

Phylogeny of North American amblemines (Bivalvia, Unionoida): prodigious polyphyly proves pervasive across genera

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Abstract. The subfamily Ambleminae is the most diverse subfamily of fresh-water mussels (order Unionoida), a globally diverse and ecologically prominent group of bivalves. About 250 amblemine species occur in North America; however, this diversity is highly imperiled, with the majority of species at risk. Assessing and protecting this diversity has been hampered by the uncertain systematics of this group. This study sought to provide an improved phylogenetic framework for the Ambleminae. Currently, 37 North American genera are recognized in Ambleminae. Previous phylogenetic studies of amblemines highlighted the need for more extensive sampling due to the uncertainties arising from polyphyly of many currently recognized taxa. The present study incorporated all amblemine genera occurring in North America north of the Rio Grande, with multiple species of most genera, including the type species for all but seven genera. A total of 192 new DNA sequences were obtained for three mitochondrial gene regions: COI, 16S, and ND1. In combination with published data, this produced a data matrix incorporating 357 gene sequences for 143 operational taxonomic units, representing 107 currently recognized species. Inclusion of published data provides additional taxa and a summary of present molecular evidence on amblemine phylogeny, if at the cost of increasing the amount of missing data. Parsimony and Bayesian analyses suggest that most amblemine genera, as currently defined, are polyphyletic. At higher taxonomic levels, the tribes Quadrulini, Lampsilini, and Pleurobemini were supported; the extent of Amblemini and the relationships of some genera previously assigned to that tribe remain unclear. The eastern North American amblemines appear monophyletic. *Gonidea* and some Eurasian taxa place as probable sister taxa for the eastern North American Ambleminae. The results also highlight problematic taxa of particular interest for further work.

Additional key words: fresh-water mussels, systematics, mtDNA

The subfamily Ambleminae is a diverse group of fresh-water bivalve mollusks found throughout North America east of the continental divide. They are also highly imperiled due to habitat specificity, limited ranges, and complex life history (Strayer et al. 2004). Many species require free-flowing medium to large rivers, a habitat type that is extensively impacted by human activity. In addition, the need for suit-

able host fish for the parasitic larval stage (the glochidium) contributes to mussels' vulnerability to habitat disturbance (Lydeard et al. 2004). As a result, $\geq 12.6\%$ of the North American species and subspecies of Ambleminae are believed to be extinct, 22.9% are federally endangered or threatened, and many others are locally or globally rare (Turgeon et al. 1998).

Conservation efforts are hampered, however, by our limited understanding of their systematics. High levels of ecophenotypic plasticity may result in intra-specific variation in shell form exceeding intergeneric

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differences (Davis 1983). Thus, Roe (2000) used molecular sequences to demonstrate that several putative specimens of highly imperiled *Ptychobranchus* species actually represented other genera. Likewise, morphological identification of glochidia is often difficult, whereas molecular data readily identify them (White et al. 1996). This morphological variability has made both taxonomic definition and identification problematic, but both are essential to establishing conservation needs.

Well-supported phylogenetic hypotheses can provide valuable information for conservation. Many aspects of the biology of rare mussel species, such as host fish choice, habitat preferences, and breeding cycles, are often inferred based on better-studied common species (e.g., many species accounts in Parmalee & Bogan [1998] have only tentative suggestions for these features, based on other species in the genus). The closest relatives of the species of interest are likely to be the best basis for such inference. If the species is misclassified, the inferences are less likely to be accurate. Also, incorrect synonymization may lead to the neglect of phylogenetically distinctive taxa. Incorrect assumptions may lead to inappropriate research or management approaches, with potentially disastrous results for the rarest species (Minton & Lydeard 2003). Likewise, an accurate general phylogenetic framework is necessary for detailed phylogenetic studies to correctly identify relevant taxa that should be included. Ecological studies may also be misled by incorrectly grouping unrelated taxa. Such issues, along with the development of new techniques, have prompted a renewed interest in the systematics of unionids (Roe & Hoeh 2003; Strayer et al. 2004). Nevertheless, molecular data for many genera and species are still lacking. Previous analyses have generally focused either on several species from a few genera or on a broad sampling of genera represented by one or two species a piece. They also have relied on only one or two genes. The present analyses double to triple the taxonomic coverage of previous studies, incorporating all presently recognized genera of North American Amblesminae.

Both molecular and morphological data have influenced the most recent classifications of the Amblesminae. Molecular studies indicate that all studied North American unionoids fall into three categories, corresponding to the family Margaritiferidae and the unionid subfamilies Amblesminae and Unioninae (Graf 2002); however, classifications in the past few decades have varied widely in detail (see the discussion below). The present paper treats the subfamily Anodontinae, referred to by earlier authors, as a tribe

within the subfamily Unioninae. Molecular data (Hoeh et al. 2001, 2002a; Graf 2002; Huang et al. 2002; Roe & Hoeh 2003) and the presence of hooked, subtriangular glochidia (Nagel et al. 1998; Hoeh et al. 2001; Roe & Hoeh 2003) suggest that *Anodonta* belongs in the same subfamily as *Unio*. Amblesminae includes ~80% of North American species and ~75% of the genera. Some East Asian taxa appear closely related to Amblesminae (Huang et al. 2002); however, other Asian taxa once assigned to Amblesminae appear to be only distantly related (Graf 2002). A few European species have also been assigned to Amblesminae (Nagel et al. 1998). However, the apparent similarities may reflect convergent shell form or plesiomorphic features rather than true affinity. The present study included data for the Asian *Hyriopsis* and *Inversidens*, and the European *Psilunio*, to represent Old World “amblesmines.” *Gonidea*, from northwestern North America, is exceptionally problematic in its affinities. If it is an amblesmine, it is the only one in Pacific drainages of North America. Existing molecular data suggest that it may be a sister taxon to the remaining North American Amblesminae (Graf 2002). *Amblesmina*, the type genus of Amblesminae, and all species that are undisputed close relatives of it occur in the Atlantic and Gulf of Mexico drainages of North America. Within Amblesminae, the North American species have been divided among the tribes Amblesmini, Pleurobemini, and Lampsilini, plus Gonideini, with some authors also recognizing Quadrulini or other taxa (Graf 2002).

The great biological diversity of North American amblesmines was first recognized in the early 1800s, by workers such as Say, Rafinesque, Lea, and Conrad. However, this work focused primarily on describing species. A few authors recognized distinct genera within what is now the Amblesminae, but others placed them, along with most of the rest of the global unionid fauna, into the single genus *Unio*. Rafinesque (1820) named the Amblesminae (as “Amblesmidia”), the only suprageneric group proposed specifically for amblesmines before 1900. The 19th century classifications were based almost entirely on shell characters. Simpson (1891, p. 86) observed that existing classifications were largely artificial but left the work of revising them “to some capable student of the future.” In fact, Simpson himself took up this challenge.

Beginning in the late 1800s, many workers recognized the distinctiveness of the American amblesmines, adding both new genera and higher taxa. Greater emphasis on anatomical characters contributed to this development. Simpson (1900) provided

the first thorough anatomy-based classification of amblemines and other Unionoida, but did not use formal subfamilial nomenclature, introducing vernacular names instead. He also grouped several taxa with disparate but relatively simple patterns of gill brooding (present Anodontini, Pleurobemini, and Margaritiferidae). Many authors have overlooked previous names and made redundant ones (e.g., 7 of the 11 families and subfamilies Modell [1964] used for North American Unionidae are junior synonyms). Different authors treat the same taxon as a tribe, subfamily, or family. Thus, the suprageneric nomenclature is confused. For consistency, the present paper treats Unioninae and Ambleminae as subfamilies of Unionidae, with both divided into tribes. Among the most widely used higher taxa in Ambleminae, albeit often under junior synonyms, are von Ihering's (1901) *Quadrulini* and *Lampsilini*, Hannibal's (1912) *Pleurobemini*, and Ortmann's (1916) *Gonideini* (all proposed as subfamilies). Additional tribes or subfamilies were proposed by several workers, especially Modell (1964 and references therein), Starobogotov (1970), and Heard & Guckert (1971), but these higher taxa have been synonymized or ignored by other workers. The proposed relationships of amblemine tribes to true Unionini (characterized by the European *Unio*, although the name has been widely misapplied to North American *Pleurobemini*), *Anodontini*, and *Alasmidontini* (now considered a synonym of *Anodontini*) also varied greatly, with many authors suggesting that Ambleminae is paraphyletic or polyphyletic. Also, authors have varied in including or excluding Eurasian and African genera in the amblemine tribes with North American types. Table 1 compares the assignments of North American amblemine genera to higher taxa in several recent classifications.

The many morphological studies have provided much additional data, but relatively few characters have shaped most classifications. Particularly important anatomical features include the structure of the gills and their modification for brooding. Other studies focused on hinge and shell features. Heard & Guckert (1971) summarized the traditional distinguishing suprageneric characters in Ambleminae from many previous sources. *Lampsilini* was distinguished by the distinctive modifications of the female posterior outer gills for brooding and by the sexually dimorphic shells. *Quadrulini* (used interchangeably with *Amblemini*) was based on the frequently sculptured shells and the use of all 4 gills for brooding ("tetrageny"). *Pleurobemini* was characterized by the generally smooth shells and the use of only the outer gills for brooding ("ectobranchy"),

without the gill specializations of *Lampsilini*. *Gonideini* was based on the lack of hinge teeth and distinctive gill anatomy. Even within this rather short list, most authors emphasized only one or two characters. For example, Modell (1964) relied heavily on beak sculpture, and Haas (1969) emphasized shell form. In contrast, Heard & Guckert (1971, p. 337) "selectively elected to ignore one entire array of characters," namely shell features, and instead emphasized reproductive features such as larval brooding periods and brooding structures of the gills.

This focus on character states for selected features produced an emphasis on grades rather than clades. Putatively primitive characters, as well as putative synapomorphies, were frequently used in defining genera and higher taxa. Thus, Hannibal (1912) proposed that *Pleurobemini* evolved from *Quadrulini* (including the present *Amblemini*), and that *Unioninae* (including the present *Gonideini*) and *Lampsilini* evolved from *Pleurobemini*. Heard & Guckert (1971) devised a very similar system, except that *Gonideini* was seen as the sister taxon to *Amblemini*. Such paraphyletic taxa are likely to be a poor guide to evolutionary relationships. Likewise, basing higher taxa on one or a few characters runs the risk of possible homoplasy in that character (Roe & Hoeh 2003). Currently used morphological characters may not provide enough data to resolve relationships within Ambleminae (Graf & Ó Foighil 2000a). Recent studies performing cladistic analyses of morphological features have found little or no resolution of relationships of Ambleminae within Unionidae, apart from the distinctive gill structures of *Lampsilini* (Hoeh et al. 2001; Roe & Hoeh 2003). This paucity of morphological characters has resulted in relatively few morphology-based hypotheses about the phylogenetic interrelationships of individual genera. Apart from general agreement about the monophyly and constituent genera of *Lampsilini*, there have been many differences between classifications. Several species have also varied in their generic assignment from author to author. Also, the use of paraphyletic taxa in non-cladistic classifications makes it unsafe to assume that previous authors thought that the genera and higher taxa that they used were monophyletic. This pattern of classification prevailed until the advent of molecular data and cladistic methodologies prompted thorough re-examination of the taxonomy.

The fossil record provides limited help in resolving the relationships of Ambleminae, due to the sporadic nature of their fossil record and the problems of convergence in shell form. Probable amblemines occur in

Table 1. Suprageneric classifications of North American Ambleminae.

Genus	Modell (1964)	Haas (1969)	Heard & Guckert (1971)
<i>Actinonaias</i>	Elliptionidae, Lampsilinae	Unionidae, Lampsilinae	Unionidae, Lampsilinae, heterogenae
<i>Amblema</i>	Elliptionidae, Ambleminae	Unionidae, Quadrulinae	Amblemidae, Ambleminae
<i>Cyclonaias</i>	Unionidae, Quadrulinae	Unionidae, Quadrulinae	Unionidae, Pleurobeminae
<i>Cyprogenia</i>	Elliptionidae, Lampsilinae	Unionidae, Lampsilinae	Unionidae, Lampsilinae, mesogenae
<i>Cyrtionaias</i>	Elliptionidae, Lampsilinae	Unionidae, Lampsilinae	Unionidae, Popenaiadinae
<i>Dromus</i>	Elliptionidae, Lampsilinae	Unionidae, Lampsilinae	Unionidae, Lampsilinae, eschatigenae
<i>Ellipsaria</i>	Elliptionidae, Lampsilinae	Unionidae, Lampsilinae	Unionidae, Lampsilinae, heterogenae
<i>Elliptio</i>	Elliptionidae, Elliptioninae	Unionidae, Unioninae	Unionidae, Pleurobeminae
<i>Elliptoides</i>	Unionidae, Quadrulinae	Unionidae, Unioninae	Amblemidae, Ambleminae
<i>Epioblasma</i>	Elliptionidae, Lampsilinae	Unionidae, Lampsilinae	Unionidae, Lampsilinae, heterogenae
<i>Fusconaia</i>	Elliptionidae, Pleurobeminae	Unionidae, Quadrulinae	Amblemidae, Ambleminae
<i>Glebulia</i>	Elliptionidae, Lampsilinae	Unionidae, Lampsilinae	Unionidae, Lampsilinae, heterogenae
<i>Gonidea</i>	Margaritiferidae, Pseudodontinae	Unionidae, Unioninae	Amblemidae, Gonideinae
<i>Hemistena</i>	Elliptionidae, Alasmidontinae	Unionidae, Unioninae	Unionidae, Pleurobeminae
<i>Lampsilis</i>	Elliptionidae, Lampsilinae	Unionidae, Lampsilinae	Unionidae, Lampsilinae, heterogenae
<i>Lemiox</i>	Elliptionidae, Lampsilinae	Unionidae, Lampsilinae	Unionidae, Lampsilinae, heterogenae
<i>Leptodea</i>	Elliptionidae, Lampsilinae	Unionidae, Lampsilinae	Unionidae, Lampsilinae, heterogenae
<i>Lexingtonia</i>	Elliptionidae, Pleurobeminae	Unionidae, Unioninae	Unionidae, Pleurobeminae
<i>Ligumia</i>	Elliptionidae, Lampsilinae	Unionidae, Lampsilinae	Unionidae, Lampsilinae, heterogenae
<i>Medionidus</i>	Elliptionidae, Lampsilinae	Unionidae, Lampsilinae	Unionidae, Lampsilinae, heterogenae
<i>Megalonaias</i>	Unionidae, Quadrulinae	Unionidae, Quadrulinae	Amblemidae, Megaloniaidinae
<i>Obliquaria</i>	Elliptionidae, Lampsilinae	Unionidae, Lampsilinae	Unionidae, Lampsilinae, mesogenae
<i>Obovaria</i>	Elliptionidae, Lampsilinae	Unionidae, Lampsilinae	Unionidae, Lampsilinae, heterogenae
<i>Plectomernus</i>	Unionidae, Quadrulinae	Unionidae, Quadrulinae	Amblemidae, Ambleminae
<i>Plethobasus</i>	Elliptionidae, Pleurobeminae	Unionidae, Unioninae	Unionidae, Pleurobeminae
<i>Pleurobema</i>	Elliptionidae, Pleurobeminae	Unionidae, Unioninae	Unionidae, Pleurobeminae
<i>Popenaias</i>	Not mentioned	Unionidae, Unioninae	Unionidae, Popenaiadinae
<i>Potamilus</i>	Elliptionidae, Lampsilinae	Unionidae, Lampsilinae	Unionidae, Lampsilinae, heterogenae
<i>Ptychobranchus</i>	Elliptionidae, Lampsilinae	Unionidae, Lampsilinae	Unionidae, Lampsilinae, ptychogenae
<i>Quadrula</i>	Unionidae, Quadrulinae	Unionidae, Quadrulinae	Amblemidae, Ambleminae
<i>Quincuncina</i>	Unionidae, Quadrulinae	Unionidae, Quadrulinae	Amblemidae, Ambleminae
<i>Toxolasma</i>	Elliptionidae, Lampsilinae	Unionidae, Lampsilinae	Unionidae, Lampsilinae, heterogenae
<i>Tritogonia</i>	Unionidae, Quadrulinae	Unionidae, Quadrulinae	Amblemidae, Ambleminae
<i>Truncilla</i>	Elliptionidae, Lampsilinae	Unionidae, Lampsilinae	Unionidae, Lampsilinae, heterogenae
<i>Uniomernus</i>	Elliptionidae, Elliptioninae	Unionidae, Unioninae	Unionidae, Pleurobeminae
<i>Venustaconcha</i>	Elliptionidae, Lampsilinae	Unionidae, Lampsilinae	Not mentioned
<i>Villosa</i>	Elliptionidae, Lampsilinae	Unionidae, Lampsilinae	Unionidae, Lampsilinae, heterogenae

Table 1. (Continued).

Genus	Starobogatov (1970)	Davis & Fuller (1981) (Unionidae)	Previous molecular studies (Unionidae)	Present study (Unionidae)
<i>Actinonaias</i>	Lampsilidae, Lampsilinae, Lampsilini	Ambleminae, Lampsilini	Ambleminae, Lampsilini	Ambleminae, Lampsilini ^a
<i>Amblesma</i>	Amblemididae, Ambleminae	Ambleminae, Amblemini	Ambleminae, Amblemini	Ambleminae, Amblemini
<i>Cyclonaias</i>	Lampsilidae, Pleurobemininae	Ambleminae, Amblemini	Ambleminae, Lampsilini?	Ambleminae, Quadrulini
<i>Cyrtionaias</i>	Lampsilidae, Cyprogeninae	Not sampled	Ambleminae, Lampsilini	Ambleminae, Lampsilini
<i>Dromus</i>	Lampsilidae, Lampsilinae, Lampsilini	Not sampled	Ambleminae, Lampsilini?	Ambleminae, Lampsilini?
<i>Ellipsaria</i>	Lampsilidae, Medionidinae, Dromini	Not sampled	Ambleminae, Lampsilini	Ambleminae, Lampsilini
<i>Elliptio</i>	Lampsilidae, Elliptioninae	Ambleminae, Pleurobemini	Ambleminae, Lampsilini	Ambleminae, Lampsilini
<i>Elliptoides</i>	Amblemididae, Ambleminae	Not sampled	Ambleminae, Pleurobemini	Ambleminae, Pleurobemini ^a
<i>Eptioblasma</i>	Lampsilidae, Lampsilinae, Pilaeni	Not sampled	Ambleminae, Lampsilini	Ambleminae, Lampsilini
<i>Fusconata</i>	Amblemididae, Quadrulinae, Quadrulini	Ambleminae, Pleurobemini ^a	Ambleminae, Pleurobemini ^a	Ambleminae, Pleurobemini ^a
<i>Glebulina</i>	Lampsilidae, Lampsilinae, Glebulini	Ambleminae, Lampsilini	Ambleminae, Lampsilini?	Ambleminae, Lampsilini
<i>Gonidea</i>	Margaritiferidae, Pseudodontinae, Pseudodontini	Ambleminae, Gonideini	Ambleminae, Gonideini	Ambleminae, Gonideini
<i>Hemistena</i>	Lampsilidae, Medionidinae, Medionidini	Not sampled	Ambleminae, Pleurobemini	Ambleminae, Pleurobemini
<i>Lampsilis</i>	Lampsilidae, Lampsilinae, Lampsilini	Ambleminae, Lampsilini	Ambleminae, Lampsilini ^a	Ambleminae, Lampsilini ^a
<i>Lemiox</i>	Lampsilidae, Lampsilinae, Lampsilini	Not sampled	Not sampled	Ambleminae, Lampsilini
<i>Leptodea</i>	Lampsilidae, Lampsilinae, Lampsilini	Ambleminae, Lampsilini	Ambleminae, Lampsilini	Ambleminae, Lampsilini
<i>Lexingtonia</i>	Lampsilidae, Pleurobemininae	Not sampled	Not sampled	Ambleminae, Pleurobemini
<i>Ligumia</i>	Lampsilidae, Lampsilinae, Lampsilini	Ambleminae, Lampsilini	Ambleminae, Lampsilini ^a	Ambleminae, Lampsilini ^a
<i>Medionidus</i>	Lampsilidae, Medionidinae, Medionidini	Not sampled	Ambleminae, Lampsilini	Ambleminae, Lampsilini
<i>Megalonaias</i>	Amblemididae, Ambleminae	Ambleminae, Amblemini	Ambleminae, Quadrulini	Ambleminae, Quadrulini
<i>Oblitquaria</i>	Lampsilidae, Cyprogeninae	Not sampled	Ambleminae, Lampsilini?	Ambleminae, Lampsilini
<i>Obovaria</i>	Lampsilidae, Lampsilinae, Glebulini	Not sampled	Ambleminae, Lampsilini ^a	Ambleminae, Lampsilini ^a
<i>Plectomerus</i>	Amblemididae, Ambleminae	Ambleminae, Amblemini	Ambleminae, Amblemini?	Ambleminae, Lampsilini?
<i>Plethobasus</i>	Lampsilidae, Pleurobemininae	Not sampled	Not sampled	Ambleminae, Pleurobemini ^a
<i>Pleurobema</i>	Lampsilidae, Pleurobemininae	Ambleminae, Pleurobemini	Ambleminae, Pleurobemini	Ambleminae, Pleurobemini ^a
<i>Popenaias</i>	Lampsilidae, Elliptioninae	Not sampled	Not sampled	Ambleminae, Pleurobemini ^a
<i>Potamilus</i>	Lampsilidae, Lampsilinae, Lampsilini	Ambleminae, Lampsilini	Ambleminae, Lampsilini	Ambleminae, Lampsilini
<i>Ptychobranchus</i>	Lampsilidae, Ptychobranchinae	Ambleminae, Lampsilini	Ambleminae, Lampsilini	Ambleminae, Lampsilini
<i>Quadrula</i>	Amblemididae, Quadrulinae, Quadrulini	Ambleminae, Amblemini	Ambleminae, Quadrulini ^a	Ambleminae, Quadrulini ^a
<i>Quincuncina</i>	Amblemididae, Quadrulinae, Quadrulini	Ambleminae, Amblemini	Ambleminae, Pleurobemini ^a	Ambleminae, Pleurobemini ^a
<i>Toxolasma</i>	Lampsilidae, Lampsilinae, Lampsilini	Ambleminae, Lampsilini	Ambleminae, Lampsilini?	Ambleminae, Lampsilini?
<i>Tritogonia</i>	Amblemididae, Quadrulinae, Quadrulini	Ambleminae, Amblemini	Ambleminae, Quadrulini	Ambleminae, Quadrulini
<i>Truncilla</i>	Lampsilidae, Lampsilinae, Glebulini	Not sampled	Ambleminae, Lampsilini	Ambleminae, Lampsilini
<i>Unionomerus</i>	Lampsilidae, Elliptioninae	Ambleminae, Pleurobemini	Ambleminae, Pleurobemini	Ambleminae, Quadrulini

Table 1. (Continued).

Genus	Starobogatov (1970)	Davis & Fuller (1981) (Unionidae)	Previous molecular studies (Unionidae)	Present study (Unionidae)
<i>Venustaconcha</i>	Lampsilidae, Lampsilinae, Lampsilini	Not sampled	Not sampled	Ambleminae, Lampsilini ^a
<i>Villosa</i>	Lampsilidae, Lampsilinae, Lampsilini	Ambleminae, Lampsilini	Ambleminae, Lampsilini	Ambleminae, Lampsilini ^a

Note that the authors used different names for some of the genera. Previous molecular studies providing information on tribal affinities or on genus monophyly include Lydeard et al. (1996, 2000), Roe & Lydeard (1998), Bogan & Hoeh (2000), Hoeh et al. (2001, 2002a), Roe et al. (2001), Graf (2002), Krebs et al. (2003), Roe & Hoeh (2003), and Serb et al. (2003).

^aIndicates polyphyly or paraphyly.

the Cretaceous faunas of central North America (Hartman 1998), suggesting that the North American amblemines have a long evolutionary history separate from other unionoids. However, these might represent unrelated, morphologically convergent taxa (Watters 2001).

The development of molecular techniques provided a novel source of information on the systematics of the Ambleminae. Some traditional classifications were supported, whereas others were called into question and new ideas were suggested. In the first molecular study to include many amblemines, Davis & Fuller (1981) used immunoelectrophoresis to assess the relationships of North American unionoid genera (Table 1). This study demonstrated the distinctiveness of Ambleminae from "Anodontinae" (Unioninae). The early immunological work was followed by numerous studies using DNA sequencing, as well as a few other genetic techniques, e.g., RFLP analysis (White et al. 1996). Rosenberg et al. (1994) found little variation in the D6 region of the 28S gene within the Unionidae, but subsequent studies have identified more variable genes (16S: Lydeard et al. 1996; COI: Roe & Lydeard 1998; ITS: King et al. 1999; D2 region of 28S: Graf & Ó Foighil 2000b; Graf 2002; ND1: Buhay et al. 2002; Serb et al. 2003; male mitotype COI: Hoeh et al. 1996, 2002b; COII: Curole & Kocher 2002; cytB: Mock et al. 2004). Studies using DNA sequencing have generally supported the higher taxa recognized by Davis & Fuller (1981), with the exception of their Amblemini, which appears to be a polyphyletic group sharing plesiomorphic features (Lydeard et al. 1996). In general, traditional species-level classification has been upheld, but genera and higher taxonomic categories often appear polyphyletic (Lydeard et al. 2000; Roe et al. 2001; Serb et al. 2003; Huff et al. 2004). However, sampling issues remain a problem, with many genera and type species unavailable to previous studies. The frequent polyphyly of genera makes data for type species especially important; otherwise, it is unclear which group of species actually belongs in the genus. Although these studies provide explicit hypotheses about the phylogenetic relationships of the included taxa, the patchy taxonomic coverage makes it impossible to extrapolate phylogenetic relationships for the Ambleminae as a whole.

The modern concept of Ambleminae as a monophyletic group has only arisen with the advent of molecular and cladistic studies. Most workers before Davis & Fuller (1981) placed Pleurobemini as closely related to, if not synonymous with, the Old World Unionini. However, Davis & Fuller (1981) found

that Unioninae (as Anodontinae) were genetically very distinct from the Ambleminae (including Pleurobemini), a conclusion substantiated by all subsequent molecular studies. In these studies (e.g., Lydeard et al. 1996; Bogan & Hoeh 2000; Graf 2002; Graf & Ó Foighil 2000a; Hoeh et al. 2002a, b; Krebs et al. 2003; Roe & Hoeh 2003), the relationships between the tribes in Ambleminae have varied. Not all studies included Gonideini or Quadrulini. Those including Gonideini placed it basal to the other tribes or outside of Ambleminae. The interrelationships of Amblemini, Quadrulini, Pleurobemini, and Lampsilini differed from analysis to analysis, and Lampsilini was not consistently monophyletic. Often, these groupings did not have strong bootstrap support, and some, if not all, of the tribes were represented by only a few taxa in each analysis.

Thus, Simpson's (1891) challenge remains a problem: what are natural groups in the Unionidae? We sought to answer this for the Ambleminae by addressing three main questions: (1) Are the North American Ambleminae a monophyletic group? (2) What are the relationships among the North American genera of Ambleminae? (3) Are these genera, as currently recognized, natural entities?

Methods

Taxa were selected to represent all 37 currently recognized North American amblemine genera. Some nomenclatural disagreements exist within the literature (e.g., Smith [2000a] synonymized *Cumberlandia* with *Margaritinopsis*; this is not supported by molecular data, however [Huff et al. 2004]). The nomenclature of Turgeon et al. (1998) is followed here for convenience of reference. Revision of the nomenclature is outside the scope of this paper, and ongoing molecular studies of several taxa make an overall revision premature. Putative amblemines from outside North America, including Asian *Hyriopsis* and *Inversidens*, and European *Psilunio*, as well as the western North American *Gonidea*, were also included in the analyses to test the monophyly of the eastern North American tribes. Outgroups included margaritiferae (*Cumberlandia* and *Margaritifera*) and unionines (Anodontini) (*Anodonta*, *Lasmigona*, *Pyganodon*, and *Strophitus*). When possible, the type species for each ingroup genus was sequenced. Suitable data were not available for the type species of *Actinonaias* (*A. sapotalensis* [LEA 1841], from Central America), *Cyrtonaias* (*C. berlandieri* [LEA 1857], from Mexico, possibly synonymous with *C. tampicoensis* analyzed herein [Howells et al. 1996]), *Epioblasma* (*E. rangiana* [LEA 1838], endangered), *Lexingtonia*

(*L. subplana* [CONRAD 1837], very rare and taxonomically problematic), *Obovaria* (although the nearly extinct *O. retusa* [LAMARCK 1819] has traditionally been considered the type species, the type designation is problematic [Graf, pers. comm.]). *Toxolasma* (*T. lividus* [RAFINESQUE 1831], failed to amplify), and *Uniomerus* (*U. tetralasmus* [SAY 1831]), failed to amplify. For the outgroups, taxa were chosen based on the availability of sequences or material. Appendix 1 lists the taxa and GenBank accession numbers, and Appendix 2 lists the locality and collection information for the new sequences. A total of 137 COI, 119 16S, and 101 ND1 sequences, representing 107 currently recognized species, were analyzed. Just over half of the sequences are new, and many of the previously published sequences had not been integrated into a single analysis.

DNA was extracted from fresh, frozen, or ethanol-preserved specimens using standard CTAB and chloroform-isoamyl alcohol protocols (Winnepenninckx et al. 1993). Foot, mantle, or adductor tissue was used to avoid the risk of sampling male mitotypes from gonadal tissue (Hoeh et al. 2002b). Portions of the COI, 16S, and ND1 genes were amplified, as they were known to show species-level variation in Ambleminae (Lydeard et al. 1996; Roe & Lydeard 1998; Buhay et al. 2002; Serb et al. 2003). The present ND1 fragment is much longer than that analyzed in previous studies and is correspondingly more informative. Primers used were:

COI: 5'-GTTCCACAAATCATAAGGATATTGG-3'
 5'-TACACCTCAGGGTGACCAAA AAACCA-3'
 16S: 5'-CCGTTCTGAACTCAGCTCATGT-3'
 5'-CGACTGTTTAACAAAAACAT-3'
 ND1: 5'-TGGCAGAAAAGTGCATCAGATTAAAGC-3'
 5'-GCTATTAGTAGGTCGTATCG-3'

(COI modified from Folmer et al. 1994; 16S from Lydeard et al. 1996; ND1 from Buhay et al. 2002 and Serb & Lydeard 2003). PCR cycle parameters for COI and ND1 were: 92°C for 2 min; 92°C for 40 s, 40°C for 40 s, 72°C for 90 s, × 5; 92°C for 40 s, 50°C for 40 s, 72°C for 90 s, × 25; 72°C for 10 min; hold 4°C. For 16S, they were: 92°C for 5 min; 92°C for 40 s, 50°C for 60 s, 68°C for 90 s, × 35; 72°C for 10 min; hold 4°C. PCR products were purified using Qiagen QIAquick PCR purification kits (Valencia, CA). Cycle sequencing used ABI Big Dye Terminator kits (Foster City, CA) with thermal cycle parameters of 1°C s⁻¹ ramp speed, starting with 1 min at 96°C followed by 26 cycles of 96°C for 10 s, 49°C for 5 s, and 60°C for 4 min, then 10 min at 60°C and hold at 4°C. The cycle sequencing products were

purified with sephadex columns or Qiagen DyeEx kits, and then run on an ABI 3100 automated sequencer.

The results for each strand were compared and aligned with published sequences using BioEdit (Hall 1999). No indels were found within the protein-coding genes. Two short variable regions in the 16S gene and all indels were excluded, as positional homology was unclear, thus excluding a total of 79 base pairs. The exact length of published sequences and of readable sequences obtained in the present study varies. We used 602 base pairs (bp) for COI, 315 bp for 16S, and 753 bp for ND1. The high evolutionary rate of mitochondrial genes may produce problems due to saturation for older divergences (Graf 2002), thus potentially limiting the resolution of higher-level relationships. However, they provide appropriate evolutionary rates to resolve relationships within Amblyminae. Sequences were analyzed using PAUP* 4.10 (Swofford 1998) and TNT (Goloboff et al. 2000). All taxa with data for at least two of the three genes were analyzed. This ensured that any two taxa would share some sequence information.

GenBank data for these genes come from several studies (COI: Hoeh et al. 1998; Roe & Lydeard 1998; King et al. 1999; Bogan & Hoeh 2000; Lydeard et al. 2000; Graf & Ó Foighil 2000a; Roe et al. 2001; Buhay et al. 2002; Giribet & Wheeler 2002; Hoeh et al. 2002b; Machordom et al. 2003; Serb & Lydeard 2003; Okazaki & Ueshima, unpubl. data; 16S: Lydeard et al. 1996; Mulvey et al. 1997; Lydeard et al. 2000; Turner et al. 2000; Roe et al. 2001; Huang et al. 2002; Krebs et al. 2003; Machordom et al. 2003; Serb & Lydeard 2003; Okazaki & Ueshima, unpubl. data; ND1: Buhay et al. 2002; Serb et al. 2003; Serb & Lydeard 2003; Okazaki & Ueshima, unpubl. data). The sequences were concatenated to provide a greater number of informative characters for the analyses. Although this approach of concatenating gene sequences produced many taxa with extensive missing data, this should not pose a problem for analyses as long as an adequate number of characters are represented in all included taxa (Wiens 2003). Concatenation of multiple sequences raises a risk of misleading results due to non-monophyly of the source taxa (Malia et al. 2003). However, in the present study, only sequences from the same species were concatenated; in many cases, a single individual supplied all the sequences. Multiple published sequences for the same species that were nearly or entirely identical and that formed a monospecific polytomy in the published analyses were eliminated as redundant. This only affected the few species that have

three or more published sequences for the same gene. Polytomies that included multiple species were retained.

To test for the compatibility of the different gene sequences, a partition homogeneity test (sensu Swofford 1998; P_{ILD} of Dowton & Austin 2002) was run in PAUP* using all taxa with data for all three genes with 100 replicates of 10 random addition replicates each. The maximum number of trees per replicate was set to 10,000. This test is sensitive to other factors, such as partition size and evolutionary model, besides data compatibility (Dowton & Austin 2002), but may provide a rough idea of agreement between data sets. Despite the problems of the ILD type of tests, no better alternative has gained wide acceptance. Sampling of 200,000 random trees yielded gI values of -0.257194 for the whole data set, -0.219740 for COI alone, -0.298611 for 16S alone, and -0.278602 for ND1 alone.

The missing data made PAUP* inefficient; however, parsimony and bootstrap analyses in TNT finished quickly. Parsimony analyses used 500 replicates of random sequence addition with TBR branch swapping, holding 10 shortest trees at each replicate. Bootstrap analysis used 1000 replicates of standard bootstrapping; each replicate used heuristic searches of 10 random addition sequence replicates.

Bayesian analysis provided a second phylogenetic technique. MrBayes 3.0b4 (Huelsenbeck & Ronquist 2001) was used to run MCMCMC searches. This was run with $Nst = 6$; rates = invgamma; partitions corresponding to the genes; revmat, shape, pinvar, and statefreq unlinked; 2,000,000 generations and 8 chains. These parameters were chosen to maximize model flexibility. Other parameters were set to default values, saving every 100th tree. Bootstrap values typically underestimate support, whereas Bayesian analyses tend to overestimate it (Erixson et al. 2003; Simmons et al. 2004), so comparison of the results of the two provides a useful check.

Results

The present data set greatly expands the molecular data available for Amblyminae. Table 2 summarizes previous molecular analyses. In comparison, our analyses include a total of 37 genera, 30 type species, and 96 species of North American Amblyminae, thus doubling to tripling the taxonomic coverage. (These tallies include *Gonidea* and accept the taxonomy within each paper in cases of disagreement, e.g., whether two forms are valid species.)

Table 2. Number of amblemine taxa in selected molecular studies.

Reference	Number of genera	Number of species	Number of type species
Davis & Fuller (1981)	21	40	15
Lydeard et al. (1996)	16	20	8
Bogan & Hoeh (2000), Hoeh et al. (2001, 2002a), and Roe & Hoeh (2003)	12	12	9
Graf & Ó Foighil (2000a)	12	17	6
Lydeard et al. (2000)	6	13	3
Krebs et al. (2003)	18	24	10
Serb et al. (2003)	17	36	13
Present study	37	96	30

Gonidea is counted, but not Eurasian species, and the taxonomy of the respective paper is followed for counting the number of taxa (e.g., if one paper synonymizes two species treated as distinct in another paper).

Among the three genes, the 16S sequence, being the shortest and most conservative, also had the fewest informative characters (240 parsimony-informative sites for COI, 132 for 16S, and 377 for ND1). The P_{ILD} test gave a value of 0.88, thus not indicating rejection of compatibility, and separate parsimony analyses for each gene yielded similar results to the patterns found with the combined data sets (not shown). This agrees with expected evolutionary similarity due to the functioning of the mitochondrial genome as a single evolutionary locus. The analyses yielded trees supporting several major clades, as well as providing resolution within these clades. Figure 1 shows the strict consensus parsimony trees with bootstrap percentages; Fig. 2 shows the Bayesian likelihood tree.

The eastern North American Ambleminae (hereafter “Ambleminae *s.s.*”) form a monophyletic group. However, *Gonidea* appears more closely related to Eurasian species than to other North American taxa. These clades had high Bayesian probability, but were unresolved in the bootstrap analysis. In turn, the clade including *Gonidea* was the sister taxon to the eastern North American Ambleminae. Within the Ambleminae *s.s.* clade, most taxa fall into Quadrulini, Pleurobemini, or Lampsilini, but a few species were not clearly assigned to one of these three main tribes. These species appear closely related to the Lampsilini+Amblemini group, but their exact relationships differed between the analyses. All phylogenetic analyses supported Lampsilini, Quadrulini, and Pleurobemini as major clades within Ambleminae, along with several subclades within these large

groups. Within the eastern North American amblemines, Quadrulini appeared as the basal taxon. Pleurobemini appears to be the sister taxon of a Lampsilini+Amblemini clade. Quadrulini and Lampsilini received 89% posterior probability from the Bayesian analysis; Pleurobemini received 100%. Although an Amblemini clade received 99% support in the Bayesian analysis, the parsimony analyses supported a different topology.

Several smaller clades occur in both the parsimony and the Bayesian analyses. Many of these clades do not correspond with current genus-level nomenclature. Twelve genera (out of 25 that have multiple species) are clearly polyphyletic, and the status of several more remains ambiguous. *Fusconaia ebena*, *F. succissa*, *Obovaria rotulata*, *Quincuncina infucata*, and *Q. kleiniana* are currently assigned to genera in the wrong tribe. Previous molecular analyses (Lydeard et al. 2000; Serb et al. 2003) indicated that they were assigned to the wrong genera, but the present data set confirms the tribe-level differences. Our analyses also suggest possible sister-taxon relationships for several genera.

Discussion

Possible extralimital amblemines

The analyses support a relationship of western North American *Gonidea*, European *Psilunio*, and Asian *Inversidens* with eastern North American Ambleminae, although this failed to receive bootstrap support. Both Bayesian and parsimony analyses placed these three taxa in a clade sister to the eastern North American amblemine clade. Within this basal amblemine group, the Eurasian *Psilunio* and the Asian *Inversidens* were supported as close relatives. *Psilunio* also resembles *Gonidea* morphologically (Nagel et al. 1998). This closely resembles the biogeographic pattern for pleurocerid gastropods (Lydeard et al. 2002). In both, the North American Pacific drainage fauna is depauperate compared with the eastern drainages and shows closest relationships with Eurasian taxa. In turn, the Eurasian–western North American clade is sister to a diverse eastern North American clade (in the Pleuroceridae, some Eurasian taxa are basal members of the eastern North American clade). The similarity between eastern Asian and western North American faunas may reflect interchange via the Bering land bridge. The pre-Pleistocene fossil record of *Gonidea* and the pleurocerid *Juga* in North America (Hannibal 1912) indicate that this interchange occurred before 4.8 million years ago (Marinkovich & Gladenkov

1999). This dating also fits with their climatic preferences. *Gonidea* and *Juga* range north only to southern British Columbia (Clarke 1981); records of *Juga* north of Washington State are dubious (Goodrich 1937). This suggests that the younger, ice-age land bridge would have been inhospitable.

The affinities of *Hyriopsis* were poorly resolved: close to Anodontini with low Bayesian probability and unresolved in the parsimony tree. Further sampling of Eurasian genera will be needed to resolve whether it is a basal amblemine, unionine, or neither. Graf (2002) also placed *Gonidea* as the sister taxon to eastern North American Amblemini.

Hyriopsis, *Inversidens*, and *Psilunio* were not included in his study; the Asian genera that he included placed outside of both Unioninae and Amblemini. As he used a more slowly evolving gene, his results are probably more reliable at this taxonomic level; conversely, he observed that his 28S data did little to resolve relationships within the eastern North American taxa. Other Eurasian taxa that appear possibly assignable to Amblemini, based on molecular evidence, include *Lamprotula*, *Ptychorhynchus*, and *Solenaiia* (Huang et al. 2002). *Inversidens* and *Psilunio* had not previously been included in phylogenetic analyses incorporating multiple amblemines.

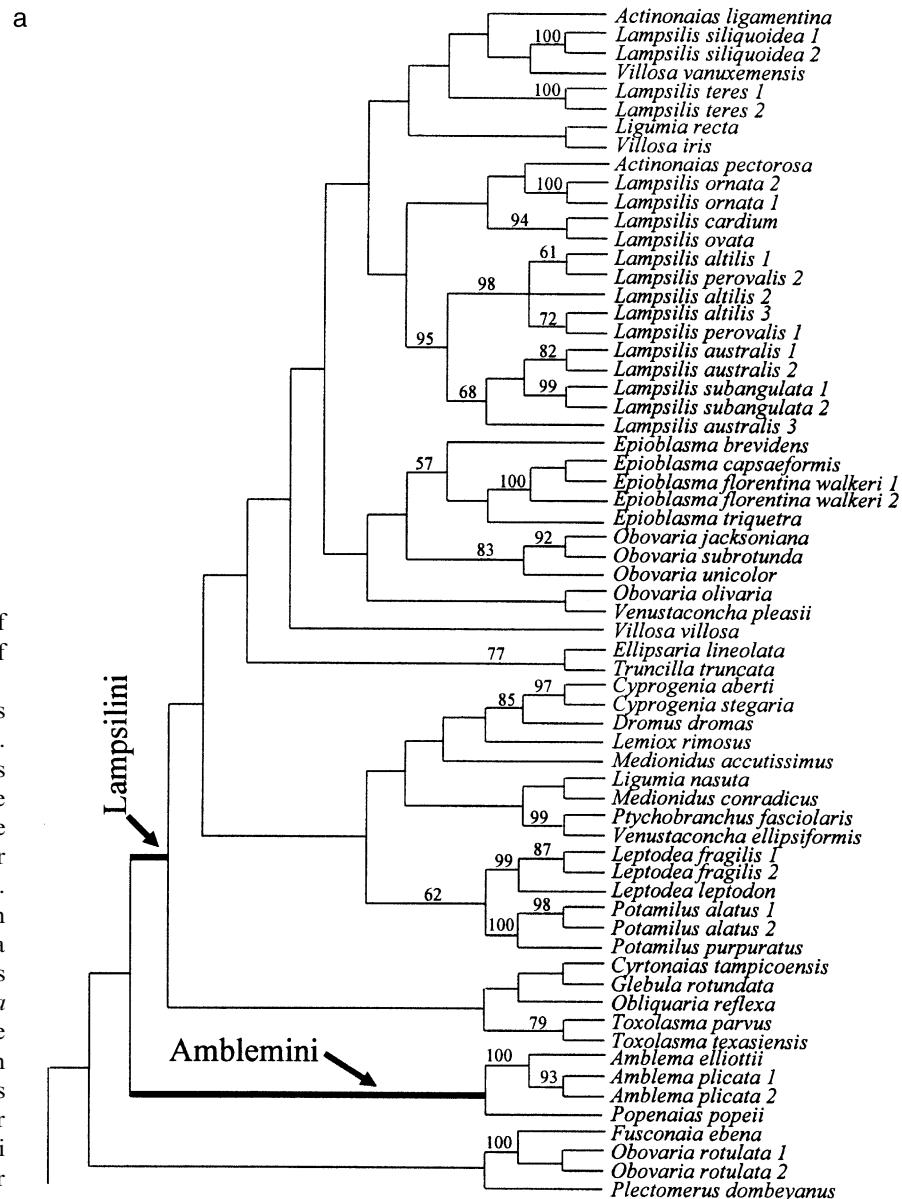


Fig. 1. a. Strict consensus of 2304 most parsimonious trees of length 7447 (CI = 0.2062, RI = 0.7938). Numbers above branches represent bootstrap percentages. Numbers after species names reflect multiple individuals of the same species. The largest clade corresponding with a particular tribe, subfamily, etc. is labeled. There is some variation between the trees as to the exact taxa included, e.g., *Plectomerus* is within Lampsilini, and *Fusconaia ebena* and *Obovaria rotulata* are within Amblemini in the Bayesian tree, but the parsimony analyses placed all three together in their own clade outside of Amblemini and of Lampsilini. See text for discussion of these taxa.

see Fig. 1b

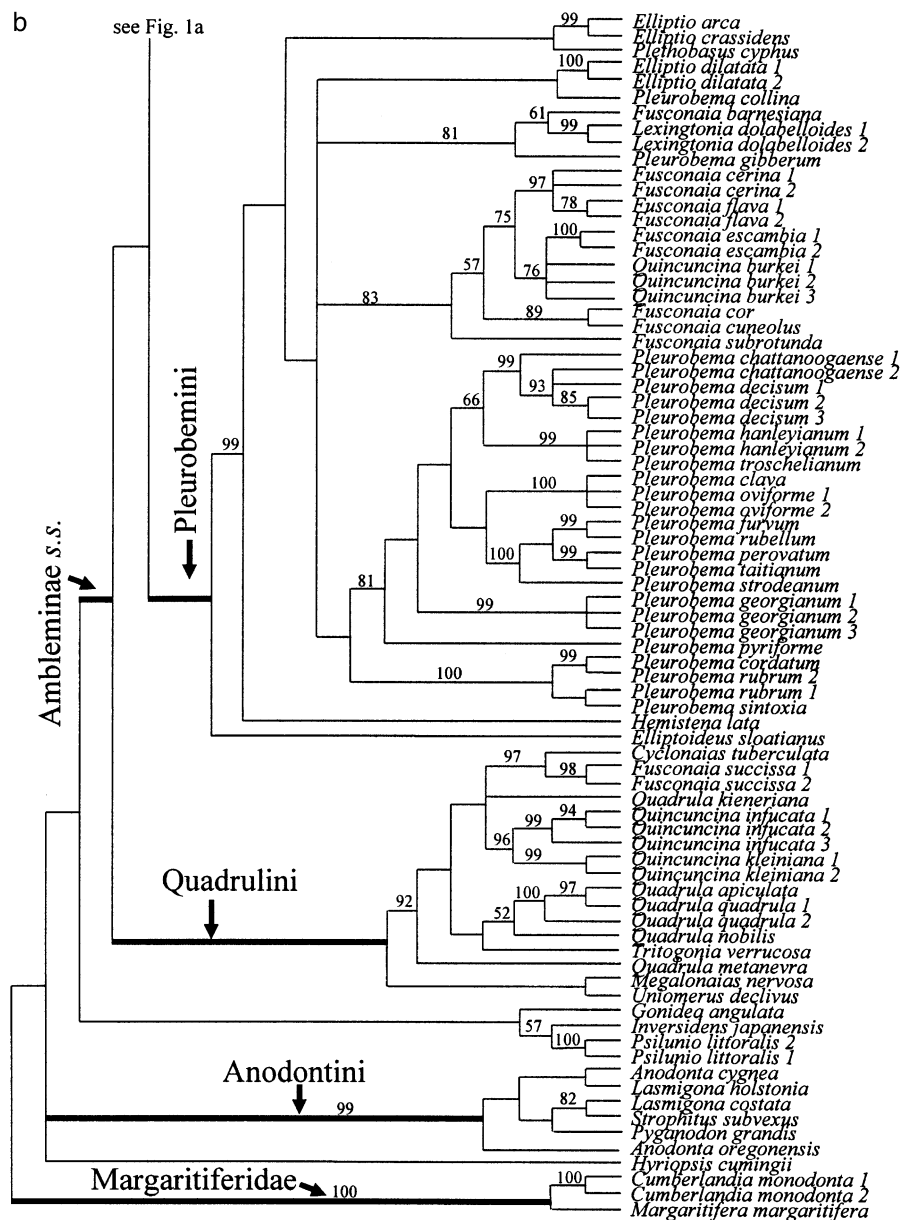


Fig. 1. b. Continuation of Fig. 1a.

Many previous classifications have assigned Eurasian and African taxa to eastern North American tribes (or ambiguous tribes, in the case of “Unionini” that included Pleurobemini). Among the sampled taxa (standardizing the taxon names to the usage in this paper), *Psilunio littoralis* was assigned to Unionini (Simpson 1900), Quadrulini (Haas 1969), and Gonideini (Nagel et al. 1998); *Hyriopsis cumingii* to Lampsilini (Simpson 1900), Unionini (Haas 1969), Anodontini (Starobogatov 1970), and Pleurobemini (Heard & Guckert 1971); and *Inversidens japonensis* to Unionini (Haas 1969) and Quadrulini (Starobogatov 1970). Modell (1964) excluded non-North

American genera from the North American amblemine tribes; however, *Gonidea* was placed in Margaritiferidae with some Asian unionids. Also, he did not unite the amblemine tribes as a monophyletic group, with Quadrulini assigned to Unioninae, and Alasmidontini (which Heard & Guckert 1971, and subsequent workers, synonymize with Anodontini) assigned to Ambleminae.

Graf (2002) suggested three reasons for the overuse of North American tribes. First, the current taxonomic framework for the unionids is based largely on the work of Simpson and Ortmann, who, working in North America, naturally relied heavily on

the regional fauna in developing their classifications. In large part, worldwide unioniids have been fit into classifications based primarily on North American taxa. Secondly, relatively few morphological characters are known for many taxa, and the characters used to group taxa are in many cases homoplasious. Although there are many disagreements as to which characters are reliable (including

molecular characters), the fact that different characters yield different classifications demonstrates that at least some of them are homoplasious. Thirdly, traditional classifications have often used symplesiomorphies as well as synapomorphies to group taxa. The present results agree with Graf (2002) and Huang et al. (2002) in excluding non-North American taxa from Quadrulini, Amblemini, Pleurobemini,

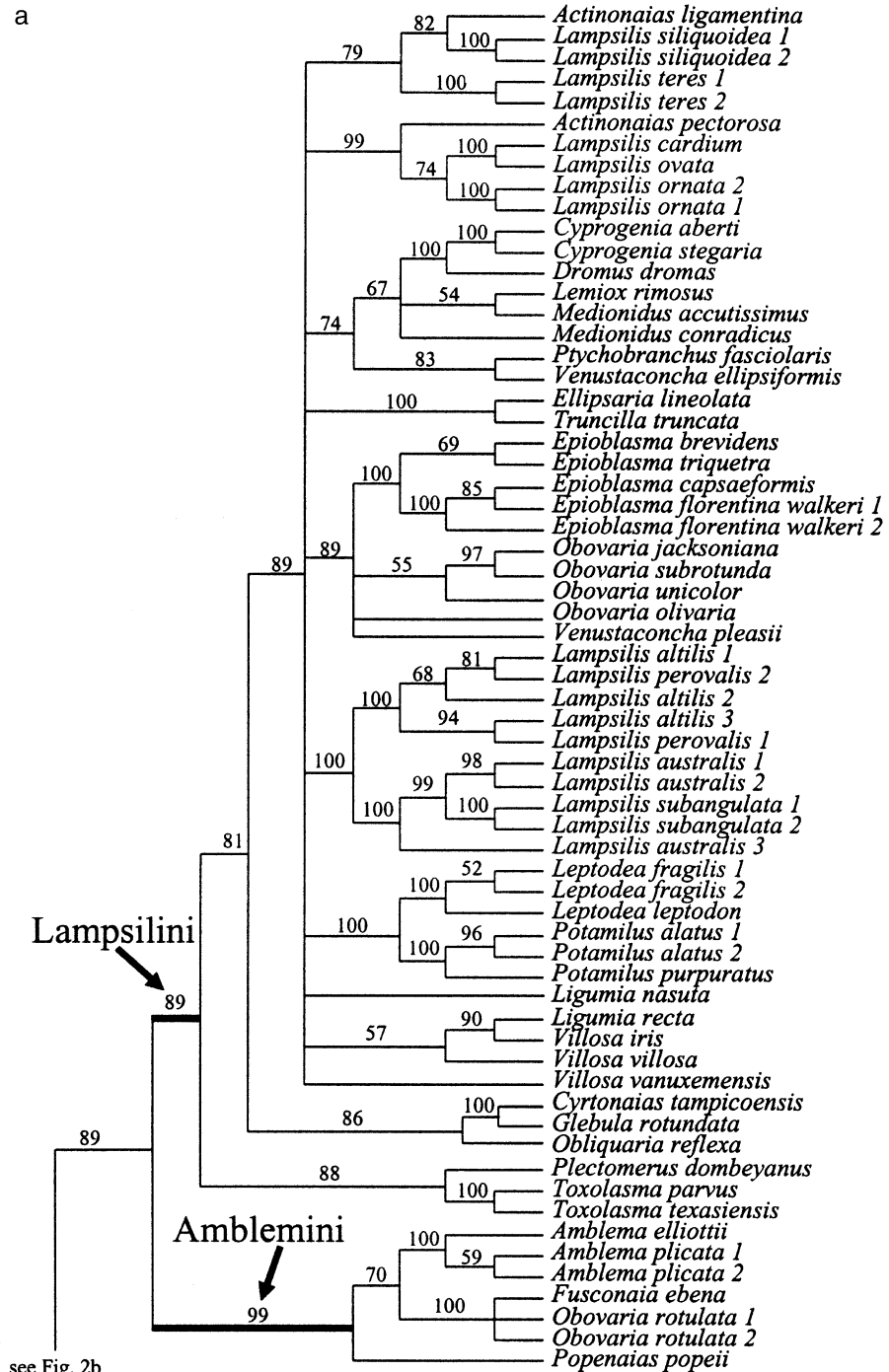


Fig. 2. a. 50% majority-rule consensus of 17,312 Bayesian likelihood trees (burn-in = 268,800, mean log likelihood = -32,760). Numbers above branches indicate posterior probabilities.

see Fig. 2b

reversal to tetrageny in *Fusconaia*), once in Lampsilini, and once in *Cyclonaias*. Although the separate origins of ectobranchy in Unioninae and Amblemini had been recognized in many previous studies (e.g., Davis & Fuller 1981; Graf 2002; Roe & Hoeh 2003), the extent of convergence within Amblemini was not previously evident.

Amblemini

Only *Amblema* can confidently be assigned to Amblemini, although *Popenaias* placed in the same clade as *Amblema* in both analyses. The affinities of *Amblema* with other genera remain poorly supported, and the composition of Amblemini remains uncertain. Both analyses suggested that Amblemini was the sister taxon to Lampsilini, but this was not strongly supported by the bootstrap. Based on the present analyses, other taxa that may be related to Amblemini include "*Fusconaia*" *ebena*, "*Obovaria*" *rotulata*, and *Plectomerus*. The relationships among these taxa, as well as their relationships with Lampsilini and Pleurobemini, varied between analyses. Previous molecular analyses likewise had difficulty placing these taxa, although no sequence data have previously been published for *Popenaias* and not all of the others were included in any one previous analysis. The present analyses placed all of these taxa, along with Lampsilini, in a large clade, sister to Pleurobemini. However, bootstrap analysis failed to support this large clade.

Although *Elliptoideus* placed basal to Pleurobemini, with 100% posterior probability, this did not receive significant bootstrap support. The association of *Elliptoideus* with Pleurobemini agrees with the results of Lydeard et al. (1996) and Krebs et al. (2003) rather than with Serb et al. (2003), who placed *Elliptoideus* and *Plectomerus* close together as possible amblemines. Morphologically, *Elliptoideus* and *Plectomerus* are similar (Modell 1964; Heard & Guckert 1971); however, they are also morphologically similar to the quadruline *Megalonaias*, which suggests that their similarities may be plesiomorphic or convergent. *Plectomerus* placed as a basal lampsiline in the Bayesian analyses, but the parsimony analysis placed it outside of Lampsilini. *Plectomerus* lacks the specialized gill structures characteristic of Lampsilini and is tetragenous rather than ectobranchous. The COI sequence obtained in this study is definitely lampsiline in its affinities; its 16S and ND1 sequences are not clearly lampsiline. *Popenaias* tends to group with *Amblema* (not always exclusive of other taxa), but this received weak bootstrap support.

Both analyses strongly supported a clade of "*Obovaria*" *rotulata* and "*Fusconaia*" *ebena*, although the exact placement of this clade varied from analysis to analysis. The close relationship of "*O.*" *rotulata* and "*F.*" *ebena* agrees with previous molecular (Lydeard et al. 2000) and morphological (Stansbery 1971; Williams & Butler 1994; Athearn 1998) studies. Davis & Fuller (1981) noted a difference between "*F.*" *ebena* and true *Fusconaia*, but did not have "*O.*" *rotulata*. However, they expressed reservations about this difference, noting the possibility of experimental error. The present results suggest that their results were correct in this regard. "*Obovaria*" *rotulata* was assigned to *Obovaria* by Simpson (1900), based on examination of the type and only known specimen at the time, a shell with no soft parts (Lydeard et al. 2000). No anatomical data have been published for "*O.*" *rotulata*, but work in progress (Garner, pers. comm.) indicates that it is anatomically similar to "*F.*" *ebena*. Both lack the strong posterior ridge and angulation characteristic of true *Fusconaia*. Although no molecular data are available for *Obovaria retusa*, the nearly extinct, putative type species of *Obovaria*, anatomical studies indicate that it is lampsiline, similar to *O. olivaria* and *O. subrotunda* (Ortmann 1911). Placement of the *ebena-rotulata* clade varied among analyses, although none put it near other species of *Fusconaia* or of *Obovaria*.

The presence of *Popenaias* among the poorly resolved taxa suggests that sampling of Mexican and Central American unionids may be necessary to resolve the relationships of the Amblemini. Likewise, the primarily tropical *Cyrtionaias* appears near the base of Lampsilini. Unfortunately, no unionid specimens from Latin America were available for molecular work.

Among these genera, *Elliptoideus* and *Plectomerus* are monotypic. "*Fusconaia*" *ebena* and "*Obovaria*" *rotulata* are clearly not closely related to *F. flava* (the type species of *Fusconaia*) nor to the other available *Obovaria* species. None of the other species of *Popenaias*, from Mexico, have been available for molecular studies. *Amblema* appears monophyletic, in agreement with the analysis of Mulvey et al. (1997).

Morphological studies had assigned taxa to the Amblemini based on the pattern of gill use in brooding (tetragenous in Amblemini versus ectobranchous in Pleurobemini and Lampsilini). However, this is evidently a plesiomorphic feature for the eastern North American Amblemini. Many recent analyses also found this (Lydeard et al. 1996; Graf & Ó Foighil 2000a; Hoeh et al. 2001, 2002a; Roe & Hoeh 2003). In fact, the morphology-based analyses that defined Amblemini based on tetrageny frequently suggested that the tribe was paraphyletic (e.g.,

Hannibal 1912; Heard & Guckert 1971; see also the cladistic morphological analyses in Graf 2000; Hoeh et al. 2001; Roe & Hoeh 2003). Graf (2002) suggested that tetrageny may be a synapomorphy for the entire Unionoidea, and thus plesiomorphic for Ambleminae; Roe & Hoeh (2003) suggested that tetrageny was plesiomorphic either for Unionoidea or the entire Unionoidea in different analyses. Reliance on this character placed *Quadrulini* and *Fusconaia* in Amblemini, contrary to the present results, which recognize *Quadrulini* as a distinct tribe and assign true *Fusconaia* to Pleurobemini. Thus, the traditional morphological character distinguishing Amblemini is not reliable for distinguishing tribes. Given the uncertainty about the included taxa, speculation on alternative morphological characters is premature.

Contrary to Davis & Fuller (1981), *Amblema*, *Megaloniaias*, and *Plectomerus* do not appear congeneric. As in the case of Margaritiferidae versus Unionidae (Smith & Wall 1984), low immunological differences apparently concealed the fact that they are on separate branches and in separate tribes.

Lampsilini

The present analyses consistently support a monophyletic Lampsilini, although with low bootstrap support. Lampsilini clearly includes the following genera: *Actinoniaias*, *Cyprogenia*, *Dromus*, *Ellipsaria*, *Epioblasma*, *Lampsilis*, *Lemiox*, *Leptodea*, *Ligumia*, *Medionidus*, *Obovaria* (except "*Obovaria*" *rotulata*), *Potamilus*, *Ptychobranchnus*, *Truncilla*, *Venustaconcha*, and *Villosa*. *Cyrtonaias*, *Glebula*, *Obliquaria*, and *Toxolasma* belong to Lampsilini based on anatomical features. The present analyses grouped these four as basal in Lampsilini. However, their association with Lampsilini did not receive significant bootstrap support, and the Bayesian analysis also placed *Plectomerus* with them as a basal lampsiline. Similarly, Lydeard et al. (1996), Bogan & Hoeh (2000), Hoeh et al. (2002a,b), and Krebs et al. (2003) found at most relatively weak bootstrap support for associating these four genera with Lampsilini; some of their analyses failed to group these genera with Lampsilini at all. (Although high neighbor-joining bootstrap values were found in some of these analyses, the phylogenetic significance of neighbor-joining bootstrap values is doubtful [Swofford et al. 1996]). No previous study on DNA sequence data included all four of the genera. Lydeard et al. (1996) and Krebs et al. (2003) also supported a close relationship of *Plectomerus* to these taxa. Hoeh et al. (2002b) found stronger support for lampsiline affinities for

Cyrtonaias when they concatenated the two analyzed gene sequences in a single analysis, suggesting that the poor resolution is mainly a function of the limited amount of data. All four of these genera have the highly modified gills and other specialized reproductive characters that characterize Lampsilini.

The variable position of *Plectomerus* may have prevented bootstrap analyses from finding strong support for any one clade involving these taxa. Although *Plectomerus* lacks the anatomical characteristics of Lampsilini, it might be the sister taxon to Lampsilini or an atavistic basal lampsiline. Analysis of individual genes indicates that the COI sequence obtained for *Plectomerus* (obtained from two different specimens) shows definite lampsiline affinities, whereas the 16S and ND1 sequences do not. Sequences for additional genes will clarify its affinities.

Within Lampsilini, both analyses supported several subclades. The phylogeny of *Epioblasma* and of the clade of superconglutinate-producing "*Lampsilis*" species (*L. altilis*, *L. australis*, *L. perovalis*, *L. subangulata*) matches the results of previous studies (Roe et al. 2001; Buhay et al. 2002). The many additional taxa in the present analysis provide much broader support for the monophyly of these two clades. However, the present analyses suggest new ideas about the relationships of *Epioblasma*, consistently supporting a close relationship with *Obovaria* and *Venustaconcha pleasii*. The inclusion of several additional taxa in the present study strengthens the conclusion of Roe et al. (2001) that the superconglutinate group is distinct from true *Lampsilis*. Although the parsimony tree placed the superconglutinate clade as sister to the clade including the type of *Lampsilis*, this was not supported by bootstrap or Bayesian analyses. *Actinoniaias pectorosa* appears closely related to *Lampsilis ovata* (the type species), *L. cardium*, and *L. ornata*. *Lemiox*, *Medionidus*, *Dromus*, *Cyprogenia*, *Ptychobranchnus*, and *Venustaconcha ellipsiformis* appeared closely related, although this group did not receive much bootstrap support. *Lampsilis teres*, *Actinoniaias ligamentina*, and *Lampsilis siliquoides* likewise formed a group with low bootstrap support; *Villosa vanuxemensis* placed in this clade in the parsimony analysis. Several pairs of genera appeared closely related, including *Cyprogenia* and *Dromus*, *Ellipsaria* and *Truncilla*, *Leptodea* and *Potamilus*, *Ptychobranchnus* and *Venustaconcha ellipsiformis* (the type), and *Cyrtonaias* and *Glebula*. In general, these groups of taxa have not previously been included in a single analysis, apart from *Leptodea* and *Potamilus* (Roe & Lydeard 1998).

High polyphyly or paraphyly of genera within the Lampsilini raises questions about the characters

currently used to define genera. A few genera (notably *Potamilus* and *Leptodea*) have very distinctive glochidia. Most genera are defined based on anatomical features, such as the form of the gill marsupium, form of the female posterior mantle edge (often modified to attract potential host fish for the glochidia), and attachment of the gill to the body (Heard & Guckert 1971; Burch 1975; Smith 2000b). However, these characters have rarely been examined in a phylogenetic context to determine whether similarities are synapomorphic, homoplaseous, or symplesiomorphic. A few genera have highly distinctive gill form (*Cyprogenia*, *Cyrtonaias*, *Dromus*, *Obliquaria*, and *Ptychobranthus*, among the sampled taxa), but the rest have been grouped as “heterogenae” (Simpson 1900; Heard & Guckert 1971; Fuller 1975; Lampsilinae of Starobogatov 1970). Even among the unusual gill types, *Cyprogenia* and *Obliquaria* do not appear closely related, despite similar gill form. Within Heterogenae, morphological characters are often problematic (Davis & Fuller 1981). Thus, some of the reproductive features used in traditional classifications probably represent symplesiomorphies for suprageneric groups within Lampsilini or convergent adaptation of similar reproductive strategies. Especially in groups based on a single feature, traditional taxa require corroboration from other lines of evidence.

Among lampsiline genera, *Dromus*, *Ellipsaria*, *Lemiox*, *Glebula*, and *Obliquaria* are monotypic, and *Cyrtonaias* and *Truncilla* are represented here by a single species. A published COI sequence (Bogan & Hoeh 2000) for the type species of *Toxolasma*, *T. lividus*, closely resembles the sequences reported on here, but no data for other genes of that species were available. *Cyprogenia*, *Leptodea*, and *Toxolasma* appear monophyletic based on species represented in our analyses. *Medionidus* appeared polyphyletic, but both species placed within the same small clade, and neither bootstrap support nor Bayesian probability for the apparent polyphyly was high. *Obovaria rotulata* is clearly not related to the other sampled species of *Obovaria*. Its placement in *Obovaria* was based solely on shell characters, and the anatomy is not distinctively lampsiline. Relationships of *Obovaria olivaria* to the other species of the *Obovaria*–*Epioblasma*–“*Venustaconcha*” *pleasii* clade were not consistently resolved between analyses, but its morphological distinctiveness (Ortmann 1919), in conjunction with the molecular results, suggest that further study is advisable.

Modest support for *Obovaria* being paraphyletic to *Epioblasma* and “*Venustaconcha*” *pleasii* makes data for the type species of the genera particularly of interest, if it can be obtained. The RFLP data of White

et al. (1996) suggest that *E. rangiana*, the type of *Epioblasma*, is genetically similar to species of *Epioblasma* represented in the present study. However, those data do not provide much resolution of its relationships. Although the currently included species of *Potamilus* form a monophyletic group, Roe & Lydeard (1998) found that *Potamilus capax* places outside of *Potamilus*. Only COI data were available for *P. capax*, so it was not included in the present analyses. *Actinonaias*, *Lampsilis*, *Ligumia*, *Venustaconcha*, and *Villosa* as presently used include taxa that are only distantly related. This finding of polyphyly agrees with Graf & Ó Foighil (2000a) for *Lampsilis* and *Ligumia*, Lydeard et al. (2000) for *Obovaria*, Roe (2000) for *Lampsilis* and *Villosa*, and Roe et al. (2001) and Krebs et al. (2003) for *Lampsilis*; an extensive survey of *Villosa* (Buhay, unpubl. data) confirms the polyphyly of this genus.

Pleurobemini

Pleurobemini includes *Elliptio*, *Fusconaia flava* (the type of the genus), and most other species currently assigned to *Fusconaia*, *Hemistena*, *Lexingtonia*, *Plethobasus*, *Pleurobema*, and *Quincuncina burkei* (the type of the genus) but not other sampled *Quincuncina* species. *Elliptioideus* placed as the sister taxon to Pleurobemini in the present analyses; however, this differs from anatomical classifications (Heard & Guckert 1971) and from the results of Serb et al. (2003). *Quincuncina burkei* appears assignable to *Fusconaia*, in agreement with previous molecular studies (Lydeard et al. 2000; Serb et al. 2003). Although the published 16S sequence for *Uniomerus carolinianus* (Lydeard et al. 1996) places with Pleurobemini, the present sequences for *Uniomerus declivis* place with Quadrulini. *Uniomerus* is taxonomically problematic, with some individuals nearly indistinguishable from *Elliptio* spp. (Davis 1983). Thus, the discrepant results for *Uniomerus* may reflect misidentification or polyphyly. The immunological analyses of Davis & Fuller (1981) and Davis (1983) suggested affinities of *Uniomerus* to Pleurobemini and Quadrulini.

The present composition of Pleurobemini thus agrees more closely with Davis & Fuller (1981) than with Heard & Guckert (1971), by including *Fusconaia* and excluding *Cyclonaias*; however, the present placement of *Uniomerus* is unexpected. Few previous studies have included more than a few taxa for Pleurobemini. Most of the species included herein have no previously published sequence data. Relationships between the genera varied among the analyses and were largely unresolved, although the genus-level clades and many groups within the genera were

well-supported. The analyses strongly supported a clade of “*Fusconaia*” *barnesiana*, “*Lexingtonia*” *dollabelloides*, and “*Pleurobema*” *gibberum*, not corresponding to any currently recognized genus.

Among the genera as currently recognized, *Hemistena* is monotypic, and only one species of *Lexingtonia* and *Plethobasus* was available. *Uniomerus* appears polyphyletic, but this is problematic in light of the taxonomic uncertainties discussed above. It may also have biochemical peculiarities, as extractions from multiple specimens persistently failed to amplify. The remaining genera in Pleurobemini are polyphyletic. However, the majority of *Fusconaia* and *Pleurobema* species each form a clade that includes the type species of the respective genus. Both *Fusconaia* and *Quincuncina*, as presently used, include species that belong in Quadrulini. This agrees with other recent molecular studies (Lydeard et al. 2000; Serb et al. 2003).

Quadrulini

Although long recognized on the basis of shell morphology, the tribe Quadrulini has been synonymized with Amblemini in most recent morphological classifications (Heard & Guckert 1971; Burch 1975) and the immunological analysis of Davis & Fuller (1981). Molecular sequencing studies, however, have supported separating it from Amblemini (Lydeard et al. 1996; Bogan & Hoeh 2000; Hoeh et al. 2001; Krebs et al. 2003; Roe & Hoeh 2003; Serb et al. 2003; see also discussion in Graf 2002). The present analyses placed Quadrulini basal within Ambleminae *s.s.*

Quadrulini includes *Cyclonaias*, “*Fusconaia*” *succissa*, *Megalonaias*, *Quadrula*, “*Quincuncina*” *infucata*, “*Quincuncina*” *kleiniana*, *Tritogonia*, and *Uniomerus*. *Megalonaias* and *Uniomerus* were the basal members of Quadrulini in all analyses. *Quadrula* appears paraphyletic to the other taxa in Quadrulini, except *Megalonaias* and *Uniomerus*. *Cyclonaias*, “*F.*” *succissa*, and the “*Quincuncina*” species appear closely related, placing with *Quadrula kieneriana* in the *pustulosa* group of Serb et al. (2003). Within *Quadrula*, *Q. metanevra* is basal to the remaining species.

These results agree closely with those of Serb et al. (2003), except in the placement of *Cyclonaias* and the prior lack of data for *Uniomerus*. Placing *Cyclonaias* in Quadrulini agrees with some morphological studies (e.g., Frierson 1927; Modell 1964) and with the results of Davis & Fuller (1981), although Heard & Guckert (1971) assigned it to Pleurobemini based on its ectobranchy. *Cyclonaias tuberculata* includes two distinctive morphologies (Parmalee & Bogan 1998); further investigation of this species is needed. Ly-

deard et al. (2000) also grouped “*Fusconaia*” *succissa*, “*Quincuncina*” *infucata*, and “*Quincuncina*” *kleiniana* with Quadrulini, and Davis & Fuller (1981) and Davis (1983) placed “*Q.*” *infucata* with Quadrulini.

Genus-level polyphyly

Most genera that currently include multiple species appear to be polyphyletic. This rampant polyphyly indicates that genera, as currently recognized, are a poor guide to phylogenetic affinity. Of the 37 currently recognized genera of North American Ambleminae (counting *Gonidea*), 12 are monotypic. The present study and previous molecular work (Roe & Lydeard 1998; Lydeard et al. 2000; Graf & Ó Foighil 2000a; Roe et al. 2001; Krebs et al. 2003; Serb et al. 2003) support paraphyly or polyphyly for 12 of the remaining genera. These include the highly diverse genera *Elliptio*, *Lampsilis*, *Pleurobema*, *Quadrula*, and *Villosa*, as well as *Actinonaias*, *Fusconaia*, *Ligumia*, *Obovaria*, *Potamilus*, *Quincuncina*, and *Venustaconcha*. *Medionidus* appeared polyphyletic, but with weakly supported relationships. *Amblema*, *Cyprogenia*, *Epioblasma*, *Leptodea*, and *Toxolasma* appeared monophyletic in the present analyses, but not all the species were included. Detailed analysis of *Cyprogenia* is in preparation (Serb, unpubl. data). Seven of the remaining genera have not yet received detailed phylogenetic study to test their monophyly. All were represented by a single sequence in the present analyses: *Cyrtonaias*, *Lexingtonia*, *Plethobasus*, *Popenaias*, *Ptychobranthus*, *Truncilla*, and *Uniomerus*. The problem of polyphyletic genera is not confined to Ambleminae as, within the outgroup, *Lasmigona* appears polyphyletic. This result agrees with King et al. (1999), who examined other *Lasmigona* species.

Thus, although the tribes Pleurobemini, Quadrulini, and Lampsilini appear to be supported by molecular data, the genera of Ambleminae appear overwhelmingly polyphyletic. A few species, such as “*Fusconaia*” *succissa* and “*F.*” *ebena*, are presently assigned to the wrong tribe. Reclassifying the species will require detailed studies on species groups, including intraspecific sampling to examine phylogeographic patterns, especially in putatively widespread, variable species. These analyses will also require a wide range of outgroups to ensure that misassigned taxa are recognized.

Problematic taxa

Some taxa show relatively poor resolution of relationships, especially many lampsilines. Most of these

are taxa with limited data; in particular, many lack ND1 data. However, other taxa seem problematic despite relatively complete data. Several possible reasons exist. First, various mistakes are possible, including misidentification and lab errors. For example, Krebs et al. (2003) noted that the 16S sequence for *Quadrula quadrula* from Lydeard et al. (1996) appeared very different from their newly generated sequence. Analysis of ND1 data for the specimen sequenced by Lydeard et al. suggested that it was in fact *Q. nobilis* (Serb et al. 2003). Although the two are genetically distinctive, they are morphologically so similar that few studies have recognized them as distinct (Howells et al. 1996). The present analyses using data for COI, 16S, and ND1 support this re-identification. The specimen is morphologically intermediate between standard *Q. nobilis* and *Q. quadrula*, and is from just outside the previously reported range of *Q. nobilis* (Howells, pers. comm.), so the molecular data provided critical evidence on the species-level affinity of the specimen. By greatly increasing the number of taxa with published sequences, the present study facilitates molecular identification in future studies.

However, it is also possible that an accurate DNA sequence may give anomalous phylogenetic results. Hybridization may produce disparate evolutionary patterns for individual genes versus for the organism as a whole (Makarenkov et al. 2004). Gene duplication and subsequent independent evolution may result in multiple similar sequences being present in a single individual. PCR may preferentially amplify one of these, producing misleading results due to non-orthology of the sampled genes (e.g., Williams & Knowlton 2001). A specialized case of non-orthologous genes results from the doubly uniparental inheritance pattern for bivalve mitochondria (Hoeh et al. 1996). The doubly uniparental inheritance pattern appears to be followed very strictly in unionoids (Curole & Kocher 2002; Hoeh et al. 2002b), although cases of mixing or switching are known in other bivalves (Hoeh et al. 1996). Because the male and female mitotypes have remained independent in unionoids, the sequences are very different (Curole & Kocher 2002; Hoeh et al. 2002b). Comparison of the present sequences with published male and female unionoid sequences confirmed that all of the present sequences group with the female sequences, as expected for DNA extracted from somatic tissue. Nevertheless, other paralogous sequences might still cause problems, such as a pseudogene derived from the female mitochondrial sequence.

Gene rearrangements may also explain the failure of some species to amplify for ND1. The forward

primer used in the present study is complementary to the tRNA immediately upstream of the ND1 gene itself. Although the two published unionid female mitochondrial genomes have only one major rearrangement, bivalves vary extensively in mitochondrial gene order (Serb & Lydeard 2003). The failure of some DNA extractions (such as *Uniomereus declivis*) to amplify for ND1, despite successful amplification using internal primers for the other genes, might reflect a different arrangement of genes from that assumed in the choice of primers. Similarly, tRNA gene remodeling (Rawlings et al. 2003) could produce a mismatch with the primer.

A few species appeared paraphyletic or polyphyletic, but with all sequences placing within a single genus or small clade (e.g., *Lampsilis attilis* and *Pleurobema rubrum*). Resolution of such problems will require further sampling of closely related species and different populations, within what is presently considered a single species. However, four species (all from monotypic genera) appeared in very different relationships based on different DNA sequences. The present study obtained identical COI sequences for two *Plectomerus dombeyanus* specimens. The newly generated 16S and ND1 sequences for *P. dombeyanus* are similar to published sequences. However, the COI sequences show strong affinity for basal lampsilines, whereas the 16S and ND1 sequences appear outside of Lampsilini when analyzed individually. Similarly, published sequences for *Obliquaria reflexa* yield different affinities, with the COI data suggesting quadrule, and 16S and ND1 suggesting basal lampsiline, affinities. The new COI and 16S sequences for *O. reflexa* differ from published sequences; the new ND1 sequence closely resembles the ND1 sequence of Serb et al. (2003). All of the new *O. reflexa* sequences support assignment to Lampsilini. Two *O. reflexa* specimens, separately sequenced, yielded the same COI sequence (Roe & Lydeard 1998; Roe et al. 2001). The use of a slightly modified primer in the present analysis may have promoted amplification of a different copy of the gene. No evidence currently available indicates which, if either, is the functional copy. Multiple independent sequences for *Plectomerus* and *Obliquaria* suggest that these disparate COI genes represent some sort of gene duplication, rather than precisely replicated lab errors. Data for additional genes or other parts of COI (to test whether the presently known partial sequences form part of a functional copy) may help identify which sequences are homologous to the amplified sequences of other amblesines. Nuclear versions of mitochondrial genes are not uncommon and may show only slight

differentiation from the mitochondrial paralogue (Zhang & Hewitt 1996).

The present *Elliptoideus sloatianus* sequences agree with the 16S sequence of Lydeard et al. (1996), but differ from the ND1 sequence of Serb et al. (2003) in grouping with Pleurobemini rather than with *Plectomerus*. The new data for *Cyclonaias tuberculata* place it in the *pustulosa* group of *Quadrula*; the sequence of Serb et al. (2003) appears lampsiline, similar to *Potamilus*. The new sequences for *Elliptoideus* and the published sequences for *Elliptoideus* and *Cyclonaias* from Serb et al. (2003) are derived from tissue clips obtained from other labs, adding more opportunities for error. All these sequences reflect single specimens, so error or non-homologous sequences are both plausible possibilities. Again, additional data for these taxa will better constrain their affinities.

Summary

The polyphyly of most currently recognized genera has important implications for all aspects of unionid research. Studies that assumed that current genera are biologically coherent units appear to have mixed apple snails and orangefoot pimplebacks. Such assumptions underlie most biological studies on unionids. Omission of key ingroups or misidentification of ingroups as outgroups may mislead phylogenetic analyses. Current genus-level taxonomy in Ambleminae conceals the relationships among species, so that taxa of interest for a particular study may lurk under multiple genera whereas putative close relatives may have little in common. Thus, a phylogenetic analysis of a genus as currently used may be nearly meaningless unless a large selection of outgroups is included. Likewise, biogeographic analyses that treat the current genera as natural groups rest on foundations that are at best misleading and at worst entirely erroneous. Exclusion of Eurasian taxa from the eastern North American tribes provides greater geographic consistency in the distribution of the tribes and new impetus to systematic work on Eurasian genera.

The improved phylogenetic understanding of the Ambleminae generated by this study will also improve our understanding of other aspects of their biology. Again, a major result is caution about interpreting the current generic assignments as necessarily indicative of close similarity. This is a particular problem because information about many taxa has been inferred from what is known about putative close relatives, due to the rarity of many species and the patchiness of our knowledge about unionid biology. However, if the presumed relationships are wrong, these inferences are also likely to be incor-

rect. Effective conservation requires protection of all aspects of the habitat that are needed at any stage of the mussel life cycle. As fish host preference may vary between populations of a single species (Haag et al., pers. comm.), inferred fish hosts based on incorrect assumptions about generic affinity will probably be incorrect. Likewise, extrapolation of ecological preferences, physiological tolerances, or breeding patterns will be more reliable in the context of a well-supported phylogenetic framework.

We hope that these results will inspire and direct further research on this diverse, imperiled clade of invertebrates, especially on taxa that remain poorly resolved.

Note added in proofs

New data indicate that specimen UAUC 136 was mislabeled, and is *Obovaria olivaria* rather than *Venustaconcha pleasii*. In addition, a paper just published proposes a new genus for some of the included taxa; *Hamiota* (Roe & Hartfield 2005) includes the superconglutinate-forming “*Lampsilis*” species.

Roe KJ & Hartfield PD 2005. *Hamiota*, a new genus of fresh-water mussel (Bivalvia: Unionidae) from the Gulf of Mexico drainages of the southeastern United States. *Nautilus* 119(1): 1–10.

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Appendices

Appendix 1. Taxa analyzed and GenBank accession numbers.

Species	Accession no.	Reference
	COI	
<i>Actinonaias ligamentina</i> (LAMARCK 1819)	AF231730	Bogan & Hoeh (2000)
<i>Actinonaias pectorosa</i> (CONRAD 1834)	AY654990	UAUC880
<i>Amblema elliotii</i> (LEA 1856)	AY654991	UAUC2511
<i>Amblema plicata</i> 1 ^T (SAY 1817)	U56841	Hoeh et al. (1998)
<i>Amblema plicata</i> 2 ^T	AF156512	Graf & Ó Foighil (2000a)
<i>Anodonta cygnea</i> ^T (LINNAEUS 1758)	U56842	Hoeh et al. (1998)
<i>Anodonta oregonensis</i> (LEA 1838)	AY493480	Mock et al. (2004)
<i>Cumberlandia monodonta</i> 1 ^T (SAY 1829)	AF156497	Graf & Ó Foighil (2000a)
<i>Cumberlandia monodonta</i> 2	AF156498	Graf & Ó Foighil (2000a)
<i>Cyprogenia stegaria</i> ^T (RAFINESQUE 1820)	AY654992	UAUC1499
<i>Cyrtonaias tampicoensis</i> (LEA 1838)	AF231749	Bogan & Hoeh (2000)
<i>Dromus dromas</i> ^T (LEA 1834)	AY654993	UAUC3156
<i>Ellipsaria lineolata</i> ^T (RAFINESQUE 1820)	AY654994	UAUC450
<i>Elliptio arca</i> (CONRAD 1834)	AY654995	UAUC498
<i>Elliptio crassidens</i> ^T (LAMARCK 1819)	AY613820	UAUC1493
<i>Elliptio dilatata</i> 1 (RAFINESQUE 1820)	AF231751	Bogan & Hoeh (2000)
<i>Elliptio dilatata</i> 2	AF156506	Graf & Ó Foighil (2000a)
<i>Elliptioideus sloatianus</i> ^T (LEA 1840)	AY613822	Specimen Es
<i>Epioblasma brevidens</i> (LEA 1831)	AF156527	Graf & Ó Foighil (2000a)
<i>Epioblasma capsaeformis</i> (LEA 1834)	AY654996	UAUC1527 (inc. AY094372 Buhay et al. 2002)
<i>Epioblasma florentina walkeri</i> 1 (WILSON & CLARK 1914)	AY094373	Buhay et al. (2002)
<i>Epioblasma florentina walkeri</i> 2	AY094374	Buhay et al. (2002)
<i>Epioblasma triquetra</i> (RAFINESQUE 1820)	AF156528	Graf & Ó Foighil (2000a)
<i>Fusconaia barnesiana</i> (LEA 1838)	AY613822	UAUC1553
<i>Fusconaia cerina</i> 1 (CONRAD 1838)	AY613823	UAUC3233
<i>Fusconaia cerina</i> 2	AF049522	Roe & Lydeard (1998)
<i>Fusconaia cor</i> (CONRAD 1834)	AY654997	UAUC2606
<i>Fusconaia cuneolus</i> (LEA 1840)	AY654998	UAUC1552
<i>Fusconaia ebena</i> (LEA 1831)	AY654999	UAUC71 (inc. AF232815 Lydeard et al. 2000)
<i>Fusconaia escambia</i> 1 (CLENCH & TURNER 1956)	AF232816	Lydeard et al. (2000)
<i>Fusconaia escambia</i> 2	AF232817	Lydeard et al. (2000)
<i>Fusconaia flava</i> 1 ^T (RAFINESQUE 1820)	AF231733	Bogan and Hoeh (2000)
<i>Fusconaia flava</i> 2 ^T	AF232822	Lydeard et al. (2000)
<i>Fusconaia subrotunda</i> (LEA 1831)	AY613824	UAUC1554
<i>Fusconaia succissa</i> 1 (LEA 1852)	AF232819	Lydeard et al. (2000)
<i>Fusconaia succissa</i> 2	AF232820	Lydeard et al. (2000)
<i>Glebula rotundata</i> ^T (LAMARCK 1819)	AF231729	Bogan and Hoeh (2000)
<i>Gonidea angulata</i> ^T (LEA 1838)	AF231755	Bogan and Hoeh (2000)
<i>Hemistena lata</i> ^T (RAFINESQUE 1820)	AY613825	UAUC2797
<i>Hyriopsis cumingii</i> (LEA 1852)	AY655000	UAUC3160
<i>Inversidens japonensis</i> (LEA 1859)	AB055625	Okazaki & Ueshima, unpubl. data
<i>Lampsilis attilis</i> 1 (CONRAD 1834)	AF385105	Roe et al. (2001)
<i>Lampsilis attilis</i> 2	AF385108	Roe et al. (2001)
<i>Lampsilis attilis</i> 3	AF385092	Roe et al. (2001)
<i>Lampsilis australis</i> 1 Simpson (1900)	AF385101	Roe et al. (2001)
<i>Lampsilis australis</i> 2	AF385098	Roe et al. (2001)
<i>Lampsilis australis</i> 3	AF385099	Roe et al. (2001)
<i>Lampsilis cardium</i> (RAFINESQUE 1820)	AF120653	Giribet & Wheeler (2002)
<i>Lampsilis ornata</i> 2	AF049520	Roe & Lydeard (1998)
<i>Lampsilis ornata</i> 1 (CONRAD 1835)	AY365193	Serb & Lydeard (2003)
<i>Lampsilis ovata</i> ^T (SAY 1817)	AY613826	UAUC108 (inc. AF385111 Roe et al. 2001)

Appendix 1. (Continued).

Species	Accession no.		Reference
	COI		
<i>Lampsilis perovalis</i> 1 (CONRAD 1834)	AF385094	Roe et al. (2001)	
<i>Lampsilis perovalis</i> 2	AF385096	Roe et al. (2001)	
<i>Lampsilis siliquoidea</i> 1 (BARNES 1823)	AF156521	Graf & Ó Foighil (2000a)	
<i>Lampsilis siliquoidea</i> 2	AF156522	Graf & Ó Foighil (2000a)	
<i>Lampsilis subangulata</i> 1 (LEA 1840)	AF385104	Roe et al. (2001)	
<i>Lampsilis subangulata</i> 2	AF385102	Roe et al. (2001)	
<i>Lampsilis teres</i> 1 (RAFINESQUE 1820)	AF385113	Roe et al. (2001)	
<i>Lampsilis teres</i> 2	AF406803	Hoeh et al. (2002b)	
<i>Lasmigona costata</i> ^T (RAFINESQUE 1820)	AF093848	King et al. (1999)	
<i>Lasmigona holstonia etowahensis</i> (LEA 1858)	AY655001	UAUC3159	
<i>Lemiox rimosus</i> ^T (RAFINESQUE 1831)	AY655002	UAUC1528	
<i>Leptodea fragilis</i> 1 (RAFINESQUE 1820)	AF049518	Roe & Lydeard (1998)	
<i>Leptodea fragilis</i> 2	AF049519	Roe & Lydeard (1998)	
<i>Leptodea leptodon</i> ^T (RAFINESQUE 1820)	AY655003	UAUC135	
<i>Lexingtonia dolabelloides</i> 1 (LEA 1840)	AY655004	UAUC1488	
<i>Lexingtonia dolabelloides</i> 2	AY613827	UAUC2819	
<i>Ligumia nasuta</i> (SAY 1817)	AF156515	Graf & Ó Foighil (2000a)	
<i>Ligumia recta</i> ^T (LAMARCK 1819)	AF385110	Roe et al. (2001)	
<i>Margaritifera margaritifera</i> ^T (LINNAEUS 1758)	U56847	Hoeh et al. (1998)	
<i>Medionidus accutissimus</i> (LEA 1831)	AY655005	UAUC82	
<i>Medionidus conradicus</i> ^T (LEA 1834)	AY655006	UAUC10	
<i>Megaloniaias nervosa</i> ^T (RAFINESQUE 1820)	AY655007	UAUC266	
<i>Obliquaria reflexa</i> ^T (RAFINESQUE 1820)	AY655008	UAUC2508	
<i>Obovaria jacksoniana</i> (FRIERSON 1912)	AY655009	UAUC680	
<i>Obovaria olivaria</i> (RAFINESQUE 1820)	AF232812	Lydeard et al. (2000)	
<i>Obovaria rotulata</i> 1 (WRIGHT 1899)	AF232814	Lydeard et al. (2000)	
<i>Obovaria rotulata</i> 2	AF232813	Lydeard et al. (2000)	
<i>Obovaria subrotunda</i> (RAFINESQUE 1820)	AY655010	UAUC2838	
<i>Obovaria unicolor</i> (LEA 1845)	AF232811	Lydeard et al. (2000)	
<i>Plectomerus dombeyanus</i> ^T (VALENCIENNES 1827)	AY655011	UAUC2536	
<i>Plethobasus cyphus</i> ^T (RAFINESQUE 1820)	AY613828	UAUC1639	
<i>Pleurobema chattanoogaense</i> 1 (LEA 1858)	AY655012	UAUC1621	
<i>Pleurobema chattanoogaense</i> 2	AY613829	UAUC3194	
<i>Pleurobema clava</i> ^T (LAMARCK 1819)	AY655013	UAUC1477	
<i>Pleurobema collina</i> (CONRAD 1837)	AY613830	UAUC1074	
<i>Pleurobema cordatum</i> (RAFINESQUE 1820)	AY613831	UAUC2572	
<i>Pleurobema decisum</i> 1 (LEA 1831)	AF232801	Lydeard et al. (2000)	
<i>Pleurobema decisum</i> 2	AY655014	UAUC253	
<i>Pleurobema decisum</i> 3	AY613832	UAUC3196	
<i>Pleurobema furvum</i> (CONRAD 1834)	AY613833	UAUC678	
<i>Pleurobema georgianum</i> 2 (LEA 1841)	AY613834	UAUC3193	
<i>Pleurobema georgianum</i> 3	AY655015	UAUC3084	
<i>Pleurobema gibberum</i> (LEA 1838)	AY613835	UAUC3319	
<i>Pleurobema hanleyianum</i> 1 (LEA 1852)	AY655016	UAUC273	
<i>Pleurobema hanleyianum</i> 2	AY613836	UAUC1622	
<i>Pleurobema oviforme</i> 1 (CONRAD 1834)	AY655017	UAUC1402	
<i>Pleurobema oviforme</i> 2	AY613837	UAUC1642	
<i>Pleurobema perovatum</i> (CONRAD 1834)	AY613838	UAUC1640	
<i>Pleurobema pyriforme</i> (LEA 1857)	AY613839	A29	
<i>Pleurobema rubellum</i> (CONRAD 1834)	AY613840	UAUC679	
<i>Pleurobema rubrum</i> 1 (RAFINESQUE 1820)	AY655018	UAUC2719	
<i>Pleurobema rubrum</i> 2	AY613841	UAUC3229	
<i>Pleurobema sintoxia</i> (RAFINESQUE 1820)	AY655019	UAUC1714	
<i>Pleurobema strodeanum</i> (WRIGHT 1898)	AY613843	UAUC1110	

Appendix 1. (Continued).

Species	Accession no.		Reference
	COI		
<i>Pleurobema taitianum</i> (LEA 1834)	AY613844	UAUC885	
<i>Pleurobema troschelium</i> (LEA 1852)	AY613845	UAUC516	
<i>Popenaias popeii</i> ^T (LEA 1857)	AY655020	UAUC3161	
<i>Potamilus alatus</i> ^T 1 (SAY 1817)	AF049510	Roe & Lydeard (1998)	
<i>Potamilus alatus</i> ^T 2	AF049511	Roe & Lydeard (1998)	
<i>Potamilus purpuratus</i> (LAMARCK 1819)	AF049507	Roe & Lydeard (1998)	
<i>Psilunio littoralis</i> ^T 1 (CUVIER 1798)	AF303348	Machordom et al. (2003)	
<i>Psilunio littoralis</i> ^T 2	AF303349	Machordom et al. (2003)	
<i>Ptychobranchnus fasciolaris</i> ^T (RAFINESQUE 1820)	AF156514	Graf & Ó Foighil (2000a)	
<i>Pyganodon grandis</i> (SAY 1829)	AF156504	Graf & Ó Foighil (2000a)	
<i>Quadrula nobilis</i> (CONRAD 1854)	AF232823	Lydeard et al. (2000)	
<i>Quadrula quadrula</i> ^T 1 (RAFINESQUE 1820)	AF231757	Bogan & Hoeh (2000)	
<i>Quadrula quadrula</i> ^T 2	AF156511	Graf & Ó Foighil (2000a)	
<i>Quincuncina burkei</i> ^T 1 (WALKER 1922)	AF232804	Lydeard et al. (2000)	
<i>Quincuncina burkei</i> ^T 2	AF232803	Lydeard et al. (2000)	
<i>Quincuncina burkei</i> ^T 3	AF232802	Lydeard et al. (2000)	
<i>Quincuncina infucata</i> 1 (CONRAD 1834)	AF232807	Lydeard et al. (2000)	
<i>Quincuncina infucata</i> 2	AF232806	Lydeard et al. (2000)	
<i>Quincuncina infucata</i> 3	AF232805	Lydeard et al. (2000)	
<i>Quincuncina kleiniana</i> 1 (LEA 1852)	AF232808	Lydeard et al. (2000)	
<i>Quincuncina kleiniana</i> 2	AF232809	Lydeard et al. (2000)	
<i>Strophitus subvexus</i> (CONRAD 1834)	AY655021	UAUC2715	
<i>Toxolasma parvum</i> (BARNES 1823)	AY655022	UAUC3331	
<i>Toxolasma texasiensis</i> (LEA 1857)	AY655023	UAUC80	
<i>Tritogonia verrucosa</i> ^T (RAFINESQUE 1820)	AY655024	UAUC3195	
<i>Truncilla truncata</i> ^T (RAFINESQUE 1820)	AF156513	Graf & Ó Foighil (2000a)	
<i>Uniomereus declivus</i> (SAY 1831)	AY613846	UAUC3290	
<i>Venustaconcha ellipsiformis</i> ^T (CONRAD 1836)	AY655025	UAUC2596-2598	
<i>Venustaconcha pleasii</i> (MARSH 1891)	AY655026	UAUC136	
<i>Villosa iris</i> (LEA 1829)	AF156524	Graf & Ó Foighil (2000a)	
<i>Villosa vanuxemensis</i> (LEA 1838)	AF156526	Graf & Ó Foighil (2000a)	
<i>Villosa villosa</i> ^T (WRIGHT 1898)	AF385109	Roe et al. (2001)	
16S			
<i>Actinonaias ligamentina</i>	AY655027	UAUC241	
<i>Actinonaias pectorosa</i>	AY655028	UAUC880	
<i>Amblesma elliottii</i>	AY655029	Mulvey et al. (1997)	
<i>Amblesma plicata</i> ^T 1	U72547	Lydeard et al. (1996)	
<i>Amblesma plicata</i> ^T 2	U72548	Lydeard et al. (1996)	
<i>Anodonta cygnea</i> ^T	AF232799	Lydeard et al. (2000)	
<i>Cumberlandia monodonta</i> ^T 1	AF232800	Lydeard et al. (2000)	
<i>Cumberlandia monodonta</i> ^T 2	U72546	Lydeard et al. (1996)	
<i>Cyclonaias tuberculata</i> ^T (RAFINESQUE 1820)	AY655030	UAUC3158	
<i>Cyprogenia aberti</i> (CONRAD 1850)	AY655031	UAUC75	
<i>Cyrtonaias tampicoensis</i>	AY655032	UAUC78	
<i>Dromus dromas</i> ^T	AY655033	UAUC3156	
<i>Ellipsaria lineolata</i> ^T	U72567	Lydeard et al. (1996)	
<i>Elliptio crassidens</i> ^T	AY655034	UAUC3150	
<i>Elliptio dilatata</i> 1	U72557	Lydeard et al. (1996)	
<i>Elliptioideus sloatianus</i> ^T	AY655035	Specimen Es	
<i>Epioblasma brevidens</i>	AY655036	UAUC509	
<i>Epioblasma capsaeformis</i>	AY655037	UAUC1527	
<i>Fusconaia barnesiana</i>	AY655038	UAUC1553	
<i>Fusconaia cerina</i> 1	AY655039	UAUC073	

Appendix 1. (Continued).

16S		
<i>Fusconaia cor</i>	AY655040	UAUC2606
<i>Fusconaia ebena</i>	AF232790	Lydeard et al. (2000)
<i>Fusconaia escambia</i> 1	AF232791	Lydeard et al. (2000)
<i>Fusconaia escambia</i> 2	AY655041	UAUC1449 (inc. AF232792 Lydeard et al. 2000)
<i>Fusconaia flava</i> ^T 1	AY238481	Krebs et al. (2003)
<i>Fusconaia flava</i> ^T 2	AY655042	UAUC146
<i>Fusconaia subrotunda</i>	AY655043	UAUC1554
<i>Fusconaia succissa</i> 1	AF232794	Lydeard et al. (2000)
<i>Fusconaia succissa</i> 2	AF232795	Lydeard et al. (2000)
<i>Glebula rotundata</i> ^T	AY655044	UAUC502
<i>Gonidea angulata</i> ^T	AY655045	UAUC3147
<i>Hemistena lata</i> ^T	AY655046	UAUC2797
<i>Hyriopsis cumingii</i>	AY655047	UAUC3160
<i>Inversidens japonensis</i>	AB055625	Okazaki & Ueshima, unpubl. data
<i>Lampsilis altilis</i> 1	AF385129	Roe et al. (2001)
<i>Lampsilis altilis</i> 2	AF385132	Roe et al. (2001)
<i>Lampsilis altilis</i> 3	AF385116	Roe et al. (2001)
<i>Lampsilis australis</i> 1	AF385125	Roe et al. (2001)
<i>Lampsilis australis</i> 2	AF385122	Roe et al. (2001)
<i>Lampsilis australis</i> 3	AF385123	Roe et al. (2001)
<i>Lampsilis cardium</i>	AF191574	Turner et al. (2000)
<i>Lampsilis ornata</i> 2	AF385136	Roe et al. (2001)
<i>Lampsilis ornata</i> 1	AY365193	Serb & Lydeard (2003)
<i>Lampsilis ovata</i> ^T	AY655048	UAUC108
<i>Lampsilis perovalis</i> 1	AF385118	Roe et al. (2001)
<i>Lampsilis perovalis</i> 2	AF385120	Roe et al. (2001)
<i>Lampsilis siliquoidea</i> 1	AF191571	Turner et al. (2000)
<i>Lampsilis siliquoidea</i> 2	U72571	Lydeard et al. (1996)
<i>Lampsilis subangulata</i> 1	AF385128	Roe et al. (2001)
<i>Lampsilis subangulata</i> 2	AF385126	Roe et al. (2001)
<i>Lampsilis teres</i> 1	AF232785	Lydeard et al. (2000)
<i>Lampsilis teres</i> 2	U72568	Lydeard et al. (1996)
<i>Lasmigona costata</i> ^T	AY238488	Krebs et al. (2003)
<i>Lemiox rimosus</i> ^T	AY655049	Unnumbered specimen
<i>Leptodea fragilis</i> 1	U72570	Lydeard et al. (1996)
<i>Leptodea fragilis</i> 2	AY238483	Krebs et al. (2003)
<i>Leptodea leptodon</i> ^T	AY655050	UAUC135
<i>Lexingtonia dolabelloides</i> 1	AY655051	UAUC3148
<i>Ligumia nasuta</i>	AY655052	No specimen data
<i>Ligumia recta</i> ^T	AY655053	UAUC89
<i>Margaritifera margaritifera</i> ^T	AF303297	Machordom et al. (2003)
<i>Medionidus accutissimus</i>	AY655054	UAUC82
<i>Medionidus conradicus</i> ^T	U72572	Lydeard et al. (1996)
<i>Megaloniaias nervosa</i> ^T	U72555	Lydeard et al. (1996)
<i>Obliquaria reflexa</i> ^T	AY655055	UAUC2508
<i>Obovaria olivaria</i>	AF232787	Lydeard et al. (2000)
<i>Obovaria rotulata</i> 1	AF232788	Lydeard et al. (2000)
<i>Obovaria rotulata</i> 2	AF232789	Lydeard et al. (2000)
<i>Obovaria subrotunda</i>	AY655056	UAUC2838
<i>Obovaria unicolor</i>	AF232786	Lydeard et al. (2000)
<i>Plectomerus dombeyanus</i> ^T	AY655057	UAUC2536
<i>Plethobasus cyphus</i> ^T	AY655058	UAUC3157
<i>Pleurobema chattanoogaense</i> 1	AY655059	UAUC1621
<i>Pleurobema clava</i> ^T	AY655060	UAUC1477
<i>Pleurobema collina</i>	AY655061	UAUC1074
<i>Pleurobema decisum</i> 1	AF232776	Lydeard et al. (2000)

Appendix 1. (Continued).

16S		
<i>Pleurobema georgianum</i> 1	AY655062	UAUC1623
<i>Pleurobema georgianum</i> 2	AY655063	UAUC3193
<i>Pleurobema gibberum</i>	AY655064	UAUC3153
<i>Pleurobema hanleyianum</i> 1	AY655065	UAUC273
<i>Pleurobema hanleyianum</i> 2	AY655066	UAUC1622
<i>Pleurobema oviforme</i> 1	AY655067	UAUC3238
<i>Pleurobema oviforme</i> 2	AY655068	UAUC1642
<i>Pleurobema rubellum</i>	AY655069	UAUC679
<i>Pleurobema strodeanum</i>	AY655070	UAUC1818
<i>Pleurobema taitianum</i>	AY655071	UAUC885
<i>Pleurobema troschelium</i>	AY655072	UAUC516
<i>Popenaias popeii</i> ^T	AY655073	UAUC3161
<i>Potamilus alatus</i> ^T 1	AY655074	UAUC41
<i>Potamilus alatus</i> ^T 2	AY238484	Krebs et al. (2003)
<i>Potamilus purpuratus</i>	U72573	Lydeard et al. (1996)
<i>Psilunio littoralis</i> ^T 1	AF3033078	Machordom et al. (2003)
<i>Psilunio littoralis</i> ^T 2	AF3033078	Machordom et al. (2003)
<i>Ptychobranchnus fasciolaris</i> ^T	AY655075	LSC23701-001
<i>Pyganodon grandis</i>	AY238490	Krebs et al. (2003)
<i>Quadrula apiculata</i> (SAY 1829)	U72554	Lydeard et al. (1996)
<i>Quadrula kieneriana</i> (LEA 1852)	AY655076	UAUC334
<i>Quadrula metanevra</i> (RAFINESQUE 1820)	U72551	Lydeard et al. (1996)
<i>Quadrula nobilis</i>	AF232798	Lydeard et al. (2000)
<i>Quadrula quadrula</i> ^T 1	AY238485	Krebs et al. (2003)
<i>Quadrula quadrula</i> ^T 2	U72552	Lydeard et al. (1996)
<i>Quincuncina burkei</i> ^T 1	AF2327779	Lydeard et al. (2000)
<i>Quincuncina burkei</i> ^T 2	AF2327779	Lydeard et al. (2000)
<i>Quincuncina burkei</i> ^T 3	AF2327779	Lydeard et al. (2000)
<i>Quincuncina infucata</i> 1	AF232782	Lydeard et al. (2000)
<i>Quincuncina infucata</i> 2	AF232781	Lydeard et al. (2000)
<i>Quincuncina infucata</i> 3	AF232780	Lydeard et al. (2000)
<i>Quincuncina kleiniana</i> 1	AF232783	Lydeard et al. (2000)
<i>Quincuncina kleiniana</i> 2	AF232784	Lydeard et al. (2000)
<i>Strophitus subvexus</i>	AY655077	UAUC2715
<i>Toxolasma parvus</i>	AY238482	Krebs et al. (2003)
<i>Toxolasma texasiensis</i>	AY655078	UAUC80
<i>Tritogonia verrucosa</i> ^T	AY655079	UAUC3195
<i>Truncilla truncata</i> ^T	AY655080	Unnumbered specimen
<i>Uniomereus declivus</i>	AY655081	UAUC3290
<i>Venustaconcha ellipsiformis</i> ^T	AY655082	UAUC2596-2598
<i>Villosa iris</i>	AY655083	UAUC260
<i>Villosa vanuxemensis</i>	AY655084	UAUC3046
<i>Villosa villosa</i> ^T	AF385133	Roe et al. (2001)
ND1		
<i>Actinonaias ligamentina</i>	AY655085	UAUC241
<i>Amblema elliottii</i>	AY655086	UAUC2511
<i>Amblema plicata</i> ^T 1	AY158796	Serb et al. (2003)
<i>Anodonta oregonensis</i>	AY655087	UAUC3169
<i>Cyclonaias tuberculata</i> ^T	AY655088	UAUC3158
<i>Cyprogenia aberti</i>	AY158749	Serb et al. (2003)
<i>Cyprogenia stegaria</i> ^T	AY655089	UAUC1499
<i>Cyrtonaias tampicoensis</i>	AY655090	UAUC314
<i>Dromus dromas</i> ^T	AY655091	UAUC3156
<i>Ellipsaria lineolata</i> ^T	AY655092	UAUC450
<i>Elliptio arca</i>	AY655093	UAUC501

Appendix 1. (Continued).

	NDI	
<i>Elliptio crassidens</i> ^T	AY613788	UAUC3150
<i>Elliptio dilatata</i> 1	AY613789	UAUC2735
<i>Elliptio dilatata</i> 2	AY655094	UAUC2721
<i>Elliptoideus sloatianus</i> ^T	AY613790	Specimen Es
<i>Epioblasma brevidens</i>	AY094378 = AY094377	Buhay et al. (2002)
<i>Epioblasma capsaeformis</i>	AY094381 = AY094379	Buhay et al. (2002)
<i>Epioblasma florentina walkeri</i> 1	AY094383	Buhay et al. (2002)
<i>Epioblasma florentina walkeri</i> 2	AY094384	Buhay et al. (2002)
<i>Epioblasma triquetra</i>	AY094375	Buhay et al. (2002)
<i>Fusconaia barnesiana</i>	AY613791	UAUC1553
<i>Fusconaia cerina</i> 1	AY655095	UAUC073
<i>Fusconaia cerina</i> 2	AY613792	UAUC3234
<i>Fusconaia cor</i>	AY655096	UAUC2606
<i>Fusconaia cuneolus</i>	AY655097	UAUC1552
<i>Fusconaia ebena</i>	AY655098	UAUC71
<i>Fusconaia flava</i> ^T 1	AY613793	UAUC2864
<i>Fusconaia flava</i> ^T 2	AY158781	Serb et al. (2003)
<i>Fusconaia subrotunda</i>	AY613794	UAUC1554
<i>Fusconaia succissa</i> 1	AY158792	Serb et al. (2003)
<i>Fusconaia succissa</i> 2	AY158809	Serb et al. (2003)
<i>Glebula rotundata</i> ^T	AY613795	UAUC502
<i>Gonidea angulata</i> ^T	AY655099	UAUC3147
<i>Hemistena lata</i> ^T	AY613796	UAUC2797 (inc. AY158787 Serb et al. 2003)
<i>Hyriopsis cumingii</i>	AY655100	UAUC3160
<i>Inversidens japonensis</i>	AB055625	Okazaki & Ueshima, unpubl. data
<i>Lampsilis altilis</i> 1	AY655101	UAUC125
<i>Lampsilis ornata</i> 2	AY158748	Serb et al. (2003)
<i>Lampsilis ornata</i> 1	AY365193	Serb & Lydeard (2003)
<i>Lampsilis ovata</i> ^T	AY613797	UAUC1681
<i>Lampsilis siliquoidea</i> 2	AY158747	Serb et al. (2003)
<i>Lampsilis teres</i> 1	AY655102	UAUC3330
<i>Lasmigona holstonia etowahensis</i>	AY655103	UAUC3159
<i>Lemiox rimosus</i> ^T	AY655104	UAUC1528
<i>Leptodea leptodon</i> ^T	AY655105	UAUC135
<i>Lexingtonia dolabelloides</i> 1	AY613798	UAUC3148
<i>Lexingtonia dolabelloides</i> 2	AY655106	UAUC2819
<i>Medionidus accutissimus</i>	AY655107	UAUC82
<i>Medionidus conradicus</i> ^T	AY158746	Serb et al. (2003)
<i>Megalonaia nervosa</i> ^T	AY158794	Serb et al. (2003)
<i>Obliquaria reflexa</i> ^T	AY655108	UAUC2508 (incl. AY158751 Serb et al. 2003)
<i>Obovaria jacksoniana</i>	AY655109	UAUC775
<i>Obovaria rotulata</i> 2	AY158799	Serb et al. (2003)
<i>Plectomerus dombeyanus</i> ^T	AY655110	UAUC2536
<i>Plethobasus cyphus</i> ^T	AY613799	UAUC3157
<i>Pleurobema chattanoogaense</i> 1	AY655111	UAUC1621
<i>Pleurobema chattanoogaense</i> 2	AY613801	UAUC3194
<i>Pleurobema clava</i> ^T	AY613802	UAUC1477
<i>Pleurobema collina</i>	AY613803	UAUC1074
<i>Pleurobema cordatum</i>	AY613804	UAUC2572
<i>Pleurobema decisum</i> 2	AY655112	UAUC2997
<i>Pleurobema decisum</i> 3	AY613805	UAUC3196
<i>Pleurobema furvum</i>	AY613806	UAUC678
<i>Pleurobema georgianum</i> 1	AY655113	UAUC1623
<i>Pleurobema georgianum</i> 2	AY613807	UAUC3193
<i>Pleurobema georgianum</i> 3	AY655114	UAUC3084
<i>Pleurobema gibberum</i>	AY613808	UAUC3153

Appendix 1. (Continued).

	ND1	
<i>Pleurobema hanleyianum</i> 1	AY655115	UAUC273
<i>Pleurobema hanleyianum</i> 2	AY613809	UAUC1622
<i>Pleurobema oviforme</i> 1	AY613810	UAUC3238
<i>Pleurobema oviforme</i> 2	AY655116	UAUC1642
<i>Pleurobema perovatum</i>	AY613811	UAUC1640
<i>Pleurobema pyriforme</i>	AY613812	A29
<i>Pleurobema rubellum</i>	AY613813	UAUC679
<i>Pleurobema rubrum</i> 1	AY655117	UAUC2719
<i>Pleurobema rubrum</i> 2	AY613814	UAUC3229
<i>Pleurobema sintoxia</i>	AY613815	UAUC1714
<i>Pleurobema strodeanum</i>	AY613817	UAUC1110
<i>Pleurobema taitianum</i>	AY613818	UAUC885
<i>Pleurobema troschelianum</i>	AY613819	UAUC516
<i>Popenaias popeii</i> ^T	AY655118	UAUC3161
<i>Potamilus alatus</i> ^T 1	AY655119	UAUC3329
<i>Ptychobranthus fasciolaris</i> ^T	AY655120	LSC23701-001
<i>Quadrula apiculata</i>	AY158805	Serb et al. (2003)
<i>Quadrula kieneriana</i>	AY158769	Serb et al. (2003)
<i>Quadrula metanevra</i>	AY158771	Serb et al. (2003)
<i>Quadrula nobilis</i>	AY158789	Serb et al. (2003)
<i>Quadrula quadrula</i> ^T 1	AY158790	Serb et al. (2003)
<i>Quadrula quadrula</i> ^T 2	AY158774	Serb et al. (2003)
<i>Quincuncina burkei</i> ^T 3	AY158793	Serb et al. (2003)
<i>Quincuncina infucata</i> 1	AY655121	UAUC3283
<i>Quincuncina infucata</i> 3	AY158810	Serb et al. (2003)
<i>Quincuncina kleiniana</i> 1	AY158795	Serb et al. (2003)
<i>Strophitus subvexus</i>	AY655122	UAUC2715
<i>Toxolasma parvus</i>	AY655123	UAUC3331
<i>Toxolasma texasiensis</i>	AY655124	UAUC80
<i>Tritogonia verrucosa</i> ^T	AY158791	Serb et al. (2003)
<i>Truncilla truncata</i> ^T	AY655125	Unnumbered specimen
<i>Venustaconcha pleasii</i>	AY655126	UAUC136
<i>Villosa iris</i>	AY655127	UAUC2701
<i>Villosa villosa</i> ^T	AY094387	Buhay et al. (2002)

T indicates the type of a genus. If more than one sequence is listed, this indicates multiple identical sequences. For new sequences, the collection number is listed; more details about the new material are in Appendix 2. With new sequences, "inc." indicates that the new sequence is a longer version of a published sequence.

UAUC, University of Alabama Unionid Collection; LSC, Leetown Science Center.

Appendix 2. Locality and collection information for new sequences.

Species	Gene	Collection number	Collector	Locality
<i>Actinonaias ligamentina</i>	16S, ND1	UAUC241	K. J. Roe	Kankakee River, Aroma Park, T30N R13W Sec 23, Kankakee Co. IL
<i>Actinonaias pectorosa</i>	COI, 16S	UAUC880	H. McCullagh	Clinch River, Pendleton Island, Rt. 72 bridge, Ft. Blackmore, Scott Co. VA
<i>Amblema elliottii</i>	COI, ND1	UAUC2511	M. Pierson, K. Chalk, R. James	Coosa River 2.7 mi. downstream of Jordan Dam, Elmore Co. AL
<i>Anodonta oregonensis</i>	ND1	UAUC3169	T. J. Frest, E. J. Johannes	Lake Washington at Magnuson Park, Seattle, King Co. WA
<i>Cyclonaias tuberculata</i>	16S, ND1	UAUC3158	S. Ahlstedt, R. Biggins	Powell River, RM 111.8 Bales Ford, Hancock Co. TN
<i>Cyprogenia aberti</i>	16S	UAUC75	J. L. Harris, R. Doster, J. Fleming, K. Stobaugh	Saline River, downstream of Hwy 229 in Benton, Saline Co. AR
<i>Cyprogenia stegaria</i>	COI, ND1	UAUC1499	S. Ahlstedt, S. Fraley	Clinch River, Brooks Island, RM 184.5, Hancock Co. TN
<i>Cyrtonaias tampicoensis</i>	16S	UAUC78	R. G. Howells	Leon River, Belton Reservoir, Bell Co. TX
<i>Cyrtonaias tampicoensis</i>	ND1	UAUC314	R. G. Howells	Nueces River, Lake Corpus Christi, Live Oak Co. TX
<i>Dromus dromas</i>	COI, 16S, ND1	UAUC3156	Unknown	Powell River, McDowell Shoal, Hancock Co. TN
<i>Ellipsaria lineolata</i>	COI, ND1	UAUC450	S. Mcgreggor, P. O'Neil	Cahaba River, below Cooper Island, Bibb Co. AL
<i>Elliptio arca</i>	COI	UAUC498	M. Hughes	Oostanaula River, 0.8 river mi upstream from Armuchee Creek, Floyd Co. GA
<i>Elliptio arca</i>	ND1	UAUC501	P. Hartfield and others	Sipsey Fork, Black Warrior River at mouth of Hurricane Creek, Winston Co. AL
<i>Elliptio crassidens</i>	COI	UAUC1493	C. Lydeard, C. R. Merrell, J. M. Serb, J. T. Garner	Tennessee River, upstream of US Rt. 43 in Florence, Lauderdale Co. AL
<i>Elliptio crassidens</i>	16S, ND1	UAUC3150	S. Ahlstedt	Coosa River, above Wetumpka below Pipeline Shoals, Elmore Co. AL
<i>Elliptio dilatata</i>	ND1	UAUC2735	S. Ahlstedt	Obed River at Alley Ford, Morgan Co. TN
<i>Elliptio dilatata</i>	ND1	UAUC2721	S. Ahlstedt, C. Hubbs	Duck River, Venable Spring, Marshall Co. TN
<i>Elliptoideus sloatianus</i>	COI, 16S, ND1	Specimen Es	J. Brim-Box and J. D. Williams	Appalachicola River, Gadsden Co. FL
<i>Epioblasma brevidens</i>	16S	UAUC509	J. Khym	Clinch River, Kyles Ford, RM 189.6, Hancock Co. TN
<i>Epioblasma capsaeformis</i>	COI, 16S	UAUC1527	L. Koch	Duck River, Lillard Mill Dam, RM 179, Marshall Co. TN
<i>Fusconaia barnesiana</i>	COI, 16S, ND1	UAUC1553	S. Ahlstedt	Duck River, Lillard Mill Dam, RM 179, Marshall Co. TN
<i>Fusconaia cerina</i>	COI	UAUC3233	M. Gangloff	Tallapoosa River, Choctafaula Creek at FR906, Tuskegee NF, Macon Co. AL
<i>Fusconaia cerina</i>	16S, ND1	UAUC73	P. Hartfield	Tombigbee River, Coal Fire Creek at CR 26, Pickens Co. AL
<i>Fusconaia cerina</i>	ND1	UAUC3234	S. Shively	Bogue Chitto River, Little Silver Creek at Pleasant Hill Road, Washington Pa. LA
<i>Fusconaia cor</i>	COI, 16S, ND1	UAUC2606	J. Fridell, M. Cantrell, S. Fraley	Holston River, North Fork above SR633 crossing, Smyth Co. VA

Appendix 2. (Continued).

Species	Gene	Collection number	Collector	Locality
<i>Fusconaia cuneolus</i>	COI, ND1	UAUC1552	S. Ahlstedt, S. Fraley	Clinch River, Pendleton Island, Rt. 72 bridge, Ft. Blackmore, Scott Co. VA
<i>Fusconaia ebena</i>	COI, ND1	UAUC71	D. Hubbs	Tennessee River, Kentucky Reservoir, RM 88.1, Humphreys Co. TN
<i>Fusconaia escambia</i>	16S	UAUC1449	J. Williams et al.	Conecuh River, CR28 1 mi. E Goshen, Pike Co. AL
<i>Fusconaia flava</i>	16S	UAUC146	P. Morrison	Ohio River, Rosewood Bend, RM625, Harris Co. IN, Jefferson Co. KY
<i>Fusconaia flava</i>	ND1	UAUC2864	W. R. Haag, D. Thurmond, J. G. McWhirter	Big Sunflower River, end of FS Rd 717A, N of Green Ash/Greentree Reservoir, 6 mi E of Rolling Fork, Sharkey Co. MS
<i>Fusconaia subrotunda</i>	COI, 16S, ND1	UAUC1554	S. Ahlstedt, S. Fraley	Powell River, McDowell Ford, RM 106.7, Hancock Co. TN
<i>Glebula rotundata</i>	16S, ND1	UAUC502	Unknown	Apalachicola River, Rm 21.8; tip of Brickyard Island, 5 air mi SSW Sumatra, Franklin Co. FL
<i>Gonidea angulata</i>	16S, ND1	UAUC3147	T. J. Frest, E. J. Johannes	Snake River, RM 569.5 upstream of Dritch Bowler's house/studio, Gooding Co. ID
<i>Hemistena lata</i>	COI, 16S, ND1	UAUC2797	S. Ahlstedt	Clinch River, Frost Ford, RM 181.2, Hancock Co. TN
<i>Hyriopsis cumingii</i>	COI, 16S, ND1	UAUC3160	H. Liu	Poyang Lake, Jiangxi Province, China
<i>Lampsilis altilis</i>	ND1	UAUC125	K. J. Roe et al.	Etowah River, Shoal Ck., Pine Glen Recreation Area, Talladega NF, Cleburne Co. AL
<i>Lampsilis ovata</i>	COI, 16S	UAUC108	J. Garner	Elk River, fish trap above Hwy 127 near state line, Limestone Co. AL
<i>Lampsilis ovata</i>	ND1	UAUC1681	H. McCullagh	Clinch River, near Pendleton Island and Rt. 72 bridge, Ft. Blackmore, Scott Co. VA
<i>Lampsilis teres</i>	ND1	UAUC3330	S. Clark	Tennessee River, Decatur, Morgan Co. AL
<i>Lasmigona holstonia etowahensis</i>	COI, ND1	UAUC3159	P. Johnson	Conasauga River, Poplar Spring Creek, Whitfield Co. GA
<i>Lemiox rimosus</i>	COI, ND1	UAUC1528	L. Koch	Duck River at Lillard Mill Dam, Marshall Co. TN
<i>Lemiox rimosus</i>	16S	Unnumbered	Unknown	Duck River, TN
<i>Leptodea leptodon</i>	COI, 16S, ND1	UAUC135	A. Roberts	Meramec River, MO
<i>Lexingtonia dolabelloides</i>	COI, ND1	UAUC2819	S. Ahlstedt	Duck River, Lillard Mill Dam, RM 179, Marshall Co. TN
<i>Lexingtonia dolabelloides</i>	COI, 16S, ND1	UAUC3148	S. Ahlstedt	Elk River, RM 105.5 Dickey Bridge, Lincoln Co. TN
<i>Ligumia recta</i>	16S	UAUC89	Unknown	Ohio River, near Louisville, Jefferson Co. KY
<i>Medionidus accutissimus</i>	COI, 16S, ND1	UAUC82	Unknown	Tombigbee River, Lubbub Ck. at CR 24, Pickens Co. AL
<i>Medionidus conradicus</i>	COI	UAUC10	C. Lydeard	Clinch River, Kyles Ford, RM 189.6, Hancock Co. TN
<i>Megalonaias nervosa</i>	COI	UAUC266	K. Roe	Coosa River, near Leesburg, downstream from mouth of Terrapin Ck., Cherokee Co. AL

Appendix 2. (Continued).

Species	Gene	Collection number	Collector	Locality
<i>Obliquaria reflexa</i>	COI, 16S, ND1	UAUC2508	M. Pierson, K. Chalk, R. James	Coosa River 2.7 mi downstream of Jordan Dam, Elmore Co. AL
<i>Obovaria jacksoniana</i>	COI	UAUC680	D. N. Sheldon	Pascagoula River, confluence of Brewton Lake, Jackson Co. MS
<i>Obovaria jacksoniana</i>	ND1	UAUC775	H. McCullagh, C. Lydeard	Sipsey River at CR2, Pickens Co. AL
<i>Obovaria subrotunda</i>	COI, 16S	UAUC2838	S. Ahlstedt	Duck River, Lillard Mill Dam, RM 179, Marshall Co. TN
<i>Plectomerus dombeyanus</i>	COI, 16S, ND1	UAUC2536	M. Pierson, K. Chalk, R. James	Coosa River 2.7 mi downstream of Jordan Dam, Elmore Co. AL
<i>Plethobasus cyphus</i>	COI	UAUC1639	S. Ahlstedt, S. Fraley	Clinch River, Brooks Island, RM 184.5, Hancock Co. TN
<i>Plethobasus cyphus</i>	16S, ND1	UAUC3157	S. Ahlstedt	Clinch River, Frost Ford, RM 181.2, Hancock Co. TN
<i>Pleurobema chattanoogaense</i>	COI, 16S, ND1	UAUC1621	S. A. Ahlstedt, R. R. Evans	Conasauga River, below Mitchell Bridge, Murray Co. GA
<i>Pleurobema chattanoogaense</i>	COI, ND1	UAUC3194	P. Johnson	Dead River, 500 m below Terrapin Creek confluence, Cherokee Co. AL
<i>Pleurobema clava</i>	COI, 16S, ND1	UAUC1477	Unknown	Allegheny River, Kennerdell and Clear Ck. SP, Venango Co. PA
<i>Pleurobema collina</i>	COI, 16S, ND1	UAUC1074	M. A. McGregor, P. Burgess	James River, Wards Creek, CR 665 1.5 mi NE Millington, Albemarle Co. VA
<i>Pleurobema cordatum</i>	COI, ND1	UAUC2572	J. Buhay, A. Wethington	Green River, Munfordville, River Road, Hart Co. KY
<i>Pleurobema decisum</i>	COI	UAUC253	P. Hartfield	Tallapoosa River, Chewacla Ck. S of CR 22 bridge crossing, 5 mi E Tuskegee, Macon Co. AL
<i>Pleurobema decisum</i>	ND1	UAUC2997	H. McCullagh	Tallapoosa River, Chewacla Creek at Rt 71 bridge, 5 mi E Tuskegee, Macon Co. AL
<i>Pleurobema decisum</i>	COI, ND1	UAUC3196	P. Johnson	Dead River, 500 m below Terrapin Creek confluence, Cherokee Co. AL
<i>Pleurobema furvum</i>	COI, ND1	UAUC678	P. Hartfield et al.	Black Warrior River, Brushy Creek, FS Rd 255, upstream from Capsey Creek, Bankhead NF, Winston Co. AL
<i>Pleurobema georgianum</i>	16S, ND1	UAUC1623	S. A. Ahlstedt, R. R. Evans	Conasauga River, Holly Creek, N Hwy 52 bridge, Murray Co. GA
<i>Pleurobema georgianum</i>	COI, 16S, ND1	UAUC3193	P. Johnson	Conasauga River 200m above Jacks river confluence, Murray Co. GA
<i>Pleurobema georgianum</i>	COI, ND1	UAUC3084	M. Gangloff	Coosa River, Big Canoe Creek between CR 36 & Rt 231, St. Clair Co. AL
<i>Pleurobema gibberum</i>	COI	UAUC3319	S. Ahlstedt, B. Butler, Rob Towes	Collins River, Barren fork, Hwy 287 Bridge, Trousdale, Warren Co. TN
<i>Pleurobema gibberum</i>	16S, ND1	UAUC3153	S. Ahlstedt, B. Butler, Rob Towes	Collins River, Barren fork, Hwy 287 Bridge, Trousdale, Warren Co. TN
<i>Pleurobema hanleyianum</i>	COI, 16S, ND1	UAUC273	K. J. Roe et al.	Coosa River, near Leesburg, downstream from mouth of Terrapin creek, Cherokee Co. AL
<i>Pleurobema hanleyianum</i>	COI, 16S, ND1	UAUC1622	P. D. Johnson, R. R. Evans	Conasauga River, below Beavertdale crossing (GA 2), Upper Kings Bridge, Murray Co. GA
<i>Pleurobema oviforme</i>	16S, ND1	UAUC3238	Steve Ahlstedt, Steve Bakalety	Big South Fork Cumberland River, Lower Rough Shoals, Scott Co. TN

Appendix 2. (Continued).

Species	Gene	Collection number	Collector	Locality
<i>Pleurobema oviforme</i>	COI	UAUC1402	Steve Ahlstedt, Steve Bakalety	Big South Fork Cumberland River, Lower Rough Shoals, Scott Co. TN
<i>Pleurobema oviforme</i>	COI, 16S, ND1	UAUC1642	S. Fraley, R Butler	Holston River, Beech Creek near Keplar Elementary at Webster Road, Hawkins Co. TN
<i>Pleurobema perovatum</i>	COI, ND1	UAUC1640	J. Williams et al.	Alabama River, Sturdivant Creek, Hwy 10 2.4 mi E Awin, Wilcox Co. AL
<i>Pleurobema pyriforme</i>	COI, ND1	A29	J. Williams	Chipola River, Big Creek, Houston Co. AL
<i>Pleurobema rubellum</i>	COI, 16S, ND1	UAUC679	P. Hartfield et al.	Black Warrior River, Brushy Creek, FS Rd 255, upstream from Capsey Creek, Bankhead NF, Winston Co. AL
<i>Pleurobema rubrum</i>	COI, ND1	UAUC2719	S. Ahlstedt, C. Hubbs	Duck River, Venable Spring, Marshall Co. TN
<i>Pleurobema rubrum</i>	COI, ND1	UAUC3229	W. R. Haag, A. M. Commens	St. Francis River, Hwy 64 bridge at Parkin, Cross Co. AR
<i>Pleurobema sintoxia</i>	COI, ND1	UAUC1714	S. Ahlstedt	Cumberland River, Big South Fork at Station Camp Creek, Scott Co. TN
<i>Pleurobema strodeanum</i>	COI, ND1	UAUC1110	K. J. Roe et al.	Choctawhatchee River, West Fork, Hwy 10, Blue Springs SP, Barbour Co. AL
<i>Pleurobema strodeanum</i>	16S	UAUC1818	K. J. Roe, K. S. Cummings	Pea River, CR 77 2 mi NW Ariton, Barbour Co. AL
<i>Pleurobema taitianum</i>	COI, 16S, ND1	UAUC885	J. T. Garner	Alabama River, Selma just below AL Hwy 80 bypass, E bank, Dallas Co. AL
<i>Pleurobema troschelium</i>	COI, 16S, ND1	UAUC516	A. Wyss, M. Hughes	Conasauga River, RM49.05, 0.8 RM upstream of Sumac Ck., E of Sumac, Murray Co. GA
<i>Popenaias popeii</i>	COI, 16S, ND1	UAUC3161	Tom Miller	Rio Grande River, Lincoln-Juarez Bridge, Laredo, Webb Co. TX
<i>Potamilus alatus</i>	16S	UAUC41	Unknown	Elk River, Limestone Co. AL
<i>Potamilus alatus</i>	ND1	UAUC3329	S. Clark	Tennessee River, Decatur, Morgan Co. AL
<i>Ptychobranhus fasciolaris</i>	16S, ND1	LSC23701- 001	W. Tolin	Elk River, Clendenin, Kanawah Co. WV
<i>Quadrula kieneriana</i>	16S	UAUC334	M. Hughes et al.	Coosawattee River 2.5 mi upstream from Hwy 225 bridge, Gordon Co. GA
<i>Quincuncina infucata</i>	ND1	UAUC3283	C. O'Brien	Flint River, Cooleewahee Ck., GA Rt. 91 bridge, Baker Co. GA
<i>Strophitus subvexus</i>	COI, 16S, ND1	UAUC2715	S. Fraley, J. Baxter	Tombigbee River, Sucarnoochie Creek, Old Scooba Crossing, Kemper Co. MS
<i>Toxolasma parvus</i>	COI, ND1	UAUC3331	D. Willis	Tennessee River, Decatur, Morgan Co. AL
<i>Toxolasma texasiensis</i>	COI, 16S, ND1	UAUC80	R. G. Howells	Colorado River, Giddings State School Lake, Lee Co. TX
<i>Tritogonia verrucosa</i>	COI, 16S	UAUC3195	P. Johnson	Conasauga River, below Mitchell Bridge, Whitfield Co. GA
<i>Truncilla truncata</i>	16S, ND1	Unnumbered	B. Sietman	Mississippi River, Hannibal, Marion Co. MO
<i>Uniomerus declivus</i>	COI, 16S	UAUC3290	W. R. Haag, J. L. Stanton	Big Sunflower River, Farrell Rd. Bridge, ~5 mi N of Clarksdale, Coahoma Co. MS
<i>Venustaconcha ellipsiformis</i>	COI, 16S	UAUC2596-8	B. Sietman	Big Piney River, Texas Co. MO

Appendix 2. (Continued).

Species	Gene	Collection number	Collector	Locality
<i>Venustaconcha pleasii</i>	COI, ND1	UAUC136	Unknown	Meramec River, Fish Trap Rapids, Franklin Co. MO
<i>Villosa iris</i>	16S	UAUC260	Louis Levine	Collins River, Highway 56 bridge near Beersheba Springs, Grundy Co. TN
<i>Villosa iris</i>	ND1	UAUC2701	S. Ahlstedt	Duck River, Lillard Mill Dam, RM 179, Marshall Co. TN
<i>Villosa vanuxemensis</i>	16S	UAUC3046	S. J. Fraley	Little River, Telb at old Wallard Hwy access 1.5 RM upstream of Melrose Rd, Blount Co. TN

UAUC, University of Alabama Unionid Collection; LSC, Leetown Science Center.