MALACOLOGIA, 1998, 39(1–2): 195–205

MOLECULAR SYSTEMATICS OF THE FRESHWATER MUSSEL GENUS POTAMILUS (BIVALVIA: UNIONIDAE)

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ABSTRACT

Few explicit hypotheses for the relationships of unionid mussels exist. The absence of explicit phylogenetic hypotheses is problematic and is in part responsible for the lack of taxonomic stability seen in this group. In this paper we examine the relationships of mussels in the genus *Potamilus,* based upon the DNA sequences of a 600 base pair portion of the first subunit of the mitochondrial cytochrome *c* oxidase (COI) gene. We also examine the genetic distinctiveness of populations of the inflated heelsplitter *P. inflatus.* The molecular phylogeny indicates that *Potamilus* is paraphyletic with *Leptodea fragilis* and *Lampsilis ornata* nested between *P. capax* and the remaining *Potamilus* species. With the exception of *P. capax,* the remaining *Potamilus* species are depicted as monophyletic and form three distinct clades: (1) a reciprocally monophyletic *P. inflatus* clade; (2) a *P. ohiensis/P. amphichaenus* clade; and (3) a *P. purpuratus/P. p. coloradoensis/P. alatus* clade. While bootstrap values indicate a high degree of support for these three clades, relationships among these three clades are not as strongly supported.

The genetic distinctiveness of two populations of the inflated heelsplitter exceeds that seen between some other species in the genus. These populations represent geographically isolated, genetically distinct entities, and we therefore recommend the recognition of both the Amite and the Black Warrior populations of *P. inflatus* as separate species.

Key words: Unionidae, *Potamilus*, cytochrome *c* oxidase subunit I.

INTRODUCTION

The freshwater mussel genus *Potamilus* Rafinesque, 1818 (Bivalvia: Unionidae), currently contains six species: *P. alatus* (Say, 1817), *P. amphichaenus* (Frierson, 1898), *P. capax* (Green, 1832), *P. inflatus* (I. Lea, 1831), *P. ohiensis* (Rafinesque, 1820), and *P. purpuratus* (Lamarck, 1819) (Turgeon et al., 1988; Williams et al., 1992). In addition to these taxa, Simpson (1914) included *P.* (*Lampsilis*) *coloradoensis* (I. Lea, 1856), which is now generally considered a western form of *P. purpuratus. Potamilus* is distributed in the St. Lawrence and Mississippi drainages and in Gulf drainages from Alabama to Texas (Valentine & Stansbery, 1971; Burch, 1975; Clarke, 1981). The type species for the genus was designated as *Unio alatus* Say, 1817, by Morrison (1969).

The genus *Potamilus* in its current form was first recognized as a natural assemblage of species by Frierson (1927) in the synonymous genus *Proptera* Rafinesque, 1819. Several researchers have proposed classifications that render the genus paraphyletic (Simpson, 1914; Hoggarth, 1988; Burch, 1975) and have placed mussels currently assigned to *Potamilus* in the genus *Lampsilis* (*P. capax*) (Simpson, 1914), the genus *Leptodea* (*P. laevissima* [= *ohiensis*], *P. amphichaenus*) (Burch, 1975) or the resurrected genus *Lastena* (Hoggarth, 1988). Whereas *Potamilus* is generally perceived as a natural group by freshwater malacologists, it has not yet achieved taxonomic and nomenclatural stability, as evidenced by the continual change in generic assignments over the last 170 years. Even after successful petitioning by Bogan et al. (1990) of the International Commission on Zoological Nomenclature for the retention of *Potamilus* (BZN, 1992), *Proptera,* a junior synonym of *Potamilus,* appears in publications as late as 1993 (e.g., McMahon, 1993). While many descriptions of the genus include the presence of a posterior wing as diagnostic, this character alone does not discriminate members of *Potamilus* from their putative sister genus *Leptodea* (Ortmann, 1912; Valentine & Stansbery, 1971). Ortmann's (1912) statement that "this genus (*Potamilus*) stands in all characters except the glochidia, by that

of *Paraptera* [= *Leptodea*]," supports the similarity of these two genera. Valentine & Stansbery (1971) stated that the only unique feature that defines *Potamilus* is the possession of axe-head shaped or ligulate glochidium (Fig. 1), and Utterback (1915) noted that with the exception of the unique glochidia and the more developed hinge, "this genus (*Potamilus*) stands with *Lasmonos* [= *Leptodea*]." A phenetic analysis by Hoggarth (1988) of the utility of glochidia morphology for deducing the relationships among North American freshwater mussels indicated that *Potamilus* is not a monophyletic group and that *P. ohiensis* and *P. amphichaenus* are more closely related to mussels in the genus *Leptodea* than to other members of *Potamilus*. Hoggarth's analysis indicated two distinct groups of mussels within *Potamilus:* those with lateral hooks on the ventral valve edges (*alatus, capax, purpuratus*) and those without such hooks (*ohiensis, amphichaenus, inflatus*). He concluded that the glochidia bore only a superficial resemblance to each other, and implied that the axe-head shaped glochidia were not homologs.

The historic lack of taxonomic stability of *Potamilus* reflects the fact that no detailed or comprehensive cladistically based study has been conducted on this genus. Despite increasing interest in freshwater mussels, only a few cladistically based analyses have been published to date (Hoeh, 1990; Hoeh et al., 1996; Lydeard et al., 1996; Mulvey et al., 1997). The primary objective of this study is to test the monophyly of *Potamilus* using a molecular data set composed of the DNA sequences of a portion of the first subunit of the mitochondrial cytochrome *c* oxidase (COI) gene, and develop hypotheses for relationships within the genus.

Additionally, we wish to examine the level of intraspecific genetic variation in the inflated heelsplitter, *P. inflatus. Potamilus inflatus* was known from the Amite and Tangipahoa rivers in Louisiana, the Pearl River in Mississippi, and the Black Warrior, Coosa, and Tombigbee rivers in Alabama. Presently, it is limited to the lower and middle reaches of the Amite River, and a portion of the Black Warrior River. In 1990, the U.S. Fish and Wildlife Service listed *P. inflatus* as a threatened species, because of its diminished range and potential threats to its continued survival in those rivers where it still occurs (USFWS, 1992). Knowledge of how genetic variation is partitioned in *P. inflatus* will aid in making management decisions concerning this species.

MATERIALS AND METHODS

Twenty-four specimens representing ten species and five genera were included in the analysis (Table 1). Genomic DNA was isolated from fresh frozen or ethanol preserved tissues using the QIAamp Tissue Kit (QIAGEN #29304) following manufacturers instructions. Care was taken to use only somatic tissues as unionid mussels exhibit bi-parental inheritance of mitochondria (Hoeh et al., 1996; Liu et al., 1996b). Double-stranded and single-stranded DNA was generated via the polymerase chain reaction (PCR) using the primers LCO1490 and HCO2198 (Folmer et al., 1994). Approximately 100 ng of genomic DNA provided the template for double stranded reactions performed in a 25 µl solution containing each dNTP at 0.1 mM, each primer at 1.0 µM, 40 mM $MgCl_2$, 2.5µl 10X Taq buffer, and 0.6 units of AmpliTaq polymerase. Reactions were amplified for 32 cycles at 94° for 40 sec, 55° for 60 sec, and 72° for 90 sec. The amplified DNA was gel purified and then used as template for single-stranded amplification (Gyllensten & Erlich, 1988) using the same conditions and primer pair, with the H-primer used in limited quantity. Single stranded DNA was concentrated on Millipore Ultrafree MC filters, and sequenced using the Sequenase version 2.0 kit (U.S. Biochemical) and ^{35}S-labeled dATP following the manufacturers instructions. The heavy strand was sequenced using overlapping primers: HCO2198 (5'-taaacttcagggtgaccaaaaaatca-3'), UNICOIH (5'-tcagcaaccaacccaggag-3'), and HUNICOIC (5'-aacaacactctctaccaaag-3').

DNA sequences were visualized via autoradiography, and aligned by eye using the software package XESEE (Cabot & Beckenbach, 1989). P-distances (uncorrected for multiple hits) and Kimura's "two parameter" distances (Kimura, 1980) were calculated using the software package MEGA (Kumar et al., 1993). Prior to phylogenetic analysis, the DNA sequences were examined for evidence of saturation by plotting the number of transversions and transitions at each codon position vs. p-distance. Trees were generated under maximum parsimony using PAUP version 3.1.1 (Swofford, 1993). Trees were rooted using *Fusconaia cerina* (Conrad, 1838) and

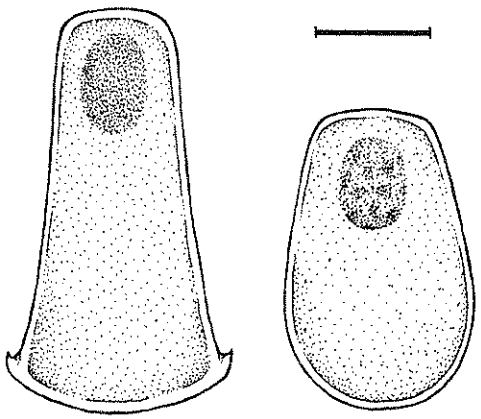

FIG. 1. (A) Glochidia of *Potamilus purpuratus*, showing the axe-head shape and lateral hooks. Redrawn from Surber (1915). (B) Glochidia of *Lampsilis cardium* for comparison. Redrawn from Surber (1912). Bar = 100 μm.

Obliquaria reflexa (Rafinesque, 1820). Bootstrapping (Felsentein, 1985) was employed to measure the internal stability of the topologies generated using 200 iterations. Skewness of tree-length distributions as a measure of phylogenetic signal (Hillis & Huelsenbeck, 1992) was estimated by generating 10,000 random trees.

RESULTS

Sequence Variation

DNA sequencing procedures yielded ~600 base pairs of COI sequence for 24 taxa for a total of 14,400 nucleotides (Genbank accession numbers AFO 49499-AFO 49522). Preliminary analysis of the sequence data revealed 182 variable sites, 151 of which were phylogenetically informative. Of those sites that were phylogenetically informative 16 were at the first position, 10 were at the second position, and 125 were at the third. Translation of codons into amino acids indicates 23 variable residues. Pairwise percent sequence differences corrected for multiple hits using the "two parameter" model (Kimura, 1980) ranged from 0 to 2.6% for intraspecific comparisons. Values for interspecific comparisons within *Potamilus* were between 1.2% and 14.5%. Pairwise comparisons for all taxa are presented in Table 2.

Scatterplots of pairwise genetic sequence differences versus the absolute number of transitions and transversions are presented for each codon position in Figure 2. Trends revealed by the scatterplots are typical for those seen in other protein coding genes (Roe et al., 1997a; Lydeard & Roe, 1997), transversions were relatively rare at first and second positions, not exceeding four and two substitutions respectively for any comparison. Transversions were considerably more common at the third codon position. A slight decrease in the number of transitions relative to the number of transversions at the third position provides evidence that some saturation is present. Saturation has the potential to affect phylogenetic analyses, therefore differential weighting of substitutions in the third codon position was employed.

Phylogenetic Analyses

Based on the analysis of nucleotide substitution patterns, phylogenetic analyses were performed under maximum parsimony using equal weighting and weighting transversions 2x transitions at the third codon position. The g^1 values (−0.362894, −0.625367) for weighted and equal weight analyses indicate the presence of significant phylogenetic signal ($p = 0.01$). Parsimony analysis of the data using equal weighting of transitions and transversions resulted in five equally parsimonious trees (CI = 0.636, RC = 0.517, 352 steps), the strict consensus of which is presented in Figure 3. Analysis of the data weighting transversions 2× transitions resulted in two equally parsimonious trees, which are presented in Figure 4. With the exception of the equivocal placement of *P. p. coloradoensis*, the two trees from the weighted analysis represent a single topology, identical to two of the five trees from the equal weight analysis. Whereas differences exist between the trees generated using transversion weighted and equal weighted parsimony analysis, all topologies depict *Potamilus* as paraphyletic. In addition, all topologies support the monophyly of all species with the exception of the *P. purpuratus* clade. All topologies also support the sister relationships of *P. ohiensis* and *P. amphichaenus*, and the reciprocal monophyly of the Amite and Black Warrior populations of *P.*

TABLE 1. Localities and number of specimens included in this study.

SPECIES	# INDIVIDUALS	LOCALITY
Potamilus alatus[1]	1	Elk River, Limestone Co., AL., 29 September 1994.
P. alatus[2]	1	Clinch River, Hancock Co., TN., 12 August 1994
P. amphichaenus[1]	1	B.A. Steinhagen Resevoir, Neches River Dr., Tyler Co., TX., 28 January 1996.
P. amphichaenus[2]	1	Sabine River, at US Highway 59, Panola Co., TX., 5 July 1995.
P. capax	2	Iron Mines Ck., ~1.25 mi. W. of AR. Highway. 140 and Red Oak Baptist Church, Poinsett Co., AR., 26 October 1994.
P. ohiensis[1]	1	St. Francis floodway, near Wittsburg, Cross Co., AR., 16 July 1995.
P. ohiensis[2]	1	Lake Arrowhead, Little Wichita River, Red River Dr., Clay Co., TX., 12 July 1994.
P. purpuratus[1]	2	Cahaba River, below Cooper Island, Bibb Co., AL., 15 September 1994.
P. purpuratus[2]	1	Cahaba River, ~1 mi. downstream of Hwy. 24, Bibb Co., AL., 30 June 1993.
P. p. coloradoensis	1	Twin Buttes Resevoir, Concho River Dr., Tom Green Co., TX., 30 August 1993.
P. inflatus	4	Amite River, above Port Vincent, Baton Rouge Pa., LA., 3–4 August 1994.
P. inflatus	4	Black Warrior River, (river mile 327.3), Tuscaloosa Co., AL., 15 October 1994.
Leptodea fragilis[1]	1	Cahaba, River, above AL. Highway 58, Centreville, Bibb Co., AL., 14 November 1994.
L. fragilis[2]	1	Elk River, upstream of AL Highway 127, Limestone Co., AL., 14 October 1996.
Lampsilis ornata	1	Cahaba, River, above AL. Highway 58, Centreville, Bibb Co., AL., 14 November 1994.
Obliquaria reflexa	1	Cahaba, River, above AL. Highway 58, Centreville, Bibb Co., AL., 14 November 1994.
Fusconaia cerina	1	Cahaba River, ~1 mi. downstream of Hwy. 24, Bibb Co., AL., 30, June 1993.

inflatus. Weaker support was found for some deeper nodes as evidenced by the low bootstrap values.

DISCUSSION

Phylogenetic Analysis

The COI data do not support the recognition of *Potamilus* as a monophyletic group. Whereas the majority of the species of *Potamilus* form a natural assemblage, the placement of *Lampsilis ornata* and *Leptodea fragilis* nested between *P. capax* and the remaining members of *Potamilus* renders the genus paraphyletic. The single morphological character that serves to unite members of *Potamilus* is the possession of axe-head shaped glochidia. Hoggarth (1988) suggested only a "superficial resemblance" between the glochidia of *P. amphichaenus, P. ohiensis* and those of *P. alatus, P. purpuratus* and *P. capax,* and recommended that mussels with axe-head shaped glochidia possessing hooks (*alatus, capax* and *purpuratus*) should remain in *Potamilus,* while those that lacked hooks (*amphichaenus, inflatus* and *ohiensis*) should be placed in the resurrected genus *Lastena* Rafinesque, 1820. Hoggarth had not examined the glochidia of *P. inflatus* and placed it in *Lastena* on the basis of the morphology of adult shells. His phenetic analysis indicated that *Lastena* was more closely allied to *Leptodea* than to *Potamilus.* Within *Lastena,* Hoggarth placed *P. ohiensis* and *P. amphichaenus* as sister to *P. inflatus.* However, recent examination of the glochidia of *P. inflatus* revealed the presence of large supernumerary hooks (Roe et al., 1997b). Based on Hoggarth's criteria, *P. inflatus* should have been placed in a group containing *P. alatus, P. purpuratus* and *P. capax,* all of which have glochidia that possess hooks. The molecular phylogeny (Fig. 4) agrees with the classification of Hoggarth (1988) in the recognition of *P.*

TABLE 2. Pairwise genetic distances based on Kimura's "two parameter" model. Values are percentages.

	P. inf.w1	P. inf.w2	P. inf.w3	P. inf.w4	P. inf.a1	P. inf.a2	P. inf.a3	P. inf.a4	P. purp1	P. purp.2	P. purp.c.	P. alatus1
P. inf.w1		0.00	0.00	0.34	2.46	2.44	2.62	2.08	9.68	10.16	10.55	10.16
P. inf.w2			0.00	0.34	2.12	2.29	2.47	1.93	9.55	9.51	9.70	9.88
P. inf.w3				0.34	2.29	2.45	2.62	2.09	9.49	9.82	10.39	10.00
P. inf.w4					2.46	2.08	2.26	2.07	9.26	9.72	10.10	9.72
P. inf.a1						0.35	0.35	0.17	9.36	9.32	9.49	9.10
P. inf.a2							0.17	0.17	9.13	9.58	10.17	9.18
P. inf.a3								0.34	9.49	9.82	10.19	9.39
P. inf.a4									9.09	9.53	10.32	9.34
P. purp.1										0.00	1.40	1.22
P. purp.2											1.55	1.38
P. purp.col.												1.20
P. alatus1												
P. alatus2												
P. capax1												
P. capax2												
P. ohien.1												
P. ohien.2												
P. amph.1												
P. amph.2												
L. frag.1												
L. frag.2												
L. ornata												
O. reflexa												
F. cerina												

	P. alatus2	P. capax1	P. capax2	P. ohien1	P. ohien.2	P. amph.1	P. amph2	L. frag.1	L. frag.2	L. ornata	O. reflexa	F. cerina
P. inf.w1	10.11	14.40	14.48	12.40	13.02	12.88	12.80	9.95	9.61	14.48	16.43	14.92
P. inf.w2	9.83	14.42	14.48	12.39	13.00	12.89	12.81	9.90	9.55	14.70	16.69	14.93
P. inf.w3	9.92	14.28	14.31	12.47	13.09	12.96	12.88	9.81	9.46	14.34	16.53	14.79
P. inf.w4	9.66	13.91	14.02	12.16	12.76	12.64	12.57	9.51	9.18	13.98	16.15	14.65
P. inf.a1	9.02	14.24	13.88	11.13	11.75	11.59	11.54	9.09	8.74	12.95	16.26	14.95
P. inf.a2	9.11	14.25	14.11	11.20	11.82	11.66	11.61	8.98	8.64	12.98	16.02	15.17
P. inf.a3	9.31	14.08	13.89	11.02	11.63	11.47	11.42	9.40	9.06	12.82	15.86	15.45
P. inf.a4	9.27	13.99	13.88	10.98	11.56	11.42	11.38	9.13	8.79	12.94	15.55	15.11
P. purp.1	1.23	13.79	13.62	10.18	10.56	11.64	11.61	7.16	7.21	11.74	14.51	14.37
P. purp.2	1.40	14.19	13.88	10.82	11.20	12.28	12.24	7.62	7.67	12.16	14.91	14.55
P. purp. c.	1.22	13.52	13.18	10.19	10.56	11.66	11.61	8.20	8.25	12.35	16.84	16.27
P. alatus1	0.00	13.12	12.99	9.98	10.37	11.27	11.22	7.24	7.29	11.33	15.75	14.79
P. alatus2		13.33	13.16	9.92	10.32	11.22	11.18	7.36	7.41	11.32	16.03	14.83
P. capax1			0.00	13.54	14.15	14.28	14.17	11.42	11.29	13.87	16.77	17.44
P. capax2				13.40	14.04	14.14	14.04	11.30	11.16	13.98	16.89	17.57
P. ohien.1					0.34	4.68	4.39	9.76	9.42	14.49	17.10	16.96
P. ohien.2						5.24	4.94	10.34	10.00	14.66	17.52	17.59
P. amph.1							0.17	10.78	10.44	16.65	17.84	17.37
P. amph.2								10.77	10.43	16.26	17.63	17.19
L. frag. 1									1.03	9.87	13.41	13.64
L. frag. 2										9.33	13.49	12.67
L. ornata											15.02	16.62
O. reflexa												13.44
F. cerina												

Note Taxon abbreviations: P. inf.w1–4, *Potamilus inflatus*-Black Warrior River; P. inf.a1–4, *Potamilus inflatus*-Amite River; P. purp.1–2, *Potamilus purpuratus;* P. purp. col., *Potamilus purpuratus coloradoensis;* P. alatus1–2, *Potamilus alatus;* P. capax1–2, *Potamilus capax;* P. ohien.1–2, *Potamilus ohiensis;* P. amph.1–2, *Potamilus amphichaenus;* L. frag.1–2, *Leptodea fragilis;* L.ornata, *Lampsilis ornata;* O.reflexa, *Obliquaria reflexa;* F. cerina, *Fusconaia cerina.*

amphichaenus, P. inflatus, and *P. ohiensis* as a natural group; however, it is not due to the shared absence of hooks. Clearly, given the homoplastic nature of hook development this character appears to be of limited phylogenetically utility.

The phylogenetic position of *P. capax* is problematic. In analyses of the molecular data, *P. capax* is depicted as the most basal member of the in-group in the weighted analysis, and is the most basal or second most basal member in the equal weight analysis.

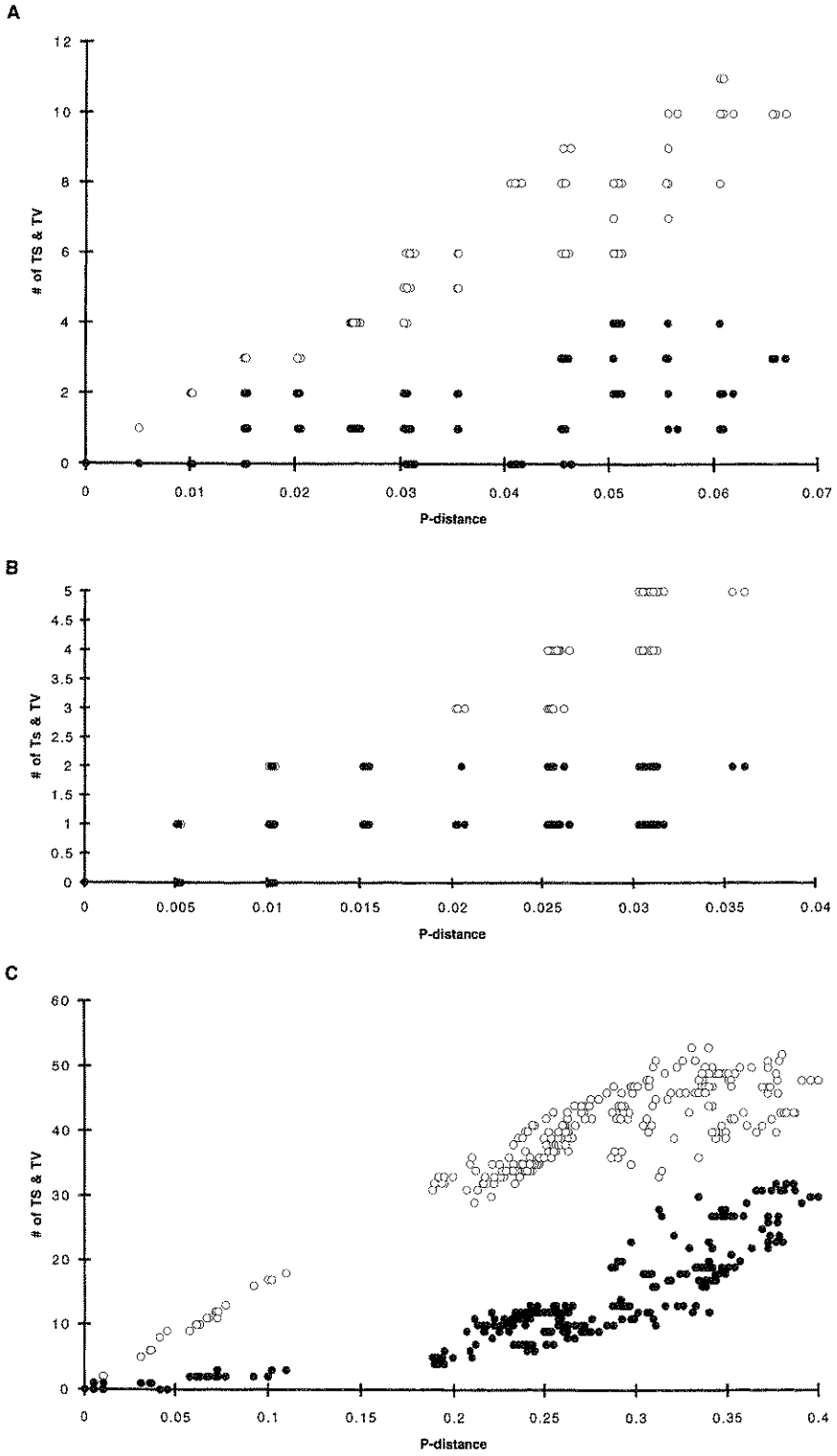

FIG. 2. Scatter plots of number of nucleotide substitutions (transitions (TS) = open circles, transversions (TV) = filled circles) versus genetic difference (p-distance) at (A) first, (B) second and (C) third codon positions.

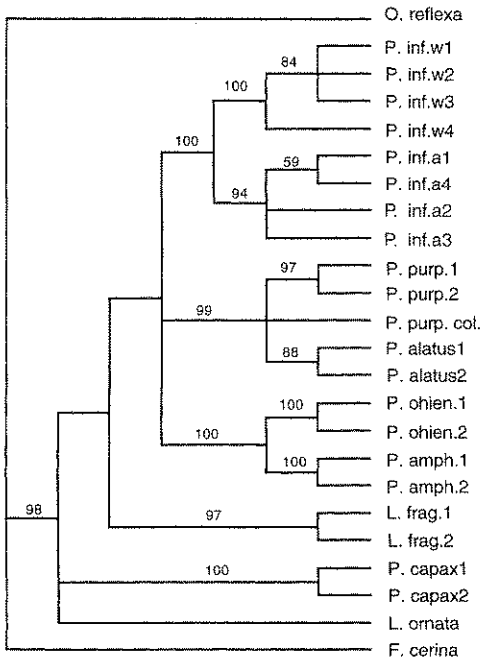

FIG. 3. Strict consensus tree for five equally parsimonious cladograms based on maximum parsimony analysis using equal weighting of all substitutions. Numbers correspond to the percentage of bootstrap replicates where the clade was found (200 total replications). Only values greater than 50% are shown. Taxon labels follow Table 2.

The placement of *P. capax* outside the remaining members of *Potamilus* indicates possible affinities with other genera. *Potamilus capax* had been placed in *Lampsilis* by Simpson (1914) based on similarities of the adult shells, particularly *L. satura* (I. Lea, 1852) (Valentine & Stansbery, 1971). Based on glochidia morphology, Coker & Surber (1911) indicated that *capax* was not a *Lampsilis* but a *Potamilus.* The molecular evidence presented here indicate no support for the placement of *P. capax* in *Potamilus;* for the present, we withhold a formal recommendation concerning the generic affinity of *P. capax* until a more inclusive analysis can be performed, including the type species of both *Leptodea* and *Lampsilis.*

Our analyses suggest that *P. p. coloradoensis* may represent a species distinct from *P. purpuratus* (Fig. 4B). Simpson (1914) also recognized *P. coloradoensis* (I. Lea, 1856) as a distinct species, although he admitted he was doubtful of its validity. The placement of the specimen referable to *P. coloradoensis* in our analysis is equivocal, either being sister to *P. purpuratus* or *P. alatus.* Examination of adult shells reveals differences in periostracum and nacre color between *P. p. coloradoensis* and *P. purpuratus* shells from east of the Mississippi River. Specimens of *P. alatus* are generally distinguishable from those of *P. purpuratus,* but examination of the glochidia of representatives of these taxa reveals no detectable differences. Based upon genetic distances *P. p. coloradoensis* is phenetically more similar to *P. alatus* (1.2%) than to *P. purpuratus* (1.5%). Genetic distances between these taxa exceed the intraspecific variation observed in all other species included in the study, with the exception of *P. inflatus.* Further research involving representatives of *P. purpuratus* and *P. alatus* from throughout their respective ranges is necessary to resolve the relationships of this clade. For the present, we recommend caution in treating *P. p. coloradoensis* and *P. purpuratus* as the same evolutionary entity.

Both *P. ohiensis* and *P. amphichaenus* were placed in the genus *Leptodea* by Burch (1975), however no support for the sister relationships of *Leptodea* and these taxa is found in this analysis. The molecular data do provide strong support for the sister relationships of *P. ohiensis* and *P. amphichaenus,* and indicate they represent distinct evolutionary entities, more closely related to other members of *Potamilus* than to *L. fragilis.*

The paraphyletic nature of *Potamilus* raises questions about the monophyly of other closely related unionid genera, such as *Leptodea. Leptodea* contains three species: *L. fragilis, L. ochracea* and *L. leptodon.* Of these, *L. ochracea* was assigned to *Lampsilis* by several authors (Simpson, 1914; Johnson, 1970; Burch, 1975) because of similarities in appearance of adult shells, particularly to *Lampsilis cariosa.* Morrison (1975) placed it in *Leptodea* because it lacked the mantle flaps often seen in species of *Lampsilis.* Hoggarth (1988) found the glochidia of *L. ochracea* to be more similar to *L. fragilis* and recommended retaining it in *Leptodea.* The type species, *Leptodea leptodon,* was originally assigned to *Leptodea* by Rafinesque (1820). It was also placed in *Lampsilis* by Simpson (1914). This species has always been considered rare (Oesch, 1984) and has become very difficult to find recently. Ultimately, any taxonomic revision of these taxa must include type species. Future phylogenetic analyses including these and other allied taxa are needed in order to more fully resolve relationships among these genera.

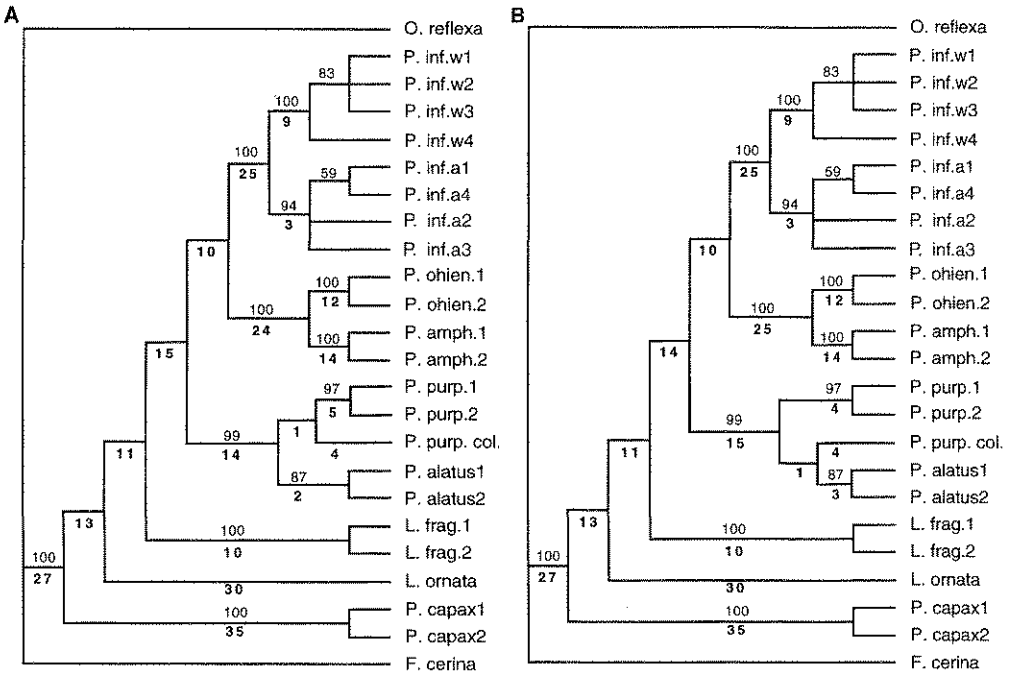

FIG. 4. (A, B). Two equally parsimonious cladograms based on maximum parsimony analysis weighting transversions 2× transitions at the third codon position. Numbers above the branches correspond to the percentage of bootstrap replicates where the clade was found (200 total replications). Only values greater than 50% are shown. Boldface numbers below the branches correspond to the number of nucleotide substitutions at those nodes. Taxon labels follow Table 2.

Conservation Genetics of *Potamilus inflatus*

DNA sequence data have been used to clarify relationships both between and within species for a large variety of organisms from whales (Milinkovitch et al., 1993) to hermit crabs (Cunningham et al., 1992). However, very few intraspecific comparisons of DNA sequences exist for studies involving unionids (Liu et al., 1996a; Mulvey et al., 1997).

Intraspecific studies are necessary for wise management decisions concerning endangered and threatened species. Phylogenetic analysis of sequence data of the COI gene indicates that populations of *P. inflatus* from the Amite River, Louisiana, and the Black Warrior River, Alabama, are reciprocally monophyletic (Figs. 3, 4) and represent distinct evolutionary entities (Moritz, 1994; Mayden & Wood, 1995). Genetic distances and the number of nucleotide substitutions that separate these two populations were compared with the number of substitutions that separate well-established species. Examination of genetic distances reveals that the two populations of *P. inflatus* are more distinct genetically than *P. purpuratus* is from *P. alatus* (Table 2). Examination of nucleotide substitution patterns reveals that a total of 12 diagnostic substitutions separate the two populations of *P. inflatus,* whereas *P. alatus* and *P. purpuratus* are separated from each other by eight substitutions. In another comparison of congenerics, *P. ohiensis* and *P. amphichaenus* are separated by 26 substitutions.

Nucleotide substitutions are considered by some researchers to accumulate at a similar rate for closely related taxa (Wilson et al., 1987; Vigilant et al., 1991; Wayne et al., 1991; Li, 1993). If this is true for *Potamilus,* it would indicate a more distant divergence time for the two populations of *P. inflatus* than that for some conspecifics. Alternatively, the differences observed could indicate an increased rate in nucleotide substitutions for the *inflatus* clade. In either case, based on these data, a strong argument can be made for the recognition of the Black Warrior and Amite popula-

tions of *P. inflatus* as distinct species. To date no conchological characters have been found that support the molecular data, and discrimination between these two species is based solely upon DNA sequence data. The recognition of cryptic unionid species is not without precedent. Davis (1983) identified allozymic differences for two phenotypically similar species of *Uniomerus*. The degree of genetic differentiation observed between populations of *P. inflatus* was greater than that seen in a comparison of two other morphologically distinct species of *Potamilus* and exceeded intraspecific values for all other species. The current geographic isolation of these two populations can only lead to further genetic differentiation of these entities and has serious implications for any plans to reintroduce *P. inflatus* in areas where it once occurred. Other studies involving mitochondrial DNA variation in unionids have come to similar conclusions regarding the protection of genetically distinct forms. For example, in a study of the conservation genetics of two unionid genera, Mulvey et al. (1997) confirmed the distinctiveness of *Amblema neislerii* (I. Lea, 1858) and *A. plicata* (Say, 1817) using allozyme and DNA sequence data. Mulvey et al. (1997) recommended additional protection for *A. neislerii* because of its restricted range and particular habitat requirements. In another study, Liu et al. (1996b) urged caution regarding any efforts aimed at re-establishing populations of the giant floater, *Pyganodon grandis*, in Colorado, because of observed mitochondrial DNA differentiation between different river drainages. Given the unique genetic status of the Amite and Black Warrior forms of *P. inflatus*, we recommend that each should be managed as a distinct evolutionary entity.

The utility of the COI gene for elucidating relationships at the species level in our study is based primarily on the relatively high number of substitutions at the third codon position. The relative lack of support, as measured by bootstrapping, for deeper nodes in the phylogeny is due in part to the smaller number of variable sites at the first and second positions. It is possible that sequencing a larger portion of the COI gene would result in higher support for these internal nodes. Lydeard & Roe (1997) found that the complete cytochrome *b* gene proved useful for diagnosing relationships of representative actinopterygian fishes, contrary to previous studies based on only a portion of the gene. These studies questioned the usefulness of this particular gene for resolving deeper phylogenetic relationships (Hillis & Huelsenbeck, 1992; Graybeal, 1993), but merely lacked sufficient data to address the question at hand.

Historically, much of the uncertainty surrounding the placement of particular unionid species in one genus or another can be attributed to the use of characters of unknown phylogenetic utility and the absence of any objective analysis. In the case of *Potamilus*, the phylogenetic analysis of an independent molecular data set indicates that such characters as glochidia shape and spines on glochidia may be homoplastic and thus not useful in diagnosing natural groups of mussels. Further investigations involving *Potamilus* and other genera are warranted and should include morphological as well as molecular characters. Davis (1983) recommended the use of multiple data sets for resolving relationships between unionid taxa. The use of multiple data sets, such as morphological and molecular characters, both independently and in a total evidence approach (Kluge, 1989) would provide a more accurate test of the phylogenetic utility of molecular and traditional morphological characters in an evolutionary context and provide much needed insight into the evolution of these traits.

ACKNOWLEDGMENTS

We wish to thank the following individuals for their assistance in procuring specimens: R. Howells, Texas Parks and Wildlife; J. Harris and R. Doster, Arkansas State Highway and Transportation Department, and P. Hartfield, USFWS. A special thanks to P. Hartfield for bringing the *P. inflatus* question to our attention. This research was supported by the Conchologists of America, the Hawaiian Malacological Society (to K. J. R.) and USFW Contract #43910-5-0098, National Science Foundation DEB-9527758 and 9707623 (to C. L.). We also thank David Neely for the glochidium illustration and A. M. Simons, A. Bogan, P. Harris and two anonymous reviewers for their comments on drafts of this manuscript.

LITERATURE CITED

BOGAN, A. E., J. D. WILLIAMS & S. L. H. FULLER, 1990, Comments on the proposed conservation of *Proptera* Rafinesque, 1819 (Mollusca:

Bivalvia). *Bulletin of Zoological Nomenclature,* 47: 206–207.

BURCH, J. B., 1975, *Freshwater unionacean clams (Mollusca: Pelecypoda) of North America* revised edition. Malacological Publications, Hamburg, Michigan 204 pp.

BZN, 1992, Opinion 1665, *Potamilus* Rafinesque, 1818 (Mollusca, Bivalvia): not suppressed. *Bulletin of Zoological Nomenclature,* 49: 81–82.

CABOT, E. L. & A. T. BECKENBACH, 1989, Simultaneous editing of multiple nucleic acid and protein sequences with ESEE. *Computer Applications in the Biosciences,* 5: 233–234.

CLARKE, A. H., 1981, *The freshwater molluscs of Canada.* National Museum of Natural Sciences, Ottawa, Canada. 446 pp.

COKER, R. E. & T. SURBER, 1911, A note on the metamorphosis of the mussel *Lampsilis laevissimus. Biological Bulletin,* 20: 179–182.

CONRAD, T. A., 1838, *Monography of the family Unionidae or naiades of Lamarck.* J. Dobson, Philadelphia, Pennsylvania. p. 95, pl. 52.

CUNNINGHAM, C. W., N. W. BLACKSTONE & L. W. BUSS, 1992, Evolution of king crabs from hermit crab ancestors. *Nature,* 355: 539–542.

DAVIS, G. M., 1983, Relative roles of molecular genetics, anatomy, morphometrics and ecology in assessing relationships among North American Unionidae (Bivalvia). Systematics Association Special Volume No. 24., *Protein polymorphism: adaptive and taxonomic significance.* G. S. OXFORD & D. ROLLINSON, eds. Academic Press, London, pp. 193–222.

FELSENSTEIN, J. F., 1985, Confidence limits on phylogenies: an approach using the bootstrap. *Evolution,* 39: 783–791.

FOLMER, O., W. R. HOEH, M. B. BLACK & R. L. VRIJENHOEK, 1994, DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from metazoan invertebrates. *Molecular Marine Biology and Biotechnology,* 3(5): 294–299.

FRIERSON, L. S., 1898, *Unio (Lampsilis) amphichaenus.* n. sp. *Nautilus* 11: 109–110.

FRIERSON, L. S., 1927, *A classified and annotated checklist of the North American naiades.* Baylor University Press, Waco, Texas. 111 pp.

GREEN, J., 1832, Notes of a naturalist. *Cabinet of Natural History and American Rural Sports,* 2: 209–291.

GRAYBEAL, A., 1993, The phylogenetic utility of cytochrome *b:* lessons from Bufonid frogs. *Molecular Phylogenetics and Evolution,* 2: 256–269.

GYLLENSTEN, U. B. & H. A. ERLICH, 1988, Generation of single stranded DNA by the polymerase chain reaction and its application to direct sequencing of the HLA-DQA locus. *Proceedings of the National Academy of Sciences, USA,* 85: 7652–7656.

HILLIS, D. M. & J. P. HUELSENBECK, 1992, Signal, noise, and reliability in molecular phylogenetic analyses. *Journal of Heredity,* 83: 189–195.

HOEH, W. R., 1990, Phylogenetic relationships among eastern North American *Anodonta* (Bivalvia: Unionidae). *Malacological Review,* 23: 63–82.

HOEH, W. R., D. T. STEWART, B. W. SUTHERLAND & E. ZOUROS, 1996, Multiple origins of gender-associated mitochondrial DNA lineages in bivalves (Mollusca: Bivalvia). *Evolution,* 50: 2276–2286.

HOGGARTH, M. A., 1988, *The use of glochidia in the systematics of the Unionidae (Mollusca: Bivalvia).* Ph.D. Dissertation, The Ohio State University, Columbus, 340 pp.

JOHNSON, R. I., 1970, The systematics and zoogeography of the Unionidae (Mollusca: Bivalvia) of the southeastern Atlantic slope. *Bulletin of the Museum of Comparative Zoology,* 140: 263–450.

KIMURA, M., 1980, A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution,* 16: 111–120.

KLUGE, A. G., 1989, A concern for evidence and a phylogenetic hypotheses of relationships among Epicrates (Boidae, Serpentes). *Systematic Zoology* 18: 1–32.

KUMAR, S., K. TAMURA & M. NEI, 1993, *MEGA: Molecular evolutionary genetics analysis.* Version 1.01. The Pennsylvania State University, University Park, 130 pp.

LAMARCK, J. B. P. A., 1819, *Histoire naturelle des animaux sans vertebrès.* Vol. VI, p. 71.

LEA, I., 1831, Observations on the naiades and descriptions of new species of that and other families. *Transactions of the American Philosophical Society,* 4: 63–121.

LEA, I., 1852, Descriptions of new species of the family Unionidae. *Proceedings of the American Philosophical Society,* 5: 252.

LEA, I., 1856, Descriptions of four new exotic uniones. *Proceedings of the Academy of Natural Sciences of Philadelphia,* 8: 103.

LEA, I., 1858, Descriptions of twelve new uniones of the United States. *Proceedings of the Academy of Natural Sciences of Philadelphia,* 2: 165.

LI, W.-H., 1993, So, what about the molecular clock hypothesis?. *Current Opinion in Genetics and Development,* 3: 896–901.

LIU, H-P., J. B. MITTON & S. J. HERRMANN, 1996a, Genetic differentiation in and management recommendations for the freshwater mussel, *Pyganodon grandis* (Say, 1829). *American Malacological Bulletin,* 13: 117–124.

LIU, H-P., J. B. MITTON & S-K. WU, 1996b, Paternal mitochondrial DNA differentiation far exceeds maternal mitochondrial DNA and allozyme differentiation in the freshwater mussel, *Anodonta grandis grandis. Evolution,* 50(2): 952–957.

LYDEARD, C., M. MULVEY & G. M. DAVIS, 1996, Molecular systematics and evolution of reproductive traits of North American freshwater unionacean mussels (Mollusca: Bivalvia) as inferred from 16*s* rRNA DNA sequences. *Transactions of the Royal Society (London)* (B) 351: 1593–1603.

LYDEARD, C. & K. J. ROE, 1997, The phylogenetic

utility of the mitochondrial cytochrome b gene for inferring relationships of actinopterygian fishes. Pp. 281–299, in: C. STEPIAN & T. KOCHER, eds., *Molecular biology of fishes,* Academic Press.

MAYDEN, R. L. & R. M. WOOD, 1995, Systematics, species concepts, and the evolutionarily significant unit in biodiversity and conservation biology. *American Fisheries Society Symposium,* 17: 58–113.

McMAHON, R. F., 1993, Mollusca: Bivalvia. Pp. 315–399, in: J. H. THORPE, & A. P. COVITCH, eds., *Ecology and classification of North American freshwater invertebrates.* Academic Press, San Diego.

MILINKOVITCH, M. C., G. ORTI & A. MEYER, 1993, Revised phylogeny of whales suggested by mitochondrial ribosomal DNA sequences. *Nature,* 361: 346–348.

MORITZ, C. T., 1994, Defining 'evolutionarily significant units' for conservation. *Trends in Ecology and Evolution,* 9: 373–375.

MORRISON, J. P. E., 1969, The earliest names for North American naiads. *American Malacological Union Annual Reports,* for 1969: 22–24.

MORRISON, J. P. E., 1975, Maryland and Virginia mussels of Lister. *Bulletin of the American Malacological Union,* for 1974: 36–39.

MULVEY, M., C. LYDEARD, D. L. PEYER, K. M. HICKS, J. BRIMBOX, J. D. WILLIAMS & R. S. BUTLER, 1997, Conservation genetics of North American freshwater mussels *Amblema* and *Megalonaias. Conservation Biology,* 11: 868–878.

OESCH, R. D., 1984, *Missouri naiades: a guide to the mussels of Missouri.* Missouri Department of Conservation. Jefferson City, Missouri 270 pp.

ORTMANN, A. E., 1912, Notes upon the families and genera of the naiades. *Annals of the Carnegie Museum,* 8: 222–365.

RAFINESQUE, C. S., 1818, Discoveries in natural history, made during a journey through the western region of the United States, by Constantine Samuel Rafinesque, esq. addressed to Samuel L. Mitchill, president and other members of the Lyceum of Natural History, in a letter dated at Louisville, falls of Ohio, 20th July, 1818. *American Monthly Magazine and Critical Review,* 3: 354–355.

RAFINESQUE, C. S., 1819, Prodrome de 70 nouveaux genres d'animaux découverts dans l'intériens des Etats-Unis d'Amérique, durant l'année 1818. *Journal de Physique,* 88: 417–429.

RAFINESQUE, C. S., 1820, Monographie des coquilles bivalves fluviatiles de la Riviere Ohio, contenant douze genres et soixantehuit especies. *Annales Generales des Sciences Physiques, Bruxelles,* 5: 287–322.

ROE, K. J., D. CONKEL & C. LYDEARD, 1997a, Molecular systematics of middle american cichlid fishes and the evolution of trophic types in '*Cichlasoma* (*Amphilophus*)' and '*C.* (*Thorichthys*)'. *Molecular Phylogenetics and Evolution,* 7: 366–376.

ROE, K. J., A. M. SIMONS & P. HARTFIELD, 1997b, Identification of a fish host of the inflated heelsplitter *Potamilus inflatus* (Bivalvia: Unionidae) with a description of its glochidium. *American Midland Naturalist,* 138: 48–54.

SAY, T., 1817, Conchology. in: William Nicholson, *American edition of the British encyclopedia,* 1st edition. Samuel A. Mitchell and Horace Ames, Philadelphia.

SIMPSON, C. T., 1914, A *descriptive catalog of the naiades, or pearly freshwater mussels.* 1540 pp., Bryant Walker, Detroit, Michigan.

SWOFFORD, D. L., 1993, *PAUP: phylogenetic analysis using parsimony.* Version 3.1.1 Illinois Natural History Survey Champaign.

SURBER, T., 1912, *Identification of the glochidia of freshwater mussels.* U.S. Bureau of Fisheries Document 771: 10 pp., 3 pl.

SURBER, T., 1915, *Identification of the glochidia of freshwater mussels.* U.S. Bureau of Fisheries Document 813: 11 pp., 1 pl.

TURGEON, D. D., A. E. BOGAN, E. V. COAN, W. K. EMERSON, W. G. LYONS, W. L. PRATT, C. F. E. ROPER, A. SCHELTEMA, F. G. THOMPSON & G. D. WILLIAMS, 1988, *Common and scientific names of aquatic invertebrates from the United States and Canada: mollusks.* American Fisheries Society Special Publication 16:vii + 277 pp., 12 pls.

U.S. FISH AND WILDLIFE SERVICE, 1992, *Inflated heelsplitter, (Potamilus inflatus) technical agency draft recovery plan.* U.S. Fish and Wildlife Service. Jackson, Mississippi. 20 pp.

UTTERBACK, W. I., 1915, The naiades of Missouri. *American Midland Naturalist,* 4: 1–29, 41–53, 97–152, 181–204, 244–273.

VALENTINE, B. D. & D. H. STANSBERY, 1971, An introduction to the naiads of the Lake Texoma region, with notes on the Red River fauna. *Sterkiana,* 42: 1–40.

VIGILANT, L., M. STONEKING, H. HARPENDING, K. HAWKES & A. C. WILSON, 1991, African populations and the evolution of human mitochondrial DNA. *Science,* 253: 1503–1507.

WAYNE, R. K., S. K. GEORGE, D. GILBERT, P. W. COLLINS, S. D. KOVACH, D. GIRMAN & N. LEHMAN, 1991, Molecular distance and divergence time in carnivores and primates. *Molecular Biology and Evolution,* 8: 297–319.

WILLIAMS, J. D., M. L. WARREN Jr., K. S. CUMMINGS, J. L. HARRIS & R. J. NEVES, 1992, Conservation Status of the freshwater mussels of the United States and Canada. *Fisheries,* 18(9): 6–22.

WILSON, A. C., H. OCHMAN & E. M. PRAGER, 1987, Molecular time scale for evolution. *Trends in Genetics,* 3: 241–247.

Revised ms. accepted 21 October 1997