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Molecular systematics of the North American freshwater bivalve genus *Quadrula* (Unionidae: Ambleminae) based on mitochondrial ND1 sequences

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Abstract

A molecular phylogeny of the bivalve genus *Quadrula* (Unionidae) was constructed based on nucleotide sequences of the mitochondrial ND1 gene. Phylogenetic analysis of 66 specimens representing 17 of the 20 currently recognized taxa within *Quadrula*, three closely allied species, and 16 outgroup taxa reveals a non-monophyletic *Quadrula* due to the placement of *Tritogonia verrucosa*, '*Fusconaia*' *succissa*, and '*Quincuncina*' *infucata*. We suggest that the taxonomic description of the genus *Quadrula* be expanded to include these species. Within the genus, we continue to recognize three monophyletic species groups (the *quadrula*, *metanvera*, and *pustulosa* species groups), as historically described; however, the *pustulosa* species group must include '*F.*' *succissa* and '*Quincuncina*' *infucata*. Finally, while our findings place the monotypic genus *Tritogonia* within *Quadrula*, its relationship to members within the genus *Quadrula* remains unresolved.

Keywords: Unionidae; *Quadrula*; mtDNA; ND1; Phylogeny

1. Introduction

The unionid bivalve genus *Quadrula* is comprised of 20 recognized taxa distributed throughout the rivers and streams of eastern North America (Turgeon et al., 1998; Williams et al., 1993). Five species are federally listed as endangered, five non-listed species are considered imperiled, and three species are presumed extinct (Table 1). Species ranges within the genus vary greatly, some are widely distributed (the entire Mississippi River Basin) while others are highly endemic. Considerable shell variation also exists within and among species of *Quadrula*, and is in part responsible for the taxonomic confusion surrounding the number of species that belong to this genus. Shells are thick and solid and round, quadrate, subrhomboidal, or elongate in shape. The shell surface can be completely smooth, but more often highly sculptured with pustules, tubercles, or ridges (Fig. 1). The combination of shell shape and surface texture have been hypothesized to function either as anchors to hold mussels in position within the substrate or anti-scouring devices that reduce the movement of substrate surrounding the shell (Watters, 1994). Clinal variation in shell morphology from the headwaters to downstream reaches appears to occur in some amblemines, including species of *Quadrula* (Clarke, 1982; Eagar, 1950; Ortmann, 1920). This degree of apparent phenotypic plasticity has been used as an argument against the sole use of morphology to identify species or evolutionary lineages (Mulvey et al., 1997; Stansbery, 1983; Williams and Mulvey, 1994).

Several higher-level phylogenetic studies conducted on the Unionidae have included only a couple of representatives of *Quadrula* (Davis and Fuller, 1981; Graf and O'Foighil, 2000; Lydeard et al., 1996, 2000); however, no species-level phylogeny for the genus has been proposed. The genus has been placed in the subfamily Ambleminae and polyphyletic tribe Amblemini, apparently sister to the monotypic genus *Mega-*

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Table 1
List of *Quadrula* taxa, listed by their respective species groups

	Sample size	Conservation status
Quadrula species group		
Quadrula apiculata (Say, 1829)	1	CS
Quadrula fragosa (Conrad, 1835)	—	E, 1991
Quadrula nobilis (Conrad, 1854)	2	Not currently recognized
Quadrula quadrula (Rafinesque, 1820)	5	CS
Quadrula rumphiana (Lea, 1852)	4	SC
Pustulosa species group		
Quadrula asperata (Lea, 1861)	5	CS
Quadrula aurea (Lea, 1859)	2	SC
Quadrula couchiana (Lea, 1860)	—	I*
Quadrula houstonensis (Lea, 1859)	—	I
Quadrula keineriana (Lea, 1852)	1	Not currently recognized
Quadrula nodulata (Rafinesque, 1820)	2	CS
Quadrula petrina (Gould, 1855)	1	I
Quadrula pustulosa (Lea, 1831)	8	CS
Quadrula mortoni (Conrad, 1835)	2	CS
Quadrula refulgens (Lea, 1868)	1	SC
Metanevra species group		
Quadrula c. cylindrica (Say, 1817)	1	I
Quadrula c. strigillata (Wright, 1898)	1	E, 1997
Quadrula intermedia (Conrad, 1836)	3	E, 1976
Quadrula metanevra (Rafinesque, 1820)	3	CS
Quadrula sparsa (Lea, 1841)	2	E, 1976
Quadrula stapes (Lea, 1831)	—	E*, 1987
Quadrula tuberosa (Lea, 1840)	—	I*

Sample size refers to number of individuals used in this study. Conservation status based on Williams et al. (1993) and Lydeard et al. (1999). CS, currently stable; SC, special concern; I, imperiled (endangered or threatened, but not federally listed); E, federally endangered with listing date; *, presumed extinct.

lonaias (Lydeard et al., 1996). Simpson (1900) recognized three species groups within *Quadrula* based on shell shape. These are now referred to as the *quadrula*, *metanevra*, and *pustulosa* species groups. Other authors, such as Frierson (1927), recognized these groups as subgenera. See Table 1 for list of *Quadrula* species in their respective species groups. It is unknown whether these species groups reflect actual phylogenetic entities. Lydeard et al. (2000) found preliminary evidence to suggest that two species, *Fusconaia succissa* and *Quincuncina infucata*, may actually be members of the genus *Quadrula*. Because of their questionable taxonomic placement, the generic names of '*Fusconaia*' *succissa* and '*Quincuncina*' *infucata* will be in single quotes.

In the present study, DNA sequences coding for the first subunit of the mitochondrial NADH dehydrogenase (ND1) gene were used to estimate the relationships within the genus *Quadrula* and among its closely allied species. Specifically, this study tests the monophyly of the genus *Quadrula*, compares morphologically diagnosed species groups to molecular phylogenetic hypotheses, and estimates the phylogenetic relationships and validity of some of the currently recognized species. These data also provide a useful evolutionary framework for future intraspecific studies within *Quadrula*.

2. Materials and methods

2.1. Specimens and vouchers

We examined 45 specimens representing 17 taxa of *Quadrula* and 19 other unionid species for our study (Materials examined). The sampling strategy included members from all three *Quadrula* species groups. Whenever possible, two specimens for each ingroup species were sequenced. We were unable to obtain specimens or tissue clips of *Q. fragosa*, which is federally listed as endangered or *Q. houstonensis*, which is an imperiled species. In addition, *Q. couchiana*, *Q. stapes*, and *Q. tuberosa* are presumed extinct (R.G. Howells, pers. comm.; Williams et al., 1993; Turgeon et al., 1998, respectively). Outgroup species were chosen based on the results of Lydeard et al. (1996, 2000) and hypothesized relationships based on morphology (Simpson, 1900; Sterki, 1907; Ortmann, 1912). In particular, *Cyclonaias tuberculata*, *Megalonaias nervosa*, *Plectomerus dombyanus*, '*Fusconaia*' *succissa*, '*Quincuncina*' *infucata*, and *Tritogonia verrucosa* were included to test the monophyly of *Quadrula*. Voucher specimens are deposited at the University of Alabama Unionid Collection (UAUC) or the Illinois Natural History Survey (INHS) (see Materials examined).

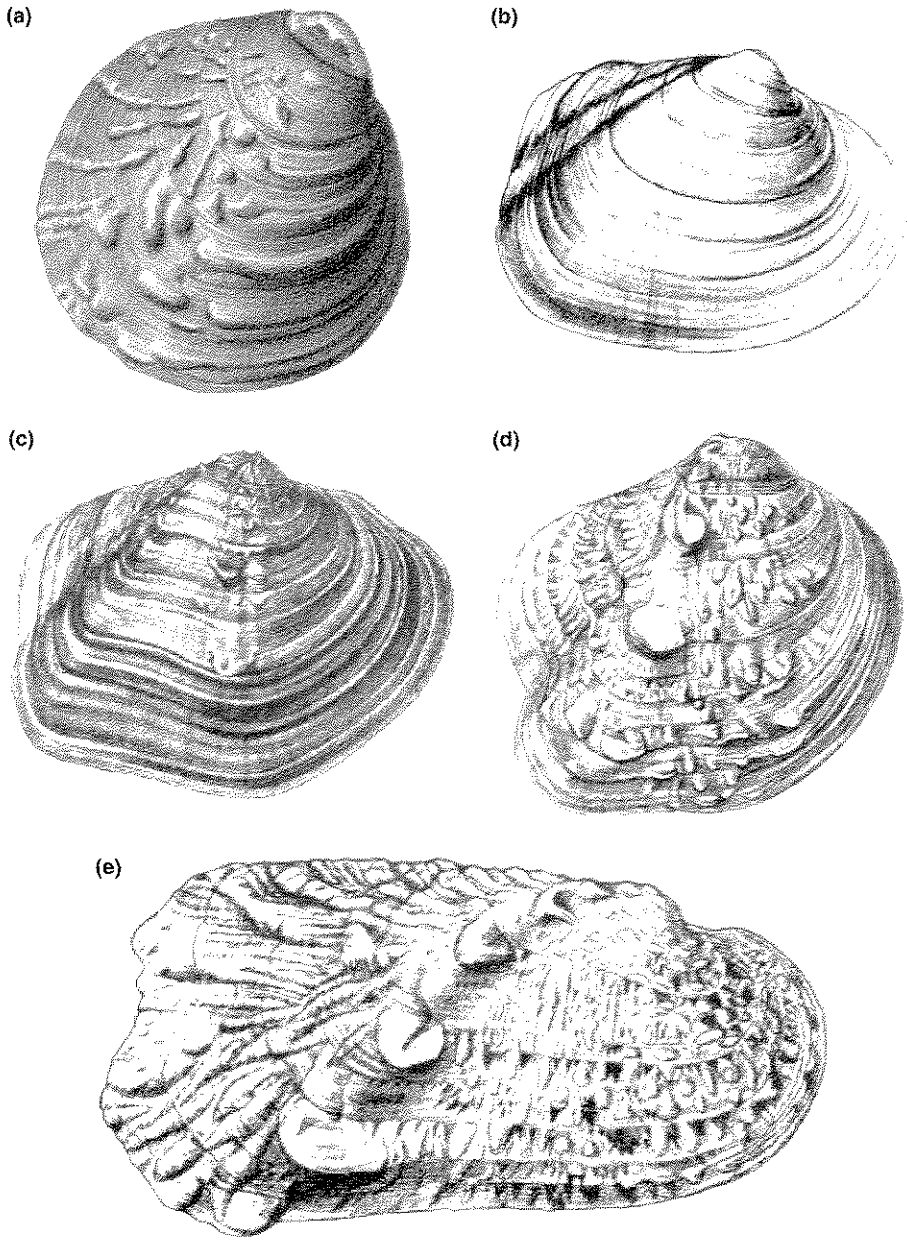

Fig. 1. Representative conchological variation within the genus *Quadrula*: (a) *Q. asperata* and (b) *Q. aurea* (the *pustulosa* species group); (c) *Q. quadrula* (the *quadrula* species group); (d) *Q. metanevra* and (e) *Q. cylindrica* (the *metanevra* species group). Drawings modified from Lea (1862) and Burch (1975).

2.2. DNA extraction, amplification, and sequencing

Whole genomic DNA was extracted from mantle tissue from frozen or ethanol-preserved specimens using standard proteinase K/SDS digest (Roe and Lydeard, 1998) or CTAB (Shahjahan et al., 1995) extraction methods followed by phenol/chloroform isolation and ethanol precipitation. A 700 basepair (bp) region of the 5′-end of the first subunit of the NADH dehydrogenase (ND1) gene was amplified using primers Leu-uurF (5′-TG GCAGAAAAGTGCATCAGATTAAAGC-3′) and NIJ-12073 (5′-TCGGAATTCTCCTTCTGCAAAGTC-3′). Genes flanking ND1, which were previously unknown for unionid species, were determined from the complete mitochondrial genome sequence of *Lampsilis ornata* (Serb and Lydeard, in prep). Leu-uurF was designed from an alignment of tRNA-Leu (uur) sequences from *Lampsilis ornata*, *Drosophila melanogaster*, and various molluscan mt genomes available on GenBank (http://www.ncbi.nlm.nih.gov). NIJ-12073 was modified from N1-N-12051 (Simon et al., 1994). For problematic taxa, a more reliable 3′-end primer was designed from the flanking the tRNA-Gly gene (LoGlyR; 5′-CCTGCT TGGAAGGCAAGTGTACT-3′). This primer pair

(Leu-uurF and LoGlyR) amplified the complete ND1 gene (~1000 bp). PCR components followed Roe and Lydeard (1998). Thermal cycling for double-stranded amplification used the following conditions: 34 cycles of denaturing (94–98 °C, 40 s), annealing (50–58 °C, 1 min), and elongation (68–72 °C, 1.5 min).

PCR products were either gel-isolated (QIAquick gel extraction kit, QIAgen) or purified using spin filtration columns (Millipore ultra-free-mc No. UFC3 LTK00). Purified PCR products were used as template for cycle sequencing reactions with the ABI Prism Big Dye Terminator kit (vers. 2.0; Applied Biosystems). Cycle sequencing reactions were cleaned by DyeEx Spin kit (QIAgen), resuspended in 10 μL of formamide, and read by an ABI 3100 automated sequencer.

Sequences were initially entered into the software program XESEE (vers. 3.0; Cabot and Beckenback, 1989) and visually aligned. The complete dataset was converted into amino acid sequence in BioEdit (Hall, 1999) to check the accuracy of the nucleotide sequence. The aligned data matrix is available electronically on the World Wide Web (http://www.bama.ua.edu/~clydeard) and individual sequences have been submitted to GenBank (see Materials examined for GenBank accession numbers). Aligned sequences were analyzed in PAUP* (vers. 4.0b10; Swofford, 2002) using maximum parsimony (MP) and maximum likelihood (ML) criteria to infer phylogenetic relationships. Maximum parsimony analyses were conducted using 100 random addition replicates of the heuristic search option with ACCTRAN, MULPARS, and TBR options. Only minimum-length trees were retained and zero-length branches were collapsed. Gaps within the tRNA-Leu (uur) alignment were coded as a fifth base. All characters were treated as unordered and of equal weight for the phylogenetic analyses due to the presumably close phylogenetic affinity of the ingroup taxa (Lydeard et al., 1996). Support for the individual nodes of the resulting phylogenetic hypotheses was assessed from decay index values (Bremer, 1988, 1994) using AutoDecay (vers. 4.0.2; Eriksson, 1999) and bootstrap values using the FAST stepwise addition option (1000 replicates) in PAUP*. A sequence evolution model was chosen using Modeltest 3.0 (Posada and Crandall, 1998) and used in the maximum likelihood analysis. Starting trees for ML were obtained via neighbor-joining (NJ). Bootstrap values using the FAST stepwise addition option (200 replicates) in PAUP* were calculated to assess support for the individual nodes. Trees were rooted in all analyses with *Lampsilis ornata*. Pairwise genetic distances were calculated across all taxa using uncorrected p-distance. Although p-distance is an underestimate of genetic divergence if saturation is occurring, p-distance values are provided for comparative purposes with other unionid studies.

To further test the monophyly of *Quadrula*, an a posteriori backbone constraint tree was constructed in MacClade 4.0 (Maddison and Maddison, 2000) and implemented in PAUP*. Only the monophyly of *Quadrula* was enforced in MP analyses. Alternative phylogenetic hypotheses (MP, ML, and constraint) were statistically compared using the Shimodaira–Hasegawa (SH) test (Shimodaira and Hasegawa, 1999) using bootstrap (1000 replicates) and RELL optimization in PAUP*.

The validity of currently recognized species was tested by employing the Phylogenetic Species Concept (PSC) (Mishler and Brandon, 1987; de Queiroz and Donoghue, 1988, 1990; Mayden, 1997). The PSC is historically based and includes the criterion of monophyly in the general sense (de Quieroz and Donoghue, 1988) or 'exclusivity,' where an exclusive group of organisms is one whose members are more closely related to each other than they are to any organisms outside the group (Baum and Donoghue, 1995).

3. Results

3.1. Sequence data

Sequence alignment of the combined tRNA-Leu and ND1 gene portions yielded 658 bp of ND1 for 66 individuals. Within the complete alignment (tRNA-Leu + ND1), 338 sites were polymorphic, 287 of which were phylogenetically informative under maximum parsimony. The first 45 bp of the alignment was sequence from the tRNA-Leu (uur), of which 28 sites were variable (20 parsimony informative). Within the ND1 gene (613 bp), 188 sites were variable at the third codon position (180 parsimony informative), 83 (59 parsimony informative) sites, and 39 (28 parsimony informative) sites at the first and second codon position, respectively.

Intraspecific uncorrected p-distance values ranged from 0.15 to 3.29%. Several interspecific pairwise comparisons yielded differences within this range including *Quadrula pustulosa* vs. *Q. aurea*, *Q. keineriana* vs. *Q. asperata*, *Q. nobilis* vs. *Q. quadrula*, *Q. apiculata* vs. *Q. quadrula*, *Q. pustulosa* vs. *Q. refulgens*, and '*Fusconaia*' *succissa* vs. *Q. refulgens*. Most interspecific pairwise uncorrected p-distance values, however, ranged from 3.65% (*Q. metanevra* vs. *Q. sparsa*) to 15.35%. Intergeneric pairwise genetic differences of taxa (excluding '*F.*' *succissa*, '*Quincuncina*' *infucata*, and *Tritogonia verrucosa*) compared with *Quadrula* ranged from 15.36% (*Megalonaias* vs. *Quadrula*) to 27.09% (lampsiline species vs. *Quadrula*). '*Fusconaia*' *succissa*, '*Quincuncina*' *infucata*, and *T. verrucosa* exhibited genetic distances comparable to intraspecific or interspecific values within *Quadrula*.

The sequence evolution model chosen by Modeltest and used for ML analysis was the general time-reversal model with among-site rate heterogeneity parameters, which allows a proportion of the sites to be invariable (I) and the remaining to vary according to a gamma distribution (Γ). Base frequencies were unequal ($A = 0.36$; $C = 0.27$; $G = 0.09$; $T = 0.28$) with variable rates of substitution among sites ($\alpha = 1.5$) and invariable sites ($I = 0.42$).

3.2. Phylogenetic analysis

The maximum parsimony analyses of the tRNA-Leu and ND1 sequence data resulted in four trees of 1508 length (CI = 0.347; RI = 0.724) (Fig. 2). Most variation among the MP trees occurred at the intraspecific level. *Quadrula* is paraphyletic (using definition of Farris, 1974) because of the placement of '*Fusconaia*' *succissa*, '*Quincuncina*' *infucata*, and *Tritogonia verrucosa*. The placement of the two former species is consistent to the findings of Lydeard et al. (2000) where these two species form a clade with *Q. quadrula*. The inclusion of '*F.*' *succissa*, '*Quincuncina*' *infucata* and *T. verrucosa* in the genus *Quadrula* is well-supported by both bootstrap (98%) and decay index (18) values.

Under MP, our sequence data support Simpson's (1900) division of *Quadrula* into three species groups, *quadrula*, *metanevra*, and *pustulosa*. The *quadrula* and *metanevra* species groups are monophyletic whereas the *pustulosa* species group includes '*F.*' *succissa* as the sister group to *Q. pustulosa*/*aurea* + *Q. mortoni*. *Quadrula nodulata* is the sister group to *Q. pustulosa*/*aurea* + *Q. mortoni* + '*F.*' *succissa*, and '*Quincuncina*' *infucata* forms a polytomy with the species group. Two Mobile River Drainage endemics, *Q. asperata* and *Q. keineriana*, form a separate clade and are the sister group to the remaining *pustulosa* species group members. A distinct Mobile River Drainage form is also seen in the *quadrula* species group (*Q. rumphiana*). The *quadrula* species group contains a monophyletic *Q. rumphiana* which is the sister group to *Q. quadrula* specimen (UAUC 1045) from the Red River (Ohio River Drainage), Kentucky. The remaining *Q. quadrula* specimens do not form a monophyletic group, even though several are from the Ohio River Drainage. A single specimen of *Q. apiculata* is contained within the *Q. rumphiana* + *Q. quadrula* clade. *Quadrula nobilis* is a monophyletic group and is the sister clade to another *Q. quadrula* (UAUC 145) from the Ohio River. The monotypic *Tritogonia verrucosa* is resolved as the sister taxon to the *quadrula* species group. The *metanevra* species group is moderately supported and is the sister group to the *pustulosa* + *quadrula* species groups. Within the *metanevra* species group, *Q. cylindrica* is the most basal taxon with all currently recognized species forming monophyletic clades (Fig. 2).

Relationships differ somewhat under the ML analysis (Fig. 3). Again, there was strong support for a *Quadrula* clade, including the same taxa as described in the MP analysis. Within *Quadrula*, the species group relationship change, where the *pustulosa* species group is the sister clade to the *metanevra* species group, and relationships within the *metanevra* species group also are altered. In addition, the *quadrula* species group is not recovered as monophyletic. Instead, *Q. nobilis* + *Q. quadrula* (UAUC 145) is the sister group to *T. verrucosa*, and this clade is basal to the remaining *Quadrula* and allied taxa.

Topologies that constrained the monophyly of *Quadrula* required 18 additional steps and were significantly different ($P < 0.001$) from both the MP and ML topologies. Variation in phylogenetic relationship between the MP and ML topologies was not significantly different ($P = 0.196$) under the SH test (Table 2).

4. Discussion

Historically, the genus *Quadrula* has been diagnosed by conchological characters, such as shell shape and sculpture, and reproductive structures, including conglutinates and the number of gills utilized as marsupia. However, data on reproductive structures of gravid females have not always been available at the time of species descriptions. In addition, variation in shell morphology has resulted in a plethora of names that have been assigned to each morphological variant and subsequently, many of these names have been synonomized. Mitochondrial markers provide an independent dataset for phylogenetic analysis and have been recently championed as a method to identify species among putative ecotypic variants and stabilize the taxonomy (Mulvey et al., 1997). Our mtDNA sequence data from the tRNA-Leu (uur) and ND1 genes provided a phylogenetic hypothesis for relationships within *Quadrula* and among its closely allied taxa, and is the first published study on unionid taxa that utilize these gene portions.

4.1. Re-examination of the genus Quadrula

All three non-*Quadrula* species ('*Fusconaia*' *succissa*, '*Quincuncina*' *infucata*, and *Tritogonia verrucosa*) that were placed within the genus in this study historically have been considered close allies to *Quadrula*. For example, the species *T. verrucosa* has been repeatedly placed in either *Quadrula* or *Tritogonia* since its description by Rafinesque (1820). Both Sterki (1907) and Ortmann (1912) recommended returning *Tritogonia* to the genus *Quadrula* based on similarity of soft parts and correlation of shell shape and sculpture. Molecular data support this relationship and further agree with

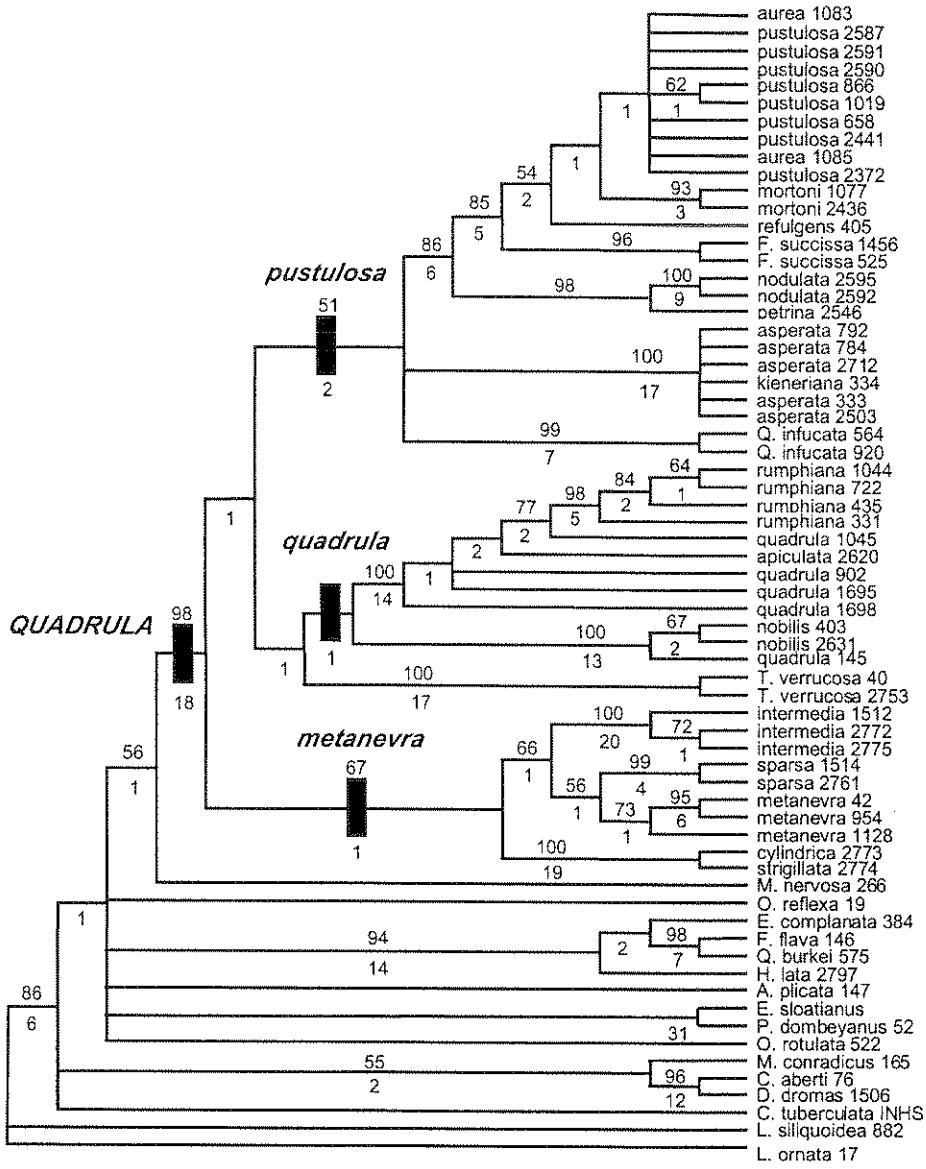

Fig. 2. Strict consensus of four trees recovered in maximum-parsimony analysis (tree length = 1508, CI = 0.347, RI = 0.724). Numbers above branches represent bootstrap support (2000 replicates) and numbers below branches indicate decay index values. Species currently recognized as *Quadrula* are listed by specific epithet. Non-*Quadrula* species are listed by the full species name. Species groups within *Quadrula* are labeled at appropriate nodes. Numbers included with the taxon label are museum accession numbers (see Materials examined).

Ortmann's (1912) placement of *T. verrucosa* as a close ally to the *quadrula* species group.

'*Quincuncina*' *infucata* was initially assigned to *Quadrula* (Simpson, 1900); however, it was placed in the newly recognized genus *Quincuncina* by Ortmann and Walker (1922) with the description of *Quincuncina burkei*. All three *Quincuncina* species possess shell sculpture with chevron-shaped nodules arranged in a quincuncial pattern. The newly described genus was distinguished from *Quadrula* by the packaging of glochidia in subcylindrical conglutinates, while *Quadrula* members possess compressed, lanceolate conglutinates (Ortmann and Walker, 1922). No conglutinate material from gravid females was available of '*Quincuncina*' *infucata* at the time of the description, and subsequently, no additional data have been published on the reproductive structures of this species (Brim-Box and Williams, 2000). Davis (1984) suggested a close phylogenetic affinity of '*Quincuncina*' *infucata* with *Quadrula* based on a phenetic analysis of allozyme data; however, his taxonomic sampling was not sufficient to fully resolve the issue. Lydeard et al. (2000) hypothesized that these diagnostic traits were homoplaseous and that the genus *Quincuncina* was polyphyletic. Here, we clearly show '*Quincuncina*' *infucata* is a member of the genus *Quadrula*.

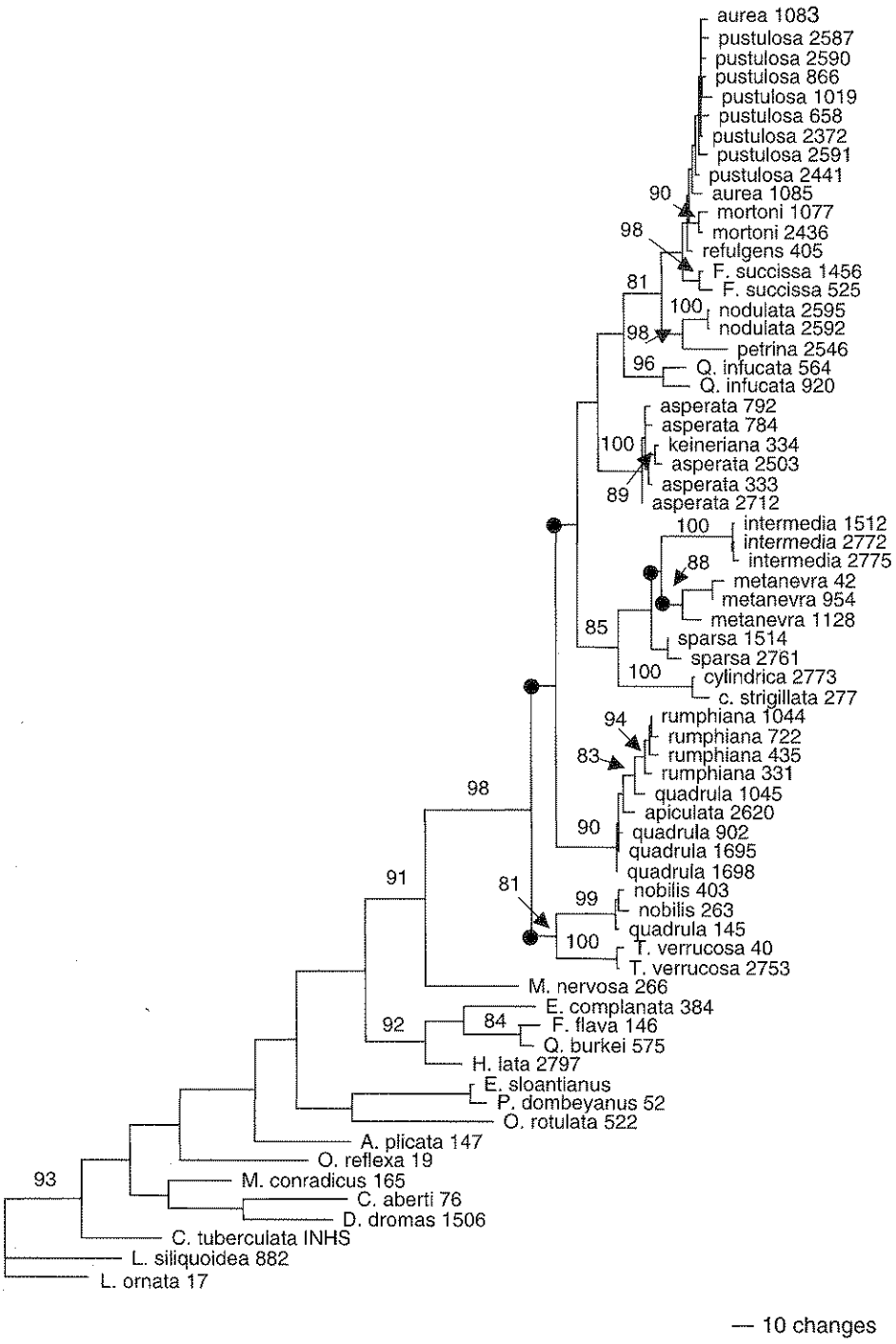

Fig. 3. Maximum likelihood tree. Branch lengths are proportional to the inferred nucleotide divergence. Bootstrap support (200 replications) greater than 80% is shown above branches. Solid circles placed on five nodes indicate variation in phylogenetic relationship between the maximum parsimony and maximum likelihood analyses. Species currently recognized as *Quadrula* are listed by specific epithet. Non-*Quadrula* species are listed by the full species name. Species groups within *Quadrula* are labeled at nodes. Numbers included with the taxon label are museum accession numbers (see Materials examined).

'*Fusconaia*' *succissa* was resolved within the *pustulosa* species group. The genus *Fusconaia* was once included as a taxonomic section within *Quadrula* (Simpson, 1900), but was elevated to a genus by Ortmann (1912) based on shape, color, and solid form of the conglutinates. At the time of description of '*F.*' *succissa*, shape and color of conglutinate material was unknown, but Ortmann (1923) felt that conchological characters of '*F.*' *succissa* did not resemble *Quadrula* species, which are highly sculptured (Ortmann, 1923). Davis and Fuller

Table 2
Summary of SH tests of alternative topologies

Topology	−ln*L*	ln*L* (diff.)	*P*
Maximum parsimony (Fig. 2)	7296.97	18.68	0.196
Maximum likelihood (Fig. 3)	7278.29	Best	—
Quadrula monophyletic	7342.90	64.00	<0.001*

Statistically significant differences between topologies are indicated by an asterisk.

(1981) suggested that '*F.*' *succissa* and *F. flava* belong to different genera based on a high genetic distance value evaluated by immunological data. Lydeard et al. (2000) recovered a polyphyletic *Fusconaia* using mtDNA sequence data (16S rRNA and COI). Our ND1 sequence data corroborates the previous hypotheses of Davis and Fuller (1981) and Lydeard et al. (2000). Although we only examined two species of *Fusconaia*, *F. flava* is the type species of the genus (Ortmann, 1912) and is placed with other members of the tribe Pleurobemini (*sensu* Davis and Fuller, 1981). Our molecular phylogeny supports the association of *Elliptio* and *Fusconaia* as Davis and Fuller (1981) reported based on extremely low immunological differences. Interestingly, the close phylogenetic relationships of the ectobranchous *Elliptio complanata*, *Hemistena lata* and the tetragenous *Fusconaia flava* and *Quincuncina burkei* supports the idea that these reproductive features do not necessarily denote close phylogenetic relationships (Davis and Fuller, 1981; Graf and O'Foighil, 2000; Lydeard et al., 1996).

As the type species of the genus *Fusconaia*, *F. flava*, appears to be basal to the *Quadrula* clade, we recommend recognizing '*F.*' *succissa* as a member of *Quadrula*. The type species for the polyphyletic genus *Quincuncina* (*Q. burkei*) is also placed within the Pleurobinini, suggesting that '*Q.*' *infucata* belongs in the genus *Quadrula*. Based on the results of our analyses, we recommend amending the genus *Quadrula* to include the aforementioned taxa and *T. verrucosa* (see Materials examined).

4.2. Phylogenetic relationships of species groups within *Quadrula*

A second goal for this study was to examine the three species groups within *Quadrula*. Our analyses supported the recognition of all three groups: monophyletic *quadrula* and *metanevra* species groups and a *pustulosa* species group including '*F.*' *succissa* and '*Quincuncina*' *infucata*. Although intraspecific sampling within each of the species groups was low, support exists for the recognition of several species. Additional studies employing denser geographic sampling is necessary to delineate species and their geographic boundaries. Interestingly, repeated geographic patterns were recovered, in particular, in the *pustulosa* and *quadrula* species groups, where there appears to be a relationship between Mississippi River and western Gulf Coast drainages versus the Mobile River System. It is expected that phylogeographic studies will yield taxa differentiated by drainage and not necessarily by current taxonomic views, especially within the *quadrula* species group.

Sequence data from the ND1 gene portion did not resolve relationships among populations of *Q. asperata* and *Q. keineriana* or *Q. aurea* and *Q. pustulosa*. Additional data will be necessary to test the validity of these taxonomic entities. Future studies on these taxa will need to include other forms within the Mobile System (*Q. aspera* and *Q. archeri*) and western Gulf Coast drainages (*Q. houstonensis*), respectively, and utilize a denser sampling scheme.

In contrast, the ND1 sequence data support taxa that were once recognized by morphological characters, but have not recognized under current taxonomic schemes (Turgeon et al., 1998). Recent studies (e.g., Watters, 1994; Williams and Mulvey, 1994) suggest that variation in shell morphology is extremely plastic due to the interaction of environmental and genetic factors. These studies suggest that shell morphology alone can be insufficient for species recognition; however, our mtDNA sequence data supports the validity of several taxa that were originally diagnosed by shell characters, but are currently unrecognized. For example, while *Q. mortoni* (in the *pustulosa* species group) was reduced to a subspecies of *Q. pustulosa* (Turgeon et al., 1998), the ND1 sequence data recovered a monophyletic *Q. mortoni*, which is the sister group to *Q. pustulosa*. This suggests that *Q. mortoni* is a valid species under the PSC. Additional sampling of the *Q. mortoni* type locality will be necessary before any taxonomic changes can be made. The placement of *Q. refulgens* between the *pustulosa* or *quadrula* species groups has been highly contested (R.G. Howells, P.D. Hartfield, J.D. Williams, pers. comm.). Our study supports the placement of *Q. refulgens* within the *pustulosa* species group. In the *quadrula* species group, *Q. nobilis* was not recognized by Turgeon et al. (1998). Our data suggest that *Q. nobilis* may be a valid entity, in particular by the placement of this taxon as a basal *Quadrula* lineage in the ML cladogram (but see below).

Except for *Q. rumphiana,* relationships within the *quadrula* species group are unresolved. In particular, the *Q. quadrula* specimens are confusing. Our study included three specimens from the Ohio River, but these appear in multiple points in the *quadrula* species group.

Most confounding is the placement of *Q. quadrula* from the Ohio River (UAUC 145) with *Q. nobilis* from the Neches and Pascagoula rivers. Denser taxonomic sampling will be needed to test the validity of *Q. quadrula* and *Q. apiculata.*

All four recognized taxa of the *metanevra* species group were recovered with good support. The federally endangered *Q. sparsa* and presumably extinct *Q. turberosa* occasionally have been synonymized under the name *Q. intermedia* (Ortmann, 1918) or treated as ecophenotypes of *Q. metanevra* (Simpson, 1914). In our molecular analysis, *Q. intermedia* and *Q. sparsa* appear to be phylogenetic species; however, relationships among *Q. metanevra*, *Q. sparsa*, and *Q. intermedia* do not agree between the MP and ML topologies. Thus, we are unable to present a strong hypothesis for phylogenetic relationship among these species. *Quadrula cylindrica* is monophyletic; however, not enough samples were examined to test the taxonomic validity of the federally endangered *Q. cylindrica strigillata.*

4.3. Taxonomic suggestions for the genus Quadrula and species groups

Analyses of mtDNA sequence support a paraphyletic *Quadrula*. We suggest that the taxonomic description of the genus *Quadrula* be expanded to include '*Tritogonia*' *verrucosa*, '*Fusconaia*' *succissa*, and '*Quincuncina*' *infucata*. This more inclusive clade is monophyletic and well-supported by the molecular data. Within the genus, we continue to recognize three species groups as described by Simpson (1900); however, the *pustulosa* species group must include '*F.*' *succissa* and '*Q.*' *infucata*. The previously recognized genus *Tritogonia* is synonymized within *Quadrula*, but its placement is unresolved between different analyses. Additional data are necessary to resolve the phylogenetic placement of the species within *Quadrula*.

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Appendix A. Materials examined

Voucher specimens are deposited at the University of Alabama Unionid Collection (UAUC) or the Illinois Natural History Survey (INHS). Museum catalog numbers, GenBank accession numbers (in parentheses), localities, and collectors are as follows: ***Quadrula apiculata***: UAUC 2620 (AY158805) Neches R., Tyler Co., Texas, R.G. Howells. ***Q. asperata***: UAUC 333 (AY158779) Coosawattee R., Gordon-Murray Co., Georgia, J.D. Williams. UAUC 784 (AY158758) Alabama R., Wilcox Co., Alabama, J.T. Garner & P.D. Hartfield. UAUC 792 (AY158757) Alabama R., Wilcox Co., Alabama, J.T. Garner & P.D. Hartfield. UAUC 2503 (AY158806) Coosa R., Elmore Co., Alabama, J.M. Pierson. UAUC 2712 (AY158768) Sucarnoochie Ck., Kemper Co., Mississippi, S.J. Fraley & J. T. Baxter. ***Q. aurea***: UAUC 1083 (AY158745) and UAUC 1085 (AY158765) Lake Corpus Christi, Live Oak Co., Texas, R.G. Howells. ***Q. c. cylindrica***: UAUC 2773 (AY158785) Duck R., Marshall Co., Tennessee, S.J. Ahlstedt. ***Q. c. strigillata***: UAUC 2774 (AY158800) Clinch R., Hancock Co., Tennessee, S.J. Ahlstedt. ***Q. intermedia***: UAUC 1512 (AY158760) Powell R., Lee Co., Virginia, S. J. Ahlstedt & S.J. Fraley. UAUC 2772 (AY158782) and UAUC 2775 (AY158783) Duck R., Marshall Co., Tennessee, S.J. Ahlstedt. ***Q. keineriana***: UAUC 334 (AY158769) Coosawattee R., Gordon-Murray Co., Georgia, J. D. Williams. ***Q. metanevra***: UAUC 42 (AY158771) Elk R., Limestone Co., Alabama, K.J. Roe. UAUC 954 (AY158803) Tennessee R., Hardin Co., Tennessee, J.T. Garner & D. Hubbs. UAUC 1128 (AY158802) Cahaba R., Dallas Co., Alabama, C. Lydeard & H. McCullagh. ***Q. mortoni***: UAUC 1077 (AY158764) Big Cypress Bayou, Marion Co., Texas, R.G. Howells. UAUC 2436 (AY158778) Lake Lewisville, Denton Co., Texas, M. Eisthen. ***Q. nobilis***: UAUC 403 (AY158786) Pascagoula R., Jackson Co., Mississippi, D. N. Shelton. UAUC 2631 (AY158804) Neches R., Tyler Co., Texas, R.G. Howells. ***Q. nodulata***: UAUC 2592 (AY158756) Mississippi R., Marion Co., Missouri, B. Sietman. UAUC 2595 (AY158755) Neches R., Tyler Co., Texas, R.G. Howells. ***Q. petrina***: UAUC 2546 (AY158798) Concho R., Concho Co., Texas, R.G. Howells. ***Q. pustulosa***: UAUC 658 (AY158762) Wolf R., Fayette Co., Tennessee, D.H. Kesler. UAUC 866 (AY158759) St. Croix R., Wisconsin, D.J. Hornbach. UAUC 1019 (AY158767) Mississippi R., Rock Island

Co., Illinois, B. Sietman. UAUC 2372 (AY158766) Amite R., East Baton Rogue/Livingston Pa., Louisiana, S. H. Shively & J. Ernst. UAUC 2441 (AY158763) Ohio R., Henderson Co., Kentucky, P. Morrison. UAUC 2587 (AY158752) and UAUC 2591 (AY158753) Ouachita R., Ouachita Co., Arkansas, J. L. Harris. UAUC 2590 (AY158754) Mississippi R., Marion Co., Missouri, B. Sietman. ***Q. quadrula***: UAUC 145 (AY158789) Ohio R., Henderson Co., Kentucky, P. Morrison. UAUC 902 (AY158772) Muskingum R., Washington Co., Ohio, B. Sietman. UAUC 1045 (AY158790) Red R., Powell Co., Kentucky, R. Cicerello. UAUC 1695 (AY158774) Ohio R., Vanderburgh Co., Indiana, M. Smith. UAUC 1698 (AY158773) Spring R., Cherokee Co., Kansas, M. Smith. ***Q. refulgens***: UAUC 405 (AY158788) Pascagoula R., Jackson Co., Mississippi, D.N. Shelton. ***Q. rumphiana***: UAUC 331 (AY158777) Coosawattee R., Gordon Co., Georgia, M.H. Hughes. UAUC 435 (AY158776) Oostanaula R., Gordon Co., Georgia, J.D. Williams. UAUC 722 (AY158775) Sipsey R., Pickens Co., Alabama, H. McCullagh & C. Lydeard. UAUC 1044 (AY158770) Black Warrior R., Jefferson Co., Alabama, J.T. Garner & P.D. Elema. ***Q. sparsa***: UAUC 1514 (AY158761) Powell R., Lee Co., Virginia, S.J. Ahlstedt & S.J. Fraley. UAUC 2761 (AY158784) Powell R., Hancock Co., Tennessee, S.J. Ahlstedt & R.G. Biggins.

Outgroup taxa: ***Amblema plicata***: UAUC 147 (AY158796) Ohio R., Kentucky, P. Morrison. ***Cyclonaias tuberculata***: INHS 20590 (AY158808) Jordan Ck., Vermilion Co., Illinois, K.S. Cummings. ***Cyprogenia aberti***: UAUC 76 (AY158749) Saline R., Saline Co., Arkansas, J.L. Harris. ***Dromus dromas***: UAUC 1506 (AY158750) Clinch R., Hancock Co., Tennessee, S.J. Ahlstedt & S.J. Fraley. ***Elliptio complanata***: UAUC 384 (AY158780) Connecticut R., Massachusetts, A.M. Simons. ***Elliptoideus sloatianus***: (AY158797) Apalachicola R., Gadsden Co., Florida, J. Brim-Box & J.D. Williams. ***Fusconaia flava***: UAUC 146 (AY158781) Ohio R., Kentucky, P. Morrison. ***F. succissa***: UAUC 1456 (AY158792) Conecuh R., Pike Co., Alabama, J.D. Williams. UAUC 525 (AY158809) Pea R., Geneva Co., Alabama, J. D. Williams. ***Hemistena lata***: UAUC 2797 (AY158787) Clinch R., Hancock Co., Tennessee, S.J. Ahlstedt. ***Lampsilis ornata***: UAUC 17 (AY158748) Cahaba R., Bibb Co., Alabama, K.J. Roe & A.M. Simons. ***L. siliquoidea***: UAUC 882 (AY158747) Douglas Lake, Cheboygan Co., Michigan, A.G.A. Pinoswka. ***Medionidus conradicus***: UAUC 165 (AY158746) Clinch R., Hancock Co., Tennessee, No collector. ***Megalonaias nervosa***: UAUC 266 (AY158794) Coosa R., Cherokee Co., Alabama, K.J. Roe. ***Obliquaria reflexa***: UAUC 19 (AY158751) Cahaba R., Bibb Co., Alabama, K. J. Roe & A. M. Simons. ***Obovaria rotulata***: UAUC 522 (AY158799) Conecuh R., Escambia Co., Alabama, J.D. Williams. ***Plectomerus dombeyanus***: UAUC 52 (AY158801) Black Warrior R., Hale Co., Alabama, K.J. Roe & A.M. Simons. ***Quincuncina burkei***: UAUC 575 (AY158793) Limestone Ck., Walton Co., Florida, J.D. Williams. ***Q. infucata***: UAUC 564 (AY158795) New R., Union/Bradford Co., Florida, J.D. Williams. UAUC 920 (AY158810) Ochlocknee R., Leon Co., Florida, C. O' Brian. ***Tritogonia verrucosa***: UAUC 40 (AY158791) Elk R., Limestone Co., Alabama, K.J. Roe. UAUC 2753 (AY158807) Cumberland R., Scott Co., Tennessee, S.J. Ahlstedt.

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