ABSTRACT

A PHYLOGENETIC PERSPECTIVE ON THE EVOLUTION OF THE UNIONOIDA (MOLLUSCA BIVALVIA PALAEOHETERODONTA): USING PATTERN TO TEST HYPOTHESES OF MACROEVOLUTIONARY PROCESS

by

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Chair: Diarmaid Ó Foighil

The Unionoida, commonly known as freshwater pearly mussels or naiades, is a diverse, ancient order of strictly continental, freshwater bivalves. Most previous discussions of freshwater mussel evolution pre-dated the widespread acceptance of phylogenetic systematics and modern biogeographic theory. As a result, our understanding of the macroevolutionary processes behind the present diversity has been limited to untested narratives. This is unfortunate, as the age, distribution, and diversity of freshwater mussels makes them useful for studying divergences ranging from the Mesozoic to the Quaternary.

This dissertation documents four cladistic studies of the Unionoida. Because no single character or taxon set is appropriate across all levels of freshwater mussel phylogeny, four specific topics are addressed using separate but overlapping analyses of morphology and mitochondrial and nuclear DNA.

The first analysis (Chapter 2) applies a strictly morphological character set to test the position of the Hyriidae among the Unionoida. Chapters 3 and 4 address the phylogeny and brooding character evolution among the Nearctic Unionidae using a combined (mitochondrial + nuclear) character set. Chapter 5 returns to the Hyriidae of the Australasian and Neotropical regions, using molecular characters to test hypotheses of biogeographic process. Chapter 6, the last analytical chapter, tests the position of the Nearctic genera relative to the global Unionoidea.

The seventh chapter reviews the macroevolution of the Unionoida from a phylogenetic perspective based on the results of these four studies.

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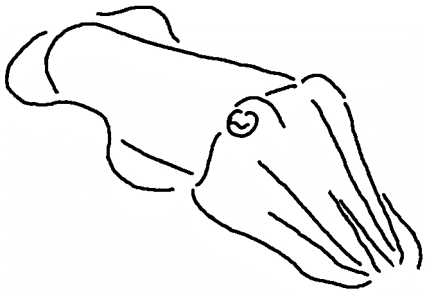

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This volume is dedicated to the handful of special people that have mentored and encouraged me during my studies

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CHAPTER 1

INTRODUCTION

AND THE CURRENT STATE OF FRESHWATER MUSSEL AFFAIRS

The Unionoida, commonly known as freshwater pearly mussels or naiads, is a diverse order of bivalved mollusks. Comprised of over 150 genera and flung widely upon all continents except Antarctica, the Unionoida is a conspicuous member of the macrobenthos of the world's rivers and stable lacustrine habitats (Haas, 1969a). Over the last 20 years, there has been a renaissance in freshwater mussel study, especially in North America. Most of that research has dwelt on such topics as fine-scale, intra-drainage distribution patterns and life history traits relevant to applied conservation and propagation issues (see numerous references in Burch, 1975 and Watters, 1994b). This buzz of ecological work focusing on contemporary processes among freshwater mussels has tended to overlook freshwater mussel evolution and the role of historical processes on their current patterns of global morphological diversity and biogeography. This dissertation will present the results of investigations into different aspects of the evolution of the Unionoida. These studies drastically alter the story of freshwater mussel diversification found in the modern biological and paleontological literature. The objectives of this introductory chapter are (1) to provide the necessary background information on freshwater mussel biology by reviewing the pertinent aspects of their life history, classification, and distribution; and (2) to furnish the scientific framework for the analytical chapters that follow.

It is necessary to first orient ourselves on the Tree of Life. Although there has been some incongruence among molluscan classification schemes, most arrangements are

consistent with the Bivalvia split among two subtaxa: Protobranchia and Autobranchia (= Isofilibranchia + Pteriomorpha + Anomalodesmata + Heterodonta + Palaeoheterodonta). According to the current consensus (*e.g.*, Newell, 1969; Boss, 1982; Brusca & Brusca, 1990), the Unionoida belong to the latter in the subclass Palaeoheterodonta (**Table 1.1**). The Recent Palaeoheterodonta, however, receives only a single non-unionoid genus, the marine *Neotrigonia*. The divisions among the Autobranchia and the inclusion of the Unionoida among the Palaeoheterodonta have been based, traditionally, upon hinge morphology (Thiele, 1934; see **Appendix I**).

Freshwater Mussel Life History

As their common name implies, unionoids are confined to freshwater environments. In most other respects, the adult mussel behaves just as one would expect a clam to behave: freshwater mussels are sedentary filter feeders. The reproductive end of their life history, however, is truly extraordinary among the Bivalvia. Freshwater mussel larvae are parasitic, generally upon fishes. Exceptions have been reported wherein some species have completed metamorphosis without a host (Howard & Anson, 1923; Allen, 1924; Parodiz & Bonetto, 1963; Kondo, 1990), and one species, *Simpsonaias ambigua* (Say, 1825), is known to naturally infect *Necturus*, an amphibian (Howard, 1915; Clarke, 1985). Excellent reviews of the freshwater mussel life cycle can be found in Coker *et al.* (1921) and Kat (1984). However, as discussed in Graf (1998), these synopses tend to overlook the more subtle evolutionary consequences of the mussels' parasitic larval life-style.

Freshwater mussels are generally gonochoristic, although there are exceptions (van der Schalie, 1970; Hoeh *et al.*, 1996b). While broadcast spawning is typical for their marine counterparts (and other marine/aquatic invertebrates) (Brusca & Brusca, 1990), fertilization of unionoid ova occurs within the mantle cavity of the female. The

male ejects his sperm directly to the water, and these must be entrained in a female's respiratory current to reach their target. Interestingly, the sperm are packaged in spermatozeugmata (Edgar, 1965; Lynn, 1994; Waller & Lasee, 1997) — also known as "sperm spheres" (Ishibashi *et al.*, 2000). This is similar to the way sperm are packaged in brooding oysters (Ó Foighil, 1989). Spermatozeugmata presumably retard the dilution of the sperm in turbulent water.

The embryos are brooded within the interlamellar spaces of the females' ctenidial demibranchs (figured in Ortmann, 1911c). For the vast majority of species, the brooded embryos develop into parasitic larvae; however, the morphology can differ drastically among the major freshwater mussel taxa (Parodiz & Bonetto, 1963; Heard & Guckert, 1971). When ripe, the larvae are released to meet a host. The larvae generally reach their host passively (Lefevre & Curtis, 1910), although some genera, such as *Lampsilis*, have evolved morphologies and behaviors (reviewed in Kat, 1984; see also O'Brien & Brim Box, 1999) for attracting potential hosts to the female before she releases her brood.

It is while encysted in the gill or fin epithelium of a suitable host that the larva can complete its metamorphosis into a free-living juvenile (Lefevre & Curtis, 1910, 1912). That is, the mussel begins formation of its postlarval/juvenile shell and internal organs. When metamorphosis is complete, the unionoid uses its new foot to break free of the cyst, falls to the bottom, and takes on the habits of an adult bivalve. The juvenile freshwater mussel may sediment-feed for a period before its ctenidia are fully matured, and it can begin suspension feeding (Yeager *et al.*, 1994; Gatenby *et al.*, 1997).

To counteract the inefficiency of their complicated life cycle, freshwater mussels tend to live for long periods — decades to over a century in some cases (Ziuganov *et al.*, 1994; Bauer, 2001). While the parasitic aspect of freshwater mussel reproduction certainly drives microevolutionary phenomena (Graf, 1998), an important macroevolutionary consequence of this interaction (*i.e.*, reliance upon freshwater fishes for not only survival but also dispersal) is that the Unionoida is (and apparently always

has been) constrained to freshwater. The mussels have no overland vagility (Graf, 1997b) and little opportunity or tolerance for marine dispersal (Sepkoski & Rex, 1974; Atrill *et al.*, 1996; but see discussions in Kat, 1983 and Strayer, 1987). Thus, the Unionoida is a strictly continental clade.

The basic reproductive information summarized in the preceding paragraphs is widely known, and it has been recapitulated or figured in the introductory chapters of many handbooks on freshwater mussels (*e.g.*, Oesch, 1984; Cummings & Mayer, 1992; Parmalee & Bogan, 1998). While a detailed knowledge of the specific life cycle (*i.e.*, duration, hosts) for many species is wanting, the scuttle for life history information has tended to overshadow, in recent years, evolutionary studies of freshwater mussel natural history. This is unfortunate as freshwater mussels present many interesting phylogenetic and biogeographic patterns.

Freshwater Mussel Classification

The most recent, comprehensive treatments of the Unionoida were those of Haas (1969a, b), and those two different perspectives — diversity and stratigraphy — upon the same topic illuminate a wealth of unexplored relationships, distributions, and other historical, evolutionary patterns. The Recent global diversity, continental ranges, and paleontological stratigraphy based on Haas's work are presented in **Table 1.2**. Graphic representations of the taxonomic and geographic diversity of the Unionoida are shown in **Figure 1.1**. The taxonomic 'arrangement' (*sensu* Wiley, 1980) has been updated to be consistent among the numerous available systems (see below) and is explained in greater detail in **Appendix II**.

The general consensus, based on various malacological schools of taxonomy (*e.g.*, Simpson, 1900, 1914; Ortmann, 1910a, 1911a, b, 1912b, 1921a; Frierson, 1927; Modell, 1942, 1949, 1964; Morrison, 1956, 1973; McMichael & Hiscock, 1958; Pain &

Woodward, 1961; Parodiz & Bonetto, 1963; Haas, 1969a, b; Heard & Guckert, 1971; Davis & Fuller, 1981; Boss, 1982; Korniushin, 1998), is that the Unionoida is composed of six families: Margaritiferidae, Unionidae, Hyriidae, Iridinidae, Mycetopodidae, and Etheriidae. While there seems to be widespread agreement upon the recognition of these taxa (reviewed in **Appendix II**), there is confusion regarding their precise generic composition and phylogeny.

The diagnoses of these six families are generally based on soft-anatomical and life history characters. Although other malacologists at other times (*e.g.*, Simpson, 1900, 1914; Modell, 1942, 1949, 1964) have relied more heavily upon shell morphology, it is the influence of Arnold E. Ortmann's (1909-1924) malacological-vs.-conchological perspective that has persisted into the modern era of freshwater mussel systematics (*i.e.*, Haas, 1969a, b; Heard & Guckert, 1971; Davis & Fuller, 1981). While there have been disagreements regarding the details of the family-level arrangement of the Unionoida, a meaningful consensus can be derived (**Table 1.2**).

The Unionidae, Margaritiferidae, and Hyriidae have, in recent decades, been associated as the Superfamily Unionoidea based upon their shared possession of glochidium-type parasitic larvae (Parodiz & Bonetto, 1963). Glochidia are small (60-350 μm), bivalved larvae (**Figure III.3**). Besides the morphological differences among the glochidia of the three families, the Unionidae, Margaritiferidae, and Hyriidae are readily distinguishable based upon their adult anatomy (Ortmann, 1911a, 1912b, 1921a; Heard & Guckert, 1971).

The Unionidae is the most diverse and widespread family of freshwater mussels, with over 670 species in roughly 120 genera (**Table 1.2 & Figure 1.1**). This taxonomic and geographic diversity has led to ample infra-familial classification (see Modell, 1942 or Pain & Woodward, 1968, for example). The most recent re-analysis of the system of the Unionidae is that of Davis & Fuller (1981). Most subsequent considerations (*e.g.*, Lydeard *et al.*, 1996; Parmalee & Bogan, 1998) have agreed that the numerous unionid

tribes should be divided among two subfamilies, Anodontinae (**Figure 1.2**) and Unioninae (**Figures 1.3-6**). The classification of the Unionidae is discussed in more detail in **Appendix II**, and the morphology of the Unionoida in general is described in **Appendix III**.

Three morphological characters have been used to diagnose the Unionidae:

(1) the presence of a supra-anal aperture,

(2) what is known as a 'slightly incomplete' diaphragm dividing the inhalant from the exhalent chambers of the mantle cavity, and

(3) possession of either a tetragenous or ectobranchous marsupial arrangement.

A supra-anal aperture is formed by a short, pallial fusion dorsal to the excurrent aperture. This fusion creates a third opening and is the only fusion of the left and right lobes of the mantle among the Unionidae (with the exception of where the lobes are joined beneath the umbo). The absence of fusion of the mantle is considered primitive among the Bivalvia (Waller, 1998).

The absence of pallial fusion between the incurrent and excurrent apertures contributes to the incomplete nature of the diaphragm dividing the infrabranchial/inhalant and suprabranchial/exhalent chambers of the posterior mantle cavity. However, the diaphragm is only 'slightly incomplete' (Davis & Fuller, 1981) since the isolation of these two chambers is accomplished by the ctenidia. The ascending lamellae of the inner demibranchs of the ctenidia are fused to each other behind the foot, and the ascending lamellae of the outer demibranchs are fused to the mantle along their entire length. Thus, without *actual* fusion, the lobes of the mantle between the incurrent and excurrent apertures are brought into contact.

The parasitic glochidia are often brooded in only a portion of the females' two pairs of demibranchs. Sometimes only the outer pair of demibranchs serve as marsupia (ectobranchy), or only the inner pair (endobranchy), or, in other cases, all four (tetrageny). Among the Unionidae, most genera use either just the outer demibranchs for

brooding, or they use all four. There are, however, rare exceptions of endobranchous unionids (Kondo, 1984, 1990). The marsupial arrangement of the demibranchs has been considered of prime importance for classification within the Unionidae, as well as for the Unionoida generally (Ortmann, 1912b; Heard & Guckert, 1971).

The nearly unanimous assumption has been that the Margaritiferidae (**Figure 1.7**) are primitive among the Unionoidea based upon the relatively simple morphology of its constituent genera (Heard & Guckert, 1971; Heard, 1974; Davis & Fuller, 1981; Lydeard *et al.*, 1996). For the most part, the family has been diagnosed by its *lack* of characters. Margaritiferids lack pallial fusion dorsal to the excurrent aperture, and, thus, they have no supra-anal aperture. The diaphragm dividing the infra- from the suprabranchial chamber is grossly incomplete, without posterior fusion of the ascending lamellae of the outer demibranchs to the adjacent mantle in addition to the absence of fusion between the incurrent and excurrent apertures. Limited separation of the infra- from the suprabranchial chamber is accomplished by 'diaphragmatic septa' emanating from the walls of the mantle cavity (Smith, 1980).

In contrast, the Hyriidae (**Figure 1.8**) exhibit several elaborations to the unionid condition (Ortmann, 1921a). The diaphragm is complete, achieving separation of the infra- from the suprabranchial chambers not only through fusion of the ctenidia (to each other and to the mantle) but also by fusion of the left and right mantle lobes in-between the incurrent and excurrent apertures. The hyriid marsupium occupies only the inner demibranchs; thus, the Hyriidae is exclusively endobranchous. Also in contrast to the Unionidae, the pallial fusion dorsal to the excurrent aperture is complete. That is, rather than re-opening to produce a supra-anal aperture, the postero-dorsal margin is closed. The adult morphology of the Hyriidae is similar to that of the Iridinidae, Mycetopodidae, and Etheriidae, but those latter three families are widely considered to comprise the second unionoid superfamily, the Etherioidea.

Whereas the Unionoidea possess glochidia, the parasitic larval form of the Etherioidea is known as a lasidium. Lasidium larvae, like glochidia, are also small (85-150 μm), but they are of a wholly different morphology. The lasidia are univalved, non-calcareous, and possess a conspicuous posterior ribbon. Parodiz & Bonetto (1963), after examining the freshwater mussels of South America, identified larval morphology (glochidium vs. lasidium) as the essential character with which to divide the Unionoida, and their scheme has been followed ever since (Haas, 1969a, b; Heard & Guckert, 1971; Boss, 1982).

The adult morphology of the etherioidean families is less variable than among the families of the Unionoidea. The soft-anatomy of the Mycetopodidae (**Figure 1.9**) is very similar to that described above for the Hyriidae, with the exception that there is usually no pallial fusion dorsal to the excurrent aperture (Parodiz & Bonetto, 1963; Boss, 1982). The three genera of the Etheriidae are of like morphology to the mycetopodids, the former family differing principally in their cementing habit and consequently asymmetrical valves (**Figure 1.9**) (Heard & Vail, 1976a).

The last unionoid family, the Iridinidae (**Figure 1.10**), shares several adult anatomical characters with the Mycetopodidae, Etheriidae and Hyriidae (Unionoidea):

(1) larval brooding is done in the inner pair of demibranchs (endobranchy),

(2) the diaphragm is complete, formed by fusion among the ctenidia and the lobes of the mantle, and

(3) pallial fusion above the excurrent aperture is complete.

These points of posterior pallial fusion are further developed in the Iridinidae than among hyriids. Iridinids generally possess siphons that are similar to those described among many veneroid genera, and these siphons are often complete with pallial sinuses (Bloomer, 1932; Heard & Dougherty, 1980).

Macroevolutionary Patterns Among the Unionoida

Table 1.2 lists the biogeographic and stratigraphic distributions reported for the six families of the Unionoida. The Margaritiferidae and Unionidae are generally Holarctic, including the Oriental region, and the latter family also occurs in Africa and has a single genus (*Haasodonta*) on New Guinea in the Australasian region. The four other families are more provincial in their distributions and, at present, restricted to the fragments of Gondwana (Brown & Lomolino, 1998): the Hyriidae on South America, Australia, and New Zealand; Neotropical Mycetopodidae; Iridinidae limited to the Ethiopian region; and Etheriidae, the pandemic exception, occurring on South America, Africa, and India.

There seems to be less recognizable pattern among the known stratigraphic ranges of these families. What can be stated is that while the Order probably has its origin in the early Mesozoic or latest Paleozoic, the extant families generally appear to have had their origins in the late Mesozoic or soon thereafter (**Table 1.2**).

The preceding descriptions of the life history, character distributions, biogeography, and stratigraphic ranges of the Margaritiferidae, Unionidae, Hyriidae, Iridinidae, Mycetopodidae, and Etheriidae can be summed up as follows: The Unionoida is an ancient, cosmopolitan, strictly continental group of freshwater bivalves. While a conceptual framework is available to propel the study of freshwater mussels analytically forward (*i.e.*, phylogenetic systematics), a comprehensive re-evaluation of the evolution of the Unionoida is well beyond the scope of a single dissertation. Rather than an exhaustive sweep over the broad spectrum of freshwater mussel evolution, I have followed a more heuristic program of applying different taxon and character sets to limited components of the larger problem. My general approach has been to apply phylogenetic systematics to test specific hypotheses dealing with freshwater mussel character evolution or biogeography. These individual studies overlap to some extent,

providing multiple tests of certain questions from different angles, and the sum of their results improves our understanding of the whole of unionoid macroevolution.

Chapter 2 employs a strictly morphological character set to test the composition of the two unionoid superfamilies. Before Parodiz & Bonetto (1963) emphasized larval characters to align the Hyriidae with the Margaritiferidae and Unionidae, Ortmann (1912b) and others (*e.g.*, Thiele, 1934; McMichael & Hiscock, 1958) placed the Hyriidae among the Etherioidea based upon their adult morphology. My parsimony analysis of 38 morphological characters — both larval and adult — provides a test of the position of the Hyriidae and an assessment of the value of morphological characters for recovering phylogeny among the Unionoida in general.

Chapter 3 addresses the problem of applying molecular characters to the phylogeny of the Unionoida. In recent years, the most comprehensive studies of freshwater mussel phylogeny have applied strictly mitochondrial data sets. In **Chapter 3**, I set out to test if those data are indeed the most appropriate for recovering family-group-level divergences among the Unionoida by addressing the specific problem of the relationships among the Nearctic unionids.

Brooding characters have traditionally figured prominently in the classification of the Unionidae (Heard & Guckert, 1971; Davis & Fuller, 1981), especially brooding period (bradytictia vs. tachytictia) and the number of marsupial demibranchs (tetrageny vs. ectobranchy). In **Chapter 4**, as an independent test of the value of brooding characters to freshwater mussel phylogenetics, I employ the phylogenies recovered in **Chapter 3** and parsimony to trace twelve brooding characters onto the resultant cladogram. The results of these two chapters not only clarify our understanding of the evolution of certain characters among the Unionoidea, but they also tests certain aspects of the classification of those freshwater mussels.

Chapter 5 returns to the Hyriidae, focusing on the biogeography of the Australasian Hyridellinae. In addition to the obvious South America-Australia

disjunction observed between the Hyriinae and Hyridellinae, respectively, there is a dramatic disjunction within the Australasian group. The hyridellines occur not only on Australia, New Guinea, and Tasmania on the western side of the Tasman Sea, but they also inhabit New Zealand to the east. To test alternative hypotheses of vicariance vs. dispersal to explain the Australasian disjunction — or, more correctly, to attempt to reject vicariance — I used a fragment of 28S nuclear rDNA to recover the relationships among the tribes of the Hyriidae from Australia, New Zealand, and South America. Based upon the robust topology recovered, I was able to not only refine the historical biogeography of the Hyriidae but also to lend some temporal perspective to their diversification.

The final analytical chapter, **Chapter 6**, covers the specific problem of the classification of the Family Unionidae. However, whereas the analysis presented in **Chapter 3** focused upon the generic relationships of the Unionoidea of eastern North America, **Chapter 6** takes a broader view of the global position of the Nearctic clades. By spotlighting the positions of two traditionally problematic genera, *Unio* and *Gonidea*, I have identified some of the weak links in the present classification of the Unionoidea.

In the closing section, **Chapter 7**, I use the combined results of the phylogenetic studies in **Chapters 2-6** to construct a Unionoida Super Tree. The Super Tree topology is then applied to the long-held evolutionary patterns presented here in **Chapter 1** to provide a modern perspective on the evolution of the Unionoida.

The results and discussions presented in this dissertation are far from the complete story of the Evolution of the Unionoida. The greatest contribution of my work here is to provide a little new data and a lot of modern scientific perspective to an old problem. Hopefully, the studies presented here will spark greater interest than there has been in unionoid evolution and systematics.

Table 1.1. Neontological Classification of the Unionoida Among the Mollusca. The table shows the relative position of the Unionoida among the Mollusca. The classification largely follows Newell (1969), Boss (1982), and Brusca & Brusca (1990). More recently, some bivalve paleontologists have suggested a radically different classification of the Bivalvia (discussed in **Appendix I**).

PHYLUM MOLLUSCA

- Class Aplacophora (= Solenogastres + Caudofoveata)
- Class Polyplacophora
- Class Monoplacophora
- Class Gastropoda
- Class Cephalopoda
- Class Scaphopoda
- **Class Bivalvia**
  - Subclass Protobranchia
  - Subclass Isofilibranchia
  - Subclass Pteriomorpha
  - Subclass Anomalodesmata
  - Subclass Heterodonta
  - **Subclass Palaeoheterodonta**
    - Order Trigonioida
    - **Order Unionoida**

Table 1.2. Global Diversity, Recent Biogeography, and Stratigraphy of the Unionoida. The data presented generally follows the works of Haas (1969a, b), the latest comprehensive compendia of unionoid diversity. Generic and specific diversities do not reflect subsequent taxonomic opinions; however, these numbers likely reflect the actual relative diversities. The quotes around the record of the Unionidae in the Australasian region refers to the single genus, *Haasodonta*, which occurs only on New Guinea (McMichael, 1956). The sources for the family-level classification are given in **Appendix II**. Geographic abbreviations: Au = Australasian; Et = Ethiopian; Na = Nearctic; Nt = Neotropical; Or = Oriental; Pa = Palearctic.

	Diversity	Biogeography						Oldest
Taxon	#Genera (spp.)	Na	Nt	Au	Et	Or	Pa	Fossil
Unionoida	159 (829)							
Unionoidea								
Margaritiferidae	2 (5)	X				X	X	Up. Cret.
Unionidae								
Anodontinae	14 (61)	X				X	X	Up. Cret.
Unioninae	106 (615)	X		"X"	X	X	X	Trias.
Hyriidae								
Hyriinae	10 (55)		X					Cret.[1]
Hyridellinae	8 (27)			X				Cret.[2]
Etherioidea								
Iridinidae	6 (22)				X			Cret.[3]
Mycetopodidae	10 (40)		X					Tert.
Etheriidae	3 (4)		X		X	X		Plio.

Table 1.2 (continued). Global Diversity, Recent Biogeography, and Stratigraphy of the Unionoida.

Notes:

[1] In addition to the Mesozoic hyriid fossils discovered within the boundaries of the family's current distribution, Triassic fossils historically attributed to the Hyriidae are known from the northeastern and southwestern regions of North America (Henderson, 1935; Good, 1998).

[2] Haas (1969b) does not list any hyridelline fossils; the Cretaceous record is from McMichael (1957).

[3] Cretaceous (Hell Creek), North American iridinid fossils have been reported (Morris & Williamson, 1988; Watters, 2001).

Figure 1.1. Taxonomic and Geographic Generic Diversity of the Unionoida. The top pie chart depicts the number of genera in each of the six freshwater mussel families. The slightly offset wedge indicates the Etherioidea; the remainder of the pie represents the diversity of the Unionoidea. The generic representation in each of the geographic provinces is illustrated in the bottom pie chart. The offset wedges there represent Gondwanan regions (note that the Oriental Province is split among the northern and Gondwanan regions due to the inclusion of India). See **Table 1.2** for notes and references.

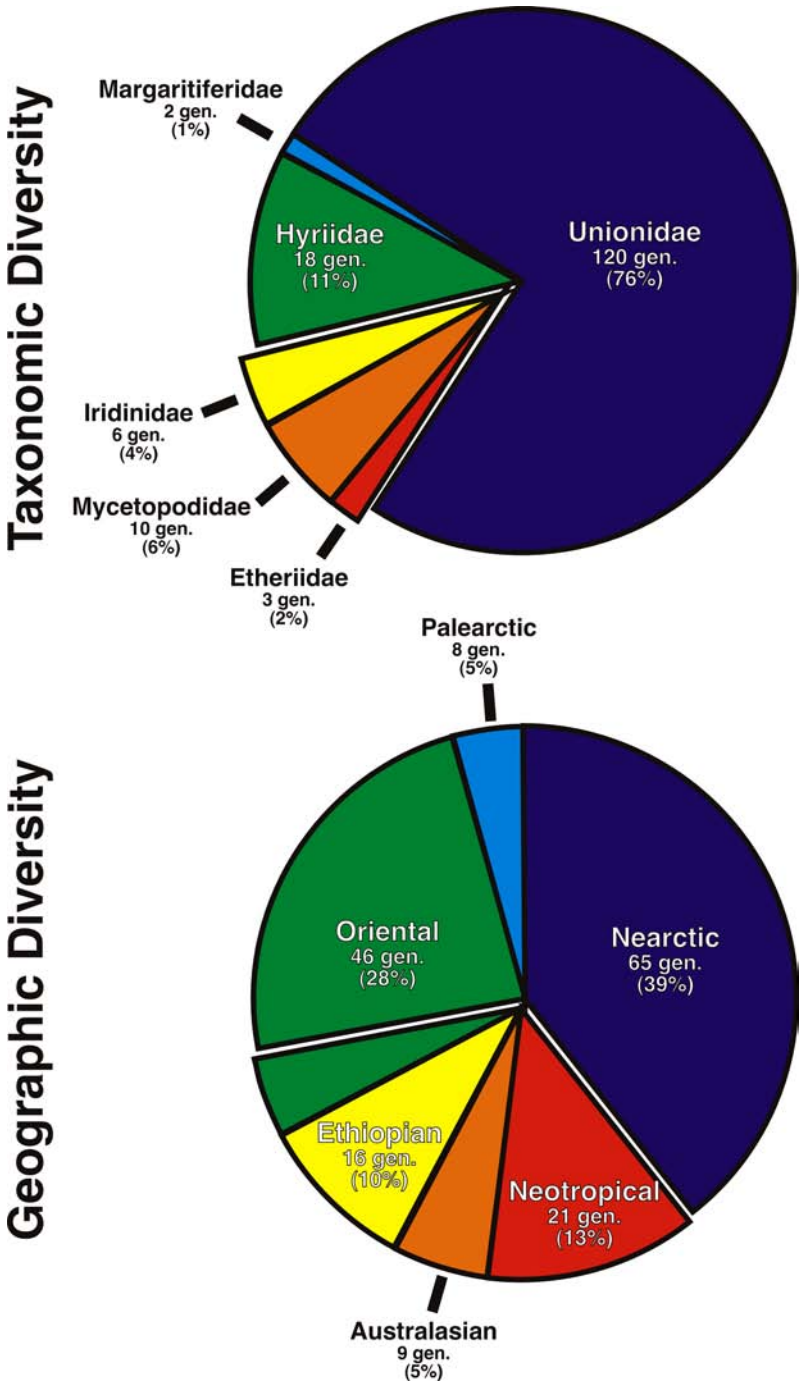


Figure 1.1. Generic Diversity of the Unionoida

Figure 1.2. Representative Unionidae: Anodontinae. All shells roughly natural size.

Anodontinae

Alasmidonta marginata Say, 1819. UMMZ 165060. Grand River, Ionia Co., Michigan, USA.

Lasmigona compressa (Lea, 1829). UMMZ 104085. Oneida Creek, Oneida Co., New York, USA.

Pyganodon grandis (Say, 1829). UMMZ 205535. Portage Creek, Kalamazoo Co., Michigan, USA.

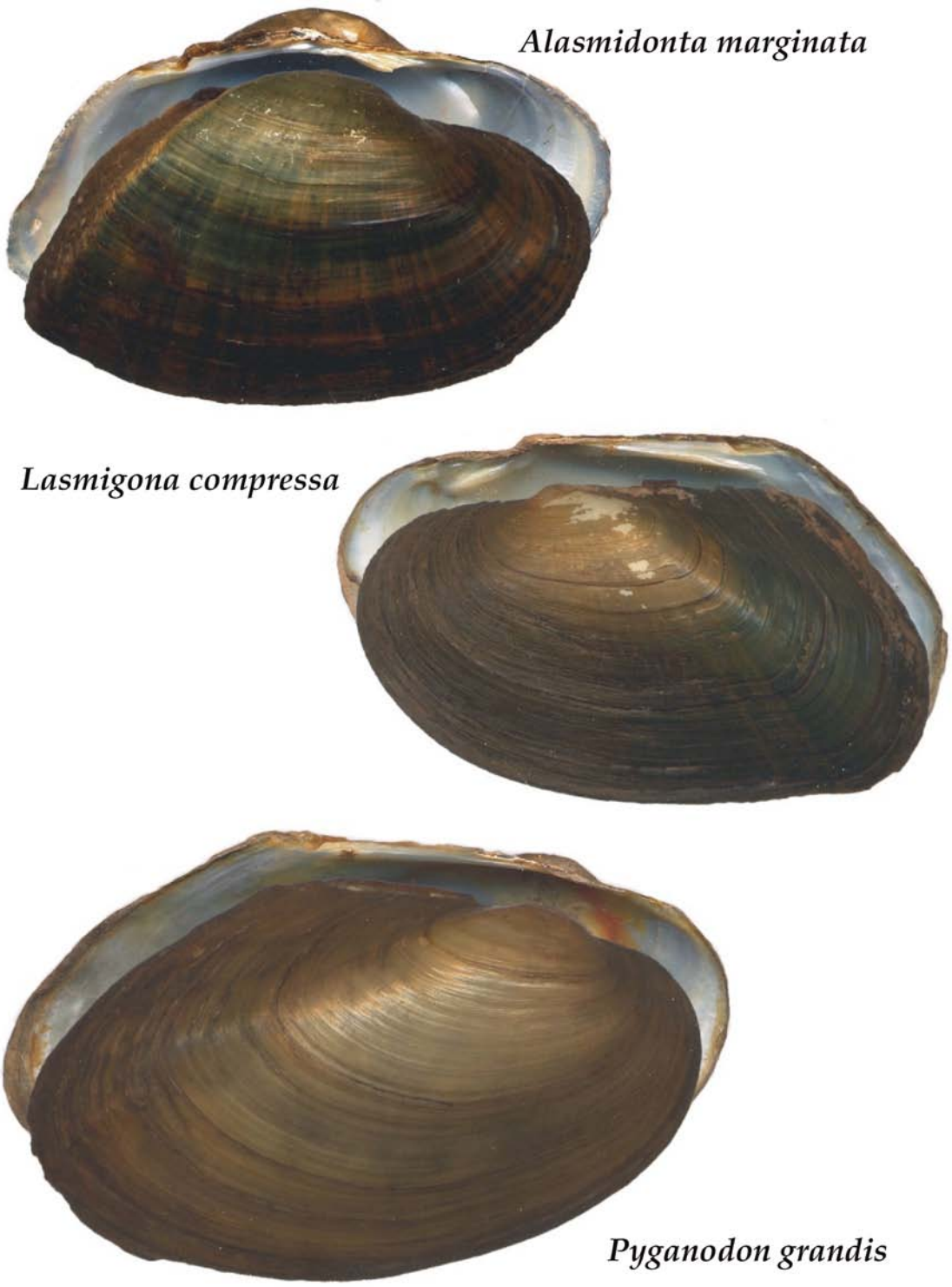


Figure 1.2. Representative Unionidae: Anodontinae

Figure 1.3. Representative Unionidae: Unioninae, Tribes Unionini and Pleurobemini. All shells roughly natural size unless otherwise noted.

Unioninae

Unionini

Unio s.s. pictorum (Linnaeus, 1758). UMMZ 9320. Birmingham, England.

Unio (Cafferia) caffer Krauss, 1848. UMMZ 60409. Irene, Transvaal, South Africa.

Pleurobemini

Pleurobema coccineum (Conrad, 1834). UMMZ 81547. Scioto River, Ohio, USA. Shell *ca.* 75% natural size.

Elliptio dilatata (Rafinesque, 1820). UMMZ 205584. Kalamazoo River, Jackson Co., Michigan, USA.

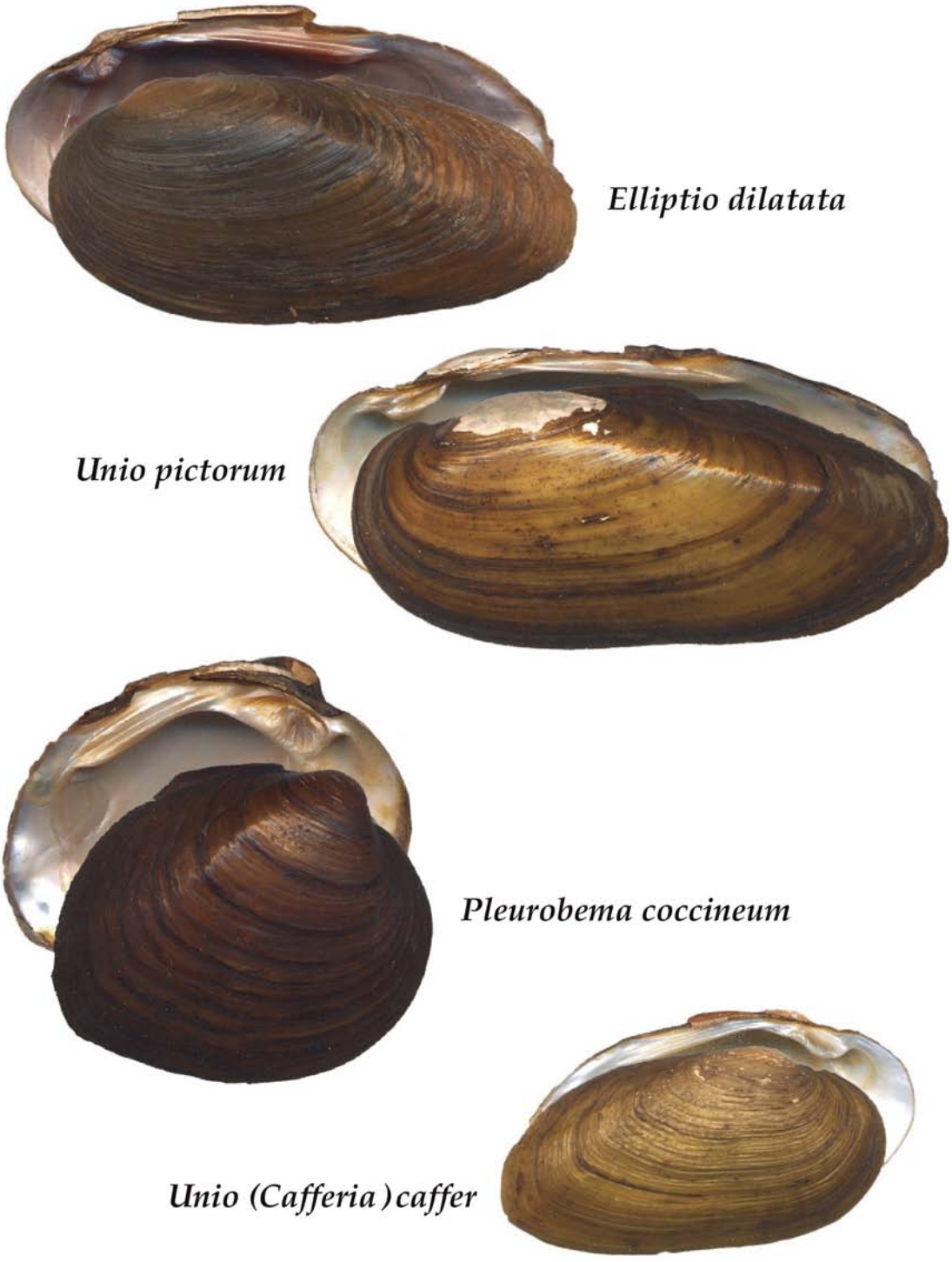


Figure 1.3. Representative Unionidae: Unioninae: Unionini and Pleurobemini

Figure 1.4. Representative Unionidae: Unioninae, Tribes Amblemini, Parreysiini, and Caelaturini. All shells roughly natural size unless otherwise noted.

Unioninae

Amblemini

Amblema plicata (Say, 1817). UMMZ 58893. Watonwan River, Blue Earth Co., Minnesota, USA. Shell *ca.* 75% natural size.

Quadrula quadrula (Rafinesque, 1820). UMMZ 76750. Mississippi River, Spring Lake, Bay Island, Illinois, USA.

Tritogonia verrucosa (Rafinesque, 1820). UMMZ 129161. Mississippi River, Lake Pepin, Minnesota, USA.

Parreysiini

Parreysia corrugata (Muller, 1774). UMMZ 110263. Sri Lanka.

Caelaturini

Grandidieria burtoni (Woodward, 1859). UMMZ 248778. Lake Tanganyika, Zambia.

Figure 1.4. Representative Unionidae: Unioninae: Amblemini, Parreysiini, and Caelaturini

Figure 1.5. Representative Unionidae: Unioninae, Tribes Gonideini, Pseudodontini, and Rectidentini. All shells roughly natural size.

Unioninae

Gonideini

Gonidea angulata (Lea, 1838). UMMZ 107910. Coyote Creek, San Jose Co., California, USA.

Pseudodontini

Pseudodon vondembuschianus (Lea, 1840). UMMZ 110149. Java [Indonesia].

Pilsbryoconcha exilis (Lea, 1839). UMMZ 110521. Singapore, "Straits Settlement."

Rectidentini

Uniandra contradens (Lea, 1838). UMMZ 51781. Kali Paprik, Java [Indonesia].

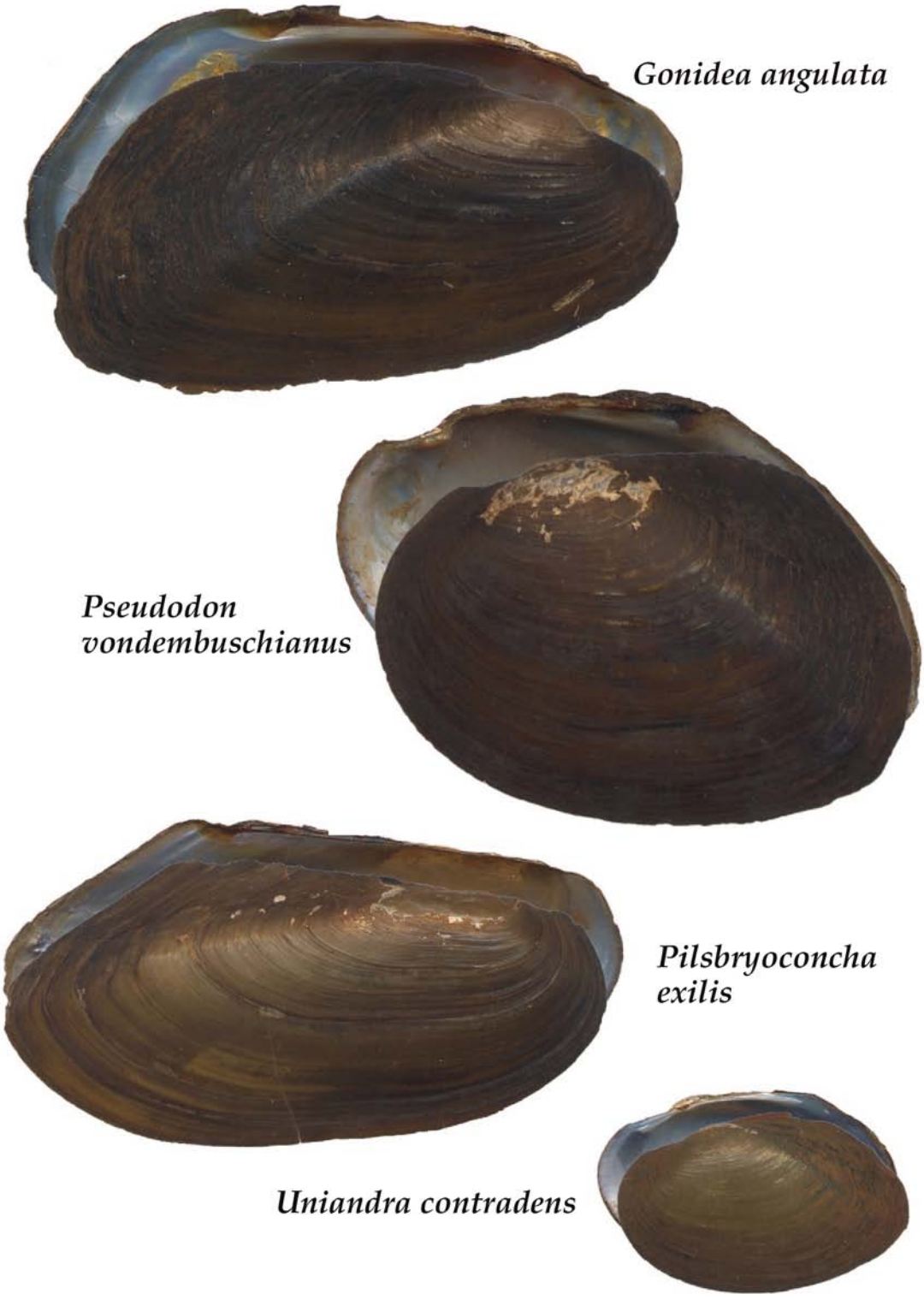


Figure 1.5. Representative Unionidae: Unioninae: Gonideini, Pseudodontini, and Rectidentini

Figure 1.6. Representative Unionidae: Unioninae, Tribe Lampsilini. All Shells roughly natural size.

Unioninae

Lampsilini

Lampsilis cardium (Rafinesque, 1820). UMMZ 130005. Mississippi River, Wabash Co., Minnesota, USA.

Epioblasma flexuosa (Rafinesque, 1820). UMMZ 91453. Ohio River, Ohio, USA.

Obliquaria reflexa Rafinesque, 1820. UMMZ 246948. St. Croix River, Stillwater, Washington Co., Minnesota, USA.

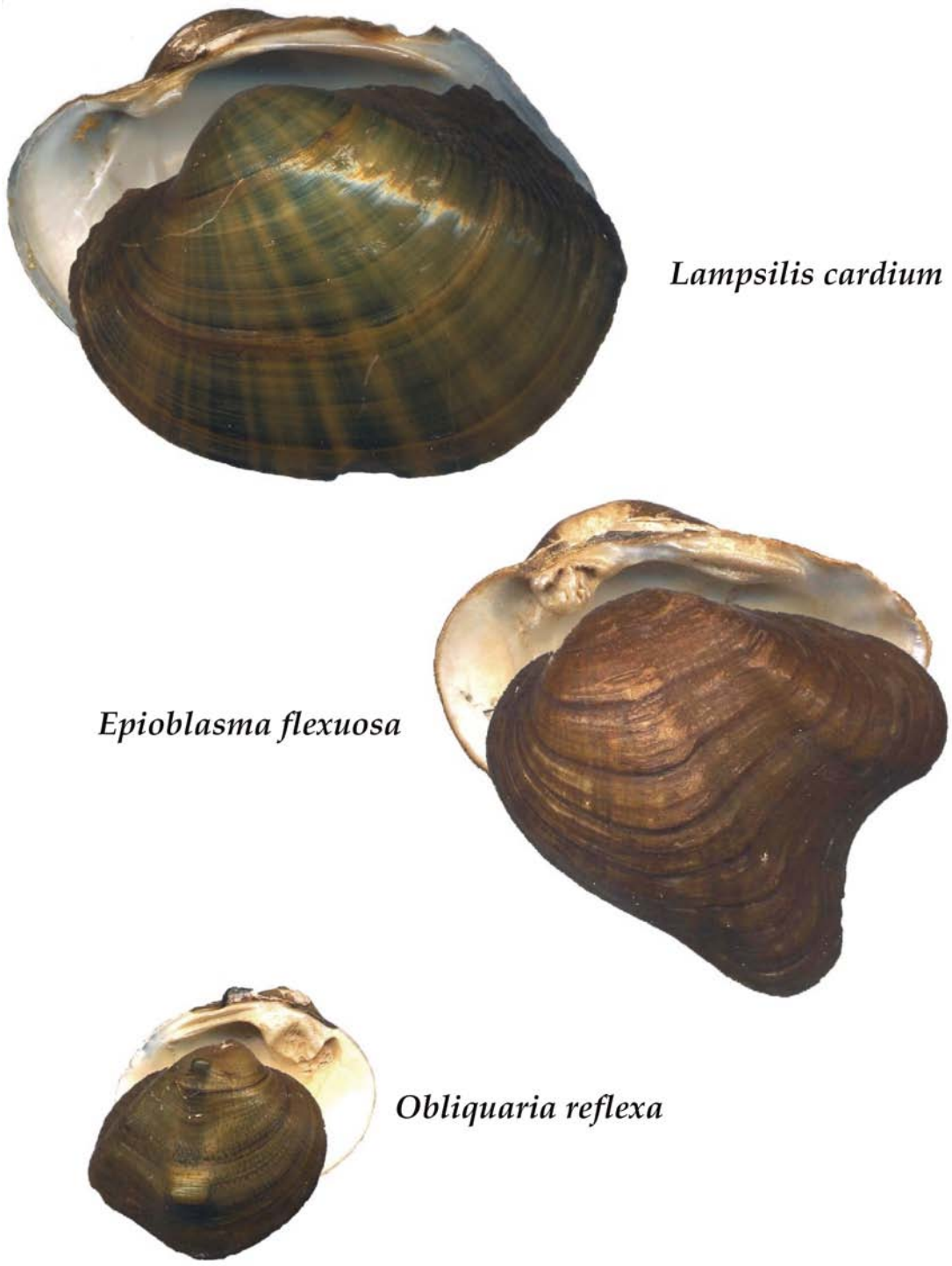


Figure 1.6. Representative Unionidae: Unioninae: Lampsilini

Figure 1.7. Representative Margaritiferidae, Subfamilies Margaritiferinae and Cumberlandiinae. All shells roughly natural size.

Margaritiferinae

Margaritifera margaritifera (Linnaeus, 1838). UMMZ 4338. St. Lawrence River, New York, USA.

Margaritifera hembeli (Conrad, 1838). UMMZ 107633. Alexandria, Rapides Parish, Louisiana, USA.

Cumberlandiinae

Cumberlandia monodonta (Say, 1829). UMMZ 107648. Clinch River, Anderson Co., Tennessee.

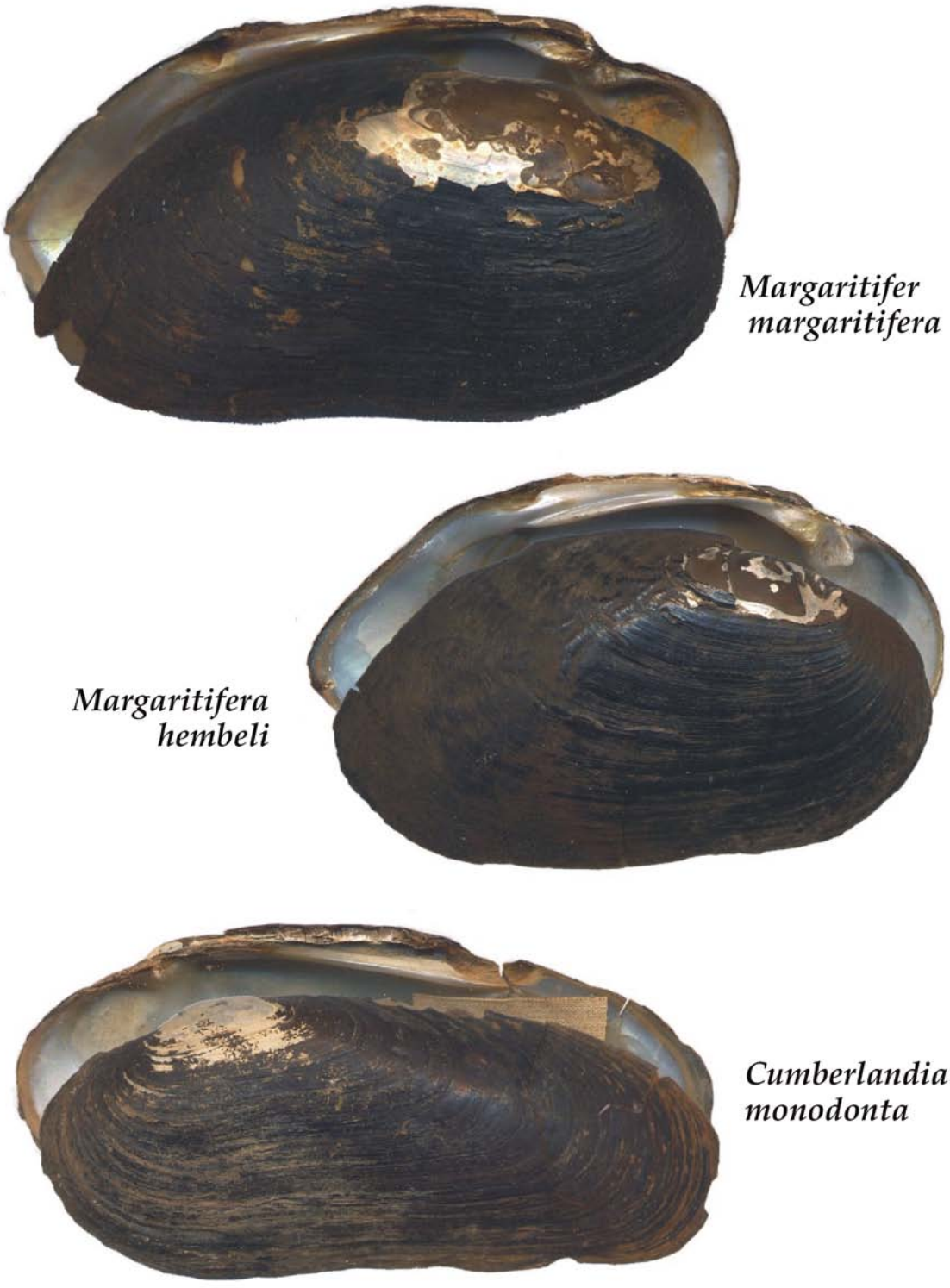


Figure 1.7. Representative Margaritiferidae

Figure 1.8. Representative Hyriidae, Subfamilies Hyriinae and Hyridellinae. All shells roughly natural size unless otherwise noted.

Hyriinae

Hyriini

Prisodon obliquus Schumacher, 1817. UMMZ 110938. Amazonias. Shell *ca.* 75% natural size.

Diplodontini

Diplodon paranensis (Lea, 1834). UMMZ 248835. Buenos Aires, Argentina.

Castaliini

Castalina martensi (von Ihering, 1891). UMMZ 110907. Rio Grande do Sul, Brazil. Shell *ca.* 75% natural size.

Hyridellinae

Hyridellini

Hyridella australis (Lamarck, 1819). UMMZ 111296. Australia.

Hyridella menziesi (Gray, 1843). [Not catalogued]. South Island, New Zealand.

Cucumerunionini

Cucumerunio novaehollandiae (Gray, 1834). Boogong Creek, New South Wales. Australia. Shell *ca.* 75% natural size.

Velesunioinini

Velesunio ambigua (Philippi, 1847). UMMZ 111839. Murray River, Australia.

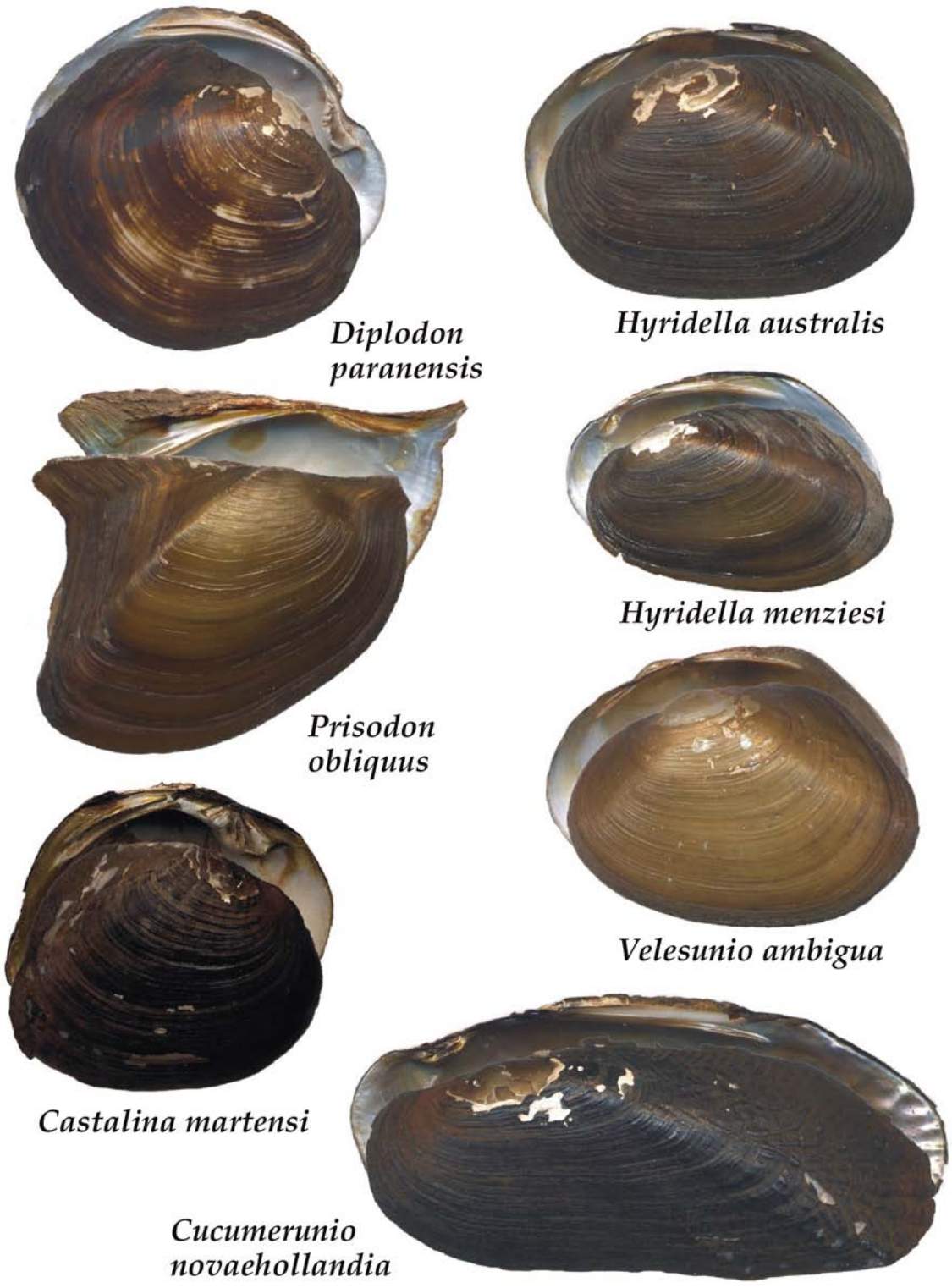


Figure 1.8. Representative Hyriidae

Figure 1.9. Representative Etheriidae and Mycetopodidae. All shells roughly natural size unless otherwise noted.

Etheriidae

Etheria elliptica (Lamarck, 1807). UMMZ 43223. White Nile River, Africa. Shell *ca.* 75% natural size.

Acostaea rivoli (Deshayes, 1827). UMMZ 112660. Amazon River, South America. Shell *ca.* 75% natural size.

Mycetopodidae

Mycetopoda siliquosa (Spix, 1827). UMMZ 112645. Marañon, Peru.

Anodontites crispata (Bruguière, 1792). UMMZ 112391. Upper Daule River, Ecuador.

Anodontites trapesialis (Lamarck, 1819). UMMZ 112429. Rio Grande do Sul, Brazil. Shell *ca.* 75% natural size.

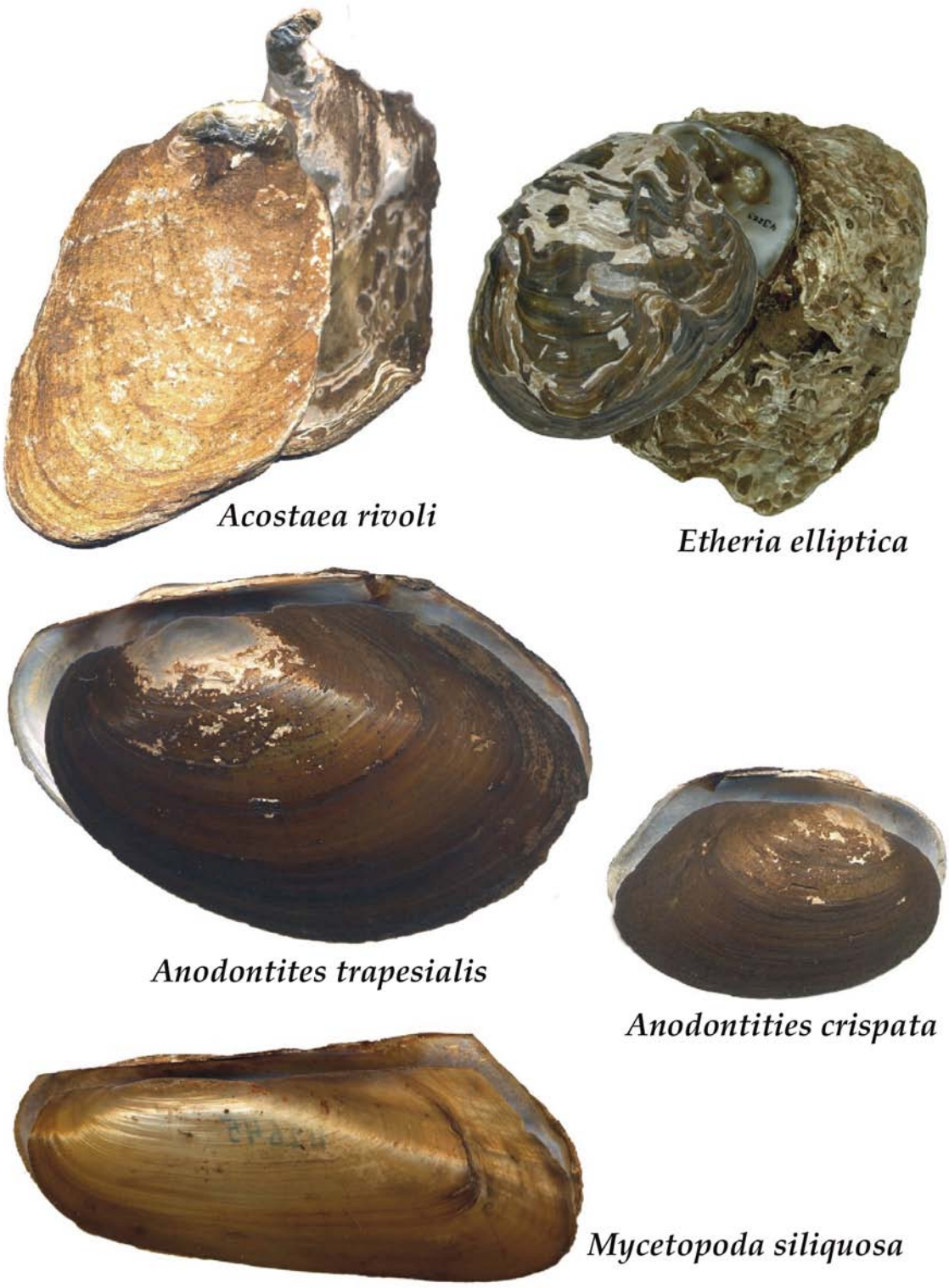


Figure 1.9. Representative Etheriidae and Mycetopodidae

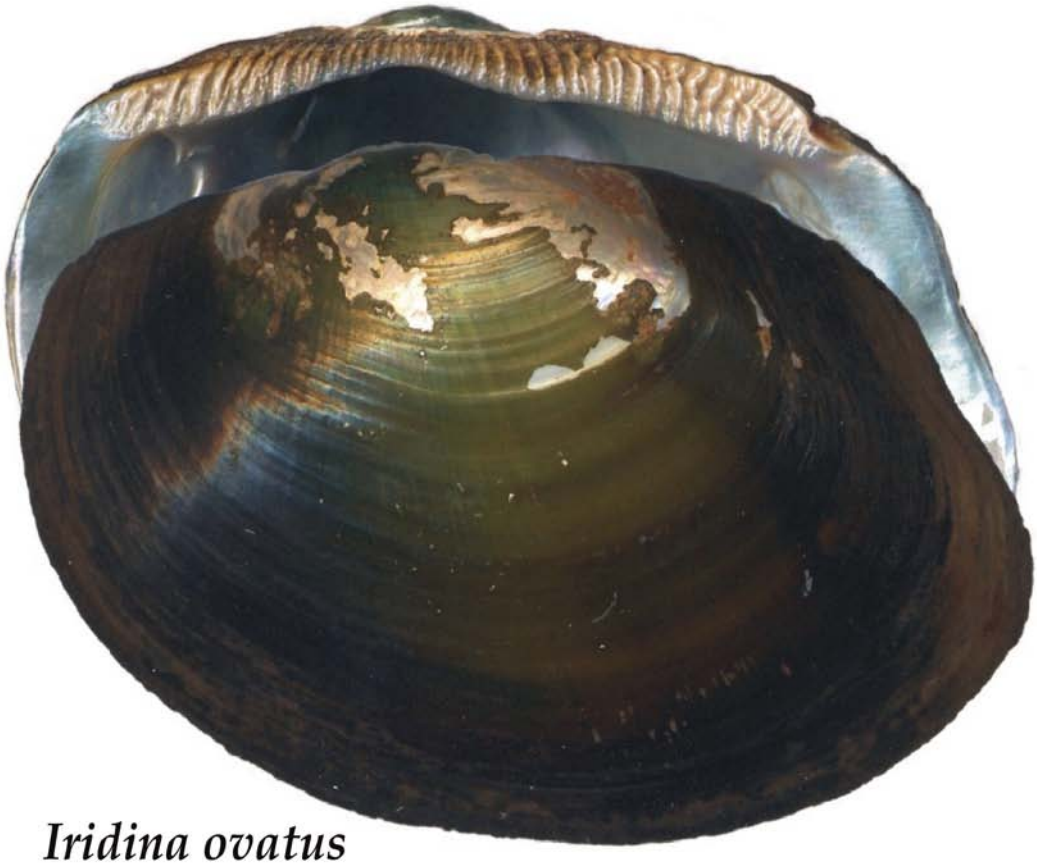
Figure 1.10. Representative Iridinidae. All shells roughly natural size.

Iridinidae

Mutela dubia (Gmelin, 1793). UMMZ 111979. Nile River, Africa.

Iridina ovatus Swainson, 1823. UMMZ 112006. Senegal.

Mutela dubia



Iridina ovatus

Figure 1.10. Representative Iridinidae

CHAPTER 2

USE OF MORPHOLOGICAL CHARACTERS TO TEST THE POSITION OF THE HYRIIDAE

Recently, there has been an increased awareness of the phylogenetic paradox created by disparity among evolutionary hypotheses derived from different semaphoronts. This is especially so among marine invertebrates (*e.g.*, Strathmann & Eernisse, 1994; Wray, 1996). Phylogenies recovered using adult morphology may not necessarily reflect the same evolutionary history suggested by comparisons of larval or juvenile characters (Wiley, 1980). Such is also the case among the Unionoida. The order has been divided into two superfamilies based solely upon larval type: the Unionoidea have glochidia, the Etherioidea (= Muteloidea) have lasidia (Parodiz & Bonetto, 1963; Boss, 1982). The objective of this chapter is to phylogenetically re-evaluate the monophyly of the Etherioidea, based upon both larval and adult morphological characters. A study of these taxa is timely in light of the lack of phylogenetic testing afforded the etherioideans and the recent nomenclatural revisions proposed by Kabat (1997). I have previously published these results (Graf, 2000a).

As discussed in **Chapter 1**, the Order Unionoida is widely considered to be composed of six nominal families (*e.g.*, Ortmann, 1912b, 1921a; Haas, 1969a, b; Heard & Guckert, 1971; Davis & Fuller, 1981; Boss, 1982): Margaritiferidae, Unionidae, Hyriidae, Iridinidae (= Mutelidae), Mycetopodidae, and Etheriidae (**Table 2.1**; see also **Appendix II**). The order is probably monophyletic, diagnosed by its restriction to freshwater, ovovivipary, and a parasitic larval stage that must infect an appropriate host to complete its metamorphosis (Boss, 1982; Kat, 1984). But, until very recently

(Rosenberg *et al.*, 1994, 1997; Lydeard *et al.*, 1996; Graf & Ó Foighil, 2000, 2001), the interrelationships of these families had not been tested phylogenetically. This phylogenetic analysis differs from all previous studies in that the character set is exclusively composed of morphological characters, rather than nucleic acid sequences alone or a combination of molecules with a limited morphological data set (but see Hoeh *et al.*, 2001).

Before the 1960's the Margaritiferidae and Unionidae were considered to comprise the Unionoidea, whereas the remaining families fell into the Etherioidea (Ortmann, 1912b, 1921a; Thiele, 1934; McMichael & Hiscock, 1958). The Unionoidea was diagnosed by:

(1) presence of a supra-anal aperture dorsal to the excurrent aperture,

(2) having inner demibranchs that connect to the visceral mass distant from the labial palps,

(3) having an incomplete or 'slightly incomplete' diaphragm (*i.e.*, composed only of the demibranchs without pallial fusion; Davis & Fuller, 1981) dividing the mantle cavity, and

(4) use of the outer pair of demibranchs (or both pairs) as marsupia for brooding (Ortmann, 1912b).

According to Ortmann (1921a), the Etherioidea (= Hyriidae + Iridinidae + Mycetopodidae + Etheriidae) was diagnosed by:

(1) pallial fusion between the incurrent and excurrent apertures (thus, a complete diaphragm),

(2) having inner demibranchs with an anterior attachment adjacent to or in contact with the labial palps, and

(3) use of only the inner pair of demibranchs for brooding.

Further enforcing this separation was the zoogeography of the two taxa, with unionoideans on the northern continents, etherioideans presenting a Gondwanan distribution (Ortmann, 1921a).

The subsequent systematic arrangement proposed by Parodiz & Bonetto (1963), however, emphasized the discordant distribution of larval types among these taxa. Although non-parasitic life cycles have been reported for a handful of freshwater mussel genera (Howard, 1915; Parodiz & Bonetto, 1963; Kondo, 1990), most have larvae that are parasitic upon fish or, infrequently, amphibians (Hoggarth, 1992; Watters, 1994b). Parasitic mussel larvae fall into two general types. Whereas the Unionoidea possess only glochidia, the Etherioidea *sensu* Ortmann had either glochidia or lasidia.

Glochidia are small (70-350 μm), composed of a single adductor muscle and mantle cells enclosed by a calcareous, bivalved shell. They attach to host tissue by clamping their valves over exposed gill or fin epithelium. The host tissue encysts the mussel larvae (Arey, 1921), and it is within this cyst that the glochidia undergo metamorphosis into juveniles (Kat, 1984; Graf, 1998). Glochidia generally belong to one of two morphological varieties: (1) sub-circular to sub-ovate and unhooked or (2) sub-triangular and hooked (Coker *et al.*, 1921; Hoggarth, 1999), although there is variation within these types (*e.g.*, *Potamilus*; Roe & Lydeard, 1998). Lasidia are also small (85-150 μm, not including the 'larval thread') but are tri-lobed larvae with a univalved, uncalcified shell. Just as with the glochidia, they come in two flavors: (1) lasidium-type and (2) haustorium-type (Bonetto & Ezcurra, 1965a). Although differing in morphology and size, the fundamental distinction between the two varieties of lasidia is that whereas the former attaches to the host by forming cysts, the haustorium-type attaches via tubular appendages (Fryer, 1954, 1961; Parodiz & Bonetto, 1963: Table 1; Wächtler *et al.*, 2001).

Parodiz & Bonetto (1963: 185) argued that,

> "The two different types of larvae, *i.e.*, glochidium and lasidium, cannot be considered to be derived one from the other or from any hypothetical direct ancestry."

They advocated the re-assignment of the Hyriidae, the only etherioideans to have glochidia, to the Unionoidea, and this scheme has been largely followed in subsequent classifications (**Table 2.1**): Unionoidea = Margaritiferidae + Unionidae + Hyriidae; and Etherioidea *sensu* Parodiz and Bonetto = Iridinidae + Mycetopodidae + Etheriidae.

The relatively recent consensus that classifications should be constructed from natural groups reflecting the pattern of phylogeny requires that supraspecific taxa be monophyletic and suggests that these should be diagnosable by shared, derived homologies (*i.e.*, synapomorphies) (Wiley, 1980). From a cladistic standpoint, the systematic hypothesis of Parodiz & Bonetto (1963) suggests that the Unionoidea and Etherioidea are each reciprocally monophyletic, as diagnosed by their larval type, but this has never been tested. Rather, arrangement has been largely authoritarian.

In order to test the monophyly of the Etherioidea *sensu* Parodiz & Bonetto (1963) and the placement of the Hyriidae among the Unionoida, I coded 38 shell and soft-anatomy characters of 18 taxa for cladistic analysis under the optimality criterion of maximum parsimony. These characters are largely those considered important by freshwater mussel systematists. Results indicate that the Unionoidea is not monophyletic and that the Hyriidae is part of a natural taxon when included among the Etherioidea. This has implications not only for the classification of the Etherioidea, but also the historical biogeography and character evolution of the freshwater pearly mussels as a whole.

Methods & Materials

Instead of limiting the analysis to solely adult or larval characters, a combined evidence approach (Kluge, 1989) was applied that included morphological and life history traits of both semaphoronts. One to six representatives of each of the six

consensus families were chosen for analysis (**Table 2.1**), with an emphasis on genera representing different infra-familial taxa. Also included was the marine *Neotrigonia*, the solitary surviving genus of the once-mighty Trigonioida (Bivalvia: Palaeoheterodonta). *Neotrigonia* is the presumed sister-group to all freshwater mussels (Thiele, 1934; Taylor *et al.*, 1969; Healy, 1989; Hoeh *et al.*, 1998, 2001, Graf & Ó Foighil, 2000; but see Newell & Boyd, 1975 and Morton, 1987; see also **Chapter 4**).

Thirty-eight characters were coded from the literature and corroborated by personal examination of specimens deposited in the University of Michigan Museum of Zoology, Ann Arbor, Michigan, USA. The specimens examined and accompanying literature references are listed in **Table 2.2**, and many of those shells were figured in **Chapter 1**. The characters analyzed include those of the shell (1-11), gross soft-anatomy (12-22), brooding and life history (23-34), and larval morphology (35-38), and these are principally the characters deemed significant by previous mussel systematists (*e.g.*, Ortmann, 1912b; Parodiz & Bonetto, 1963; Heard & Guckert, 1971). Character diagnoses are discussed in **Appendix III**.

Table 2.3 shows the analyzed matrix. Inapplicable characters (*e.g.*, marsupial characters in non-brooding taxa, hinge characters in edentulous taxa, *etc.*) were coded as dashes ('-'), and missing data with question marks ('?'). When a particular character varied intragenerically, the character state of the type species was given precedence over assuming monophyly of the genus and coding the character as polymorphic. Phylogenetic analyses (branch-and-bound) were performed with PAUP* 4.0b3 (Swofford, 1998). Dashes were treated as missing data. *Neotrigonia* was designated as the outgroup, and the ingroup was constrained to be monophyletic in order to root the phylogeny. Character transformation series were traced using MacClade 3.07 (Maddison & Maddison, 1997) and PAUP*. To gauge the 'robustness' of each node, Bremer-Decay Index (Bremer, 1995) values were calculated using TreeRot (Sorenson, 1999), and a

jackknife resampling analysis (200 replicates, heuristic searches with 10 random sequence additions each) was performed using PAUP*.

Results

Parsimony analysis recovered a single, most-parsimonious tree 62 steps long (25 parsimony informative characters, *CI* = 0.625, *RC* = 0.561) (**Figure 2.1**). The Margaritiferidae (= *Margaritifera*) is sister to the remaining Unionoida. The other families are each recovered as monophyletic with the exceptions of the "Unionidae" and the "Mycetopodidae" (= Etheriidae; see below). The Hyriidae is sister to the Etherioidea *sensu* Parodiz & Bonetto (1963), not the "Unionoidea." Thus, cladistic analysis of the characters traditionally employed to diagnose unionoid taxa rejects the hypothesis that glochidia diagnose a monophyletic clade composed of the Margaritiferidae, Unionidae, and Hyriidae. The jackknife 50% consensus tree (not shown) differs from **Figure 2.1** in that the unionids collapse into a polytomy. The (Hyriidae + (Iridinidae + Etheriidae)) clade, however, is resolved by the jackknife analysis. **Figure 2.2** illustrates all character transformations, and these are also described in **Table 2.4**.

The recovered phylogeny finds no support for a monophyletic "Mycetopodidae" (**Figure 2.1**). Heard & Vail (1976a), discovering no soft-anatomical synapomorphies to distinguish *Etheria* from the mycetopodids, synonymized Etheriidae with Mycetopodidae. Kabat (1997) demonstrated that, of the two, Etheriidae has priority. Parodiz & Bonetto (1963) recognized several subfamilies within the "Mycetopodidae," but the monophyly of these nor their interrelationships have not been tested.

Discussion

The phylogenetic reconstruction of Parodiz & Bonetto (1963; also Haas, 1969a, b; Heard & Guckert, 1971) divided the Unionoida into two groups based solely upon larval

type. That hypothesis is rejected by the present analysis of the morphological characters considered essential by previous mussel systematists. In the most parsimonious reconstruction, glochidium-type parasitic larvae are considered synapomorphic at the ordinal level — a glochidium is the plesiomorphic, parasitic larval stage among the Unionoida. In the lineage leading to the (Iridinidae + Etheriidae) clade (= Etherioidea *sensu* Parodiz and Bonetto), the glochidium was modified into a lasidium (**Figure 2.2**). This is in direct contradiction to Parodiz & Bonetto (1963) who could not imagine one larval type being derived from the other (see quote above). The definition of the Etherioidea should be expanded to include the Hyriidae.

The Etherioidea *sensu lato* [= (Hyriidae + (Iridinidae + Etheriidae))] is synonymous with the Mutelidae of Ortmann (1912b, 1921a), Thiele (1934), and McMichael & Hiscock (1958). That clade is diagnosed by at least three morphological synapomorphies:

(1) pallial closure above the excurrent aperture [character 18, see **Table 2.4** and **Appendix III**],

(2) attachment of the inner demibranchs to the visceral mass near to or in contact with the labial palps [15], and

(3) pallial fusion between the incurrent and excurrent apertures creating a complete diaphragm [17] (**Figure 2.2**).

Given the condition of these characters in *Neotrigonia* (discussed in **Chapter 7**), each of these characters would have had to evolve twice — once as a synapomorphy of the Hyriidae, once as a synapomorphy of (Iridinidae + Etheriidae) — under Parodiz & Bonetto's (1963) scenario of unionoid evolution. According to the present analysis, the tendency for complete fusion of the inner demibranchs to the visceral mass [14] and parasitism via a glochidium [35, 36] are plesiomorphic; they define natural taxa with levels of universality higher than the Etherioidea.

Endobranchy (the use of only the inner demibranchs for brooding) is also plesiomorphic among the Etherioidea, as suggested by the present analysis (**Figure 2.2**, character 25). This result, however, hinges on the position of *Grandidieria* and the apparent paraphyly of the Unionidae (**Figure 2.1**). While parsimony analysis of this data set weakly supports the paraphyly of the unionoideans, more extensive molecular phylogenetic analyses support the monophyly of that group (*e.g.*, Graf & Ó Foighil, 2000; **Chapter 3**; but see **Chapter 6**). Presuming the Unionidae are monophyletic, the endobranchous condition of *Grandidieria*, as well as certain other unionids (*e.g.*, *Moncetia*; Kondo, 1984), should not be considered homologous with that of the Etherioidea. Thus, endobranchy may be a fourth synapomorphy diagnosing the (Hyriidae + (Iridinidae + Etheriidae)) clade.

The inclusion of the Hyriidae among the Etherioidea is circumstantially supported by the biogeography of the superfamily. The Unionoida is an ancient group, the extant families extending perhaps as far back as the Triassic (Henderson, 1935; Haas, 1969b; Good, 1998; Watters, 2001). It is expected that the evolution of an ancient, continental taxon should reflect the break up of Pangaea. This expectation is realized in the phylogeny recovered here. While there is evidence for Mesozoic etherioideans in North America (Pilsbry *in* Wanner, 1921; Morris & Williamson, 1988), the present-day Etherioidea are limited to the southern continents of the former Gondwana, and unionoideans occur almost exclusively on the northern continents (**Table 2.1**). Etherioideans and unionoideans are sympatric only in areas of secondary contact: Central America, Africa, and India. The problem of hyriid disjunction across the Pacific Ocean is dealt with in **Chapter 5**.

The results of this analysis bear directly upon the taxonomy of the Unionoida. A revised system of the Etherioidea, based on this and other studies, is presented in **Table 2.5**. An important aspect of this classification — equally as important as the hierarchical arrangement of taxa within it — is its falsifiability. It is based upon explicit statements of

character homology (**Appendix III**) and the single, best-corroborated pattern of nested synapomorphies (**Figure 2.1**). However, further testing is necessary. Recent results (Rosenberg *et al.*, 1994, 1997; Lydeard *et al.*, 1996; Hoeh *et al.*, 1998, 2001; Graf & Ó Foighil, 2000, 2001) demonstrate the suitability of molecular characters to test hypotheses of freshwater mussel phylogeny and morphological character evolution with a large, independent data set. Combined-matrices of DNA sequences and anatomical data from all life history stages coupled with global taxon sampling may further refine these hypotheses of the pattern of evolution among the Etherioidea. Molecular phylogenetic analyses of both the Unionoida and the Etherioidea are the subjects of the following analytical chapters.

Table 2.1. Summary of the Parodiz & Bonetto (1963) Suprageneric Taxonomy of the Unionoida. The genera listed are those included in the present phylogenetic analysis. This scheme has been followed in most subsequent classifications (See **Table 1.2** and **Appendix II**), but it represents a shift from the view before the work of Parodiz & Bonetto (1963) that the Hyriidae should be placed among the Etherioidea (Ortmann, 1912b, 1921a; Thiele, 1934; McMichael & Hiscock, 1958). Also provided are the large-scale distributions of the genera analyzed (Brown & Lomolino, 1998).

Taxon	Distribution
Unionoidea	
Unionidae	
Unio	Palearctic - Ethiopian
Elliptio	Nearctic
Lampsilis	Nearctic
Pyganodon	Nearctic
Parreysia	India
Grandidieria	Ethiopian
Margaritiferidae	
Margaritifera	Holarctic
Hyriidae	
Castalina	Neotropical
Diplodon	Neotropical
Hyridella	Australian
Velesunio	Australian

Table 2.1 (continued). Summary of the Parodiz & Bonetto (1963) Suprageneric Taxonomy of the Unionoida.

Taxon	Distribution
Etherioidea (= Muteloidea)	
Etheriidae	
Etheria	Ethiopian
Acostaea	Neotropical
Mycetopodidae	
Anodontites	Neotropical
Mycetopoda	Neotropical
Iridinidae (= Mutelidae)	
Mutela	Ethiopian
Iridina (= *Pleiodon*)	Ethiopian

Table 2.2. Genera Analyzed, Specimen Lots Examined, and Relevant Literature References to Anatomical Descriptions of Larvae and/or Adults. Genera are listed alphabetically. All specimens are deposited in the University of Michigan Museum of Zoology (UMMZ), Ann Arbor, Michigan. A dagger ('†') identifies the type species of each genus. Many of the specimens examined were figured in **Chapter 1**.

Acostaea d'Orbigny, 1851. — †*A. rivoli* (Deshayes, 1827) UMMZ 112660 Amazon River, South America. — Yonge (1978), Arteaga (1994).

Anodontites Bruguière, 1792. — †*A. crispata* (Brug., 1792) UMMZ 112391 upper Daule River, Ecuador. *A. trapesialis* (Lam., 1819) UMMZ 112429 Rio Grande do Sul, Brazil. — Ortmann (1921a), Bonetto & Ezcurra (1962, 1965b).

Castalina von Ihering, 1891. — †*C. martensi* (von Ihering, 1891) UMMZ 110907 Rio Grande do Sul, Brazil. *C. undosa* (von Martens, 1885) UMMZ 110905 Sao Paulo, Brazil. — Ortmann (1911a, 1921a), Bonetto (1961b).

Diplodon Spix, 1827. — †*D. ellipticum* (Spix, 1827) none examined. *D. paranensis* (Lea, 1834) UMMZ 248835 Buenos Aires, Argentina. *D. fontainianus* (d'Orbigny, 1835) UMMZ 111280 Rio Grande do Sul, Brazil. — Ortmann (1921a), Bonetto (1951, 1961a, 1962a).

Elliptio Rafinesque, 1819. — †*E. crassidens* (Lam., 1819) UMMZ 129451 Mississippi River, Wabasha Co., Minnesota, USA. *E. dilatata* (Rafinesque, 1820) UMMZ 205584 Kalamazoo River, Jackson Co., Michigan, USA. — Ortmann (1912b), Baker (1928).

Etheria Lamarck, 1807. — †*E. elliptica* (Lam., 1807) UMMZ 43223 White Nile River, Africa. — Yonge (1962), Heard & Vail (1976a).

Table 2.2 (continued). Genera Analyzed, Specimen Lots Examined, and Relevant Literature References to Anatomical Descriptions of Larvae and/or Adults.

Grandidieria Bourguignat, 1885. — †*G. burtoni* (Woodward, 1859) UMMZ 248778 Lake Tanganyika, Zambia; UMMZ 110105 Lake Tanganyika, Zaire. — Bloomer (1933), Kondo (1990).

Hyridella Swainson, 1840. — †*H. australis* (Lam., 1819) UMMZ 111296 Australia. *H. depressa* (Lam., 1819) UMMZ 111827 Paramalta River, New South Wales, Australia. — McMichael & Hiscock (1958), Jones *et al.* (1986), Jupiter & Byrne (1997).

Iridina Lamarck, 1819 [= *Pleiodon* Conrad, 1834]. — †*I. exotica* Lam., 1819 UMMZ 111973 Africa. *I. ovatus* Swainson, 1823 UMMZ 112006 Senegal. — Heard & Dougherty (1980).

Lampsilis Rafinesque, 1820. — †*L. ovata* (Say, 1817) [= *L. cardium* Raf., 1820] UMMZ 50637 St. Joseph River, Berrien Co., Michigan, USA; UMMZ 130005 Mississippi River, Wabasha Co., Minnesota, USA. — Ortmann (1912b), Baker (1928), Kraemer (1970).

Margaritifera Schumacher, 1816. — †*M. margaritifera* (Linn., 1758) UMMZ 4338 St. Lawrence River, New York, USA. *M. hembeli* (Conrad, 1838) UMMZ 107633 Alexandria, Rapides Parish, Louisiana, USA. — Ortmann (1911b, 1912b), Smith (1979).

Mutela Scopoli, 1777. — †*M. dubia* (Gmelin, 1793) UMMZ 111979 Nile River, Africa. *M. nilotica* (Cailliaud, 1823) UMMZ 111984 Mahmoudich, Egypt. — Bloomer (1932), Fryer (1954, 1961).

Table 2.2 (continued). Genera Analyzed, Specimen Lots Examined, and Relevant Literature References to Anatomical Descriptions of Larvae and/or Adults.

Mycetopoda d'Orbigny, 1835. — †*M. soleniformis* d'Orbigny, 1835 none examined. *M. siliquosa* (Spix, 1827) UMMZ 112645 Marañon, Peru. — Ortmann (1921a), Bonetto (1962b), Bonetto & Ezcurra (1965b).

Neotrigonia Cossmann, 1912. — †*N. pectinata* (Lam., 1819) none examined. *N. margaritacea* (Lam., 1804) UMMZ 253004 Tasmania. — Tevesz (1975), Morton (1987), Darragh (1998), Ó Foighil & Graf (2000).

Parreysia Conrad, 1853. — †*P. corrugata* (Müller, 1774) UMMZ 110263 Sri Lanka. — Ortmann (1910b, 1911a), Pilsbry & Bequaert (1927).

Pyganodon Crosse & Fischer, 1894. — †*P. grandis* (Say, 1829) UMMZ 205535 Portage Creek, Kalamazoo Co., Michigan, USA. *P. cataracta* (Say, 1817) UMMZ 101840 Framingham, Middlesex Co., Massachusetts, USA. — Ortmann (1912b), Baker (1928).

Unio Philipsson, 1788. — †*U. pictorum* (Linn., 1758) UMMZ 79230 Birmingham, England; UMMZ 79213 River Saale, Jena, Germany. *U. caffer* Krauss, 1848 UMMZ 60409 Irene, Transvaal, South Africa; UMMZ 234710 Lundi River, 14 mi S Chiredzi, Zimbabwe. — Ortmann (1912b, 1918a, b), Heard and Vail (1976b).

Velesunio Iredale, 1934. — †*V. ambigua* (Philippi, 1847) UMMZ 111839 Murray River, Australia. — Ortmann (1912c), McMichael & Hiscock (1958), Bonetto & Ezcurra (1965a).

Table 2.3. Adult and Larval Unionoid Morphology Data Matrix. *0* indicates the hypothesized plesiomorphic condition of the Palaeoheterodonta. Character diagnoses can be found in **Appendix III**.

	1 2 3 12345678901234567890123456789012345678
Neotrigonia	001000000-00000000--0000---0------0---
Margaritifera	01-0010010010000000--00110--00-0000100-
Pyganodon	01-2--0010111000010-001120-1210100101-
Lampsilis	0000000010111100010-011121-1200111100-
Elliptio	0000110010111000011-001120-1200000100-
Unio	0000000010111000010-001120-1100000101-
Parreysia	0001000011111100010-00110--1?00000100-
Grandidieria	0000000001111100010-00111-011000002---
Hyridella	0001000011111111012-100111-12100000101-
Velesunio	000100001011111012-100111-12100000101-
Diplodon	0001000011111111012-000111-10100000101-
Castalina	0010110011111111112-000111-10100000101-
Mutela	01-2--001011111112-000111-0120100011-0
Pleiodon	1-----001011111112-000111-01201000????
Anodontites	01-2--001011111010-000111-0120100011-1
Mycetopoda	01-2--001011111010-000111-0120100011-1
Etheria	01-2--10--11111010-010111-01201000????
Acostaea	01-2--11--11111010-010111-0120100011-1

Table 2.4. Character Transformations Suggested by this Phylogenetic Analysis. The character numbers are the same as in **Table 2.3**. An *s* refers to the number of transformations (*i.e.*, steps) each character undergoes; *CI* and *RC* are the Consistency and Rescaled Consistency Indices, respectively. A dagger ('†') indicates that the *RC* is taken to be unity when the Retention Index is undefined (Farris, 1989). Nomenclature follows the taxonomy listed in **Table 2.5**, and character transformations are mapped in **Figure 2.2**

1. Synapomorphy of *Iridina* (*s* = 1, *CI* = 1.0, *RC* = 1.0†).
2. There are three independent reductions in *Margaritifera*, *Pyganodon*, and (Iridinidae, Etheriidae) (*s* = 3, *CI* = 0.333, *RC* = 0.222).
3. Arises as independent synapomorphies of both *Neotrigonia* and *Castalina* (*s* = 2, *CI* = 0.500, *RC* = 0).
4. Independent synapomorphies of *Parreysia* and (*Diplodon,* (*Hyridella*, *Velesunio*)), with two independent transformations to *2* in *Pyganodon* and (Iridinidae, Etheriidae) (*s* = 4, *CI* = 0.500, *RC* = 0.375).
5. Arises as independent synapomorphies of both *Elliptio* and *Castalina* (*s* = 2, *CI* = 0.500, *RC* = 0).
6. Arises as independent synapomorphies of *Margaritifera*, *Elliptio*, and *Castalina* (*s* = 3, *CI* = 0.333, *RC* = 0).
7. Synapomorphy of (*Etheria*, *Acostaea*) (*s* = 1, *CI* = 1.0, *RC* = 1.0).
8. Autapomorphy of *Acostaea* (*s* = 1, *CI* = 1.0, *RC* = 1.0†).
9. Synapomorphy of the Unionoida with an reversal to *0* in *Grandidieria* (*s* = 2, *CI* = 0.500, *RC* = 0).
10. Synapomorphy of (*Parreysia*, (*Grandidieria*, Etherioidea)), with independent reversions to *0* in *Velesunio* and (Iridinidae, Etheriidae) (*s* = 3, *CI* = 0.333, *RC* = 0.167).

Table 2.4 (continued). Character Transformations Suggested by this Phylogenetic Analysis.

11.	Synapomorphy of ("Unionidae," Etherioidea) (*s* = 1, *CI* = 1.0, *RC* = 1.0).
12.	Synapomorphy of the Unionoida. (*s* = 1, *CI* = 1.0, *RC* = 1.0†).
13.	Synapomorphy of ("Unionidae," Etherioidea) (*s* = 1, *CI* = 1.0, *RC* = 1.0).
14.	Arises independently as synapomorphies of (*Parreysia*, (*Grandidieria*, Etherioidea)) and *Lampsilis* (*s* = 2, *CI* = 0.500, *RC* = 0.375).
15.	Synapomorphy of the Etherioidea (*s* = 1, *CI* = 1.0, *RC* = 1.0).
16.	Arises independently as synapomorphies of the Iridinidae and *Castalina* (*s* = 2, *CI* = 0.500, *RC* = 0.250).
17.	Synapomorphy of the Etherioidea (*s* = 1, *CI* = 1.0, *RC* = 1.0).
18.	Synapomorphy of ("Unionidae," Etherioidea). Transformation to *2* in the Etherioidea and reversion to *0* in the Etheriidae (*s* = 3, *CI* = 0.667, *RC* = 0.600).
19.	Synapomorphy of *Elliptio* (*s* = 1, *CI* = 1.0, *RC* = 1.0†).
20.	Synapomorphy of (*Hyridella*, *Velesunio*) (*s* = 1, *CI* = 1.0, *RC* = 1.0).
21.	Synapomorphy of (*Etheria*, *Acostaea*) (*s* = 1, *CI* = 1.0, *RC* = 1.0).
22.	Synapomorphy of *Lampsilis* (*s* = 1, *CI* = 1.0, *RC* = 1.0†).
23.	Synapomorphy of the Unionoida (*s* = 1, *CI* = 1.0, *RC* = 1.0†).
24.	Synapomorphy of the Unionoida (*s* = 1, *CI* = 1.0, *RC* = 1.0†).
25.	Synapomorphy of (*Grandidieria*, Etherioidea), and *2* is a synapomorphy of (*Unio,* (*Elliptio* (*Lampsilis*, *Pyganodon*))) (*s* = 2, *CI* = 1.0, *RC* = 1.0).
26.	Synapomorphy of *Lampsilis* (*s* = 1, *CI* = 1.0, *RC* = 1.0†).
27.	Synapomorphy of Hyriidae (*s* = 1, *CI* = 1.0, *RC* = 1.0).
28.	Synapomorphy of ("Unionidae," Etherioidea). Reversion to *0* in the Hyriidae, but with *2* a synapomorphy of (*Hyridella*, *Velesunio*) (*s* = 3, *CI* = 0.667, *RC* = 0.500).

Table 2.4 (continued). Character Transformations Suggested by this Phylogenetic Analysis.

29.	Synapomorphy of ("Unionidae," Etherioidea), with independent transformations to *2* in (*Elliptio*, (*Lampsilis*, *Pyganodon*)) and (Iridinidae, Etheriidae) (*s* = 3, *CI* = 0.667, *RC* = 0.533).
30.	Synapomorphy of *Pyganodon* (*s* = 1, *CI* = 1.0, *RC* = 1.0†).
31.	Synapomorphy of (Iridinidae, Etheriidae) (*s* = 1, *CI* = 1.0, *RC* = 1.0).
32.	Synapomorphy of (*Pyganodon*, *Lampsilis*) (*s* = 1, *CI* = 1.0, *RC* = 1.0).
33.	Synapomorphy of *Lampsilis* (*s* = 1, *CI* = 1.0, *RC* = 1.0†).
34.	Synapomorphy of *Lampsilis* (*s* = 1, *CI* = 1.0, *RC* = 1.0†).
35.	Synapomorphy of the Unionoida, with a transformation to *2* in *Grandidieria* (*s* = 2, *CI* = 1.0, *RC* = 1.0†).
36.	Synapomorphy of (Iridinidae, Etheriidae) (*s* = 1, *CI* = 1.0, *RC* = 1.0).
37.	Arises as independent synapomorphies of in *Pyganodon*, *Unio*, and the Hyriidae (*s* = 3, *CI* = 0.333, *RC* = 0.111).
38.	Synapomorphy of the Etheriidae (*s* = 1, *CI* = 1.0, *RC* = 1.0†).

Table 2.5. Revised Taxonomy of the Etherioidea. The classification has been compiled from the results of this study as well as various syntheses. Although *Leila* was not included in the present analysis, what is known of its anatomy suggests that it may belong among the Iridinidae (Parodiz & Bonetto, 1963; Bonetto, 1963). This analysis does not support a monophyletic Hyriinae. However, these data are insufficient to revise the taxonomy of the Hyriidae. That topic is addressed in **Chapter 5**.

Etherioidea Deshayes, 1830

Etheriidae *s.s.*

- Etheriinae *s.s.* — *Etheria* Lamarck, 1807; *Acostaea* d'Orbigny, 1851; *Pseudomulleria* Anthony, 1907. (Heard & Vail, 1976a; Yonge, 1978)
- Mycetopodinae Gray, 1840 — *Mycetopoda* d'Orbigny 1835; *Mycetopodella* Marshall, 1927. (Parodiz & Bonetto, 1963)
- Monoconylaeinae Modell, 1942 — *Monocondylaea* d'Orbigny, 1835; *Haasica* Stans, 1932; *Fossula* Lea, 1870; *Tamsiella* Haas, 1931. (Parodiz & Bonetto, 1963)
- Anodontitinae Modell, 1942 — *Anodontites* Bruguière, 1792.

Iridinidae Swainson, 1840

- Iridininae *s.s.* — *Mutela* Scopoli, 1777; *Aspatharia* Bourguignat, 1885 [= *Spathopsis* Simpson, 1900]; *Iridina* Lamarck, 1819 [= *Pleiodon* Conrad, 1834]. (Haas, 1969b)
- ?? Leilinae Morretes, 1949 — *Leila* Gray, 1840.

Hyriidae Swainson, 1840

- Hyriinae *s.s.* (Parodiz & Bonetto, 1963)
  - Hyriini *s.s.* — *Prisodon* Schumacher, 1817 [= *Hyria* Lamarck, 1819]; *Paxyodon* Schumacher, 1817.

Table 2.5 (continued). Revised Taxonomy of the Etherioidea.

Castaliini Parodiz & Bonetto, 1963 — *Castalia* Lamarck , 1819; *Castalina* von Ihering, 1891; *Callonaia* Simpson, 1900; *Castaliella* Simpson, 1900.

Diplodontini Parodiz & Bonetto, 1963 — *Diplodon* Spix, 1827; *Diplodontites* Marshall, 1922.

Velesunioninae Iredale, 1934 — *Velesunio* Iredale, 1934; *Microdontia* Tapparone-Canefri, 1883; *Alathyria* Iredale, 1934; *Westralunio* Iredale, 1934. (McMichael & Hiscock, 1958)

Lortiellinae Iredale, 1934 — *Lortiella* Iredale, 1934.

Hyridellinae Iredale, 1934 — *Hyridella* Swainson, 1840.

Cucumerunioninae Iredale, 1934 — *Cucumerunio* Iredale, 1934; *Virgus* Simpson, 1900.

Figure 2.1. Single Tree Recovered by Parsimony Analysis. Tree statistics: 62 steps, *CI* = 0.625, *RC* = 0.561. Taxonomy reflects the classification listed in **Table 2.1**. The "Unionidae," "Mycetopodidae," and "Unionoidea" are shown not to be monophyletic. The revised taxonomy of the Etherioidea is listed in **Table 2.5** and depicted in **Figure 2.2**. Numbers above the branches indicate branch lengths, those below are jackknife and Bremer-Decay Index values, respectively. BDI <2 are not shown.

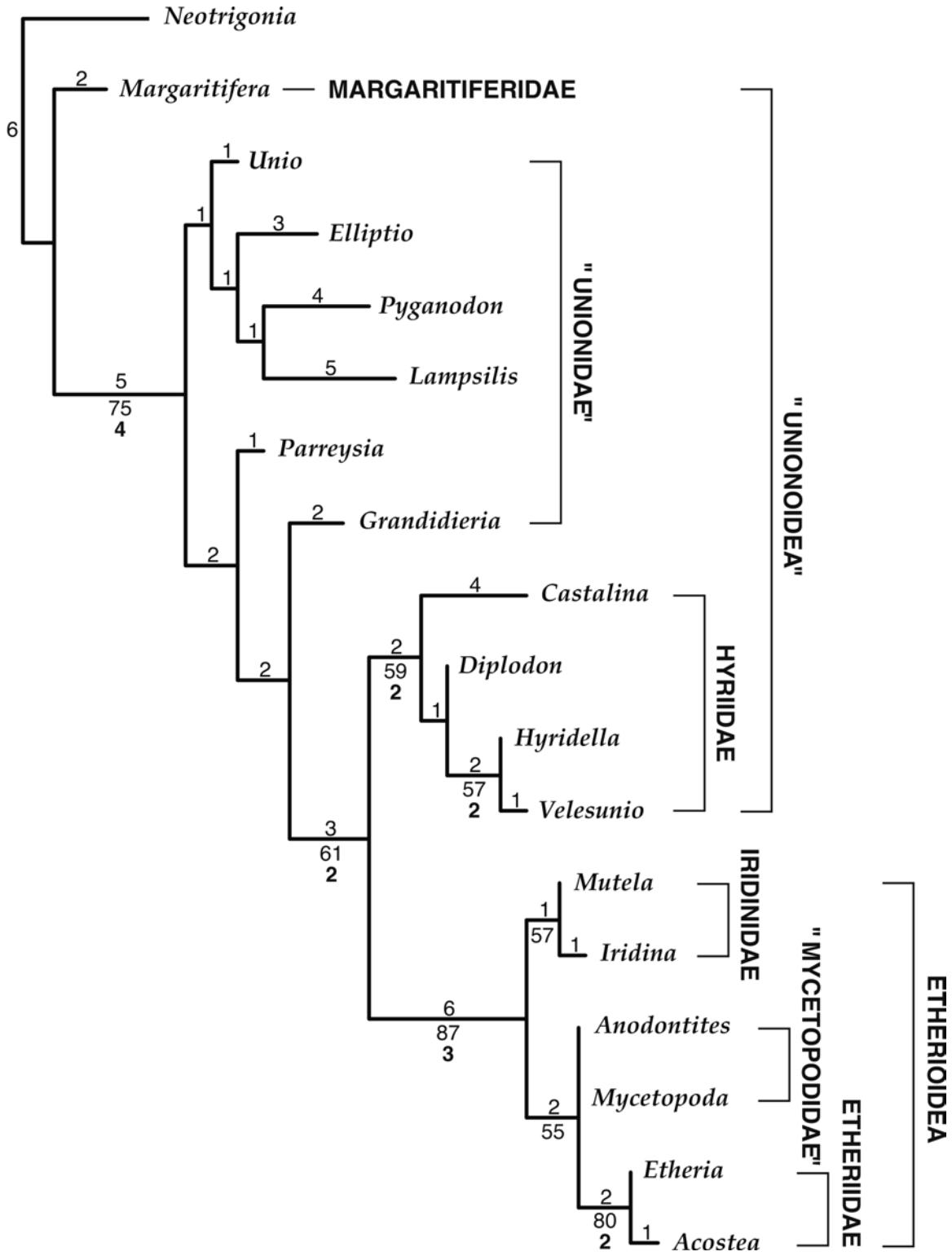


Figure 2.1. Most Parsimonious Tree

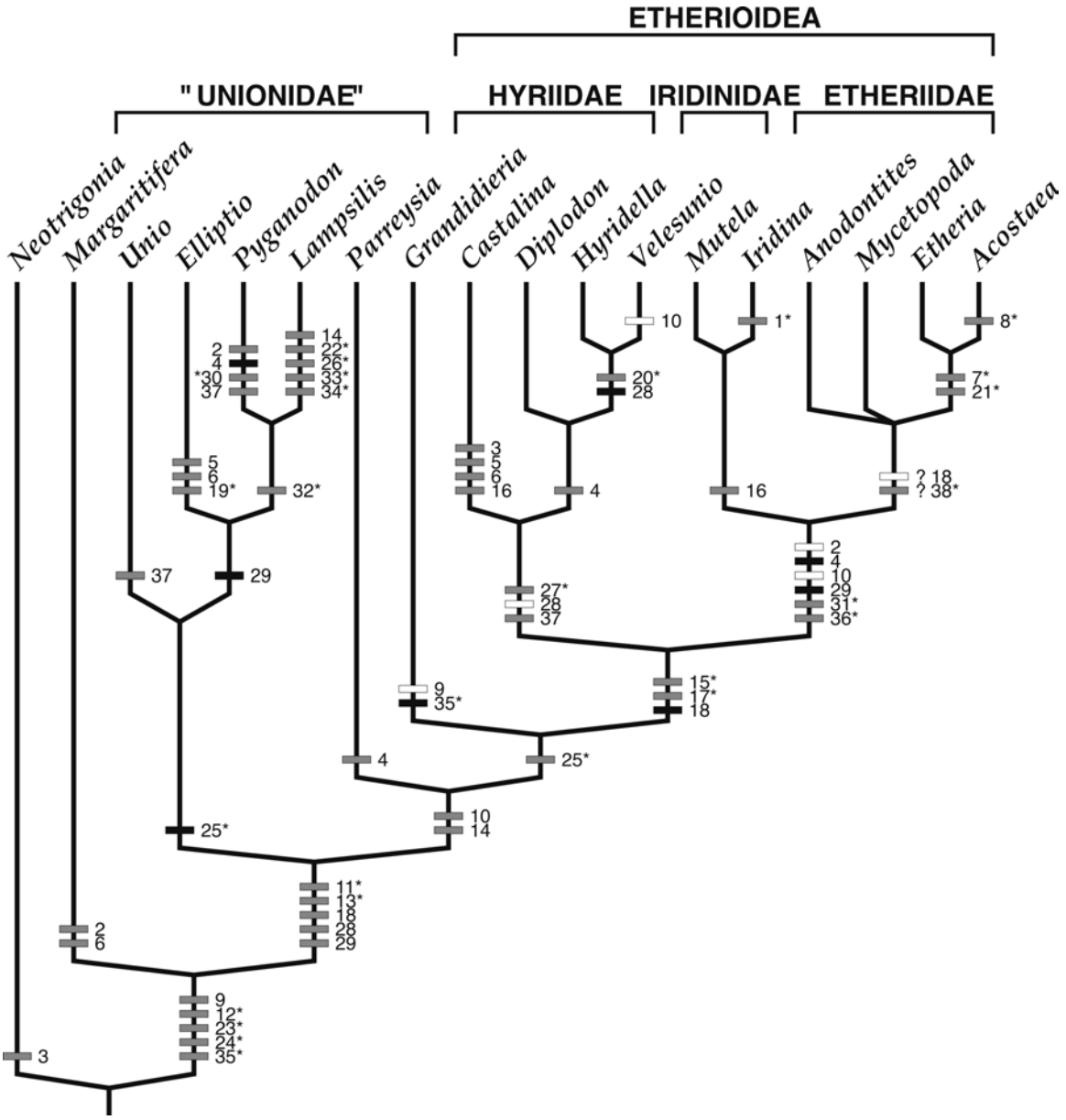


Figure 2.2. Cladogram Depicting Character Transformations and the Revised Taxonomy of the Etherioidea. Character numbers refer to those listed in **Tables 2.3-4**. Shaded boxes indicate character acquisition (gray and black for states *1* and *2*, respectively), white boxes identify proposed character losses (character state *0*). Unambiguous character transformations (*CI* = 1.0) are labeled with an asterisk ('*').

CHAPTER 3

A COMPARISON OF NUCLEAR AND MITOCHONDRIAL DATA SETS FOR THE RECOVERY OF THE PHYLOGENY OF THE UNIONOIDA

The analyses presented in **Chapter 2** demonstrated that for the character set available at this time, morphology seems to be of little value for recovering phylogenetic patterns among the Unionoidea *s.l.* (= Margaritiferidae + Unionidae). While a few previous studies have attempted to improve the resolution of freshwater mussel intergeneric relationships by applying molecular characters (*e.g.*, Rosenberg *et al.*, 1994, 1997; Lydeard *et al.*, 1996; Graf & Ó Foighil, 2000; Hoeh *et al.*, 2001), each of these analyses presented shortcomings that weakened their conclusions. The objective of this chapter will be to evaluate the effectiveness of two molecular data sets derived from different freshwater mussel genomes for recovering family-group-level phylogenies among the Unionoida. I have addressed this topic previously, although in a different context, in Graf & Sparks (2000).

One of the most under-appreciated aspects of molecular phylogenetic analyses is the choice of data set. There has been much debate in the literature with regard to the trade-offs inherent in phylogenetic analyses — *e.g.*, Is it better to have lots of taxa or lots of characters (see Graybeal, 1998; Naylor & Brown, 1998; Hillis, 1998)? This wide interest in the *quantity* of phylogenetic characters has not carried over into concern for the *quality* of phylogenetic characters. As should be obvious, no single DNA fragment should be applicable across all levels of phylogenetic inquiry, and it is up to the molecular systematist to choose an appropriate 'gene' for the hypotheses that he or see is testing. However, most molecular systematists, when concerned at all, seem content to

base their phylogenetic quality-estimates on the 'hierarchical structure' of the data set through 'skewness' (*e.g.*, g_1 statistic) or permutation test (*e.g.*, PTP) analyses (Swofford *et al.*, 1996). Statistics like these, however, may be too liberal and over-look certain well-known shortcomings of data sets toward particular questions.

The problem of 'saturation' among molecular characters is among these well-known shortcomings. Because (1) substitutions in the DNA sequence are Markovian processes and (2) there is no way, except through phylogenetic analysis, to deduce homology among apparently identical states at the same position, the initial alignment of DNA sequences is often tainted with homoplasies (*i.e.*, multiple transformations to the same character state at the same site in the DNA alignment). As character transformations of higher generality are over-written by younger substitutions, the data set becomes saturated. That is, there has been *too much* change to recover the deeper relationships of the taxa being studied — there is hierarchical structure to the characters, but not for all levels of the hierarchy.

As a molecular systematist and freshwater malacologist, the question for me thus becomes, "Are there higher quality character sets for the Unionoida than have been previously applied?" The most comprehensive analyses of freshwater mussel intergeneric relationships are the mitochondrial (mtDNA) studies of the Unionidae and Margaritiferidae by Lydeard *et al.* (1996) and Graf & Ó Foighil (2000) using the large ribosomal subunit (16S) and cytochrome oxidase subunit I, respectively. The topological results of phylogenetic analyses upon these two data sets largely concurred. However, the branch support, based upon bootstrap analysis, was weak for deeper, family-level nodes. While Lydeard *et al.* (1996: 1602) reported that "No substantial degree of saturation was indicated" by their tests, Graf & Ó Foighil (2000) suggested that perhaps saturation was a confounding factor in their study.

Besides these mitochondrial phylogenetic analyses, which potentially exhibited a more rapid rate of nucleotide substitution, there were the earlier analyses of Rosenberg *et*

al. (1994, 1997). Rather than a fragment of mtDNA, these studies employed a short fragment (domain 6) of the large, nuclear ribosomal subunit (28S). As regards the relationships of unionoids in their studies, there was not nearly the number of phylogenetically informative characters to resolve the intergeneric relationships of freshwater mussel genera.

In order to test if the substitution rate of mtDNA is the most appropriate of the gene fragments that are easily available for recovering the relationships among the Nearctic Unionoidea (= Margaritiferidae + Unionidae), I compared the phylogenetic performance of COI to that of 28S, domain 2. 28S (D2) has been applied to the phylogeny of similarly divergent heterodont bivalves by Park & Ó Foighil (2000). I predicted that if COI is as appropriate or better than 28S (D2) for recovering the family-group-level relationships among the Unionidae and Margaritiferidae of North America:

(1) COI should display a degree of saturation less than or equal to 28S;

(2) if COI is appropriate for recovering these relationships at all (*i.e.*, not just relative to 28S), the majority of characters should trace to the branches in question (*i.e.*, the inter-family-group branches); and

(2) branch support values, as measured by jackknifing and decay indices, for the COI analysis should be higher or comparable to those for 28S.

Methods & Materials

Acquisition of Nucleotide Sequences

Cytochrome oxidase subunit I (COI) mtDNA and nuclear 28S domain 2 [28S (D2)] rDNA sequences were obtained from 28 unionoid species following the methods described in **Appendix IV**. I had previously published many of these sequences (Graf & Ó Foighil, 2000, 2001), but I also acquired several novel unionoid sequences (COI n = 6,

28S n = 13) for these analyses. In addition to the Unionoida sequences, a novel 28S sequence was also acquired for *Neotrigonia margaritacea*; a COI sequence for *N. margaritacea* was available on GENBANK (National Center for Biotechnology Information, National Institutes of Health). GENBANK sequences were also available for three outgroup bivalves. The taxa employed in these analyses are listed in **Table 3.1**, and the relevant GENBANK accession numbers are listed in **APPENDIX IV**.

Initially, for the novel sequences, only females were utilized as sources of mtDNA in order to avoid potential complications associated with doubly-uniparental mitochondrial inheritance among freshwater mussels (Hoeh *et al*., 1996c). Once heteroplasmy was determined not to be a problem for direct sequencing of somatic tissue (*e.g.*, foot, mantle), males and mussels of undetermined sex were also included. Multisequence alignments were compiled and manipulated using Sequence Monkey 2.8 (Graf, 2000b) and Clustal_X (Thompson *et al.*, 1994, 1997), and these were refined manually where necessary.

Phylogenetic Analyses

Three molecular data sets were analyzed under maximum parsimony (MP): Combined (28S + COI), 28S solo, and COI solo. MP analyses of each data set (heuristic searches, 100 random sequence additions, tree-bisection-reconnection) were performed unrooted using *PAUP* 4.0b3* (Swofford, 1998). A protocol of iterative reweighting of characters based on their Rescaled Consistency Index (*RC*) was followed (Farris, 1969, 1989) when multiple most-parsimonious topologies were recovered. In all analyses, *Ostrea, Rangia,* and *Mercenaria* were designated as outgroups.

Skewness statistics (g_1) and PTP tests for each data set were conducted using PAUP*.

Saturation Analyses

Two different analyses were undertaken to illustrate the degree of saturation in the two data sets, COI and 28S.

The first analysis illustrated the difference in the degrees of divergence among pairwise comparisons for each of the two data sets. For both COI and 28S, PAUP* was used to determine the absolute number of character differences for each pairwise comparison of *Unio pictorum* and half the other taxa, and *Villosa iris* and the other half. For COI, the number of changes was determined for each codon position separately. The COI differences were plotted against those of 28S.

The second analysis was not independent of phylogeny (unlike the first) — that is, no tree search was necessary, nor does it allow a direct comparison of saturation levels. Instead, for each of the same pairwise comparisons of taxa as the previous analysis, the number of absolute differences was plotted against the number of MP steps between each pair. For both COI and 28S, the number of MP transformations was counted over the topology of the Combined (COI + 28S) phylogeny. The difference between the absolute changes and the MP steps between each pair of species represents a minimum estimate of the degree of saturation.

Character Distributions

The number of misinformative characters was estimated as the sum of the characters traced, under the accelerated transformation (ACCTRAN) model, to terminal branches having a $CI < 1.00$, minus the total number of uninformative characters. The proportion of misinformative transformations of the total estimates the upper bound for the number of characters that contribute only homoplasy to the analysis (Graf & Sparks, 2000).

The 'stemminess' values for the various previously recognized clades were calculated following the methods of Graf & Sparks (2000). The 'stemminess' of a particular clade was quantified as the mean of the supporting branch to terminal branch ratios of all the taxa in a clade (after Fiala & Sokal, 1985; as modified by Salisbury, 1999). The 'stemminess' of a clade gives an indication of the distribution of characters within and supporting the node in question. A low 'stemminess' (*i.e.*, 'leafiness') suggests that there is low support for that particular clade and warns of long-terminal-branch/short-internal-branch problems.

Assessment of Branch Support

To gauge the 'robustness' of the resulting trees, both jackknife resampling and Bremer-Decay Index values were calculated for each data set. Jackknifing (50% character deletion each replication; at least 200 replications, heuristic searches of 10 random additions) provides a rough quantification, as a percentage, of the support throughout the data set for a particular node. Bremer-Decay Indices (BDI) were calculated using TreeRot (Sorenson, 1999), which creates a command file for PAUP*. For each node, BDI indicate the difference in length of the next shortest tree without that node. The greater the BDI, the better the support for that node (Bremer, 1995).

Results

Sequences of both 28S (D2) and COI were obtained for a total of 32 species, including outgroups. These were aligned into a matrix of 1135 total characters (COI nt = 652, 28S nt = 483). Both data sets were skewed, although 28S was more so (COI g_1 = -0.687, 28S g_1 = -1.100), and both data sets contained significant hierarchical structure based on the PTP test ($p < 0.005$ for both). The three cladograms recovered from parsimony analysis of the three data sets are shown in **Figures 3.1-3**. Phylograms

depicting branch lengths are shown in **Figure 3.4**; data set and tree statistics are shown in **Table 3.2**; and levels of support (*i.e.*, jackknife, BDI, and stemminess) are listed for the major clades in **Tables 3.3-4**.

The Combined (28S + COI) phylogeny is shown in **Figure 3.1**. This topology agrees in many respects with the strictly mitochondrial phylogenies published by Lydeard *et al.* (1996) and Graf & Ó Foighil (2000) for the taxa they included. The Palaeoheterodonta (*Neotrigonia* + Unionoida) were found to be monophyletic, and this result supports a sister-group relationship between the Trigonioida and the Unionoida (Thiele, 1934; Taylor *et al.*, 1969; Boss, 1982; Smith, 1983; Healy, 1989, Hoeh *et al.*, 1998, 2001).

According to the Combined analysis, the Unionidae is composed of three clades:

(1) Rectidentini, represented by *Uniandra* in this study;

(2) a (Unionini + Anodontinae) clade composed of ((*Unio* + *Cafferia*) + (*Pyganodon* + *Strophitus* + *Alasmidonta* + *Lasmigona*)); and

(3) a third group composed of the remaining Unionidae.

The latter clade had been further subdivided by Davis & Fuller (1981) into the Lampsilini, Pleurobemini, and "Amblemini." These are synonymous with the Lampsilinae, Pleurobeminae, and "Ambleminae" of Heard & Guckert (1971), respectively. Of these, only the Lampsilini and Pleurobemini are here recovered as monophyletic. No support is found for the familial taxa of Heard & Guckert (1971) or the inclusion of the North American Pleurobemini, *Quadrula*, or *Amblema* among the Unioninae of Ortmann (1912b). The Unionidae is sister to the Margaritiferidae (= *Cumberlandia*), and the two comprise a monophyletic Unionoidea.

The topology of the 28S solo analysis (**Figure 3.2**) differs principally from the Combined analysis in

(1) the lack of resolution among the more-shallowly divergent clades and

(2) the position of Rectidentini (= *Uniandra*) as sister to the (*Gonidea* + "Amblemini" + Pleurobemini + Lampsilini) clade.

The COI topology (**Figure 3.3**) differs in several respects:

(1) the intergeneric relationships among the Lampsilini and Anodontini;

(2) the branching order of *Amblema, Quadrula,* and *Tritogonia*;

(3) the position of *Gonidea* as sister to the Unionini; and

(4) the sister relationship among *Cumberlandia* and (*Gonidea* + Unionini), and, thus, the non-monophyly of the Unionidae,

These differences in topology among the combined and partitioned analyses reflect the ability of the individual mitochondrial and nuclear data sets to resolve different regions of the phylogeny of the freshwater mussels.

Figure 3.5 shows a plot of pairwise COI differences (by codon position) vs. 28S differences. That figure shows that (1) the majority of changes in COI occur in the 3rd codon position, and (2) the rate of COI change slows dramatically and appears to level-off relative to 28S divergence among the same taxa. The secondary axis on that figure charts a 'best-fit' curve for the ratio of total COI: 28S differences over the range of 28S differences. This curve clearly shows that among shallowly divergent freshwater mussels species, COI differences exceed those of 28S by >20x. This proportion drops rapidly as the divergence (as measured by 28S differences) among taxa deepens. This is consistent with a higher degree of saturation in the COI character alignment than that of 28S.

The upper graph in **Figure 3.6** charts the actual, pairwise 28S differences vs. the number of steps between the same pairs of species on the Combined analysis topology. If there were no saturation in this data set (and the Combined topology is a reasonable estimation of the phylogeny of Nearctic Unionidae), the dots would be expected to plot along the diagonal. The dots do not, however, so there is some saturation in the 28S data set. The same can be said for the bottom figure, which shows the analogous comparison for the COI character set. What these graphs clearly show is the difference in the degree

of saturation evident among the two data sets. For 28S, all comparisons among unionids (*i.e.*, all divergences less than or equal to *Unio* vs. *Uniandra*) hold tightly to the diagonal, and the majority of unionid-unionid points exhibit less than 25% saturation. The COI graph, on the other hand, shows that most unionid-unionid points lie to the right of the 25% boundary, and, indeed, they are largely indistinguishable from many of the unionid-non-unionid points. This is consistent with a higher degree of saturation in the COI character set than the 28S data set.

As shown in **Table 3.2**, overall, the majority of COI characters (>60%), whether using the Combined or COI topology, trace to the terminal branches (under ACCTRAN) but they are not uninformative (*i.e.*, $CI < 1.00$); thus, these transformations are misinformative (Graf & Sparks, 2000). The opposite pattern is seen for the 28S MP steps, the minority of which (< 40%) are misinformative. The same basic character distributions are also evident for individual clades on the Combined topology (**Table 3.3**): for nearly all of the clades of interest, 28S has a greater relative proportion of characters supporting clades than within clades than does COI. That is, the 28S topology is generally stemmier for the nodes of interest. That is not to say that all clades on the Combined topology are stemmy with regard to 28S, only that they are *stemmier* than COI (up to 7.9x).

Table 3.4 lists the levels of branch support (BDI and jackknife) for the two character sets for the clades of interest. The overall trend of these values is that branch support indices for 28S tend to be greater for deep nodes, and COI tends to better support more shallow, tribal relationships. For the subfamilial relationships among the Unionidae, the COI partition in the Combined analysis had negative BDI. The negative (Unionidae + Margaritiferidae) BDI for the 28S partition is discussed below.

Discussion

The recent resurgence in interest in the relationships of freshwater mussels has relied upon mtDNA for comprehensive, molecular phylogenetic studies (Hoeh *et al*., 1996a, 2001; Lydeard *et al.*, 1996; Graf & Ó Foighil, 2000). However, the assumption that mtDNA is the most appropriate character set for family-group-level divergences has not heretofore been tested relative to the performance of another data set for the same taxa. The analyses of this chapter sought to test the hypothesis that cytochrome oxidase subunit I mtDNA is just as good or better than domain 2 of 28S for recovering the interfamilial relationships of the Nearctic Unionidae. Three predictions were deduced from this hypothesis regarding the relative degrees of saturation, character distribution, and branch support for the two data sets. The results of these analyses of freshwater mussel 28S and COI are not consistent with these three predictions. I conclude that the hypothesis that COI is the most appropriate fragment of DNA for phylogenetic studies of this sort is false.

With regard to the interfamilial relationships of the Unionidae, COI is saturated, whereas 28S does not appear to be. The degree of COI saturation is best illustrated in **Figures 3.5-6**. All positions, but especially third positions, at first increase rapidly in divergence as 28S divergence increases reflecting the high substitution rate of mtDNA over nuclear rDNA. However, the rate of COI substitutions slows to almost nothing (relative to 28S), presumably because more recent mutations occurred at the same sites as previous ones. Mild saturation is also evident for the 28S data set, but not to the same degree as the mtDNA (**Figure 3.6**). These results falsify the prediction that COI should display a lesser or equal degree of saturation than 28S (for phylogenetic analyses of the clades of interest).

The distribution of characters on a phylogenetic hypothesis can be extremely telling as to the utility of that data set for answering a particular question. Simply stated,

the relationship of a particular clade to another is dependent upon the characters of higher generality than the branch supporting the two clades. Those characters that trace to levels of lesser universality at best are uninformative to the question at hand and in all other cases are misinformative (Graf & Sparks, 2000). For the present study, misinformative character transformations were tallied for the entire tree. As shown in **Table 3.2**, the majority (>60%) of COI steps on the MP trees are misinformative, whereas the minority (<40%) of 28S characters are.

Character distribution can also be studied for particular clades (rather than the whole tree, which contains long outgroup branches that may inflate the number of misinformative characters) by examining their 'stemminess.' The stemminess index describes the relationship between the number of characters supporting a clade relative to those within it. **Table 3.3** lists the stemminess values for individual clades among the Nearctic Unionidae and Margaritiferidae. With only a few exceptions (*i.e.*, Unionini, Anodontinae, and Pleurobemini), the COI branch lengths within these clades are roughly 3x longer than the branches supporting them. 28S, on the other hand, tends to have longer or only slightly shorter supporting branches than the terminals. These results falsify the prediction that the majority of COI characters should trace to the branches of interest; instead, the majority trace to the terminal branches.

Branch support is another useful quantifier of phylogenetic utility. The goal of any phylogenetic analysis should be a robust test of the hypotheses in question — in this case, the family-group-level phylogeny of the Nearctic Unionidae. Assuming that the Combined analysis topology (**Figure 3.1**) is a reasonable approximation of the 'true' phylogeny, the branch support results for the COI and 28S analyses seem to be performance opposites. As regards 28S, (1) with few exceptions, the topology of the 28S Solo strict-consensus tree (**Figure 3.2**) is consistent with the Combined topology, and (2) the branch support tends to be greatest toward the higher-level relationships. In contrast, for COI, (1) Solo analysis (**Figure 3.3**) failed to recover many of the clades found in the

Combined topology and (2) branch support is best for more shallow relationships (*i.e.*, tribal).

Partitioned BDI values on the Combined analysis topology are even more informative in that they provide negative values. As seen in **Table 3.4**, COI has negative BDI for all subfamily and higher unionoidean clades except (Unionidae + Margaritiferidae). This anomaly is discussed below. The results of the branch support analyses are inconsistent with the prediction that COI should have better branch support for family-group-level clades than does 28S; with the exception of the tribes, 28S at least resolves the family-level relationships and, in some cases, with strong branch support, where COI does not.

Although the falsification of the branch support prediction seems equivocal based on these data relative to the robust rejection of the other two predictions, the sum of these results is, in my view, sufficient to reject the hypothesis that COI is at least as appropriate or better than 28S for recovering family-group-level relationships among the Unionoida. COI is saturated beyond reliability above intra-subfamilial branches. This pattern has been has been observed before (Brown *et al.*, 1982, *etc.*) and has been suggested to confound phylogenetic analyses, especially parsimony (Felsenstein, 1978; Meyer, 1994). Other researchers (Yang, 1998; Wenzel & Siddall, 1999), however, have argued that this sort of 'noise' has little effect on recovering phylogeny (but see Graf & Sparks, 2000). Even though the skewness and PTP tests suggest that analyses of both data sets might be fruitful (but see Pere-Noto & Marques, 2001), it seems clear that saturation within the COI character set led to weak topologies among higher family-group-level taxa. 28S, in contrast, robustly resolves these higher relationships. Where 28S does fall short is for many of the shallower nodes where COI seems to excel (**Table 3.4** and **Figures 3.2-3**). Based on these analyses, my recommendation for future work on the molecular phylogenetics of freshwater mussels is to apply mtDNA for strictly intergeneric analyses,

28S for deeper relationships within the Unionoida, and a combination of the two when the resolution of a wide range of divergences is necessary.

The results of the Combined analysis, relative to Solo analyses of the two partitions, had interesting results that bear upon not only the analyses presented in this chapter but the whole of this dissertation. One important result was the position of the Margaritiferidae. Individually, and in concert, COI and 28S placed the Margaritiferidae as sister to the Unionidae (**Figures 3.1-3**). This is not surprising, given that that topology has been previously preferred (Davis & Fuller, 1981; Hoeh *et al.*, 1996a; Lydeard *et al.*, 1996; Graf & Ó Foighil, 2000). The results of this Combined analysis do become interesting in the context of the 28S data set (with a different taxon set) presented in **Chapter 6**. That analysis places the Margaritiferidae as sister to the Hyriidae (**Figure 6.1**). This is especially eye-catching with regard to the negative BDI for the 28S partition at that node in the Combined analysis (**Table 3.4**). Thus, 28S harbors signals for various interpretations of the position of the Margaritiferidae. These issues are discussed further in **Chapter 7**.

Finally, the topological results of the Combined analysis are in conflict with the consensus classification presented in **Table 1.2**. In that table, the Unionidae was divided into two subfamilies: Unioninae and Anodontinae. However, with the Combined topology (**Figure 3.1**), neither *Unio* nor *Uniandra* is recovered among the "Unioninae": the former is sister to the Anodontinae and *Uniandra* is sister to the remaining Unionidae in this taxon set. Thus, these genera require three subfamilies of the Unionidae:

(1) Unioninae = ((*Unio* + *Cafferia*) + "Anodontinae"),

(2) Ambleminae = (*Gonidea* + "Amblemini" + Pleurobemini + Lampsilini), and

(3) Rectidentinae = *Uniandra*.

This updated classification will be applied in **Chapter 4** and taken up in greater detail in **Chapters 6-7**.

Table 3.1. Taxa for which Cytochrome *c* Oxidase Subunit I and 28S Fragments were Obtained. The classification of the Bivalvia and the Unionoida follows the consensus classifications of **Tables 1.1-2**; tribal determinations follow Brandt (1974) and Davis & Fuller (1981). GenBank accession numbers and molecular sequence acquisition protocols are listed in **Appendix IV**.

Pteriomorpha

Ostrea chilensis

Heterodonta

Mercenaria mercenaria, *Rangia cuneata*

Palaeoheterodonta: Trigonioida

Neotrigonia margaritacea

Unionoida: Hyriidae

Hydrella depressa, *H. australis, H. menziesi*, *Velesunio ambigua*, *Diplodon chilensis*

Margaritiferidae

Cumberlandia monodonta

Unionidae: Anodontinae

Strophitus undulatus, *Alasmidonta marginata*, *Lasmigona compressa*, *Pyganodon grandis*

Unioninae: Unionini

Unio (s.s.) pictorum, *Unio (Cafferia) caffer*

Amblemini

Amblema plicata, *Quadrula quadrula*, *Tritogonia verrucosa*

Gonideini

Gonidea angulata

Table 3.1 (continued). Taxa for which Cytochrome *c* Oxidase Subunit I and 28S Fragments were Obtained.

Rectidentini
Uniandra contradens
Pleurobemini
Elliptio dilatata, *Pleurobema coccineum*
Lampsilini
Actinonaias carinata, *Epioblasma triquetra*, *Lampsilis cardium*, *Ligumia nasuta*, *Ligumia recta*, *Ptychobranchus fasciolaris*, *Truncilla truncata*, *Villosa iris*, *Villosa vanuxemensis*

Table 3.2. Molecular Character and Tree Statistics. Character statistics are based on the three data sets: combined 28S + COI and the two partitions. Tree statistics are derived from both the MP analyses of the three data sets and by tracing the individual partitions on the combined-data topology. Abbreviations: N = number of characters; Cons = number of constant characters; Uninf = uninformative characters; Inf = informative characters; Trees = number of trees recovered by unweighted MP; S = length in steps of MP tree(s); *CI* = Consistency Index; *RC* = Rescaled Consistency Index; Misinf = proportion of misinformative characters.

		Character Statistics			
Analysis	Figure	N	Cons	Uninf	Inf
Combined	**3.1**	1135	434	217	484
28S Solo	**3.2**	483	196	120	167
COI Solo	**3.3**	652	238	97	317

	Tree Statistics				
Analysis	Trees	S	*CI*	*RC*	Misinf
Combined	1	2565	0.457	0.226	0.557
28S Solo	4439[a]	592	0.735	0.567	0.377[b]
28S (combo)	—	604	0.720	0.543	0.389
COI Solo	1	1954	0.378	0.156	0.613
COI (combo)	—	1961	0.376	0.154	0.609

[a] By iteratively re-weighting the 28S characters by their *RC*s, the number of trees was reduced to 509.

[b] Misinformative characters were calculated from a random single tree of the original 4439 (**Figure 3.4**).

Table 3.3. 'Stemminess' of the Major Clades Among the Nearctic Unionidae. 'Stemminess' values were calculated from the 28S and COI partitions on the Combo phylogeny (**Figure 3.1**). The 28S: COI 'stemminess' ratio shows the improvement in informative characters gained from 28S over COI. A dash indicates that the clade was not recovered.

	'stemminess'		
Clade	28S	COI	28S: COI
Unionini	2.00 ± 1.41	4.22 ± 0.31	0.474
Anodontinae	1.02 ± 0.70	0.57 ± 0.28	1.771
Unionini + Anodontinae	2.46 ± 0.76	0.32 ± 0.11	7.783
Amblemini	—	—	—
Pleurobemini	0.00	0.62 ± 0.09	0
Lampsilini	2.17 ± 1.15	0.28 ± 0.08	7.872
Ambl. + Pleurob. + Lamps.	0.95 ± 0.49	0.27 ± 0.09	3.524
ditto. + *Gonidea*	0.82 ± 0.19	0.14 ± 0.05	5.851
Unionidae	1.14 ± 0.15	0.26 ± 0.09	4.390
Unionidae + Margaritiferidae	0.27 ± 0.05	0.19 ± 0.09	1.388

Table 3.4. Support Values the Major Clades Among the Nearctic Unionidae. BDI and jackknife support indices refer to the MP tree(s) of three analyses (**Figures 3.1-3**). The $28S_p$ and COI_p are the respective partitioned BDI on the Combo phylogeny. A dash indicates that the particular clade was not recovered.

	Bremer					**jackknife**		
Clade	Combo	$28S_p$	COI_p	28S	COI	Combo	28S	COI
Unionini	31	4	27	3	29	100	84	100
Anodontinae	6	1	5	—	8	78	—	82
Unionini + Anodontinae	7	2	5	3	—	87	85	—
Amblemini	—			—	—	—	—	—
Pleurobemini	4	6	-2	—	3	72	—	74
Lampsilini	4	3.5	0.5	1	1	61	<50	<50
Ambl. + Pleurob. + Lamps.	4	2	2	1	4	70	<50	<50
ditto. + *Gonidea*	3	5.6	-2.6	3	—	<50	70	—
Unionidae	15	17	-2	14	—	100	99	—
Unionid. + Margaritiferid.	9	-5	14	2	4	86	60	<50

Figure 3.1. Cladogram Derived from the Combined Parsimony Analysis of 28S and COI. The cladogram depicts the single most-parsimonious topology (2565 steps, *CI* = 0.457, *RC* = 0.226). Character and tree statistics are also listed in **Table 3.2**. Values shown above the branches are Bremer-Decay Indices; those below are jackknife resampling percentages. BDI <2 are not shown.

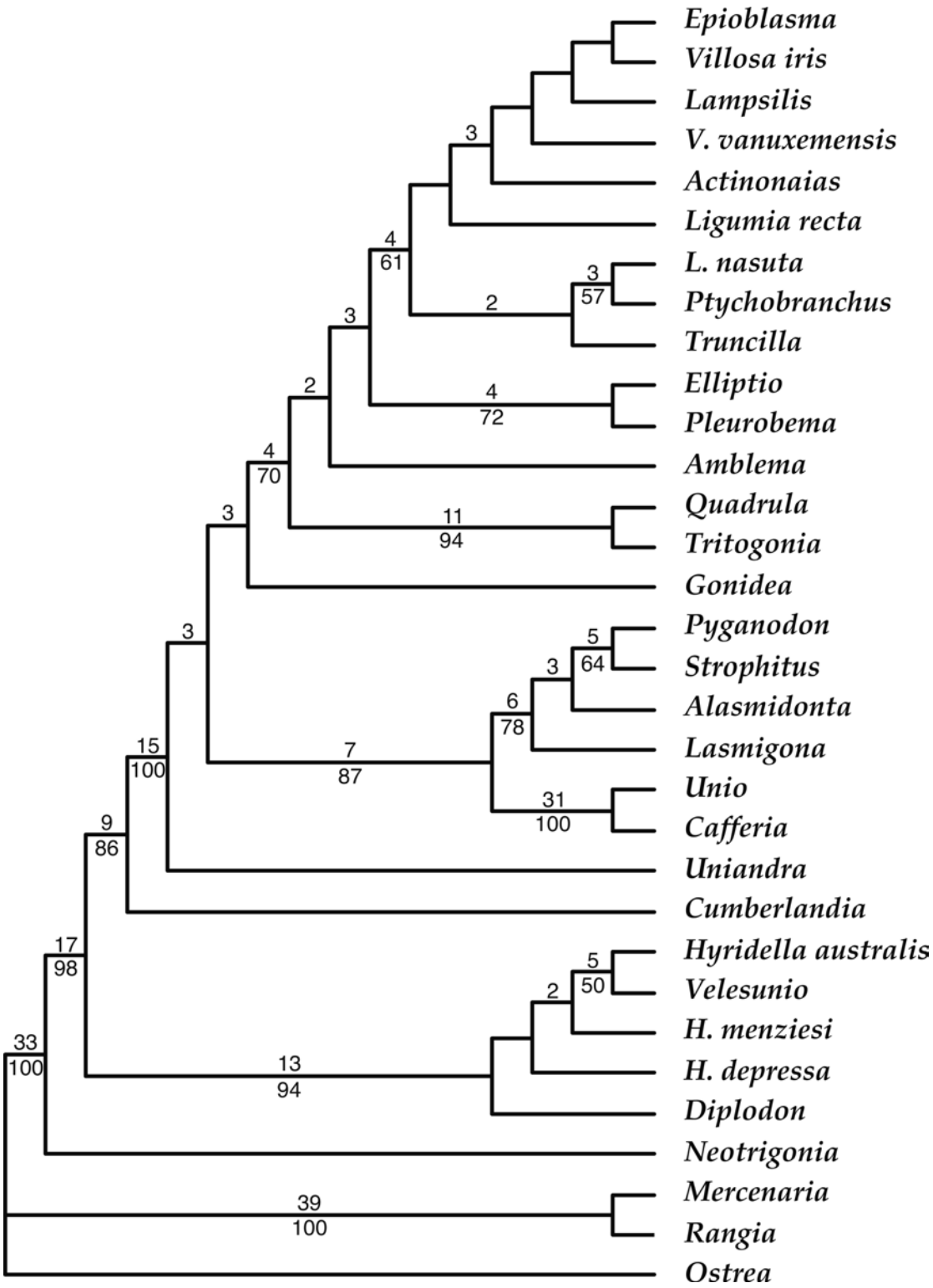


Figure 3.1. Combined Analysis Cladogram

Figure 3.2. Consensus Cladogram Recovered from Reweighted Parsimony Analysis of 28S. Parsimony analysis of the 28S partition recovered 4439 equally most-parsimonious topologies (all trees 592 steps, *CI* = 0.735, *RC* = 0.567). Iteratively reweighting the characters by their *RC* reduces the number of trees to 509, all of length 592 unweighted steps (359.6 weighted steps; 102 characters with weight <1). The figure shows the strict consensus of the 509 reweighted parsimony trees. Dashed lines indicate the branches that collapse in the unweighted consensus. Character and tree statistics are also listed in **Table 3.2**. Values shown above the branches are Bremer-Decay Indices; those below are jackknife resampling percentages. BDI <2 are not shown.

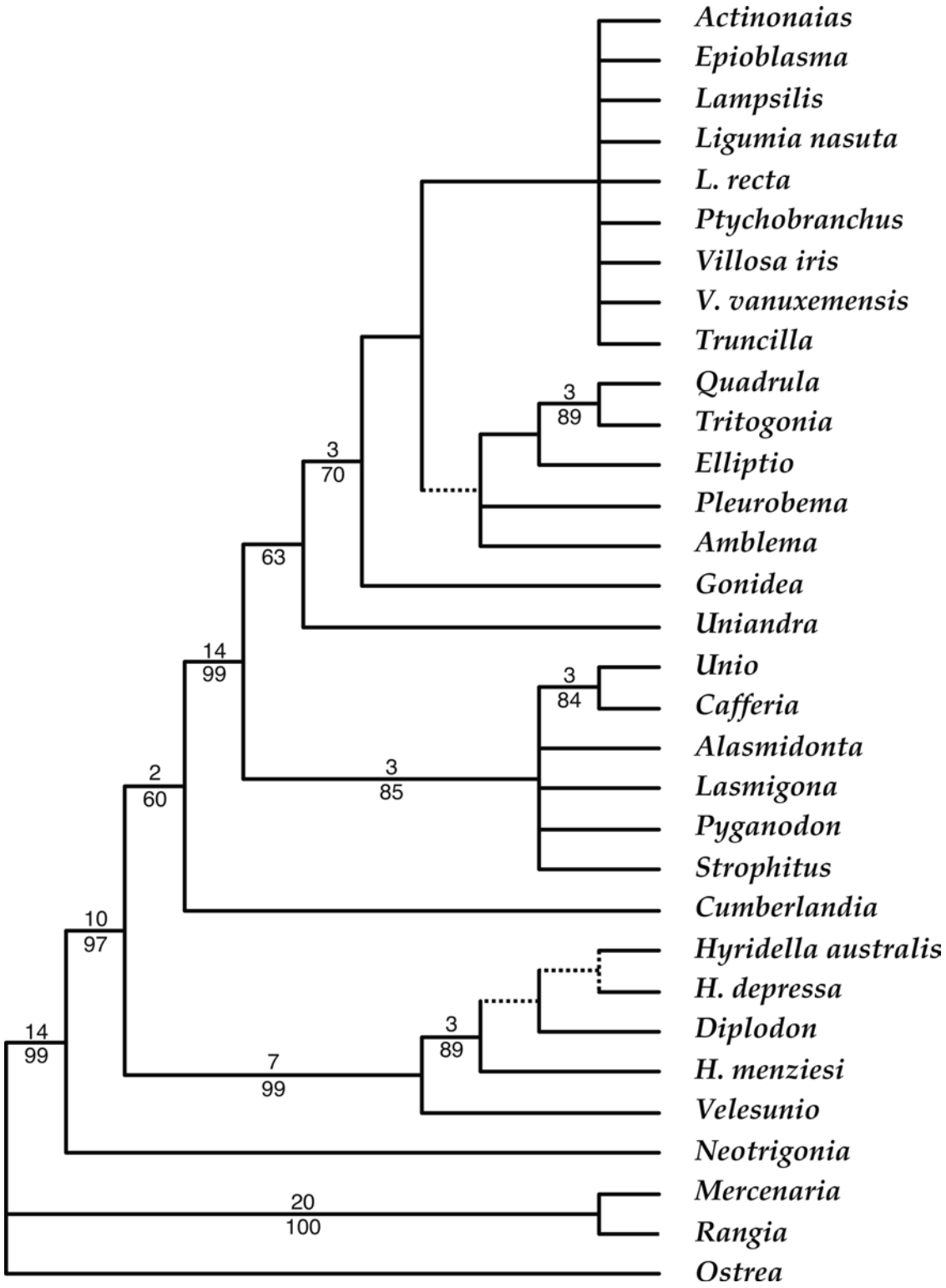


Figure 3.2. 28S Analysis Consensus Cladogram

Figure 3.3. Cladogram Derived from the Parsimony Analysis of COI. The cladogram depicts the single most-parsimonious topology (1954 steps, *CI* = 0.378, *RC* = 0.156). Character and tree statistics are also listed in **Table 3.2**. Values shown above the branches are Bremer-Decay Indices; those below are jackknife resampling percentages. BDI <2 are not shown.

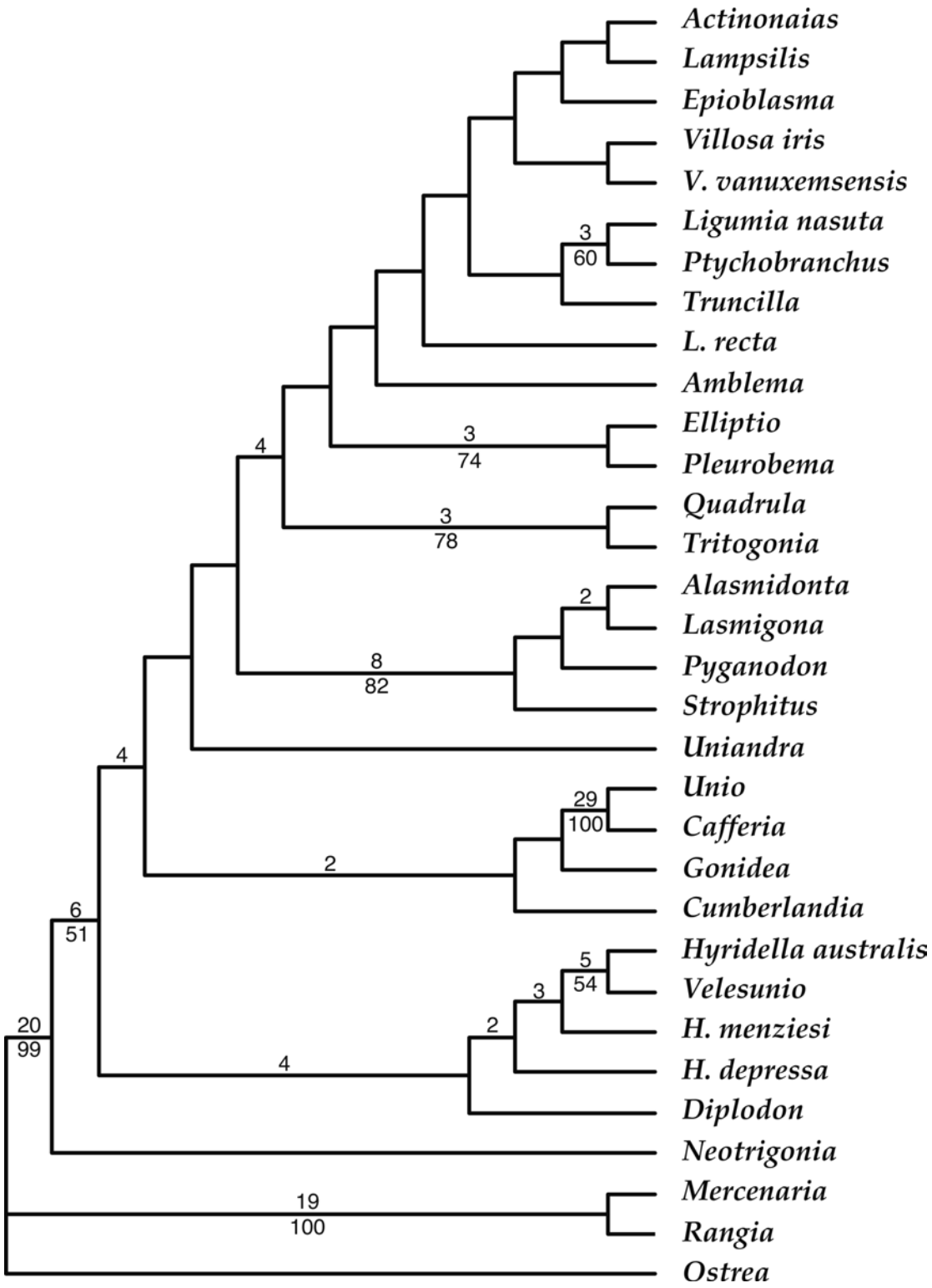


Figure 3.3. COI Analysis Cladogram

Figure 3.4. Combined, COI, and 28S Phylograms Depicting Relative Branch Lengths. Values listed above the branches are ACCTRAN branch lengths. A random topology of the 4439 most-parsimonious solo 28S trees was selected as representative of the lot.

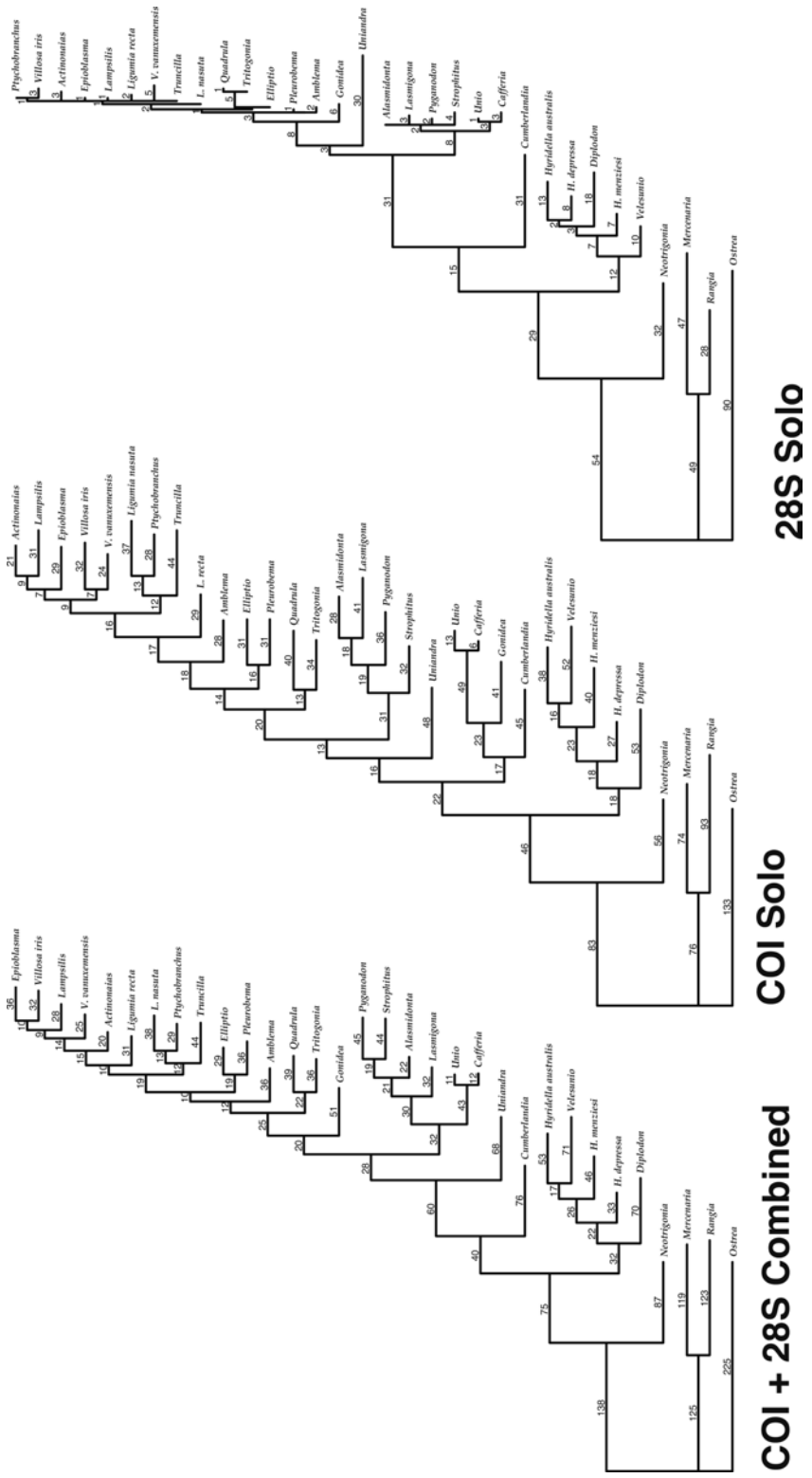


Figure 3.4. Representative Phylograms

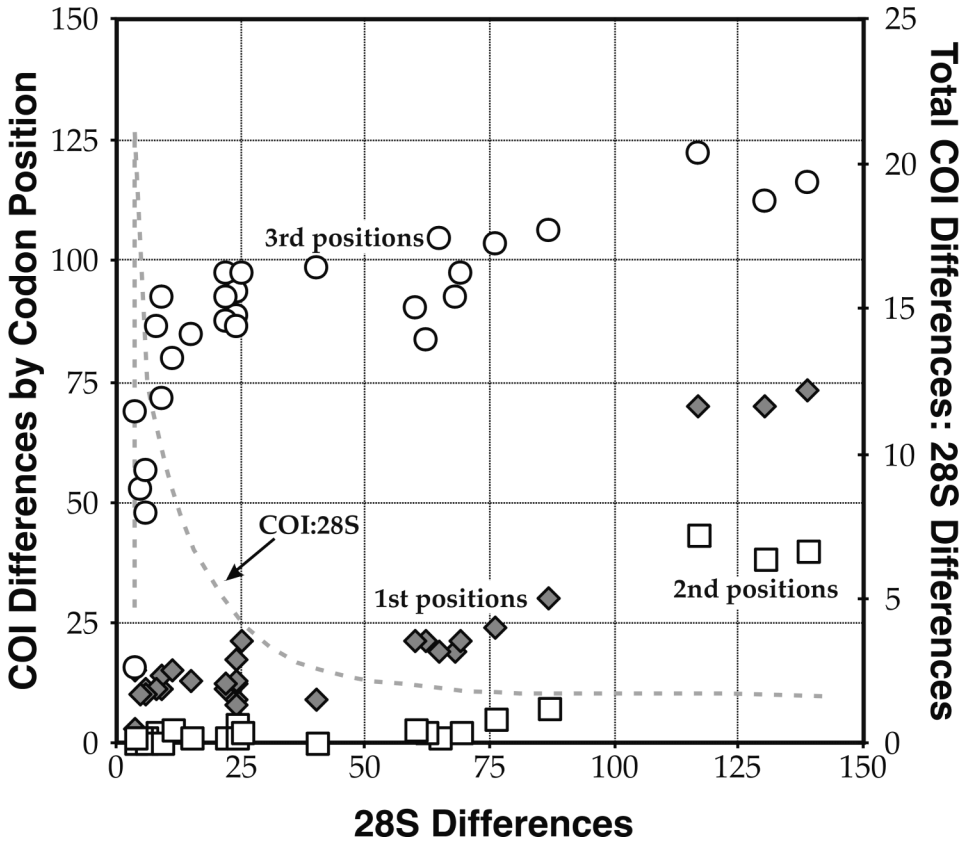


Figure 3.5. Pairwise COI Differences vs. Pairwise 28S Differences for Two Unionid Species. The primary axis (left) graphs the pairwise comparisons of *Unio pictorum* from half of the other taxa analyzed and *Villosa iris* from the other half. The secondary axis (right) shows the ratio of COI: 28S. Open circles = COI 3rd positions; shaded diamonds = COI 1st positions; open squares = 2nd positions; and the dotted line = best fit of the COI: 28S ratio.

Figure 3.6. Pairwise Difference vs. MP Steps for Two Unionid Species. Both the upper (28S) and lower (COI) charts graph the pairwise comparisons of *Unio pictorum* from half of the taxa analyzed and *Villosa iris* from the other half. If there is no saturation in the data set and the combined phylogeny is a reasonable estimation of the phylogeny of Nearctic Unionoida, then the pairwise comparisons of actual differences vs. MP differences should follow the heavy, dotted diagonal. The shaded areas indicate the boundaries of 25% and 35% saturation.

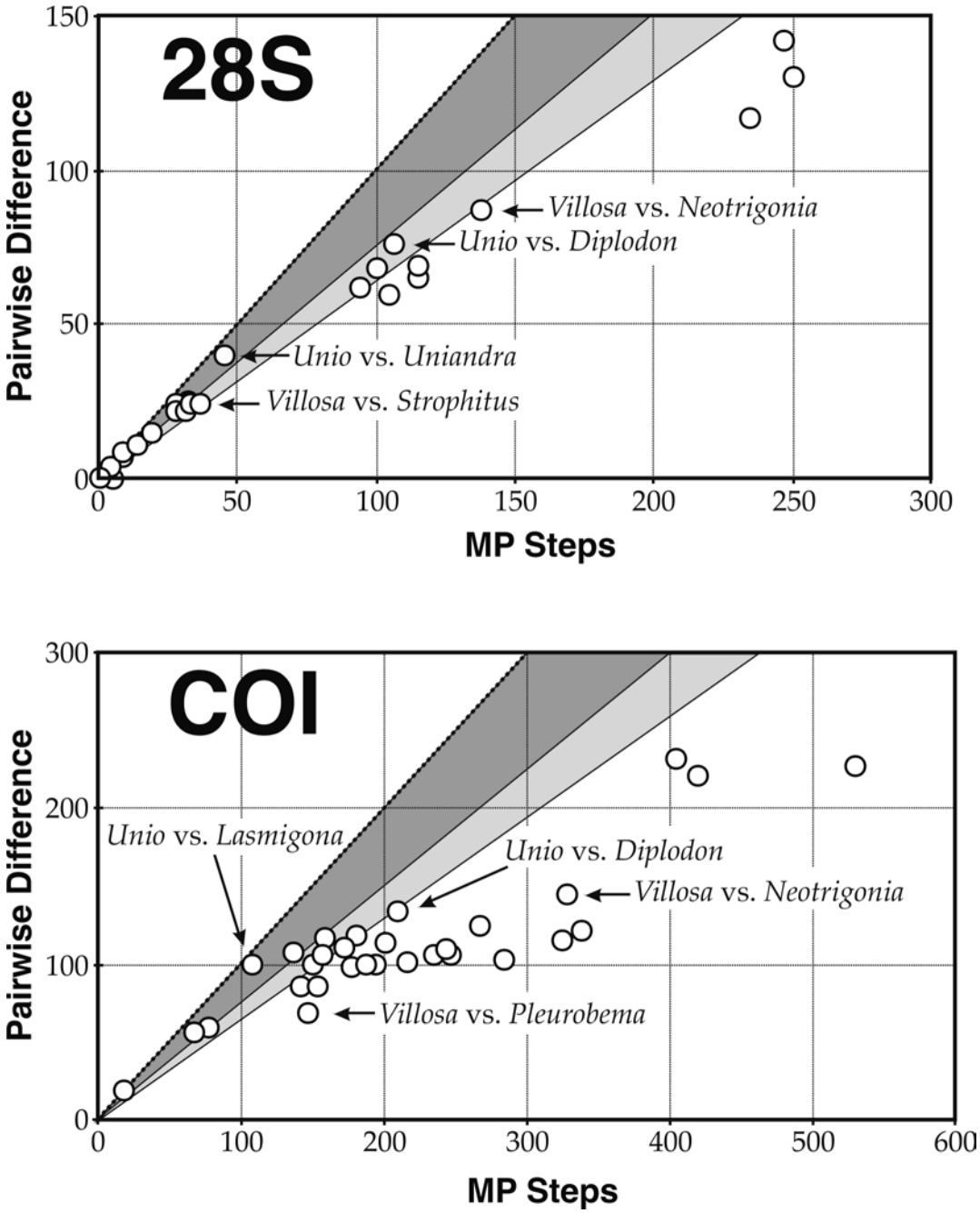


Figure 3.6. Pairwise Difference vs. MP Steps

CHAPTER 4

THE EVOLUTION OF BROODING CHARACTERS AMONG THE NEARCTIC UNIONOIDA

The extraordinary life cycle of the Unionoidea (Bivalvia: Palaeoheterodonta) has been well-studied (*e.g.*, Coker *et al.*, 1921; Kat, 1984; Graf, 1998), and much has been made of the systematic value of variation in both the mechanics of their life history and morphology of their various semaphoronts (Simpson, 1900, 1914; Ortmann, 1911a, 1912b; Parodiz & Bonetto, 1963; Haas, 1969a, b; Heard & Guckert, 1971; Davis & Fuller, 1981; Lydeard *et al.*, 1996; Roe & Lydeard, 1998; Hoeh *et al.*, 2001 see **Chapter 2**). The characters associated with parental care, and reproduction, in general, have been widely employed to diagnose taxa within the Unionoidea. Especially important in many previous classifications of the more than 300 species of North American freshwater mussels were brooding period (*i.e.*, the length of time embryos and larvae are brooded) and arrangement of the marsupium within the females' demibranchs. The objective of this chapter is to test hypotheses of brooding character evolution in order to evaluate their effectiveness in recovering phylogeny — Do similarities in brooding characters among Nearctic freshwater mussels represent homology or homoplasy? Hypotheses of brooding character evolution will be tested using the phylogenetic patterns recovered in **Chapter 3**.

Two general patterns of brooding have long been recognized among the temperate Unionoidea of North America: short-term and long-term (Sterki, 1895, 1898; Ortmann, 1909; reviewed in Graf, 1997a and Heard, 1998). Ortmann (1911c) coined the terms tachytictic and bradytictic for each of these brooding types, respectively. Tachytictic

(short-term brooding) mussels spawn their gametes in the spring, with embryos and larvae brooded in the females' marsupial demibranchs only until they have fully developed into parasitic larvae, the glochidia. The larvae are then released to the water to infect their host fish and complete their metamorphosis. The whole sequence of events is generally completed over the course of the late spring and summer, with certain exceptions (see below). Bradytictic (long-term brooding) mussels, in contrast, spawn in the late summer, brood their glochidia over the winter, and release them in the early spring. The fundamental distinction is that bradytictic mussels continue to brood their larvae long after they are infectious (Coker *et al.*, 1921; Kat, 1984). Variation in the brooding patterns of North American mussels has been attributed to climate, especially ice ages (Sterki, 1903; Ortmann, 1909; Graf, 1997a), as well as to synchronize with seasonal host activity (Zale & Neves, 1982).

There is also significant variation in the morphological characters associated with parental care. In the Unionoidea, as with the freshwater Sphaeriidae and Corbiculidae (both Bivalvia: Heterodonta), larvae are brooded within the interlamellar spaces of the ctenidia (McMahon, 1991). The portion of the female's ctenidia that serve as brood spaces, the marsupium, varies from only a limited portion of the outer demibranchs, to the entire outer pair, to all four demibranchs (Ortmann, 1911c, 1912b). There are also fine structural differences in the development of interlamellar connections among and within the types of different marsupial arrangements (Ortmann, 1911c; Heard & Vail, 1976b).

Early on, malacologists recognized the correlation between brooding period and morphology, and they felt that anatomical specializations associated with long-term ovovivipary of larvae were of special systematic significance (**Table 4.1**).

> "Having correlated physiological function with anatomical and morphological structures, we may rest assured, that we have discovered an essential principle in the development of the Najades, and we may say with all confidence that a systematic arrangement, which is founded upon

> such structures, which we are able to understand, must be the correct one." (Ortmann, 1911c: 305)

The extent to which these characters were perceived as homologies, however, varied from taxonomist to taxonomist. This is reflected in their disparate classifications (**Table 4.2**; reviewed in Davis & Fuller, 1981 and **Appendix II**). There has, however, been widespread agreement that margaritiferids, because of their morphological simplicity, are the most 'primitive' unionoideans (Ortmann, 1912b; Heard & Guckert, 1971; Davis & Fuller, 1981). The brooding characters of the Margaritiferidae, thus, have been taken to be the plesiomorphic condition among the freshwater mussels in general. That assumption has not been tested phylogenetically.

Any discussion of character evolution within the Unionoidea must be based on a phylogenetic hypothesis that reflects the evolutionary history of the group. Although the classifications of Ortmann (1911c, 1912b), Heard & Guckert (1971), and Davis & Fuller (1981) each have strong points, no single one of these is suitable to test hypotheses of brooding character evolution among the Nearctic freshwater mussels. A fundamental drawback of these studies is their lack of outgroups to objectively polarize the direction of character evolution (Wiley, 1980). Also, interpreting the classifications of the authors cited above from a phylogenetic perspective may not always be appropriate. After all, it may not have been their intention to recognize only monophyletic taxa. For example, the classification of Heard & Guckert (1971), from a cladistic vantage, is at odds with their own evolutionary tree (their Figure 1).

Rosenberg *et al.* (1994, 1997) published the first cladistic phylogeny of the Unionoidea, followed soon afterward by the more comprehensive study of Lydeard *et al.* (1996). The latter study greatly improved the resolution of intergeneric relationships among the freshwater mussels of North America and also supported certain aspects of Davis & Fuller's (1981) classification. However, their use of the edible blue mussel, *Mytilus edulis* (Linnaeus), as the sole outgroup does not allow for a discussion of

brooding character evolution among freshwater mussels. Although possibly a meaningful outgroup for molecular characters, no logical criterion exists to make homology statements about the morphological characters of the Unionoida and those of *Mytilus* (their Table 3).

A fundamental difficulty of arranging the freshwater mussels of North America into natural groups is the apparent lack of informative morphological characters. Shell, adult and larval gross anatomical, and, especially, brooding characters have been exploited in the past, but these are of poor quantity and quality (see **Chapter 2**).

The molecular phylogenies presented in **Chapter 3** overcome the outgroup problems of Lydeard *et al.* (1996). For those analyses, I sampled a wide range of taxa (**Table 4.3**). Not only representatives of the major groups of North American unionoideans, but also *Unio* (from both Europe and Africa), *Uniandra* (from Southeast Asia), non-unionoidean freshwater pearly mussels, and a handful of marine bivalves. These latter taxa include *Neotrigonia*, the marine outgroup of all freshwater Unionoida (Thiele, 1934; Newell, 1969; Boss, 1982; Hoeh *et al.*, 1998; but see Newell & Boyd, 1975 and Morton, 1987). Tracing brooding characters onto the best-corroborated molecular phylogeny allows independent tests of hypotheses of morphological evolution. Specifically, I set out to test the homology of bradytictia and of morphological modifications of the ctenidia associated with parental care among the Lampsilini and Anodontinae.

Methods & Materials

The phylogeny upon which the brooding characters were traced was derived from the topologies recovered in **Chapter 3**. Based on the three different topologies recovered from the Combined (28S + COI) (**Figure 3.1**), Solo 28S (**Figure 3.2**), and Solo COI

(**Figure 3.3**) analyses, a single 'best-estimate' topology for the Nearctic Unionidae was derived which played to the strengths of the different data sets involved.

Twelve brooding characters (**Tables 4.4-5**) were traced onto the 'best-estimate' phylogeny using PAUP* (Swofford, 1998). Eleven of the 12 brooding characters correspond to characters 22-26, 28-30, and 32-34 applied in **Chapter 2** and described in **Appendix III**. The remaining brooding character examined (character 11, **Table 4.4**) refers to unionid brooding period (long- vs. short-term) and was not addressed in **Chapter 2**. Transformation series were followed using both PAUP* and MacClade 3.07 (Maddison & Maddison, 1997).

Results

Based on the three phylogenetic analyses performed in the previous chapter — Combined 28S + COI and the two individual partitions — the 'best-estimate' phylogeny of the Nearctic Unionoidea is a chimera of the Combined topology (**Figure 3.1**) to represent the deeper splits, and the COI topology (**Figure 3.3**) for only the Lampsilini. The principal difference depicted among the Lampsilini in these two topologies is that COI recovers a monophyletic *Villosa* (= *V. iris* + *V. vanuxemensis*). **Figure 4.1** shows what I am taking to be the 'best-estimate' cladogram, based on these data.

Assuming that the 'best-estimate' topology is a reasonable estimate of the 'true' tree, **Figure 4.1** depicts the pattern of character evolution among the 12 brooding characters listed in **Table 4.4**. Character transformations are described in the **Table 4.6**. Six brooding characters are shown to be unambiguous synapomorphies (*CI* = 1.0): freshwater habitat [character 1, see **Table 4.4**] and brooding [2] (synapomorphies of the Unionoida); tripartite water tubes [7] (Anodontini); restriction of the marsupium to a portion of the outer demibranchs [4], ventral extension of the marsupium [9], and larval discharge through the ventral margin of the marsupium [10] (Lampsilini). The six

remaining characters exhibit homoplasy in varying degrees, including brooding period [11] (*CI* = 0.500) and number of marsupial demibranchs [3] (*CI* = 0.500) which have figured prominently in past classifications (Ortmann, 1912b; Heard & Guckert, 1971).

Discussion

Evolution of Brooding Among Freshwater Bivalves

The evolution of brooding among bivalves is correlated with colonization of freshwater habitats from a marine environment. There have been at least four, independent heteroconch (= Palaeoheterodonta + Heterodonta) invasions of freshwater: Unionoida, Corbiculidae, Sphaeriidae, and *Dreissena* (*e.g.*, Park & Ó Foighil, 2000). Among these taxa, only the latter has not evolved ovovivipary; it retains its plesiomorphic veliger. However, *Dreissena* may have infiltrated freshwater environments only as recently as the Pleistocene (McMahon, 1991).

In marine environments, the stereotypical bivalve larval form is a planktonic veliger (Brusca & Brusca, 1990; Waller, 1998), and passive dispersal of this veliger or other planktonic larva is the principle means of distribution. In a freshwater stream environment, such a strategy is disadvantageous — reliance upon buoyant, microscopic larvae for dispersal would allow bivalves to colonize only downstream habitats and eventually fall back into the ocean. Sphaeriids and corbiculids have overcome this problem by abandoning a planktonic larval stage in favor of direct-development of offspring within their brood chambers (McMahon, 1991). Indirect development has persisted among the Unionoida, although passive dispersal by water currents has been swapped for distribution by the host fishes of their parasitic glochidia (Coker *et al.*, 1921; Kat, 1984). As shown in **Chapter 2**, parasitic larvae in the form of a glochidium is a synapomorphy of the Unionoida; other palaeoheterodonts (*i.e.*, Trigonioida) are

apparently obligate planktotrophs (Ó Foighil & Graf, 2000). Direct development has been secondarily derived in only a few unionoid lineages (*e.g.*, Kondo, 1990; Parodiz & Bonetto, 1963).

Evolution of Brooding Pattern Among Nearctic Unionoidea

Among the Unionoidea of North America, two general patterns of brooding have been observed: bradytictia (long-term brooding) and tachytictia (short-term brooding) (reviewed in Graf, 1997a and Heard, 1998). Sterki (1903), Ortmann (1912b), and Heard & Guckert (1971) considered brooding period to be of principle importance in their classifications of the freshwater mussels of North America. Davis & Fuller (1981) and Lydeard *et al.* (1996: 1601) argued that brooding period lacked value as a phylogenetic character, suggesting that, "the bradytictic and tachytictic conditions have evolved several times." The data presented here (**Figure 4.1**) clearly indicate that, among Nearctic freshwater mussels, bradytictia is a derived condition, having evolved twice independently: once in the Anodontini and once in the Lampsilini. The plesiomorphic condition among the Unionidae is tachytictia, as suggested by Heard (1998). The brooding data on the non-North American taxa is sparse, but the Hyriidae and tropical Unionidae apparently breed all year or during the austral summer (reviewed in Watters, 1994b and Heard, 1974).

As discussed by Graf (1997a), much of the confusion regarding the systematic value of brooding period has been compounded by differing definitions of long-term and short-term brooding applied among freshwater malacologists, especially by confusing them with their original descriptors: winter-brooding and summer-brooding, respectively (Lefevre & Curtis, 1910, 1912). For example, *Megalonaias* has been regularly listed

among the bradytictic mussels (Utterback, 1916; Heard & Guckert, 1971; Lydeard *et al.*, 1996; Heard, 1998) because it broods in the late fall and winter (Woody & Holland-Bartels, 1993). However, it is a short-term brooder (*i.e.*, glochidia are not brooded after they are infectious) and might thus be dubbed 'winter-tachytictic.' Although not included in this analysis, Lydeard *et al.* (1996) found *Megalonaias* to be sister to *Quadrula*, another tachytictic genus.

There has been similar confusion surrounding the Margaritiferidae. Heard & Guckert (1971), Davis & Fuller (1981), and Lydeard *et al.* (1996) considered them to be bradytictic, while Sterki (1903), Connor (1909), and Ortmann (1912b) considered those mussels to be tachytictic. Watter's (1994b) review of infection periods for margaritiferids, as well as Howard (1915) and Gordon & Smith's (1990) reports of multiple broods for *Cumberlandia*, suggest that margaritiferids are facultatively bradytictic. Heard (1998) considered the Margaritiferidae to be 'sequentially tachytictic' while Graf (1997a) suggested that unionid terminology might best be reserved solely for the Unionidae. Obviously, more life history data are needed from margaritiferids and other mussel species to resolve this problem. For this analysis, margaritiferid brooding pattern was coded as unknown (character 11 in **Table 4.4**).

Evolution of Marsupium Morphologies

As noted by Ortmann (1911c, 1912b; also see Heard & Guckert, 1971 and Graf, 1997a), certain morphological novelties have been associated with bradytictia. Among these are the number and arrangement of marsupial demibranchs as well as modifications of the marsupium to facilitate long-term brooding (**Table 4.1**). This study (**Figure 4.1 & Table 4.6**), however, suggests that (1) tachytictia and tetrageny (use of all four demibranchs for brooding) are not correlated and that (2) the brooding modifications of the bradytictic clades are not homologous.

The plesiomorphic condition of the Unionidae is a tachytictic [11] mussel employing only the outer pair of demibranchs for brooding [3] (**Figure 4.1**). Graf & Ó Foighil (2000: Figure 3) found that within the Unionidae, use of all four demibranchs is shown to be a derived condition among certain Ambleminae. Ortmann (1912b) noted in *Amblema* and *Quadrula* that the septa of the outer marsupial demibranchs are more crowded than those of the inner demibranchs. This supports the hypothesis that tetrageny evolved secondarily from an ectobranchous (*i.e.*, using only the outer demibranchs as marsupia) condition in which the septa are more crowded in marsupial demibranchs than they are in those that are non-marsupial (Ortmann, 1911c, 1912b).

The cladogram in **Figure 4.1** seems to simplify the complex pattern of evolution described in Graf & Ó Foighil (2000). Within the Unionidae, tetrageny apparently arose a single time in the ancestor of the Ambleminae (*Gonidea* + ("amblemini" + Pleurobemini + Lampsilini) and was then reversed back to ectobranchy as a synapomorphy of (Pleurobemini + Lampsilini). The difficulty derives from the uncertain branching pattern of the Ambleminae. Of the relevant molecular studies, neither Lydeard *et al.* (1996), Graf & Ó Foighil (2000) nor Hoeh *et al.* (2001) found a sister relationship among the Pleurobemini and Lampsilini (**Figure 4.2**), a challenge to the hypothesis that marsupia composed of only the outer demibranchs is a 'synapomorphy' of that 'clade.' In addition, tetrageny occurs among other genera not included in this analysis, *e.g.*, *Fusconaia* (Graf & Ó Foighil, 2000) and *Elliptoideus* (Lydeard *et al.*, 1996), both considered Pleurobemini. Thus, an alternative to the hypothesis that tetrageny is a synapomorphy of the Ambleminae is that use of all four demibranchs as marsupia arose multiple times among the primitively ectobranchous freshwater mussels.

The plesiomorphic marsupial arrangement of the Unionoidea is ambiguous (**Figure 4.1 & Table 4.6**). This phylogenetic analyses suggest that the 'primitive' margaritiferid condition may actually be derived. Besides being tetragenous [3], *Cumberlandia* has reduced the septa of its demibranchs to sparse interlamellar junctions

[5, 6]. A reduction hypothesis would also apply to other presumed 'primitive' characters among the Margaritiferidae, such as loss of a supra-anal aperture and atrophy of the diaphragm dividing the mantle cavity (Ortmann, 1912b; Baker, 1928; Heard & Guckert, 1971; Davis & Fuller, 1981; Graf, 2000a; see **Chapter 7**).

This hypothesis of margaritiferid specialization vs. plesiomorphy could be tested by adding more taxa to the analysis that would intersect the branch between the Hyriidae and (Margaritiferidae + Unionidae). Hoeh *et al.* (1996a, 2001) reported that the African *Caelatura* was the most basal unionoidean in their analysis. *Caelatura* is tetragenous, as are *Pseudodon, Brazzaea,* and *Parreysia* of Southeast Asia, Africa, and India, respectively (Bloomer, 1931a, 1932; Ortmann, 1911a, 1916a; Heard, 1974), but some other tropical Unionoidea are known to use only one or the other pair of demibranchs as marsupia (Ortmann, 1911a; Brandt, 1974; Kondo, 1990). These freshwater mussels all have septa dividing the demibranchs into water tubes, though the septa are perforated. Contrary to present classification (*e.g.*, Haas, 1969a, b; Brandt, 1974; Boss, 1982), these tropical unionoideans (including *Uniandra*) may represent a radiation independent of the temperate taxa that I have examined here and may provide insights into the plesiomorphic condition of the Unionoidea. This topic is addressed in more detail in **Chapters 6-7**.

Having their gas exchange and feeding organs clogged with developing offspring for extended periods is an obvious physiological disadvantage to a gravid mussel (*e.g.*, Tankersley, 1996). Besides gross morphological changes in marsupial arrangement, the freshwater mussels of North America have also undergone several structural specializations to alleviate this strain. Both the Anodontini and Lampsilini have convergently augmented the base of the lamellae of the marsupium with tissue to allow for great expansion when the mussel is gravid [8]. In the case of the Lampsilini, this tissue is further modified to allow the marsupium to extend beyond the ventral margin of the demibranch [9] and for the expulsion of glochidia through that tissue [10] rather than

via the suprabranchial space. In most Lampsilini, the marsupium is limited to only a portion of the marsupial demibranch [4], but the actual configuration varies among genera (numerous figures in Ortmann, 1912b).

While the Lampsilini tend to limit the number of water tubes reserved for brooding, the Anodontini divides the water tubes themselves. Each water tube of the gravid marsupium is divided by a pair of lateral septa running parallel to the axis of the ctenidium (figured in Ortmann, 1911c). These 'tripartite' water tubes [7], with the embryos and larvae brooded only in the center compartment, allow the respiratory and feeding current to flow freely through the lateral compartments. And so, as long-term brooding has evolved separately in the two bradytictic clades, each has derived unique specializations to accommodate it.

Conclusions and Comments

Some brooding characters were found to be synapomorphies diagnosing clades within the Palaeoheterodonta and Unionoida. However, brooding period and, especially, the arrangement of marsupial demibranchs were found to be of limited systematic value. Bradytictia evolved independently in both the Anodontini and Lampsilini (**Figure 4.1**), so long-term brooding can not be considered homologous among all bradytictic mussels. Rather, long-term brooding may be a convergent adaptation to temperate winters in these two clades (see discussions in Graf, 1997a and Heard, 1998).

It is interesting to consider the biogeography of long-term brooding among the Unionoidea. The Lampsilini are strictly North American, and the Anodontini are distributed holarcticly (Haas, 1969a). Bradytictia has evolved twice among northern, temperate clades. Both Sterki (1903) and Ortmann (1909) speculated that bradytictia was favored during harsh climatic regimes, and Graf (1997a) suggested that over-wintering larvae within the marsupium allowed bradytictic mussels to more effectively colonize the

formerly glaciated basins of eastern North America. Bradytictia may be an adaptation to long winters and the deteriorating climate of the Late Tertiary and Quaternary, and this may explain the radiation of anodontine and lampsiline mussels in North America. Heard (1998) also provided examples of bradytictic genera taking on a tachytictic habit in the warm temperate southern United States.

Marsupial arrangement has figured prominently in past classifications of the North American Unionoidea. Although the plesiomorphic marsupial arrangement of the Unionoidea is ambiguous, the hypothesis that tetrageny is the primitive condition among the Unionoida can be rejected. My analysis suggests that using all four demibranchs for brooding may be a derived condition, but this hypothesis is in need of further testing. This may be best achieved by including tropical unionoideans in future phylogenetic analyses.

Table 4.1. Brooding Characters of Bradytictic and Tachytictic Freshwater Mussels.

	bradytictia	tachytictia
brooding period	long	short
marsupial demibranchs	the outer pair or less	the outer pair, or sometimes all four
ctenidial brooding modifications	gravid marsupium expands, tripartite water-tubes, *etc.*	none

Table 4.2. Synopsis of the classifications of Ortmann (1912b), Heard & Guckert (1971), and Davis & Fuller (1981; Lydeard *et al.*, 1996) for North American Unionoidea.

Ortmann	Heard & Guckert	Davis & Fuller
MARGARITIFERIDAE	MARGARITIFERIDAE	UNIONIDAE
UNIONIDAE	Margaritiferinae	Margaritiferinae
Unioninae	Cumberlandinae	Anodontinae
Anodontinae	AMBLEMIDAE	Ambleminae
Lampsilinae	Ambleminae	Amblemini
	Megalonaiadinae	Pleurobemini
	UNIONIDAE	Lampsilini
	Unioninae	
	Pleurobeminae	
	Anodontinae	
	Lampsilinae	

Table 4.3. Taxa for which Brooding Characters were Determined and Traced on the 'Best-Estimate' Phylogeny. Taxonomy follows the consensus classifications of **Tables 1.1-2**, as modified by the analyses of **Chapter 3**.

Pteriomorpha

Ostrea chilensis

Heterodonta

Mercenaria mercenaria, *Rangia cuneata*

Palaeoheterodonta: Trigonioida

Neotrigonia margaritacea

Unionoida: Hyriidae

Hydrella depressa, *H. australis, H. menziesi* (South Island, New Zealand), *Velesunio ambigua*, *Diplodon chilensis*

Margaritiferidae

Cumberlandia monodonta

Unionidae: Unioninae: Unionini

Unio (s.s.) pictorum, *Unio (Cafferia) caffer*

Anodontini

Strophitus undulatus, *Alasmidonta marginata*, *Lasmigona compressa*, *Pyganodon grandis*

Ambleminae: "Amblemini"

Amblema plicata, *Quadrula quadrula*, *Tritogonia verrucosa*

Gonideini

Gonidea angulata

Table 4.3 (continued). Taxa for which Brooding Characters were Determined and Traced on the 'Best-Estimate' Phylogeny.

Rectidentini
Uniandra contradens
Pleurobemini
Elliptio dilatata, *Pleurobema coccineum*
Lampsilini
Actinonaias carinata, *Epioblasma triquetra*, *Lampsilis cardium*, *Ligumia nasuta*, *Ligumia recta*, *Ptychobranchus fasciolaris*, *Truncilla truncata*, *Villosa iris*, *Villosa vanuxemensis*

Table 4.4. Diagnoses of Brooding Characters Among the Palaeoheterodonta. The brooding and life history characters are described in detail in **Appendix III**. The matrix of character states for the genera analyzed is shown in **Table 4.5**.

Brooding and Life History Characters

1. Habitat. — *0* = Marine. *1* = Freshwater.
2. Parental care. — *0* = None; fertilization is presumably external. *1* = Female broods embryos and larvae in ctenidial marsupium.
3. Demibranchs occupied by marsupium. — *0* = All four. *1* = Inner pair only. *2* = Outer pair only.
4. Outer marsupial demibranch. — *0* = Entire demibranch marsupial or nearly so. *1* = A restricted portion of the demibranch marsupial.
5. Interlamellar connections of non-marsupial demibranchs, including those of males. — *0* = None or scattered. *1* = Complete septa. *2* = Perforated septa.
6. Interlamellar connection of marsupial demibranchs. — *0* = Absent or scattered. *1* = Complete septa. *2* = Perforated septa.
7. Marsupial water tubes. — *0* = Undivided. *1*= Divided by lateral septa ("tripartite").
8. Edge of marsupium. — *0* = Remains sharp when gravid. *1* = Expands greatly when gravid.
9. Ventral extent of marsupium. — *0* = Ventral margin of marsupium does not extend past the non-marsupial portion. *1* = Ventral margin of marsupium extends past the non-marsupial portion.
10. Larval discharged. — *0* = Larvae discharged out the excurrent aperture with the respiratory current. *1* = Larvae discharge through the ventral margin of the demibranch and out the incurrent aperture.

Table 4.4 (continued). Diagnoses of Brooding Characters Among the Palaeoheterodonta.

Brooding and Life History Characters	
11.	Brooding period. — *0* = Tachytictic (short). *1* = Bradytictic (long)
12.	Mantle ventral to the incurrent aperture. — *0* = Smooth or weakly elaborated. *1* = Elaborated with conspicuous papillae or a ribbon-like flap.

Table 4.5. Matrix of Brooding Characters Among the Palaeoheterodonta. Character states were determined from direct observation of specimens and from the literature (Baker, 1928; Bloomer, 1932; Darragh, 1998; Heard & Vail, 1976b; Kraemer, 1970; McMichael & Hiscock, 1958; Morton, 1987; Ortmann, 1911b, 1912a, b, 1913-1916, 1918a, b, c, 1921b, 1923-1924; Smith, 1979). Character numbers refer to those in **Table 4.4**. See text for a discussion of character coding.

	1	2	3	4	5	6	7	8	9	10	11	12
Trigonioida	0	0	?	?	0	?	?	?	?	0	?	0
Hyriidae	1	1	1	?	2	2	0	0	0	0	?	0
Margaritiferidae	1	1	0	?	0	0	0	0	0	0	?	0
Unionidae												
Unio	1	1	2	0	1	2	0	0	0	0	0	0
Cafferia	1	1	2	0	1	2	0	0	0	0	0	0
Strophitus	1	1	2	0	1	1	1	1	0	0	1	0
Alasmidonta	1	1	2	0	1	1	1	1	0	0	1	0
Lasmigona	1	1	2	0	1	1	1	1	0	0	1	0
Pyganodon	1	1	2	0	1	1	1	1	0	0	1	0
Amblema	1	1	0	?	1	1	0	0	0	0	0	0
Quadrula	1	1	0	?	1	1	0	0	0	0	0	0
Tritogonia	1	1	0	?	1	1	0	0	0	0	0	0
Gonidea	1	1	0	?	2	2	0	0	0	0	0	0
Uniandra	1	1	2	0	2	2	0	0	0	0	0	0
Elliptio	1	1	2	0	1	1	0	0	0	0	0	0
Pleurobema	1	1	2	0	1	1	0	0	0	0	0	0

Table 4.5 (continued). Matrix of Brooding Characters Among the Palaeoheterodonta.

	1	2	3	4	5	6	7	8	9	10	11	12
Truncilla	1	1	2	1	1	1	0	1	1	1	1	0
Ptychobranchus	1	1	2	1	1	1	0	1	1	1	1	0
Lampsilis	1	1	2	1	1	1	0	1	1	1	1	1
Ligumia nasuta	1	1	2	1	1	1	0	1	1	1	1	1
L. recta	1	1	2	1	1	1	0	1	1	1	1	1
Villosa	1	1	2	1	1	1	0	1	1	1	1	1
Actinonaias	1	1	2	1	1	1	0	1	1	1	1	0
Epioblasma	1	1	2	1	1	1	0	1	1	1	1	1

Table 4.6. Brooding Character Transformations. The character numbers are the same as in **Table 4.4**. An *s* refers to the number of transformations (*i.e.*, steps) each character undergoes; *CI* and *RC* are the Consistency and Rescaled Consistency Indices, respectively. A dagger ('†') indicates that the *RC* is taken to be unity when the Retension Index is undefined (Farris, 1989). Brooding character transformations are mapped in **Figure 4.1**.

1. Synapomorphy of Unionoida (Hyriidae + (Margaritiferidae + Unionidae)) (*s* = 1, *CI* = 1.0, *RC* = 1.0†).
2. Synapomorphy of Unionoida (*s* = 1, *CI* = 1.0, *RC* = 1.0†).
3. *1* synapomorphy of Hyriidae.

   2 synapomorphy of Unionidae, with reversal to *0* in (*Gonidea* + Ambleminae) and reacquisition by (Pleurobemini + Lampsilini) (*s* = 4, *CI* = 0.500, *RC* = 0.250).
4. Synapomorphy of Lampsilini (*s* = 1, *CI* = 1.0, *RC* = 1.0).
5. *2* synapomorphy of Unionoida, reversed to *0* in Margaritiferidae, transition to *1* in (Unioninae + Ambleminae), and reversed to *2* in *Gonidea*.

   Or, *2* synapomorphy of Unionoida, reversed to *0* in Margaritiferidae, independent transformations to *1* in Unioninae and ("amblemini" + Pleurobemini + Lampsilini) (*s* = 4, *CI* = 0.500, *RC* = 0.167).
6. *2* plesiomorphic among Unionoida.

   0 synapomorphy of Margaritiferidae.

   1 independent synapomorphies of Anodontini and ("amblemini" + Pleurobemini + Lampsilini) (*s* = 3, *CI* = 0.667, *RC* = 0.500).
7. Synapomorphy of Anodontini (*s* = 1, *CI* = 1.0, *RC* = 1.0).
8. Independent synapomorphies of Anodontini and Lampsilini (*s* = 2, *CI* = 0.500, *RC* = 0.450).

Table 4.6 (continued). Brooding Character Transformations.

9.	Synapomorphy of Lampsilini (*s* = 1, *CI* = 1.0, *RC* = 1.0).
10.	Synapomorphy of Lampsilini (*s* = 1, *CI* = 1.0, *RC* = 1.0).
11.	Independent synapomorphies of Anodontini and Lampsilini (*s* = 2, *CI* = 0.500, *RC* = 0.438).
12.	Independent synapomorphies of *Ligumia nasuta* and (*L. recta* + (*Villosa* + (*Epioblasma* + (*Lampsilis* + *Actinonaias*)))), with reversal to *0* in *Actinonaias* (*s* = 3, *CI* = 0.333, *RC* = 0.200).

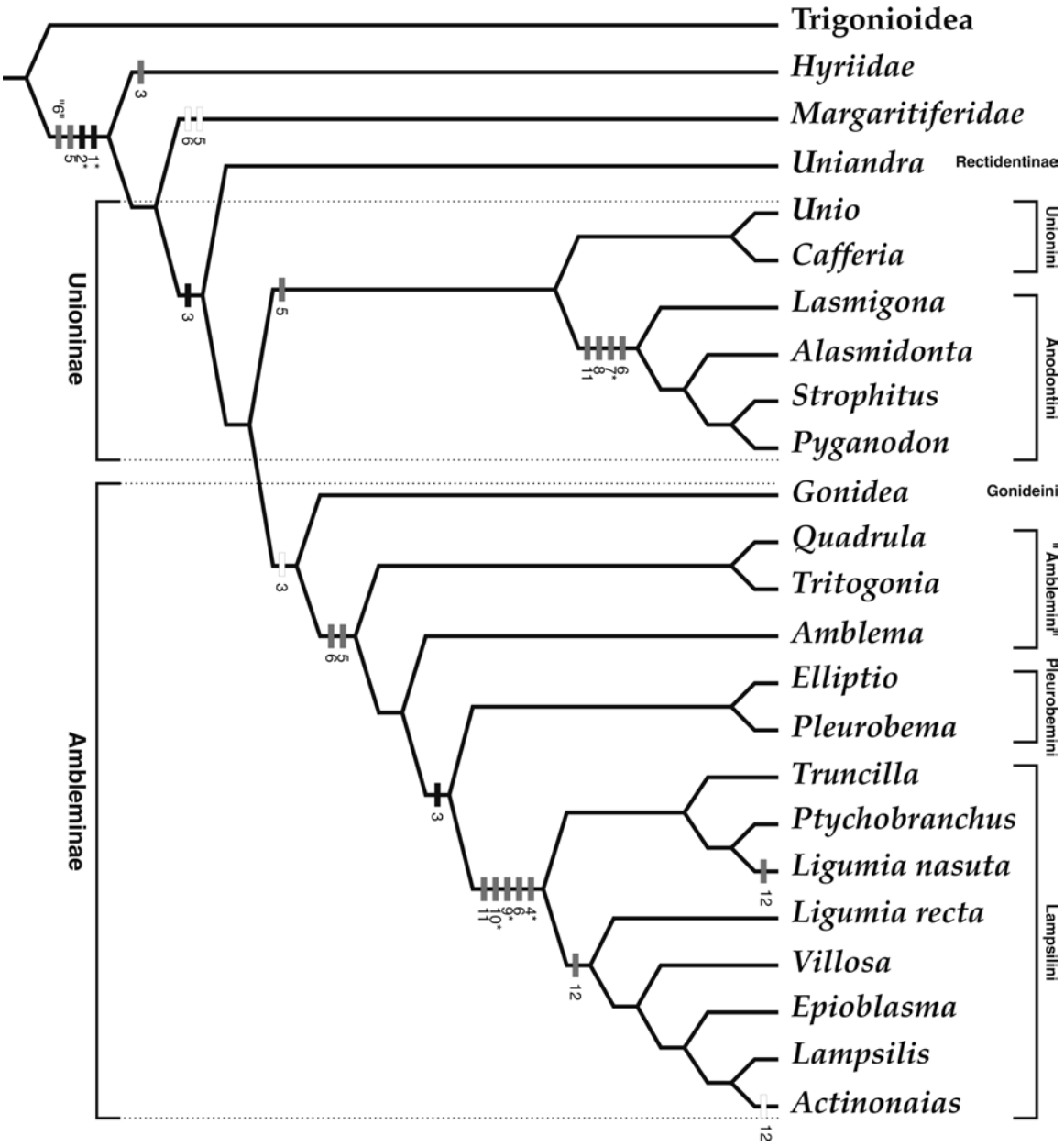


Figure 4.1. Trace of Brooding Characters on the 'Best-Estimate' Cladogram. See text for a discussion of how the 'best-estimate' tree was chosen. Unambiguous character transformations (*CI* = 1.0) are labeled with an asterisk ('*').

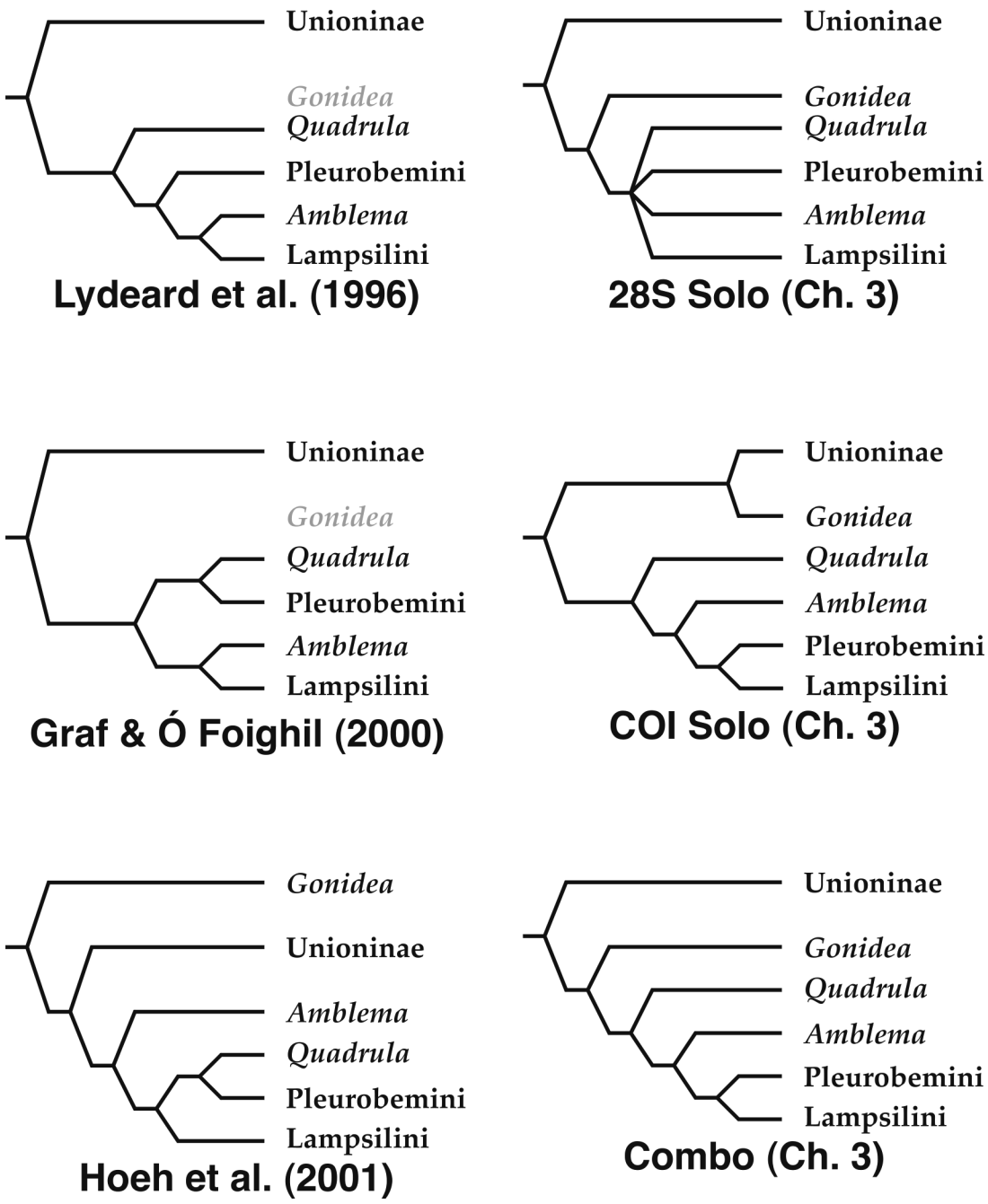


Figure 4.2. Alternative arrangements of the Ambleminae. These five cladograms compare the branching pattern of the Ambleminae from three previously published molecular studies (Lydeard *et al.*, 1996; Graf & Ó Foighil, 2000; Hoeh *et al.*, 2001) and the three molecular character sets studied in the previous chapter (**Figures 3.1-3**).

CHAPTER 5

USE OF 28S TO TEST THE PHYLOGENY OF THE AUSTRALIAN HYRIIDAE

As discussed in **Chapter 1**, freshwater mussels (Unionoida) are a globally distributed, ancient group of strictly continental bivalves. Their diversity and unique parasitic larvae have attracted a great deal of ecological study, especially from a conservation perspective [see Kat (1984) and Watters (1994b) and references cited therein]. Much of that research was focused on the Nearctic mussel assemblage and, until very recently, has lacked a modern evolutionary context (Graf & Ó Foighil, 2000). This is surprising given that the age, distribution, and diversity of the Unionoida provide ample pattern with which to test hypotheses of macroevolutionary processes such as character evolution and biogeography.

Of special interest is the zoogeography of the freshwater mussel families confined to the Southern Hemisphere: Etheriidae, Iridinidae, and Hyriidae (Etherioidea). These mussels are presently restricted to the southern continents, although there are paleontological arguments suggesting their Mesozoic inhabitance of North America (Henderson, 1935; Morris & Williamson, 1988; Good, 1998). The modern distribution of the Etherioidea, as determined in **Chapter 2**, easily leads to speculation about the influence of the disintegration of Gondwana on the evolution of those mussels. That morphological cladistic analysis principally tested the relationships of the Hyriidae (**Figure 2.1**), a widespread family found in both South American and Australasian fresh waters. However, that data set did little to clarify the relationships within the Hyriidae (**Table 2.5**). The object of this chapter is to address the evolution and biogeography of Australasian hyriid clades, especially the problem of disjunction of New Zealand

freshwater mussels across the Tasman Sea. This analysis has been previously published (Graf & Ó Foighil, 2001).

Based upon the strictly morphological cladistic analysis of **Chapter 2**, there seems little doubt that the Hyriidae is monophyletic (Graf, 2000a). If this is true, however, their present distribution presents a dramatic disjunction: the Neotropical Hyriinae and the Australasian Hyridellinae (**Table 5.1**). Unfortunately, nearly all evolutionary discussion of these families pre-dated both (1) the widespread acceptance of phylogenetic systematics as a scientific means to discover organismal relationships and (2) the recognition of continental drift as a potential mechanism for vicariance. Thus, the narrative of hyriid macroevolution has yet to be formally purged of problematic hypotheses involving waif dispersal or migration of hypothetical 'ancestral stocks' across post-Mesozoic 'land bridges' (*e.g.*, Ortmann, 1921a; Modell, 1942; McMichael & Hiscock, 1958; McMichael & Iredale, 1959; Parodiz & Bonetto, 1963).

While the Hyriinae is restricted to South America, the Hyridellinae presents its own disjunction. The Australian Hyriidae occur in the rivers and lakes of Australia, Tasmania, New Guinea, and the Solomons on the western and northern sides of the Tasman Sea, and New Zealand on the eastern side. Two of the eight genera that inhabit the region occur on New Zealand: *Hyridella menziesi*, *H. aucklandica*, and *Cucumerunio websteri* (McMichael, 1958). The consensus has been that the observed disjunction among the Hyridellinae is due to late Tertiary long-distance dispersal via phoresy upon migratory birds (McMichael, 1954, 1958; McMichael & Hiscock, 1958) or host fish (Walker *et al.*, 2001) from Australia.

Until the relatively recent acceptance of continental drift among western biogeographers (Wegener, 1966), transoceanic dispersal or migration across 'land bridges' would have been the only options available to explain the disjunctions of the Hyriidae (*e.g.*, Darlington, 1957). Modern biogeographic theory suggests an alternative. The Hyriidae may have been distributed widely on the Mesozoic Gondwana, the southern

supercontinent composed of what are now South America, Africa, Madagascar, India, Antarctica, Australia, and New Zealand. When that landmass rifted apart, the respective hyriid faunas of South America, Australia, and New Zealand were isolated and have persisted into modern times.

A review and chronology of the disintegration of Gondwana can be found in Storey (1995: Figure 1) and Brown & Lomolino (1998: Figure 6.17). In summary, rifting and sea floor spreading around 160 million years ago (Mya) (Jurassic) split South America and Africa from the rest of Gondwana. However, southern South America remained in close proximity to Antarctica into the Tertiary. On the other side of Antarctica around 100 Mya (Cretaceous), Australia began to separate from Antarctica. New Zealand remained locked to both Australia and Antarctica until roughly 90 Mya. Since 80 Mya, New Zealand has been isolated from Australia; and since 60 Mya, the two have been separated by a minimum distance of over 1000 km (Cooper *et al.*, 1993). Vicariance hypotheses to explain the distributions of southern continent freshwater mussels have received almost no attention and have gone largely untested (McMichael, 1967; Graf, 2000a; Walker *et al*., 2001).

For this study, I attempted to falsify vicariance as the biogeographic mechanism of hyriid disjunction across the Tasman Sea. From the alternative biogeographic process hypotheses (*i.e.*, dispersal vs. vicariance), I derived predictions of molecular phylogenetic pattern. If the vicariance hypothesis is true, the origin of the genus *Hyridella* must predate the barrier. Thus, if the New Zealand hyriids achieved their present distribution by vicariance 80 Mya (*i.e.,* the rifting of New Zealand from Australia and Antarctica), then the branch lengths separating New Zealand *Hyridella* spp. from their Australian congeners should be long relative to the internal branch supporting the clade. I presumed that the length of these terminal branches should be of the same order (or longer) as the branch lengths presented by other late Mesozoic freshwater mussel splits. This model assumes a reasonably constant rate of molecular evolution, allowing branch length to

serve as a loose proxy for time (*i.e.*, long branch ≈ long time, and vice versa). Strictly speaking, clades containing both New Zealand and Australian species should be 'leafy' (Graf & Sparks, 2000; see **Chapter 3**). Short terminal branch lengths for New Zealand hyriids or a 'stemmy' topology would reject a vicariance hypothesis and support more recent dispersal.

Several recent studies have demonstrated the value of nucleic acid characters in recovering the family-level phylogeny of freshwater mussels (Rosenberg *et al.*, 1994, 1997; Lydeard *et al.*, 1996; Hoeh *et al.*, 1998; Graf & Ó Foighil, 2000), especially where morphological characters have fallen short (Graf, 2000a). The results of **Chapter 3**, based on the relative branch lengths of the limited hyriids included in that analysis (**Figure 3.4**), suggest that 28S is the more appropriate molecule to address the question at hand (see discussion in that chapter as to the appropriateness of molecular data for phylogenetic studies). I acquired domain 2 of 28S rDNA from South American, Australian, and New Zealand hyriids, as well as representative northern continent unionoids to serve as outgroups and for branch length comparisons. That gene fragment has been successfully employed to recover late Mesozoic phylogenetic branching patterns among the Bivalvia (Park & Ó Foighil, 2000). During preliminary analyses, I discovered some hyriids exhibit unexpectedly high levels of intraspecific 28S rDNA variation. As an independent test of branch lengths among the Hyridellinae, I also obtained a stretch of cytochrome oxidase subunit I (COI) for the Australian and New Zealand species. My results allow a re-evaluation of the story of the evolution of the Hyriidae from a modern biogeographic perspective.

Methods & Materials

Domain 2 of 28S [28S (D2)] rDNA sequences were obtained from 11 species of freshwater mussels, including five outgroups (**Table 5.2**) following the methods

described in **Appendix IV**. Multisequence alignments were compiled and manipulated using Sequence Monkey 2.8.0 (Graf, 2000b) and Clustal_X (Thompson *et al.*, 1994, 1997) and refined manually where necessary.

Phylogenetic analyses were carried out using *PAUP* 4.0b3* (Swofford, 1998). Maximum Parsimony (MP) and Maximum Likelihood (ML) optimality criteria were both applied to recover the phylogeny of the Hyriidae. MP searches ran as branch-and-bound with gaps in the alignment treated as missing data. ML searches (heuristic searches, 5 random sequence additions) were performed under the HKY model (Hasegawa *et al.*, 1985) with rate heterogeneity. The transition: transversion ratio, proportion of invariable sites, and gamma shape parameter were estimated by maximum likelihood. Analogous searches were performed for both 28S and COI. However, the COI matrix was composed only of the hyridellid taxa from Australia and New Zealand and was intended solely as an independent test of the branch lengths obtained from the 28S phylogeny.

To gauge the 'robustness' of the topology recovered from the 28S MP analysis, Jackknife resampling analysis (50% character deletion each replication; 1000 replications, heuristic searches, 10 random additions each) was run using PAUP*. Also, Bremer-Decay Index (BDI) values were calculated using TreeRot (Sorensen, 1999), which creates a constraint file for PAUP*. For each node, BDI indicates the difference in length of the next shortest tree without that node. The larger the BDI, the better the support (Bremer, 1995).

Stemminess of the *Hyridella* clade was calculated as explained in **Chapter 3**, where stemminess equals the average internal to terminal branch length ratio. Stemminess was estimated from the branch lengths assigned by PAUP* (ACCTRAN character-state optimization) on the 28S phylogeny for both MP and ML. Stemminess values greater than 1.0 indicate a 'stemmy' topology with short terminals relative to the internal branch; less than 1.0 indicates a 'leafy' topology with long terminal branches.

Alternative topologies were constructed using MacClade 3.07 (Maddison & Maddison, 1997). These were scored with PAUP* under both parsimony and the likelihood model derived from the ML search. Kishino & Hasegawa (1989) tests were used to gauge the significance of alternative topologies. The likelihood of the optimal 28S phylogeny was also analyzed both with and without a molecular clock enforced, and a Likelihood Ratio Test (LRT) was applied to test the significance between the two models. The molecular clock analysis was rooted between the Hyriidae and (*Cumberlandia*, Unionidae) based on Graf (2000a; Graf & Ó Foighil, 2000; see **Chapters 2-3**).

Results

Fourteen partial 28S rDNA sequences acquired from 11 species were aligned into a matrix of 446 characters. On average, outgroup sequences were slightly longer than those of the ingroup. Whereas the five outgroup sequences averaged 430.2±2.2 nucleotides (nt) in length, the median ingroup sequence length was 412 nt, with only *Diplodon* (413 nt), *Castalia* sp. (387), and one of the *Hyridella menziesi* (307) deviating. In the case of *Castalia*, the missing nt were from the ends of the gene fragment; I truncated these due to a high number of ambiguous nucleotides. The *H. menziesi* from the North Island of New Zealand, on the other hand, had a deletion extending from positions 234 to 349 in the aligned matrix. In addition to sequence length variation, I also uncovered unexpected intraspecific sequence divergence among the Australasian hyriids: *H. australis* (4.4%), *H. menziesi* (3.3%), and *Velesunio ambigua* (0.7%). All three *H. depressa* individuals that I analyzed had the same 28S (D2) rDNA sequence.

MP and ML analysis of the 28S (D2) data set recovered a single phylogeny (**Figure 5.1**). The Hyriidae were recovered as monophyletic. However, contrary to the strictly morphological analysis (**Figure 2.1**), the Australasian 'hyridellinae' were

paraphyletic relative to the Hyriinae. In addition, *H. australis* was recovered as paraphyletic. Analysis of an alternate topology with a monophyletic *H. australis* was found to be insignificantly different from the optimal tree under both MP and ML, while a monophyletic Hyridellinae was significantly different from the optimal topology only under MP (**Table 5.3**). The ML model is provided in **Table 5.3**. The 28S (D2) terminal branch lengths among *Hyridella* spp. are long relative to the internal branch supporting that clade (**Figure 5.1**). The 'stemminess' value for *Hyridella* is decidedly 'leafy,' regardless of whether it is calculated from MP (0.22) or ML (0.17) branch lengths (**Table 5.5**). These 28S data are not consistent with a perfect molecular clock (**Table 5.3**).

Seven COI sequences were obtained from nine individuals representing four species of Australian and New Zealand hyriids (**Table 5.2**). We aligned these into a matrix of 638 nt that contained no insertion-deletions. Mitochondrial DNA, as suggested by Graf & Sparks (2000) and the combined 28S + COI analysis presented in **Chapter IV**, proved of little value in recovering the potentially late Mesozoic divergences of the Australian region Hyridellinae, as evidenced by the insignificantly different topologies favored by the two optimality criteria. Results of phylogenetic analyses of COI are presented in **Table 5.4** and **Figure 5.2**. COI did, however, serve to put the observed 28S rDNA variation into perspective. For example, whereas the 28S sequences of the two *H. australis* differed by >4%, the COI haplotypes of these two individual mussels were identical. In addition, the mean uncorrected, interspecific, pairwise distance among the COI haplotypes was 14.0±1.0%. This corresponds to the average divergence between the Pleurobemini and Lampsilini (Graf & Ó Foighil, unpublished), a suspected Cretaceous split (Haas, 1969b; Lydeard *et al.*, 1996).

Discussion

The patterns recovered by these phylogenetic analyses cast a new light on the evolution of the Hyriidae (**Figure 5.1**). This is relevant not only to the limited biogeographic problem among *Hyridella* spp. in Australasia, but also to the evolution of the Hyriidae on the southern continents. These results are consistent with ancient vicariance caused by the rifting of New Zealand from Australia and Antarctica as the mechanism behind the disjunction of freshwater mussels across the Tasman Sea. I find this result incompatible with late Tertiary dispersal as suggested by McMichael (1958; McMichael & Hiscock, 1958). Other available evidence, inconsistent with the dispersal model, is reviewed below.

Most systematists of the Hyriidae have considered the Hyridellinae of Australasia to be a "well-defined unit" (McMichael & Hiscock, 1958: 496). An ancestral hyriid invaded the region from southeastern Asia (Iredale, 1934; McMichael & Hiscock, 1958) or South America via Antarctica (Parodiz & Bonetto, 1963), and the present endemic diversity resulted from speciation on the isolated continent. This model predicts that the Hyridellinae are monophyletic (assuming a single invasion) and that the ancestor of the clade was limited to Australasia. This was supported by the morphological analysis of the Etherioidea documented in **Chapter 2** (Graf, 2000a).

The results of this molecular phylogenetic study, however, lead me to reject this model. **Figure 5.1** shows that the Australasian 'hyridellinae' are paraphyletic relative to the South American Hyriinae and that this result is robust. From a vicariance perspective, this topology suggests that hyriids pre-date the disintegration of Gondwana and that they were widespread on that supercontinent. The present endemism of the two Australasian tribes represented in my analysis, Velesunionini and Hyridellini, is due to persistence rather than cladogenesis on an isolated continent. Ortmann's (1921a; also

Modell, 1942) 'land bridge' scenario is also consistent with our phylogenetic results: the Hyriidae arose on Australia and spread via Antarctica to South America.

McMichael (1954, 1958; McMichael & Hiscock, 1958; McMichael & Iredale, 1959) argued for freshwater mussel phoresy upon migratory birds as the mechanism of dispersal to New Zealand from Australia. Based on the conchological similarity of the New Zealand mussel species to those of modern Australia, he (1958: 430) argued for "fairly recent" dispersal, but did not elaborate on the timing beyond Tertiary. More recently, Walker *et al.* (2001) have also allowed for transoceanic dispersal of hyriids upon their host fish. The relationships presented here (**Figure 5.1**), however, are not consistent with these models, at least as far as *H. menziesi* is concerned.

The 28S phylogeny in **Figure 5.1** shows a 'leafy' *Hyridella* clade. Although this result is complicated by unexpected rapid, intraspecific evolution in *H. menziesi* and *H. australis*, as well as by the unconvincing branching order within *Hyridella* (**Table 5.3**), my COI results corroborate the long 28S (D2) branches (**Figure 5.2**). Intraspecific variation in nuclear rDNA is not uncommon but is peculiar under the current paradigm of paralogous sequence homogenization by 'concerted evolution' (Hillis & Dixon, 1991; Park & Ó Foighil, 2000). My results suggest that perhaps 28S rDNA sequence evolution is mediated by another mechanism in the Hyriidae.

Any correlation between sequence divergence and time would have extremely wide confidence limits (Hillis *et al.*, 1996). But with that in mind, it is interesting to note that the observed 28S and COI branch lengths between *H. menziesi* and its Australian congeners generally match or exceed those among other mussel taxa also suspected of late Mesozoic divergence [(*Unio* + *Pyganodon*) and (*Amblema* + *Lampsilis*) in **Figure 5.1**; Graf & Ó Foighil, unpublished]. This is consistent with vicariance due to the break up of Gondwana as an explanation for the disjunction of *Hyridella* across the Tasman Sea. In the same vein, these data also suggest that the split among *H. depressa* and *H. australis* dates to a similarly ancient time.

Based upon these tree topologies, I reject the hypotheses of late-Tertiary/Quaternary dispersal of freshwater mussels by birds or fish across the Tasman Sea — at least for *Hyridella menziesi*. The philosophical hurdle that must be addressed when applying dispersal hypotheses to problems of disjunction is that dispersal, as a biogeographical mechanism, is generally not testable (Croizat *et al.*, 1974; Ball, 1976). Succinctly put, for each realized falsifiable prediction supporting a vicariance hypothesis, a consistent *ad hoc* dispersalist scenario can also be concocted. This is not to say that individual dispersal hypotheses can not be rejected. Fortunately, vicariance hypotheses are generally falsifiable. They differ fundamentally from dispersal hypotheses in that they describe a temporally and spatially discrete vicariance event: the formation of a barrier. Dispersal, on the other hand, refers to an essentially infinite series of improbable events spanning the entire history of the barrier. My molecular phylogenetic results alone are not inconsistent with ancient transoceanic dispersal, but post-Gondwana migration is in conflict with other lines of evidence, namely the fossil record, unionoid life history, and the distributions of other southern continent taxa.

While the reality of continental drift had not yet taken hold by the late 1950s, hypotheses of past connection between the southern continents, especially based on their shared floras (*e.g.*, Hooker, 1867), enjoyed wide recognition (Brown & Lomolino, 1998). McMichael (1958; McMichael & Hiscock, 1958) was correct to predict that, for a vicariance (*i.e.*, land bridge) hypothesis, the common ancestor of the New Zealand and Australian hyridellids must have (1) pre-dated the formation of the Tasman Sea (Cretaceous) and (2) been found on both sides of that barrier (Platnick & Nelson, 1978). He was, however, incorrect in his assessment of the available fossil evidence. The terrestrial paleontological record for New Zealand is far from complete (Cooper *et al.*, 1993; Daugherty *et al.*, 1993), and McMichael's (1958) rejection of continuous mussel occupation of New Zealand since the Mesozoic based on the lack of a uninterrupted transitional series seems unfounded. Ideally, the nearest common ancestor of Australian

and New Zealand *Hyridella* would be identified from the fossil record. In practice, that is unlikely. The bottom line is that there are Mesozoic fossil hyriids on New Zealand, including at least one tantalizingly hyridelline specimen (McMichael, 1957). While inconclusive, this is still consistent with the vicariance hypothesis.

Among the most damning evidence against long-distance avian dispersal of freshwater mussels is that it has never been observed— it is purely hypothetical. Contrary to historical anecdotes (*e.g.*, Call, 1878; Kew, 1893; Simpson, 1893; Cotton, 1934), all available evidence suggests that freshwater mussels are dispersed only via their host fish (Johnson, 1970; Graf, 1997b, 1998). It has been suggested by Walker *et al.* (2001) that *H. menziesi* might have reached New Zealand via *Anguilla*, which that mussel has been reported to infect (Hine, 1978). Infection alone, however, is not a convincing indication of parasitism (Graf, 1998), and anguillid catadromy and semelparity make this hypothesis a non-starter (Lake, 1971; Bastrop *et al.*, 2000). As discussed above, these *ad hoc* dispersal hypotheses are difficult to falsify and, in light of the evidence supporting vicariance, unnecessary. One hundred years ago, mussel phoresy upon migratory birds or fish may have seemed more likely than the possibility of a dynamic continental crust. Our improved understanding of unionoid life histories and historical geology no longer supports this assumption.

It is beyond the scope of this analysis to provide a detailed discussion of these biogeographic patterns in the context of vicariant distributions among other New Zealand taxa (*e.g.*, Platnick & Nelson, 1978; Rosen, 1978; Craw, 1985). Suffice it to say that New Zealand is home to more than a few Gondwanan 'relics'— taxa incapable of transoceanic dispersal like frogs (*Leiopelma*), tuataras (*Sphenodon*), onycophorans (*Peripatus*), beeches (*Nothofagus*), *etc.* [reviewed in Cooper *et al.* (1993), Daugherty *et al.* (1993), and Humphries & Parenti (1986)]. *Hyridella* should be added to that list.

Based on my molecular phylogenetic analyses, I failed to reject the hypothesis that vicariance due to the break up of Gondwana was the biogeographic mechanism of

hyriid disjunction across the Tasman Sea. Taken together, the results of these molecular phylogenetic analyses and my review of the data for and against long-distance dispersal provide a compelling case for vicariance, and, at the same time, demonstrate that there really are no data in favor of the long-distance dispersal model. However, this examination was based on only a single clade: *Hyridella menziesi* on New Zealand and a limited sample of its congeners on Australia. Further testing is necessary, especially with regard to the generality of our conclusions to the other two New Zealand freshwater mussels, *H. aucklandica* and *Cucumerunio websteri*.

Table 5.1. Taxonomy and Distribution of the Hyriidae. The nomenclature of the Hyriidae has been updated to standardize the views of Iredale (1934; McMichael & Hiscock, 1958), Parodiz & Bonetto (1963), and Graf (2000a): the Australian family and subfamilies have been demoted to a subfamily with four tribes. † Indicates presence on Tasmania.

Taxon	New Guinea[1,2,3]	Australia[1,2]	New Zealand[1,2,4]	South America[5]
Hyridellinae				
Hyridellini	X	X†	X	
Cucumerunionini	X	X	X	
Velesunionini	X	X†		
Lortiellini		X		
Hyriinae				
Hyriini [= Prisodontini]				X
Diplodontini				X
Castaliini				X

Distribution data references: [1]McMichael & Hiscock (1958), [2]Walker *et al.* (2001), [3]McMichael (1956), [4]McMichael (1958), [5]Parodiz & Bonetto (1963).

Table 5.2. Taxa from Which Sequences Were Acquired. See **Appendix IV** for protocol and references.

Taxon	Locality
Velesunionini	
Velesunio ambigua (n = 2)	New South Wales, Australia
Hyridellini	
Hyridella australis (n = 2)	New South Wales, Australia
H. depressa (n = 2)	New South Wales, Australia
H. menziesi	North Island, New Zealand
H. menziesi	South Island, New Zealand
Diplodontini	
Diplodon chilensis	Chile, South America
Castaliini	
Castalia sp.	Paraguay, South America
outgroups	
Cumberlandia monodonta	Minnesota, USA
Unio pictorum	Austria
Pyganodon grandis	Michigan, USA
Amblema plicata	Michigan, USA
Lampsilis cardium	Michigan, USA

Table 5.3. Examination of Alternative 28S rDNA Phylogenetic Topologies and Models. Alternative topologies, constraining the monophyly of taxa not recovered in the optimal tree (Figure 1), were tested using the Kishino-Hasegawa Test, and the alternative likelihood models (molecular clock vs. no molecular clock) were tested using an LRT. CI is the Consistency Index; *p* indicates the probability of getting a more extreme statistic under the null hypothesis (*i.e.*, no difference between the two trees or models). * Indicates statistical significance at the 95% level.

		Parsimony		Maximum Likelihood	
Topology	Length	CI	*p*	-ln L	*p*
optimal	209	0.890	-	1718.86	-
H. australis monophyletic	210	0.886	0.318	1721.06	0.465
Australian *Hyridella* monophyletic	210	0.886	0.564	1719.57	0.884
Hyridellinae monophyletic	214	0.869	0.025*	1726.77	0.141

Model	ln L	*p*
Molecular clock not enforced	-1718.86	-
Molecular clock enforced	-1736.07	<0.05*

ML model: HKY with rate heterogeneity, ti/tv = 1.261, proportion of invariable sites = 0.108, gamma parameter = 0.909.

Table 5.4. Examination of Alternate COI Phylogenetic Topologies. Abbreviations and statistics are as in **Table 5.3.** * Indicates statistical significance.

Alternative topologies

MP tree: (*Velesunio* + (*Hyridella menziesi* + (*H. australis* + *H. depressa*)))

ML tree: (*Velesunio* + (*Hyridella australis* + (*H. depressa* + *H. menziesi*)))

		Parsimony		Maximum Likelihood	
Topology	Length	CI	*p*	ln L	*p*
MP	206	0.903	-	-1772.38	0.64
ML	210	0.886	0.37	-1771.40	-

ML model: HKY with rate heterogeneity, ti/tv = 3.91, proportion of invariable sites = 0.69, gamma parameter = ∞ (set to maximum allowable value: 300).

Table 5.5. MP and ML stemminess values for *Hyridella* 28S rDNA. IBL is the internal branch length; TBL is the terminal branch length. See text for an explanation of the stemminess calculation.

	Parsimony			Maximum Likelihood		
species	IBL	TBL	Stemminess	IBL	TBL	Stemminess
(1) *H. menziesi*	2	21	0.10	4.2x10-3	6.5x10-2	0.06
(2) *H. menziesi*	2	11	0.18	4.2x10-3	2.9x10-2	0.15
H. depressa	2	8	0.25	4.2x10-3	2.1x10-2	0.20
(1) *H. australis*	2	13	0.15	4.2x10-3	3.5x10-2	0.12
(2) *H. australis*	2	5	0.40	4.2x10-3	1.3X10-2	0.32
Mean			**0.22±0.12**			**0.17±0.10**

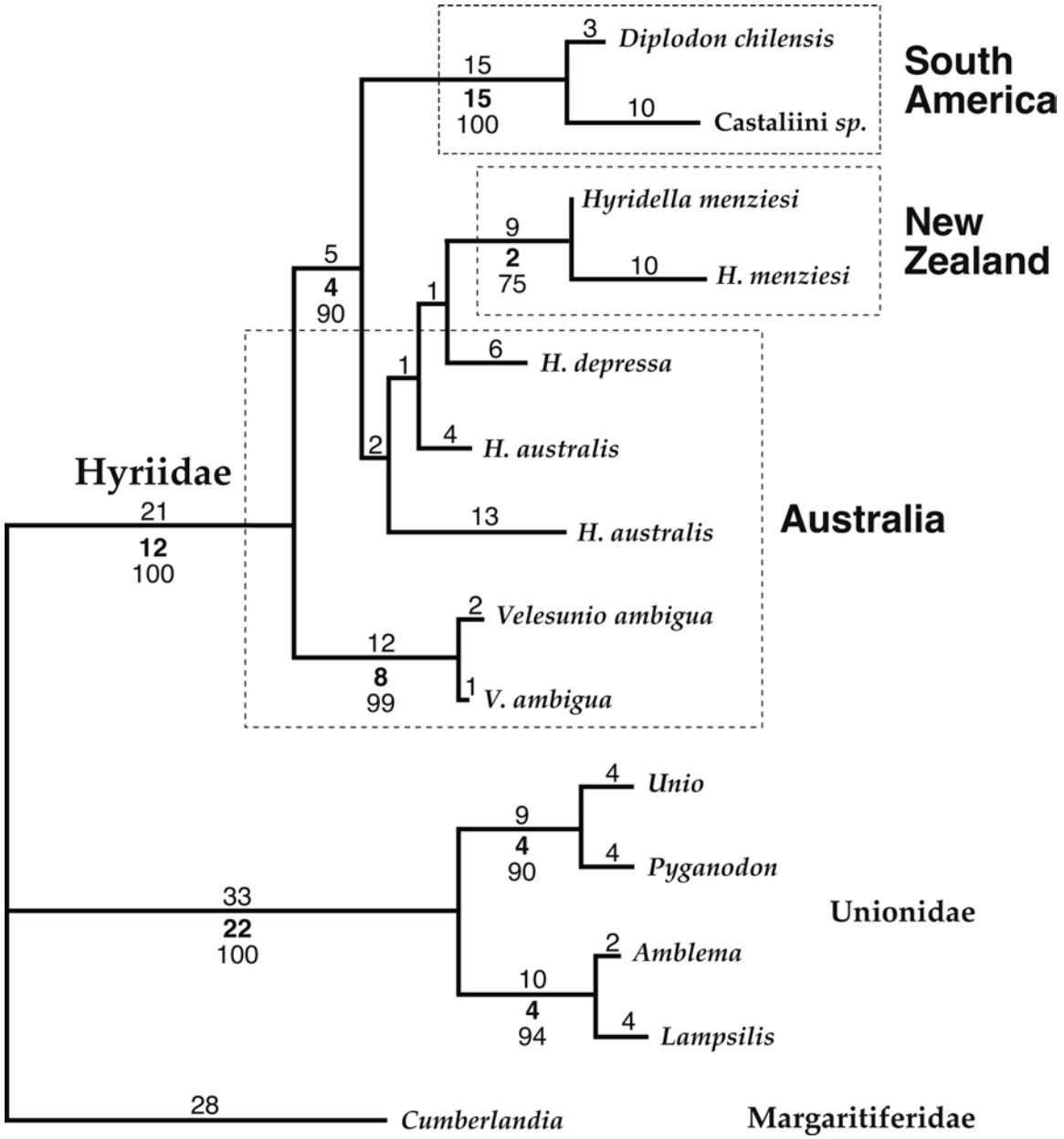


Figure 5.1. 28S rDNA Domain 2 Phylogeny of the Hyriidae. Numbers above the branches are MP branch lengths; those below are **BDI** and jackknife resampling values also from the MP analysis. The ML analysis recovered the same branching order. BDI <2 not shown.

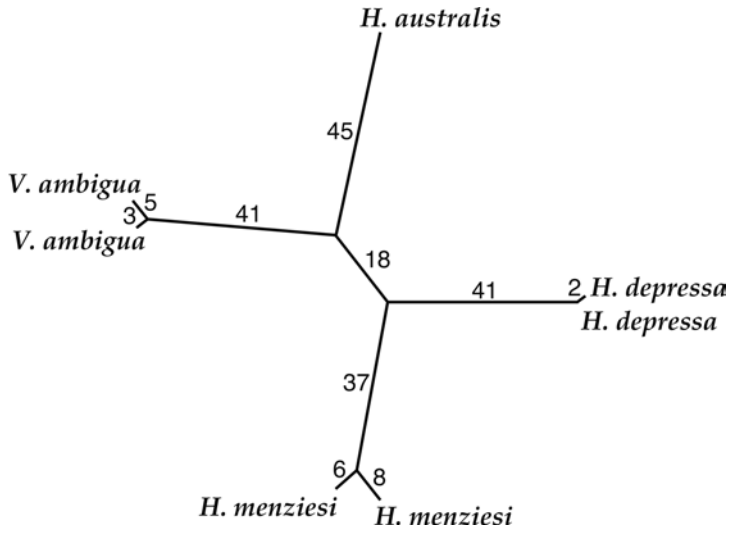


Figure 5.2. COI MP phylogeny of the Representative Hyridellinae. Numbers associated with the branches are MP branch lengths. The ML phylogeny has a different topology (**Table 5.4**), but the terminal branch lengths are of a similar magnitude relative to the internal branch.

CHAPTER 6

THE PHYLOGENETIC POSITIONS OF *UNIO* AND *GONIDEA*: A TEST OF THE CLASSIFICATION OF THE NEARCTIC UNIONIDAE

The Nearctic freshwater pearly mussels, or naiades, (Bivalvia: Unionoidea: Unionidae and Margaritiferidae) are a diverse assemblage of over 300 species and subspecies (Haas, 1969a; Burch, 1975; Williams *et al.*, 1993). While the generic relationships among the eastern North American mussels have been comprehensively addressed multiple times (see references cited below), the lack of a cosmopolitan, phylogenetic perspective has hampered macroevolutionary studies of the Unionoidea (discussed in **Chapter 4**). The object of this chapter is a molecular cladistic test of the system of the Nearctic Unionidae focusing on two traditionally problematic taxa: the Old World *Unio*, and *Gonidea*, a monotypic genus confined to the Pacific drainages of the western United States and Canada.

The various classifications of the Nearctic Unionidae have been reviewed by Heard & Guckert (1971), Davis & Fuller (1981), and in **Appendix II**. The majority of those systems that considered *Unio* and/or *Gonidea* lacked a phylogenetic perspective and were largely authoritarian narratives (*e.g.*, Simpson, 1900, 1914; Ortmann, 1912b, 1916a; Modell, 1942, 1949, 1964; Haas, 1969a, b; Starobogatov, 1970; Heard & Guckert, 1971). Although their non-phylogenetic perspective does not necessarily make them 'wrong,' it does mean that their methods were not scientific, at least in the Popperian sense.

Unio has generally been considered similar to the North American *Pleurobema* and *Elliptio*. Ortmann (1912b) lists several characters shared by those genera, for example:

(1) larval brooding is confined to the outer demibranchs,

(2) the marsupium does not distend greatly when gravid,

(3) brooding is for a short period during the northern summer (i.e., tachytictia), and

(4) there are non-tripartite water-tubes dividing the interlamellar spaces.

But, enumerating those mussels' similarities in this fashion is misleading. *Unio* and the Pleurobemini (*sensu* Davis & Fuller, 1981) actually display the *lack* of those characters diagnostic of anodontine and lampsiline mussels, and each is potentially plesiomorphic among the Unionidae. In addition, highlighting these particular characters ignores the similarity among the hooked-type larvae of *Unio* and the Anodontinae (Hoggarth, 1999).

Gonidea shares some characters with genera like *Amblema*, such as brooding in all four demibranchs (*i.e.*, tetrageny) and 'unhooked'-type glochidia. *Gonidea*, though, is unique among the Nearctic Ambleminae in that

(1) its non-tripartite septa partitioning the interlamellar spaces are perforated and

(2) its hinge dentition is greatly reduced (Ortmann, 1916a; Heard & Guckert, 1971; Davis & Fuller, 1981).

While this combination of attributes is otherwise unknown in North America, it is common among the freshwater mussels of Southeast Asia, and an affinity between *Gonidea* and genera like *Pseudodon* and *Pilsbryoconcha* has been previously suggested (Modell, 1942; Heard, 1974).

Davis (Davis & Fuller, 1981; Davis *et al.*, 1981) initiated the analytical study of mussel classification, and Lydeard *et al.* (1996) followed-up on that work from a cladistic vantage using 16S ribosomal mtDNA. While Davis & Fuller's (1981) phenetic study included *Gonidea*, Lydeard *et al*. did not. Graf & Ó Foighil (2000) included *Unio* in their

cytochrome oxidase subunit I phylogeny, but the rapid rate of mtDNA evolution hindered the recovery of a robust family-level phylogeny (Graf & Sparks, 2000). Rosenberg *et al.* (1994, 1997) included both *Unio* and *Gonidea* in their studies, but their analyses were also unable to resolve the relationships within the Unionidae. Graf's (2000a) morphological phylogenetic analysis of the Etherioidea (see **Chapter 2**) included several unionids, among them *Unio*, but the morphological character set was too homoplastic to reliably resolve the relationships among those lineages of the northern continents. Thus, despite the recent surge in cladistic analyses of the Unionidae, the sister groups of either *Unio* or *Gonidea* remain a guess.

For this phylogenetic test of the positions of *Unio* and *Gonidea*, I not only included representatives of the Margaritiferidae, anodontines and North American amblemids (*Lampsilis*, *Obliquaria*, *Amblema*, and *Elliptio*) but also the southeast Asian *Uniandra*, *Pseudodon*, and *Pilsbryoconcha*. These taxa represent genera with which *Unio* and *Gonidea* have been previously affiliated. In addition to those, I have accounted for Australian and South American hyriids, which have traditionally been considered unionoideans (Parodiz & Bonetto, 1963; Heard & Guckert, 1971; but see **Chapter 2**). These taxa, as well as my character set derived from 28S nuclear rDNA (domain 2), allow a robust test of the interfamilial relationships of the Nearctic freshwater mussel assemblage and a new context for the generic phylogenies of Lydeard *et al.* (1996) and Graf & Ó Foighil (2000).

Methods & Materials

Domain 2 of 28S rDNA [28S (D2)] sequences were harvested from 17 species of freshwater mussels and *Neotrigonia margaritacea*, an extant trigoniid and the marine sister taxon of the Unionoida (Hoeh *et al.*, 1998), following the methods described in **Appendix IV**. Nine of those sequences had been included in an earlier study (Graf & Ó

Foighil, 2001). Ingroup taxon choice focused on representing (1) the monophyletic tribal lineages previously studied by Lydeard *et al.* (1996) and Graf & Ó Foighil (2000) and (2) the Asian families traditionally placed near *Gonidea* (Modell, 1942; Heard, 1974). Two non-palaeoheterodont bivalve sequences were obtained from GENBANK (National Center for Biotechnology Information, National Institutes of Health) (**Table 6.1**). Multisequence alignments were compiled and manipulated using Sequence Monkey 2.8 (Graf, 2000b) and Clustal_X (Thompson *et al.*, 1994, 1997) and refined manually where necessary.

Phylogenetic analyses were carried out using PAUP* 4.0b3 (Swofford, 1998). Maximum parsimony (MP) and maximum likelihood (ML) optimality criteria were both applied to test the branching pattern among the Unionoidea. MP searches ran as branch-and-bound with gaps (*i.e.*, insertion-deletions) in the alignment treated as missing data. For MP analyses, *Mytilus* and *Astarte* were designated as outgroups but trees were left unrooted (**Table 6.1**). ML analysis was performed unrooted with a subset of the ingroup (**Table 6.1**). ML searches (heuristic searches, 5 random sequence additions) employed the HKY model (Hasegawa, Kishino & Yano, 1985) with rate heterogeneity, empirical nucleotide frequencies, and the transition: transversion ratio, proportion of invariable sites, and gamma shape parameter estimated by ML.

To gauge the 'robustness' of the recovered phylogeny, jackknife resampling analysis (50% character deletion each replication; 1000 replications, heuristic searches, 10 random additions each) was performed under MP using PAUP*. Bremer-Decay Index (BDI) values were calculated using a combination of TreeRot (Sorenson, 1999) and PAUP*. For each node, BDI indicates the difference in length of the next-shortest tree without that node. The greater the BDI, the more distant the next shortest tree and the better the support for that node (Bremer, 1995).

Results

Eighteen partial 28S rDNA sequences were acquired from 17 species of freshwater mussels and *Neotrigonia*. These sequences, plus the two outgroups were aligned into a matrix of 455 nucleotides, including gaps and uninformative characters. MP analysis recovered a single phylogeny (**Figure 6.1**). The branching pattern is robust— only a single branch in the MP tree, the one leading to the (*Elliptio* + *Amblema*) clade, collapses in a Jackknife 70% majority-rule consensus tree. The ingroup branching pattern is corroborated by the ML phylogeny as well (not shown; -ln L = 1613.233, ti:tv = 1.320, proportion invariant sites = 0.000, gamma shape parameter = 0.629).

The 28S (D2) phylogeny depicted in **Figure 6.1** supports the monophyly of the unionid tribes listed in **Table 6.1**, at least for the four with more than one taxon (*i.e.*, Unionini, Anodontini, Lampsilini, and Pseudodontini). The Unionidae and Hyriidae were each recovered as monophyletic. The Margaritiferidae, represented by only *Cumberlandia*, was found to be sister to the Hyriidae (in contrast to the phylogenies recovered in **Chapters 3-4**). The paraphyly of the Australian Hyriidae— in this case, *Velesunio* and *Hyridella*— was discussed in **Chapter 5**.

Within the Unionidae, three distinct lineages were recovered: the paraphyletic Asian tribes Pseudodontini and Rectidentini, and a unionid clade composed of the remaining tribes (**Figure 6.1**). The Unionini was well-supported as sister to the Anodontini, and *Gonidea* was placed sister to an (*Amblema* + *Elliptio* + *Obliquaria* + *Lampsilis*) clade. The paraphyly of the Asian tribes was reasonably well-supported by jackknife resampling, but moving *Uniandra* to a position sister to the Pseudodontini results in a phylogeny only three steps longer.

Discussion

Historically, both *Unio* and *Gonidea* have been problematic with regard to classification. In general, this difficulty has simply been a problem of perspective. Among the comprehensive systematists of the Unionoida (cited above), all but Hans Modell (1942) considered their locally reasonable taxonomies as applicable elsewhere. The schemes that they used to divide the Nearctic Unionoidea into families and subfamilies was applied to the rest of the world and not vice versa. Thus, the relationships of North American mussel genera to those of other regions have been arrived at circularly: All genera must belong to Nearctic families because that is the system of the Unionoidea. Thus, while *Unio* and *Gonidea* have been classified relative to the eastern North American Unionidae, so have all other unionids, and there has been little consensus regarding their specific sister relationships.

The type genus of the order has been difficult to place, the irony of which should not be overlooked. By tradition, (*i.e.*, Simpson, 1900, 1914; Ortmann, 1912b; Haas, 1969a, b; Heard & Guckert, 1971; Davis & Fuller, 1981), *Unio* has generally been assumed to be closely related to *Elliptio*, *Pleurobema*, and similar unionid genera that lack the diagnostic anatomical characters of anodontine or lampsiline mussels (see above). By this definition, any taxon comprised of *Unio*, *Elliptio*, *Pleurobema*, *etc.* seems potentially unnatural in an Aristotelian sense: The group is defined by what it *is not* (Eldredge & Cracraft, 1980). Ortmann (1916a) eventually recognized the catch-all nature of his Unioninae and the distinctiveness of *Unio* from the Nearctic genera.

> "...Certain groups of my "Unioninae" should be elevated to the rank of subfamilies. Of the genera treated by myself in 1912, eight (*Fusconaia* to *Uniomerus*) should form the subfamily Quadrulinae; the European *Unio* should form the subfamily Unioninae, *Parreysia* and *Lamellidens* probably should form a third subfamily. In addition, another new subfamily should be erected for [*Gonidea*], that of the Gonideinae..." (Ortmann, 1916a: 53).

Rosenberg *et al.* (1994, 1997) included *Unio* (and *Gonidea*) in their phylogenetic analysis of molluscan 28S rDNA, domain 6. This study suffered from exactly the opposite problem of Graf & Ó Foighil's analysis (2000)— while the latter study was limited by too much character evolution, Rosenberg *et al.* (1994, 1997) did not have nearly enough informative change among the Unionoidea. My analysis of 28S (D2) places the Unionini as sister to the Anodontini. This result has also been obtained by preliminary examinations of unionoid 28S (D2) (Graf & Sparks, 2000; Graf & Ó Foighil, 2001; see **Chapters 3 & 5**) and corresponds to the classifications of Hannibal (1912) and Morrison (1956).

That *Unio* shares a closer common ancestor with *Pyganodon* than *Elliptio* is not surprising from an anatomical standpoint, although the few morphologists that recognized it (*e.g.*, Hannibal, 1912; Morrison, 1956) apparently failed to make a very strong case. The principle morphological synapomorphy diagnostic of the (Unionini + Anodontini) clade is an ontogeny including a sub-triangular, hooked glochidium (Ortmann, 1912b; Heard & Vail, 1976b). Ignoring its larvae, *Unio* appears plesiomorphic in all other respects and presents no objection to deposition in Ortmann's "Unioninae" potpourri. However, the Unionini and Anodontini are the only two tribes among the Unionidae to have hooked glochidia. A convergent larval type occurs among the Hyriidae (Parodiz & Bonetto, 1963; see **Chapter 2**).

Gonidea has generally been placed near to *Amblema*, *Megalonaias*, *etc.* but in its own subfamily or tribe (Ortmann, 1916a; Heard & Guckert, 1971; Davis & Fuller, 1981). There have been exceptions to this, generally due to the emphasis of shell characters over soft-anatomy. Hannibal (1912) considered *Gonidea* to be anodontine, and Modell (1942) placed *Gonidea* among the Margaritiferidae. As quoted above, Ortmann's (1916a) detailed examination of *Gonidea* gave him pause enough to re-think his system of non-anodontine/non-lampsiline unionids. In his subsequent papers on the anatomy of

Hyriopsis (Ortmann, 1916b) and *Uniandra* (Ortmann, 1917), Ortmann avoided explicit statements of taxonomy beyond placing those genera in his original Unioninae.

These differing taxonomic opinions potentially agree on *Gonidea* standing close to the anatomically similar forms of eastern and southeastern Asia, specifically *Pseudodon* and *Pilsbryoconcha* (Modell, 1942; Heard, 1974). By logical extension, the *Gonidea* + Asian group should be close the Nearctic *Amblema*, *Megalonaias*, *etc.* (Brandt, 1974; Davis & Fuller, 1981). According to the present analysis, *Gonidea* is sister to the (Amblemini + Pleurobemini + Lampsilini) clade. The two Asian lineages (Rectidentini and Pseudodontini in **Table 6.1**), however, are paraphyletic relative to each other and distinct from the remaining Unionidae here studied (**Figure 6.1**). Although this is a novel result, it is, as with the position of *Unio*, not especially surprising.

The proposed affiliation of *Gonidea*, *Pseudodon*, and *Pilsbryoconcha* was based largely upon their shared possession of four anatomical characters:

(1) perforated septa,

(2) tetrageny,

(3) tachytictia, and

(4) reduced or absent hinge dentition (Ortmann, 1916a; Modell, 1942; Heard, 1974)

None of these characters is itself a convincing synapomorphy. Graf & Ó Foighil (2000: Figure 3) found that tetrageny (*i.e.*, use of all four demibranchs as a brooding marsupium) has arisen multiple times, and tachytictia (*i.e.*, short-term brooding) is plesiomorphic among the Unionoidea. The reduction of hinge dentition also occurs convergently among the Anodontini, Lampsilini (*e.g.*, *Leptodea*), Margaritiferidae, and (Iridinidae + Etheriidae). Although no comprehensive cladistic analysis of unionoidean morphology has been undertaken, perforated septa dividing the water-tubes of at least the marsupial demibranchs appear to be plesiomorphic among the Unionoida (**Figures 2.2 & 4.1**; see **Chapter 7**). Besides the three genera under consideration, perforated septa have been

described for *Unio* (Heard & Vail, 1976b), *Brazzaea* (Bloomer, 1931a), *Caelatura* (Bloomer, 1932), *Grandidieria* (Bloomer, 1933), *Hyriopsis* (Ortmann, 1916b), *Lamellidens* (Bloomer, 1931b), *Uniandra* (Ortmann, 1917), and many others including the Hyriidae (Ortmann, 1921a; McMichael & Hiscock, 1958).

The imprecise phylogenetic language of previous mussel classifications can be credited to the historical separation of systematics, evolution, and hypothesis-testing by freshwater malacologists. A classification based upon cladistic methods is an obvious scientific improvement as it is firmly grounded in modern evolutionary theory and explicit, testable hypotheses rather than the subjective emphasis of characters assumed to be essential (Wiley, 1980). The results of this molecular phylogenetic study of *Unio* and *Gonidea* bear directly upon the classification of the North American Unionidae, and conservative revisions in the accepted system are warranted (**Table 6.2**). The taxonomy follows the more contemporary classification of Davis & Fuller (1981; Lydeard *et al.*, **1996**) in recognizing two North American unionid subfamilies divided into tribes, rather than Heard & Guckert's (1971) system of two families partitioned into subfamilies. The distinction between these two views is arbitrary, solely one of nomenclature (Principle of Coordination, Art. 36 ICZN, 1999), and has no evolutionary basis (but see discussion in Davis & Fuller, 1981).

The classification presented in **Table 6.2** differs from that of Davis & Fuller (1981) by the inclusion of the Unionini among their Anodontinae. The Principle of Priority (Art. 23, ICZN, 1999) requires that the subfamily be named the Unioninae, and the Anodontinae is demoted to a tribe within it. The classification of Heard & Guckert (1971; Boss, 1982) differs only in the displacement of the Lampsilini and Pleurobemini to the Ambleminae. The Unionini has no representatives in North America, but the Anodontini is Holarctic.

A second difference from both of those previous classifications is the recognition that the Ambleminae (including *Gonidea*) is apparently endemic to the Nearctic province.

This may change, however, with the future inclusion of additional non-North American genera. The Ambleminae of eastern North America (Lampsilini + Pleurobemini + Quadrulini + Amblemini, *etc.*) appear to be monophyletic and diagnosed by the possession of complete septa (*i.e.*, septa lacking perforations). The Rectidentinae and Pseudodontinae are distinct from the (Unioninae + Ambleminae) clade (**Table 6.2**), and Hoeh *et al.* (1996a, 2001) reported the same relative position for *Caelatura*. It is likely that as cladistic analyses of the Unionidae progress, many of Hans Modell's familial nomina will be born again.

Lastly among the deviations from recent systems is the non-monophyly of the "Amblemini" *sensu* Davis & Fuller (1981). Neither of the independent, mtDNA studies of Lydeard *et al.* (1996) nor Graf & Ó Foighil (2000) recovered an exclusive, amblemine clade containing both *Amblema* and *Quadrula*. The 28S (D2) data set, unfortunately, will not be able to shed any light on this issue (see discussion in **Chapter 3**). As evidenced by the short branch lengths among the Ambleminae in **Figure 6.1**, the rate of evolution is insufficient to robustly resolve intra-amblemine relations. For the classification, at least two tribes need to be recognized at this time: Amblemini and Quadrulini (and perhaps a third for *Plectomerus* after Lydeard *et al.*, 1996: figures 3-4). Further phylogenetic work is necessary to test the placement of heretofore unanalyzed genera.

My analysis of 28S (D2) (**Figure 6.1**) has solidified the deeper branching order of the Unionidae and, combined with previous morphological analyses (numerous studies cited above) and the mtDNA phylogenies of Lydeard *et al.* (1996) and Graf & Ó Foighil (2000), has facilitated a taxonomic revision of the family (**Table 6.2**). While the recovered phylogeny certainly indicates future work within the Unionidae (*e.g.*, the positions of other non-North American genera, the branching pattern within the Nearctic Ambleminae), the placement of the Margaritiferidae as sister to the Hyriidae is a novel result that should also be pursued. Building upon the morphological cladistic results of the preceding chapters, the 28S (D2) phylogeny presented here (**Figure 6.1**) suggests that

the Margaritiferidae are sister to the Etherioidea. This hypothesis could be tested by adding representatives of the Iridinidae and Etheriidae to the present data set.

Table 6.1. Taxa from Which Sequences Were Acquired. Unionid taxonomy applied here divides the family into tribes assumed to be monophyletic based on previous studies (Lydeard *et* al., 1996; Graf & Ó Foighil, 2000) or, in the case of the southeast Asian genera, convenience (Brandt, 1974). A '†' marks those taxa included in the maximum likelihood analysis to represent subfamilial or tribal lineages. GenBank accession numbers are listed in **Appendix IV**.

Taxon	Locality
Unionidae	
Unionini	
† *Unio s.s. pictorum*	Austria
Unio (Cafferia) caffer	South Africa
Anodontini	
† *Pyganodon grandis*	Michigan, USA
Alasmidonta marginata	Michigan, USA
Lampsilini	
† *Lampsilis cardium*	Michigan, USA
Obliquaria reflexa	Minnesota, USA
Pleurobemini	
Elliptio dilatata	Michigan, USA
Amblemini	
† *Amblema plicata*	Michigan, USA
Gonideini	
† *Gonidea angulata*	western USA
Rectidentini	
† *Uniandra contradens*	Thailand

Table 6.1 (continued). Taxa from Which Sequences Were Acquired.

Taxon	Locality
Pseudodontini	
Pilsbryoconcha exilis	Thailand
† *Pseudodon vondembuschianus*	Thailand
Margaritiferidae	
† *Cumberlandia monodonta*	Minnesota, USA
Hyriidae	
† *Diplodon chilensis*	Chile, South America
Castalia sp.	Paraguay, South America
† *Hyridella depressa*	New South Wales, Australia
Velesunio ambigua	New South Wales, Australia
Trigoniidae	
Neotrigonia margaritacea	Tasmania, Australia
outgroups	
Pteriomorpha	
Mytilus edulis	
Heterodonta	
Astarte castanea	

Table 6.2. Re-classification of the Nearctic Unionidae. Taxa occurring in North America are listed in **bold**. Classification of the Unionoida follows Graf (2000a). See text for discussion.

Class Bivalvia

Subclass Palaeoheterodonta

Order Unionoida

Family **Unionidae**

Subfamily **Unioninae**

Tribe Unionini

Tribe **Anodontini**

Subfamily **Ambleminae**

Tribes **Amblemini**, **Quadrulini**, *etc.*

Tribe **Pleurobemini**

Tribe **Lampsilini**

Tribe **Gonideini**

Subfamilies Rectidentinae, Pseudodontinae, *etc.*

Family Margaritiferidae

Family Hyriidae

Family Iridinidae

Family Etheriidae

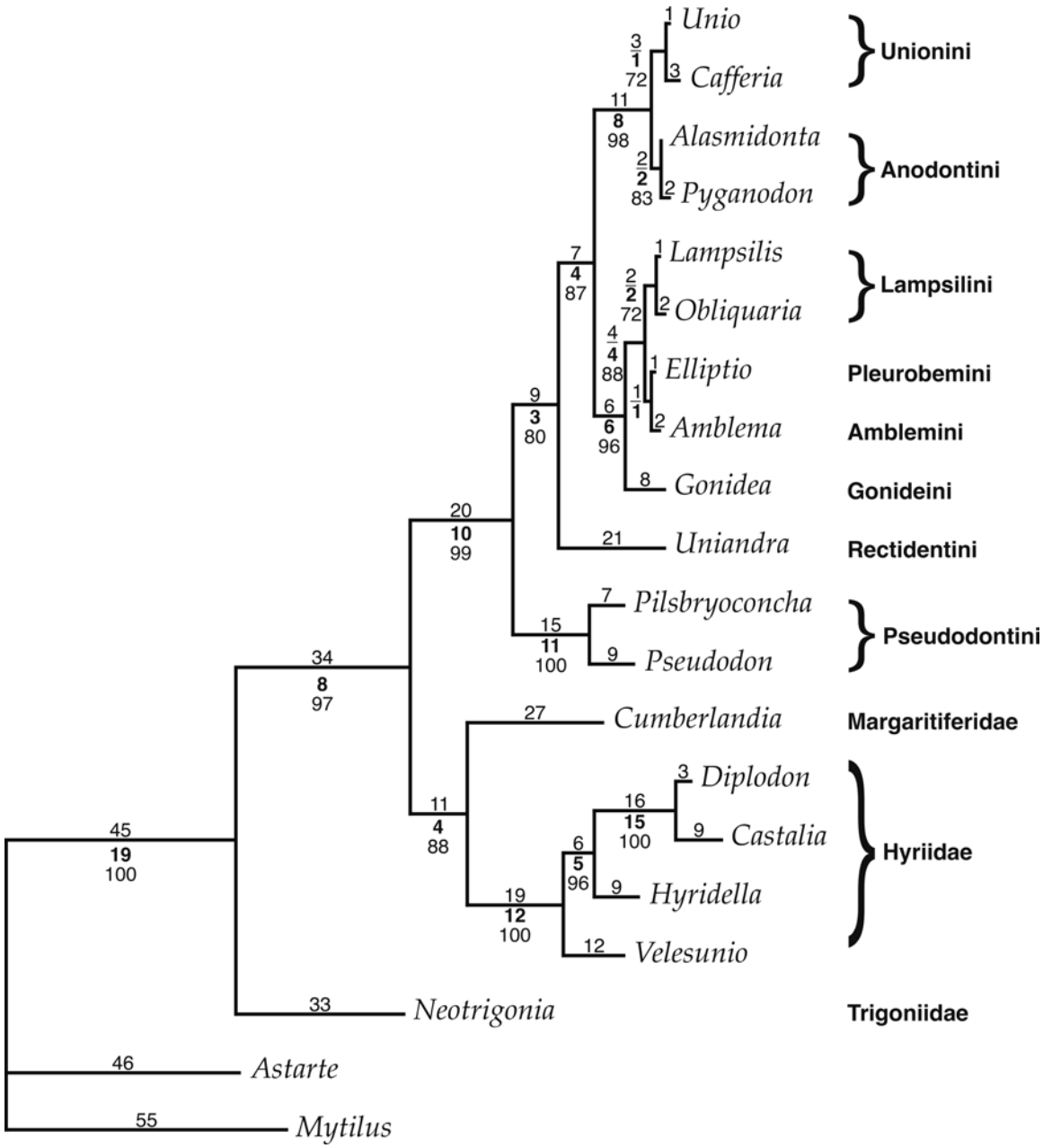


Figure 6.1. 28S Phylogram Depicting the Relationships Among the Freshwater Mussel Tribes Analyzed. Numbers above the branches are branch length, those below are **BDI** and Jackknife indices.

CHAPTER 7

SUMMATION AND CONCLUSIONS: A PHYLOGENETIC PERSPECTIVE ON THE EVOLUTION OF THE UNIONOIDA

In recent years, the most intensive study of freshwater mussels has gone on in the United States under the rubric of conservation and propagation (see numerous references in Burch, 1975; Watters, 1994b). These research lines are inarguably necessary, as the Nearctic unionoid fauna is both among the most diverse in the world (**Figure 1.1**) and also among the most imperiled (Williams *et al.*, 1993; Bogan, 1993). However, this flurry of ecological work emphasizing contemporary processes has displaced evolutionary studies of the Unionoida to a back burner. In the introductory chapter of this dissertation, I reviewed the then-current understanding of freshwater mussel macroevolutionary patterns — *i.e.*, classification, morphological character distributions, and biogeography. It is the purpose of this closing chapter to re-examine those same patterns through the new lens of unionoid phylogeny acquired in **Chapters 2-6** and other literature (see below): A new perspective for a new millennium. As evidenced by the length of this summary, the studies here reviewed have led to a considerable overhaul of unionoid evolutionary biology.

Freshwater mussel life history bears upon the evolution of the group — and is extremely interesting, so a brief review is appropriate. The larvae of the Unionoida are obligate parasites upon vertebrates (generally freshwater fishes) (Coker *et al.*, 1921; Kat, 1984). Most unionoid larvae encyst in host epithelium, usually on the gills or fins, and it is within this cyst that the freshwater mussel undergoes metamorphosis (Wächtler *et al.*,

2001). While this association certainly drives microevolutionary processes (Graf, 1998), the dependence of mussels upon freshwater fishes also has broader consequences. Much has been made of the inability of unionoids to cross terrestrial barriers (*e.g.*, van der Schalie, 1939, 1945; Johnson, 1970; Graf, 1997b, in press). Freshwater mussels have equally restricted opportunities and tolerance for marine dispersal (Kat, 1983; Sepkoski & Rex, 1974; Atrill *et al.*, 1996). Thus, the Unionoida is a strictly continental taxon that is dispersed by its hosts and generally confined to stable, freshwater environments.

A consensus of the 20th century view of freshwater mussel evolution is summarized in **Figure 7.1**. The taxonomy is a chimera of several malacological schools spanning the last century (*e.g.*, Simpson, 1900, 1914; Ortmann, 1910a, 1911a, b, 1912b, 1921a; Frierson, 1927; Modell, 1942, 1949, 1964; Morrison, 1956, 1973; McMichael & Hiscock, 1958; Pain & Woodward, 1961; Parodiz and Bonetto, 1963; Haas, 1969a, b; Starobogatov, 1970; Heard & Guckert, 1971; Davis & Fuller, 1981; Boss, 1982; Korniushin, 1998). Although there have been disagreements regarding ranking and precise generic contents, most freshwater mussels systematists have historically recognized six families of freshwater mussels: Margaritiferidae, Unionidae (= Anodontinae + Unioninae), Hyriidae, Iridinidae, Mycetopodidae, and Etheriidae. The first three of these families have been united under the Unionoidea, and the last three belong to the superfamily Etherioidea. These superfamilies have been diagnosed solely by their larval type: the Unionoidea have glochidia and the Etherioidea have lasidia (Parodiz & Bonetto, 1963). The families themselves have been recognized using such anatomical characters as which demibranchs are used for brooding, whether or not the diaphragm dividing the mantle cavity is complete, and the presence or absence of a 'supra-anal' aperture (Ortmann, 1912b).

The biogeographic and stratigraphic distributions of the six families are also shown in **Figure 7.1**. The Unionoida presently occurs on every continent except Antarctica, although that continent does harbor Paleozoic fossil freshwater mussels

(Anthracosioidea) (Bradshaw, 1984). The global distributions of the extant families presented in **Figure 7.1** should be self-explanatory with the exceptions of the four, dotted circles. In the cases of the Hyriidae and Iridinidae, these represent purely fossil distributions. The fourth accounts for the single unionid genus that inhabits the Australasian region: *Haasodonta*. The stratigraphic patterns are those of Haas (1969b) as listed and modified in **Table 1.2**. Few conclusions have been made regarding the deep history of the Unionoida other than that the order probably had its origins in the early Triassic, and most of the extant groups first appear in Cretaceous or later strata.

Freshwater mussel research during the 20th century uncovered a *Lagerstätte* of macroevolutionary patterns (**Figure 7.1**). The sum of these patterns can be condensed as such: The Unionoida is a cosmopolitan, ancient, and strictly continental group of freshwater bivalves. Given that these patterns are accurate and that geographic separation contributes to the diversification of organisms (Mayr, 1963), it would be expected that the biogeography, character evolution, as well as phylogeny of the freshwater mussels should reflect the Mesozoic and Cenozoic evolution of the continental landmasses. Historically, mammals have been preferred over bivalves as the currency of continental macroevolutionary study (Simpson, 1944). Because of this, it has gone too long unrecognized that the Unionoida may be among the ideal taxa for testing hypotheses of macroevolutionary processes at scales ranging from family-level splits due to the break-up of Pangaea during the Mesozoic, to generic diversification associated with Tertiary continental watershed evolution, right up to the population-level effects of Pleistocene glaciation of the northern continents.

Reflecting upon the current state of our knowledge accumulated over 200 years of freshwater mussel research (documented in **Figure 7.1**), as many questions are raised as are answered. Are the superfamilies monophyletic? Are the families? What are the morphological synapomorphies of the clades? How did the continental distributions of the families come to be?

The reason that so many fundamental questions have gone untested appears to be quite simple. Nearly all evolutionary discussions of freshwater mussels from a global and/or deep-time perspective (*e.g.*, Simpson, 1896; Walker, 1917; Modell, 1942; Parodiz & Bonetto, 1963; Heard & Guckert, 1971) predate the widespread acceptance of both (1) continental drift as a mechanism of vicariance and (2) phylogenetic systematics (*i.e.*, cladistics) as a scientific methodology to test hypotheses of evolutionary pattern (Brown & Lomolino, 1998). Those earlier, out-moded views, while representing substantial contributions to the narrative phase of freshwater malacology, have colored the world-view of freshwater mussels into the modern era.

With the 21st century upon us, a new perspective of freshwater mussel evolution is available through the application of cladistics — the philosophy that taxa should be natural units (*i.e.*, monophyletic) and diagnosed only by synapomorphies, or *shared, derived* homologies. The analyses of freshwater mussel phylogenetics presented in **Chapters 2-6**, as well as recent, published cladistic studies (Lydeard *et al.*, 1996; Graf, 2000a, in prep.; Graf & Ó Foighil, 2000, 2001; Hoeh *et al.*, 2001) provide a new system of unionoid evolution with which macroevolutionary process hypotheses can be inter-subjectively evaluated (Popper, 1968). For this closing chapter of my dissertation, I will use the phylogenetic patterns tested to-date to discuss the problems of:

(1) the classification of the Unionoida,

(2) the synapomorphies of the Unionoida, and

(3) application of these phylogenetic patterns to questions of biogeography and stratigraphy.

The Unionoida (Palaeoheterodonta) Super Tree

As discussed in **Chapter 2** and explicitly tested in **Chapter 3**, no single character set is applicable to recover all levels of the phylogeny of the Unionoida. Different

workers have, in recent years, applied various phylogenetic data sets to the problem of freshwater mussel phylogeny: 16S mt-rDNA (Lydeard *et al.*, 1996), COI mtDNA (Hoeh *et al.*, 1998, 2001; Graf & Ó Foighil, 2000; see also **Chapter 3**), 28S n-rDNA(Rosenberg *et al.*, 1994, 1997; Graf & Ó Foighil, 2001; Graf, in prep.; see also **Chapters 3 & 5-6**), and morphology (Lydeard *et al.*, 1996; Graf, 2000a; Graf & Ó Foighil, 2000; Hoeh *et al.*, 2001; **Chapters 2 & 4**). In several cases, more than one of these character sets have been combined (Lydeard *et al.*, 1996; Graf & Ó Foighil, 2000; Hoeh *et al.*, 2001; see also **Chapter 3**). While there is a reasonable degree of agreement among topologies recovered through phylogenetic analysis of these various data sets, the ranges of their individual utilities appear to be extremely narrow. For example, Graf (2000a; **Chapter 2**) found that morphological characters were extremely useful for distinguishing among the superfamilies but that these same characters provided almost no meaningful resolution among the Unionoidea.

One way, then, to recover the grand system of freshwater mussels could be a 'total evidence' approach (Kluge, 1989). All of the individual character sets — morphology, 16S, COI and 28S — could be combined into a single matrix, and a grand analysis could be undertaken. The problems with this method are two-fold. First, while many of the same higher taxa are replicated in each of the different character sets, the exact terminals are not. Second, and most importantly, compounding the strengths of different data sets across the wide range of unionoid evolution also compounds the weaknesses of those same characters. For example, while the analyses of **Chapter 3** demonstrated that COI out-performed 28S for the more shallow relationships among the Unionidae, the former also confounded the latter regarding the deeper nodes.

I have taken an alternative approach to acquire the grand system of freshwater mussel evolution. Each of the phylogenetic analyses listed above provided tests of the monophyly of specific unionoid clades. Some of these tests were more robust than others but many have been replicated through multiple analyses. By assessing the proposed

sister relationships on a case by case basis, a grand scheme — a Super Tree — of the phylogeny of the Unionoida can be constructed that is the best-estimate of freshwater mussel phylogeny based on the data available and a bold hypothesis that is eminently falsifiable.

The Super Tree cladogram representing the overall results of phylogenetic analyses upon four different data sets or some combination thereof is shown in **Figure 7.2** (see references above and in **Table 7.1**). The taxa shown are meant only to be representative of the clades to which they belong. The color-coding of that diagram reflects the principle evidence supporting each node; those are also listed more thoroughly in **Table 7.1**.

Generally, more-terminal (*i.e.*, less divergent) clades were better supported by COI, while the deeper nodes received their support from 28S. This follows from the findings of **Chapter 3** where COI was found to be highly saturated deeper than the tribe or subfamily level, and 28S was determined to have a substitution rate too slow to have accumulated changes among the more recent taxa. The study of freshwater mussel 28S and COI by Graf & Sparks (2000) suggested that, chronologically, Cenozoic divergences were better resolved by mtDNA, and the same fragment of nuclear rDNA was more appropriate for recovering Mesozoic splits.

The exceptions to this overall pattern are the sources for support for a sister relationship between the Unionidae and Margaritiferidae (marked with a 'U' on **Figure 7.2**). Whereas most COI analyses supported that clade, 28S gave mixed results (**Table 7.1**). The rDNA alone (**Figure 3.2**) and in combination with mtDNA (**Figure 3.1**) supported a (Unionoida + Margaritiferidae) clade, based upon the topologies recovered. Conversely, as indicated by the 28S analysis in **Chapter 6** and the partitioned Bremer-Decay Index in the combined analysis of 28S and COI (**Table 3.4**), 28S also harbors support for a (Margaritiferidae + Etherioidea) clade (**Figure 7.2**). Based on those ambiguous data, it is difficult to reject the traditional view of a sister relationship

between Unionidae and Margaritiferidae (*i.e.*, Unionoidea). These analyses are taken *a fortiori* to support a (Margaritiferidae + Unionidae) clade, but this placement is tenuous.

The monophyly of the (Hyriidae + (Iridinidae + "mycetopodidae" + Etheriidae)) clade on the Super Tree topology (labeled with an 'E' on **Figure 7.2**) was resolved by morphological analyses (Graf, 2000a; Hoeh *et al.*, 2001; see **Table 7.1**). That topology was not corroborated by analyses of COI alone (Graf & Ó Foighil, 2000) or in combination with morphological characters (Hoeh *et al.*, 2001). However, based on the depth of the divergences among these families (see below) and given the relatively rapid saturation rate of COI (see **Chapter 3**), it is difficult to consider the results of those latter analyses as definitive. This is especially so considering the robust results obtained through morphological phylogenetics where the individual characters were more carefully considered. Although coincidental, it worked out well for me and my dissertation research that the unionoids that would be the most difficult to acquire had such robust literature-based characters for phylogenetic analyses.

Phylogenetic Classification of the Unionoida (Palaeoheterodonta)

The topology of the Super Tree is not consistent with the traditional classification of the Unionoida (**Table 1.2**). The previous 'arrangement' (*sensu* Wiley *et al.*, 1991) of the Unionoida had been based upon the authoritarian narratives of 100 years of freshwater malacology (see references above) which obviously lacked a phylogenetic perspective. While several studies have depicted unionoid relationships as branching diagrams (*e.g.*, Modell, 1942; Heard & Guckert, 1971; Davis & Fuller, 1981), there has been almost no concern, until recently, for the naturalness of freshwater mussel taxa — *i.e.*, their monophyly and their diagnosis by shared, *derived* homologies.

The traditional arrangement of the Unionoida (**Table 1.2**), when interpreted from a cladistic standpoint, is certainly a valid hypothesis of freshwater mussel relationships.

The Super Tree topology constitutes a test of that hypothesis. **Figure 7.3** is a phylogenetic re-interpretation of freshwater mussel classification based upon the correct family-group names (**Appendix II**) and the results of the several recent phylogenetic analyses that comprise the Super Tree (see **Table 7.1**).

Ideally, a phylogenetic classification that is logically consistent should be representable by only a single phylogeny (Hull, 1964; Wiley *et al.*, 1991). 'Ideally' is emphasized because, as our knowledge of the phylogeny of the Unionoida is far from complete, I am opting not to attempt this lofty goal. At this time, based on the work done to-date, there are unresolved clades [*e.g.*, (Lampsilini + Pleurobemini + Quadrulini + Amblemini)], hesitantly placed branches (*e.g.*, the sister group of the Margaritiferidae), and simply unanalyzed family-level branches, such as the overwhelming majority of non-North American taxa. Given all this, it seems imprudent to attempt to name every node with a formal moniker. My recommended re-classification of the extant Palaeoheterodonta is listed in **Table 7.2**.

The classification presented in **Table 7.2** is a conservative, phylogenetic (*i.e.*, natural) re-organization of the traditional arrangement of the Unionoida presented in the introductory chapter of this dissertation. Although the classification is mostly self-explanatory, a few novel and interesting reconstructions require a brief discussion. Traditionally, *Neotrigonia*, the only extant trigonioid, has been placed as sister to the freshwater mussels (Thiele, 1934; Taylor *et al.*, 1969; Healy, 1989; Hoeh *et al.*, 1998, Graf & Ó Foighil, 2000; but see Newell & Boyd, 1975 and Morton, 1987). That hypothesis has recently been supported by phylogenetic analysis of molecular characters (Hoeh *et al.*, 1998; Graf & Ó Foighil, 2000; Graf, in prep.; **Chapters 3 & 6**). The sister group of the Unionoida bears directly upon the determination of the synapomorphies of freshwater mussel taxa (see discussion below).

Within the order of freshwater mussels, the division into two superfamilies, Unionoidea and Etherioidea (= Mutelacea), remains (Parodiz & Bonetto, 1963; Boss,

1982); the composition of those taxa, however, has changed. The key shift is the removal of the Hyriidae from the Unionoidea to the Etherioidea. The latter superfamily is now made up of three families — (Hyriidae + (Iridinidae + Etheriidae)), and, based upon the lack of unique synapomorphies (Heard & Vail, 1976a), the Etheriidae now receives the several subfamilies of the "mycetopodidae" (Graf, 2000a; **Chapter 2**).

Within the Hyriidae, there are now at least three subfamilies. Traditionally, the hyriids were divided into two groups: the Australasian Hyridellinae and the Neotropical Hyriinae. However, whereas molecular phylogenetic analyses have supported the monophyly of the Hyriinae (**Table 7.1**), the "hyridellinae" has been found to be paraphyletic relative to the South American clade (Graf & Ó Foighil, 2001; **Chapter 5**). The Australasian taxa examined in this dissertation have been retained at the rank of subfamily, Velesunioninae and Hyridellinae, although the monophyly of those traditional groups (*sensu* McMichael & Hiscock, 1958) has not been tested. Thus, the phylogeny of the Hyriidae is far from completely resolved.

The Unionoidea is composed of two families: the Margaritiferidae and Unionidae (**Figure 7.3**). The former family, well supported as monophyletic (**Table 7.1**), is the same phylogenetic taxon as it has been traditionally. Smith (2001), however, has recently suggested some nomenclatural revisions within the family. The Unionidae, in contrast, has suffered a major makeover.

As was discussed in **Chapter 6** and **Appendix II**, there have been differing views regarding the taxonomy of the Unionidae. Ortmann (1912b, 1916a, *etc.*), Modell (1942, 1949, 1964), and Haas (1969a, b) advocated multiple subfamilies of unclear phylogenetic relationship. Heard & Guckert (1971) favored a two family system: Unionidae and Amblemidae; and Davis & Fuller (1981) more recently have recognized a single family and two subfamilies. In actuality, Davis & Fuller (1981) considered the Margaritiferinae as the third subfamily of the Unionidae, but this view has been rejected by most systematists following Smith & Wall (1984). The consensus classification presented in

Chapter 1 (and **Appendix II**) follows Davis & Fuller's (1981) classification of the Unionidae: Anodontinae and Unioninae (= Ambleminae). Their "Ambleminae" was renamed Unioninae as they admitted the Palearctic genus *Unio* into that subfamily.

The classification of the Unionidae derived from the Super Tree topology (**Table 7.2**) is somewhat distinct from previous classifications. The Super Tree (**Figure 7.3**) shows the *Unio* clade as sister to Davis & Fuller's (1981) Anodontinae. As *Unio* is the type genus of the family, displacing that genus to a new clade takes its name with it. Thus, the new subfamily is the Unioninae, with two tribes: Unionini and Anodontini.

The clade from which *Unio* was removed reverts to the next available family-group-level name: Ambleminae (see **Appendix II**). Most researchers, relying on the combined agenda of Heard & Guckert (1971) and Davis & Fuller (1981), have used the Ambleminae as a catchall for the remaining Unionidae of the globe (*i.e.*, Heard, 1974; Brandt, 1974; Nagel *et al.*, 1998; Nagel & Badino, 2001). Traditionally, that clade — as a means to launder non-Nearctic genera into a well-recognized unit — is potentially unnatural.

The Super Tree classification confirms that suspicion (**Table 7.2**). The Ambleminae *sensu* Heard & Guckert (1971) and Davis & Fuller (1981) is paraphyletic, with the Ambleminae *sensu lato* composed solely of North American genera, and the exotic genera here analyzed are dispersed among two subfamilies, Rectidentinae and Pseudodontinae. Only a single rectidentine, *Uniandra*, has been studied here, so the monophyly of that group (*sensu* Brandt, 1974) has not been tested. The single exotic unionid genus analyzed by Hoeh *et al.* (2001: *Caelatura*) and the two examined by Graf (2000a, **Chapter 2**: *Parreysia* and *Grandidieria*) confirm the general distinctness of non-Nearctic "amblemines" from the core (Unioninae + Ambleminae) clade. It is likely that as more and more Old World genera are studied phylogenetically, many of the family-group names of Hans Modell (1942) will rise from the ashes of synonymy.

Despite the most intensive phylogenetic study of any freshwater mussel clade — or maybe because of it! — the relationships within the Ambleminae *sensu lato* are unresolved. The best evidence (**Figures 7.2-3**) supports the monotypic Pacific-drainage Tribe Gonideini as sister to the eastern North American tribes (informally named 'Amblemini Tribe Group'); however, the relationships within that latter clade are unclear. There is good evidence for the monophyly of the constituent tribes thus far studied: Lampsilini, Pleurobemini, Quadrulini, and Amblemini (monotypic) (**Table 7.2**). But, nearly every analysis has recovered a different branching pattern for these taxa (**Figure 4.2**). Indeed, there are still many eastern North American genera yet to be studied, and it is highly likely that additional tribes may need to be recognized to maintain the logical consistency of the classification (Graf, in prep.; **Chapter 6**).

The Synapomorphies of the Unionoida

Besides propelling the classification of the Unionoida analytically onward to a goal of a completely natural, logically consistent classification (**Table 7.2**), the Super Tree topology (**Figure 7.1**), derived from the several studies in this dissertation and the published literature (see **Table 7.1**), can also be applied to test hypotheses of morphological character evolution among the freshwater mussels and even the whole extant Palaeoheterodonta. Towards this end, I have refined the morphological data set used by Graf (2000a; **Chapter 2**) into 26 morphological characters (18 binary, 8 unordered multistate) for the terminals represented on the Super Tree, and the transformations of the characters were traced onto that topology under maximum parsimony using PAUP* (Swofford, 1998). The determination of the synapomorphies, where they are available, of the freshwater mussel clades allows the placement of genera not specifically addressed into the new system of the Unionoida (**Table 7.2**).

The specific characters employed are basically those of **Chapter 2**, but their coding has been reconsidered to reflect the new information gained through subsequent phylogenetic analyses (Graf, 2000a, in prep.; Graf & Ó Foighil, 2000; Hoeh *et al*., 2001). The character diagnoses are listed in **Table 7.3**, and they are grouped as in **Chapter 2** (and **Appendix III**): Shell; Gross Soft Anatomy; Brooding and Life History; and Larvae. The matrix of characters and taxa can be found in **Table 7.4**.

This new character set was optimized on the Super Tree topology under two different parsimony criteria: accelerated transformation (ACCTRAN) and delayed transformation (DELTRAN). A good discussion and examples of the differences between these two models and their application can be found in Wiley *et al.* (1991), but the basic distinction is that, in cases of ambiguous character placement, ACCTRAN favors early evolution and then subsequent character loss, whereas DELTRAN suggests later, convergent evolution of the 'same' character state. The majority of transformations of this refined character set on the Super Tree are unambiguous — they trace to the same branches under both ACCTRAN or DELTRAN. In most cases of ambiguous transformations, I have preferred ACCTRAN, as consistent with Hennig's Auxiliary Principle, "Never assume convergence or parallel evolution, always assume homology in the absence of contrary evidence." (Wiley *et al.*, 1991: 14). I take this principle as a null hypothesis to be falsified when trying to understand the synapomorphies on a particular topology. However, in cases where the derived character state is the reduction or loss of a particular character, DELTRAN and ACCTRAN optimizations are reversed. The 55 transformations (*i.e.*, steps) of these 26 morphological characters traced onto the Super Tree topology are shown in **Figure 7.4**. The individual and group character transformation statistics are given in **Tables 7.5 & 7.6**, respectively.

When considering the synapomorphies — shared, *derived* homologies — of the Unionoida and its constituent clades, it is necessary to also consider the plesiomorphies, the *ancestral* characters. That is, characters can be relied upon as derived only relative to

the ancestral state *from which* they were derived. Among the orders of bivalves, the Unionoida is exceptional in having such a clear, well-supported sister group, the genus *Neotrigonia*. That genus is *the* relic of the predominantly Mesozoic Trigonioida. Together, the extant members of those two orders, Unionoida and Trigonioida, form a monophyletic Palaeoheterodonta (Hoeh *et al.*, 1998; Graf & Ó Foighil, 2000; Graf, in prep.; **Chapters 3 & 6**). In contrast, the Palaeoheterodonta is absolutely typical among the Bivalvia in that its specific sister taxon remains largely a guess.

Only two published phylogenetic analyses have tested the position of the Palaeoheterodonta among the Bivalvia. Salvini-Plawen & Steiner's (1996) morphological analysis did not recover a monophyletic (Trigonioida + Unionoida) clade, but their study was in every way preliminary. My own re-evaluation (unpublished) of their matrix failed to replicate their character coding, most-parsimonious topology, or character optimization (even on their own "preferred" tree). Adamkewicz *et al.* (1997) applied molecular characters (a fragment of the nuclear, small ribosomal subunit rDNA). Overall, their topology (**Figure I.2**) was weak, and it required several decidedly perverse relationships among the Bivalvia. It did, however, support a sister relationship between palaeoheterodonts and the heterodonts. Their results are at least in agreement with the traditional view that the Palaeoheterodonta and Heterodonta constitute a more universal taxon variously labeled Heteroconchia or Eulamellibranchia (Thiele, 1934; Cox, 1960; Pojeta, 1978; Brusca & Brusca, 1990; Waller, 1998). See **Appendix I** for a brief review of the history and methods of the placement of the Palaeoheterodonta among the Bivalvia.

The difficulty created by this situation can be stated quite succinctly: As most of the important phylogenetic characters of the Unionoida are neontological (*i.e.*, soft anatomy, life history, *etc.*), the plesiomorphic condition of those characters must be judged through comparison only with the genus *Neotrigonia*. The synapomorphies traced to the ingroup branch can only be placed there under the assumption that the character

states presented by *Neotrigonia* are indeed plesiomorphic. Fortunately, even in the absence of quality phylogenetic analyses to explicitly trace characters to the outgroup branch (and deeper), the assumption of *Neotrigonia* plesiomorphy, though necessary, is reasonable.

Table 7.7 lists the presumed plesiomorphic states for the characters listed in **Table 7.3** as well as those traditionally applied to diagnose the Palaeoheterodonta. Each of these characters matches the conditions observed in *Neotrigonia* (**Chapter 2**) and, based upon their distributions among the Bivalvia (Salvini-Plawen & Steiner, 1996; Waller, 1998), few if any of these characters could even be considered synapomorphies of the Palaeoheterodonta. This includes many of the characters previously designated to diagnose the palaeoheterodonts: nacreous, aragonitic, equivalved shell with periostracum; external, opisthodentic ligament; and a broadly unfused mantle (Thiele, 1934; Newell, 1969; Truemen *in* Cox, 1969; Boss, 1982).

The single possible exception might be the shared possession of schizodont hinge dentition among the Palaeoheterodonta. The lamellar teeth of *Neotrigonia* and the Unionoida are unique among the extant Bivalvia (Thiele, 1934), and, as such, are potentially synapomorphic. However, paleontological data raise some serious doubts about this view. Cox (1969) and others (*e.g.*, Scarlato & Starobogatov, 1979) have argued that the schizodont teeth of the Palaeoheterodonta are simply minor, convergent modifications of the actinodont dentition seen among the Cycloconchidae, Lyrodesmatidae, and Myomorphidae of the early and middle Paleozoic (Pojeta, 1971, 1978; Morris, 1978; Cope, 1996a; but see Newell & Boyd, 1975). This confusion may be resolved once a modern, phylogenetic perspective is brought to the early evolution of the Bivalvia (but see Newell & Boyd, 1978 for an argument against applying cladistics to fossil bivalves).

Given that *Neotrigonia* truly represents the plesiomorphic conditions for the characters listed in **Table 7.3** and traced on **Figure 7.4**, the synapomorphies of the

Unionoida and its family-group-level taxa studied in this dissertation are listed in **Table 7.8**. Based upon the consistency indices of the various character groups (**Table 7.6**), larval characters are the most conserved, whereas shell characters are worst — *i.e.*, the most homoplastic. The poor quality of shell characters among the Unionoida explains the difficulty of inferring evolutionary patterns from paleontological data (see discussion below). Of special concern among the Unionoida are the evolution of larval and brooding characters generally, and the difficulty of resolving clades among the Unionoidea using morphological characters.

The evolution of larval characters among the Unionoida was discussed in by Graf (2000a; **Chapter 2**). Parodiz & Bonetto (1963) had previously argued that the two different parasitic larval types observed among freshwater mussels — glochidia and lasidia — could not even be imagined to be homologous; the two larval types apparently evolved from some unknown ancestral type. According to phylogenetic analysis, however, possession of a parasitic glochidium is a synapomorphy of the Unionoida; the glochidium was then modified into a lasidium along the branch leading to the (Iridinidae + Etheriidae) clade (**Table 7.8**).

Among the lasidum bearing mussels (*i.e.*, Iridinidae + Etheriidae), it is unclear which of the two morphologies — 'lasidium'-type or 'haustorium'-type is plesiomorphic. The principle differences between the two parasitic larvae are (1) how they attach to their hosts and (2) their size. Lasidia, as described for most etheriids are smaller and attach to their host by encystment, while haustoria tend to be larger and attach via tubular appendages (Fryer, 1954, 1961; Parodiz & Bonetto, 1963; Wächtler *et al*., 2001); scale drawings of lasidia, haustoria, and other unionoid larval types are shown in **Figure III.3**. For the sake of this analysis, 'lasidium'-type lasidia have been coded as ancestral in an additive binary pair, but this was largely arbitrary and had no real effect on character optimization on the Super Tree topology (**Table 7.4** and **Figure 7.4**).

Among the glochidium-bearing mussels, the plesiomorphic condition among the Unionoida is a glochidium of the subcircular, unhooked variety; there have, thus, been two independent evolutions of 'triangular hooked' glochidia: once as a synapomorphy of the Unioninae and a second time among the Etherioidea (**Figure 7.4**). Although it may seem unlikely, at first glance, that such a specialized condition should evolution twice independently, there are distinct morphological differences between the unionine hooked glochidia and those known from the Hyriidae: the former posses numerous smaller spines in addition to their hook and the latter's hooks are often bifurcated or S-shaped (Jupiter & Byrne, 1997; Hoggarth, 1999; see **Figure III.3**). This is exactly the opposite interpretation of glochidial evolution implied by Wächtler *et al.* (2001: 101) who emphasized the hooked glochidia found among Palearctic and Neotropical unionoids as "... the more general and widely distributed type, whereas the smaller hookless ones represent the more specialized type with restricted occurrence."

Brooding characters were discussed in detail by Graf & Ó Foighil (2001; **Chapter 4**) and Hoeh *et al.* (2001). Brooding among the Unionoida is closely associated with the invasion of freshwater, as it has been in other freshwater bivalves (McMahon, 1991); ovovivipary allows freshwater mussels to successfully inhabit rivers, whereas a planktonic larval stage (as is typical among the Bivalvia; Waller, 1998) would be passively returned to the ocean. The number of marsupial demibranchs in which larvae are brooded has been considered important by most mussel systematists (reviewed in **Chapter 4**). Among the Unionoidea, use of only the inner demibranchs for larval brooding (*i.e.*, endobranchy) is limited to a few African genera (*i.e. Grandidieria* and *Moncetia*; Bloomer, 1933; Kondo, 1984, 1990). That condition is considered derived and not homologous with the endobranchy observed among the Hyriidae, Iridinidae, and Etheriidae (Graf, 2000a). Based on the tetragenous condition of the Margaritiferidae, most mussel systematists have taken the use of all four demibranchs for brooding as the 'primitive' condition among the Unionidae (Davis & Fuller, 1981; Lydeard *et al.*, 1996).

The recognition that the margaritiferids may present a derived suite of characters challenges this view (see below), and the precise plesiomorphic marsupium of the Unionidae (and the Unionoida, in general) will have to wait until more non-North American taxa are placed on the unionoidean phylogeny and the relationships within the Amblemini Tribe Group are resolved.

Whereas the branches leading to the Unionoida, Etherioidea, and (Iridinidae + Etheriidae) clades are each supported by several unambiguous synapomorphies, only two unionoidean lineages are so well defined: Anodontini and Lampsilini (**Figure 7.4**). Each of these clades is supported by several synapomorphies generally associated with brooding (**Table 7.8**). The Unionidae is diagnosed by only a single, unambiguous (as coded) synapomorphy — the presence of a supra-anal aperture, and no morphological characters can be traced to the Unionoidea (= Unionidae + Margaritiferidae) branch (**Figure 7.4**). Hoeh *et al.* (2001: Figure 14.8) charted eight characters as homoplastic synapomorphies (many ambiguous) of the Unionoidea. These are purely a function of their tree topology, which is fundamentally different than that of the Super Tree (**Figure 7.2**).

As discussed in **Chapter 4**, most systematists of the Unionoida have *a priori* assumed the Margaritiferidae to be 'primitive' based on the lack of several derived unionoid characters: lack of pallial fusion between the mantle lobes, lack of 'water-tubes' for brooding, an incomplete diaphragm dividing the infrabranchial from the suprabranchial space, and reduced hinge dentition (Heard & Guckert, 1971; Davis & Fuller, 1981; Lydeard *et al.*, 1996). Implicit in this assumption is that these characters are thus retained from a proto-unionoid ancestor and that the derived conditions observed in both the Unionidae and Etherioidea are convergent (*i.e.*, DELTRAN). However, as argued above, this model, though implicitly favored historically, is superceded by the ACCTRAN optimization of those characters.

The hypotheses of absence of unionoidean synapomorphies and presence of derived, degenerate margaritiferid synapomorphies are derived from Hennig's Auxiliary Principle (quoted above): unless there is contrary evidence, the characters shared among the various palaeoheterodont groups are homologous. Having assumed that (1) hinge teeth (both lateral and pseudocardinal), (2) interlamellar 'water-tubes' for brooding, and (3) fusion of the outer demibranchs to the mantle along their entire length are plesiomorphic among the freshwater mussels, the interpretation is that (1) the Unionidae are simply plesiomorphic for those character states and (2) the Margaritiferidae is degenerate the same characters.

The character transformations abstracted above and summarized in **Table 7.8** and **Figure 7.4** are far from rock solid. The inclusion of new characters and taxa may not only clear up some of the ambiguities I have alluded to, it may in fact overturn many of the hypotheses that seem, based upon the data at hand, unquestionable. However, at the present time, the Super Tree phylogeny (**Figure 7.2**), based upon the several analyses of freshwater mussel relationships to date (**Table 7.1**), is the best estimate of the 'true' phylogeny of the Unionoida. As many of our ideas about the last 200 million years of unionoid evolution have been based on the old view of freshwater mussel classification, it is likely that our understanding of the distributions of freshwater mussels in space and in time will require the same overhaul.

Distributions of Freshwater Mussels in Space and in Time

The pattern of freshwater mussel evolution, as represented by the Super Tree phylogeny of the Unionoida (**Figure 7.2**), is just that: pattern. But, as demonstrated in the preceding discussion, this pattern can be used to test hypotheses of evolutionary process (*e.g.*, character evolution). Hypotheses about the evolution of heritable, *intrinsic* properties are testable with phylogeny alone — indeed they are the basis of the

phylogeny itself. Hypotheses regarding the evolutionary history of the *extrinsic* properties of organisms (*i.e.*, biogeography, stratigraphy) are often more difficult to test. Phylogeny is not sufficient in those cases, but it is necessary.

As mentioned above, freshwater mussels are widespread, presently occurring on all non-glaciated continents (**Table 7.9**), and they are ancient, dating from the Mesozoic, or perhaps even earlier (Haas, 1969b; Good, 1998). Examination of **Table 7.9** reveals a broad paradox — All mussel families exhibit some degree of endemism (*i.e.*, none is found in all biogeographic regions), but most have wide ranges that span terrestrial and marine barriers. How did these distributional patterns come to be?

While many freshwater malacologists have been interested in that question, nearly all discussions of the topic — even 'modern' ones — have lacked a modern scientific perspective (*sensu* Popper, 1968). The authoritarian paradigm of the global history of the Unionoida was decided early (*e.g.*, Simpson, 1896; Walker, 1917; Modell, 1942) and many subsequent treatments have only reviewed their finds in that context (*e.g.*, Parodiz & Bonetto, 1963; Davis & Fuller, 1981; Good, 1998; Watters, 2001). What those early treatises lacked was:

(1) accurate knowledge of earth history and continental drift (*e.g.*, Wegener, 1966; Brown & Lomolino, 1998),

(2) an analytical framework with which to conceptualize biogeographic processes (*e.g.*, Platnick & Nelson, 1978; Nelson & Rosen, 1981), and, finally,

(3) a robust phylogeny of the Unionoida.

Today, we have all three.

As argued by Graf (2000a; **Chapter 2**), the distributions of the freshwater mussel families are consistent with an origin on Pangaea, and consequent diversification with further continental disintegration. At present, the Etherioidea is limited to the southern components of the former Gondwana, only unionoideans occur on the northern continents, and the two superfamilies are sympatric only in areas of secondary contact

(*i.e.*, Central America, southeastern Asia, and Africa) (**Table 7.9**). But it is within the Etherioidea, that we find the most dramatic transoceanic disjunctions.

Among the best defined of these are the disjunctions presented by the Hyriidae (Graf & Ó Foighil, 2001; **Chapter 5**). The present isolation of the two traditional "subfamilies" has long been known: the hyridellines (*sensu* **Table 1.2**) in Australasia and the Neotropical Hyriinae (Ortmann, 1921a; McMichael & Hiscock, 1958; Parodiz & Bonetto, 1963). Of more localized interest has been the seeming impassable barrier dividing the range of the Australasian group: the Tasman Sea. To the west of that body of water, hyridellines occur on Australia, Tasmania, and New Guinea; three species in two genera inhabit New Zealand on the eastern side. The freshwater mussels of New Zealand have traditionally been placed in Australian genera (*Hyridella* and *Cucumerunio*), but the two land masses have been isolated for roughly 80 million years (Storey, 1995)!

Historically, there have been two schools of thought on the subject of the origins of the New Zealand mussel fauna. South American malacologists have viewed the whole of the Australasian hyridellines as a Neotropical derivative via land bridges or some such thing through Antarctica (*e.g.*, Parodiz & Bonetto, 1963). The Australians have favored independent origins of these two groups of hyriids (McMichael & Hiscock, 1958; Walker *et al.*, 2001; also Modell, 1942 and Ortmann, 1921a). The Australian freshwater mussels were hypothesized to have originated by dispersal from unknown ancestors in Southeast Asia, and New Zealand was populated by subsequent transoceanic dispersal from the 'mainland' (see **Chapter 5**). Neither of these scenarios is supported by the Super Tree topology (**Figure 7.2**).

As discussed in detail by Graf & Ó Foighil (2001; **Chapter 5**), general dispersal hypotheses like those suggested in the previous paragraph are (generally) difficult to falsify owing to the imprecise nature of the predictions they offer. Vicariance hypotheses, because they refer to specific events, are generally falsifiable. Graf & Ó

Foighil (2001; **Chapter 5**) predicted that if vicariance due to the break up of Gondwana was the cause of the disjunction observed among the hyridellines, then a molecular phylogenetic analysis should produce a long branch (or branches) leading to the New Zealand taxa, consistent with ca. 80 million years of evolution on an isolated microcontinent. A short branch, relative to other freshwater mussel splits of similar age, would potentially falsify the vicariance hypothesis.

Based upon the topology of the Super Tree (**Figure 7.2**) and the branch lengths found by Graf & Ó Foighil (2001; **Figures 5.1-2**), the vicariance hypothesis can not be rejected. The paraphyly of the "Hyridellinae" (*i.e.*, the entire Australasian mussel fauna; *Hyridella* and *Velesunio* on **Figure 7.2**) rejects both the South American- and Asian-origin hypotheses of Parodiz & Bonetto (1963) and McMichael & Hiscock (1958), respectively. Those Australian freshwater mussels are Gondwanan relics (Graf & Ó Foighil, 2001; **Chapter 5**). The long branch leading to the New Zealand *Hyridella menziesi* from its Australian congeners also supports a Gondwanan origin of the New Zealand mussel fauna. The *lack* of data in support of transoceanic dispersal was discussed in **Chapter 5**. The data are not yet available to so explicitly test the biogeographic process hypotheses behind the distributions of the other etherioidean families.

To the north, the Holarctic ranges of both the Unionidae and Margaritiferidae tend to obscure many of the possible biogeographic processes which caused those distributions. The literature is full of stories detailing intercontinental dispersal via low sea level, *etc.* (*e.g.*, Walker, 1917; Modell, 1942; Ziuganov *et al.*, 1994; Watters, 2001). These hypotheses have been largely untested and were based, conveniently, on the absence of phylogeny and no shortage of circular reasoning. However, one particularly interesting biogeographic question relating to unionoidean distributions was addressed in this dissertation: the problem of *Gonidea*.

As discussed by Graf (in prep.; **Chapter 6**), *Gonidea* is a monotypic genus confined to the Pacific-drainages of North America. While anatomically quite distinct from the freshwater mussel genera of eastern North America (Ortmann, 1916a; Heard & Guckert, 1971; Davis & Fuller, 1981), *Gonidea* is similar in certain respects to many of the genera presently restricted to eastern Asia — all have reduced hinge teeth and perforated septa dividing the interlamellar spaces (Heard, 1974). Based upon this pattern, it has been hypothesized that *Gonidea* should have a closer common ancestor among the Southeast Asia genera than to any in eastern North America (Davis & Fuller, 1981). Watters (2001) recently 'supported' this hypothesis by re-stating the evidence in its favor. However, the Super Tree Topology (**Figure 7.3**) rejects this hypothesis. *Gonidea* forms the 'basal' member of a monophyletic Ambleminae *sensu lato* and is distinct from either of the Rectidentinae or Pseudodontinae. The distributions of the morphological characters suggest that genera like *Gonidea, Pilsbryoconcha*, and *Pseudodon*, traditionally believed to be closely related (*e.g.*, Heard, 1974), are united only by symplesiomorphies (**Figure 7.4**). The amblemines, including *Gonidea*, are presently limited to North America. What is clear is that more Old World mussels will need to be added to the phylogeny before we can truly test hypotheses of the origin of *Gonidea*.

The same general methodology has historically been applied to understand mussel distributions through time: concoct *ad hoc* 'just-so' stories to explain the stratigraphic distributions of the Unionoida. The two most recent reviews of fossil unionoids (*i.e.*, Good, 1998; Watters, 2001) are only slight improvements on the narratives spun 100 years ago (*e.g.*, Simpson, 1896; Walker, 1917). The Super Tree topology, however, can serve to clarify paleontological issues (somewhat).

Haas (1969b) and Watters (2001) reported the temporal distributions of the extant unionoid groups. Unfortunately, their arrangements of mussel relationships were largely independent of phylogeny, and it is difficult to reconcile them with the Super Tree topology (**Figure 7.3**) or each other. However, two points of consensus can serve as a

starting point. Both of these synopses agreed that (1) anodontines arose in the Cretaceous and (2) modern-type amblemines (= Amblemini Tribe Group = Quadrulinae *sensu* Haas, 1969b = Ambleminae *sensu* Watters, 2001) also made their first appearance in the Cretaceous.

Both Haas (1969b) and Watters (2001) perpetuated the traditional, catchall nature of *Unio*, the genus under which nearly all fossil Unionoida were originally described (Henderson, 1935). The modern retention of these forms under *'Unio'* is merely an indication that no one has decided where they should go, rather than a statement of relationships. Some modern paleontologists (*e.g.*, Good, 1998; Hartman, 1998) have opted for *Protelliptio* for North American fossil taxa previously assigned to *Unio* but unplaceable in modern genera.

As shown in the revised classification of the Unionoida (**Table 7.2** vs. **Table 1.2**), modern *Unio* (and related genera with hooked glochidia; *e.g.*, *Cafferia, Nodularia*) are sister to the Anodontini rather than among the amblemines with which they have traditionally been affiliated. Based on the first fossil appearance of both the sister to the Unionini (*i.e.*, Anodontini) and Unioninae (*e.g.*, Ambleminae) in the Cretaceous, it seems likely that the modern Unionini are also Cretaceous (or later) in origin. This certainly raises a red flag regarding the affinity of Cenozoic *Unio* with those *'Unio'* of the middle and early Mesozoic.

Based upon the branching pattern of the Super Tree (**Figure 7.3**) and the relatively plesiomorphic nature of southeastern Asian and African unionids — *Brazzaea* (Bloomer, 1931a), *Caelatura* (Bloomer, 1932), *Grandidieria* (Bloomer, 1933), *Hyriopsis* (Ortmann, 1916b), *Lamellidens* (Bloomer, 1931b), *Uniandra* (Ortmann, 1917), *etc.*, it is reasonable to hypothesize that those lineages represent relic lines of pre-Cretaceous Unionidae. It is interesting to note in this context that nearly all fossil *'Unio'* with hinge teeth have the plesiomorphic, delicate hinge teeth of the extant Old World Unionidae.

This same dentition also occurs among the freshwater Triassic 'vetulonaians' (Watters, 2001) and the Jurassic and Cretaceous Trigonioidoidea (Gu, 1998; Guo, 1998).

In some cases, biogeographic data have stratigraphic value. The minimum ages of the etherioidean clades can be estimated based upon Gondwanan events as well as from dated fossil strata. As all families within the superfamily are found on the fragments of Gondwana, the Etherioidea likely pre-date the break up of the southern supercontinent during the Jurassic (Storey, 1995). The Iridinidae is presently limited to Africa, but fossil Cretaceous *Iridina* have been reported from both North and South America (Haas, 1969b; Morris & Williamson, 1988). The present far-flung distribution (**Table 7.9**) and limited fossil record of the Etheriidae (= Mycetopodidae) also suggests that they had their origins on Gondwana (Haas, 1969b; Watters, 2001).

As shown in **Chapter 5**, the Hyriidae must have originated by the Cretaceous, as evidenced by their Gondwanan distribution. 'Hyriids' have been reported from the Triassic of North America (Pilsbry *in* Wanner, 1921; Henderson, 1935; Parodiz & Bonetto, 1963; Good, 1998; Watters, 2001). The assignment of those taxa to the modern family was based solely upon homoplastic, angular shell sculpture found not only among the extant Hyriidae, but also fossil Unionidae (Watters, 2001) and modern *Scabies* (Brandt, 1974), *Parreysia* (Ortmann, 1910b), and others. Despite the convictions of recent reviewers, those Triassic specimens can not be placed with any confidence in the Hyriidae.

Finally, the branching pattern of the Super Tree (**Figure 7.3**) and the fossil record of the Unionidae (see above) suggests that the Margaritiferidae are Triassic in origin. This agrees with the general thesis of Ma (1996) that the Margaritiferidae arose in the Late Triassic and were most prosperous during the Early and Middle Jurassic.

The 21st Century View of Freshwater Mussel Evolution

Although far from completely resolved, the 20th century view of freshwater mussel evolution (**Figure 7.1**) has been improved by the studies I have undertaken for this dissertation. These have been discussed and elaborated in the pages since I first introduced that figure in this chapter. An improved version of the figure is now due, the 21st Century View of Freshwater Mussel Evolution, shown in **Figure 7.5**. This refined perspective on unionoid classification, character evolution, biogeography, and stratigraphy, while expressing these various aspects of freshwater mussel evolution in a testable, phylogenetic way, also suggests lines for future research. I would like to conclude this dissertation with my thoughts on some of the more interesting potential future research avenues on the phylogeny of the Unionoida.

A pivotal blind spot brought to my attention by my research of the last four years is the sister group of the Margaritiferidae. As discussed above, the Super Tree topology (**Figure 7.3**) places the Margaritiferidae as sister to the Unionidae, but there was also some limited 'signal' in certain data sets pointing the Margaritiferidae toward the Etherioidea (**Figure 7.2**). The early divergence of the Unionoida needs to be firmly understood before we can be certain about the plesiomorphic conditions of freshwater mussel anatomical and life-history characters. It is likely that molecular characters will be the most informative at resolving the problem, ideally a character set even more conservative than the fragment of 28S that I have analyzed (see **Chapter 3**).

Within the Unionidae, the positions of most Old World genera remain a guess. The analyses reviewed here (see **Table 7.1**) demolished the two-subfamily system so-long applied to the Unionidae, and these studies suggested that even more novel lineages may require recognition before the classification of freshwater mussels is entirely natural. Playing conservative-but-informative, I have simply left **Figure 7.5** with a paraphyletic "Old World unios" group. The combination of the unavailability of unambiguous

synapomorphies for the Old World genera here studied and the number of unstudied genera (especially in Southeast Asia) for which the soft anatomy and brooding patterns are unknown means that the true biogeographic ranges of the Unionini, Ambleminae, and "Old World unios" are not thoroughly understood. The nuclear, large ribosomal subunit (*i.e.*, 28S) character set seems to be a good candidate to test the deeper branching order of the Unionidae as specimens before available (see **Chapters 3 & 6**).

On the Etherioidea side of the phylogeny, the relationships among the families are well supported by morphological characters (Graf, 2000a; Hoeh *et al.*, 2001; **Chapter 2**). However, these need to be further tested using molecular characters. The analyses presented in **Chapter 3** as well as the results of Graf & Ó Foighil (2000) and Hoeh *et al.* (2001) demonstrated that cytochrome oxidase subunit I mtDNA is certainly not the most appropriate tool for assessing the Mesozoic divergences (see Graf & Sparks, 2000). Nuclear rDNA, however, as specimens become available, may prove suitable. Of special phylogenetic interest within the Etherioidea are (1) the relationships among the cementing Etheriidae of India, Africa, and South America to the non-cementing "mycetopodids" and (2) the position of the enigmatic genus *Leila*, a Neotropical genus with sharing several important characters with the African Iridinidae (Bonetto, 1963).

As I stated in the introduction to this, my Ph.D. dissertation, my goal has been to progress the study of freshwater mussel evolution through the application of phylogenetic systematics. I believe that I have done that — *I* have certainly learned a great deal about the last 200 million years of the evolution of the Unionoida. But, as I have hinted in the paragraphs immediately preceding, there are still several interesting questions remaining to be answered. I hope my work will inspire interest in refining our phylogenetic perspective on the evolution of the freshwater mussels.

Table 7.1. Synopsis of the Evidence Supporting the Super Tree Topology of Unionoid Relationships. The clade names are those used in **Table 7.2**. The genera listed are not only those shown on the Super Tree (large) but also those others which have been included in the phylogenetic studies considered. The various studies are indicated with letters A-J: **A** = 16S (Lydeard *et al.*, 1996); **B** = COI (**Chapter 3**); **C** = COI (Graf & Ó Foighil, 2000); **D** = 28S (**Chapter 3**); **E** = 28S (**Chapter 5**; Graf & Ó Foighil, 2001); **F** = 28S (**Chapter 6**; Graf, in prep.); **G** = morphology (**Chapter 2**; Graf, 2000a); **H** = morphology (Hoeh *et al.*, 2001); **I** = COI + 28S (**Chapter 3**); **J** = COI + Morphology (Hoeh *et al.*, 2001). The symbols for the contribution of each study to each clade in the Super Tree: + = supports the clade; — = does not support the clade; Ø = although multiple terminals of the clade were included in the analysis, it does not constitute a test of family-group monophyly; ? = not resolved.

		mtDNA			nrDNA			Morph		Combo	
Clade	Genera	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Unionini	*Unio, Cafferia*	·	+	+	+	·	+	·	Ø	+	Ø
Anodontini	*Alasmidonta, Pyganodon*	+	+	+	?	·	+	·	+	+	+
	Anodonta, Anodontoides, Lasmigona, Strophitus, Utterbackia										
Unioninae	Unionini + Anodontini	·	—	—	+	+	+	—	—	+	+
Lampsilini	*Lampsilis, Obliquaria*	+	+	+	+	·	+	·	—	+	+
	Actinonaias, Cyrtonaias, Ellipsaria, Glebula, Leptodea, Ligumia, Medionidus,										
	Potamilus, Ptychobranchus, Toxolasma, Truncilla, Villosa										

Table 7.1 (continued). Synopsis of the Evidence Supporting the Super Tree Topology of Unionoid Relationships.

		mtDNA			nrDNA			Morph		Combo	
Clade	Genera	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Pleurobemini	Pleurobema	+	+	+	—	·	·	·	?	+	+
	Elliptio, Fusconaia, and perhaps *Ellipsoideus*?										
Quadrulini	*Quadrula, Tritogonia*	+	+	·	+	·	·	·	·	+	.
	Megalonaias										
Lampsilini + Pleurobemini + Quadrulini + *Amblema*		Ø	+	Ø	—	Ø	+	+	—	+	+
Ambleminae	Lampsilini + Pleurobemini + Quadrulini + *Amblema* + *Gonidea*	·	—	·	+	·	+	—	—	+	—
(Unioninae + Ambleminae)		Ø	—	Ø	—	Ø	+	+	—	+	+
(Unioninae + Ambleminae + *Uniandra*)		·	—	·	+	·	+	·	·	+	·
Pseudodontinae	*Pseudodon, Pilsbryoconcha*	·	·	·	·	·	+	·	·	·	·
Unionidae	Unioninae + Ambleminae + *Uniandra* + Pseudodontinae	+	—	+	+	Ø	+	—	—	+	—

Table 7.1 (continued). Synopsis of the Evidence Supporting the Super Tree Topology of Unionoid Relationships.

		mtDNA			nrDNA			Morph		Combo	
Clade	Genera	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Margaritiferidae	*Cumberlandia*	+	·	+	·	·	·	·	+	·	+
	Margaritifera										
Unionoidea	Unionidae	Ø	+	+	+	·	—	—	—	+	+
	+ Margaritiferidae										
Hyriinae	*Diplodon*	·	·	·	·	+	+	—	—	·	+
	Castalia, Castalina										
Hyriidae	Hyriinae + *Diplodon*	·	+	·	+	+	+	+	+	+	+
	+ *Velesunio*										
	Lortiella										
Iridinidae	*Iridina, Mutela*	·	·	·	·	·	·	+	Ø	·	Ø
Etheriidae	*Etheria, Acostaea,*	·	·	·	·	·	·	+	+	·	+
	Anodontites, Mycetopoda										
	Monocondylaea										
(Iridinidae + Etheriidae)		·	·	·	·	·	·	+	+	·	+

Table 7.1 (continued). Synopsis of the Evidence Supporting the Super Tree Topology of Unionoid Relationships.

		mtDNA			nrDNA			Morph		Combo	
Clade	Genera	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Etherioidea	Hyriidae + Iridinidae + Etheriidae	·	·	—	·	·	·	+	+	·	—
Unionoida	Unionoidea + Etherioidea	·	+	+	+	·	+	+	+	+	+

Table 7.2. A Natural Classification of the Extant Palaeoheterodonta Analyzed in this Dissertation. A dagger ('†') indicates that the monophyly of a taxon has not been tested. This classification follows Wiley's (1980) conventions for annotated Linnaean classifications (*e.g.*, except where indicated, taxon order reflects branching order). Informal taxa are enclosed in brackets.

Class BIVALVIA

Subclass PALAEOHETERODONTA

Order TRIGONIOIDA

Order UNIONOIDA

Superfamily UNIONOIDEA Rafinesque, 1820

Family UNIONIDAE *s.s.*

Subfamily PSEUDODONTINAE Frierson, 1927

Subfamily RECTIDENTINAE Modell, 1942 †

Subfamily AMBLEMINAE Rafinesque, 1820

Tribe GONIDEINI Ortmann, 1916

[Amblemini Tribe Group, all *incertae mutabilis*]

Tribe AMBLEMINI *s.s.*

Tribe QUADRULINI von Ihering, 1901

Tribe PLEUROBEMINI Hannibal, 1912

Tribe LAMPSILINI von Ihering, 1901

Subfamily UNIONINAE *s.s.*

Tribe UNIONINI *s.s.*

Tribe ANODONTINI Rafinesque, 1820

Family MARGARITIFERIDAE Haas, 1940

Table 7.2 (continued). A Natural Classification of the Extant Palaeoheterodonta Analyzed in this Dissertation.

Superfamily ETHERIOIDEA Deshayes, 1830
Family HYRIIDAE Swainson, 1840
Subfamily VELESUNIONINAE Iredale, 1934 †
Subfamily HYRIDELLINAE McMichael, 1956 (1934) †
Subfamily HYRIINAE *s.s.*
Family IRIDINIDAE Swainson, 1840
Family ETHERIIDAE *s.s.*

Table 7.3. Diagnoses of Refined Morphological Characters. These characters are derived from those used in **Chapter 2** (Graf, 2000a). For all characters, state *0* is hypothesized to be primitive based on previous analyses (see text). Superscript numbers refer to the same characters in **Appendix III**.

Shell Characters

1. Hinge characters. — *0* = Schizodont dentition or edentulous. *1* = Pseudotaxodont dentition.[1]
2. Posterior (lateral) hinge teeth. — *0* = Well-developed. *1* = Reduced or absent.[2]
3. Anterior (pseudocardinal) hinge teeth. — *0* = Resembling the posterior teeth, but shorter; often with two teeth on the right valve and one on the left. *1* = Shorter and more robust; with two teeth on the left, one on the right. *2* = reduced or absent.[4-6]
4. Beak/post-larval sculpture. — *0* = Concentric, double-looped, or absent; *not* angular. *1* = Zigzag or 'radial' sculpture.[9-10]
5. Valve symmetry. — *0* = Equivalved. *1* = Valves asymmetrical due to cementation; sometimes monomyarian.[7-8]

Gross-Anatomical Characters

6. Ctenidial filaments. — *0* = Filibranch. *1* = Eulamellibranch.[12]
7. Fusion of the ascending lamella of the outer demibranch. — *0* = Not fused to the mantle along its entire length. *1* = Fused to the mantle along its entire length or nearly so.[13]
8. Fusion of the ascending lamella of the inner demibranch. — *0* = Tending not to be fused to the visceral mass. *1* = Tending to be fused to the visceral mass.[14]

Table 7.3 (continued). Diagnoses of Refined Morphological Characters.

Gross-Anatomical Characters (continued)

9. Attachment of the anterior end of the inner demibranch. — *0* = Attaches distant from the labial palps. *1* = Attaches close to or in contact with the labial palps.[15]
10. Pallial fusion ventral to the incurrent aperture. — *0* = None. *1* = Short, with extensive pedal gape; generally associated with 'siphons' including a pallial sinus.[16]
11. Pallial fusion in-between the incurrent and excurrent apertures. — *0* = None; integrity of the incurrent and excurrent apertures is accomplished by fusion of the ctenidia to the mantle or via 'pallial ridges.' *1* = Pallial fusion present.[17]
12. Diaphragm dividing infra- and supra-branchial chambers. — *0* = Not perforated. *1* = Perforated.[20]
13. Pallial fusion dorsal to the excurrent aperture. — *0* = None. *1* = Short, allowing supra-anal aperture. *2* = Continuous, without supra-aperture.[18]
14. Length of the pallial fusion between the supra-anal and excurrent apertures. — *0* = Not distinctly shorter than the excurrent aperture. *1* = Distinctly shorter than the excurrent aperture.[19]
15. Mantle elaborations ventral o the incurrent aperture. — *0* = Elaborations lacking. *1* = Posteroventral mantle elaborations with conspicuous papillae or a ribbon-like flap.[22]

Brooding Characters

16. Habitat. — *0* = Marine. *1* = Freshwater.[23]
17. Parental care. — *0* = No parental care. *1* = Larvae brooded in ctenidial marsupia.[24]

Table 7.3 (continued). Diagnoses of Refined Morphological Characters.

Brooding Characters (continued)

18. Demibranchs occupied by marsupium. — *0* = Tetragenous (all four demibranchs). *1* = Endobranchous (inner pair of demibranchs only). *2* = Ectobranchous (outer pair of demibranchs only).[25]
19. Portion of the outer demibranch that is marsupial. — *0* = Entire outer demibranch. *1* = Only a central or posterior portion is utilized for brooding.[26]
20. Interlamellar connections. — *0* = None or scattered. *1* = Perforated septa in at least the brooding demibranchs. *2* = Continuous (*i.e.*, imperforate) septa.[28-29]
21. Marsupial water-tubes. — *0* = Not divided. *1* = Divided by lateral septa (*i.e.*, tripartite). 2 = Interlamellar septa bearing a 'marked swelling.'[30-31]
22. Expansion of the marsupial demibranchs when gravid. — *0* = Not expanded; ventral edge remains sharp. *1* = Ventral mantle edge augmented with tissue to allow for expansion only laterally. *2* = Mantle capable of expansion ventrally as well as laterally.[32-33]
23. Discharge of Larvae. — *0* = Larvae released through the excurrent aperture. *1* = Larvae forcibly expelled through the ventral margin of the marsupium.[34]

Larval Characters

24. Larvae. — *0* = Free-living. *1* = Parasitic glochidium. *2* = Parasitic lasidium.[35-36]
25. Glochidium morphological type. — *0* = Subcircular, unhooked. *1* = Subtriangular, hooked, without spines. 2 = Subtriangular, hooked, with numerous spines.[37]
26. Lasidium morphological type. — *0* = Lasidium-type. *1* = Haustorium-type.[38]

Table 7.4. Matrix of Taxa and Characters. Character numbering and states follows that of **Table 7.1**. Taxonomy is that of **Table 7.2**. A question mark ('?') indicates unknown or inapplicable character states. See text for discussion and references.

	1 2 12345678901234567890123456
Unionidae	
Unio	00110110000?1001120100012?
Cafferia	00110110000?1001120100012?
Alasmidonta	01210111000?1001120211012?
Pyganodon	01210110000?1001120211012?
Lampsilis	00110111000?1011121202110?
Obliquaria	00110110000?1001121202110?
Pleurobema	00110110000?1101120200010?
Quadrula	00110110000?110110?200010?
Tritogonia	00110110000?110110?200010?
Amblema	00110110000?110110?200010?
Gonidea	01210110000?100110?100010?
Uniandra	00000110000?1001120100010?
Pilsbryoconcha	01210110000?100110?100010?
Pseudodon	01210110000?100110?100010?
Margaritiferidae	
Cumberlandia	01110100000?0?0110?0?00010?

Table 7.4 (continued). Matrix of Taxa and Characters.

	1 2 12345678901234567890123456
Hyriidae	
Diplodon	00000111101O2?0111?100011?
Hyridella	00000111101I2?0111?100011?
Velesunio	00010111101I2?0111?100011?
Iridinidae	
Iridina	11210111111O2?0111?220????
Mutela	01210111111O2?0111?22002?1
Etheriidae	
Anodontites	01210111101O0?0111?22002?0
Mycetopoda	01210111101O0?0111?22002?0
Etheria	01211111101O0?0111?220????
Acostaea	01211111101O0?0111?22002?0
Trigonioida	
Neotrigonia	000?0000000?0?000??0??00?

Table 7.5. Character Diagnostics of the Refined Morphological Characters. Character numbers refer to those listed in **Table 7.3**. Characters are traced to cladograms in **Figure 7.4**. Unambiguous characters refer to those that trace the same under both ACCTRAN and DELTRAN optimization. For each character, s = number of steps on the Super Tree, *CI* = Consistency Index, *RC* = Rescaled Consistency Index, and Optimization refers to the preferred optimization criterion for ambiguous transformations. A dagger ('†') indicates that the RC is taken to be unity when undefined (Farris, 1989).

Character	Ubamb.?	s	*CI*	*RC*	Optimization
1.	Y	1	1.00	1.00†	—
2.	N	5	0.20	0.13	DELTRAN
3.	N	6	0.33	0.22	DELTRAN
4.	Y	2	0.50	0.25	—
5.	Y	1	1.00	1.00	—
6.	Y	1	1.00	1.00†	—
7.	N	2	0.50	0.00	ACCTRAN
8.	Y	3	0.33	0.27	—
9.	Y	1	1.00	1.00	—
10.	Y	1	1.00	1.00	—
11.	Y	1	1.00	1.00	—
12.	N	2	0.50	0.00	ACCTRAN
13.	N	3	0.67	0.59	ACCTRAN
14.	Y	2	0.50	0.33	—
15.	Y	1	1.00	1.00†	—
16.	Y	1	1.00	1.00†	—
17.	Y	1	1.00	1.00†	—

Table 7.5 (continued). Character Diagnostics of the Refined Morphological Characters.

Character	Ubamb.?	s	*CI*	*RC*	Optimization
18.	N	5	0.40	0.31	ACCTRAN
19.	Y	1	1.00	1.00	—
20.	N	5	0.40	0.27	ACCTRAN
21.	Y	2	1.00	1.00	—
22.	Y	2	1.00	1.00	—
23.	Y	1	1.00	1.00	—
24.	Y	2	1.00	1.00	—
25.	N	2	1.00	1.00	DELTRAN
26.	N	1	1.00	1.00†	ACCTRAN

Table 7.6. Length and Consistency Statistics of Character Sets. Upper values reflect the values derived from tracing the characters in **Table 7.3** on the Super Tree Topology (**Figures 7.4**). The bottom set are the statistics for each of the 98 trees recovered by an MP search on the total morphological data set (tree not shown). Individual character diagnostics as traced on the Super Tree are giving in **Table 7.5**. Abbreviations are the same as in **Table 7.5**; n = number of characters.

Character Set	n	s	CI	RC
Shell	5	15	0.400	0.262
Soft-Anatomy	10	17	0.647	0.552
Brooding	8	18	0.667	0.542
Larvae	3	5	1.000	1.000
TOTAL	**26**	**55**	**0.618**	**0.497**
each of 98 MP trees	26	47	0.7234	0.6355

Table 7.7. Plesiomorphies of the Unionoida. Plesiomorphies were inferred based on the characters present in *Neotrigonia* and other bivalves (Salvini-Plawen & Steiner, 1996; Waller, 1998). Superscript numbers refer to characters listed in **Table 7.3**. See text for discussion and references.

Plesiomorphy
Marine[16]
Nacreous, aragonitic shell with periostracum
Opisthodentic, exterior ligament
Degenerate byssus in the adult
Equivalved[5]
Schizodont dentition[1-3]
Filibranch ctenidia[6]
Ctenidia posteriorly free of the mantle and anteriorly free of the visceral mass except for the extreme anterior insertion[7-8]
Extreme anterior insertion of inner demibranch on the visceral mass distant from the attachment of the labial palps[9]
Mantle lobes unfused except beneath the umbos[10-14]
Gonochoristic
No parental care (*i.e.*, non-brooding)[17-19, 22]
Without interlamellar connections[20-21]
Larvae discharged from the excurrent aperture[23]
Free-living, non-parasitic larvae[24-26]

Table 7.8. Synapomorphies of the Major Unionoid Clades Here Studied. Character numbers refer to those listed in **Table 7.3**. Unambiguous character reconstructions are given in **bold**; the preferred optimization criterion for ambiguous transformations is given in brackets. Clades without formal names are marked with a double asterisk ('**').

Order Unionoida

- **6. Eulamellibranch ctenidia**
- **16. Strictly freshwater**
- **17. Larvae brooded by the female in ctenidial marsupia composed of all four demibranchs (tetrageny)**
- **24. Larvae are parasitic, subcircular, unhooked, glochidia**
- 7. Ascending lamellae of outer demibranchs fused to the mantle along their entire length [ACCTRAN]
- 20. Interlamellar spaces divided into water-tubes by perforated septa [ACCTRAN]

Superfamily Unionoidea

- Ø. [there are no morphological synapomorphies]

Family Margaritiferidae

- **3. Robust pseudocardinal hinge teeth**
- 2. lateral hinge teeth are reduced [DELTRAN]
- 7. Ascending lamellae of outer demibranchs not fused to the mantle along their entire length; posterior ends free of the mantle [ACCTRAN]
- 20. Interlamellar connections scattered; water-tubes absent or oblique [ACCTRAN]

Table 7.8 (continued). Synapomorphies of the Major Unionoid Clades Here Studied.

Family Unionidae

13. Supra-anal aperture

Subfamily Pseudodontinae

2. Reduced lateral hinge teeth [DELTRAN]
3. Absent pseudocardinal hinge teeth [DELTRAN]

**** ((Unioninae + Ambleminae) + Rectidentinae)**

? 18. Ectobranchy [ACCTRAN]

Subfamily Rectidentinae

4. Zigzag type beak/post-larval sculpture

**** (Unioninae + Ambleminae)**

3. Robust pseudocardinal hinge teeth [DELTRAN]

Subfamily Unioninae

25. Subtriangular, hooked glochidia with numerous spines

Tribe Unionini

Ø. [there are no unambiguous morphological synapomorphies]

Table 7.8 (continued). Synapomorphies of the Major Unionoid Clades Here Studied.

Tribe Anodontini

2. **Reduced lateral hinge teeth**
20. **Interlamellar space divided into water-tubes by complete (*i.e.*, imperforate) septa**
21. **'Tripartite' water-tubes**
22. **Ventral margins of marsupial demibranchs augmented with tissue to allow for lateral expansion**
3. Reduced pseudocardinal hinge teeth [DELTRAN]

Subfamily Ambleminae

? 18. Tetrageny [ACCTRAN]

Tribe Gonideini

2. **Absent lateral hinge teeth**
3. Absent pseudocardinal hinge teeth [DELTRAN]

**** Amblemini Tribe Group (= Amblemini, Quadrulini, Pleurobemini, Lampsilini)**

14. **Pallial fusion between the supra-anal and excurrent apertures is distinctly shorter than the excurrent aperture**
20. **Interlamellar spaces divided into water-tubes by complete (*i.e.*, imperforate) septa**

Table 7.8 (continued). Synapomorphies of the Major Unionoid Clades Here Studied.

Tribes Amblemini & Quadrulini

Ø. [there are no morphological synapomorphies for either tribe]

Tribe Pleurobemini

? 18. Ectobranchy

Tribe Lampsilini

14. Pallial fusion between the supra-anal and excurrent apertures is *not* distinctly shorter than the excurrent aperture

? 18. Ectobranchy

19. Only a portion of the outer demibranchs is used for brooding

22. When gravid, the marsupium extends both laterally and ventrally

23. Larvae are forcibly discharged through the incurrent aperture

Superfamily Etherioidea

8. Ascending lamellae of the inner demibranchs tend to be fused to the visceral mass

9. Anterior end of the inner demibranch attaches to the visceral mass close to or in contact with the labial palps

11. Pallial fusion between the incurrent and excurrent apertures

18. Endobranchy

13.Continuous pallial fusion dorsal to the excurrent aperture (*i.e.*, without supra-anal aperture) [ACCTRAN]

Table 7.8 (continued). Synapomorphies of the Major Unionoid Clades Here Studied.

Family Hyriidae

12. Perforated diaphragm dividing the infra- and the supra-branchial chambers [ACCTRAN]

? 25. Subtriangular, hooked glochidia lacking numerous spines [DELTRAN]

**** (Iridinidae + Etheriidae)**

20. Interlamellar spaces divided into water-tubes by complete (*i.e.*, imperforate) septa

21. Marsupial interlamellar septa bearing a 'marked swelling'

24. Larvae is parasitic lasidium

2. Absent lateral hinge teeth [DELTRAN]

3. Absent pseudocardinal hinge teeth [DELTRAN]

Family Iridinidae

10. Short pallial fusion ventral to the incurrent aperture, with an extensive pedal gape

? 26. Haustorium-type lasidium larvae [ACCTRAN]

Family Etheriidae

13. Pallial fusion dorsal to the excurrent aperture is lost [ACCTRAN]

Table 7.9. The Present Global Distributions of Freshwater Mussel Families Based on the Super Tree Phylogeny. Taxonomy is as in **Table 7.2**. Distributions follow Haas (1969a). The single quotes around the record of the Unionidae in the Australasian region represents the single, typically Southeast Asian genus *Haasodonta* which occurs on New Guinea.

Taxon	Nearc.	Palearc.	Orient.	Ethiop.	Neotrop.	Austral.
Unionoida						
Unionoidea						
Unionidae	X	X	X	X		'X'
Margaritiferidae	X	X	X	X		
Etherioidea						
Hyriidae					X	X
Iridinidae				X		
Etherioidea			X	X	X	

Figure 7.1. 20th Century View of Freshwater Mussel Evolution. The figure is an overview of unionoid systematics derived from the traditional taxonomic, stratigraphic, and evolutionary narrative. The two different color schemes, light blue and purplish, distinguish the two superfamilies, Unionoidea and Etherioidea. The dotted circles among the biogeographic distributions represent apparently anomalous fossil or recent distributions. The stratigraphy is based on the data in **Table 1.2**, and character evolution patterns are discussed in **Chapter 1**.

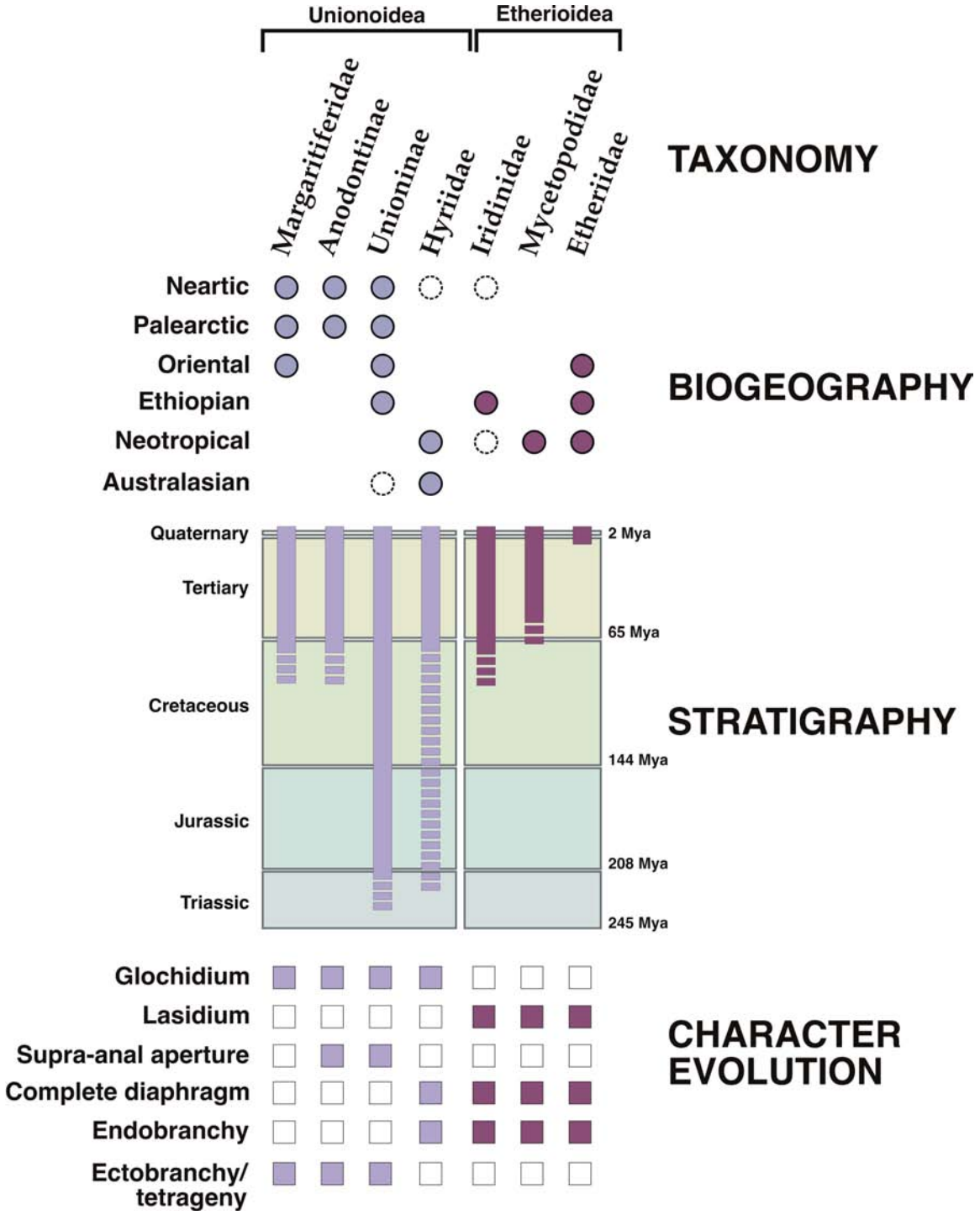


Figure 7.1. 20th Century View of Freshwater Mussel Evolution

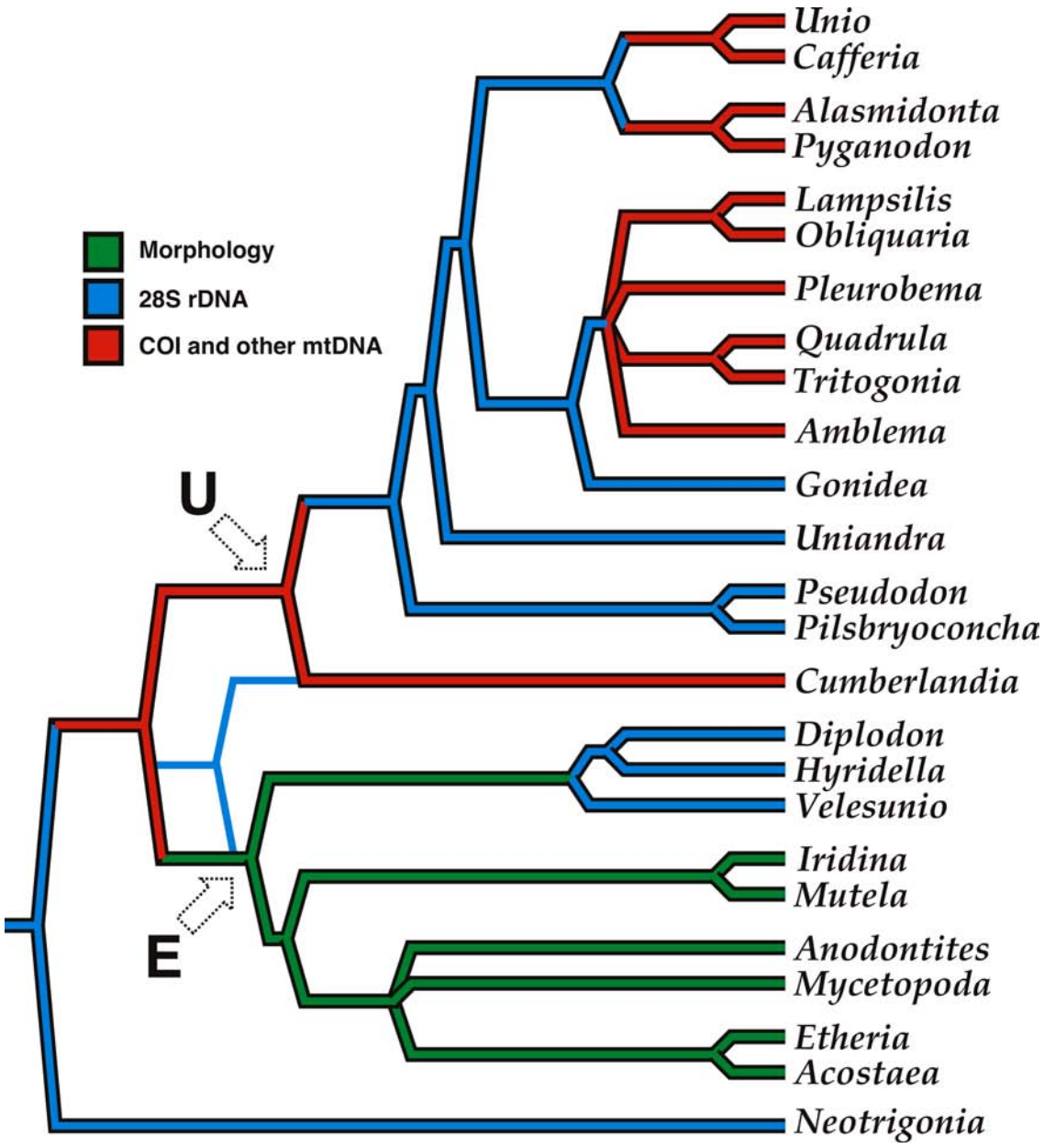


Figure 7.2. Unionoida Super Tree Showing Evidence. The Super Tree Topology is based on the results of the several phylogenetic analyses upon different data sets listed in **Table 7.1**. The color-coding of the branches reflects the principle data sets supporting the branches. 'U' indicates the Unionoidea node; 'E' indicates the Etherioidea node. See text for discussion.

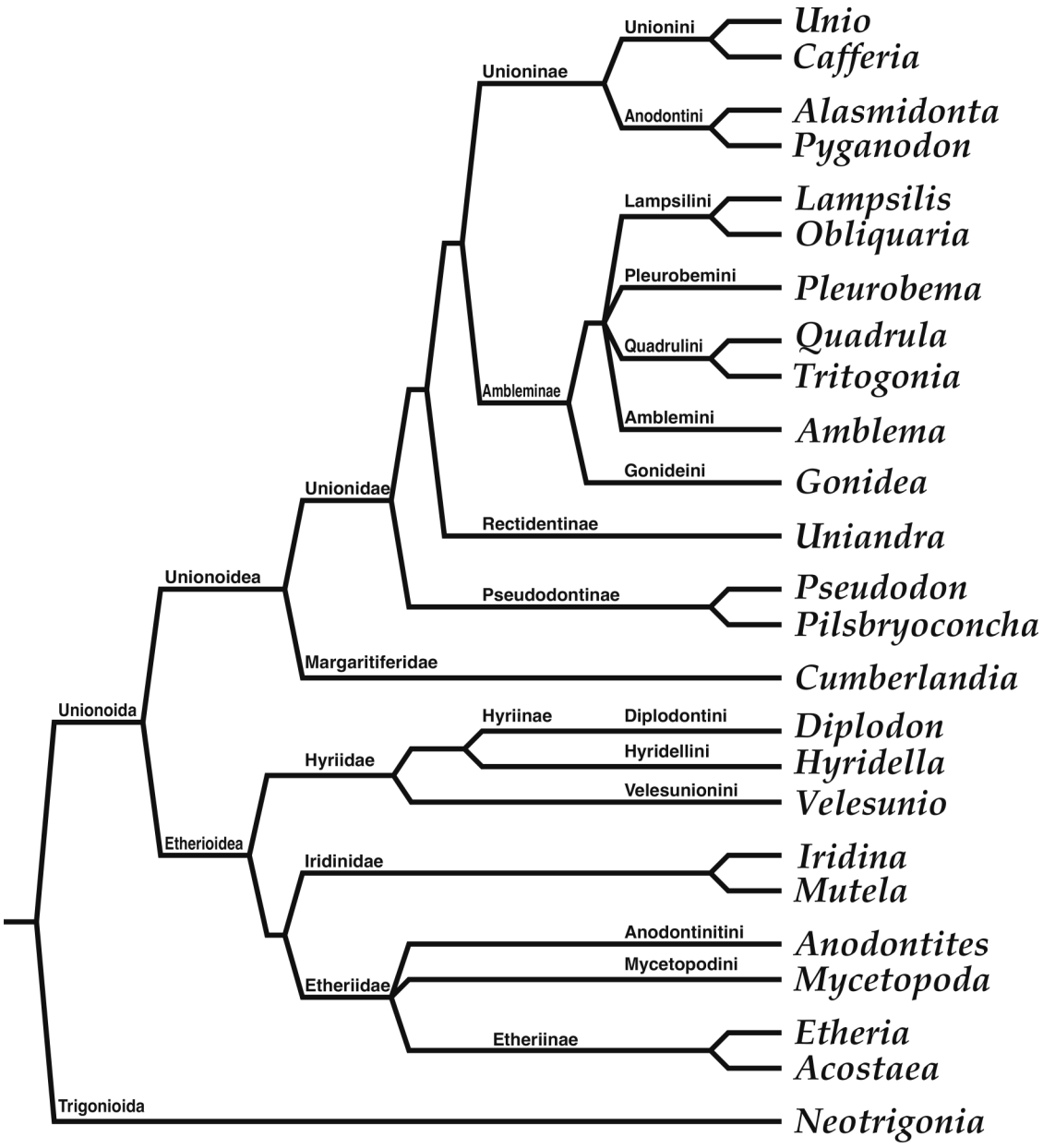


Figure 7.3. Phylogenetic View of Mussel Classification. The classification is derived from the Super Tree topology in **Figure 7.2**. See text for discussion.

Figure 7.4. Freshwater Mussel Characters Transformations. The transformations of the characters in **Table 7.3** were traced under both ACCTRAN and DELTRAN optimizations; unambiguous transformations are those that trace the same under both. Diamonds represent non-homoplastic characters (*i.e.*, *CI* = 1.0); squares for all others. See text for discussion.

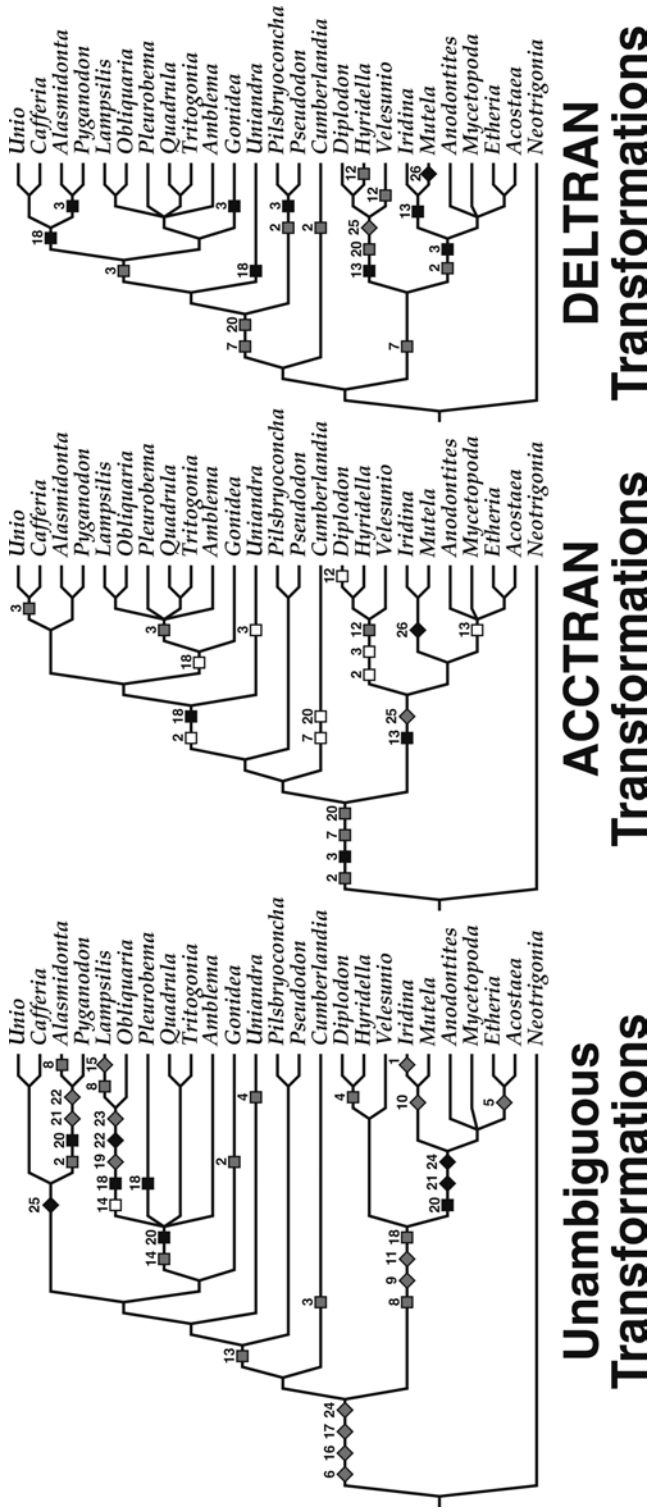


Figure 7.4. Mussel Character Transformations

Figure 7.5. 21st Century View of Freshwater Mussel Evolution. The figure is an overview of unionoid systematics as updated by the studies here described. Conventions follow those in **Figure 7.1**. A 'P' under the Character Evolution chart reflects that a traditionally diagnostic character is plesiomorphic. See text for discussion.

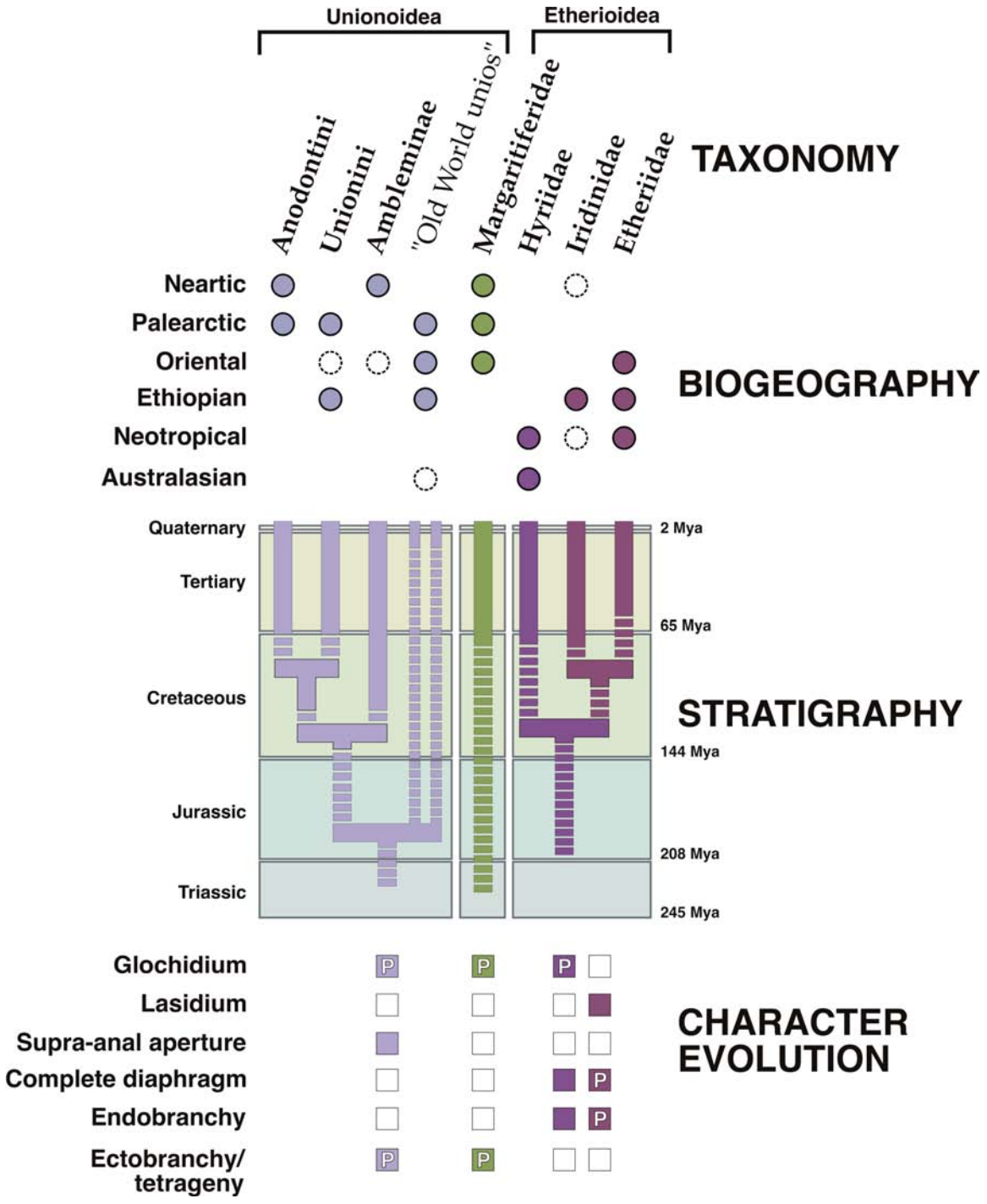


Figure 7.5. 21st Century View of Freshwater Mussel Evolution

APPENDICES

APPENDIX I
THE POSITION OF THE PALAEOHETERODONTA AMONG THE BIVALVIA

The position of the Palaeoheterodonta (= Unionoida + Trigonioida) among the other bivalves can, at present, only be discussed in the vaguest of terms. This is due to the dirth of quality phylogenetic characters available to diagnose ordinal and higher taxa. Despite the incomplete nature of our understanding of bivalve phylogeny, the approximate position of the freshwater pearly mussels among the other bivalve orders is of obvious interest in the context of the evolution of the Unionoida. The principle difficulties have apparently been (1) the lack of congruence among different lines of phylogenetic evidence, (2) the near absence of a modern cladistic perspective, and (3) the difference in perspectives among modern neontologists and paleontologists.

The history of bivalve classification is long and convoluted, but has been excellently reviewed by Cox (1960) and Newell (1965, 1969). In overview, beginning with Linnaeus and lasting into the middle 20th Century, the *modus operandi* of bivalve systematists had been to focus on single organ systems to the disregard of all others. Among other over-emphasized characters were the degree of mantle fusion (Yonge, 1957, 1982), development of adductor muscles (Yonge, 1953), gross ctenidial morphology (Pelseneer, 1891; Ridgewood; 1903), ctenidial ciliation (Atkins, 1936-1938), labial palp attachments (Stasek, 1961, 1963), and stomach anatomy (Owen, 1959; Purcheon, 1956-1960, 1987). Paleontologists, on the other hand, tended (for obvious reasons) to focus on hard- rather than soft-parts (Cox, 1960; Newell, 1965). The sum of these different schemes is a largely irreconcilable system of bivalve character convergence (Newell, 1969: Table 1).

The previous generation's character descriptions fueled half a century of narratives expounding upon bivalve evolutionary history (*e.g.*, Cox, 1960; Newell, 1969;

Nevesskaya *et al.*, 1971; Scarlato & Starobogatov, 1978, 1979; Allen, 1985; Starobogatov, 1992; Morton, 1996). While these works built upon the earlier malacological treatises of Dall (1913) and Thiele (1934), for the most part they represented Kipling-esque 'Just-So' stories. For example, Brian Morton (1996: 337) described his objective as, "... to try and present a *personal view* of the evolution of the Bivalvia." (My emphasis) Whether due to its quality or simply the wide circulation of the *Treatise on Invertebrate Phylogeny* (Moore, ed., 1969), Newell's (1969) system of bivalve higher taxonomy has been followed in many subsequent classifications — especially among neontologists (*e.g.*, Vokes, 1980, Boss, 1982; Vaught, 1989; Brusca & Brusca, 1990).

The position of the mostly freshwater Palaeoheterodonta has not been a priority of bivalve systematists; the focus has traditionally been on marine taxa. **Figure I.1** compares and contrasts the major 20th century systems of the Bivalvia. The arrangements of Thiele (1934), Cox (1960), Newell (1965, 1969), Scarlato & Starobogatov (1978, 1979), Boss (1982), and Brusca & Brusca (1990) present a natural progression, and there is more agreement among them than may appear. Most divide the Bivalvia into three subtaxa: Protobranchia, Pteriomorpha, and Heteroconchia (= Eulamellibranchia *sensu* Thiele, 1934 *non* Brusca & Brusca, 1990). The greatest area of incongruence seems to be whether the eulamellibranch orders should be paired with those with filibranch ctenidia or with the septibranchs.

Following the recognition that the late-Early Cambrian *Fordilla* was a bivalve (and not a conchostracan crustacean) (Newell & Boyd, 1978), and with the discovery of *Pojetaia* from the same time period (Jell, 1980), many paleontologists revised their view of bivalve evolution. Rather than shoe-horning extinct taxa into a modern classification, as had been done in the *Treatise* (Moore, ed., 1969), Pojeta (1971, 1978; Pojeta & Runnegar, 1985) and others (*e.g.*, Morris, 1978; Tunnicliff, 1982; Runnegar & Pojeta, 1992; Babin & Gutiérrez-Marco, 1991) took a 'bottom up' approach fundamentally based

upon hard-part characters (*e.g.*, muscle scars, hinge morphology). Pojeta's (1978) system of seven early Paleozoic subclasses is broadly reconcilable with Newell's (1969) six subclasses (**Figure I.1**), and many paleontologists of the Bivalvia have perpetuated the nomenclature of the *Treatise* (*e.g.*, Cope, 1996a, b, 1999). Overall, with regard to the position of the Palaeoheterodonta (≈ Actinodonta), most authors have agreed that Trigonioida and Unionoida share some affinity with the Heterodonta (includes Veneroida, Myoida, *etc.*) (Boss, 1982). However, these authoritarian treatments of bivalve systematics hardly constitute a test of any particular hypothesis of freshwater mussel relationships.

Malacology in general has been slow to adopt modern scientific methods of phylogenetic reconstruction (Wiley, 1980). Purcheon (1978) launched the analytical phase of bivalve systematics, and his unresolved phenogram clustered the Palaeoheterodonta with different veneroid superfamilies. The first true phylogenetic treatments (*i.e.*, seeking monophyletic taxa and diagrammed on strictly bifurcating cladograms) of deeper bivalve relationships did not appear until the 1990s, but these were little improvement over the previous generation's authoritarianism. Four comprehensive 'analyses' have dealt with the position of the Palaeoheterodonta, and their resultant phylogenies are presented in **Figure I.2**. Of these, Salvini-Plawen & Steiner (1996), Morton (1996), and Waller (1998) applied strictly morphological characters. Actually, only Salvini-Plawen & Steiner (1996) searched among possible trees; the other two came up with a tree by other means (see Morton's quote above). There is little agreement among these cladograms regarding the sister group (or even the monophyly) of the Palaeoheterodonta (**Figure I.2**).

The fourth of these studies was a molecular phylogenetic analysis of bivalve small-subunit nuclear rDNA (18S) (**Figure I.2**): Adamkewicz *et al.* (1997). They found the Palaeoheterodonta (represented only by the Unionoida) to be sister to the Heterodonta (Adamkewicz *et al.*, 1997: figure 3). However, the plethora of perverse relationships

among the taxa they studied, does not allow much confidence in their palaeoheterodont results. Other molecular studies that have included Palaeoheterodonta (*i.e.*, Rosenberg *et al.*, 1997 [28S]; Hoeh *et al.*, 1998, 2001 [COI]; Distel, 2000 [18S]) contribute little more in this context.

Based on the current state of bivalve systematics, as lamented above, the phylogenetic position of the Palaeoheterodonta can only be stated in the vaguest of terms. Recognizing that the authoritarian arrangements reviewed above (**Figure I.1**) at least provided a framework in which modern cladists could work their craft, that latter group has been largely unsuccessful (**Figure I.2**). See **Chapter 7** for continued discussion of the position of the Palaeoheterodonta among the Bivalvia, especially as it regards the determination of the plesiomorphic states for unionoid synapomorphies.

Figure I.1. Major 20th Century Higher Classifications of the Bivalvia. The classification of the Bivalvia up to 1960 has been excellently reviewed by Cox (1960). This figure shows Cox's scheme relative to the six other major bivalve systems of the last century: Thiele (1934), Newell (1965, 1969), Scarlato & Starobogatov (1978, 1979), Boss (1982), Brusca & Brusca (1990), and Pojeta (1971, 1978). The shaded boxes indicate the placement of the freshwater mussels among the other taxa. See text for further discussion.

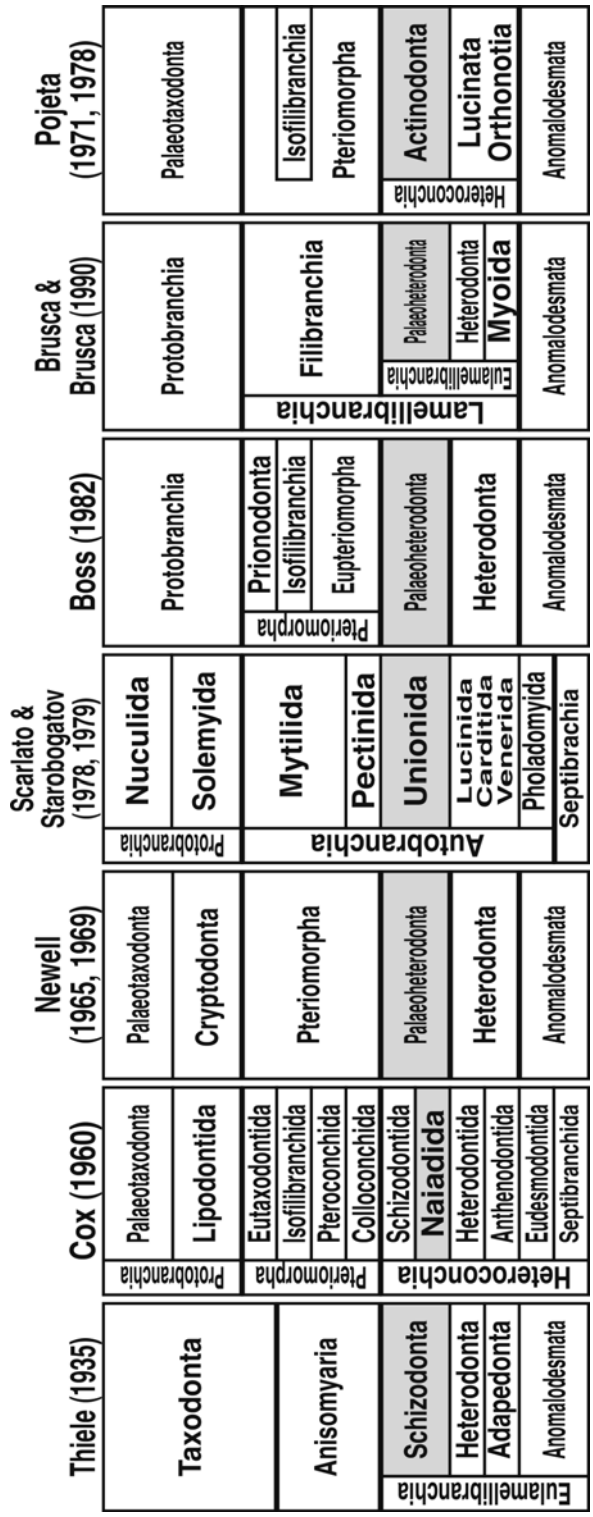


Figure I.1. Higher Classifications of the Bivalvia

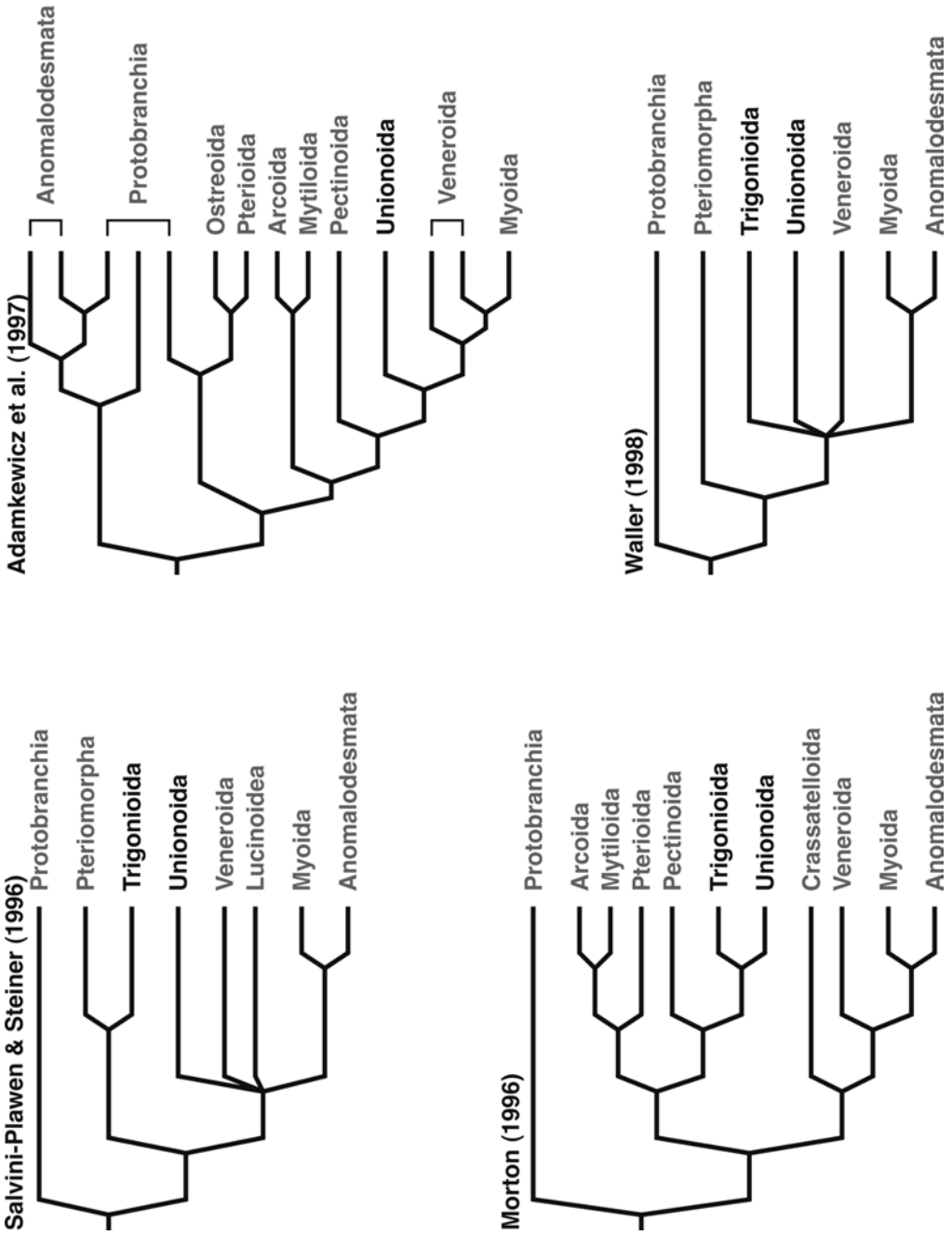


Figure I.2. Phylogenetic Interpretations of Bivalve Evolution. The figure shows four cladograms of bivalve evolution based on different data sets and analytical methods. Only Adamkewicz *et al.* (1997) applied molecular characters; the other three based their phylogenies upon morphology and 'their feelings.' See text for discussion.

APPENDIX II
CORRECT FAMILY-GROUP NAMES OF THE UNIONOIDA, THOSE APPLIED IN THIS DISSERTATION, AND A CONSENSUS CLASSIFICATION

There has been some confusion regarding the correct names of unionoid families, or, in some cases, there *should have been* some confusion but it has never been recognized. The likely reason has been the general lack of concern among freshwater malacologists to establish the correct familial nomenclature (as opposed to generic and trivial nomina) and disinterest in applying the recommendations of the ICZN. The lexicon of freshwater mussel families names presented here is not meant to be complete but rather a guide to the nomenclature applied in this dissertation. Thus, the numerous unionid lineages that I do not consider in analyses are not listed; the majority of those ignored names presumably date from Modell (1942). The correct names of the Etheriidae, Iridinidae, and Mycetopodidae have been dealt with in detail by Kabat (1997).

All family-group names are listed in **Table II.1**. These names are largely non-problematical from a nomenclatural perspective, with two exceptions: Margaritiferidae and Diplodontidae. Margaritiferidae Haas, 1940 has been added to the official list of family-group names (Melville & Smith, 1987) based on ICZN O.495 (Hemming, 1957). This ruling ignores Henderson's (1935) earlier, correct application of that nominum. However, because (1) Margaritiferidae is one of only two freshwater mussel family-group nomina on the official list and (2) changing the authority will have no effect on unionoid nomenclature, the family-group name for *Margaritifera* will be maintained as Margaritiferidae Haas, 1940.

In the case of Diplodontidae von Ihering, 1901, the historically incorrect usage of that name is continued in this dissertation for the sake of consistency. Parodiz & Bonetto

(1963: 198-199) discussed the application of the family-group names Diplodontidae and Prisodontidae to the Hyriidae of South America:

> "The family name Diplodontidae Ihering 1901... is not valid, being preoccupied by Diplodontidae Dall [1895], created for marine bivalves. Prisodontini Modell 1942 included the genus *Hyria* (= *Prisodon*) which cannot be separated as a subfamily by itself. The name Hyriidae Swainson 1840 has priority, but Diplodontini and Prisodontini can be used as tribal denominations."

While Parodiz & Bonetto (1963) clearly recognized the problems with Diplodontidae and Prisodontidae, their designations did nothing to correct them. It seems clear from the quote above that they did not consider "tribal denominations" as family-groups names. They are, however, according to the ICZN (Art. 36). In the case of Prisodontini, that nominum is simply a junior synonym of Hyriini.

Dall (1895: 545) purposefully introduced Diplodontidae as a junior synonym of Ungulinidae H. & A. Adams, 1857, stating, "The name *Ungulinidæ* has been used for very different assemblages of genera, and I prefer to use a name for the family about which there can be no uncertainty." Despite the poor reasoning for introducing a new name, Diplodontidae Dall, 1895 is still available as a family-group name. Thus, either Dall's or von Ihering's use of that name will need to be suppressed. Such an act of nomenclatural providence is beyond the scope of this dissertation, and the incorrect use of Diplodontidae von Ihering, 1901 for the genus *Diplodon* will be retained.

Table II.2 is a consensus classification of the Unionoida based on the 'correct' family-group names in **Table II.1** and differing schools of malacological thought (*e.g.*, Simpson, 1900, 1914; Ortmann, 1910a, 1911a, b, 1912b, 1921a; Frierson, 1927; Modell, 1942, 1949, 1964; Morrison, 1956, 1973; McMichael & Hiscock, 1958; Pain & Woodward, 1961; Parodiz and Bonetto, 1963; Haas, 1969a, b; Heard & Guckert, 1971; Davis & Fuller, 1981; Boss, 1982; Korniushin, 1998). Although there is widespread disagreement as to the ranks of the various family-group taxa, there are several points of agreement. Most malacologists since Parodiz & Bonetto (1963), have agreed that the

Unionoida should be divided into two superfamilies: Unionoidea and Etherioidea. The taxa with glochidium-type larvae — Margaritiferidae, Unionidae, and Hyriidae — comprise the Unionoidea, and in the Etherioidea resides those families with lasidium-type larvae: Iridinidae (= Mutelidae), Mycetopodidae, and Etheriidae. With the exception of the Unionidae (as listed in **Table II.2**), there seems to be general agreement with regard to the family-level taxa.

The two most recent classifications of the Unionoidea —Heard & Guckert (1971) and Davis & Fuller (1981; also Lydeard *et al.*, 1996) — differ with regard to whether or not the Unionidae should be regarded as a single family or as two. However, this difference seems only to be a question of taxon rank, and the system of Davis & Fuller (1981) with one family divided into two subfamilies is analogous to Heard & Guckert's (1971: Figure 1) two family system. Heard & Guckert (1971) included the genus *Unio* in the same family as *Anodonta*; thus, their two families were Unionidae and Amblemidae. Davis & Fuller (1981) and others (Ortmann, 1910a, 1912b) placed *Unio* in the same family as *Amblema*; thus, their two subfamilies were Anodontinae and "Ambleminae." The classification presented in **Table II.2** follows Davis & Fuller's (1981) system except that "Ambleminae" is replaced with the correct nominum: Unioninae.

The Russian school of Unionoida taxonomy *(e.g.*, Starobogatov, 1970) can be reconciled with the Western consensus. The Russian system — reviewed by Shikov & Zatravkin, 1991 and Korniushin, 1998 — is mostly conchological and aimed at 'splitting,' emphasizing differences among taxa rather than their similarities. Their proliferation of genera and families, however, still follows the basic divisions of the Unionoida shown in **Table II.2**.

Totally separate and irreconcilable with this consensus classification is the system proposed by Hans Modell (1942, 1949, 1964). Admirably, Modell (translated by Stansbery & Soehnagen, 1964: 3) sought to objectively approach a "natural" system of the Unionoida, explaining,

> "It is almost as if I had before me the material obtained on an expedition to an unexplored planet and I have used on it the experiences of a biological nature which I have obtained in better than 20 years of collecting."

Reaching the misconceived conclusion that, "Simpson [1900, 1914], as Ortmann emphasized in 1912, paid too little attention to the shell and especially to the sculpture of the beaks..." (Stansbury & Soehnagen, 1964: 2), Modell used these latter two characters to divide the Unionoida into better than 30 subfamilies in four families. A glance at Modell's phylogeny (1942, plate 5) suggests that he had no intention of discovering "natural" (*i.e.*, monophyletic) suprageneric categories.

The consensus arrangement shown in **Table II.2** serves to place the 'classification' of the Unionoida in a logically consistent framework. The 'logical consistency of classification' I am referring to is *not* in the sense of Wiley (1980) (*i.e.*, classification should accurately reflect phylogeny). Instead, I am referring to idea that all of the disparate systems of some portion of the Unionoida are reconciled to make an arrangement of the whole. The 'logical consistency' of the natural classification *sensu* Wiley (1980) is dealt with in **Chapter 7**.

Table II.1. Family-Group Names of the Unionoida. All names are listed alphabetically by their family-level rank (Principle of Coordination, ICZN Art. 36). For the lineages traditionally placed among the UNIONIDAE (**Table I.2**), only those specific lineages dealt with in this dissertation have been included. That is, among that group, only family-group names for genera here analyzed or discussed are listed. The family-group names under the ETHERIOIDEA (**Table I.2**), nomenclature follows Kabat (1997).

ACOSTAEIDAE Morrison, 1973.

ACOSTAEIDAE Morrison (1973: 45).

ALASMIDONTIDAE Swainson, 1840. — Spelling emended by Frierson (1927).

ALASMODONTINÆ Swainson (1840: 268, 381).

ALASMIDONTINAE Frierson (1927: 8-9, 18).

AMBLEMIDAE Rafinesque, 1820.

AMBLEMIDIA *Les Amblémides* Rafinesque (1820: 44, 1964: 46).

AMBLEMINAE Modell (1942: 180, 1949: 41, 1964: 90).

ANODONTIDAE Rafinesque, 1820.

ANODONTIDIA *Les Anodontides* Rafinesque (1820: 50, 1964: 48).

ANODONTINÆ Swainson (1840: 286, 381).

ANODONTINÆ Ortmann (1910a: 117, 1912b: 224).

ANODONTITIDAE Modell, 1942.

ANODONTITINAE Modell (1942: 175, 1949: 38, 1964: 81).

GLABARINAE Modell (1942: 175, 1949: 38, 1964: 81).

Table II.1 (continued). Family-Group Names of the Unionoida.

CAELATURIDAE Modell, 1942.

CAELATURINAE Modell (1942: 190, 1949: 46, 1964: 116).

CAFFERIIDAE Modell, 1942.

CAFFERIINAE Modell (1942: 188, 1949: 45).

CASTALIIDAE Morretes, 1949.

CASTALIINAE Morretes (1949: 21).

CASTALIINI Parodiz & Bonetto (1963: 201, 204).

CUCUMERUNIONIDAE Iredale, 1934. — Iredale's (1934) incorrect suffix emended by Modell (1942) in accordance with ICZN Article 29.

CUCUMERUNIONAE Iredale (1934: 58, 77, 1943a: 191).

CUCUMERUNIONINAE (Iredale, 1934) Modell (1942: 184, 1949: 42).

CUMBERLANDIIDAE Heard & Guckert, 1971. — The suffix applied by Heard & Guckert (1971) is not in accordance with ICZN Art. 29. It was corrected by Davis & Fuller (1981).

CUMBERLANDINAE Heard & Guckert (1971: 338).

CUMBERLANDIINAE Davis & Fuller (1981: 237, 250).

Table II.1 (continued). Family-Group Names of the Unionoida.

DIPLODONTIDAE von Ihering, 1901. — This name is a junior homonym of the DIPLODONTIDAE Dall, 1895 (= UNGULINIDAE H. & A. Adams, 1858). In the future, one of these nomina should be suppressed and renamed.

DIPLODONTIDAE von Ihering (1901: 52-53).

DIPLODONTINAE Morretes (1949: 17).

DIPLODONTINI Parodiz & Bonetto (1963: 199, 205).

ELLIPTIONIDAE Modell, 1942.

ELLIPTIONIDAE Modell (1942: 178, 180, 1949: 40, 1964: 88).

ETHERIIDAE Deshayes, 1830.

Ethéries Deshayes (1830: table, fam. 20).

ETHERIDÆ Swainson (1840: 258, 390).

FOSSULIDAE Bonetto, 1966.

FOSSULINI Bonetto (1966: 3, 5).

GONIDEIDAE Ortmann, 1916.

GONIDEINAE Ortmann (1916a: 53).

Table II.1 (continued). Family-Group Names of the Unionoida.

HYRIDELLIDAE McMichael, 1956 (1934). — The priority of HYRIDELLIDAE over PROPEHYRIDELLIDAE due to priority of the type genus is valid according to ICZN Article 40.2 because the replacement occurred before 1961.

PROPEHYRIDELLIDAE Iredale (1934: 58, 76-77, 1943a: 189-190, 1943b: 87).

HYRIDELLINAE McMichael (1956: 42, 1958: 427; McMichael & Hiscock, 1958: 435).

HYRIIDAE Swainson, 1840. — Swainson's (1840) incorrect suffix was emended by Ortmann (1910a) in accordance with ICZN Article 29. HYRIIDAE is retained over PRISODONTIDAE following ICZN Article 40.1.

HYRINÆ Swainson (1840: 268, 282). — Modell (1964: 103).

HYRIANÆ Swainson (1840: 379). — Agassiz (1845: 43).

HYRIOIDÆ Agassiz (1846: 192).

HYRIINÆ Ortmann (1910a: 115, 1911a: 108, 120, 130, 1912b: 225, 1912c: 103, 1921a: 457). — Modell (1942: 186, 1949: 43).

PRISODONTINAE Modell (1942: 174, 1949: 38, 1964: 80).

PRISODONTIDAE Morretes (1949: 17, 23).

PRISODONTINI Parodiz & Bonetto (1963: 201, 204).

IRIDINIDAE Swainson, 1840

IRIDININÆ Swainson (1840: 261, 286, 380).

Table II.1 (continued). Family-Group Names of the Unionoida.

LAMPSILIDAE von Ihering, 1901.

LAMPSILINÆ von Ihering (1901: 53).

LAMPSILINÆ Ortmann (1910a: 118, 1912b: 224).

LEILIDAE Morretes, 1949.

LEILINAE Morretes (1949: 28).

LORTIELLIDAE Iredale, 1934.

LORTIELLINAE Iredale (1934: 58, 77, 1943a: 190).

MARGARITIFERIDAE Haas, 1940. — The ICZN (O.495 Hemming, 1957) ruled that MARGARITIFERIDAE Haas, 1940 was the official family name following suppression of MARGARITANIDAE Ortmann, 1910a (Melville & Smith, 1987).

MARGARITANINÆ Ortmann (1910a: 114, 1912b: 223).

MARGARITIFERINAE Henderson (1935: 68).

MARGARITIFERIDAE Haas (1940: 119).

MARGARITIFERINAE Modell (1942: 184, 1949: 42, 1964: 97).

MEGALONAIADINAE Heard & Guckert, 1971.

MEGALONAIADINAE Heard & Guckert (1971: 338).

MONOCONDYLAEIDAE Modell, 1942.

MONOCONDYLAEINAE Modell (1942: 175, 1949: 38, 1964: 81).

Table II.1 (continued). Family-Group Names of the Unionoida.

MUTELIDAE Gray, 1847.

MUTELADAE Gray (1847:197).

MYCETOPODIDAE Gray, 1840.

MYCETOPODIDAE Gray (1840: 142, 155).

PARREYSIIDAE Henderson, 1935.

PARREYSIINAE Henderson (1935: 69). — Modell (1964: 107).

PARREYSIINAE Modell (1942: 186, 1949: 43).

PLEUROBEMIDAE Hannibal, 1912.

PLEUROBEMINÆ Hannibal (1912: 119). — Modell (1949: 40).

PLEUROBEMINAE Modell (1942: 179, 1964: 88).

PSEUDODONTIDAE Frierson, 1927.

PSEUDODONTINAE Frierson (1927: 67).

PSEUDOMULLERIIDAE Starobogatov, 1970.

PSEUDOMULLERIIDAE Starobogatov (1970: 75, 288).

PSEUDOMULLERIDAE Morrison (1973: 46).

Table II.1 (continued). Family-Group Names of the Unionoida.

QUADRULIDAE von Ihering, 1901.

QUADRULINÆ von Ihering (1901: 53). — Hannibal (1912: 119). — Modell (1949: 44, 1964: 106).

QUADRULINAE Haas (1929: 333). — Modell (1942: 187).

RECTIDENTIDAE Modell, 1942. — The genus *Uniandra* has been placed in CONTRADENTINAE and ANODONTINAE by Modell (1942, 1949, 1964). CONTRADENTINAE, the type genus of which is *Uniandra*, was considered a synonym of the RECTIDENTINAE by Brandt (1974).

RECTIDENTINAE Modell (1942: 189, 1949: 45, 1964: 113). — Brandt (1974: 287).

CONTRADENTINAE Modell (1942: 189, 1949: 45).

STROPHITIDAE Starobogatov, 1970.

STROPHITINAE Starobogatov (1970: 69, 287). — Bogan (1985: 141-142).

STROPHITINI Gordon (1981: 58, 1985: 8).

UNIONIDAE Rafinesque, 1820. — Originally, the ICZN (O.495 Hemming, 1957) had ruled the UNIONIDAE Fleming, 1828 was the official family name. This was subsequently corrected by Melville & Smith (1987).

UNIODIA *Les Uniodés* Rafinesque (1820: 24, 1964: 35).

UNIONIDAE Fleming (1828: 408, 415).

UNIONIDÆ Swainson (1840: 257, 264, 377).

Table II.1 (continued). Family-Group Names of the Unionoida.

VELESUNIONIDAE Iredale, 1934. — Iredale's (1934) incorrect suffix emended by Modell (1942) in accordance with ICZN Article 29.
VELESUNIONAE Iredale (1934: 58, 76, 1943a: 189).
VELESUNIONINAE (Iredale, 1934) Modell (1942: 178, 1949: 39, 1964: 87).

Table II.2. An Annotated Consensus Classification of the Unionoida. See text for discussion.

Order UNIONOIDA

Superf. UNIONOIDEA Rafinesque, 1820.

Fam. UNIONIDAE *s.s.*

Subf. UNIONINAE *s.s.*

Tr. UNIONINI *s.s.* (= CAFFERIINI Modell, 1942)

Tr. AMBLEMINI Rafinesque, 1820

(= MEGALONAIADINAE Heard & Guckert, 1971)

(= QUADRULINI von Ihering, 1901).

Tr. CAELATURINI Modell, 1942.

Tr. GONIDEINI Ortmann, 1916a.

(=PSEUDODONTINI Frierson, 1927).

Tr. LAMPSILINI von Ihering, 1901.

Tr. PARREYSIINI Henderson, 1935.

Tr. PLEUROBEMINI Hannibal, 1912

(= ELLIPTIONINI Modell, 1942).

Tr. PSEUDODONTINI Frierson, 1927.

Tr. RECTIDENTINI Modell, 1942.

Subf. ANODONTINAE Rafinesque, 1820.

Tr. ANODONTINI *s.s.*

Tr. ALASMIDONTINI Swainson, 1840.

Tr. STROPHITINI Starobogatov, 1970.

Table II.2 (continued). An Annotated Consensus Classification of the Unionoida.

Fam. MARGARITIFERIDAE Haas, 1940.

Subf. MARGARITIFERINAE *s.s.*

Subf. CUMBERLANDIINAE Heard & Guckert, 1971.

Fam. HYRIIDAE Swainson, 1840.

Subf. HYRIINAE *s.s.*

Tr. HYRIINI *s.s.*

Tr. CASTALIINI Morretes, 1949.

Tr. DIPLODONTINI von Ihering, 1901.

Subf. HYRIDELLINAE McMichael, 1956 (1934).

Tr. HYRIDELLINI *s.s.*

Tr. CUCUMERUNIONINI Iredale, 1934.

Tr. LORTIELLINI Iredale, 1934.

Tr. VELESUNIONINI Iredale, 1934.

Superf. ETHERIOIDEA Deshayes, 1830.

Fam. ETHERIIDAE *s.s.*

Subf. ETHERIINAE *s.s*

Subf. ACOSTAEINAE Morrison, 1973.

(= PSEUDOMULLERIINAE Starobogatov, 1970).

Fam. MYCETOPODIDAE Gray, 1840.

Subf. MYCETOPODINAE *s.s.*

Subf. ANODONTITINAE Modell, 1942.

Subf. LEILINAE Morretes, 1949.

Subf. MONOCONDYLAEINAE Modell, 1942.

Table II.2 (continued). An Annotated Consensus Classification of the Unionoida.

Tr. MONOCONDYLAEINI *s.s.*

Tr. FOSSULINI Bonetto, 1966.

Fam. IRIDINIDAE Swainson, 1840.

(= MUTELINAE Gray, 1847).

APPENDIX III
MORPHOLOGICAL CHARACTERS OF THE UNIONOIDA USED FOR PHYLOGENETIC ANALYSES

Although the general anatomy and various reproductive specializations of freshwater pearly mussels have been described and contrasted elsewhere (*e.g.*, Lefevre & Curtis, 1910; Coker *et al.*, 1921; Baker, 1928; Ortmann, 1911c; Graf, 1998), they are worth reviewing in the context of the present analyses. The following is a synopsis of the characters and character states of the taxa here included. Character numbers refer to those listed in **Table 2.4** and indicated on **Figure 2.2**. Superscript numbers refer to the numbering used in **Chapter 4** for the brooding character analysis. The characters described below are grouped into four classes: Shell, Anatomical, Brooding, and Larval Characters. See **Chapter 2** for an explanation of phylogenetic methodology. In all cases, *0* is hypothesized to be the plesiomorphic condition of the Palaeoheterodonta (see **Chapter 7** for further discussion).

Shell Characters

The general Bauplan of a freshwater mussel is that of a typical autobranch bivalve. The gross morphology has been more-than-adequately reviewed and figured for both autobranchs, in general (Brusca & Brusca, 1990) and freshwater mussels (Ortmann, 1911c). Unionoids possess an aragonitic, bivalved shell lined externally by a proteinaceous periostractum and internally by a pearly layer of nacre (Boss, 1982; Brusca & Brusca, 1990). The two valves are connected by an external ligament, forming a hinge. Characters 1-6 deal with hinge morphology.

1. Hinge type. — *0* = Schizodont. *1* = Taxodont.

Hinge dentition is generally "schizodont" (Thiele, 1934; but see discussions in Cox, 1969 and Morton, 1987), although it may be edentulous or taxodont. The dentition of a schizodont hinge is divided into posterior lateral teeth behind the umbo and pseudocardinal teeth located directly ventral or anterior to the umbo. Hinges **A-F** shown in **Figure III.1** are schizodont; **G** is taxodont.

2. **Development of posterior (lateral) hinge teeth. — *0* = Well-developed. *1* = Reduced or absent.**
3. **Morphology of posterior (lateral) hinge teeth. — *0* = Smooth. *1* = Serrate.**

Taxa with well-developed lateral teeth have a double lateral in the left valve and an interlocking single tooth in the right, although the right hinge may also be double. **Figure III.1F** shows the hinge of *Pseudodon* with a reduced lateral (and pseudocardinal, see below) teeth. Lateral teeth may be marked by perpendicular serrations, as in *Neotrigonia* and some South American hyriines (von Ihering, 1910; Ortmann, 1921a; Hoeh et al., 1996a; see **Figure III.1A**).

4. **Development of anterior (pseudocardinal) hinge teeth. — *0* = Robust, generally with two teeth on the left, one on the right. *1* = Delicate, generally with one on the left, two on the right. *2* = Reduced or absent.**
5. **Angle between posterior and anterior hinge teeth. — *0* = Obtuse. *1* = Acute.**
6. **Morphology of anterior (pseudocardinal) hinge teeth. — *0* = Tab-like. *1* = Peg-like.**

Well-developed pseudocardinal dentition tends to be much shorter and heavier than the laterals, with two teeth and a socket in the left valve and the reciprocal on the right. Delicate pseudocardinals are not as strongly developed and more often resemble an anterior set of laterals; this type may often have reversed dentition, with two teeth in the right valve and one on the left (**Figure III.1D**).

Pseudocardinal teeth vary in their orientation relative to the lateral teeth and tend to be either obtuse or more acute. This is best measured in the left valve. The angle, θ, is determined at the intersection of a tangential line through the posterior teeth and a line normal to orientation of the anterior pseudocardinals (**Figure III.1B**). The angle is considered more acute if θ is substantially less than 90°.

Regardless of orientation, pseudocardinal teeth may be peg-like or tab-like, with corresponding holes or slots in the opposite valves. Robust, peg-like pseudocardinals, as in Pleurobema, Quadrula, and Elliptio are depicted in **Figure III.1B**, **C**, and **E**, respectively.

7. Shell shape. — *0* = Bilaterally symmetrical. *1* = Asymmetrical due to cementation.

8. Adult adductor scars. — *0* = Dimyarian. *1* = Monomyarian.

The shell is equivalved and inequilateral, bearing prosogyrous umbos (often extending above the hinge line) and varying in outline from sub-ovate and globose to lanceolate and compressed to trigonal (figures of external morphology can be found in Haas, 1969b). Or, the valves may be completely asymmetrical, resembling those of an oyster. Such a morphology is due to their cementation rather than a typical, infaunal habit. Conchological convergence with ostreids may be so great that some genera, like *Acostaea* are monomyarian as adults (Yonge, 1978 With the exception of the monomyarian shells of *Acostaea*, the Unionoida are dimyarian with well-impressed anterior adductor scars and variably impressed posterior scars. The pallial line is generally simple but is sinuate in *Leila* Gray (not available for the present analyses), which has contractile siphons (Boss, 1982). Examples of asymmetrical shells can be seen in **Figure 1.10.**

9. External shell. — *0* = Sculptured. *1* = Smooth.

While many unionoids are smooth externally, others possess sculpturing on the disk of the shell ranging from many small pustules or nodules to a few large nodules to oblique folds; some have more than one of these in combination. Each of these different sculpture types has been hypothesized to be derived from a trigonioid ancestor (e.g., Stanley, 1970; Watters, 1994a). It is therefore reasonable to assume that all external shell sculpturing in the Unionoida and *Neotrigonia* is homologous. Ortmann (1912b) considered external sculpturing, along with most aspects of shell morphology, to be of no suprageneric taxonomic value. This was primarily due to the extreme ecophenotypic variation characteristic of the Unionoida (see discussion in Graf, 1997c). **Figures 1.2-10** show examples of sculptured and unsculptured shells.

10. Beak (umbo) sculpture. — *0* = Absent, concentric or double-looped. *1* = Angular or zigzag (including 'radial').

Ortmann (1912b) did recognize beak sculpture, umbonal corrugations retained from the juvenile shell, to be of systematic significance, especially at the generic level. Modell (1942) and Frierson (1909, 1911) considered beak sculpture to be of utmost importance. Most umbonal sculpturing can be hypothetically derived from a simple concentric-type (Ortmann, 1912b), and every gradation between simple and double-looped is seen in the taxa included in the present study [illustrations of different beak sculptures can be found in Marshall (1890), Bonetto (1962a), and Clarke (1973)]. However, angular, zigzag sculpture is seen in a number of genera (*e.g.*, Pilsbry & Bequaert, 1927; Prashad, 1930; Brandt, 1974); this includes the apparently radial beak sculpture of *Castalina* and *Diplodon* (Bonetto, 1962a; Watters, 1994a).

11. Mantle muscle scars. — *0* = Present. *1* = Absent.

Smith (1983) has drawn attention to small mantle muscle scars within the pallial line on the shells of *Neotrigonia* and the Margaritiferidae.

Gross Soft-Anatomy Characters

The gross, soft-tissue anatomy of the Unionoida is that of a typical lamellibranch bivalve (*e.g.*, Brusca & Brusca, 1990). The visceral mass bears the labial palps, laterally-compressed foot, and a pair of bipectinate ctenidia. All four demibranchs are present, an inner pair and a somewhat smaller outer pair.

12. Ctenidial morphology. — *0* = Filibranch. *1* = Lamellibranch.

Demibranchs of freshwater mussels are of the eulamellibranch type, with adjacent filaments connected by tissue-grade, interfilamental junctions bearing ostia for the passage of water. This is opposed to the filibranch type of *Neotrigonia* (Morton, 1987; Smith, 1998) in which individual ctenidial filaments are loosely associated by interlocking tufts of cilia (Brusca & Brusca, 1990: 736: figures 31C-F).

13. Association of ascending lamellae of outer demibranchs with the mantle. — *0* = Completely free (unfused) or free posteriorly , but separation of the infrabranchial from the suprabranchial chamber is achieved by a 'pallial ridge' or 'diaphragmatic septum.' *1* = Fused to mantle along entire length or nearly so.

Ascending lamellae of the outer demibranchs tend to be fused to the mantle along their entire length, although in some taxa they are entirely or partly free of the mantle; the diaphragm dividing the infrabranchial from the suprabranchial chamber as such ranges from complete to grossly incomplete. For those taxa in which the ctenidia are completely, or only posteriorly, free of the mantle (*e.g.*, *Neotrigonia* and *Margaritifera*), the two chambers of the mantle cavity are divided by a 'pallial ridge' or 'diaphragmatic

septum' formed by extensions of the posterior mantle (Gould & Jones, 1974; Smith, 1980), and there is generally no clear demarcation externally separating the incurrent and excurrent apertures (Ortmann, 1912a, b).

14. Association of ascending lamellae of inner demibranchs with the visceral mass. — *0* = Tending to be free of the visceral mass except at the anterior end. *1* = Tending to be complete fused to the visceral mass.

15. Anterior attachment of the inner demibranchs to the visceral mass. — *0* = Distant from labial palps. *1* = In contact with or adjacent to the labial palps.

The ascending lamellae of the inner demibranchs are fused to each other behind the foot. They may be completely or partially fused to the visceral mass, or they may be free of it except at the anterior end. As shown in **Figure III.2**, the inner demibranchs may attach to the visceral mass in contact with or close to the labial palps, or the palps and inner demibranchs may be separated by a wide gap (Ortmann, 1911c, 1921a; McMahon, 1991). The latter condition is considered typical of the Palaeoheterodonta ('category I' *sensu* Stasek, 1963).

16. Pallial fusion ventral to the incurrent aperture. — *0* = Absent. *1* = Short.

17. Pallial fusion between the incurrent and excurrent apertures. — *0* = Absent. *1* = Present.

18. Pallial fusion dorsal to excurrent aperture. — *0* = Absent. *1* = Present but re-opening to form a supra-anal aperture. *2* = Present but without a supra-anal aperture.

19. Pallial fusion between supra-anal and excurrent aperture. — *0* = Equal in length to or longer than the excurrent aperture. *1* = Distinctly shorter than the excurrent aperture or secondarily absent.

20. Diaphragm formed by fusion of mantle with ctenidia. — *0* = Imperforate. *1* = Perforate.

Covering the visceral mass, foot, ctenidia, and labial palps and lining the two valves is the mantle. These two halves are broadly unfused ventrally, although there many be pallial fusion ventral to the incurrent aperture, between the incurrent and excurrent apertures, or dorsal to the excurrent aperture. In genera where there is pallial fusion dorsal to the excurrent aperture, this connection may reopen to allow for a "supra-anal" aperture, and the connection between the excurrent and supra-anal may be shorter (and often missing) or longer (Ortmann, 1912b, 1921a; see **Figure III.2A-B**). In other taxa, the pallial fusion continues dorsally without allowing a supra-anal aperture (**Figure III.2C-D**).

When pallial fusion divides the incurrent and excurrent apertures, the diaphragm separating the infrabranchial from the suprabranchial chamber is formed by both the ctenidia and the mantle (**Figure III.2C-D**). In the Australian Hyriinae, the connection between ctenidia and mantle is perforated (McMichael & Hiscock, 1958). Without pallial fusion between the apertures, the mantle margins are drawn into opposition by a diaphragm formed solely by the ctenidia. This condition of the diaphragm was referred to as "slightly incomplete" by Davis & Fuller (1981).

21. Ctenidial filament morphology. — *0* = Homorhabdic (ctenidia smooth). *1* = Heterorhabdic (ctenidia plicate).

The ctenidia of most Unionoida are smooth, the individual filaments being all the same size (homorhabdic). Some genera, such as *Etheria*, possess heterorhabdic ctenidia with a plicate appearance (Heard & Vail, 1976a). Heterorhabdic ctenidia among the Etheriidae are yet another character convergent with the Ostreidae.

22.[12] Mantle ventral to the incurrent aperture. — *0* = Smooth or weakly elaborated. *1* = Elaborated with conspicuous papillae or a ribbon-like flap.

In certain freshwater mussel genera, the mantle margin directly anteroventral to the incurrent aperture is modified. It bears elaborations in the form of conspicuous papillae or a ribbon-like flap, as with *Villosa* and *Lampsilis* (Ortmann, 1912b; Kraemer, 1970; see **Figure III.2B**); otherwise, it is only weakly crenulated or smooth.

Brooding and Life History Characters

23.[1] Habitat. — *0* = Marine. *1* = Freshwater.

24.[2] Parental care. — *0* = None, fertilization is external. *1* = Female broods embryos and larvae in ctenidial marsupium.

25.[3] Demibranchs occupied by marsupium. — *0* = All four. *1* = Inner pair only. *2* = Outer pair only.

26.[4] Outer marsupial demibranch. — *0* = Entire demibranch marsupial or nearly so. *1* = A restricted portion of the demibranch marsupial.

27. Inner marsupial demibranch. — *0* = Entire demibranch marsupial or nearly so. *1* = A restricted portion of the demibranch marsupial.

As their common name implies, freshwater mussels occur exclusively in freshwater. Their life history revolves around their maternal care and unique parasitic larval stage (Coker *et al.*, 1921; Kat, 1984; Graf, 1998). Ova are fertilized internally, and developing embryos are retained in marsupia comprised of the female's ctenidial demibranchs; such ovovivipary differs from the primitive bivalve strategy of external fertilization followed by a presumably pelagic larval stage, as seen in *Neotrigonia* (Darragh, 1998; Ó Foighil & Graf, 2000). The embryos may be brooded in all four

demibranchs, only a single pair, or only a portion of one pair (Ortmann, 1911a, 1912b, 1921a).

28.[5] Interlamellar connections of non-marsupial demibranchs, including those of males. — *0* = None or scattered. *1* = Complete septa. *2* = Perforated septa.

29.[6] Interlamellar connection of marsupial demibranchs. — *0* = Absent or scattered. *1* = Perforated septa. *2* = Complete septa.

The Unionoida, and other bivalves, possess variously developed interlamellar connections between the lamellae of each demibranch (Brusca and Brusca, 1990; McMahon, 1991). These interlamellar connections may be sparse and scattered as in Neotrigonia and the Margaritiferinae (Boss, 1982), or they may be arranged into rows of septa running parallel to the ctenidial filaments as with other Unionoida. The septa, whether perforated or complete (i.e., imperforate), divide the interlamellar space into a series of "waters tubes" in which the embryos are brooded.

In many genera, septa of the marsupium are modified from those of non-marsupial demibranchs. This can clearly be seen in those taxa in which marsupial septa are complete and non-marsupial septa are perforated, or vice versa (*e.g.*, *Velesunio*, *Lamellidens*; McMichael & Hiscock, 1958; Ortmann, 1911a)

30.[7] Marsupial water tubes. — *0* = Undivided. *1* = Divided by lateral septa ('tripartite').

31. Interlamellar septa of marsupium. — *0* = Without a swelling protruding into the water tubes. *1* = Bearing a 'marked swelling.'

There are also modifications unique to marsupial septa. Genera such as *Anodonta* possess "tripartite" marsupial septa, in which the water tubes are divided by lateral septa perpendicular to the interlamellar septa (Ortmann, 1910a, d, 1911c). The lateral septa separate the lumen of each water tube into three compartments, the middle of which is

used for brooding. In other taxa (*e.g.*, *Iridina*, *Mycetopoda*), the water tubes are incompletely partitioned by a swelling formed by the presence of large blood vessel (Heard & Dougherty, 1980: Figures 3-4).

32.[8] Edge of marsupium. — *0* = Remains sharp when gravid. *1* = Expands greatly when gravid.

33.[9] Ventral extent of marsupium. — *0* = Ventral margin of marsupium does not extend past the non-marsupial portion. *1* = Ventral margin of marsupium extends past the non-marsupial portion.

The ventral margin of the marsupium may be modified to allow for expansion of the demibranchs when gravid, otherwise it remains sharp (Ortmann, 1911c). In lampsiline genera, the expansion of the marsupium usually extends ventrally past the margin of the non-marsupial portion of the demibranch; the marsupium itself is permanently differentiated from the non-marsupial demibranch and visible in non-gravid females (Lefevre & Curtis, 1910; see **Figure III.2B**).

34.[10] Larval discharged. — *0* = Larvae discharged out the excurrent aperture with the respiratory current. *1* = Larvae discharge through the ventral margin of the demibranch and out the incurrent aperture.

The marsupium may be further modified for expulsion of larvae through the ventral margin of the demibranch and out the incurrent aperture. Typically, larvae are evacuated out the open tops of the water tubes into the suprabranchial chamber and exit through the excurrent aperture (Ortmann, 1910c, 1911c; Kraemer, 1970).

Larval Characters

35. Larval type. — *0* = Free-living. *1* = Parasitic. *2* = Secondarily non-parasitic.

36. Parasitic larval type. — *0* = Glochidium. *1* = Lasidium.

Most of the Unionoida possess parasitic larvae, although a few are direct-developers (Lefevre & Curtis, 1912; Parodiz & Bonetto, 1963; Kondo, 1990). For those parasitic taxa, the host is generally a fish, although a small number infect amphibians (*e.g.*, Howard, 1915; Fryer, 1954; Kondo, 1984; Hoggarth, 1992; Watters, 1994b). The parasitic type of larva comes in two varieties: a glochidium or lasidium (**Figure III.3**).

37. Glochidium morphology. — *0* = Unhooked. *1* = Hooked.

Glochidia are small (50-350 μm), bivalved larvae composed of a calcareous shell, a single adductor muscle, and mantle cells; they attach to fish or other host tissue by clamping their valves upon exposed gill or fin epithelium (**Figure III.3A-D**). The host tissue encysts the mussel larvae (Arey, 1921), and it is within this cyst that the glochidia undergo metamorphosis into juveniles (Kat, 1984; Graf, 1998). Glochidia generally belong to one of three morphological types: (1) semi-circular and unhooked (**Figure III.3C**), (2) spade-shaped or sub-triangular and hooked (**Figure III.3A-B**), or (3) ax-head shaped (which is a modified, unhooked glochidium; **Figure III.3D**). However, variation exists within each of these three glochidial morphologies (Baker, 1928; Ortmann, 1912a, 1917, 1921a; Parodiz & Bonetto, 1963; Jones *et al.*, 1986; Roe & Lydeard, 1998).

38. Lasidium morphology. — *0* = Attaches by tubular appendages (haustorium-type). 1 = Attaches by forming a cyst (lasidium-type).

The lasidium larvae are small (85-150 μm, not including the 'larval thread'), tri-lobed larvae with a univalve, uncalcified shell (**Figure III.3E-G**). Just as with the glochidia, there is more than one type of lasidium, although Parodiz and Bonetto (1963)

seemed to over-emphasize the differences between the (1) lasidium-type (**Figure III.3E**) and the (2) haustoria-type (**Figure III.3G**) described by Fryer (1954, 1961). Although distinguishable by size and other morphological characteristics (Parodiz & Bonetto, 1963: Table 1), the fundamental distinction between the two types is that the former attaches to the host by forming cysts, the haustoria-type attaches via tubular appendages.

Figure III.1. Palaeoheterodont Hinge Morphology. The figure illustrates exemplar hinge morphologies from seven palaeoheterodont species: **A** *Neotrigonia margaritacea*, **B** *Pleurobema coccineum*, **C** *Quadrula quadrula*, **D** *Velesunio ambigua*, **E** *Elliptio dilatata*, **F** *Pseudodon vondembuschianus*, and **G** *Iridina ovatus*. Each photo shows the hinge of the right valve except for **B**, *P. coccineum*. Numbers refer to characters and are used to mark the derived condition among the hinge morphologies depicted. Shells are approximately natural size. See text for discussion.

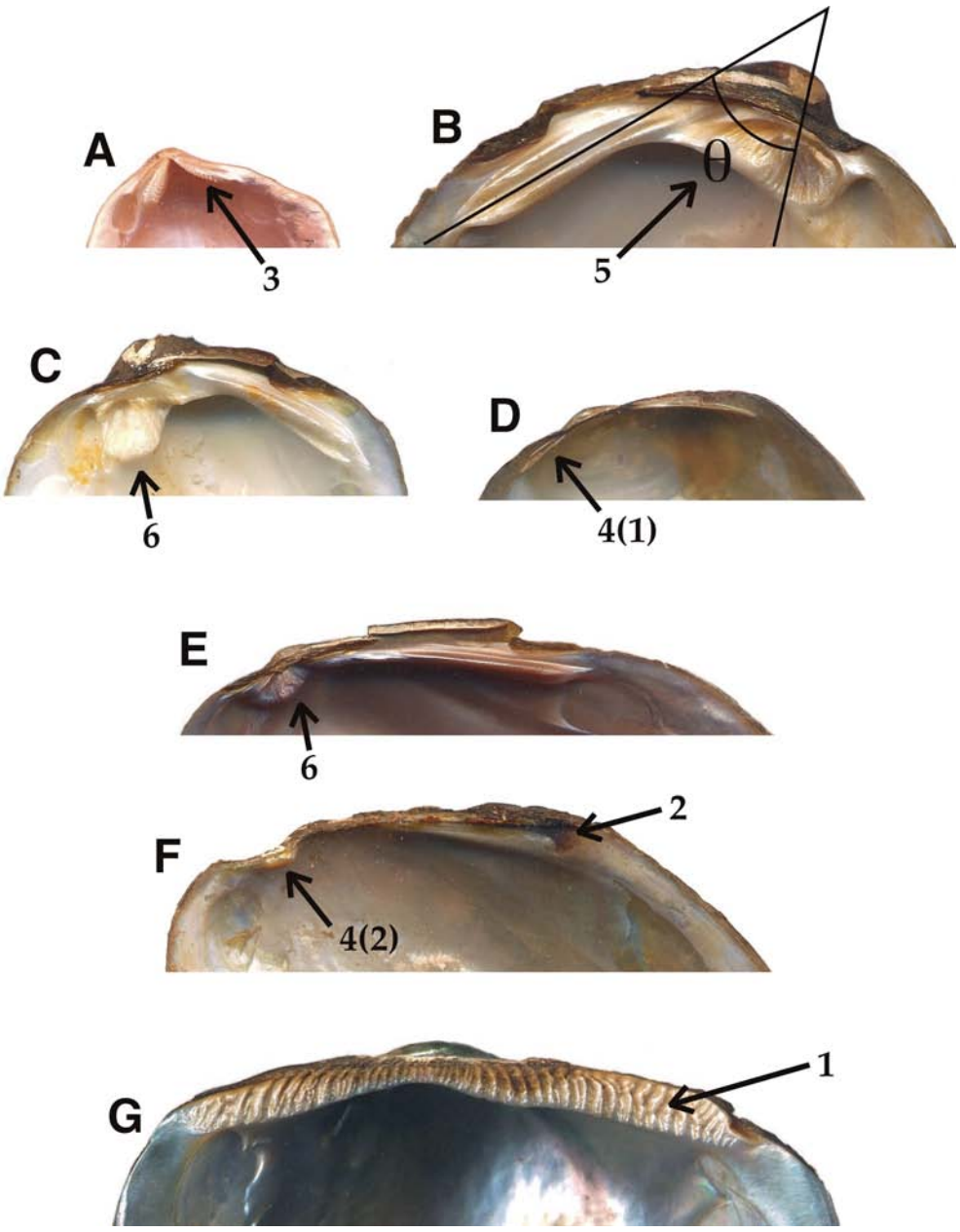


Figure III.1. Palaeoheterodont Hinge Morphology

Figure III.2. Unionoida Gross Soft-Anatomy. The figure shows schematic diagrams of the soft anatomy of mussel genera: **A** *Fusconaia*, **B** *Lampsilis*, **C** *Castalina*, and **D** *Mutela*. Numbers refer to characters and are used to mark the derived condition among the bodies illustrated. See text for discussion. Figures A, C, and D were re-drawn from Ortmann (1911a); B was re-drawn from Ortmann (1912b).

Key. — *aa* = Anterior adductor/retractor muscle complex. *ex* = Excurrent aperture. *f* = Foot. *i* = Inner demibranch. *in* = Incurrent aperture. *l* = Labial palp. *m* = Mantle. *o* = Outer demibranch. *pa* = Posterior adductor muscle. *pp* = Pedal protractor muscle. *pr* = Posterior pedal retractor muscle. *sa* = Supra-anal aperture.

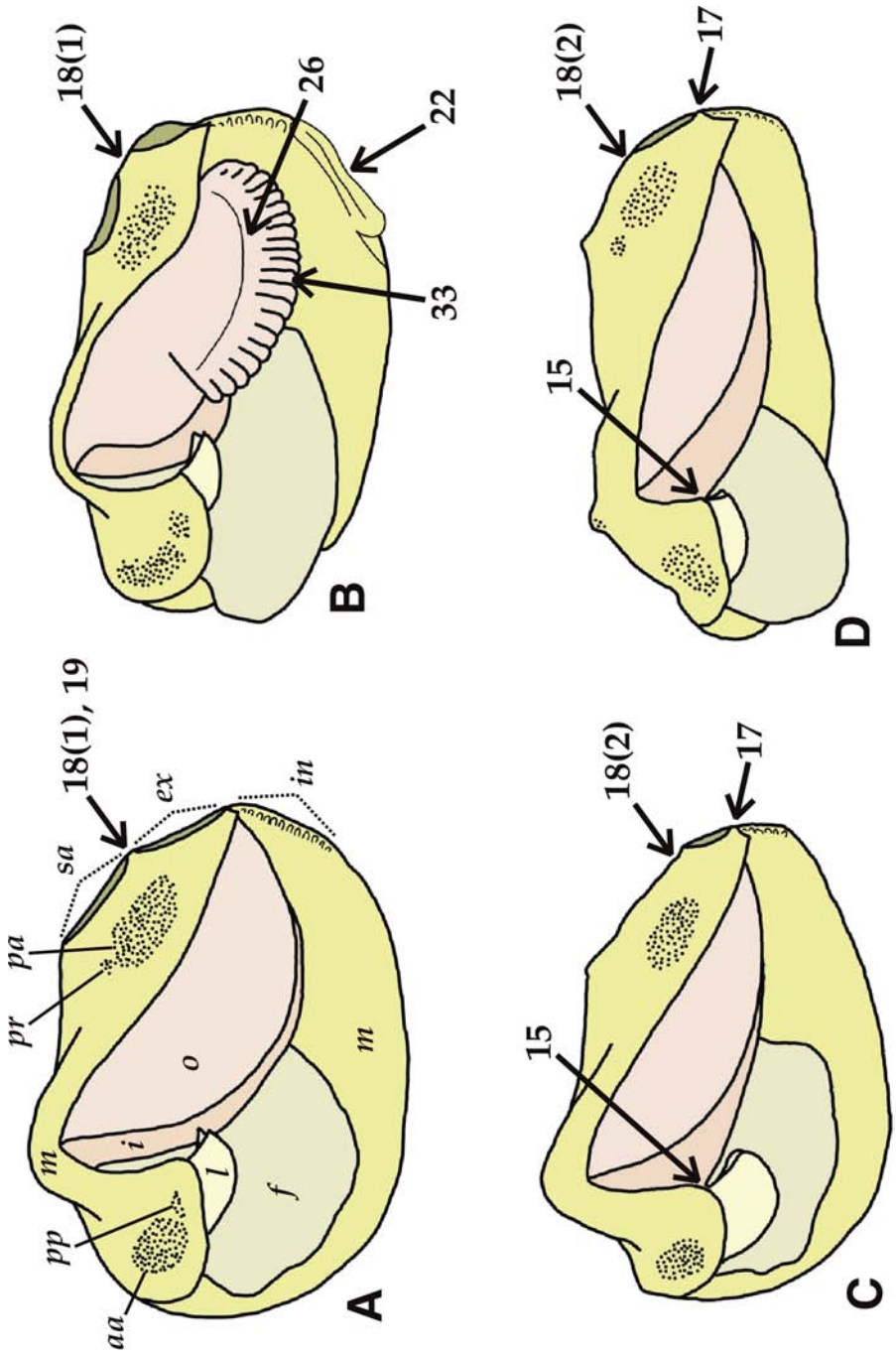


Figure III.2. Unionoida Gross Soft-Anatomy

Figure III.3. Unionoida Parasitic Larval Types. The figure shows scale drawings of various freshwater parasitic larval morphologies: **A** hooked-type glochidium of *Alasmidonta marginata*, **B** hooked-type glochidium of *Prisodon corrugatus*, **C** unhooked-type glochidium of *Villosa iris*, **D** ax-head-type glochidium of *Potamilus alatus*, **E** 'lasidium'-type lasidium of *Monocondylaea paraguayana*, **F** 'lasidium'-type?? lasidium of *Leila blainvilleana*, and **G** 'haustorium'-type lasidium of *Mutela bourguignati*. See text for discussion. Figures A, C, and D were re-drawn from Baker (1928); B and E were re-drawn from Bonetto & Ezcurra (1963); F was re-drawn from Bonetto (1963); and G was re-drawn from Fryer (1954).

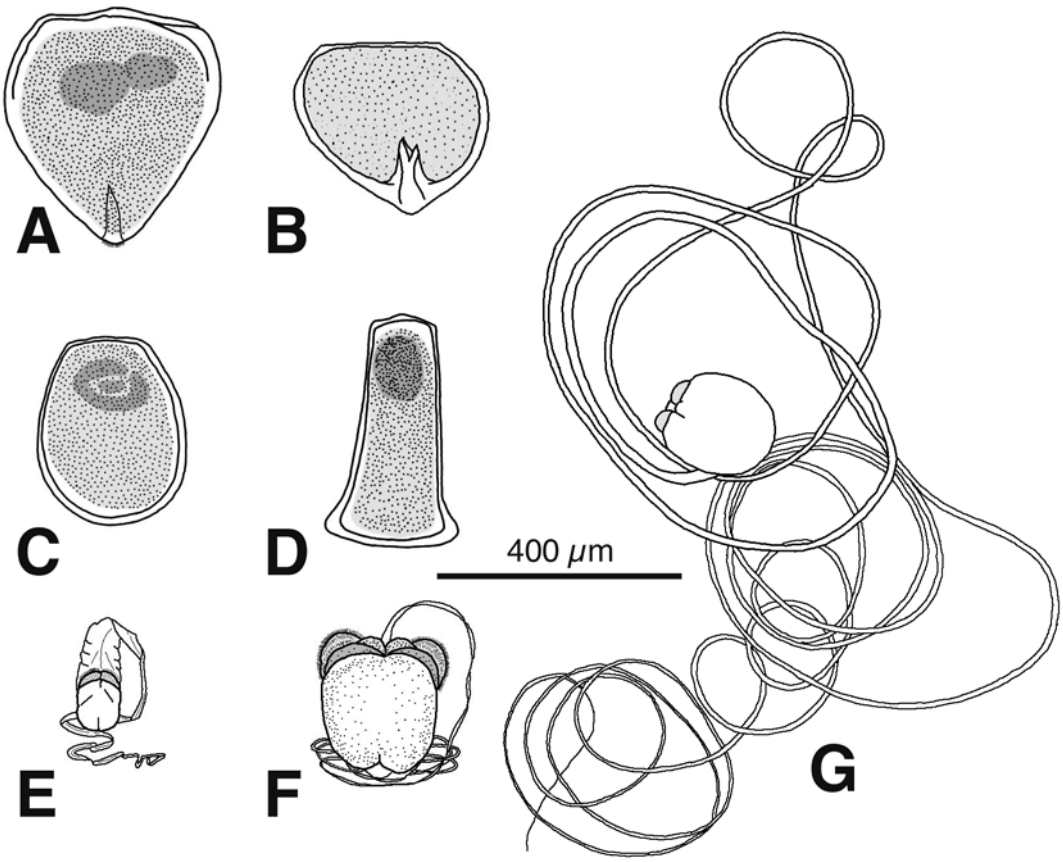


Figure III.3. Unionoida Parasitic Larval Types

APPENDIX IV
Sources of Molecular Sequences

The protocol for the acquisition of novel mussel domain 2 of 28S nuclear rDNA [28S (D2)] (and large flanking stretches of domains 1 and 3) and mtDNA cytochrome oxidase subunit I (COI) DNA sequences was as follows. Total genomic DNA was extracted from mantle or foot tissue using a QIAmp Tissue Kit (QIAGEN). Target sequences were amplified using the Polymerase Chain Reaction (PCR). COI primers were those of Folmer *et al.* (1994): LCO1490 5'—ggtcaacaaatcataaagatattgg—3' and HCO2198 5'—taaacttcagggtgaccaaaaaatca—3'; the optimal annealing temperature was 43° C. 28S primers were those of Park & Ó Foighil (2000) for 28S (D2): D23F 5'—gagagttcaagagtacgtg—3' and D4RB 5'—tgttagactccttggtccgtgt—3'; the optimal annealing temperature was 50° C. A three-step PCR protocol was employed (30 sec 94 °C denaturing, ≥30 sec annealing, 60 sec 72 °C extension) for a total of 40 cycles. For all reactions, the initial annealing temperatures were incrementally reduced from 60 °C (1 ° per cycle) to the optimal annealing temperature. Once the optimal temperature was reached, the annealing temperature was held constant for the remaining cycles ("touch down" procedure *sensu* Palumbi, 1996:228).

Double-stranded PCR products were stained with ethidium bromide, isolated on 1% agarose gels, excised under UV light, and purified using a QIAquick (QIAGEN) Gel Extraction Kit. Both strands of amplified product were directly cycle-sequenced using a "Big Dye" Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer Applied Biosystems, Inc.) with the respective PCR primers and electrophoresed on an ABI 377 automated DNA sequencer.

Table IV.1 is a list of the taxa for which molecular sequences were obtained. For the most part, these DNA sequences for COI and 28S have been published previously.

However, those from the studies of Graf & Ó Foighil (2000, 2001), Graf (in prep.), and Park & Ó Foighil (2000) were generated using the protocol described in the preceding paragraphs. All sequences are available from the author upon request.

Table IV.1. Sources and References for Cytochrome Oxidase Subunit I and 28S (Domain 2) Sequences. Where multiple same-species, same-gene sequences were available for a particular study in this dissertation, preference was always given to the first one listed in this table. **NS** refers to a novel sequence not previously published.

Taxon	COI	28S	Reference
Actinonaias carinata	AF156517	**NS**	Graf & Ó Foighil (2000)
Alasmidonta marginata	AF156502	Pending	Graf (in prep.) Graf & Ó Foighil (2000)
Amblema plicata	AF156512	AF305385	Graf & Ó Foighil (2000, 2001)
Astarte castanea		AF131001	Park & Ó Foighil (2000)
Castalia sp.		AF305381	Graf & Ó Foighil (2001)
Cumberlandia monodonta	AF156498	AF305382	Graf & Ó Foighil (2000, 2001)
Diplodon chilensis	**NS**	AF305380	Graf & Ó Foighil (2001)
Elliptio dilatata	AF156506	Pending	Graf (in prep.) Graf & Ó Foighil (2000)
Epioblasma triquetra	AF156528	**NS**	Graf & Ó Foighil (2000)
Gonidea angulata	**NS**	Pending	Graf (in prep.)
Hydrella depressa	AF305368	AF305375	Graf & Ó Foighil (2000, 2001)
Hyridella australis	AF305367	AF305373-4	Graf & Ó Foighil (2001)

Table IV.1 (continued). Sources and References for Cytochrome Oxidase Subunit I and 28S (Domain 2) Sequences.

Taxon	COI	28S	Reference
Hyridella menziesi[1]	AF305369-70	AF305376-7	Graf & Ó Foighil (2001)
Lampsilis cardium	AF156518	AF305386	Graf & Ó Foighil (2000, 2001)
Lasmigona compressa	AF156503	**NS**	Graf & Ó Foighil (2000)
Ligumia nasuta	AF156515	**NS**	Graf & Ó Foighil (2000)
Ligumia recta	AF156516	**NS**	Graf & Ó Foighil (2000)
Mercenaria mercenaria	U47648	AF131019	Park & Ó Foighil (2000) Baldwin *et al.* (1996)
Mytilus edulis		Z29550	Littlewood (1994)
Neotrigonia margaritacea	U56850	Pending	Graf (in prep.) Hoeh *et al.* (1998)
Obliquaria reflexa		Pending	Graf (in prep.)
Ostrea chilensis	AF112286	AF137045	Ó Foighil *et al.* (1999) Ó Foighil & Taylor (2000)
Pilsbryoconcha exilis		Pending	Graf (in prep.)
Pleurobema coccineum	AF156508	**NS**	Graf & Ó Foighil (2000)
Pseudodon vondembuschianus		Pending	Graf (in prep.)

Table IV.1 (continued). Sources and References for Cytochrome Oxidase Subunit I and 28S (Domain 2) Sequences.

Taxon	COI	28S	Reference
Ptychobranchus fasciolaris	AF156514	**NS**	Graf & Ó Foighil (2000)
Pyganodon grandis	AF156504	AF305384	Graf & Ó Foighil (2000, 2001)
Quadrula quadrula	AF156511	**NS**	Graf & Ó Foighil (2000)
Rangia cuneata	U47652	AF131002	Park & Ó Foighil (2000) Baldwin *et al.* (1996)
Strophitus undulatus	AF156505	**NS**	Graf & Ó Foighil (2000)
Tritogonia verrucosa	**NS**	**NS**	
Truncilla truncata	AF156513	**NS**	Graf & Ó Foighil (2000)
Uniandra contradens	**NS**	Pending	Graf (in prep.)
Unio (Cafferia) caffer	AF156500	Pending	Graf (in prep.) Graf & Ó Foighil (2000)
Unio (s.s.) pictorum	AF156499	AF305383	Graf & Ó Foighil (2000, 2001)
Velesunio ambigua	AF305371-2	AF305378-9	Graf & Ó Foighil (2001)
Villosa iris	AF156523	**NS**	Graf & Ó Foighil (2000)
Villosa vanuxemensis	AF156525	**NS**	Graf & Ó Foighil (2000)

Table IV.1 (continued). Sources and References for Cytochrome Oxidase Subunit I and 28S (Domain 2) Sequences.

Taxon	COI	28S	Reference

[1] The four *H. menziesi* sequences were harvested from the same two individuals. The first individual of both pairs was collected on the North Island of New Zealand, the second individual from the South Island.

BIBLIOGRAPHY

BIBLIOGRAPHY

Adamkewicz, S.L., M.G. Harasewych, J. Blake, D. Saudek, & C.J. Bul. 1997. A molecular phylogeny of the bivalve mollusks. *Molecular Biology & Evolution* **14**: 619-629.

Adams, H. & A. Adams. 1858. *The Genera of Recent Mollusca; Arranged According to their Organization.* Volume 2. John van Voorst, London.

Agassiz, L. 1845. *Nomenclator Zoologicus… Nomina Systematica Generum Molluscorum.* Soloduri, sumtibus typis Jent et Gaussmann.

Agassiz, L. 1846. *Nomenclator Zoologicus… Indicem Universalem.* Soloduri, sumtibus typis Jent et Gaussmann.

Allen, E. 1924. The existence of a short reproductive cycle in *Anodonta imbecillis*. *Biological Bulletin* **46**: 88-94.

Allen, J.A. 1985. The Recent Bivalvia; their form and evolution. pp. 337-403. [In] E.R. Trueman & M.R. Clarke (eds.). *The Mollusca,* **10***: Evolution.* Academic Press, Inc., Harcourt Brace Jovanovich Publishers, Orlando, Florida.

Arey, L. B. 1921. An experimental study on glochidia and the factors underlying encystment. *Journal of Experimental Zoology* **33**: 463-499.

Arteaga, S. E. 1994. El lasidium de *Acostaea rivoli* Deshayes, 1827 (Mollusca: Bivalvia: Etheriidae) y su importancia para la ubicacian taxonomica de esta especie. *Boletin Ecotropica* **27**: 1-9.

Atkins, D. 1936-1938. On the ciliary mechanisms and interrelationships of the lamellibranchs. *Quarterly Journal of Microscopical Science* **79**: 181-308, 339-445; **80**: 321-436.

Attrill, M.J., S.D. Rundle, & R.M. Thomas. 1996. The influence of drought-induced low freshwater flow on an upper-estuarine macroinvertebrate community. *Water Research* **30**: 261-268.

Babin, C. & J.-C. Gutiérrez-Marco. 1991. Middle Ordovician bivalves from Spain and their phyletic and palaeogeographic significance. *Palaeontology* **34**: 109-147.

Baker, F. C. 1928. The freshwater Mollusca of Wisconsin. *Wisconsin Geological and Natural History Survey Bulletin* **70**: 1-495.

Baldwin, B.S., M. Black., O. Sanjur, R. Gustafson, R.A. Lutz, & Vrijenhoek, R.C. 1996. A diagnostic molecular marker for zebra mussels (*Dreissena polymorpha*) and potentially co-occurring bivalves: mitochondrial COI. *Molecular Marine Biology and Biotechnology* **5**: 9-14.

Ball, I.R. 1976. Nature and formulation of biogeographic hypotheses. *Systematic Zoology* **24**: 407-430.

Bastrop, R., B. Strehlow, K. Jurss, & C. Sturmbauer. 2000. A new molecular phylogenetic hypothesis for the evolution of freshwater eels. *Molecular Phylogenetics and Evolution* **14**: 250-258.

Bauer, G. 2001. Life-history variation on different taxonomic levels of naiads. pp. 83-91. [In] G. Bauer & K. Wächtler (eds.). *Ecology and Evolution of the Freshwater Mussels Unionoida.* Ecological Studies, Volume 145. Springer-Verlag, Berlin, Germany.

Bloomer, H.H. 1931a. On the anatomy of *Brazzaea anceyi*, Bourguignat. *Proceedings of the Malacological Society of London* **19**: 228-223.

Bloomer, H.H. 1931b. A note on the anatomy of *Lamellidens marginalis*, Lamarck and *L. thwaitesii*, Lea. *Proceedings of the Malacological Society of London* **19**: 270-272.

Bloomer, H.H. 1932. Notes on the anatomy of some African Naiades— Part I. *Proceedings of the Malacological Society of London* **20**: 166-173.

Bloomer, H.H. 1933. Notes on the anatomy of some African Naiades— Part II. *Proceedings of the Malacological Society of London* **20**: 237-241.

Bogan, A.E. 1985. A comment on Strophitinae Gordon, 1984 (Unionidae, Bivalvia). *Nautilus* **99**: 141-142.

Bogan, A.E. 1993. Freshwater bivalve extinctions (Mollusca: Unionoida): a search for causes. *American Zoologist* **33**: 599-609.

Bonetto, A.A. 1951. Acera de las formas larvales de Mutelidae Ortmann. *Jornadas Icticas* **1**: 1-8.

Bonetto, A.A. 1961a. Investigaciones acera de las formas larvales en el genera "*Diplodon*" y su aplicacion a los estudios sistematicos. *Direccion General de Recursos Naturales, Provincia de Santa Fe Ministerio de Agricultura y Ganaderia.* 48 pp.

Bonetto, A.A. 1961b. Notas sobre los generos *Castalina* y *Castalia* en el Parana Medio e Inferior. *Direccion General de Recursos Naturales, Provincia de Santa Fe Ministerio de Agricultura y Ganaderia*. 11 pp.

Bonetto, A.A. 1962a. Notas sobre *Diplodon charruanus* (Orb.) y *Diplodon rhuacoicus* (Orb.). *Direccion General de Recursos Naturales, Provincia de Santa Fe Ministerio de Agricultura y Ganaderia, Publicación Técnica* (10): 35-44.

Bonetto, A.A. 1962b. Especies del genera "*Mycetopoda*" en el sistema hidrografico del Rio de la Plata. *Revista del Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Ciencias Zoológicas* **8**: 173-182.

Bonetto, A.A. 1963. Contribución al conocimiento de *Leila blainvilleana* (Lea) (Mollusca: Pelecypoda). *Physis* **24**: 11-16.

Bonetto, A.A. 1966. Especies de la subfamilia Monocondylaeinae en las aguas del sistema del Rio de la Plata. *Archiv für Molluskenkunde* **95**: 3-14.

Bonetto, A.A. & I.D. Ezcurra. 1962. El desarrollo de lasidium de *Anodontites trapesialis forbesianus* (Lea) (Moll. Lamell.). *Physis* **23**: 195-203.

Bonetto, A.A. & I.D. Ezcurra. 1963. Notas malacológicas. I. 1) El desarrollo del glochidium de *Diplodon delodontus delodontus* (Lam.). 2) El glochidium de *Prisodon (Triplodon) corrugatus* Lam. 3) El lasidium de *Monocondylaea paraguayana* D'Orbigny. *Physis* **24**: 17-21.

Bonetto, A.A. & I.D. Ezcurra. 1965a. Estudio comparado de las formas larvales de Mutelidae Ortmann y su significacion sistematica y zoogeografica. *Anais Do II Congresso Latino Americano de Zoologia* (S. Paulo, 1962). p. 55-71.

Bonetto, A.A. & I.D. Ezcurra. 1965b. Notas malacológicas. III. 5) la escultura del periostraco en el genero *Anodontites*. 6) el lasidium de *Anodontites trapezeus* (Spix). 7) el lasidium de *Mycetopoda siliquosa* (Spix). *Physis* **25**: 197-204.

Boss, K.J. 1982. Mollusca. pp. 945-1166. [In] S.P. Parker (ed.). *Synopsis and Classification of Living Organisms*. Volume 1. McGraw-Hill Book Company, New York.

Boss, K.J. & M.K. Jacobson. 1985. General evolutionary patterns and the system of the Class Bivalvia. Special Occasional Publication of the Department of Mollusks, Harvard University (5). [English translation of Scarlato & Starobogatov (1979)]

Bradshaw, M. 1984. Permian non-marine bivalves from the Ohio Range, Antarctica. *Alcheringa* **8**: 305-309.

Brandt, R.A.M. 1974. The non-marine aquatic Mollusca of Thailand. *Archiv für Molluskenkunde* **105**: 1-423.

Bremer, K. 1995. Branch support and tree stability. *Cladistics* **10**: 295-304.

Brown, J. H. & M.V. Lomolino. 1998. *Biogeography*, 2nd Edition. Sinauer Associates, Inc., Sunderland, Massachusetts.

Brown, W.M., E.M. Prager, A. Wang, & A.C. Wilson. 1982. Mitochondrial DNA sequences of primates: tempo and mode of evolution. *Journal of Molecular Evolution* **18**: 225-239.

Brusca, R.C. & G.J. Brusca. 1990. *Invertebrates*. Sinauer Associates, Inc. Sunderland, Massachusetts.

Burch, J.B. 1975. *Freshwater Unionacean Clams (Mollusca: Pelecypoda) of North America,* revised edition. Malacological Publications, Inc., Hamburg, Michigan.

Call, R.E. 1878. Mode of distribution of fresh-water mussels. *American Naturalist* **12**:472-473.

Clarke, A.H. 1985. The tribe Alasmidontini (Unionidae: Anodontinae), Part II: *Lasmigona* and *Simpsonaias*. *Smithsonian Contributions to Zoology* (399): 1-75.

Clarke, A.H. 1973. The freshwater mollusks of the Canadian Interior Basin. *Malacologia* **13**: 1-509.

Coker, R.E., A.F. Shira, H.W. Clark, & A.D. Howard. 1921. Natural history and propagation of freshwater mussels. *Bulletin of the Bureau of Fisheries* **37**: 77-81. [Reprinted as Bureau of Fisheries Document No. 893.]

Conner, C.H. 1909. Supplementary notes on the breeding seasons of the Unionidae. *Nautilus* **22**: 111-112.

Cooper, A., I.A.E. Atkinson, W.G. Lee, & T.H. Worthy. 1993. The New Zealand biota—historical background and new research. *Trends in Ecology & Evolution* **8**: 429-433.

Cope, J.C.W. 1996a. The early evolution of the Bivalvia. pp. 361-370. [in] J.D. Taylor (ed.). *Origin and Evolutionary Radiation of the Mollusca*. Oxford University Press, New York, New York.

Cope, J.C.W. 1996b. Early Ordovician (Arenig) bivalves from the Llangynog Inlier, South Wales. *Palaeontology* **39**: 979-1025.

Cope, J.C.W. 1999. Middle Ordovician bivalves from mid-Wales and the Welsh borderland. *Palaeontology* **42**: 467-499.

Cotton, B.C. 1934. A freshwater mussel attached to a duck's foot. *South Australian Naturalist* **15**: 113.

Cox, L.R. 1960. Thoughts on the classification of the Bivalvia. *Proceedings of the Malacological Society of London* **34**: 60-88.

Cox, L.R. 1969. General features of the Bivalvia. pp. N2-N129. [In] R.C. Moore (ed.). *Treatise on Invertebrate Paleontology,* Part N, Mollusca, 6. Volume 1: Bivalvia. Geological Society of America and University of Kansas Press, Lawrence, Kansas.

Craw, R.C. 1985. Classic problems of southern hemisphere biogeography re-examined. *Zeitschrift für Zoologische Systematik und Evolutionsforschung* **23**: 1-10.

Croizat, L., G. Nelson, & D.E. Rosen. 1974. Centers of origin and related concepts. *Systematic Zoology* **23**: 265-287.

Cummings, K.S. & C.A. Mayer. 1992. *Field Guide to Freshwater Mussels of the Midwest.* Illinois Natural History Survey Manual (5), Champaign, Illinois.

Dall, W.H. 1895. Contributions to the Tertiary fauna of Florida, with special reference to the Miocene silx-beds of Tampa and the Pliocene beds of the Caloosahatchie River. Part III. A new classification of the Pelecypoda. *Transactions of the Wagner Free Institute of Science, Philadelphia* **3**: 483-570.

Dall, W.H. 1913. Pelecypoda. pp. 421-507. [In] C.R. Eastman (ed.). [English translation of] K.A. von Zittel. *Textbook of Palaeontology,* **1**. Macmillan & Co., New York, New York.

Darlington, P.J., jr. 1957. *Zoogeography: the Geographical Distribution of Animals*. John Wiley & Sons, New York, New York.

Darragh, T.A. 1998. Order Trigonioida. pp. 294-296. [In] P.L. Beesley, G.J.B. Ross, & A. Wells (eds.). *Mollusca: the Southern Synthesis.* Fauna of Australia Volume 5. CSIRO Publishing, Melbourne, Australia.

Daugherty, C.H., G.W. Gibbs, & R.A. Hitchmough. 1993. Mega-island or micro-continent— New Zealand and its fauna. *Trends in Ecology & Evolution* **8**: 437-442.

Davis, G.M. & S.L.H. Fuller. 1981. Genetic relationships among Recent Unionacea (Bivalvia) of North America. *Malacologia* **20**: 217-253.

Davis, G.M., W.H. Heard, S.L.H. Fuller, & C. Hesterman. 1981. Molecular genetics and speciation in *Elliptio* and its relationships to other taxa of North American Unionidae (Bivalvia). *Biological Journal of the Linnean Society* **15**: 131-150.

Deshayes, G.P. 1792-1832. Histoire Naturalle des Vers. *Encyclopédie Méthodique* **2**: 1-594.

Distel, D.L. 2000. Phylogenetic relationships among Mytilidae (Bivalvia): 18S rRNA data suggest convergence in mytilid bodyplans. *Molecular Phylogenetics & Evolution* **15**: 25-33.

Edgar, A.L. 1965. Observations on the sperm of the pelecypod *Anodontoides ferussacianus* (Lea). *Transactions of the American Microscopical Society* **84**: 228-230.

Eldredge, N. & J. Cracraft. 1980. *Phylogenetic Patterns and the Evolutionary Process : Method and Theory in Comparative Biology.* Columbia University Press, New York.

Farris, J.S. 1969. A successive approximations approach to character weighting. *Systematic Zoology* **18**: 374-385.

Farris, J.S. 1989. The Retention Index and the Rescaled Consistency Index. *Cladistics* **5**: 417-419.

Felsenstein, J. 1978. Cases in which parsimony and compatibility methods will be positively misleading. *Systematic Zoology* **27**: 401-410.

Fiala, K.L. & R.R. Sokal. 1985. Factors determining the accuracy of cladogram estimation: Evaluation using computer simulation. *Evolution* **39**: 609–622.

Fleming, J. 1828. *A History of British Animals, Exhibiting the Descriptive Characters and Systematical Arrangement of the Genera and Species of Quadrupeds, Birds, Reptiles, Fishes, Mollusca, and Radiata of the United Kingdom; Including Indigenous, Extirpated, and Extinct Kinds; Together with Periodical and Occasional Visitants,* in two volumes. Bell & Bradfute, Edinburgh.

Folmer, O., M. Black, W. Hoeh, R. Lutz, & R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology **3**:294–299.

Frierson, S.L. 1909. Remarks on the subfamilies Hyriinae and Unioninae. *Nautilus* **22**: 106-107.

Frierson, S.L. 1911. Notes on Oriental Unionidae. *Nautilus* **24**: 97-98.

Frierson, L.S. 1927. *A Classified and Annotated Check List of the North American Naiades.* Baylor University Press, Waco, Texas.

Fryer, G. 1954. Development in a mutelid Lamellibranch. *Nature* **183**: 1342-1343.

Fryer, G.. 1961. The developmental history of *Mutela bourguignati* (Ancey) Bourguignat (Mollusca: Bivalvia). *Philosophical Transactions of the Royal Society of London* B **244**: 259-298.

Gatenby, C.M., B.C. Parker, & R.J. Neves. 1997. Growth and survival of juvenile rainbow mussels, *Villosa iris* (Lea, 1829) (Bivalvia: Unionidae), reared on algal diets and sediment. *American Malacological Bulletin* **14**: 57-66.

Good, S.C. 1998. Freshwater bivalve fauna of the Late Triassic (Carnian-Norian) Chinle, Dockum, and Dolores Formations in the Southwest United States. pp. 223-249. [In] P.A. Johnston & J.W. Haggart (eds.). *Bivalves: An Eon of Evolution — Paleobiological Studies Honoring Norman D. Newell.* University of Calgary Press, Calgary.

Gordon, M.E. 1981. Recent Mollusca of Arkansas with annotations to systematics and zoogeography. *Proceedings of the Arkansas Academy of Science* **34**(162): 58-62.

Gordon, M.E. 1985. Mollusca of Frog Bayou, Arkansas. *Nautilus* **99**: 6-9.

Gordon, M.E. & D.G. Smith. 1990. Autumnal reproduction in *Cumberlandia monodonta* (Unionoidea: Margaritiferidae). *Transactions of the American Microscopical Society* **109**: 407-411.

Gould, S.J., & C.C. Jones. 1974. The pallial ridge of *Neotrigonia*: functional siphons without mantle fusion. *Veliger* **17**: 1-7.

Graf, D.L. 1997a. The effect of breeding period on the biogeography of freshwater mussels (Bivalvia: Unionoidea) in the Minnesota region of North America. *Occasional Papers on Mollusks* **5**: 393-407.

Graf, D.L. 1997b. Northern redistribution of freshwater pearly mussels (Bivalvia: Unionoidea) during Wisconsin deglaciation in the southern Glacial Lake Agassiz region: a review. *American Midland Naturalist* **138**: 37-47.

Graf, D.L. 1997c. *Morphology, Zoogeography, and Taxonomy of* Fusconaia flava *(Rafinesque, 1820) (Mollusca: Bivalvia: Unionidae) in the Upper Mississippi, Great Lakes, and Nelson River Basins.* M.S. Thesis, Department of Biology, Northeastern University, Boston, Massachusetts.

Graf, D.L. 1998. Sympatric speciation of freshwater mussels (Bivalvia: Unionoidea): a model. *American Malacological Bulletin* **14**: 35-40.

Graf, D.L. 2000a. The Etherioidea revisited: a phylogenetic analysis of hyriid relationships (Mollusca: Bivalvia: Paleoheterodonta: Unionoida). *Occasional Papers of the University of Michigan Museum of Zoology* (729): 1-21.

Graf, D.L. 2000b. *Sequence Monkey.* Freeware and documentation available at http://www.monkeysoftwerks.com.

Graf, D.L. In press. Historical biogeographic and late glacial origin of the freshwater pearly mussel (Bivalvia: Unionidae) faunas of Lake Erie, North America. *Occasional Papers on Mollusks*.

Graf, D.L. In preparation. A molecular phylogenetic analysis of two problematic freshwater mussel genera (*Unio* and *Gonidea*) and a re-evaluation of the classification of Nearctic Unionidae (Bivalvia: Paleoheterodonta: Unionoida).

Graf, D.L. & D. Ó Foighil. 2000. The evolution of brooding characters among the freshwater pearly mussels (Mollusca: Bivalvia: Unionoidea) of North America. *Journal of Molluscan Studies* **66**: 157-170.

Graf, D.L. & D. Ó Foighil. 2001. Molecular phylogenetic analysis of 28S rDNA supports a Gondwanan origin for Australasian Hyriidae (Mollusca: Bivalvia: Unionoida). *Vie et Milieu*, in press.

Graf, D.L. & J.S. Sparks. 2000. Sometimes Too Much is Not Enough: A comment on the use of mtDNA to recover ancient relationships. *Naive Verificationist* **1**: 1-13. [On-line publication available at http:// www.monkeysoftwerks.com.]

Gray, J.E. 1840. [Mollusca]. pp. 86-89, 106-151. [In:] *Synopsis of the Contents of the British Museum,* 42nd edition. G. Woodfall and Son, London.

Gray, J.E. 1847. A list of the genera of Recent Mollusca, their synonyma and types. *Proceedings of the Zoological Society of London* **15**: 129-219.

Graybeal, A. 1998. Is it better to add taxa or characters to a difficult phylogenetic problem? *Systematic Biology* **47**:9–17.

Gu, Z. 1998. Evolutionary trends in non-marine Cretaceous bivalves of Northeast China. pp. 267-276. [In] P.A. Johnston & J.W. Haggart (eds.). *Bivalves: An Eon of Evolution — Paleobiological Studies Honoring Norman D. Newell.* University of Calgary Press, Calgary.

Guo, F. 1998. Origin and phylogeny of the Trigonioidoidea (non-marine Cretaceous bivalves). pp. 277-289. [In] P.A. Johnston & J.W. Haggart (eds.). *Bivalves: An Eon of Evolution — Paleobiological Studies Honoring Norman D. Newell.* University of Calgary Press, Calgary.

Haas, F. 1929. Bemerkungen uber mittleamerikanische Najaden. *Senkenbergiana* **11**: 310-344.

Haas, F. 1940. A tentative classification of the Palearctic unionids. *Zoological Series of Field Museum of Natural History* **24**: 115-141.

Haas, F. 1969a. Superfamilia Unionacea. [In] R. Martens and W. Hennig (eds.). *Das Tierrich*, Lieferung 88, 663 pp. Walter de Gruyter and Co., Berlin.

Haas, F. 1969b. Superfamily Unionacea. pp. N411-N470. [In] R.C. Moore (ed.). *Treatise on Invertebrate Paleontology,* Part N, Mollusca, 6. Volume 1: Bivalvia. Geological Society of America and University of Kansas Press, Lawrence, Kansas.

Hannibal, H. 1912. A synopsis of the Recent and Tertiary freshwater Mollusca of the California Province, based upon an ontogenic classification. *Proceedings of the Malacological Society of London* **10**: 112-211, pls. 5-8.

Hartmann, J.H. 1998. The biostratigraphy and paleontology of Latest Cretaceous and Paleocene freshwater bivalves from the western Williston Basin, Montana, U.S.A. pp. 317-345. [In] P.A. Johnston & J.W. Haggart (eds.). *Bivalves: An Eon of Evolution — Paleobiological Studies Honoring Norman D. Newell.* University of Calgary Press, Calgary.

Hasegawa, M., H. Kishino, & T. Yano. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **21**: 160-174.

Healy, J.M. 1989. Spermiogenesis and spermatozoa in the relict genus *Neotrigonia*: relevance to trigonioid relationships, particularly Unionoidea. *Marine Biology* **103**: 75-85.

Heard, W.H. 1974. Anatomical systematics of freshwater mussels. *Malacological Review* **7**: 41-42.

Heard, W.H. 1998. Brooding patterns in freshwater mussels. *Bivalvia* **1**, *Malacological Review, Suppliment* (7): 105-121.

Heard, W.H. & R.H. Guckert. 1971. A re-evaluation of the Recent Unionacea (Pelecypoda) of North America. *Malacologia* **10**: 333-355.

Heard, W.H. & B.J. Dougherty. 1980. An anatomical redescription of *Pleiodon (Cameronia) spekii* (Pelecypoda: Mutelidae: Pleiodoninae). *Malacological Review* **13**: 45-53.

Heard, W.H., & V.A. Vail. 1976a. Anatomical systematics of *Etheria elliptica* (Pelecypoda: Mycetopodidae). *Malacological Review* **9**: 15-24.

Heard, W.H. & V.A. Vail. 1976b. The systematic position of *Unio caffer* (Pelecypoda: Unionoida: Unionidae). *Zoologica Africana* **11**: 45-58.

Hemming, F. (ed.). 1957. Opinion 495 — Designation under plenary powers of a type species in harmony with accustomed usage for the nominal genus *Unio* Philipsson, 1788 (Class Pelecypoda) and validation under the same powers of the family-group name MARGARITIFERIDAE Haas, 1940. *Opinions and Declarations Rendered by the International Commission on Zoological Nomenclature,* Volume **17**. International Trust for Zoological Nomenclature, London.

Henderson, J. 1935. Fossil non-marine Mollusca of North America. *Geological Society of America Special Papers* **3**: 1-313.

Hillis, D.M. 1998. Taxonomic sampling, phylogenetic accuracy, and investigator bias. *Systematic Biology* **47**:3–8.

Hillis, D.M. & M.T. Dixon. 1991. Ribosomal DNA: Molecular evolution and phylogenetic inference. *Quarterly Review of Biology* **66**: 411–453.

Hillis, D.M., B.K. Mable, & C. Moritz. 1996. Application of molecular systematics: The state of the field and a look to the future. pp. 515-543. [In] D.M. Hillis, C. Moritz, & B.K. Mable (eds.). *Molecular Systematics*, 2nd edition. Sinauer Associates, Inc., Sunderland, Massachusetts.

Hine, P.M. 1978. Distribution of some parasites of freshwater eels in New Zealand. *New Zealand Journal of Marine and Freshwater Research* **12**: 179-187.

Hoeh, W.R., M.B. Black, R.G. Gustafson, A.E. Bogan, R.A. Lutz, & R.C. Vrijenhoek. 1998. Testing alternative hypotheses of *Neotrigonia* (Bivalvia: Trigonioida) phylogenetic relationships using cytochrome c oxidase subunit I DNA sequences. *Malacologia* **40**: 267-278.

Hoeh, W.R., A.E. Bogan, K.S. Cummings, M.B. Black, & R.C. Vrijenhoek. 1996a. The origin and evolutionary relationships of the freshwater mussels (Bivalvia: Unionoida) as assessed by mitochondrial DNA comparisons. *American Malacological Union, 62nd Annual Meeting, Chicago, Illinois*. p. 39 [abstract].

Hoeh, W.R., A.E. Bogan, & W.H. Heard. 2001. A phylogenetic perspective on the evolution of morphological and reproductive characteristics in the Unionoida. pp. 257-280. [In] G. Bauer & K. Wächtler (eds.). *Ecology and Evolution of the Freshwater Mussels Unionoida.* Ecological Studies, Volume 145. Springer-Verlag, Berlin, Germany.

Hoeh, W.R., K.S. Frazer, E. Naranjo-García, & R.J. Trdan. 1996b. A phylogenetic perspective on the evolution of simultaneous hermaphrodism in a freshwater mussel clade (Bivalvia: Unionidae: *Utterbackia*). *Malacological Review* **28**: 25-42.

Hoeh, W.R., D.T. Stewart, B.W. Sutherland, & E. Zouros. 1996c. Multiple origins of gender-associated mitochondrial DNA lineages in bivalves (Mollusca: Bivalvia). *Evolution* **50**: 2276-2286.

Hoggarth, M.A. 1992. An examination of the glochidia-host relationships reported in the literature for North American species of Unionacea (Mollusca: Bivalvia). *Malacology Data Net* **3**: 1-30.

Hoggarth, M.A. 1999. Descriptions of some of the glochidia of the Unionidae (Mollusca: Bivalvia). *Malacologia* **41**: 180-298.

Hooker, J.D. 1867. Lecture on Insular Floras. London. Delivered before the British Association for the Advancement of Science at Nottingham, 27 August 1866.

Howard, A.D. 1915. Some exceptional cases of breeding among Unionidae. *Nautilus* **29**: 4-11.

Howard, A.D. & B.J. Anson. 1923. Phases of parasitism of the Unionidae. *Journal of Parasitology* **9**: 68-82.

Hull, D.L. 1964. Consistency and monophyly. *Systematic Zoology* **13**: 1-11.

Humphries, C.J. & L.R. Parenti. 1986. *Cladistic Biogeography*. Clarendon Press, Oxford.

Ihering, H. von. 1901. The Unionidae of North America. *Nautilus* **15**: 37-39, 50-53.

International Commission on Zoological Nomenclature (ICZN). 1999. *International Code of Zoological Nomenclature,* 4th edition. International Trust for Zoological Nomenclature, London, UK.

Iredale, T. 1934. The freshwater mussels of Australia. *Australian Zoologist* **8**: 57-78.

Iredale, T. 1943a. A basic list of the fresh-water Mollusca of Australia. *Australian Zoologist* **10**: 188-230.

Iredale, T. 1943b. Guide to the freshwater shells of New South Wales. Part I. *Australian Naturalist* **11**: 85-95.

Ishibashi. R., A. Komaru, & T. Kondo. 2000. Sperm sphere in unionid mussels (Bivalvia: Unionidae). *Zoological Science* **17**: 947-950.

Jell, P.A. 1980. Earliest known pelecypod on Earth — a new Early Cambrian genus from South Australia. *Alcheringa* **4**: 233-239.

Johnson, R.I. 1970. The systematics and zoogeography of the Unionidae (Mollusca: Bivalvia) of the southern Atlantic Slope region. *Bulletin of the Museum of Comparative Zoology* **140**:263-450.

Jones, H.A., R.D. Simpson, & C.L. Humphry. 1986. The reproductive cycles and glochidia of fresh-water mussels (Bivalvia: Hyriidae) of the Macleay River, northern New South Wales, Australia. *Malacologia* **27**: 185-202.

Jupiter, S.D., & M. Byrne. 1997. Light and scanning electron microscopy of the embryos and glochidia larvae of the Australian freshwater bivalve *Hyridella depressa* (Hyriidae). *Invertebrate Reproduction and Development* **32**: 177-186.

Kabat, A.R. 1997. Correct family names for the freshwater "muteloid" bivalves (Unionoida: Etherioidea). *Occasional Papers on Mollusks* **5**: 379-392.

Kat, P.W. 1983. Morphological Divergence, genetics, and speciation among *Lampsilis* (Bivalvia: Unionidae). *Journal of Molluscan Studies* **49**: 133-145.

Kat, P. W. 1984. Parasitism and the Unionacea (Bivalvia). *Biological Reviews* **59**: 189-207.

Kew, H.W. 1893. *The Dispersal of Shells: an Inquiry into the Means of Dispersal Possessed by Fresh-Water and Land Mollusca.* Kegan Paul, Trench, Trübner and Co., London.

Kishino, H. & M. Hasegawa. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order of the Hominoidea. *Journal of Molecular Evolution* **29**: 170-179.

Kluge, A.G. 1989. A concern for evidence and a phylogenetic hypothesis of relationships among *Epicrates* (Boidae, Serpentes). *Systematic Zoology* **38**: 7-25.

Kondo, T. 1984. Hosts of the larvae of *Moncetia lavigeriana* (Bivalvia: Mutelidae) in Lake Tanganyika. *Venus* **43**: 347-352.

Kondo, T. 1990. Reproductive biology of a small bivalve *Grandidieria burtoni* in Lake Tanganyika. *Venus* **49**: 120-125.

Korniushin, A.V. 1998. Review of the studies on freshwater bivalve mollusc systematics carried out by the Russian taxonomic school. *Bivalvia* **1**, *Malacological Review, Suppliment (7)*: 65-82.

Kraemer, L.R. 1970. The mantle flap in three species of *Lampsilis* (Pelecypoda: Unionidae). *Malacologia* **10**: 225-282.

Lake, J.S. 1971. *Freshwater Fishes and Rivers of Australia.* Thomas Nelson & Sons Ltd, Melbourne, Australia.

La Rocque, A. 1964. A Rafinesque portrait, and monograph of the fluviatile bivalve shells of the Ohio River, containing twelve genera and sixty-eight species, by Mr. C.S. Rafinesque; a new translation. *Sterkiana* (16): 1-52. [English translation of Rafinesque (1820)]

Lefevre, G. & W.C. Curtis. 1910. Reproduction and parasitism in the Unionidae. *Journal of Experimental Zoology* **9**: 79-115, pls. 1-5.

Lefevre, G. & W.C. Curtis. 1912. Studies on the reproduction and artificial propagation of freshwater mussels. *Bulletin of the United States Bureau of Fisheries* **30**: 105-201.

Littlewood, D.T. 1994. Molecular phylogenetics of cupped oysters based on partial 28S rRNA gene sequences. *Molecular Phylogenetics and Evolution* **3**: 221-229.

Lydeard, C., M. Mulvey, & G.M. Davis. 1996. Molecular systematics and evolution of reproductive traits in North American freshwater unionacean mussels (Mollusca: Bivalvia) as inferred from 16S rRNA gene sequences. *Philosophical Transactions of the Royal Society of London* B **351**: 1593-1603.

Lynn, J.W. 1994. The ultrastructure of the sperm and motile spermatozeugmata released from the freshwater mussel *Anodonta grandis* (Mollusca: Bivalvia: Unionidae). *Canadian Journal of Zoology* **72**: 1452-1461.

Ma, Q.H. 1996. Revision of Mesozoic Margaritiferidae in China and their development. *Acta Palaeontologica Sinica* **35**: 408-429.

Maddison, W.P. & D.R. Maddison. 1997. *MacClade: Analysis of Phylogeny and Character Evolution, Version 3.07*. Sinauer Associates, Inc., Sunderland, Massachusetts.

Marshall, W.B. 1890. Beaks of the Unionidae inhabiting the vicinity of Albany, New York. *Bulletin of the New York State Museum* **2**: 169-189.

Mayr, E. 1963. *Animal Species and Evolution.* Belknap Press of Harvard University Press, Cambridge, Massachusetts.

McMahon, R. 1991. Mollusca: Bivalvia. pp. 315-399. [In] J.H. Thorp & A.P. Covich (eds.). *Ecology and Classification of North American Freshwater Invertebrates*. Academic Press, Inc., San Diego, California.

McMichael, D.F. 1954. Gene exchanges in freshwater mussel populations. *American Malacological Union Annual Reports for 1954*: 11-12. Reprinted in *Mimeograph Reprint Series*, Columbus, Ohio (5).

McMichael, D.F. 1956. Notes on the freshwater mussels of New Guinea. *Nautilus* **70**: 38-48.

McMichael, D.F. 1957. A review of the fossil freshwater mussels (Mollusca, Pelecypoda) of Australasia. *Proceedings of the Linnean Society of New South Wales* **81**: 222-244.

McMichael, D.F. 1958. The nature and origin of the New Zealand freshwater mussel fauna. *Transactions of the Royal Society of New Zealand* **85**: 427-432.

McMichael, D.F. 1967. Australian freshwater Mollusca and their probable evolutionary relationships: A summary of present knowledge. [In] A.H. Weatherley (ed.). *Australian Inland Waters and their Fauna.* Australian National University Press, Canberra.

McMichael, D.F. & I.D. Hiscock. 1958. A monograph of freshwater mussels (Mollusca: Pelecypoda) of the Australian region. *Australian Journal of Marine and Freshwater Research* **9**: 372-508.

McMichael, D.F. & T. Iredale. 1959. The land and freshwater Mollusca of Australia. *Biogeography and Ecology in Australia, Monographie Biologicae* **8**: 224-245.

Melville, R.V. & J.D.D. Smith (eds.). 1987. *Official Lists and Indexes of Names and Works in Zoology.* International Trust for Zoological Nomenclature, London.

Meyer, A. 1994. Shortcomings of the cytochrome *b* gene as a molecular marker. *Trends in Ecology and Evolution* **9**: 278-280.

Modell, H. 1942. Das natürliche System der Najaden. *Archiv für Molluskenkunde* **74**: 161-191 [in German; translated by Stansbery & Soehnagen, 1964]

Modell, H. 1949. Das natürliche System der Najaden. *Archiv für Molluskenkunde* **78**: 29-48.

Modell, H. 1964. Das natürliche System der Najaden. *Archiv für Molluskenkunde* **93**: 71-129.

Moore, R.C. (ed.). 1969. *Treatise on Invertebrate Paleontology,* Part N, Mollusca, 6. Volume 1: Bivalvia. Geological Society of America and University of Kansas Press, Lawrence, Kansas.

Morretes, F.L. de. 1949. Ensaio de catálogo dos moluscos do Brasil. *Arquivos do Museu Parananense* **7**: 5-216.

Morris, N.J. 1978. The infaunal descendants of the Cycloconchidae: an outline of the evolutionary history of the Heteroconchia, superfamilies Cycloconchacea to Chamacea. *Philosophical Transactions of the Royal Society of London B* **284**: 259-275.

Morris, P.J. & P.G. Williamson. 1988. *Pleiodon* (Conrad) (Bivalvia Mutelidae Pleiodoninae) from the Late Cretaceous of Montana— a 1st North-American record for the Mutelidae. *Journal of Paleontology* **62**: 758-765

Morrison, J.P.E. 1956. Family relationships of the North American fresh water mussels. *American Malacological Union Annual Reports for 1955:* 16-17.

Morrison, J.P.E. 1973. The families of the pearly freshwater mussels. *Bulletin of the American Malacological Union, Inc.* **38**: 45-46.

Morton, B. 1987. Functional morphology of *Neotrigonia margaritacea* (Bivalvia: Trigoniacea), with a discussion of phylogenetic affinities. *Records of the Australian Museum* **39**: 339-354.

Morton, B. 1996. The evolutionary history of the Bivalvia. pp. 337-359. [in] J.D. Taylor (ed.). *Origin and Evolutionary Radiation of the Mollusca*. Oxford University Press, New York, New York.

Nagel, K.-O., G. Badino, G. Celebrano. 1998. Systematics of European naiades (Bivalvia: Margaritiferidae and Unionindae): a review and some new aspects. *Bivalvia* **1**, *Malacological Review, Suppliment* (7): 83-104.

Nagel, K.-O. & G. Badino. 2001. Population genetics and systematics of European Unionoida. pp. 51-80. [In] G. Bauer & K. Wächtler (eds.). *Ecology and Evolution of the Freshwater Mussels Unionoida.* Ecological Studies, Volume 145. Springer-Verlag, Berlin, Germany.

Naylor, G. J. P., and W. M. Brown. 1998. *Amphioxus* mitochondrial DNA, chordate phylogeny, and the limits of inference based on comparisons of sequences. *Systematic Biology* **47**:61–76.

Nelson, G. & D.E. Rosen (eds.). 1981. *Vicariance Biogeography: A Critique.* Columbia University Press, New York, New York.

Nevesskaya, L.A., O.A. Scarlato, Y.I. Starobogatov, & A.G. Eberzin. 1971. New ideas on bivalve systematics. *Paleontological Journal* **5**: 141-155.

Newell, N.D. 1965. Classification of the Bivalvia. *American Museum Noviates* (2206): 1-25.

Newell, N.D. 1969. Classification of Bivalvia. pp. N205-N224. [In] R.C. Moore (ed.). *Treatise on Invertebrate Paleontology,* Part N, Mollusca, 6. Volume 1: Bivalvia. Geological Society of America and University of Kansas Press, Lawrence, Kansas.

Newell, N.D. & D.W. Boyd. 1975. Parallel evolution in early trigonacean bivalves. *Bulletin of the American Museum of Natural History* **154**: 53-162.

Newell, N.D. & D.W. Boyd. 1978. A palaeontologists view of Bivalve phylogeny. *Philosophical Transactions of the Royal Society of London B* **284**: 203-215.

O'Brien, C. & J. Brim Box. 1999. Reproductive biology and juvenile recruitment of the shinyrayed pocketbook, *Lampsilis subangulata* (Bivalvia: Unionidae) in the Gulf Coastal Plain. *American Midland Naturalist* **142**: 129-140.

Oesch, R.D. 1984. *Missouri Naiades: A Guide to the Mussels of Missouri.* Missouri Department of Conservation, Jefferson City, Missouri.

Ó Foighil, D. 1989. Role of spermatozeugmata in the spawning ecology of the brooding oyster *Ostrea edulis*. *Gamete Research* **24**: 219-228.

Ó Foighil, D. & D.L. Graf. 2000. Prodissoconch morphology of the relict marine paleoheterodont *Neotrigonia margaritacea* (Mollusca: Bivalvia) indicates a non-planktotrophic prejuvenile ontogeny. *Journal of the Marine Biological Association of the United Kingdom* **80**: 175-176.

Ó Foighil, D., B.A. Marshall, T.J. Hillbish, & M.A. Pino. 1999. Trans-Pacific range extension by rafting is inferred for the flat oyster *Ostrea chilensis*. *Biological Bulletin* **196**: 122-126.

Ó Foighil, D. & D. Taylor. 2000. Evolution of parental care and ovulation behavior in oysters. *Molecular Phylogenetics and Evolution* **15**: 301-303.

Ortmann, A.E. 1909. The breeding season of Unionidae in Pennsylvania. *Nautilus* **22**: 91-95, 99-103.

Ortmann, A.E. 1910a. A new system of the Unionidae. *Nautilus* **23**: 114-120

Ortmann, A.E. 1910b. The systematic position of the unionid-genus *Parreysia*. *Nautilus* **23**: 139-142.

Ortmann, A.E. 1910c The discharge of glochidia in the Unionidae. *Nautilus* **24**: 94-95.

Ortmann, A.E. 1910d. The marsupium of the Anodontinae. *Biological Bulletin* **19**: 217.

Ortmann, A.E. 1911a. The anatomical structure of certain exotic Najades compared with that of the North American forms. *Nautilus* **24**: 103-108, 114-120, 127-131.

Ortmann, A.E. 1911b. The classification of the European Naiades. *Nautilus* **25**: 5-7, 20-23.

Ortmann, A.E. 1911c. Monograph of the Najades of Pennsylvania. Part I. Anatomical investigations. Part II. The system of North American Najades. *Memoirs of the Carnegie Museum* **4**: 279-347.

Ortmann, A.E. 1912a. *Cumberlandia*, a new genus of Naiades. *Nautilus* **26**: 13-14.

Ortmann, A.E. 1912b. Notes upon the families and genera of the Najades. *Annals of the Carnegie Museum* **8**: 222-365.

Ortmann, A.E. 1912c. The anatomy of the Najad *Hyridella australis* (Lamarck) (= *Diplodon australis*). *Nautilus* **25**: 100-103.

Ortmann, A.E. 1913-1916. Studies in Najades. *Nautilus* **27** [1913]: 88-91; **28** [1914]: 20-22, 28-34, 41-47, 65-69; **28** [1915]: 129-131, 141-143; **29** [1915]: 63-67; **30** [1916]: 54-57.

Ortmann, A.E. 1916a. The anatomical structure of *Gonidea angulata* (Lea). *Nautilus* **30**: 50-53.

Ortmann, A.E. 1916b. The anatomy of the Nayad *Hyriopsis myersiana* (Lea). *Nautilus* **30**: 85-87.

Ortmann, A.E. 1917. The anatomy of *Contradens cambojensis* (Sowerby). *Nautilus* **30**: 106-108.

Ortmann, A.E. 1918a. The anatomy of two African Nayades, *Unio caffer* and *Spatha wahlbergi*. *Nautilus* **31**: 75-78.

Ortmann, A.E. 1918b. The identity of the Nayad-genus *Nodularia* Conrad with *Unio* Retzius. *Nautilus* **31**: 128-131.

Ortmann, A.E. 1918c. The systematic position of two species of mussels from the Ozarks. *Nautilus* **32**: 13-15.

Ortmann, A.E. 1921a. South American Naiades; a contribution to the knowledge of the freshwater mussels of South America. *Memoirs of the Carnegie Museum* **8**: 451-670.

Ortmann, A.E. 1921b. The anatomy of certain mussels from the upper Tennessee. *Nautilus* **34**: 81-91.

Ortmann, A.E. 1923-1924. Notes on the anatomy and taxonomy of certain Lampsilinae from the Gulf Drainage. *Nautilus* **37**: 56-60, 99-105, 137-144.

Owen, G. 1959. The ligament and digestive system in the taxodont bivalves. *Proceedings of the Malacological Society of London* **33**: 215-223.

Pain, T., & F.R. Woodward. 1961. A revision of freshwater mussels of the family Etheriidae. *Journal of Conchology* **25**: 2-8.

Pain, T., F.R. Woodward. 1968. A monograph of the African bivalves of the genera *Brazzaea* Bourguignat, *Mweruella* Haas, *Prisodontopsis* Tomlin and *Pseudospatha* Simpson. *Revue de Zoologie et de Botanique Africaines* **77**: 190-220.

Palumbi, S.R. 1996. Nucleic Acids II: the polymerase chain reaction. pp. 205–247. [In] D.M. Hillis, C. Moritz, & B.K. Mable (eds.). *Molecular Systematics*, 2nd edition. Sinauer Associates, Inc., Sunderland, Massachusetts.

Park, J.-K. & D. Ó Foighil. 2000. Sphaeriid and corbiculid clams represent separate heterodont bivalve radiations into freshwater environments. *Molecular Phylogenetics and Evolution* **14**: 75–88.

Parmalee, P.W. & A.E. Bogan. 1998. *The Freshwater Mussels of Tennessee*. University of Tennessee Press, Knoxville, Tennessee.

Parodiz, J.J. & A.A. Bonetto. 1963. Taxonomy and zoogeographic relationships of the South American Naiades (Pelecypoda: Unionacea and Mutelacea). *Malacologia* **1**: 179-214.

Pelseneer, P. 1891. Contribution à l'étude des lamellibranches. *Archives de Biologie* **11**: 147-312.

Peres-Noto, P.R. & F. Marques. 2001. When are random data not random, or is the PTP test useful? *Cladistics* **16**: 420-424.

Pilsbry, H. A. & J. Bequaert. 1927. The aquatic mollusks of the Belgian Congo, with a geographical and ecological account of Congo malacology. *Bulletin of the American Museum of Natural History* **3**: 69-602.

Platnick, N.I. & G.J. Nelson. 1978. A method of analysis for historical biogeography. *Systematic Zoology* **27**:1-16.

Pojeta, J., jr. 1971. Review of Ordovician Pelecypoda. *U.S. Geological Survey Professional Paper* (695). 46 pp.

Pojeta, J., jr. 1978. The origin and early taxonomic diversification of pelecypods. *Philosophical Transactions of the Royal Society of London B* **284**: 225-246.

Pojeta, J., jr. & B. Runnegar. 1985. The early evolution of diasome molluscs. pp. 295-336. [In] E.R. Trueman & M.R. Clarke (eds.). *The Mollusca,* **10***: Evolution.* Academic Press, Inc., Harcourt Brace Jovanovich Publishers, Orlando, Florida.

Popper, K. 1968. *The Logic of Scientific Discovery,* 2nd Edition. Harper & Row Publishers, New York, New York.

Purcheon, R.D. 1956. The stomach in the Protobranchia and Septibranchia (Lamallibranchia). *Proceedings of the Zoological Society of London* **127**: 511-515.

Purcheon, R.D. 1957 The stomach in the Filibranchia and Pseudolamellibranchia. *Proceedings of the Zoological Society of London* **129**: 27-60.

Purcheon, R.D. 1960. The stomach in the Eulamellibranchia: stomach types IV and V: *Proceedings of the Zoological Society of London* **135**: 431-489.

Purcheon, R.D. 1978. An analytical approach to a classification of the Bivalvia. *Philosophical Transactions of the Royal Society of London B* **284**: 425-436.

Purcheon, R.D. 1987. The stomach in the Bivalvia. *Philosophical Transactions of the Royal Society of London B* **316**: 183-276.

Rafinesque, C.S. 1820. Monographie des coquilles bivalves et fluviatiles de la Rivière Ohio, contenant douze genres et soixante-huit espèces. *Annales Générales des Sciences Physiques, Bruxelles* **5**: 287-322. [in French; translated by La Rocque (1964)]

Ridgewood, W.G. 1903. On the structure of the gills of lamellibranchs. *Philosophical Transactions of the Royal Society of London B* **194**: 147-284.

Roe, K. J. & C. Lydeard. 1998. Molecular systematics of the freshwater mussel genus *Potamilus* (Bivalvia: Unionidae). *Malacologia* **39**: 195-205.

Rosen, D.E. 1978. Vicariance patterns and historical explanations in biogeography. *Systematic Zoology* **27**: 159-188.

Rosenberg, G., G.M. Davis, G.S. Kuncio, & M.G. Harasewych. 1994. Preliminary ribosomal RNA phylogeny of gastropod and unionoidean bivalve mollusks. *Nautilus Suppliment* **2**: 111-121.

Rosenberg, G., S. Tillier, A. Tillier, G.S. Kuncio, R.T. Hanlon, M.Masselot, & C.J. Williams. 1997. Ribosomal RNA phylogeny of selected major clades in the Mollusca. *Journal of Molluscan Studies* **63**: 301-309.

Runnegar, B. & J. Pojeta. 1992. The earliest bivalves and their Ordovician descendants. *American Malacological Bulletin* **9**: 117-122.

Salisbury, B. 1999. Misinformative characters and phylogeny shape. *Systematic Biology* **48**: 153–169.

Salvini-Plawen, L.v. & G. Steiner. 1996. Synapomorphies and plesiomorphies in higher Classification of Mollusca. pp. 29-51. [in] J.D. Taylor (ed.). *Origin and Evolutionary Radiation of the Mollusca*. Oxford University Press, New York, New York.

Scarlato, O.A. & Y.I. Starobogatov. 1978. Phylogenetic relations and the early evolution of the Class Bivalvia. *Philosophical Transactions of the Royal Society of London B* **284**: 217-224.

Scarlato, O.A. & Y.I. Starobogatov. 1979. Osnovnye Cherty Evolyutsii i sistema klassa Bivalvia morfologiya, Sistematika, i filogeniya Mollyuskov. *Trudy Zoologicheskogo Instituta Akademiya Nauk SSSR* **80**: 5-38. [in Russian; translated in Boss & Jacobson (1985)]

Sepkoski, J.J., jr. & M.A. Rex. 1974. Distribution of freshwater mussels: Coastal rivers as biogeographic islands. *Systematic Zoology* **23**: 165-188.

Shikov, E.V. & M.N. Zatravkin. 1991. The comparative method of taxonomic study of Bivalvia used by Soviet malacologists. *Malakologische Abhandlungen* **15**: 149-159.

Simpson, C.T. 1893. On the relationships and distribution of the North American Unionidae, with notes on the west coast species. *American Naturalist* **27**:353-358.

Simpson, C.T. 1896. The classification and geographic distribution of the pearly fresh-water mussels. *Proceedings of the U.S. National Museum* **18**: 295-343.

Simpson, C.T. 1900. Synopsis of the Naiades, or pearly freshwater mussels. *Proceedings of the. U.S. National Museum* **22**: 501-1044.

Simpson, C.T. 1914. *A Descriptive Catalogue of the Naiades or Pearly Freshwater Mussels, Volumes I to III.* Privately published by Bryant Walker, Detroit, Michigan.

Simpson, G.G. 1944. *Tempo and Mode in Evolution*. Columbia University Press, New York.

Smith, B.J. 1998. Order Unionoida. pp. 296-298. [In] P.L. Beesley, G.J.B. Ross, & A. Wells (eds.). *Mollusca: the Southern Synthesis.* Fauna of Australia Volume 5. CSIRO Publishing, Melbourne, Australia.

Smith, D.C. 1979. Marsupial anatomy of the demibranch of *Margaritifera margaritifera* (Lin.) in northeastern North America (Pelecypoda: Unionacea). *Journal of Molluscan Studies* **45**: 39-44.

Smith, D.C. 1980. Anatomical studies on *Margaritifera margaritifera* and *Cumberlandia monodonta* (Mollusca: Pelecypoda: Margaritiferidae). *Zoological Journal of the Linnean Society* **69**: 257-270.

Smith, D.C. 1983. On the so-called mantle muscle scars on the shells of the Margaritiferidae (Mollusca, Pelecypoda), with observations on the mantle-shell attachment in the Unionoida and Trigonioida. *Zoologica Scripta* **12**: 67-71.

Smith, D.C. 2001. Systematics and distribution of the Recent Margaritiferidae. pp. 34-49. [In] G. Bauer & K. Wächtler (eds.). *Ecology and Evolution of the Freshwater Mussels Unionoida.* Ecological Studies, Volume 145. Springer-Verlag, Berlin, Germany.

Smith, D.C. & W.P. Wall. 1984. The Margaritiferidae reinstated: a reply to Davis and Fuller (1981), "Genetic relationships among Recent Unionacea (Bivalvia) of North America." *Occasional Papers on Mollusks* **4**: 321-330.

Sorenson, M.D. 1999. *TreeRot,* v. 2. Boston University, Boston, Massachusetts. Freeware and documentation available from http://mightyduck.bu.edu/TreeRot.

Stanley, S.M. 1970. Relation of shell form to life habits in the Bivalvia. *Memoirs of the Geological Society of America* **125**: 1-296.

Stansbery, D.H. & U. Soehnagen. 1964. Hans Modell — The natural system of the Naiades. *Sterkiana* (14): 1-18. [English translation of Modell (1942)]

Starobogatov, Y.I. 1970. *Fauna Molliuskov i Zoogeograficheskoe Raionirovanie Kontinental'nykh Vodoemov Zemnogo Shara [Fauna of Mollusks and Zoogeographical Provinces of Continental Waters of the World].* Nauka, Leningrad.

Starobogatov, Y.I. 1992. Morphological basis for phylogeny and classification of Bivalvia. *Ruthenica* **2**: 1-25.

Stasek, C.R. 1961. The ciliation and function of the labial palps of *Acila castrensis* (Protobranchia, Nuculidae), with an evaluation of the role of the protobranch organs of feedings in the evolution of the Bivalvia. *Proceedings of the Zoological Society of London* **137**: 511-538.

Stasek, C.R. 1963. Synopsis and discussion of the association of ctenidia and labial palps in the bivalved Mollusca. *Veliger* **6**: 91-97.

Sterki, V. 1895. Some notes on the genital organs of Unionidae, with reference to systematics. *Nautilus* **9**: 91-94.

Sterki, V. 1898. Some observations on the genital organs of Unionidae, with reference to classification. *Nautilus* **12**: 18-21, 28-32.

Sterki, V. 1903. Notes on the Unionidae and their classification. *American Naturalist* **37**: 103-113.

Storey, B.C. 1995. The role of mantle plumes in continental breakup: Case histories from Gondwanaland. *Nature* **377**: 301-308.

Strathmann, R.R. & D. J. Eernisse. 1994. What molecular phylogenies tell us about the evolution of larval forms. *American Zoologist* **34**: 502-512.

Strayer, D. 1987. Ecology and zoogeography of the freshwater mollusks of the Hudson River basin. *Malacological Review* **20**: 1-68.

Swainson, W. 1840. *A Treatise on Malacology, or the Natural Classification of Shells and Shell-Fish.* [Part of the *The Cabinet Cyclopædia*]. Longman, Orme, Brown, Green, & Longmans, and John Taylor, London.

Swofford, D.L., G.J. Olsen, P.J. Waddell, & D.M. Hillis. 1996. Phylogenetic inference. pp. 407-514. [In] D.M. Hillis, C. Moritz, & B.K. Mable (eds.). *Molecular Systematics*, 2nd edition. Sinauer Associates, Inc., Sunderland, Massachusetts.

Swofford, D.L. 1998. *PAUP* 4.0b3: Phylogenetic Analysis Using Parsimony, version 4.0*. Sinauer Associates, Inc., Sunderland, Massachusetts.

Tankersley, R.A. 1996. Multipurpose gills: effect of larval brooding on the feeding physiology of freshwater unionid mussels. *Invertebrate Biology* **115**: 243-255.

Taylor, J.D., W.J. Kennedy, & A. Hall. 1969. The shell structure and mineralogy of the Bivalvia. Introduction. Nuculacea - Trigonacea [*sic*]. *Bulletin of the British Museum of Natural History (Zoology), Supplement* **3**: 1-125.

Tevesz, M.J.S. 1975. Structure and habits of the "living fossil" pelecypod *Neotrigonia*. *Lethaia* **8**: 321-327.

Thiele, J. 1934. *Handbuch der Systematischen Weichtierkunde*, Part 3: 779-1022. Jena, Gustav Fischer.

Thompson, J.D., D.G. Higgins, & T.J. Gibson. 1994. CLUSTAL-W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**:4673–4680.

Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin, & D.G. Higgins. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25**: 4876–4882. Freeware available from http://ncbi.nlm.nih.gov.

Tunnicliff, S.P. 1982. A revision of Late Ordovician bivalves from Pomeroy Co., Tyrone, Ireland. *Palaeontology* **25**: 43-88.

Utterback, W.I. 1916. Breeding records of Missouri mussels. *Nautilus* **30**: 13-21.

van der Schalie, H. 1939. Distributional studies of the naiades as related to Geomorphology. *Journal of Geomorphology* **2**: 251-257.

van der Schalie, H. 1945. The value of mussel distribution in tracing stream confluence. *Papers of the Michigan Academy of Science, Arts and Letters* **30**: 355-373.

van der Schalie, H. 1970. Hermaphrodism among North American freshwater mussels. *Malacologia* **10**: 93-112.

Vaught, K.C. 1989. *A Classification of the Living Mollusca.* American Malacologists, Inc., Melbourne, Florida.

Vokes, H.E. 1980. *Genera of the Bivalvia: A Systematic and Bibliographic Catalogue (Revised and Updated).* Paleontological Research Institution, Ithaca, New York.

von Ihering, H. 1910. Über Brasilianische Najaden. *Senckenbergische Naturforchende Gesellschaft, Abhandlungen* **32**: 113-140.

Wächtler, K., M.C. Dreher-Mansur, & T. Richter. 2001. Larval types and early postlarval biology in Naiads (Unionoida). pp. 93-125. [In] G. Bauer & K. Wächtler (eds.). *Ecology and Evolution of the Freshwater Mussels Unionoida.* Ecological Studies, Volume 145. Springer-Verlag, Berlin, Germany.

Walker, B. 1917. The method of evolution in the Unionidæ. *Occasional Papers of the Museum of Zoology, University of Michigan* (45): 1-10.

Walker, K.F., M. Byrne, C.W. Hickey, & D.S. Roper. 2001. Freshwater mussels (Hyriidae, Unionidae) of Australasia. pp. 3-31. [In] G. Bauer & K. Wächtler (eds.). *Ecology and Evolution of the Freshwater Mussels Unionoida.* Ecological Studies, Volume 145. Springer-Verlag, Berlin, Germany.

Waller, D.L. & B.A. Lasee. 1997. External morphology of spermatozoa and spermatozeugmata of the freshwater mussel *Truncilla truncata* (Mollusca: Bivalvia: Unionidae). *American Midland Naturalist* **138**: 220-223.

Waller, T.R. 1998. Origin of the molluscan class Bivalvia and a phylogeny of major groups. pp. 1-45. [In] P.A. Johnston & J.W. Haggart (eds.). *Bivalves: An Eon of Evolution — Paleobiological Studies Honoring Norman D. Newell.* University of Calgary Press, Calgary.

Wanner, H. E. 1921. Some faunal remains from the Trias of York County, Pennsylvania. *Proceedings of the Academy of Natural Sciences, Philadelphia* **73**: 30-36.

Watters, G. T. 1994a. Form and function of unionoidean shell sculpture and shape (Bivalvia). *American Malacological* **11**: 1-20.

Watters, G. T. 1994b. An Annotated Bibliography of the Reproduction and Propagation of the Unionoidea (Primarily of North America). *Ohio Biological Survey Miscellaneous Contribution* **1**. Columbus, Ohio.

Watters, G.T. 2001. The evolution of the Unionacea in North America, and its implications for the worldwide fauna. pp. 281-307. [In] G. Bauer & K. Wächtler (eds.). *Ecology and Evolution of the Freshwater Mussels Unionoida.* Ecological Studies, Volume 145. Springer-Verlag, Berlin, Germany.

Wegener, A. 1966. *The Origin of Continents and Oceans*. Translation of the 1929 edition by J. Biram. Dover Publications, New York, New York.

Wenzel, J.W. & M.E. Siddall. 1999. Noise. *Cladistics* **15**: 61-64.

Wiley, E.O. 1980. *Phylogenetics: the Theory and Practice of Phylogenetic Systematics.* Wiley and Sons, Inc., New York.

Wiley, E.O., D. Siegel-Causey, D.R. Brooks, & V.A. Funk. 1991. *The Complete Cladist: A Primer of Phylogenetic Procedures.* University of Kansas Museum of Natural History Special Publication (19). Lawrence, Kansas.

Williams, J.D., M.L. Warren, K.S. Cummings, J.L. Harris, & R.J. Neves. 1993. Conservation status of the freshwater mussels of the United States and Canada. *Fisheries, A Bulletin of the American Fisheries Society* **18**(9): 6-22.

Woody, C.A. & L. Holland-Bartels. 1993. Reproductive characters of a population of the Washboard Mussels *Megalonaias nervosa* (Rafinesque 1820) in the Upper Mississippi River. *Journal of Freshwater Ecology* **8**: 57-66.

Wray, G.A. 1996. Parallel evolution of nonfeeding larvae in echinoids. *Systematic Zoology* **45**: 308-322.

Yang, Z. 1998. On the best evolutionary rate for phylogenetic analysis. *Systematic Biology* **47**: 125-133.

Yeager, M.M., D.S. Cherry, & R.J. Neves. 1994. Feeding and burrowing behaviors of juvenile rainbow mussels, Villosa iris (Bivalvia: Unionidae). *Journal of the North American Benthological Society* **13**: 217-222.

Yonge, C.M. 1953. The monomyarian condition in the Lamellibranchia. *Transactions of the Royal Society of Edinburgh* **62**: 443-478.

Yonge, C.M. 1957. Mantle fusion in the Lamellibranchia. *Pubblicazioni di Stazione Zoologica di Napoli* **29**: 151-171.

Yonge, C.M. 1962. On Etheria elliptica Lam. and the course of evolution, including the assumption of monomyarianism, in the family Etheriidae (Bivalvia: Unionacea). *Philosophical Transactions of the Royal Society of London B* **244**: 423-458.

Yonge, C.M. 1978. On the monomyarian, *Acostaea rivoli* and evolution in the family Etheriidae. *Journal of Zoology* **184**: 429-448.

Yonge, C.M. 1982. Mantle margins with a revision of siphonal types in the Bivalvia. *Journal of Molluscan Studies* **48**: 102-103.

Zale, A.V. & R.J. Neves. 1982. Fish hosts of four species of lampsiline mussels (Mollusca: Unionidae) in Big Moccasin Creek. *Canadian Journal of Zoology* **60**: 2535-2542.

Ziuganov, V., A. Zotin, L. Nezlin, & V. Tretiakov. 1994. *The Freshwater Pearly Mussels and Their Relationships with Salmonid Fish.* Vniro Publishing House, Moscow.