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Genetic diversity of two common freshwater mussel species, *Lampsilis cardium* and *Quadrula pustulosa* (Bivalvia: Unionidae), in a large federally protected waterway (St. Croix River, Minnesota/Wisconsin, U.S.A.)

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Abstract: Freshwater mussels in the family Unionidae have suffered severe population declines because of severe anthropogenic disturbances, such as habitat destruction and habitat alteration. Understanding the genetic diversity of healthy unionid populations is crucial to developing informed management plans for imperilled mussels. Here, we characterize the genetic diversity of two common species, *Lampsilis cardium* Rafinesque, 1820 and *Quadrula pustulosa* (I. Lea, 1831), using the mitochondrial gene ND1. Populations of *Q. pustulosa* contained more numerous and more highly divergent haplotypes than populations of *L. cardium*. This disparity in genetic diversity could be because of several factors, including differences in population size and location and extent of refugia during the Last Glacial Maximum. For both species, AMOVA analysis indicated no genetic structuring based on location within the river, with genetic diversity concentrated within rather than between populations. This finding is consistent with patterns seen for other common mussel species characteristic of large rivers.

Key words: aquatic, conservation genetics, population genetics, mtDNA, ND1

Mussels in the family Unionidae are important components of freshwater ecosystems with 680 species recognized worldwide (Graf and Cummings 2009). Alarmingly, the International Union for Conservation of Nature and Natural Resources Red List identifies 261 unionid species as extinct, endangered, or threatened (IUCN 2008). The overwhelming majority of these species reside in North America (Lydeard *et al.* 2004) and are in decline throughout their range (Williams *et al.* 1992), with 70 species in the United States listed as federally endangered or threatened (U.S. Fish and Wildlife Service 2009). To further understanding of unionid ecology and evolution, population genetic studies of unionids have proliferated in recent years, including some work on rare or endangered species (*e.g.*, Buhay *et al.* 2002, Machordom *et al.* 2003, Kelly and Rhymer 2005, Grobler *et al.* 2006, Zanatta and Murphy 2008, Grobler *et al.* 2011), as well as studies focused on common species (*e.g.,* Mock *et al.* 2004, Berg *et al.* 2007, Burdick and White 2007, Wolfe *et al.*, 2007, Elderkin *et al.* 2008).

The St. Croix River is a major drainage traversing some 164 miles in Minnesota and Wisconsin, U.S.A. Despite several major anthropogenic disturbances to the St. Croix, such as intensive logging in the late 1800s and the construction of a hydroelectric dam at the historic St. Croix Falls in 1903, it has remained a high-quality habitat for freshwater mussels. It was one of the original rivers included in the National Wild and Scenic River Act of 1968 and since then has been subjected to few human disturbances in comparison with other major rivers in the region, most notably the Mississippi. There are 48 species of freshwater mussels native to Minnesota, and 40 of them reside in the St. Croix (National Park Service 2008). These 40 species are, or were, also widely distributed throughout the upper Mississippi River drainage, and it is possible that the St. Croix may provide a refuge for a significant component of the upper Mississippi River mussel fauna. Unlike many large Midwestern drainages, the St. Croix River has yet to be infiltrated by zebra mussels throughout the majority of its length, with populations currently limited to the furthest downstream reach. Because of the global decline in number and diversity of unionids, the native mussels of the St. Croix River have received significant attention from local ecologists, including multiple long-term community monitoring studies (Hornbach *et al.* 1996, Hornbach 2001, National Park Service 2008). Understanding the genetic diversity and population structure of healthy and recruiting unionid populations, such as the diverse and well-studied mussel assemblage of the St. Croix, is particularly desirable as propagation and re-introduction efforts are undertaken for mussels extirpated from the Mississippi River.

The life cycle of unionid species involves a larval stage, the glochidium that parasitizes the gills or fins of fishes and is thought to function primarily to facilitate dispersal as fishes are much more vagile than mussels. Although the St. Croix River is less disturbed than other major drainages in the

region, the dam at St. Croix Falls probably acts as a barrier to upstream movement of host fishes, potentially affecting mussel distribution (Kelly and Rhymer 2005, Haponski *et al.* 2007, Barnhart *et al.* 2008). Fish distribution in the St. Croix River differs up and downstream of the St. Croix Falls Dam with several fishes confined to downstream areas (*e.g., Pylodictis olivaris* (Rafinesque, 1818), *Sander canadensis* (Griffith and Smith, 1834), and *Ammocrypta clara* Jordan and Meek, 1885 (Fago 1986), consistent with the idea that the dam acts as a barrier to fish dispersal. The geographic range of appropriate fish hosts is known to limit the geographic range of mussel species (Strayer 2008); therefore limits on fish movement imposed by the dam and historic falls may affect the population structure of mussels in this area. Fish host vagility is also thought to affect population structure, with more vagile hosts associated with lower levels of genetic differentiation between mussel populations (*e.g.,* Berg *et al.* 2007, Elderkin *et al.* 2008).

Previous studies have demonstrated that the mussel assemblages in areas of the St. Croix River above and below the St. Croix Falls also differ, with the subfamilies Lampsilinae (Tribe Lampsilini *sensu* Graf and Cummings 2007) and Ambleminae (Tribes Amblemini + Quadrulini + Pleurobemini *sensu* Graf and Cummings 2007) varying in prevalence above versus below the St. Croix Falls dam (Hornbach, 2001 Fig. 12.8). This variability is not likely to be a result of variation in microhabitat factors (Hornbach 2001), and it is possible that the historic falls and current dam have restricted fish movement and thereby influenced mussel community structure in the St. Croix River. Although the age of the historic falls is not known, this region of the river has been free of ice for 12,000 years (Crawford 1994) and thus a natural physical barrier to fish passage may have existed for a significant period of time prior to the construction of the St. Croix Falls dam.

In the current study, we examine the population genetic diversity and structure of two unionids common in the St. Croix River, each of which is congeneric with an endangered species from the St. Croix: *Lampsilis cardium* Rafinesque, 1820 (congeneric with *L. higginsii* (I. Lea, 1857), the endangered Higgins eye) and *Quadrula pustulosa* (I. Lea, 1831) (congeneric with *Q. fragosa* (Conrad, 1835), the critically endangered winged mapleleaf). Therefore, the data presented here can serve as a point of comparison for conservation managers interested in preserving the genetic diversity of *L. higginsii* and *Q. fragosa*. Characterization of healthy populations is crucial to ensuring that conservation decisions are made from an informed perspective with defined goals in mind. In addition, we compared the genetic diversity of the two species and performed tests for population genetic structure associated with the St. Croix Falls dam.

MATERIALS AND METHODS

Sample Collection

Samples were taken from June - July 2007 from four locations known to have large populations on the St. Croix River (Hornbach 2001); two locations above the St. Croix Falls dam (Wild River and Seven Islands) and two below (Peaslee and Hudson) (Fig. 1). GPS coordinates for each collection site were recorded and individuals were photographed for voucher purposes (Table 1). Voucher photographs for all specimens are publicly available through MorphoBank, (morphobank.org), project 376. At each location, a small amount of mantle tissue was clipped (approximately 1 cm^2) from each of 20 adult *Lampsilis cardium* and 20 adult *Quadrula pustulosa* mussels before returning the animals to the riverbed. Care was taken to avoid the reproductive areas of tissue donors, as cells from gonadal tissue have been shown to exhibit doubly parental inheritance mechanisms of mitochondrial DNA in some species of unionid mussels (Liu *et al.* 1996), which would complicate phylogenetic analyses based on mitochondrial genes. Tissue samples were preserved in 95% ethanol and later stored at -20 °C. Mussel nomenclature follows Turgeon *et al.* (1998).

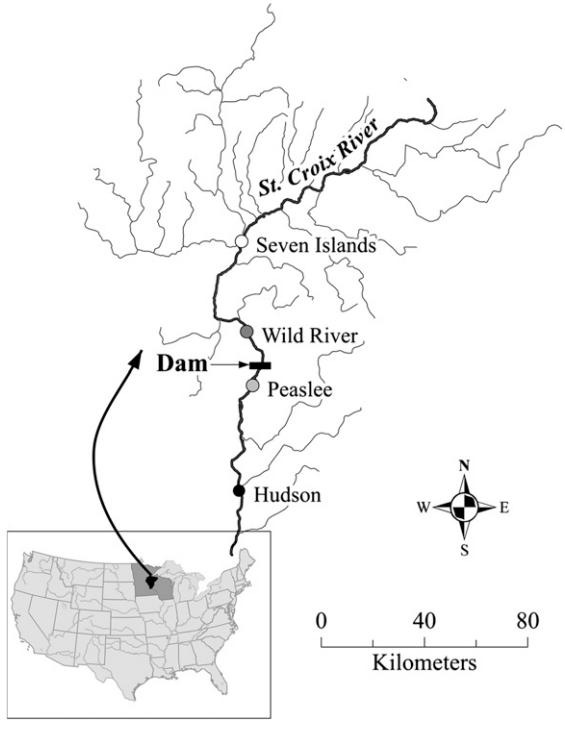

Figure 1. Tissue sample collection locations on the St. Croix River: two populations above (Seven Islands and Wild River) and two populations below (Peaslee and Hudson) the St. Croix Falls Dam were sampled; mantle tissue clippings from twenty mussels of each species, *Quadrula pustulosa* and *Lampsilis cardium*, were collected from each population.

Table 1. Tissue collection information and GenBank accession numbers. Sample ID is coded with collecting locality (H = Hudson, P = Peaslee, SI = Seven Islands, WR = Wild River), species name (C = *Lampsilis cardium*, P = *Quadrula pustulosa*), and sequential numerals. Voucher photographs for all specimens are available through MorphoBank, project 376.

	ID	Longitude (W)	Latitude (N)	GenBank Accession #
Lampsilis cardium	HC1–20	92°46′14.0″	44°58′31.7″	FJ601300–319
	PC1–7	92°42′08.0″	45°20′52.0″	FJ601320–26
	PC9–20	92°42′08.0″	45°20′52.0″	FJ601327–338
	PC21–22	92°42′14.7″	45°21′24.3″	FJ601339–340
	SIC1–3	92°44′41.8″	45°50′32.4″	FJ601281–83
	SIC4–12	92°49′54.0″	45°49′31.9″	FJ601284–292
	SIC14	92°49′54.0″	45°49′31.9″	FJ601293
	SIC15–20	92°49′54.0″	45°49′31.9″	FJ601294–99
	WRC1–12	92°43′44.4″	45°31′21.8″	FJ601341–352
	WRC14–17	92°43′44.4″	45°31′21.8″	FJ601353–56
	WRC19–20	92°43′44.4″	45°31′21.8″	FJ601357–58
Quadrula pustulosa	HP1–2	92°46′14.0″	44°58′31.7″	FJ601253–54
	HP4–7	92°46′14.0″	44°58′31.7″	FJ601255–58
	HP12–18	92°46′14.0″	44°58′31.7″	FJ601259–65
	HP20	92°46′14.0″	44°58′31.7″	FJ601266
	PP1–21	92°41′41.1″	45°22′01.1″	FJ601220–240
	PP22	92°42′14.0″	45°21′19.4″	FJ601241
	SIP6	92°49′54.0″	45°49′31.9″	FJ601242
	SIP8–9	92°49′54.0″	45°49′31.9″	FJ601243–44
	SIP11–14	92°49′54.0″	45°49′31.9″	FJ601245–48
	SIP17–20	92°49′54.0″	45°49′31.9″	FJ601249–252
	WRP1–2	92°43′44.4″	45°31′21.8″	FJ601267–68
	WRP11–20	92°43′44.4″	45°31′21.8″	FJ601269–278

Amplification of the ND1 Gene Region

Genomic DNA was extracted from approximately half of each mantle clipping with the DNeasy Tissue Kit (Qiagen) following the provided instructions for animal tissue extractions. Polymerase chain reaction was used to selectively amplify the NADH dehydrogenase subunit 1 (ND1) gene, which has been used in previous population studies of bivalves (Serb and Harris 2003, Serb *et al.* 2003, Campbell *et al.* 2005, Grobler *et al.* 2006, Grobler *et al.* 2011). We used the following primers: forward: 5′-TGG CAG AAA AGT GCA TCA GAT TAA AGC-3′ and reverse: 5′-CCT GCT TGG AAG GCA AGT GTA CT-3′ (Serb *et al*. 2003). To amplify ND1, 1X PCR buffer, 0.25 μM of each primer, 200 μM dNTPs, 1.25 units of *Taq* polymerase, and 50 ng of template DNA were combined in a 25 μl reaction. PCR reactions were performed in an ABI 2720 thermal cycler using the following profile: 98 °C denaturation for 2 min; 30 cycles of 10 s denaturation at 98 °C, 5 s annealing at 57 °C, and 1 min 15 s extension at 72 °C; final extension for 2 min at 72 °C. DNA was purified with a QIAquick PCR Purification Kit (Qiagen) and stored at -20 °C. A NanoDrop® ND-1000 Spectrophotometer was used to quantify the amplified DNA. Typical DNA concentrations ranged from approximately 30–40 ng/μl.

Cycle Sequencing

Cycle sequencing reactions consisted of 30–40 ng cleaned PCR product, 100 nM forward or reverse ND1 primer, and 8 μl DTCS Quickstart (Beckman Coulter) in a 20 μl reaction. DNA and primer were preheated in thermal cycler at 95 °C for 5 min before adding DTCS Quickstart. We used the following sequencing reaction program: 30 cycles of 96 °C denaturing for 20 sec, 50 °C annealing for 20 sec, and 60 °C extension for 4 min, and a final hold at 4 °C. A magnetic plate and magnetic beads (Beckman Coulter) were used with 3 washes of 85% ethanol to purify reactions. Automated sequencing was performed using a Beckman Coulter CEQ8000 sequencer with the LFR-1 separation method. Sequences were published on GenBank under the accession numbers listed in Table 1.

Data Preparation and Population Genetic Analyses

Sequence data from Beckman Coulter's CEQ8000 Genetic Analysis System v. 9.0 were edited and assembled

into contigs (consensus sequences) with Sequencher (version 4.8, Gene Codes Corporation). Alignments were created using MacClade (Maddison and Maddison 2001).

Population genetic analyses were performed using Arlequin version 3.01 (Excoffier *et al.* 2005). Standard diversity indices, including number of haplotypes (*Nh*), number of polymorphisms (*Np*), haplotypic diversity (*h*), and nucleotide diversity (π_n) were calculated to assess diversity within each population. A comparison of haplotypic diversity between the two species was performed using the Wilcoxon t-test as implemented in JMP (version 7, SAS Institute Inc.). An analysis of molecular variance (AMOVA, Excoffier *et al.* 1992) was performed separately for each species to test hierarchical models of genetic variance using pairwise differences as a measure of divergence (1000 permutations). The data were partitioned as follows: individuals from each locality were considered a population, and localities above versus below the dam were considered groups. An AMOVA was run to test for differentiation between populations and between groups.

Minimum spanning networks were created using statistical parsimony in TCS 1.21 (Clement *et al.* 2000). Reticulations within the haplotype networks were resolved using the principles of coalescent theory, as described by Crandall *et al.* (1994), with interior and frequently occurring haplotypes inferred to be more probable precursors to new haplotypes.

To further compare levels of genetic diversity in the two species, mean p-distances within species, within populations (*i.e.* sampling localities in Figure 1), and between populations were calculated using pairwise deletion, and standard error was assessed for each using 500 bootstrap replicates, with MEGA version 4.0 (Tamura *et al.* 2007).

Phylogenetic Analysis

We performed separate phylogenetic analyses of the two datasets. For phylogenetic analysis of the *Lampsilis cardium* dataset, we included outgroups from across the Lampsilini (*sensu* Graf and Cummings 2007) as well as one published sequence from *L. cardium*, from the Middle Maitland River (Ontario, Canada). For phylogenetic analysis of the *Quadrula pustulosa* dataset, we included outgroups from across the Quadrulini (*sensu* Graf and Cummings 2007) as well as 13 published sequences from *Q. pustulosa* from localities across the species' range (Table 2).

The model of sequence evolution was chosen separately for each dataset using ModelTest 2.3 (Posada and Crandall 1998, Nylander 2004) using the Akaike information criterion as recommended by Posada and Buckley (2004); in each case GTR+I+Γ was selected. Phylogenetic analyses were performed separately for each dataset using MrBayes 3.1 (Huelsenbeck and Ronquist 2001) under the GTR+I+Γ model. The *Quadrula pustulosa* analysis consisted of 437,900 replicates with the first 90,000 discarded as burnin. *Lampsilis cardium* Bayesian analysis included 376,500 replicates with the first 40,000 discarded as burnin. Parsimony analyses were performed in PAUP* v 4.0 (Swofford 2002). Support was assessed with 1000 bootstrap replicates. Trees were rooted based on the findings of Campbell *et al.* (2005).

Long-Term Monitoring

Mussel communities at each of our locations of interest have been monitored at 2–5 year intervals since 1992. Community analysis was undertaken by collecting 100 x ¼ m^2 quadrats at each locality as described by Hornbach *et al.* (1996). Data were collected at Hudson during 4 seasons (400 samples total), Peaslee during 5 seasons (500 samples total), Seven Islands during 2 seasons (200 samples total), and Wild River during 6 seasons (600 samples total) for a total of 1700 samples overall.

RESULTS

Samples from 78 *Lampsilis cardium* were successfully sequenced for 869 bp of the ND1 gene. There were 34 polymorphic sites, comprising 4% of the positions sequenced (Table 2). From these 78 individuals, 22 haplotypes were identified, four of which were found in all four populations and 13 (59%) of which were unique (Table 3). The average number of individuals per haplotype was 3.5, with 50% of all individuals concentrated into the two most frequent haplotypes.

Samples from 59 *Quadrula pustulosa* were successfully sequenced to 686 bp of the ND1 gene. There were 72 polymorphic sites, comprising 11% of the positions sequenced (Table 2). From these 59 individuals, 41 distinct haplotypes were identified (Table 3). None of these 41 haplotypes was found in all four populations sampled and 35 haplotypes (85%) were unique, represented by only one individual. The average number of individuals per haplotype was 1.4, with 50% of all individuals concentrated into the 12 most frequent haplotypes.

Wilcoxon *t*-test indicated that haplotypic diversity was significantly different between the two species ($P < 0.05$ for both 2-sample test with normal approximation and 1-way test with chi-square approximation). The differences in genetic diversity of *Lampsilis cardium* relative to *Quadrula pustulosa* are represented in the minimum spanning haplotype networks generated using the program TCS (Figs. 2 and 3). The *L. cardium* dataset included 19 fewer haplotypes than the *Q. pustulosa* dataset, despite a larger sample size. Branch-lengths, which represent the number of mutations between haplotypes, are shorter in the *L. cardium* network

Table 2. Genetic diversity indices for *Lampsilis cardium* and *Quadrula pustulosa* at each sampling location. *n* = sample size, *Nh* = number of haplotypes ± *SE*, *Np* = number of polymorphic sites ± *SE*, *h* = haplotypic diversity, π_n= nucleotide diversity.

Population	Species	*n*	*Nh*	*Np*	*h*	π_n
Hudson	*L. cardium*	20	10	22	0.878 ± 0.0479	0.00584 ± 0.00330
Peaslee	*L. cardium*	21	11	21	0.900 ± 0.0472	0.00628 ± 0.00352
Seven Islands	*L. cardium*	19	8	13	0.853 ± 0.0537	0.00518 ± 0.00302
Wild River	*L. cardium*	18	9	12	0.823 ± 0.0752	0.00600 ± 0.00362
Hudson	*Q. pustulosa*	14	13	22	0.989 ± 0.0314	0.00574 ± 0.00344
Peaslee	*Q. pustulosa*	22	19	45	0.974 ± 0.0276	0.01089 ± 0.00590
Seven Islands	*Q. pustulosa*	11	8	27	0.927 ± 0.0665	0.01002 ± 0.00576
Wild River	*Q. pustulosa*	12	10	22	0.954 ± 0.0569	0.00744 ± 0.00438

compared to the *Q. pustulosa* network. Mean p-distances were smaller in *L. cardium* than *Q. pustulosa,* with values of 0.006 (*SE* = 0.001) and 0.009 (*SE* = 0.002) respectively. Mean p-distances within and between followed this trend, and for each species within-population and between-population distances were equivalent in magnitude. Within populations, mean *p*-distance ranged from 0.005–0.006 (*SE* = 0.002 in each case) in *L. cardium* and from 0.007–0.011 (*SE* = 0.001–0.002) in *Q. pustulosa.* Between populations, mean p-distance ranged from 0.005–0.006 (*SE* = 0.001–0.002) in *L. cardium* and from 0.007–0.011 (*SE* = 0.001–0.002) in *Q. pustulosa*.

No geographic structuring was observed in the minimum spanning networks. Analysis of molecular variation (AMOVA) confirmed that all variation occurred within populations and no variation occurred either among groups (above versus below the dam) or among populations (collecting localities) (Table 4).

Phylogenetic analysis of the Lampsilini data indicated that all *Lampsilis cardium* individuals, including one published sequence from the Middle Maitland River in Ontario (Canada) form a well-supported clade. Within *L. cardium* no structure related to geography is evident (Fig. 4). Phylogenetic analysis of the Quadrulini data results in *Quadrula pustulosa* rendered paraphyletic with respect to *Quadrula aurea* (I. Lea, 1859) (Fig. 5). As with *L. cardium*, we found no resolution within the St. Croix River among different sampling locations. *Q. pustulosa* sequences downloaded from GenBank (Appendix 1) representing collections from Arkansas, Illinois, Kentucky, Louisiana, Missouri, Tennessee, Virginia, and Wisconsin are interspersed with samples from the St. Croix River (Fig. 5).

Long-term mussel community monitoring data are presented in Table 5. With the exception of the Seven Islands mussel assemblage, *Lampsilis cardium* was outnumbered by *Quadrula pustulosa* at every site monitored from 1992–2009, comprising on average 2.43 ± 0.02% of the total assemblage compared to 4.05 ± 0.03% for *Q. pustulosa*.

DISCUSSION

Berg *et al*. (2007) hypothesized that mussel species characteristic of large river systems exhibit large amounts of within-population genetic variation, while species that inhabit small streams are characterized by higher between-population variation. The results of our study of two abundant species within a large river system are consistent with this hypothesis. Recent analysis of parentage of *Lampsilis cardium* individuals from Ohio waterways indicates the possibility of dispersal of spermatozoa over many kilometers in this species (Ferguson 2009), providing a potential explanation for the lack of geographic structure seen in this species and other mussels in large rivers (*e.g.*, Berg *et al*. 1998, Elderkin *et al*. 2007).

In addition to finding more within-population than between-population variation in both our species of interest, we found differences in the absolute amount of variation in the ND1 gene exhibited by *Lampsilis cardium* and *Quadrula pustulosa*, with *Q. pustulosa* displaying greater variation than *L. cardium* in terms of number of haplotypes, number of polymorphic sites, haplotypic divergence, nucleotide diversity and mean p-distances within species, within populations, and between populations (Table 2, Figs. 2 and 3). We performed a Wilcoxon *t*-test to formally compare the number of haplotypes found in populations of the two species, and this test confirmed that differences were significant. Differing levels of genetic diversity could reflect differences in population size; in three of the four sampling localities in this study, *Q. pustulosa* has larger population sizes than does *L. cardium* (Table 5). Data from 17 years of monitoring the St. Croix River mussel communities show that *Q. pustulosa* makes up on average 4.05 ± 0.03% of the total mussel assemblages at the four sampling locations, whereas *L. cardium* comprises approximately 2.43 ± 0.02% of the total assemblage (n = 1700 collected quantitatively since 1992) (Table 4). As genetic diversity is correlated with population

Table 3. Location and frequency of *Lampsilis cardium and Quadrula pustulosa* haplotypes from above and below the St. Croix Falls dam. *Lampsilis cardium* haplotypes begin with L and *Quadrula pustulosa* haplotypes begin with Q. SI = Seven Islands, WR = Wild River, P = Peaslee, H = Hudson (See map in Fig. 1).

Lampsilis cardium					***Quadrula pustulosa***				
	Localities					**Localities**			
	above		**below**			**above**		**below**	
Haplotypes	**SI**	**WR**	**P**	**H**	**Haplotypes**	**SI**	**WR**	**P**	**H**
L1	2	1	2	1	Q1	3	3	4	
L2	6	7	6	5	Q2	1		1	1
L3	4	4	1	5	Q3	2			1
L4	3	1	3	3	Q4	1	1		1
L5			2	1	Q5				1
L6		1	1		Q6				1
L7	1			1	Q7				1
L9			2		Q8				1
L10		1			Q9		1		2
L11			1		Q10			1	
L12			1		Q11			1	
L13			1	1	Q12			1	
L14	1				Q13			1	
L15		1			Q14			1	
L16	1				Q15			1	
L17		1			Q16			1	
L18		1			Q17	1		1	
L19	1				Q18			1	
L20				1	Q19			1	
L21				1	Q20	1			
L22			1		Q21	1			
L23				1	Q22		1		
					Q23				1
					Q26			1	
					Q27				1
					Q28		1		
					Q29		1		
					Q30				1
					Q31				1
					Q32				1
					Q33			1	
					Q34			1	
					Q35			1	
					Q36			1	
					Q37			1	
					Q38			1	
					Q39		1		
					Q40		1		
					Q41		1		
					Q42	1			
					Q43		1		

size, the differences in population size could explain the higher genetic diversity in *Q. pustulosa* populations. However, other hypotheses could also explain this difference. For example, it is possible that the diversity of host fishes used by each species could contribute to differing amounts of genetic variation. In addition, the number and extent of refugia

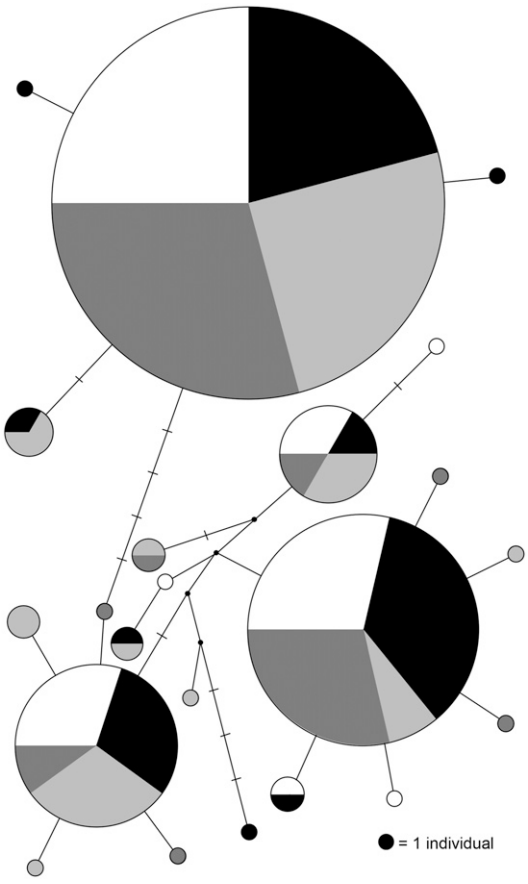

Figure 2. Minimum spanning network for *Lampsilis cardium* haplotypes based on TCS analysis: haplotype frequency is shown by proportionally scaled pie graphs, as indicated by key. Above dam locations (Seven Islands and Wild River) are shown in white and dark gray, respectively; locations below the dam (Peaslee and Hudson) are shown in light gray and black, respectively. This shading scheme matches that of Fig. 1. Hash marks on lines connecting haplotypes indicate the number of base pair changes between haplotypes, and black dots at line junctions represent unobserved haplotypes.

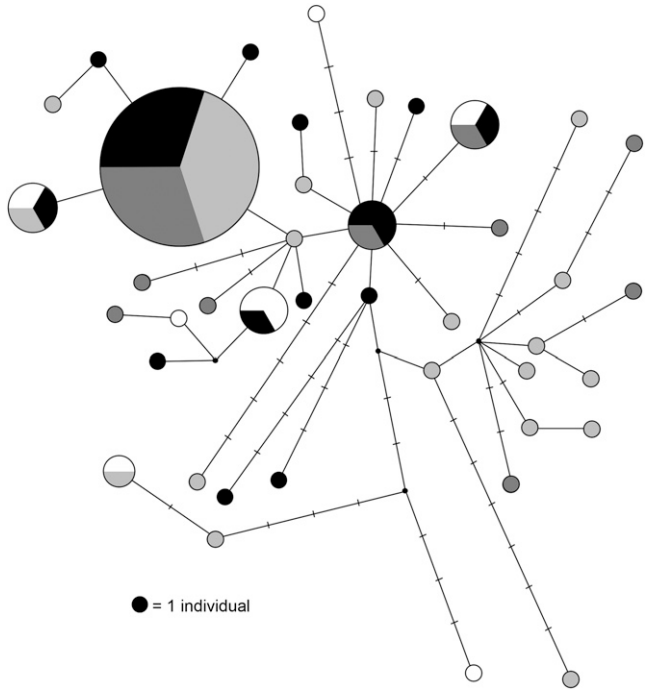

Figure 3. Minimum spanning network for *Quadrula pustulosa* haplotypes based on TCS analysis: haplotype frequency is shown by proportionally scaled pie graphs, as indicated by key. Above dam locations (Seven Islands and Wild River) are shown in white and dark gray, respectively; locations below the dam (Peaslee and Hudson) are shown in light gray and black, respectively. This shading scheme matches that of Fig. 1. Hash marks on lines connecting haplotypes indicate the number of base pair changes between haplotypes, and black dots at line junctions represent unobserved haplotypes.

occupied by these species during the Last Glacial Maximum might have been different, and could also account for the contrast in genetic diversity. It is likely a combination of the aforementioned factors that account for the observed disparities in genetic diversity between the two species.

In addition to generating data on genetic variation in healthy unionid populations within a large river, a secondary aim of this study was to determine whether the former St. Croix Falls and the 18 m high dam currently built on its location have acted as a barrier to host fish movement and thus mussel dispersal. Watters (1996) examined the impact of dams ranging in size from 1 to 17.7 m on the distribution of two species of freshwater mussels and found that animals were confined to the river sectors downstream of dams, suggesting that unionid distribution can be strongly influenced by dams. A recent study on genetic divergence in *Percina caprodes* (Rafinesque, 1818) and *Etheostoma blennioides* Rafinesque, 1819 corroborated this finding and demonstrated that dams as small as 4 m may act as unidirectional barriers to gene flow in some species of fishes (Haponski *et al.* 2007). In the present study, AMOVA revealed no structuring based on geographic distance along the river was observed, and, in fact, in both *Lampsili cardium* and *Quadrula pustulosa* several haplotypes were found at all four localities, implying that the dam and St. Croix Falls have not served as a barrier to gene flow that could be detected with the ND1 locus (Table 3).

Phylogenetic analysis of our datasets confirmed the lack of geographic structure in each species (Figures 4 and 5). Although unionid taxonomy is outside the scope of the current study, it is intriguing to note that *Quadrula pustulosa* is paraphyletic with respect to *Q. aurea* in our analyses (Figure 5), confirming the findings of Serb *et al.* (2003). *Quadrula*

Table 4. Analysis of Molecular Variance (AMOVA) indicates no genetic structuring among groups (above and below the dam), or among populations (sampling locations); variation is concentrated within populations.

Lampsilis cardium

Source of Variation	d.f.	Sum of squares	Variance components	% Variation	Fixation indices
Among groups	1	1.352	0.00516 Va	0.22	F_{SC} = -0.02788
Among populations within groups	2	2.299	-0.06614 Vb	-2.78	F_{ST} = -0.02565
Within populations	74	180.426	2.43819 Vc	102.57	F_{CT} = 0.00217
Total	77	184.077	2.37722		

Quadrula pustulosa

Source of Variation	d.f.	Sum of squares	Variance components	% Variation	Fixation indices
Among groups	1	2.881	-0.00586 Va	-0.19	F_{SC} = -0.00077
Among populations within groups	2	6.092	-0.00237 Vb	-2.78	F_{ST} = -0.00268
Within populations	55	169.399	3.07999 Vc	100.27	F_{CT} = -0.00191
Total	58	178.373	3.07176		

aurea, commonly known as the golden orb, is a critically endangered species endemic to Texas. *Quadrula aurea* and *Q. pustulosa* differ in color and presence of pustules, both of which are highly variable characters within *Q. pustulosa*. *Quadrula pustulosa* does occur in eastern Texas, and it is possible that these two morphs are not in fact reproductively isolated species, and/or have diverged very recently. A more detailed study of morphological and genetic characteristics of these two taxa could clarify their relationship.

Although it has been used in many previous population genetic studies of unionids, ND1 may be too slow-evolving to be informative about population structure associated with the construction of the dam at St. Croix Falls. Considering a longer timescale, this site has been free of glacial ice for 12,000 years (Crawford 1994), and the historic falls that existed at the dam location may therefore have acted as a barrier to fish dispersal long before the dam was constructed; however, this is not detected with the data presented here. Analysis of nuclear microsatellite loci has been used to detect population variation within unionid species that was not discernable with mitochondrial DNA (Zannatta and Murphy 2008); therefore, microsatellites may have the potential to resolve population structure related to very recent events such as the construction of the St. Croix Falls dam. However, Grobler *et al.* (2011) found genetic structure in the endangered unionid *Cyprogenia stegaria* (Rafinesque, 1820) that was detectable with the ND1 locus but not with microsatellite data. In the case of one of our species of interest, *Quadrula pustulosa*, preliminary microsatellite data have been generated, from the same set of individuals examined in the current study, for 20 loci developed to study the genetic structure of the endangered species *Q. fragosa* (Hemmingsen *et al.* 2009). These preliminary data, which are part of an unpublished study comparing genetic diversity of a common mussel (*Q. pustulosa*) and an endangered mussel (*Q. fragosa*), confirm a lack of structure associated with the St. Croix Falls dam within *Q. pustulosa* (K. Roe, unpubl. data).

The homogeneity of the genetic pool across the gradient of the St. Croix River suggests that in freshwater mussels it is normal for a high proportion of genetic diversity to occur within populations rather than between populations. Although it is important to keep in mind that our data are drawn from a single locus, this finding is consistent with the hypothesis of Berg *et al.* (2007), and indicates that conservation efforts for other *Lampsilis* and *Quadrula* species should focus not only on maintaining a large number of mussel populations per species, but also on sustaining large population sizes.

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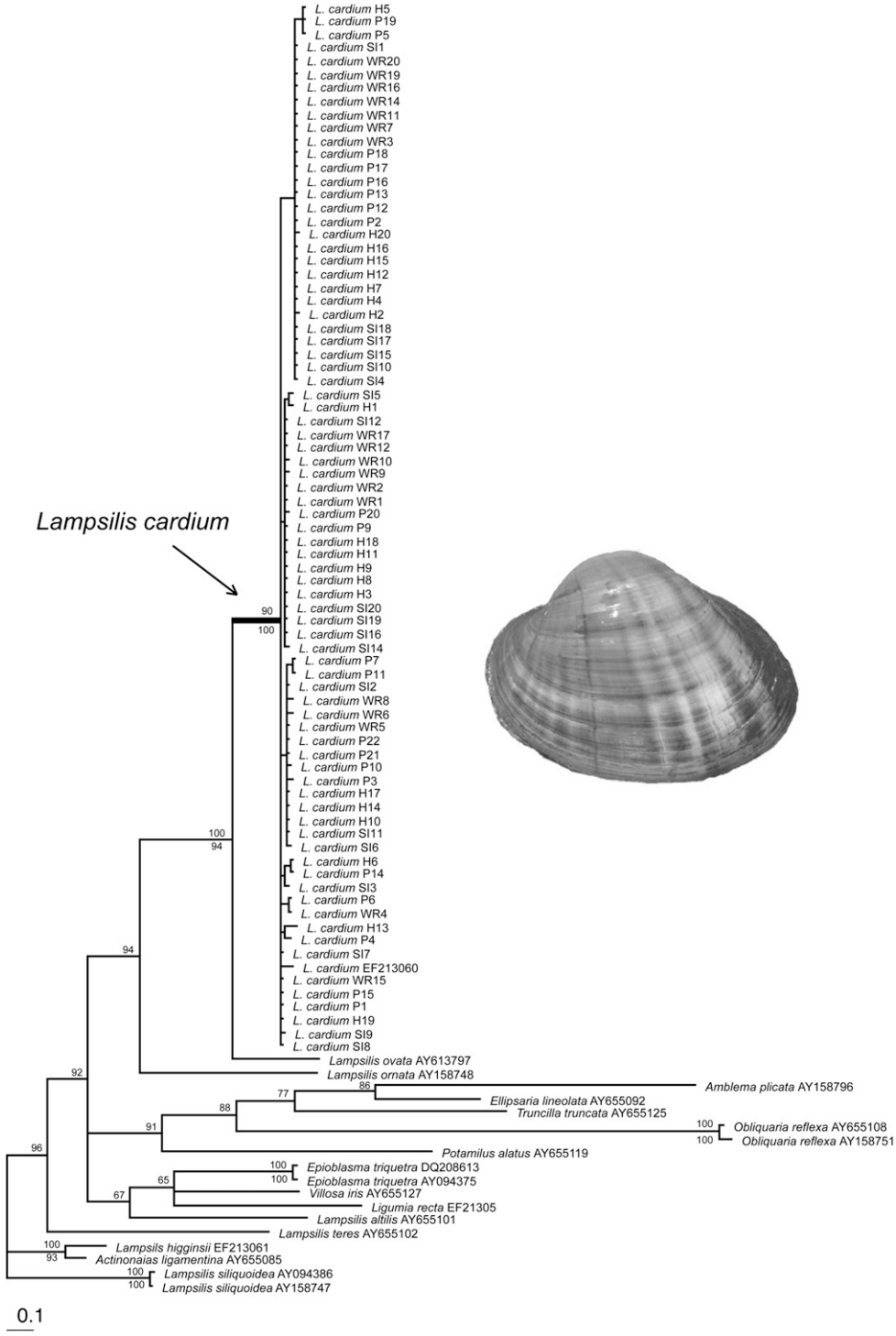

Figure 4. Phylogenetic relationships of *Lampsilis cardium.* Bayesian analysis was conducted with the GTR+I+ Γ model. Numbers on branches indicate posterior probabilities (above) and parsimony bootstrap values (below) when above 50%. Support values on short internal branches within *L. cardium* are not shown because of space constraints. The thickened branch supports the monophyly of *L. cardium.* Individuals from the St. Croix River are labelled with their collecting locality (SI, WR, P, and H) and sample number. Published outgroup sequences and *L. cardium* from outside the St. Croix River are labelled with GenBank accession numbers. See Appendix 1 for more information on published data from GenBank. The shell image represents one of our voucher photographs, for individual PC14.

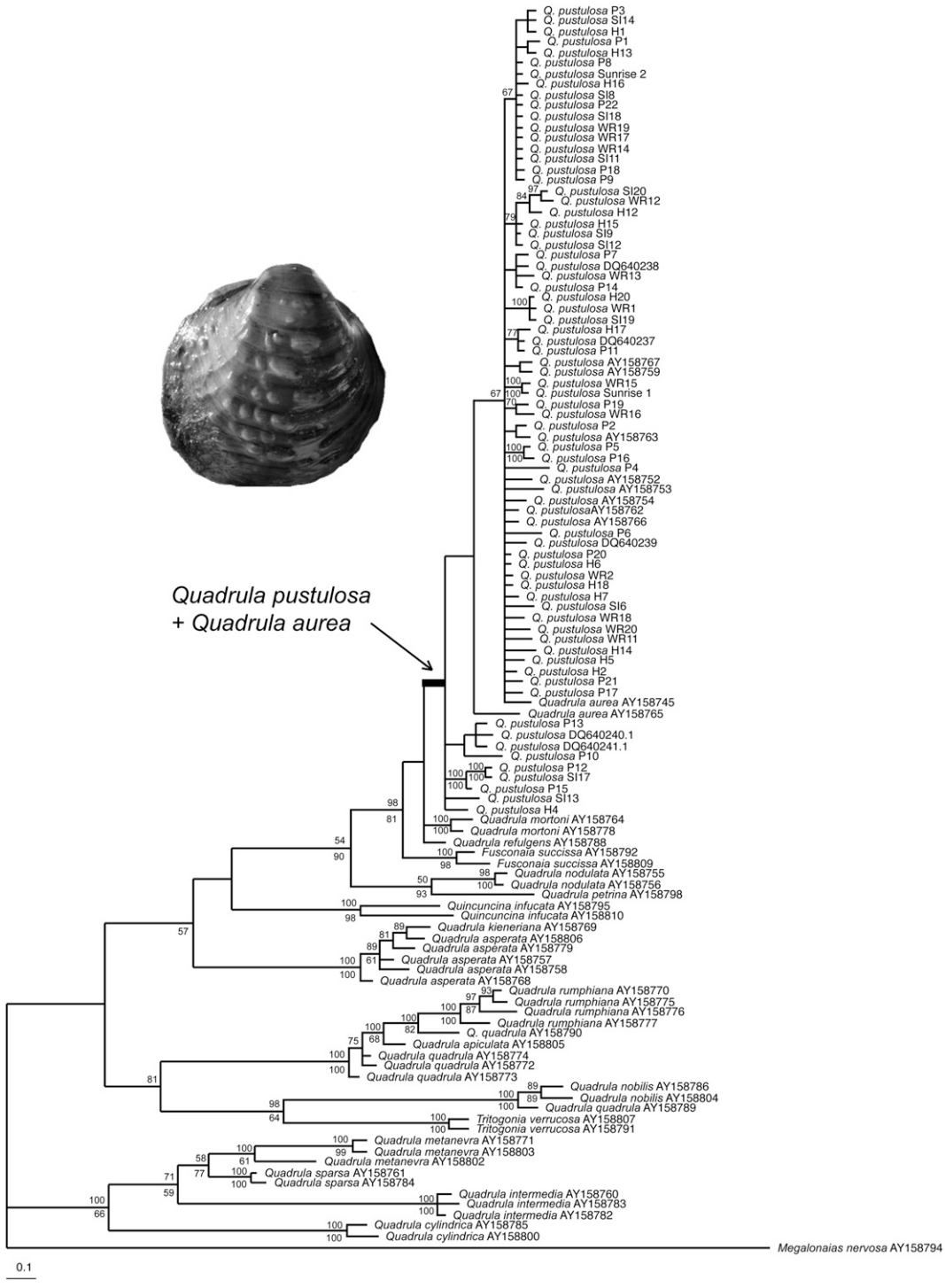

Figure 5. Phylogenetic relationships of *Quadrula pustulosa*. Bayesian analysis was conducted with the GTR+I+ Γ model. Numbers on branches indicate posterior probabilities (above) and parsimony bootstrap values (below) when above 50%. Stars are used to indicate 100% when space is limited. The thickened branch indicates the most restricted clade including all *Q. pustulosa* individuals. Individuals from the St. Croix River are labelled with their collecting locality (SI, WR, P, and H) and sample number. Published outgroup samples are labelled with the species name and GenBank accession number. See Appendix 1 for more information on published data from GenBank. The shell image represents one of our voucher photographs, for individual HP1.

Table 5. Census data from long-term monitoring of collection localities. From 1992 to 2009, 1700 mussels have been surveyed at Hudson, Peaslee, Seven Islands and Wild River. The contribution of each species to the total mussel assemblage at each locality is shown as % of assemblage. Total values indicate the average percent of the assemblage for each species over all four sampling areas

		% of Assemblage		Mussels/m^2	
Locality	**Sample size**	*L. cardium*	*Q. pustulosa*	*L. cardium*	*Q. pustulosa*
Hudson	400	0.8%	3.7%	0.11 ± 0.06	0.53 ± 0.1
Peaslee	500	0.7%	3.5%	0.06 ± 0.06	0.26 ± 0.09
Seven Islands	200	6.0%	1.5%	0.48 ± 0.09	0.12 ± 0.14
Wild River	600	2.2%	7.5%	0.55 ± 0.05	1.90 ± 0.08
Total	1700	2.43 ± 0.02%	4.05 ± 0.03%		

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Appendix 1. GenBank accession numbers, geographic information, and authors for published sequence data incorporated in phylogenetic analyses. All samples are from either the United States or Ontario, Canada. (R = river, FMCC = Freshwater Mollusk Conservation Center).

Species	GenBank Accession #	River	State or Province	Citation
Actinonaias ligamentina (Lamarck, 1819)	AY655085	Kankakee R.	Illinois	Campbell *et al.*, 2005
Amblema plicata (Say, 1817)	AY158796	Ohio R.	Kentucky	Serb *et al.*, 2003
Ellipsaria lineolata (Rafinesque, 1820)	AY655092	Cahaba R.	Alabama	Campbell *et al.*, 2005
Epioblasma triquetra (Rafinesque, 1820)	AY094375	Clinch R.	Tennessee	Buhay *et al.* 2002
Epioblasma triquetra	DQ208613	Clinch R.	Tennessee	Jones *et al.* 2006
Fusconaia succissa (I. Lea, 1852)	AY158792	Conecuh R.	Alabama	Serb *et al.*, 2003
Fusconaia succissa	AY158809	Pea R.	Alabama	Serb *et al.*, 2003
Lampsilis altilis (Conrad, 1834)	AY655101	Etowah R.	Alabama	Campbell *et al.*, 2005
Lampsilis cardium	EF213060	Middle Maitland R.	Ontario	Zanatta and Murphy
Lampsilis higginsii	EF213061	St. Croix R.	Wisconsin	Zanatta and Murphy
Lampsilis ornata (Conrad, 1835)	AY158748	Cahaba R.	Alabama	Serb *et al.*, 2003
Lampsilis ovata (Say, 1817)	AY613797	Clinch R.	Alabama	Campbell *et al.*, 2005
Lampsilis siliquoidea (Barnes, 1823)	AY094386	Douglas Lake	Michigan	Buhay *et al.* 2002
Lampsilis siliquoidea	AY158747	Douglas Lake	Michigan	Serb *et al.*, 2003
Lampsilis teres (Rafinesque, 1820)	AY655102	Tennessee R.	Alabama	Campbell *et al.*, 2005
Ligumia recta (Lamarck, 1819)	EF213055	Sydenham R.	Ontario	Zanatta and Murphy
Megalonaias nervosa (Rafinesque, 1820)	AY158794	Coosa R.	Alabama	Serb *et al.*, 2003
Obliquaria reflexa Rafinesque, 1820	AY158751	Cahaba R.	Alabama	Serb *et al.*, 2003
Obliquaria reflexa	AY655108	Coosa R.	Alabama	Campbell *et al.*, 2005
Potamilus alatus (Say, 1817)	AY655119	Tennessee R.	Alabama	Campbell *et al.*, 2005
Quadrula apiculata (Say, 1829)	AY158805	Neches R.	Texas	Serb *et al.*, 2003
Quadrula asperata (I. Lea, 1861)	AY158757	Alabama R.	Alabama	Serb *et al.*, 2003
Quadrula asperata	AY158758	Alabama R.	Alabama	Serb *et al.*, 2003
Quadrula asperata	AY158768	Sucarnoochie Ck.	Mississippi	Serb *et al.*, 2003
Quadrula asperata	AY158779	Coosawattee R.	Georgia	Serb *et al.*, 2003
Quadrula asperata	AY158806	Coosa R.	Alabama	Serb *et al.*, 2003
Quadrula aurea	AY158745	Lake Corpus Christi	Texas	Serb *et al.*, 2003
Quadrula aurea	AY158765	Lake Corpus Christi	Texas	Serb *et al.*, 2003
Quadrula cylindrica cylindrica (Say, 1817)	AY158785	Duck R.	Tennessee	Serb *et al.*, 2003
Quadrula cylindrica strigillata	AY158800	Clinch R.	Tennessee	Serb *et al.*, 2003
Quadrula intermedia (Conrad, 1836)	AY158760	Powell R.	Virginia	Serb *et al.*, 2003
Quadrula intermedia	AY158782	Duck R.	Tennessee	Serb *et al.*, 2003
Quadrula intermedia	AY158783	Duck R.	Tennessee	Serb *et al.*, 2003
Quadrula kieneriana Lea, 1852	AY158769	Coosawattee R.	Georgia	Serb *et al.*, 2003

Appendix 1. (Continued)

Species	GenBank Accession #	River	State or Province	Citation
Quadrula metanevra (Rafinesque, 1820)	AY158771	Elk R.	Alabama	Serb *et al.*, 2003
Quadrula metanevra	AY158802	Cahaba R.	Alabama	Serb *et al.*, 2003
Quadrula metanevra	AY158803	Tennessee R.	Tennessee	Serb *et al.*, 2003
Quadrula pustulosa mortoni (Conrad, 1835)	AY158764	Big Cypress Bayou	Texas	Serb *et al.*, 2003
Quadrula pustulosa mortoni	AY158778	Lake Lewisville	Texas	Serb *et al.*, 2003
Quadrula nobilis (Conrad, 1854)	AY158786	Pascagoula R.	Mississippi	Serb *et al.*, 2003
Quadrula nobilis	AY158804	Neches R.	Texas	Serb *et al.*, 2003
Quadrula nodulata (Rafinesque, 1820)	AY158755	Neches R.	Texas	Serb *et al.*, 2003
Quadrula nodulata	AY158756	Mississippi R.	Missouri	Serb *et al.*, 2003
Quadrula petrina (Gould, 1855)	AY158798	Concho R.	Texas	Serb *et al.*, 2003
Quadrula pustulosa	AY158752	Ouachita R.	Arkansas	Serb *et al.*, 2003
Quadrula pustulosa	AY158753	Ouachita R.	Arkansas	Serb *et al.*, 2003
Quadrula pustulosa	AY158754	Mississippi R.	Missouri	Serb *et al.*, 2003
Quadrula pustulosa	AY158759	St. Croix R.	Wisconsin	Serb *et al.*, 2003
Quadrula pustulosa	AY158762	Wolf R.	Tennessee	Serb *et al.*, 2003
Quadrula pustulosa	AY158763	Ohio R.	Kentucky	Serb *et al.*, 2003
Quadrula pustulosa	AY158766	Amite R.	Louisiana	Serb *et al.*, 2003
Quadrula pustulosa	AY158767	Mississippi R.	Illinois	Serb *et al.*, 2003
Quadrula pustulosa	DQ640237	FMCC, Virginia Tech	Virginia	Henley *et al.*, 2006
Quadrula pustulosa	DQ640238	FMCC, Virginia Tech	Virginia	Henley *et al.*, 2006
Quadrula pustulosa	DQ640239	FMCC, Virginia Tech	Virginia	Henley *et al.*, 2006
Quadrula pustulosa	DQ640240	FMCC, Virginia Tech	Virginia	Henley *et al.*, 2006
Quadrula pustulosa	DQ640241	FMCC, Virginia Tech	Virginia	Henley *et al.*, 2006
Quadrula quadrula (Rafinesque, 1820)	AY158772	Muskingum R.	Ohio	Serb *et al.*, 2003
Quadrula quadrula	AY158773	Spring R.	Kansas	Serb *et al.*, 2003
Quadrula quadrula	AY158774	Ohio R.	Indiana	Serb *et al.*, 2003
Quadrula quadrula	AY158789	Ohio R.	Kentucky	Serb *et al.*, 2003
Quadrula quadrula	AY158790	Red R.	Kentucky	Serb *et al.*, 2003
Quadrula refulgens (I. Lea, 1868)	AY158788	Pascagoula R.	Mississippi	Serb *et al.*, 2003
Quadrula rumphiana (I. Lea, 1852)	AY158770	Black Warrior R.	Alabama	Serb *et al.*, 2003
Quadrula *rumphiana*	AY158775	Sipsey R.	Alabama	Serb *et al.*, 2003
Quadrula rumphiana	AY158776	Oostanaula R.	Georgia	Serb *et al.*, 2003
Quadrula rumphiana	AY158777	Coosawattee R.	Georgia	Serb *et al.*, 2003
Quadrula sparsa (I. Lea, 1841)	AY158761	Powell R.	Virginia	Serb *et al.*, 2003
Quadrula sparsa	AY158784	Powell R.	Tennessee	Serb *et al.*, 2003
Quincuncina infucata (Conrad, 1834)	AY158795	New R.	Florida	Serb *et al.*, 2003
Quincuncina infucata	AY158810	Ochlocknee R.	Florida	Serb *et al.*, 2003
Truncilla truncata Rafinesque, 1820	AY655125	Mississippi R.	Missouri	Campbell *et al.*, 2005
Tritogonia verrucosa (Rafinesque, 1820)	AY158791	Elk R.	Alabama	Serb *et al.*, 2003
Tritogonia verrucosa	AY158807	Cumberland R.	Tennessee	Serb *et al.*, 2003
Villosa iris (I. Lea, 1829)	AY655127	Duck R.	Tennessee	Campbell *et al.*, 2005