig. 1). Karyological examination of the Michigan study population of Form A showed that it too was triploid ($3n=54$; Fig. 1). The Form A mt haplotype has also been recovered in European exotic populations (Renard *et al.* 2000, Fig. 1), where they occur in diploid individuals (Pfenninger *et al.* 2002).

A second mitochondrial lineage, previously isolated from the North American Form B shell morphotype (Siripattawan *et al.* 2000), also had a bicontinental distribution. Two haplotypes were encountered, one in populations from western interior USA (Rio Grande, Utah), the other, which differed by a single synonymous substitution, in the Iguazú Falls section of the Paraná drainage system (Figs. 1, 2). Both of our New World samples of the Form B morphotype, from the Utah and Rio Grande populations, were fixed for the former haplotype. However, unlike the other two New World mt genotypes, the Form B mt lineage was not exclusive to a single New World morphotype. In the Rio Grande, where Form A and Form B morphotypes were sampled in microsympatry, the 15 Form A individuals typed also had the North American Form B mt haplotype (Fig. 1). In addition, all 15 of the typed clams from Iguazú Falls had the South American Form B mt haplotype in association with a distinctive shell morphotype that most closely resembled La Plata Form C (Fig. 1). Outside of the New World, the North American haplotype is widespread in Asia (Park and Kim 2003) and has been recovered from a triploid ($3n=54$) Korean clone (Siripattawan *et al.* 2000, Park *et al.* 2000). A minor variant of this mt lineage is also present in exotic Western European populations (Renard *et al.* 2000; Fig. 1).

The third New World mt lineage was represented by a single haplotype found in clams with the morphotype Form C shell from the La Plata region of Argentina (Figs. 1, 2). Although Form A and Form C morphotypes from La Plata were sampled in sympatry (*i.e.*, the same stream), they were fixed for distinct mt genotypes (Fig. 1). The Form C haplotype was phylogenetically distinct from the available repertoire of Austral/Asian mt diversity of freshwater species of *Corbicula*, although it also occurred in exotic Western European populations (Renard *et al.* 2000, Fig. 1).

Nuclear genotype distribution

We cross-profiled a sub-sample (89 individuals) of our

Table 1. Sampling particulars for New World specimens of *Corbicula*, together with UMMZ catalog numbers and GenBank Accession numbers.

Population	Locality	Morphotype/ # sequenced for COI	Date	Collector	UMMZ#	GenBank# (COI/28S)
Michigan	Davis Creek, South Lyon, Michigan, USA. 42.462°N, 83.708°W.	Form A 15	September 10, 1999	R. S. Mulcrone	300014	AF519495 AF519513
Utah	Small stream, 1 mi. south of southern exit to Brigham City along interstate 15, Box Elder County, Utah, USA. 41.482°N, 112.062°W.	Form B 17	November 1, 1999	P. and M. Hovingh	266695	AF519509 AF519528
California	SFPUC Station 12, Upper Crystal Springs Reservoir, San Mateo County, California, USA. 37.509°N, 122.35°W.	Form A 17	August 15, 2000	J. Bielski	300015	AF519497 AF519515
North Carolina	Little Fishing Creek (Tar River Basin), 1 mi. west of White Oak, Halifax County, North Carolina, USA. 36°11'09"N, 77°52'36"W.	Form A 16	July 7, 1998	A. Bogan	266694	AF519496 AF519514
New Mexico	Rio Grande at Mesilla Bridge, Las Cruces, New Mexico, USA. 32.291°N, 106.826°W.	Form A 15 Form B 15	December 4, 2001	D. Ó Foighil	300016(A) 300017(B)	AF519510(A) AF519526(A) AF519511(B) AF519529(B)
Florida	Santa Fe River at High Springs, Alachua County, Florida, USA. 29.827°N, 82.597°W.	Form A 16	May 2001	P. Baker	300018	AF519498 AF519516
Cuba	Parque Lenin, Havana, Cuba. 23.102°N, 82.381°W.	Form A 15	January 3, 2002	A. Gutierrez	300019	AF519499 AF519517
Mexico	3 locations in Jalisco, Mexico: Balneiro de Teuchitlan, El Arco, Rancho La Huerta, Buenos Aires.	Form A 2 1 2	May 10-11, 2000	S. Webb	300020 300021 300022	AF519500 AF519518 AF519501 AF519519 AF519502 AF519520
Panama	Panama Canal System at Gamboa Bridge, Panama. 9°6'57"N, 79°41'49"W.	Form A 15	April 27, 2002	D. Ó Foighil	300023	AF519503 AF519521
Ecuador	Río Pastaza at Consuelo, Ecuador. 1°55'9"S, 77°48'52"W.	Form A 15	July 18, 1999	J. Sparks	300024	AF519504 AF519522
Ecuador	Río Pastaza at Warany, Ecuador. 2°14'9"S, 77°15'10"W.	Form A 3	July 16-17, 1999	J. Sparks	300025	AF519505 AF519523
Peru	Río Pastaza at Laguna Rimachi near Cano Rimachi, Peru. 04°25'8"S, 76°40'33"W.	Form A 1	August 14, 1999	J. Sparks	300026	AF519506 AF519524
Iguazú Falls	Isla San Martín, Iguazú Falls, Misiones Province, Argentina. 25°41'39"S, 54°25'36"W.	Iguazú Falls 15	November 8, 1995	C. F. Ituarte	300027	AF519512 AF519530
Argentina	Unnamed brook, 17 km south of La Plata City, Buenos Aires, Argentina. 34°57'37"S, 57°46'37"W.	Form A 16 Form C 16	February 18 and May 28, 2000	C. F. Ituarte	300028(A) 300029(C)	AF519507(A) AF519525(A) AF519508(C) AF519527(C)

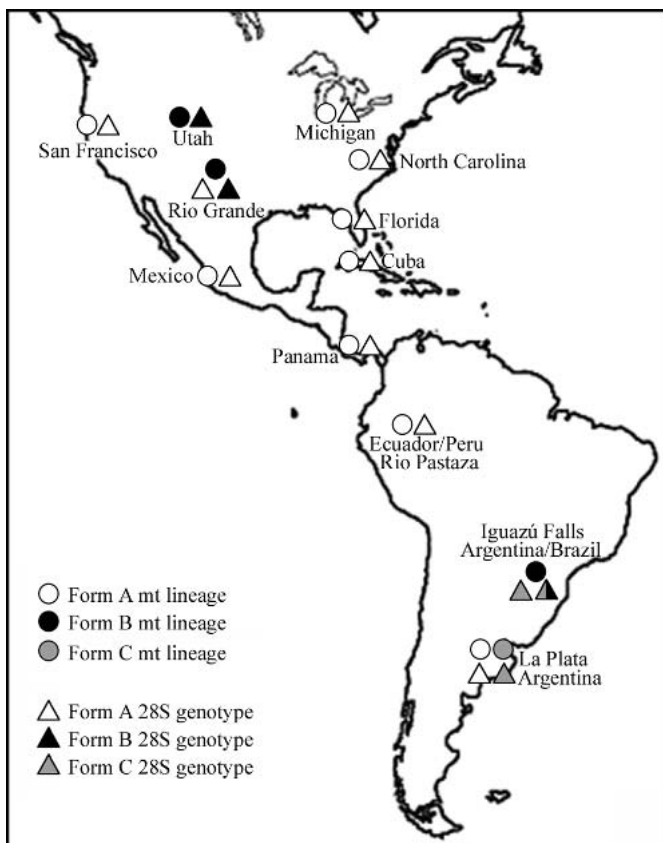


Figure 2. Summary map showing the distribution patterns of the three mt lineages and the three 28S nuclear genotypes detected among our 12 New World sampling sites of *Corbicula*.

study populations for nucleotide variation in a 412 nt segment of the large subunit (28S, Domain 2) nuclear ribosomal gene. Three 28S genotypes were detected in New World specimens of *Corbicula* (Fig. 3), and there was near-perfect congruence among shell morphotype (Fig. 1) and nuclear 28S genotype. The exception was a minority (4/15) of Iguazú Falls morphotypes that displayed heterozygous chromatograph profiles containing both Form C and Form B 28S genotypes (Fig. 2). Significantly, individuals from 9 bicontinental populations bearing the Form A mt haplotype and shell morphotype (Fig. 1) exclusively exhibited the Form A 28S genotype (Fig. 2).

We encountered evidence of nuclear/organelle genetic disjunction in two New World samples. All 15 clams of Form A shell morphotype from Rio Grande investigated had a Form A nuclear 28S genotype and a Form B mt COI genotype (Fig. 2). Correspondingly, all 15 clams of Form C shell morphotype from Iguazú Falls investigated had a Form C nuclear 28S genotype (four were also heterozygous for Form B 28S) and a Form B mt COI genotype (Fig. 2).

DISCUSSION

Our results indicate that New World populations of the invasive freshwater clam genus *Corbicula* are composed of a small number of lineages, one of which appears to be predominant in temperate, subtropical, and tropical ecosystems. Remarkably, the triploid Form A lineage present in the Huron River watershed in Ann Arbor, Michigan, shared the same COI haplotype and 28S genotype with multiple bicontinental populations of Form A, including those in such geographically and ecologically diverse locations as Cuba, the Panama Canal system, the headwaters of the Amazon, and La Plata, Argentina (Figs. 1-2). Although our preliminary results require testing with more rapidly evolving nuclear markers, they are consistent with the surprising hypothesis that the vast New World range expansion of the genus *Corbicula* may be predominantly composed of the Form A lineage, which appears to have a realized broad-spectrum ecophysiological scope.

The limited data available on reproductive mode(s) of alien New World morphs of *Corbicula* are consistent with clonality, especially for studied North American Form A and Form B populations that are triploid ($3n = 54$; confirmed for Michigan Form A in this study), lack meiotic metaphases, and produce unreduced sperm (Burch *et al.* 1998, J. B. Burch, pers. comm.). Morphs of Form A also occur in Central and South America, and share the same mt and nuclear genotypes with triploid North American samples (Fig. 2), but their ploidy status remains to be determined. However, La Plata populations of both Form A and Form C produce biflagellate sperm (C.F. Ituarte, pers. obs.), as do North American populations of Form A and Form B (Kraemer 1983, Siripattawan *et al.* 2000). This trait has been proposed as a morphological marker for clonality in lineages of *Corbicula* (Siripattawan *et al.* 2000). The rationale for this hypothesized linkage is two-fold. Biflagellate sperm are extremely rare in the Mollusca and have only been recorded, apart from occasional aberrant individual cells (J. M. Healy, pers. comm.), from hermaphroditic, freshwater lineages of *Corbicula*. Quantitative DNA analyses of biflagellate sperm from Asian 2n (Komaru *et al.* 1997, Ishibasi *et al.* 2003), Asian 3n (Komaru *et al.* 1997), Asian 4n (Qiu *et al.* 2001), hermaphroditic, freshwater lineages of *Corbicula*, and also from 3n North American exotics (Burch *et al.* 1998), have consistently found them (unlike their sexual monoflagellate congeners [Konishi *et al.* 1998]) to be unreduced, having the equivalent DNA content to somatic cells. Loss of meiosis is thought to have originated in clonal lineages of *Corbicula* by abortive cytokinesis of the 1st or 2nd meiotic divisions (Komaru *et al.* 1997). A disjunction between the various signaling pathways that control distinct aspects of cellular replication could conceivably result in a situation where some

	1		104
Form A	AAACCGCATAGAGCCAAACGGGTGGATCCGCAGAGTCGACCCGGGAAATTCAGCCCGGCCAGCGGCCTCGCAAAGTTCGGATCCGCAAGGACCGGGCCAGC		
Form BA.....		
Form CCA..A.....		
	105		208
Form A	GGGACGTCCCGTGGCAGGGTGCACCTTCTCCGGTTCGAGTGCTACGACCGGTTTCGAGGCGGTCAGAAGCCCGGGCAAAGGTAGCGCCGCCCTTCGGGGTG		
Form BCA.....		
Form CC.....		
	209		312
Form A	TCGTGTTATAGGCCCGCGGTGGACTCGCCGCGAGACCGAGGACGCTTCCCGCCGAGCGGTCGCCGGCCGCTCTGGGAGGTTTCGACCTCGGCTGCACCTGCT		
Form B		
Form C		
	313		412
Form A	CCGCAGAGCACCGTAACCGCCGCCCTAGCACGGTCCGGAGACTGCCGGCGCCTCGGGTCAGTAGCGAATCGGTCGGTCTCCACCCGACCCGCTCTTGAA	(51)	
Form B	(19)	
Form C	(19)	

Figure 3. Aligned Domain 2 genotypes of the large subunit (28S) nuclear ribosomal gene obtained from a sub-sampling of the 12 New World study populations of *Corbicula* previously typed for mt COI. A total of 89 individuals were characterised for 28S. These included all specimens from samples displaying mt/shell morphotype disjunction (30 Rio Grande and 15 Iguazú Falls specimens) plus a subsample of four individuals/morphotype/location from the remaining 10 New World study locations (GenBank AF519513-519530). Three morphotype-associated genotypes were recovered (see prefixes) and the bracketed number at the end of each sequence indicates the number of individuals encountered in our morphotype subsample bearing that genotype. Dots indicate nucleotide identity to the first sequence presented (Form A) and inferred nucleotide substitutions relative to Form A are shown.

elements of cell division (*e.g.*, nuclear division, formation of a cleavage furrow) were suppressed, whereas others (*e.g.*, centriolar replication, flagellar formation) proceeded independently. The linkage of biflagellate sperm with clonal reproduction is robust for the North American populations of Forms A, B that are known to be triploid and ameiotic (Burch *et al.* 1998, J. B. Burch, pers. comm.). It is more speculative for the South American Form C, which has yet to be karyotyped or to have the DNA content of its sperm quantified.

Clonal animal lineages are typically of hybrid origin and the introgressive events leading to their genesis may result in the uncoupling of organellar and nuclear genome genealogies (Kraus and Miyamoto 1990). We encountered evidence of qualitatively distinct nuclear/organellar genetic disjunction in two New World samples: Rio Grande Form A and the Iguazú Falls specimens (Fig. 2). In the former case, the disjunction was complete: all 15 Rio Grande Form A specimens typed had Form B mitochondria in a Form A nuclear setting (Form A 28S genotype, Form A shell morphotype). It was more nuanced in the latter case: all 15 Iguazú Falls specimens typed had Form B mitochondria in a heterogeneous nuclear setting (all had the Form C nuclear 28S genotype, four were also heterozygous for Form B 28S; shell phenotype was ambiguous). These two nuclear/mitochondrial disjunct populations might represent independent alien introductions. Another possibility, however, is that they are products of post-introduction interaction among New World clones. The latter scenario is more plausible for the Rio Grande population given that it is our sole sample of sympatric

Form A and Form B lineages and also of this particular genetic disjunction.

Parallel nuclear/mitochondrial disjunctions have also been discovered in recent studies of Korean (Park *et al.* 2002) and European (Pfenninger *et al.* 2002) freshwater lineages of *Corbicula*. Both of these studies concluded that the results were consistent with hybridization events. Hybridization is a reasonable explanation for the disjunction at Iguazú Falls due to the detection of heterozygous nuclear genotypes and the formation of a shell morphotype with putatively intermediate characteristics. The absence of the latter two features from Rio Grande Form A specimens is inconsistent with a nuclear genomic hybrid origin in this particular nuclear/mitochondrial disjunction and raises obvious difficulties given that the distribution data indicate that this condition may well have arisen *in situ*. One intriguing possibility is that the Rio Grande disjunction resulted from androgenetic capture of Form B eggs by unreduced Form A sperm in this watershed, *i.e.*, replacement of the Form B maternal nuclear genome by the Form A paternal genome. Androgenesis has been demonstrated in diploid and triploid Japanese clonal lineages of this genus (Komaru *et al.* 1998, 2000, Ishibashi *et al.* 2002, 2003) and may be widespread among freshwater hermaphroditic congeners. Inter-clone androgenetic egg capture would be confirmed by detecting embryos or larvae in broods of Rio Grande Form B bearing exclusively Form A nuclear genomes. Irrespective of the generating mechanism(s), the presence of multiple nuclear/mitochondrial disjunctions in freshwater lineages of *Corbicula* clearly signals that systematic interpretations based solely

on mitochondrial lineages (Renard *et al.* 2000, Siripattawan *et al.* 2000, Glaubrecht and Korniuschin 2003, Park and Kim 2003) could be seriously misleading. Our data, although indirect, imply that genetic interaction among clones of *Corbicula* may be mechanistically diverse.

Our results provide a phylogenetic perspective to New World populations of the genus *Corbicula*, and have implications for ecological studies of these prominent freshwater exotics and for their projected evolutionary persistence. Although our data caution against a blanket application of ecophysiological datasets to all New World populations, they facilitate meaningful comparative studies of the geographically widespread Form A lineage in bicontinental temperate, subtropical, and tropical freshwater ecosystems. Form A's presence in remote, ostensibly pristine, habitats, *e.g.*, the Río Pastaza section of the Amazonian drainage headwaters (Table 1, Fig. 2), is an indirect measure of its pronounced invasiveness. In 1999, Conservation International surveyed the aquatic diversity of a 380 km linear stretch of the Pastaza, more than 3200 km upstream from the mouth of the Amazon, and kindly forwarded us their samples of bivalve molluscs. Form A of *Corbicula* represented, by far, the numerically dominant bivalve recovered in both Andean (Ecuador) and lowland rainforest (Peruvian) Pastaza segments. The extraordinarily rapid spread of exotic lineages of *Corbicula* has been attributed to a combination of passive downstream transport via mucous drogue lines (Prezant and Charlermwat 1984, McMahon 1999), natural spread by phoresy among adjacent watersheds, and human-mediated introductions to geographically disjunct drainages. The putative clonal nature of New World lineages of *Corbicula* may act to enhance further their invasive prowess because single, distantly translocated, juvenile clams could readily found entire new populations.

There are of course potential downsides to obligate clonality. According to the Red Queen hypothesis, the evolutionary trajectory of New World clonal lineages of *Corbicula* will be truncated once the endemic parasitic biota adapts to their ecological presence (Lively and Dybdahl 2000). In this scenario, long-term evolutionary persistence of clones of *Corbicula* in the New World will depend on the generation of genetic novelty through either clonal introgression and/or the continued introduction of fresh exotic clones. The latter process may already be playing out in the La Plata region of Argentina where the formerly abundant Form C is being replaced by the now dominant Form A clone (Darrigran 1991).

The speed of the spread of *Corbicula* lineages across bicontinental New World watersheds is such as to invite its dismissal as an aberration that has little generality. Unfortunately, it may instead be a portent of things to come, and we anticipate that over the next decades a significant fraction

of the hundreds of freshwater New World invasive taxa (Benson 1999) will gradually attain extensive bicontinental distributions.

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