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4

SYSTEMATICS OF FRESHWATER MUSSELS (BIVALVIA: UNIONOIDA)

Bivalves of the order Unionoida (also called freshwater mussels or naiades) are a diverse group of freshwater organisms (about 175 genera) with a broad distribution that currently includes all continents except Antarctica (Simpson 1896, 1900, 1914; Haas 1969a; Starobogatov 1970). The group has a fossil record extending back to at least the Triassic (e.g., Henderson 1935; Haas 1969b; Waller 1990, 1998). Habitat destruction and other anthropogenic perturbations have led to rapid population declines in North America and elsewhere such that freshwater mussels may represent the most endangered group of animals (e.g., Bogan 1993, 1998; Williams et al. 1993; Neves et al. 1997). Particular genera (e.g., *Epioblasma*) have been so severely affected that most of the species contained within them are either endangered or presumed extinct (Johnson 1978; Turgeon et al. 1998; Buhay et al. 2002).

Despite the substantial amount of research conducted on them, unionoids are still poorly understood from a systematic perspective. Systematic insights can contribute markedly to unionoid evolutionary and conservation biology research initiatives (e.g., see Moritz 1996; Lydeard and Roe 1998; Roe and Lydeard 1998a; Roe et al. 2001). This chapter provides an historical review of the systematics of unionoid mussels. The review is in two sections: lower-level (genera and below) and higher-level relationships. The lower-level section is almost entirely devoted to North American taxa because virtually all published molecular systematic studies at the lower-level have focused on North American taxa. Many more lower-level studies are needed from other regions of the world. The next section presents a reexamination of higher-level unionoid relationships using an alternative coding of the morphological data (Hoeh et al. 2001). This

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review will provide a background on unionoid systematics and encourage further study of the group.

LOWER-LEVEL SYSTEMATICS

Unionoid bivalves are a diverse group of mollusks with worldwide distribution. The last decade has seen an increase in interest in all aspects of unionoid mussel biology, particularly in the United States. This increased interest has arisen primarily because of concerns about the continued survival of many species. Some estimates indicate that roughly 70% of all species of unionids in North America are threatened or endangered (Williams et al. 1993; Neves et al. 1997; Master et al. 1998). Before the advent of allied molecular techniques (allozymes, restriction fragment analysis, DNA sequencing), the history of lower-level systematics of unionoids often consisted of the description and redescription of species, largely on the basis of conchological features. The most prolific of all workers was undoubtedly Isaac Lea, who described 850 species of unionoid mussels worldwide (not including fossil taxa).

North America represents a region with a very high diversity of unionoid bivalves (Burch 1975; Lydeard and Mayden 1995; Neves et al. 1997; Turgeon et al. 1998). Despite the increase in interest in unionoids on other continents (for a recent review, see Nagel and Badino 2001), most studies involving generic or species-level relationships have been produced in the United States. Recent studies have indicated that the Unionidae is a paraphyletic group, therefore, we use the term "unionoid" when referring to freshwater mussel taxa as a whole. In this section, we review studies concerning the systematics of unionoid mussels at the generic level and below and examine the utility of various types of character data for investigating evolutionary relationships among them.

Characters

Morphology Many researchers believed that accurately determining the number of unionoid taxa was hindered by having to rely too heavily on conchological differences (e.g., Davis 1984). Although much of the current classification is based on conchological characters, the high degree of conchological variation exhibited by unionoids can make it impossible to clearly delineate the boundaries between some species based on conchology alone (Davis et al. 1981; Kat 1983a; Davis 1983; Williams and Mulvey 1994). Species delineation can be further complicated by the many conchological convergences and parallelisms in shell shape and external morphology that some think are driven by environmental variables such as substrate composition or water velocity (e.g., Johnson 1970; Watters 1994). Typical conchological characters used in the systematics of unionoids include the presence or absence and position or shape of hinge teeth, position of adductor muscle scars, presence, shape, and position of external shell sculpture (pustules, knobs, ridges, sulci, spines), shell thickness, and overall shape. Although most conchological characters used in unionoid systematics are derived from the interior and exterior valve surface, Kat (1983b) examined the taxonomic utility of the microstructure of conchiolin layers deposited within the shells of freshwater mussels. Although the external "anatomy" of unionoid bivalves has provided the bulk of the morphological characters used in systematic studies, the internal or soft anatomy has remained underexploited. Kat (1983a) examined several anatomical characters such as stomach anatomy, mantle edge characteristics, and number of papillae on the exhalant and inhalant siphons. Smith (1986) also examined stomach morphology in comparing members of the Margaritiferidae. In an earlier publication, Smith (1980) compared the mantle and neural anatomy of two margaritiferids and provided several characters that could be included in explicit tests of the relationships within the genus *Margaritifera*. In addition to adult morphology, the utility of larval morphology has also been explored, albeit on a limited basis. In a survey of the larval characters of unionid mussels, Hoggarth (1988, 1999) provided a wealth of morphological characters that have yet to be fully utilized for lower-level systematic analyses. Hoeh (1990) used some of these larval characters and additional characters from Kat (1983c) to develop a morphology-based phylogenetic hypothesis for eastern *Anodonta*.

With few exceptions, the systematic relationships presented in most of the morphological studies mentioned above were arrived at by intuition and not by explicitly analyzing the characters. Within the context of modern character-based phylogenetic analyses, only a few studies have explored the utility of morphological characters to elucidate the relationships between species and genera. Hoeh's (1990) morphology-only tree was based on 10 characters and, not surprisingly, was poorly resolved. A combined analysis of the morphological characters with 24 presumptive allozyme loci, however, did produce a well-resolved topology. Roe and Lydeard (1998b), in examining the molecular systematics of the genus *Potamilus*, tested the hypothesis that the genus was diagnosed by ax-head–shaped glochidia and that the presence of hooklike teeth on the valve edges was indicative of sister relationships of species within *Potamilus*. The results of their analyses did not support the monophyly of *Potamilus*, but indicated that it was paraphyletic with respect to its presumed sister genus *Leptodea*, and that the presence of hooklike teeth on the valve edges was a homoplastic character and not always indicative of phylogenetic relatedness within *Potamilus*. The confidence in the homology of some adult shell characters used to diagnose genera has also been called into question. The genus

Quincuncina is diagnosed primarily by the presence of chevron-shaped ridges on the disk and posterior slope. Lydeard et al. (2000) provided evidence that the chevron-shaped ridges that diagnose *Quincuncina* are not homologous and that *Quincuncina,* as currently recognized, is not a natural group.

The two studies just presented call into question the utility of morphological characters for diagnosing lower-level relationships and seem to support the contention of Davis (1984) and others that morphological characters are often too variable to be useful in determining relationships among species of unionids. It is worth reiterating that the synapomorphic status of most of the morphological characters used by malacologists to diagnose genera or species of unionoid mussels (e.g., Smith 2000) have never been tested within the framework of a modern phylogenetic analysis. Of the few characters that have undergone explicit testing in a phylogenetic context, some (e.g., larval teeth), although homoplastic in some instances, were diagnostic at some level. This indicates that not all morphological characters are too variable for use in determining species-level relationships among unionids. Wiens (2000, 2001) provides invaluable guidelines and methods for including morphological characters in phylogenetic analyses. The methods outlined by Wiens (2001) for analyzing continuous characters and Zelditch et al.'s (2000) discovery of phylogenetic characters in morphometric data seem particularly appropriate for analyzing unionoids.

Allozymes Davis et al. (1981) and Davis (1984) examined relationships between and within genera of freshwater mussels. In particular, their intrageneric examinations focused on members of the genus *Elliptio*. Davis et al. (1981) determined that sympatric populations of mussels in the *Elliptio complanata* species-group that exhibited distinct phenotypes displayed extremely low levels of genetic divergence. Despite these results, Davis et al. (1981) consistently rejected the concept that the various phenotypes observed represented a single polymorphic species. Instead they interpreted the low genetic diversity as evidence of a recent origin of these species. In a similar study, Davis (1984) found that measures of genetic polymorphism and heterozygosity indicated that the number of species of *Elliptio* has been "considerably underestimated" and that lanceolate-shaped shells appeared to have arisen at least three times in the genus *Elliptio*. Berg et al. (1998) examined intraspecific variation in *Quadrula quadrula* and also found significant differences at 3 of the 10 allozyme loci surveyed. Genetic differences in this study were small, but were positively correlated with geographic distance.

Kat (1983d) examined the amount of genetic variation among species of *Lampsilis* from the Atlantic slope and found that allozyme variation was consistent with the morphological differentiation observed in stomach anatomy. Kat and Davis (1984) tested hypotheses of dispersal by comparing the number of alleles present, allele frequencies, and heterozygosity of peripheral populations of freshwater mussels in Nova Scotia. The results of this study revealed two groups of mussels: one was characterized by low levels of heterozygosity, whereas the other group exhibited moderate levels typical of conspecifics from the northeastern United States. The authors attributed the observed differences in heterozygosity to differences in the dispersal ability of the various species, which was related to whether they used anadromous or saltwater-tolerant host species. This example illustrates how aspects of the life history of an organism can affect the genetic variation in a species. Other aspects of the complex life histories of unionoids have been found to have some effect on the genetic structure of species as well. Hoeh et al. (1998a) examined the allozymic variation among populations of the genus *Utterbackia* and found that hermaphroditic populations of *U. imbecillis* and another undescribed species of *Utterbackia* displayed low levels of within-population variation relative to the dioecious species *U. peggyae* and *U. peninsularis*. These results suggested a high degree of self-fertilization in the hermaphroditic species, and concomitantly high levels of among–population-level variation, which the authors attributed to a combination of self-fertilization and founder events.

Allozyme data have been used to develop explicit hypotheses for the systematic relationships of freshwater mussels, as they have for other organisms. Most allozyme studies of animals have focused on intrageneric relationships (e.g., Gutierrez et al. 1983; Hafner and Nadler 1988), often because at higher levels of divergence, taxa share few electromorphs. Analysis of allozyme data has changed over time. Buth (1984) provides a useful review of the application of allozyme data to systematic questions. Studies on unionoid phylogenetic relationships have used a variety of coding strategies for allozyme data. For example, Hoeh (1990) examined relationships of eastern *Anodonta* and treated the allele as the character, whereas Hoeh et al. (1995) sought to elucidate phylogenetic relationships and the evolution of simultaneous hermaphroditism in the genus *Utterbackia* and coded the locus as the character.

There has also been some debate over inferring evolutionary trees by using the allele frequencies rather than coding the presence/absence of alleles. Opponents of retaining frequency information have cited cases where ancestral taxa are reconstructed as having impossible allele frequencies. Swofford and Berlocher (1987) advocate retaining allele frequency data and have often criticized reducing frequency data to simple presence/absence as ignoring valuable phylogenetic information. Swofford and Berlocher (1987) proposed a parsimony-based method that would allow retention of allele frequency information

without the undesirable result of unrealistic estimations of ancestral frequencies.

Early allozyme-based examinations of unionoids frequently used values of genetic similarity, often derived from studies of unrelated organisms as indicators of whether the operational taxonomic units included represented distinct species, subspecies, or members of the same population. Many earlier studies that used allozyme data for examining relationships among freshwater mussels did not use character-based analyses and did not generate explicit phylogenetic hypotheses. This makes it difficult to evaluate the strength of the phylogenetic signal contained within the data. Later studies, such as Hoeh et al. (1995), reported high levels of homoplasy at some loci included in their dataset, although in this case the allozyme-based phylogeny was corroborated by an independent mitochondrial DNA (mtDNA) dataset (Knazek et al. 2001). A review of the studies presented in this section supports Hoeh's (1990) contention that phylogenetic analysis of allozyme data is informative in studies of specific and generic-level relationships.

RFLPs and RAPDs Restriction fragment length polymorphisms (RFLPs) have been widely used as molecular markers (see Dowling et al. 1996 for a review). Despite their popularity, few RFLP-based studies have been conducted on unionids (White et al. 1994, 1996). Kandl et al. (2001) used a variety of data types including RFLPs, allozyme electrophoresis, and mitochondrial cytochrome *c* oxidase subunit I (COI) sequences to assess the genetic distinctiveness of populations of several species of *Pleurobema* along the eastern Gulf Coast. The authors also wished to clarify the species status of *P. pyriforme* and putative species *P. bulbosum*, and *P. reclusum*. Whereas all data types in the study were able to discriminate between currently recognized species of *Pleurobema*, specimens referable to *P. bulbosum* and *P. reclusum* were not found to be distinct from *P. pyriforme* based on allozyme and RFLP data. However, *Pleurobema reclusum* was found to be genetically distinguishable from *P. pyriforme* using the COI sequence data.

Randomly amplified polymorphic DNA (RAPD; Welsh and McClelland 1990) involves screening genomic DNA for interpretable polymorphisms using a variety of short primers of arbitrary sequence to amplify at random using polymerase chain reaction (PCR). Lieberman (2000), in the only study to date to use RAPDs on unionids, examined the population structure of *Amblema plicata* and analyzed the results for similarities to patterns produced by freshwater fishes (Mayden 1988). The difficulty of assessing the homology of RAPD markers has generally restricted their application to population-level studies (Avise 1994; Hillis 1994), where the potential for comparing nonorthologous bands is minimized. Lieberman's (2000) results reveal some geographic structure in the data and some similarity to Mayden's (1988) results, but as the author states, "such congruence is quite incomplete." One possible explanation for the lack of congruence observed between these two area cladograms is that, unlike the fishes examined by Mayden (1988), which are generally endemic to habitats marked by cool, clear, high-gradient streams, *Amblema plicata* is a widespread species that inhabits large rivers such as the Mississippi, as well as its many tributaries. The cosmopolitan distribution of *Amblema plicata*, combined with the widespread distribution of its 13 known or suspected host fishes, would suggest a panmictic population and little or no geographic structure. That the results did show some local geographic correlation would tend to support the utility of RAPDs for more restricted studies.

DNA Sequences The advent of the polymerase chain reaction (PCR) (Saiki et al. 1985) has revolutionized both the manner and scope of systematic studies. As early as 1991, DNA sequence data accounted for approximately 25% of all systematic studies published that year (Sanderson et al. 1993). That percentage has undoubtedly increased over the past decade and, although the limitations of DNA sequence data have been realized (see below), sequence data will assuredly play a major role in systematics for many years to come.

The phenomenon of doubly uniparental inheritance (DUI) of mitochondria has been documented in unionids and some other bivalve taxa such as *Mytilus* (Hoeh et al. 1996; Chapter 2, this volume). In most animals, mtDNA is inherited along a matrilineal line, but in taxa that exhibit DUI, males are heteroplasmic, with female mitotypes in the somatic and gonadal tissues and a unique male mitotype in the gonads. Thus, to ensure that orthologous genes are being compared for a phylogenetic study, care should be taken to ensure that all female or all male mitotypes are being examined (see Quesada et al. 1996 for associated problems).

Many molecular systematists working on unionoids have used the primers developed by either Folmer et al. (1994) or Lydeard et al. (1996) that amplified portions of the first subunit of the cytochrome *c* oxidase gene (COI) and the 16S rDNA (16S) genes, respectively. Both of these genes are somewhat conserved, but the protein coding the COI gene often provides ample variation at the third codon position for resolving relationships within and among genera (e.g., Roe and Lydeard 1998b).

One of the earliest studies to include DNA sequences to examine lower-level relationships in unionoids was Mulvey et al. (1997). In this study, the authors used both DNA sequences of the 16S gene and allozyme data in an attempt to identify diagnosably distinct evolutionary entities within *Amblema* and *Megalonaias*. Their results supported the recognition of three species of *Amblema* (*A.*

ellioti, *A. neislerii*, and *A. plicata*), but only one species of *Megalonaias*. The conservation implications of these findings were significant for species in both of these commercially important genera, because two of the three species of *Amblema*—*A. neislerii* and *A. ellioti*—occupy restricted ranges. The results did not support the recognition of *Megalonaias boykiniana*, which had previously been considered for federal protection by the U.S. Fish and Wildlife Service (Butler 1993).

Roe and Lydeard (1998b) attempted to resolve the phylogenetic relationships within the genus *Potamilus* and to address the question of relationships between *Potamilus* and its putative sister taxon *Leptodea*, using a portion of the COI gene. The results of the analysis supported retaining most of the species of *Potamilus* as a natural group; however, the placement of *P. capax* rendered *Potamilus* paraphyletic. The authors also examined the degree of genetic variation between two populations of the federally threatened Alabama heelsplitter, *Potamilus inflatus*. The range of this species has been greatly reduced and known reproducing populations were restricted to the Amite River in Louisiana and the Black Warrior River in Alabama. The analyses of Roe and Lydeard (1998b) identified both populations as genetically distinct evolutionary entities and the degree of genetic divergence between these populations exceeded that observed between other species of *Potamilus*.

King et al. (1999) was the first and, to date, the only to investigate the evolutionary relationships within a unionoid genus that included DNA sequence data from a nuclear locus. The authors used sequence data from the first internal transcribed region (ITS-1), which lies between the 5.8S and 18S rDNAs in the nuclear genome. They also used mitochondrial COI gene sequences to investigate the phylogeography of the green floater, *Lasmigona subvirdis*. The authors found significant genetic differentiation between northern and southern populations of *L. subvirdis* and recommended that these populations be managed as separate conservation units. In the course of examining genetic variation within *L. subvirdis*, King et al. also presented a phylogeny of *Lasmigona* based on COI sequences that indicated the genus *Lasmigona* was not a monophyletic group.

Turner et al. (2000) compared two different approaches for determining the level of interspecific variation in *Lampsilis hydiana:* nested cladistic analysis (Templeton et al. 1995) and analysis of molecular variation (AMOVA) on a portion of the 16S gene. Turner et al. (2000) examined individual variation through single-stranded conformation polymorphism (SSCP). This method detects point mutations and insertion–deletion events that can affect the folding of the single-stranded fragments and, by doing so, alter their mobility during electrophoresis. Turner et al. (2000) also sequenced two representatives of each unique haplotype identified using the SSCP method. In general, the results indicated two "fragmentation events," one separating populations in the Arkansas River from the Saline and Ouachita rivers to the southwest and a second more recent event separating the upper Saline populations from those in the lower Saline and the Ouachita rivers. The results of the methodological comparison revealed that under some conditions, nested cladistic analysis provided less insight into the processes shaping population differentiation than traditional AMOVA.

Using sequences from both the COI and the 16S genes, Roe et al. (2001) examined the relationships among four threatened and endangered species of the genus *Lampsilis*. These four species are the only freshwater mussels known to produce superconglutinate lures that are endemic to the Gulf Coast drainages of the United States. Additional goals of the study were to compare the zoogeographic patterns of these taxa to patterns produced by other organisms. The results of the study supported the recognition of these four species as a natural group, although the monophyly of three of the four species was not supported. The authors attributed the lack of observed monophyly of the species to a combination of the recent origin of these species lineages and the conservative nature of the COI and 16S sequences. As did King et al. (1999), Roe et al. (2001) also urged that any plans for augmenting existing population through captive rearing of freshwater mussels should maintain the genetic identity of the respective populations.

As can be seen from these examples, examinations of relationships between species of unionids are often driven, at least in part, by questions relating to the conservation of freshwater mussel species. The importance of phylogenetic systematics to the conservation of freshwater mussels was outlined by Lydeard and Roe (1998). Knowledge of the genetic diversity both within and between populations is crucial to maximizing the benefits of captive breeding and reintroduction of endangered mussel species. In addition, systematic studies can identify previously unrecognized diversity (e.g., cryptic species) in need of protection.

Protecting areas of endemism is also crucial for preserving mussel diversity. Biodiversity hotspots, such as the rivers of the southeastern United States, are also in need of protection. Systematic studies that include these areas can improve estimates of biotic diversity. For example, Lydeard et al. (2000) used COI and 16S sequences in an examination of relationships of mussels from the Gulf Coast drainages. Their analyses identified several genera as polyphyletic and not representative of natural groups. Specifically, *Obovaria rotulata* was found to be more closely related to *Fusconaia ebena* than it was to its putative congeners *O. unicolor* and *O. olivaria*. Furthermore, *F. succisa* was found to be sister to *Quincuncina infucata* and *Q. burkei* sister to *F. escambia*.

Undoubtedly, DNA sequence data will continue to be a valuable source of

data for improving our estimates of species boundaries and the systematic relationships of unionoid mussels. What is required, however, is the development of additional markers better suited to the range of systematic questions that unionoids present. These new markers should include nuclear as well as mitochondrial loci and ideally will encompass a wide range of evolutionary rates.

HIGHER-LEVEL SYSTEMATICS

Most recent classifications of the Unionoida (e.g., Boss 1982; Vaught 1989; Bogan and Woodward 1992; Bonetto 1997; but see Starobogatov 1970 for an alternative classification) have recognized two superfamilies: the Unionoidea, containing three families: Hyriidae, Margaritiferidae, and Unionidae, and the Etherioidea (formerly Muteloidea, see Kabat 1997), containing three families: Etheriidae, Iridinidae, and Mycetopodidae. The current geographic ranges of these families are restricted with respect to continental landmasses (e.g., Simpson 1896; Parodiz and Bonetto 1963). Within the Unionoidea, representatives of the Unionidae are found in North America, Eurasia, and Africa. Those of the Hyriidae are restricted to Australasia and South America, and the Margaritiferidae inhabit North America, northwest Africa, and Eurasia. Within the Etherioidea, representatives of the Iridinidae (formerly Mutelidae; see Kabat 1997) are restricted to Africa, whereas those of the Mycetopodidae are found only in Central and South America. The Etheriidae, typically understood to represent the freshwater oysters, is distributed in Africa, India, and South America. However, a recent phylogenetic analysis indicated that the freshwater oysters are a polyphyletic assemblage (Bogan and Hoeh 2000).

The Unionoida contains an array of distinctive morphological characteristics, many of which are associated with reproduction. Two highly differentiated larval morphologies exist within the Unionoida. Species typically included in the Unionoidea possess a bivalved larva called a glochidium, whereas members of the Etherioidea have a strikingly distinct univalved lasidium/haustorium larva (e.g., see Bonetto 1951, 1997; Parodiz and Bonetto 1963; Wachtler et al. 2001). The morphological differences between these larval types are so extraordinary that Parodiz and Bonetto (1963) hypothesized that unionoidean and etherioidean bivalves represented two independent invasions of freshwater. The recent corroboration of unionoid bivalve monophyly (Hoeh et al. 1998b; Graf and Ó Foighil 2000b) suggests that an evaluation of the polarity of the glochidium–lasidium/haustorium transition(s) would indeed be appropriate. Another aspect of reproduction that varies among unionoid higher taxa is the location of larval brooding in modified gills called marsupia. Etherioidean and hyriid bivalves use the two inner demibranchs only (endobranchy), whereas margaritiferid bivalves use all four demibranchs (tetrageny) for brooding. Unionids use either all four or only the two outer demibranchs (ectobranchy) for brooding (e.g., Heard and Guckert 1970). Elucidating the polarity and order of evolutionary transitions among these reproductive character states depends on the availability of robust estimates of phylogeny for the Unionoida.

The particular classification presented above does not imply that a consensus has been reached regarding unionoid evolutionary relationships. Quite the contrary, unionoid bivalves have a long history of protean classification schemes. Early classifications were artificial and subjective, owing to different a priori character selections and weightings, which produced little consensus (see reviews in Heard and Guckert 1970; Davis and Fuller 1981; Parmalee and Bogan 1998). Lamarck (1805, 1812) established the family Nayades (subsequently changed to Naiades [Lamarck 1830]) for the freshwater mussels and placed all species in two genera, *Anodonta* and *Unio*. Rafinesque (1820, 1831, 1832) departed from the typical custom of placing each species in one of a very small number of genera by erecting 37 new freshwater bivalve genera and more than 100 new species. He is also credited with the first use of *Unio* as the root of a freshwater mussel higher taxon (Unioninae) and thus the current ordinal name, Unionoida, dates from Rafinesque 1820 and not Fleming 1828 as has been credited for many years (Bowden and Heppell 1968).

Isaac Lea, in his four editions of the *Synopsis* (1836, 1838, 1852, 1870), was one of the first workers to attempt an in-depth global view for the Unionoida. As was the case for most early unionoid taxonomists, Lea's classifications relied largely on conchological characteristics and placed all species in one of two large genera, *Margaron* and *Platiris*. Specifically, Lea used adult shell sculpture, shell form, and the presence or absence of dorsal shell wings, while at the same time realizing that a better understanding of anatomy would be required for a more permanent classification.

Subsequently, other workers made use of different suites of unionoid characteristics to enable their classifications. For example, Swainson (1840) used shell characteristics and a quinary system of contiguous circles (each representing a group of related species) to divide the Unionidae into five subfamilies. Gray (1840, 1847) established a classification for the freshwater mussels, based on anatomical characteristics, which contained three families: Mutelidae, Mycetopodidae, and Unionidae. Troschel (1847) used anatomical characteristics and von Ihering (1893) used larval characteristics to inform their classifications of the Unionoida. The latter author divided the freshwater mussels into two families: Mutelidae (possessing lasidial larvae) and Unionidae (possessing glochidial larvae). Troschel first noted that "*Anodonta*" from South America

(now in the genus *Anodontites*) was actually more closely related to certain African forms than to *Anodonta* of the northern hemisphere.

Charles T. Simpson made significant contributions to global unionoid systematics by producing classifications based on conchological, larval/marsupial characteristics, and the presence/absence of sexual dimorphism (Simpson 1896, 1900, 1914). Simpson (1900, 1914), following von Ihering (1893), divided the freshwater mussels into two families, the Mutelidae and Unionidae, based largely on the presence of glochidial larvae in the latter and lasidial larvae in the former (Figure 4.1A). His 1914 volume is still the most comprehensive compendium on global unionoid alpha-level taxonomy.

Arnold E. Ortmann (e.g., 1910, 1911, 1912, 1916, 1921) greatly extended the work of Simpson. Ortmann integrated all available morphological characteristics, especially adult soft part anatomy, into his classification schemes. Ortmann (1911) was the first to officially recognize the extreme anatomical distinctness of the margaritiferids from other unionoids by raising that group to familial status (e.g., Smith 2001). His later freshwater mussel classifications (1912, 1921) contained three families: Margaritanidae (now Margaritiferidae, Kennard et al. 1925; Haas 1940), Mutelidae, and Unionidae. Ortmann (1912) noted that the Margaritiferidae was, undoubtedly, the most ancient of these three families. Notwithstanding his familial elevation of the margaritiferids, Ortmann's classification (1912, 1921) is substantively distinct from the seemingly similar versions of von Ihering (1893) and Simpson (1900, 1914) in that Ortmann placed the hyriines in his Mutelidae (Figure 4.1B). The other two authors placed them in the Unionidae. The placement of the hyriines in the Unionidae was largely based on the presumed importance of a single, potentially plesiomorphic characteristic, the shared larval type (glochidium), whereas its placement in the Mutelidae was based on multiple shared apomorphic adult anatomical characteristics. Most subsequent unionoid classification schemes have included the hyriines in the Unionidae or Unionoidea (e.g., Modell 1942, 1949, 1964; Parodiz and Bonetto 1963; Haas 1969a, 1969b; Heard and Guckert 1970; Starobogatov 1970; Morrison 1973; Boss 1982; Vaught 1989; Bogan and Woodward 1992; Bonetto 1997; Walker et al. 2001), whereas relatively few have supported Ortmann's position (e.g., Hannibal 1912; Thiele 1935; McMichael and Hiscock 1958; Heard and Guckert 1970: Fig. 1).

The relatively recent integration of explicit tree-building methodologies with biochemical/molecular genetics (e.g., Hillis et al. 1996b) has contributed significantly to our current understanding of unionoid systematics because of their ability to explicitly test hypotheses of evolutionary relationships. Davis and Fuller (1981) made the initial attempt to investigate higher-level unionoid relationships using these new techniques. This investigation was prompted by a

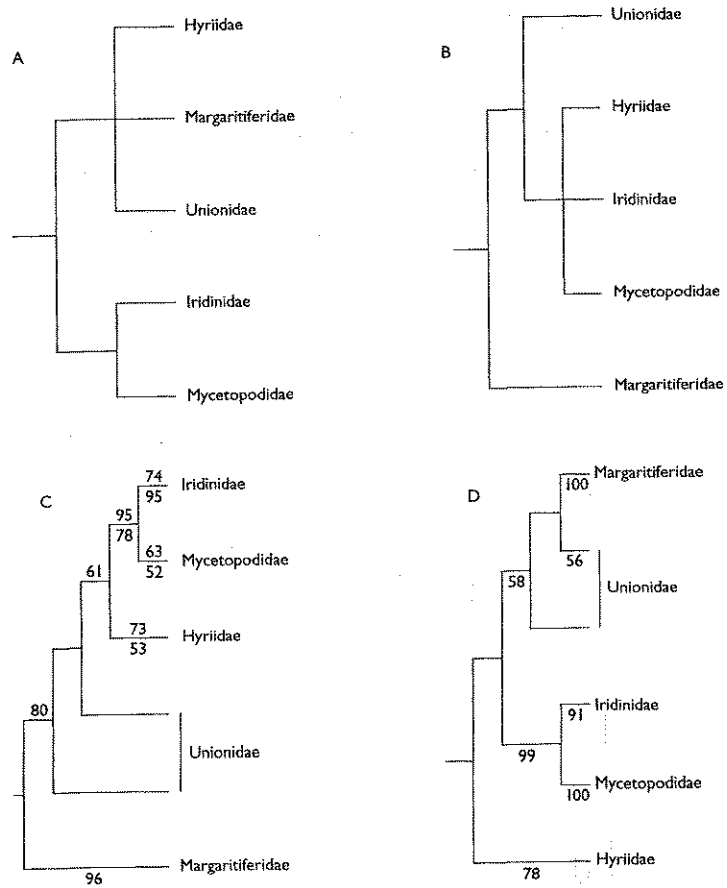

Figure 4.1. Unionoid familial relationships based on the classifications of (A) Simpson (1900) and (B) Ortmann (1912). (C) Phylogenetic analyses of morphological characters from Graf (2000) and Hoeh et al. (2001). (D) Phylogenetic analyses of morphological plus molecular characters after Hoeh et al. (2001). In C and D, bootstrap percentages above branches are from Graf (2000) and those below branches are from Hoeh et al. (2001). Unionid paraphyly is indicated in both C and D.

unionoidean classification, based largely on the duration and location of larval brooding, proposed by Heard and Guckert (1970). Davis and Fuller used immunoelectrophoretic techniques to build distance matrices for 52 species representing 27 genera of the North American Unionoidea (i.e., excluding the hyriids). The matrices were analyzed using ordination and minimum spanning tree

techniques to produce two-dimensional plots of relationships. Their results (e.g., Davis and Fuller 1981: Fig. 2) were largely consistent with the unionid classification of Ortmann (1910) in that margaritiferines, anodontines, and unionines + lampsilines were portrayed as three very distinct groups. This led Davis and Fuller (1981: Fig. 4) to propose subfamilial status, within the Unionidae, for those three groups: Margaritiferinae, Anodontinae, and Ambleminae. Additional systematic work, using allozyme data (Davis et al. 1981; Davis 1984), was largely congruent with these determinations. A principal inference from these studies was that unionid reproductive character (e.g., marsupial placement and length of the brooding season) evolution has often been homoplasious. This finding rejects the underlying basis for the unionid classification proposed by Heard and Guckert (1970).

A significant follow-up to the landmark Davis and Fuller study was that of Lydeard et al. (1996). The latter examined 29 species representing 23 genera of North American Unionoidea by comparing 16S ribosomal DNA sequences alone and in conjunction with a morphological dataset. This was the first use of DNA sequences to specifically examine freshwater mussel evolutionary relationships. Parsimony and neighbor-joining techniques were used for their phylogenetic analyses. The resulting phylogenies gave strong support for (1) unionid monophyly and (2) sister taxa status for anodontines and amblemines, thus offering general corroboration of the phylogenetic and reproductive character evolution hypotheses presented in Davis and Fuller (1981). As with the latter hypotheses, those of Lydeard et al. (1996) offer little support for the views of Heard and Guckert (1970) regarding unionid relationships.

Although both Davis and Fuller (1981) and Lydeard et al. (1996) have contributed significantly to our understanding of North American unionid evolutionary relationships, these studies displayed the same significant flaw. As with other regional studies (e.g., Nagel and Badino 2001; Walker et al. 2001), they evaluated unionoid phylogeny and character state transitions. These evaluations were based on estimates of phylogeny for a paraphyletic group (i.e., North American unionoids) given the absence of representative hyriids, iridinids, and mycetopodids from their trees and the paucity of earlier support for Unionidae + Margaritiferidae monophyly. The analyses of nonmonophyletic groups to estimate phylogeny and character state transitions can produce misleading inferences (e.g., see Eldredge and Cracraft 1980; Brooks and McLennan 1991). Thus, to obtain robust estimates of phylogeny and character state transitions for unionoids, multiple representatives from each of the suprageneric taxa within a recognized monophyletic assemblage (i.e., the Unionoida) should be included in the phylogenetic analyses (e.g., see Swofford et al. 1996). Subsequent unionoid systematic work has begun to address this problem.

Two recent parsimony analyses of nonmolecular character matrices have largely supported Ortmann's view (1912, 1921) of higher-level unionoid relationships. Both Graf (2000) and Hoeh et al. (2001) performed unweighted parsimony analyses on qualitative unionoid characteristics that were coded in a multistate fashion. Although these two matrices had only partial overlap in the characters used, the resulting tree topologies were quite similar (Figure 4.1C). Graf's (2000) analysis, based on 38 characters, found a single most parsimonious tree (Graf 2000: Fig. 1), which indicated a "basal" Margaritiferidae, paraphyletic Unionidae and Unionoidea, and a monophyletic group that contained etheriids, hyriids, mycetopodids, and iridinids. This tree's placement of hyriids as the sister taxon to etheriids + mycetopodids + iridinids is inconsistent with the classification of Simpson (1900, 1914), but consonant with that of Ortmann (1912, 1921). The Hoeh et al. (2001) morphological analysis, based on 28 morphological characters, found 240 equally parsimonious trees, where the strict consensus tree (Hoeh et al. 2001: Fig. 14.2) indicated the same major features as Graf's (2000) analysis. However, evidential support for most nodes in these two studies was meager. The general topology supported in both studies is consistent with tetragenous brooding of glochidial larvae as the ancestral unionoid condition.

Recent phylogenetic analyses of partial COI sequences confirmed the monophyly of the Paleoheterodonta (Unionoida + *Neotrigonia*) and the sister taxa status for *Neotrigonia* and the Unionoida (Hoeh et al. 1998b; Graf and Ó Foighil 2000b). Graf and Ó Foighil's (2000b: Fig. 2B) analysis of partial COI sequences did not corroborate the nonmolecular analyses of Ortmann (1912), Graf (2000), and Hoeh et al. (2001). However, the Graf and Ó Foighil study did not include a representative of the Mycetopodidae and lacked replicate taxon sampling for the iridinids and hyriids. Nevertheless, after the application of successive weighting techniques (Farris 1969), this study produced relatively high nodal support values (jackknife percentages) for the Paleoheterodonta (99), Unionoida (96), Unionoidea (86), Margaritiferidae (99), Anodontinae (76), Ambleminae (94), and Lampsilini (72).

In addition to the morphology-based analysis mentioned above, a total evidence analysis (partial COI sequences plus morphology) of unionoid phylogeny was presented in Hoeh et al. (2001: Fig. 1D). This topology suggests that both the Unionidae and Unionoidea (Hyriidae + Margaritiferidae + Unionidae), as currently conceived, are not monophyletic groups. This topology is distinct from those presented above, given the placement of the Hyriidae as a product of the earliest cladogenic event within the ancestral unionoid lineage. However, the results of this analysis and that of Graf and Ó Foighil (2000b) are consistent with the hypothesis that endobranchy is the brooding state of the ancestral

unionoid. It is worthwhile to emphasize that the family-level evolutionary relationships indicated by the non-molecule–based phylogenetic analyses (Graf 2000; Hoeh et al. 2001) have not been corroborated (or strongly rejected because of relatively weak support at crucial nodes) by these additional analyses using many more characters (partial COI DNA sequences, Graf and Ó Foighil 2000b; partial COI DNA sequences together with morphology, Hoeh et al. 2001).

Attempts at rigorous phylogenetic analyses of unionoid nonmolecular traits are hampered by a relatively small number of coded characters that can contain a significant level of homoplasy (e.g., Graf 2000; Hoeh et al. 2001). These situations can yield multiple, equally parsimonious trees with low nodal confidence scores. Although additional traits are currently being added to the matrices mentioned above, further exploration of alternative character coding and analytical techniques is warranted at this time. The appropriateness of multistate versus presence/absence coding for qualitative characteristics has recently been debated at length (e.g., Pimentel and Riggins 1987; Meier 1994; Pleijel 1995; Wilkinson 1995; Lee and Bryant 1999; Strong and Lipscomb 1999; Seitz et al. 2000), with no clear consensus emerging. The same could be said for the use of "successive weighting" in parsimony analyses (e.g., Farris 1969; Carpenter 1988; Carpenter et al. 1993; Carpenter 1994; Swofford et al. 1996; Cunningham 1997b). Presence/absence coding has yet to be explored for use in unionoid phylogenetic studies, but there is precedent for the use of successive weighting techniques (Lydeard et al. 1996; Graf and Ó Foighil 2000b). Nevertheless, further comparisons of the results using alternatively coded qualitative characteristics and *a posteriori* weighting techniques would be a useful undertaking given the disparate and relatively unresolved estimates of higher-level unionoid evolutionary history we currently have.

To explore the usefulness of alternative coding and analytical techniques for morphology-based unionoid phylogenetics, the 28-character, multistate-coded data matrix from Hoeh et al. (2001) was recoded into a 57-character, presence/absence matrix (Table 4.1) from 31 bivalve taxa (Table 4.2), and analyzed with the parsimony algorithm in PAUP* v4.0b8 (Swofford 2002) using successive weighting based on the rescaled consistency index. Ultimately, six equally parsimonious trees were found and the strict consensus tree is presented in Figure 4.2. The sister taxa relationship for the Hyriidae and Etherioidea, indicated by the non-molecule–based analyses of Graf (2000) and Hoeh et al. (2001), is supported by this tree; however, unlike the previous two analyses, Figure 4.2 indicates that the Unionidae and Margaritiferidae are sister taxa, albeit with little evidential support. Generally, the nodal support percentages, based on 10,000 replicates, are higher in this analysis than in those of the previously mentioned two studies. Nevertheless, the evolutionary propinquity of

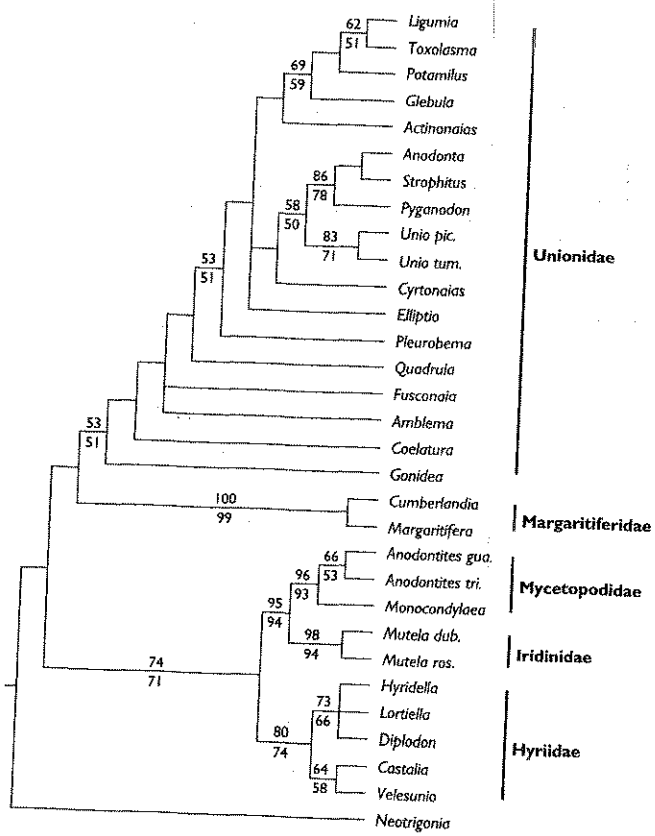

Figure 4.2. Strict consensus tree from parsimony analysis of 57 unionoid morphological traits using presence/absence character coding and successive weighting. Numbers represent bootstrap (above branch) and jackknife (below branch) percentages from 10,000 replicates using fast-heuristic searches. Only percentages ≥50% are shown.

the Hyriidae and Etherioidea was indicated when either equally weighted analyses of multistate or successively weighted analysis of presence/absence coded qualitative nonmolecular characters was used to estimate unionoid phylogeny. The amount of resolution and nodal support levels were generally higher in the latter analysis.

An examination of both nonmolecular and molecular datasets in a total evidence approach (e.g., Miyamoto 1985; Kluge 1989, 1998), using presence/absence coding of the nonmolecular characters and successive weighting tech-

Table 4.1

Presence/absence morphology data matrix for the bivalve taxa listed in Table 4.2

	Character Number					
		1111111111	2222222222	3333333333	4444444444	55555555
Taxon/Node	123456789	0123456789	0123456789	0123456789	0123456789	01234567
Neotrigonia	010000100	017?011000	1000000000	0000000001	0000000100	11001000
Actinonaias	110010000	1010010100	1001011001	0010000010	0100100010	00010100
Amblema	110010000	1010011000	1010011001	0010000100	0100101000	00010100
Anodonta	110010000	1010011000	1001011001	1010011000	0000000010	00010100
Anodontites gua.	010010000	1001010110	0100010101	0101000000	0000010000	00101000
Anodontites tri.	010010000	1001010110	0100010101	0101000000	0000010000	00101000
Castalia	001010000	1010010110	0100011010	0010011001	0100100100	01000110
Coelatura	110010000	1010011000	1010011010	0010000100	0100100001	00010177
Cumberlandia	010000001	0010101000	1010000000	0010000100	1001000010	10010100
Cyrtonaias	110010000	1010011000	1001111001	001000???0	0100101000	00010100
Diplodon	001010000	0110010110	0100011010	0010011000	1001000100	00010110
Elliptio	110010000	1010011000	1001011001	0010000010	0100100010	00010100
Fusconaia	110001000	1010011000	1010011001	0010000100	0100100010	00010100
Glebula	11001000?	??10010100	1001111001	0010000010	0100100000	00010101
Gonidea	11000100?	??10011000	1010010100	0010000100	0100100010	00010100
Hyridella	00101000?	????010101	0100011010	0010011000	1001000001	01000110
Ligumia	110010010	1010010100	1001111001	0010000010	0100101000	00100101
Lortiella	???????0?	????01????	?100011010	001001???0	1001000000	0???????
Margaritifera	010010001	0010101000	1010000000	0010000100	1001000010	10010100
Monocondylaea	001010000	0101010110	010001017?	0101000000	0000110100	00101000
Mutela dub.	00010000?	????010110	0100010101	0000100000	0010010000	00100177
Mutela ros.	00010000?	????010110	0100010101	0000100000	0010010000	00100177
Pleurobema	110001000	1010011000	1001011001	0010000010	0100100010	00010100
Potamilus	110010000	1010010100	1001111001	0010000000	0100100010	01000101
Pyganodon	110010000	1010011000	1001011001	1010011000	0000001000	00010100
Quadrula	110000100	1010011000	1010011001	0010000010	0100100010	00010100
Strophitus	110010000	1010011000	1001011001	1010011000	0000000010	00010100
Toxolasma	11001001?	??10011000	1001111001	0010000010	0100100010	01000101
Unio pic.	110010000	1010011000	1001011010	0010011000	0100101000	00010100
Unio tum.	110010000	1010011000	1001011010	0010011000	0100101000	00010100
Velesunio	0????1000?	????010101	0100011010	0010011000	0100100000	01000110

Morphological characters and character states based on literature and specimen examination:

0 = absent, 1 = present in each:

1. Supra-excurrent opening.

2. Posterior end of mantle sheets unfused, with simple incurrent and excurrent openings.

3. Posterior end of mantle sheets with a simple incurrent opening, but fused to provide a short, muscular excurrent siphon.

4. Posterior end of mantle sheets fused to provide short, muscular incurrent and excurrent siphons.

5. Simple incurrent papillae.

6. Branched incurrent papillae.

7. Arborescent incurrent papillae.

8. Mantle margin of females with specialized structures (e.g., flaps, caruncles, etc.) anteroventral to the incurrent opening.

Table 4.1 continued

9. Position of anus on the posterior adductor muscle at the dorsal aspect of posterior adductor muscle.

10. Position of anus on the posterior adductor muscle at the posterior aspect of posterior adductor muscle.

11. Position of anus on the posterior adductor muscle at the posteroventral aspect of posterior adductor muscle.

12. Intestine morphology simple and undifferentiated.

13. Intestine morphology complex, with three compartments.

14. Attachment of the dorsal margin of the outer lamella of the outer demibranchs to the inner surface of the mantle except at the posterior end of those demibranchs.

15. Attachment of the dorsal margin of the outer lamella of the outer demibranchs to the inner surface of the mantle for the entire length of those demibranchs.

16. Attachment of the dorsal margin of the inner lamella of the inner demibranchs to the visceral mass only at the anterior region of those demibranchs.

17. Attachment of the dorsal margin of the inner lamella of the inner demibranchs to the visceral mass for the entire length of the visceral mass.

18. Diaphragm (tissue separation of the suprabranchial and branchial components of the mantle cavity) complete, formed in part by the dorsal margin of the inner lamella of the inner demibranchs and in part by the siphonal musculature.

19. Diaphragm (tissue separation of the suprabranchial and branchial components of the mantle cavity) incomplete, with a single perforation in the siphonal musculature.

20. Diaphragm (tissue separation of the suprabranchial and branchial components of the mantle cavity) incomplete, formed only by the inner demibranchs.

21. Endobranchous (only the inner two gills) brooding.

22. Tetragenous (all four gills) brooding.

23. Ectobranchous (only the outer two gills) brooding.

24. Marsupial region of marsupial demibranchs seasonally extends below the ventral margin of the filaments.

25. Interlamellar space of all marsupial and nonmarsupial demibranchs divided into vertical water-tubes by vertical, transverse interlamellar septa.

26. Relative number and spacing of transverse (primary) marsupial septa greater in marsupial than in nonmarsupial regions of marsupial and in nonmarsupial demibranchs.

27. Relative number and spacing of transverse (primary) marsupial septa similar in marsupial and nonmarsupial regions of marsupial and in nonmarsupial demibranchs.

28. Transverse (primary) septa are vertically perforated in marsupial but imperforate in nonmarsupial regions of marsupial demibranchs and throughout nonmarsupial demibranchs.

29. Transverse (primary) septa are vertically imperforate throughout marsupial and nonmarsupial demibranchs.

30. Tripartite water tubes.

31. Lateral ridges on primary septa approximating secondary vertical septa.

32. Glochidium larva.

33. Lasidium larva.

34. Haustorium larva.

35. Medioventral glochidial hooks.

36. Subtriangular glochidial shape (lateral view).

37. Subcircular glochidial shape (lateral view).

38. Subovate glochidial shape (lateral view).

39. Radial disk sculpture.

40. Reduced lateral teeth dentition.

41. Full lateral teeth dentition.

42. Pseudotaxodont lateral teeth dentition.

43. Reduced pseudocardinal teeth dentition.

44. Full pseudocardinal teeth dentition.

45. V-shaped lamellar-ligament fossette.

46. Double-looped beak sculpture.

47. Radial beak sculpture.

48. Concentric barred beak sculpture.

49. Zigzag beak sculpture.

50. Lateral muscle scars.

51. Triangular shaped labial palp.

52. Semicircular to kidney shaped labial palp.

53. Falciform shaped labial palp.

54. Elongate anterior adductor muscle shape.

55. Round anterior adductor muscle shape.

56. Marsupium restricted to middle of gill.

57. Marsupium restricted to posterior of gill.

Table 4.2
Bivalve species examined in this study

Taxonomic Position
Outgroup
Order Trigonioida
Superfamily Trigonioidea
Family Trigoniidae
Neotrigonia margaritacea (Lamarck 1804)
Ingroup
Order Unionoida
Superfamily Etherioidea
Family Iridinidae
Mutela dubia (Gmelin 1791)
Mutela rostrata (Rang 1835)
Family Mycetopodidae
Anodontites guanarensis Marshall 1927
Anodontites trigonus (Spix 1827)
Monocondylaea minuana d'Orbigny 1835
Superfamily Unionoidea
Family Hyriidae
Castalia stevensi (H. B. Baker 1930)
Diplodon deceptus Simpson 1914
Hyridella menziesi (Gray 1843)
Lortiella rugata (Sowerby 1868)
Velesunio angasi (Sowerby 1867)
Family Margaritiferidae
Cumberlandia monodonta (Say 1829)
Margaritifera margaritifera (Linnaeus 1758)
Family Unionidae
Actinonaias ligamentina (Lamarck 1819)
Amblema plicata (Say 1817)
Anodonta cygnea (Linneaus 1758)
Coelatura aegyptiaca (Cailliaud 1827)
Cyrtonaias tampicoensis (Lea 1838)
Elliptio dilatata (Rafinesque 1820)
Fusconaia flava (Rafinesque 1920)
Glebula rotundata (Lamarck 1819)
Gonidea angulata (Lea 1838)
Ligumia recta (Lamarck 1819)
Pleurobema clava (Lamarck 1819)
Potamilus alatus (Say 1817)
Pyganodon grandis (Say 1829)
Quadrula quadrula (Rafinesque 1820)
Strophitus undulatus (Say 1817)
Toxolasma lividus (Rafinesque 1831)
Unio pictorum Linnaeus 1758
Unio tumidus Retzius 1788

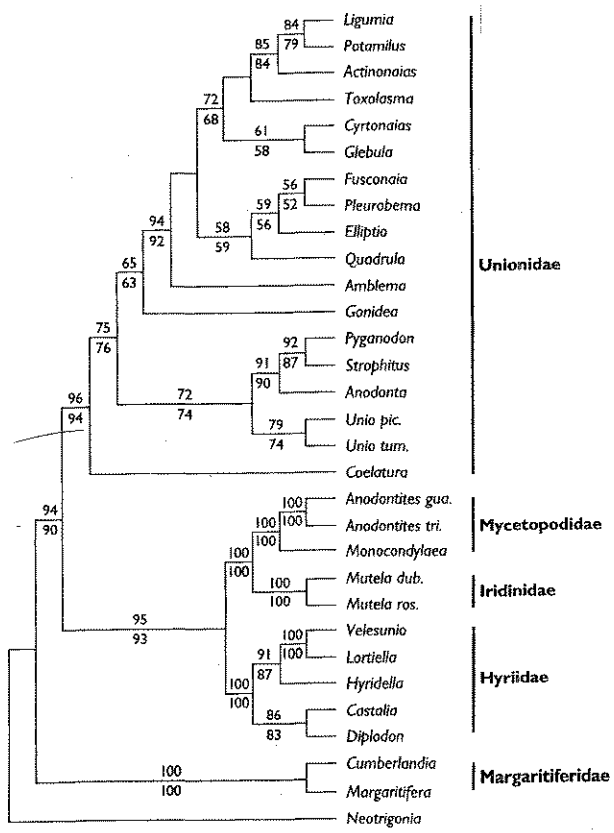

Figure 4.3. Single most parsimonious tree from a simultaneous analysis of 687 (243 parsimony-informative) unionoid morphological and molecular traits using successive weighting. Numbers represent bootstrap (above branch) and jackknife (below branch) percentages from 1,000 replicates using heuristic searches. Only percentages ≥50% are shown.

niques, could be used to evaluate the stability of the non-molecule–based topology (Figure 4.2). To that end, the 57-character presence/absence-coded morphology data matrix was combined with the 630-character transformed (only transversions coded at third positions) COI matrix, 186 parsimony-informative characters from Hoeh et al. (2001) and analyzed with successive weighting techniques using the parsimony algorithm in PAUP* v4.0b8. The single shortest tree obtained from this analysis is presented in Figure 4.3. Despite the detection of significant incongruence between the morphology and COI datasets ($p = 0.003$), using the character congruence (ILD) test (Farris et al. 1994; Chapter 3, this vol-

ume), with or without the deletion of invariant characters (Cunningham 1997a), the bootstrap support levels determined in the total evidence analysis for unionoid families and higher taxa are surprisingly high. The major unionoid family-level relationships indicated by the non-molecule–based analyses of Graf (2000) and Hoeh et al. (2001) are supported by the total evidence topology, except that here the Unionidae is monophyletic. This well-supported analysis represents the best-resolved, best-supported hypothesis of unionoid bivalve higher-order relationships produced to date. It is interesting to emphasize that despite the significant level of incongruence detected between the 57-character morphology dataset and the 630-character COI dataset, the total evidence analysis produced higher levels of bootstrap support for some of the same unionoid higher taxa clades supported in the morphology analysis. At least three of these clades were not evident in the strict consensus parsimony trees from both unweighted and successively weighted analyses of the transformed COI matrix alone (trees not shown). It seems reasonable to infer that congruence among the more consistent characters in the two datasets produced the high degree of support for the family and higher-level relationships obtained in the total evidence analysis.

If presence/absence character coding and successive weighting techniques are ultimately judged appropriate for these phylogenetic analyses, then the generally higher resolution and nodal support provided by them yield an increased level of confidence in the findings of the morphology and total evidence-based estimates of unionoid phylogeny presented here. Important evolutionary inferences regarding the Unionoida obtained from our total evidence analysis include the following: (1) tetragenous brooding is the ancestral condition, (2) the glochidium is the ancestral larval type, (3) endobranchous brooding evolved a single time from a tetragenous ancestor, and (4) ectobranchous brooding evolved at least twice from a tetragenous ancestor. Most of these hypotheses (except the fourth) are consistent with the classification of Ortmann (1912) and the unionoid tree of Heard and Guckert (1970: Fig. 1), but some run counter to findings from recent analyses based largely on mtDNA sequences as discussed above (Bogan and Hoeh 2000: Fig. 1; Graf and Ó Foighil 2000b: Fig. 3; Hoeh et al. 2001: Fig. 14.9).

Although a total evidence approach somewhat obviates their necessity, discussions regarding the existence and detection of incongruence among different data partitions are ongoing in systematics, as are discussions regarding the relative utility of morphological and molecular characters (e.g., Baker et al. 1998; Hillis and Wiens 2000; Yoder et al. 2001). The apparent incongruence between morphology and DNA-based estimates of unionoid phylogeny (e.g., Hoeh et al. 2001: Fig. 14.3) may be real in that single gene-based phylogenies need not accurately trace organismic phylogeny (e.g., Pamilo and Nei 1988; Doyle 1992; Bull et al. 1993; Hoeh et al. 1997). Because animal mtDNA typically lacks recombination (but see Awadalla et al. 1999; Hagelberg et al. 1999; Ladoukakis and Zouros 2001), which causes the genes to be inherited as a single unit, simply adding sequences from other mitochondrial genes to our database will not counter this effect if it exists. To date, DNA-based analyses of unionoid phylogeny have largely been implemented using mtDNA sequences (e.g., Lydeard et al. 1996; Hoeh et al. 1998b, 2001; Roe and Lydeard 1998b; Bogan and Hoeh 2000; Graf and Ó Foighil 2000b; Roe et al. 2001; but see King et al. 1999; Graf and Ó Foighil 2000a; Graf 2002). Alternatively, the apparent incongruence between non-molecule–based and DNA-based estimates of phylogeny could be illusory: it may simply represent inadequate sampling of taxa and the relatively small morphological datasets used to date. Further sampling of additional morphological and molecular characteristics is necessary to address these alternative hypotheses. Phylogenetic analyses of the two independently inherited mtDNA genomes contained within the Unionoida (e.g., Hoeh et al. 1996; Liu et al. 1996) may offer a unique opportunity to further our understanding of the group's phylogeny. The increased resolution and strongly supported nodes generated using a total evidence approach combined with successive weighting suggest that significant advances in our understanding of unionoid systematics may be possible with the data currently available.

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5

MOLECULAR SYSTEMATICS OF THE SCAPHOPODA

Scaphopods constitute one of the smaller molluscan classes, with approximately 520 validly described extant species (Steiner and Kabat 2001, and in press), which is composed exclusively of marine benthic infaunal "tusk shells." Although relatively minor contributors to benthic community species diversity, the group has a world-wide geographic distribution and is found from the intertidal zone (Lamprell and Healy 1998) to depths of 7,000 meters (Knudsen 1964). Current classification identifies two orders, Dentaliida (Palmer 1974) and Gadilida (Starobogatov 1974), 14 families, and 60 genera (46 extant) (Steiner and Kabat 2001) (Table 5.1).

The earliest scaphopod identified with certainty in the fossil record is a member of the Dentaliida from the Mississippian Carboniferous (362.5 million years; Yochelson 1999). However, several disputed records would place the first appearance considerably earlier; these include the Ordovician *Plagioglypta iowensis* and *Rhytiodentalium kentuckyensis* (Bretsky and Bermingham 1970; Pojeta and Runnegar 1979; Engeser and Riedel 1996; Lamprell and Healy 1998; Yochelson 1999) and several Devonian species that are also questionable (Ludbrook 1960; Emerson 1962; Yochelson 1999). The Gadilida appear much later, in the Tertiary (65 million years; Emerson 1962), but there are also earlier disputed reports, from the Permian (Yancey 1973; Yochelson 1999).

SCAPHOPOD MOLECULAR DATA

Scaphopod molecular data, as published in GenBank, has been scant compared with that available for larger molluscan classes. Scaphopoda have received only