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Bivalvia – a look at the Branches
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Palaeoheterodont diversity (Mollusca: Trigonioida + Unionoida): what we know and what we wish we knew about freshwater mussel evolution

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The Palaeoheterodonta is a diverse clade consisting of the freshwater bivalve order Unionoida and its marine sister group, *Neotrigonia*. *Neotrigonia* is the sole surviving genus of the Trigonioida, known from only six species in Australian waters. Unionoids (freshwater mussels), in contrast, are widespread on all continents except Antarctica and are represented by *c*. 900 species. Discussion is biased towards the freshwater mussel condition, but *Neotrigonia* is crucial as a 'living fossil' for establishing the plesiomorphic states of unionoid synapomorphies. *Neotrigonia* retains many of the characters of the ancestral heteroconch. Our object is to provide evidential support for the natural classification of the extant Palaeoheterodonta. A supermatrix of 50 taxa and 1183 characters was constructed from 62 previously published DNA sequences of mitochondrial cytochrome oxidase subunit I (COI) and 28S nuclear ribosomal DNA, 15 novel sequences, and 59 morphological characters. Published COI sequences for *Coelatura aegyptiaca*, *Pseudomulleria dalyi*, and *Obliquaria reflexa* were treated as potentially problematic because of their inconsistency under different methodological assumptions and conflict with other datasets. Each partition was analysed under the criterion of parsimony separately and in combined analyses; analyses were run both with and without the problematic sequences. From our 'combined evidence' topology (with problematic sequences excluded), the Unionoida is monophyletic on the basis of eight synapomorphies, including larval parasitism, brood protection, and restriction to freshwater. The order is composed of six families in two superfamilies, Unionoidea and Etherioidea: ((Unionidae + Margaritiferidae) + (Hyriidae + (Etheriidae + (Mycetopodidae + Iridinidae)))). The morphological synapomorphies of these taxa are discussed with an emphasis on both the diagnosing of taxa and highlighting areas of ambiguity and missing data. Three appendices provide descriptions of the morphological characters (Appendix 1), a diagnosis of apomorphies for all branches of the phylogeny (Appendix 2), and a family-level classification of the extant Palaeoheterodonta, including a complete synonymy (Appendix 3). © 2006 The Linnean Society of London, *Zoological Journal of the Linnean Society*, 2006, **148**, 343–394.

ADDITIONAL KEYWORDS: Bivalvia – cladistic – classification – Etheriidae – Hyriidae – Iridinidae – Margaritiferidae – Mycetopodidae – Unionidae.

'When you know a thing, to hold that you know it; and when you do not know a thing, to allow that you do not know it: this is knowledge.' Confucius

INTRODUCTION

The bivalve subclass Palaeoheterodonta was conceived in 1965 by Norman Newell for the lineage consisting of early Palaeozoic actinodonts up through to the modern Trigonioida and Unionoida. The Recent diversity is limited to these last two orders only. The once-speciose Trigonioida (Cox, 1969; Newell & Boyd, 1975) has dwindled to a single, extant genus, *Neotrigonia*, which is found in subtidal waters around Australia (Darragh, 1998). The Unionoida (= naiades or freshwater mussels), on the other hand, is widely distributed in the freshwaters of all continents except

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Antarctica and is composed of around 900 species. As a result of this disparity and our own personal biases, we treat the extant Palaeoheterodonta as freshwater mussels and their living fossil outgroup, *Neotrigonia*. We have recently set ourselves upon the task of cataloging and revising the Unionoida (the 'MUSSEL Project', http://www.mussel-project.net/), and the objective of this paper is to review and re-analyse the available phylogenetic data, especially the morphological synapomorphies of the various family-level clades of the extant Palaeoheterodonta. It is our intention for this paper to benchmark the current state of the science regarding freshwater mussel macroevolutionary patterns of diversity, and to revise their classification on the basis of the available evidence.

In many ways, palaeoheterodonts are similar to the other bivalves more familiar to marine biologists. As adults, they are sedentary, infaunal filter-feeders (McMahon, 1991). What distinguishes freshwater mussels from their marine counterparts is their unique and complex combination of life history traits. All members of the Unionoida exhibit parental care in the form of larval brood protection (Coker *et al*., 1921; Graf & Ó Foighil, 2000a). Moreover, the freshwater mussel life cycle includes larvae that are obligate parasites on freshwater fishes (Kat, 1984; Wächtler, Dreher-Mansur & Richter, 2001). Variations in the structures and behaviours associated with these traits have long been argued to be of taxonomic value (reviewed in Heard & Guckert, 1970; Lydeard, Mulvey & Davis, 1996; Graf & Ó Foighil, 2000a), but most recent effort has dwelt on mussels' ecological impact (e.g. Haag & Warren, 1998, 1999; Vaughn & Taylor, 2000; Vaughn, Gido & Spooner, 2004), especially for the North American assemblage.

Freshwater malacology has experienced a bittersweet renaissance over the last three decades. Invasive species, such as *Corbicula fluminea* (Müller, 1774) and *Dreissena polymorpha* (Pallas, 1771), and the degradation of continental habitats have drawn a great deal of attention to the imperilled status of freshwater mussels (Bogan, 1993; Williams *et al*., 1993). In the eastern USA, a hotspot of palaeoheterodont species diversity (Fig. 1), freshwater mussels are considered amongst the most imperilled taxa, with more than half of the species requiring federal or state protection (Lydeard *et al*., 2004). This recent buzz to document and explain the *contemporary* processes impinging on the maintenance of unionoid diversity has tended to overshadow the fact that the evolutionary sources of this diversity have been influenced by a long and complex *history* that ranges over the entire Earth and extends back in time over 200 million years (Haas, 1969b).

It is unfortunate for students of malacology interested in macroevolutionary patterns that the history of freshwater mussel taxonomic research has not been

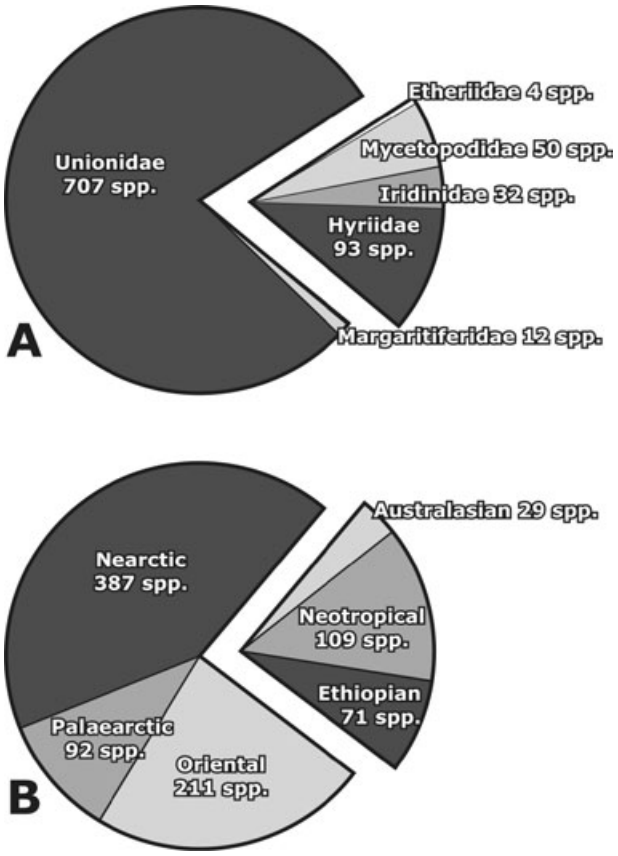

Figure 1. Taxonomic and geographical diversity of the Unionoida. Data summarized from Table 1. A, Taxonomic partitions of the Unionoida. Separate wedges represent the two superfamilies discussed in the text: Unionoidea and Etherioidea. B, Geographical distribution of the Unionoida. The smaller wedge represents the southern continents: South America, Africa, and Australasia.

a story of integration and synthesis. C.T. Simpson began the 20th century strong with a cosmopolitan review of Unionoida geographical patterns (Simpson, 1896) and a comprehensive synopsis of the previous century's alpha-taxonomy (Simpson, 1900, 1914). However, the over-arching theme of the last 100 years has been provincialism. The resulting consensus is a chimera of taxonomically and/or geographically restricted treatments: Ortmann (1912b, 1921), Frierson (1927), Haas (1940), Zhadin (1952), McMichael & Hiscock (1958), Parodiz & Bonetto (1963), Heard & Guckert (1970), Brandt (1974), Zatravkin & Bogatov (1987), Mandahl-Barth (1988), Turgeon *et al*. (1988, 1998), Subba Rao (1989), Williams *et al*. (1993), Daget (1998), Korniushin (1998), and many others. This sporadic series was periodically punctuated by global syntheses (Modell, 1942, 1949, 1964; Haas, 1969a, b; Starobogatov, 1970), but, of these, only Haas (1969a) treated all known species-level taxa. We presently

Table 1. Overview of the classification, diversity, and distribution of extant Palaeoheterodont families. Diversity values and distributions are estimated from numerous citations listed in the 'Introduction' and the references therein

Taxon	Genera	Species	Distribution
Order Trigonioida			
Family Trigoniidae	1	6	Australasian (Marine!)
Order Unionoida			
Family Unionidae	65	374	Nearctic, south through Mesoamerica
	56	295	Palaearctic, Oriental, New Guinea?
	10	38	Ethiopian
Family Margaritiferidae	3	12	Holarctic, one species in south-eastern Asia
Family Hyriidae	8	66	Neotropical
	8	27	Australasian
Family Mycetopodidae	10	50	Neotropical, and north into Mexico
Family Iridinidae	6	32	Ethiopian
Family Etheriidae	4	4	Neotropical, Ethiopian, and India
Subclass Palaeoheterodonta	171	904	Global, except Antarctica

stand at six families composed of roughly 170 genera with around 900 valid species (although there is a wide variance in this tally because of the imperfect union of these various classification schemes). The traditional arrangement and geographical diversity of the Palaeoheterodonta, including *Neotrigonia*, are enumerated in Table 1 and represented graphically in Figure 1.

We are unprepared to provide a detailed review of the palaeontology of the Palaeoheterodonta, and phylogenetic analyses combining fossil and Recent taxa are unavailable; however, it should be introduced into evidence that these bivalves have fossil representatives going back in time to nearly the beginning of multicellular life. Briefly, the common ancestor of the modern palaeoheterodonts is hypothesized to be the Ordovician genus *Lyrodesma* (Pojeta, 1978; Scarlato & Starobogatov, 1978), although this genus is believed to have been siphonate, unlike extant trigonioids and unionoids (see 'Discussion'). Newell & Boyd (1975: figs 3, 27) traced the origin of the Mesozoic 'Trigoniacea' from the Silurian genus *Schizodus*, and it was from the Triassic Pachycardiidae that they argued the modern Unionoida had their origin. The extant, crown group of the Unionoida dates apparently from the Triassic (Haas, 1969b; Good, 1998), and all of the modern families are recognizable by the Cretaceous (Watters, 2001). The age of the Palaeoheterodonta provides a wide window into bivalve evolution and a vital context for our neotological studies.

For example, let us consider the age and distribution of the Palaeoheterodonta in the context of the parasitic life history of the Unionoida. Although this association between mussels and their freshwater host fish certainly drives population-level processes (Graf, 1997c; Vaughn & Taylor, 2000), this dependence also has broader consequences. Much has been made of the inability of unionoids to cross terrestrial barriers (Johnson, 1970; Graf, 1997b, 2002b). Freshwater mussels also have restricted opportunities and tolerance for marine dispersal (Sepkoski & Rex, 1974; Kat, 1983). Thus, in general, the Unionoida is a strictly continental taxon that is dispersed by its hosts and confined to stable, freshwater environments. Such traits make these bivalves especially useful for the study of evolutionary processes over various scales of space and time, and we predict that freshwater mussel phylogeny should reflect the influence of the breakup of Pangaea in the Mesozoic, continental watershed evolution during the Tertiary, right up through the latest round of Pleistocene glaciation. Given the diversity of the Palaeoheterodonta, the kinds of macroevolutionary process questions that can be addressed are effectively limitless.

The absence of an integrated evolutionary perspective of the history and classification of the Unionoida (i.e. a phylogeny) has had two consequences. First, comprehensive treatments of clades are rare. The notable exceptions are those studies revising small groups, such as the cementing Etheriidae (Pain & Woodward, 1961a) and the Margaritiferidae (Smith, 2001), and a few considering single genera (e.g. Pain & Woodward, 1961b; 1968; Johnson, 1978; Roe & Lydeard, 1998; Roe, Hartfield & Lydeard, 2001; Serb, Buhay & Lydeard, 2003). It has sometimes happened that, in preparng a regional monograph, an endemic taxon has been dealt with in its entirety, such as the Hyridellinae of Australasia (McMichael & Hiscock, 1958), the Iridinidae of Africa (Mandahl-Barth, 1988; Daget, 1998), or the lampsiline mussels of North America (Frierson, 1927). A second consequence of this fragmented approach to palaeoheterodont sys-

tematics is that the prevailing dogma regarding freshwater mussel evolution is outdated (Walker, 1917). It is noteworthy that the major synthetic works (cited above) antedate widespread acceptance of both phylogenetic systematics (i.e. cladistics) and continental drift. As a result, the literature is replete with tales of land bridges, avian dispersal, and obviously paraphyletic taxa (for examples, see Graf & Ó Foighil, 2000b).

We regard taxonomy as the verbal presentation of phylogeny, not simply a nomenclature of convenience. Although several cladistic analyses (see below) of the Palaeoheterodonta (and the Bivalvia in general) have moved palaeoheterodont systematics forward, the implications of these various schemes have yet to trickle down to influence the nonsystematists' understanding of freshwater mussel origins and diversification. A good deal of phylogenetic data have accumulated over the last 10 years, and they are sufficient to reject some long-held hypotheses of freshwater mussel relationships and to establish new ones consistent with various lines of evidence.

Molecular phylogenetic studies of the Palaeoheterodonta to date have applied a variety of markers, but the majority have been limited in scope to interspecific relationships between Nearctic or Australasian taxa (e.g. Mulvey *et al*., 1997; Lydeard, Minton & Williams, 2000; Roe *et al*., 2001; Serb & Lydeard, 2003; Serb *et al*., 2003; Baker *et al*., 2004; Hughes *et al*., 2004; Campbell *et al*., 2005); these have been reviewed recently by Roe & Hoeh (2003). The large mitochondrial ribosomal subunit (16S) has been applied in restricted capacity to the problem of subfamilial relationships within the Unionidae of eastern North America (Lydeard *et al*., 1996; Campbell *et al*., 2005) and China (Huang *et al*., 2002). However, the handful of phylogenetic analyses with sufficient taxon and character sampling to resolve the deeper family-level branches of the Palaeoheterodonta have relied on three principal character sets: cytochrome oxidase subunit I (COI) mtDNA, domain 2 of the large nuclear ribosomal DNA (28S), and morphology.

Although multiple analyses have been performed since 2000 (Bogan & Hoeh, 2000; Graf & Ó Foighil, 2000a; Hoeh, Bogan & Heard, 2001; Hoeh *et al*., 2002a; Roe & Hoeh, 2003), in reality, only two different COI matrices have been compiled. Graf & Ó Foighil's (2000a) study of 40 palaeoheterodonts focused primarily on brooding character evolution amongst the Nearctic genera. The series of studies by Hoeh and colleagues (Bogan & Hoeh, 2000; Hoeh *et al*., 2001, 2002a; Roe & Hoeh, 2003) dwelt on the reworking of the same DNA sequences (31–34 taxa; 630 nucleotides) in combination with other data, and with different methods of analysis, to explore the relationships amongst unionoid families.

The large nuclear ribosomal subunit (28S rDNA) has been applied with mixed success. Rosenberg *et al*. (1994, 1997) employed a short, conserved segment corresponding to domain 6 of this gene, but the taxon sampling and resolution within the Unionoida were insufficient to test interfamilial relationships. More recently, Graf & Ó Foighil (2000b) and Graf (2002a) used domain 2 of 28S to test specific hypotheses within the Unionidae and Hyriidae. Unlike the rapidly evolving gene for COI in the mitochondrion, nuclear 28S provides a good measure of support for the basal branches of the Palaeoheterodonta (Graf, 2002a: fig. 2).

Morphological characters have not been given their due respect as far as phylogenetic analyses of the Palaeoheterodonta are concerned. Lydeard *et al*. (1996), Graf & Ó Foighil (2000a), and Hoeh *et al*. (2002a) each treated morphological characters in a limited capacity, and only three studies have tried to resolve freshwater mussel relationships via cladistic analysis of morphological characters: Graf (2000a), Hoeh *et al*. (2001), and Roe & Hoeh (2003).

'Nonmolecular' characters are regularly impugned simply because traditional, morphologically based classifications are often at odds with molecular phylogenetic results. However, the comparison is unfair. We concede that conventional taxonomic arrangements, based strictly on authoritarian treatments of a restricted set of morphological traits, are practically and theoretically inferior to objective and repeatable molecular phylogenetic analyses of many nucleotides. Nevertheless, it is untrue that the distinction between these two approaches is simply 'morphology vs. molecules'. The real shortcoming of such authoritarian arrangements is their noncladistic (i.e. nonscientific) methodology.

Although these different lines of evidence (COI, 28S, and morphology) have contributed novel insights into the evolution of the Palaeoheterodonta, these previous studies are not without their limitations. Cladistic analyses of morphological characters have thus far fallen short when attempting to recover phylogenetic patterns amongst the Unionidae, presumably because of high levels of homoplasy and the relatively few characters available for study (Graf, 2000a; Hoeh *et al*., 2001). Phylogenies derived from relatively fast-evolving mitochondrial genes, such as cytochrome oxidase, have been very inconsistent, often with the same data matrix supporting incongruent results under different methodological assumptions (e.g. compare Bogan & Hoeh, 2000 with Roe & Hoeh, 2003). The nuclear ribosomal DNA analysed to date, although demonstrating promise for the resolution of more basal nodes amongst the palaeoheterodonts, shows almost no informative variability amongst more recent divergences (Graf & Ó Foighil, 2000b; Graf, 2002a). It is often difficult for the nonsystematist to

know how to interpret these seemingly contradictory analytical studies, and, as a consequence, the evolutionary radiation of these fascinating bivalves into freshwater has not received the attention it deserves.

We have set out to make the most of the available phylogenetic evidence by combining these previous datasets into a 'supermatrix' of COI, 28S, and morphology (Sanderson & Driskell, 2003). Each of these three datasets has already been analysed separately in previous studies (cited above). By combining them, the results can be interpretted in a broader context, and the shortcomings and advantages of each partition can be assessed relative to the others. These are the data on which phylogenetic conclusions have been based and, although imperfect, they represent the best evidence on which to base the taxonomy of the Palaeoheterodonta. The resulting 'total evidence' phylogeny (Kluge, 1989) serves as the basis for a discussion of the known morphological synapomorphies of the various family-level taxa within the Palaeoheterodonta, and to illuminate those areas of the phylogeny for which more data would be especially welcomed. These objectives are discussed in the context of the taxonomic and morphological diversity of the various clades to serve as a benchmark for subsequent analyses and a revision of the classification.

MATERIAL AND METHODS

In order to provide a phylogeny of the Unionoida that incorporates and accommodates as much of the available phylogenetic data as possible, a 'supermatrix' of three different character partitions was constructed, largely from previously published analyses with sufficient taxon sampling (and phylogenetic information) to test family group-level hypotheses of monophyly and sister relationships. These three datasets were the partial sequence of the large nuclear ribosomal subunit DNA (28S, domain 2) (Graf & Ó Foighil, 2000b; Graf, 2002a), the Folmer *et al*. (1994) fragment of COI mtDNA (Bogan & Hoeh, 2000; Graf & Ó Foighil, 2000a; Hoeh *et al*., 2001, 2002a; Roe & Hoeh, 2003), and morphology (Lydeard *et al*., 1996; Graf & Ó Foighil, 2000a; Graf, 2000a; Hoeh *et al*., 2001; Roe & Hoeh, 2003). The previously published partial 28S sequences of Rosenberg *et al*. (1994, 1997) and those of the mtDNA marker 16S (Lydeard *et al*., 1996; Huang *et al*., 2002) were not included in the present study because the taxon sample was insufficient outside the Unionidae and, within that family, the species-level overlap with the available COI and 28S sequences was limited. However, the results of these studies are discussed in the context of our results.

Eleven 28S sequences and four COI sequences, previously analysed in Graf (2001), are introduced here. These were obtained by the standard polymerase chain reaction (PCR) and cycle sequencing methods described by Graf & Ó Foighil (2000a, b). All available applicable 28S sequences were included in the supermatrix. Existing COI sequences were included from all Old World palaeoheterodonts from the studies cited above; amongst the New World lineages, a subset of sequences was chosen to represent previously established clades and to maximize coincidence with the 28S sequences. No COI sequences were incorporated for the two outgroups, *Mytilus edulis* and *Astarte castanea*; preliminary analysis of this gene fragment revealed that the magnitude of homoplasy introduced by these outgroup sequences compromised the basal branches of the ingroup phylogeny. Between DNA sequence partitions, terminal taxa were nonchimeric at the species level, with the lone exception of *Castalia*. The taxonomy, GenBank accession numbers, and references for the sequences employed are listed in Table 2.

The morphological matrix was constructed by integrating the characters from published phylogenetic analyses of palaeoheterodont morphology (cited above) and the relevant characters from the bivalve matrix of Giribet & Wheeler (2002). The states of these characters were verified independently with published descriptions and from direct examination of specimens in various collections. Primarily, we used the Academy of Natural Sciences (ANSP, Philadelphia, PA, USA), the Illinois Natural History Survey (INHS, Champaign, IL, USA), and the University of Michigan Museum of Zoology (UMMZ, Ann Arbor, MI, USA). Additional specimens were studied at the Natural History Museum (BMNH, London, UK), the Museum of Comparative Zoology (MCZ, Cambridge, MA, USA), and the Field Museum of Natural History (FMNH, Chicago, IL, USA). References to exemplar vouchers and anatomical descriptions are listed in Table 2. Where necessary (and possible), character states were coded from congeneric species. Nine previously unconsidered characters were added to the morphological matrix (characters 9, 15, 16, 21, 33, 51, 53, 58, and 59). Furthermore, two taxa for which there are no available nucleotide characters, *Chambardia wahlbergi* and *Aspatharia rugifera*, were included with only morphological data to round out the Iridinidae. The complete morphological matrix is shown in Table 3, and character descriptions and explanations of their coding are listed in Appendix 1.

Molecular sequence alignments were derived using CLUSTAL_X (Thompson *et al*., 1997) and refined manually; all datasets were formatted for analysis using a combination of Sequence Monkey (Graf, 2000b) and MacClade 4.05 (Maddison & Maddison, 2002). The heterogeneity of the phylogenetic signal amongst the three character partitions (COI, 28S, and morphology) was tested with the partition homo-

Table 2. Taxa analysed. GenBank accession numbers are provided for the sequences considered here. Vouchers refer to morphological exemplars used to confirm morphological states; other specimens are cited in figure captions. Citations refer to those studies in which the sequences were originally introduced and those from which morphological data were obtained

Taxon	COI	28S	Morphology vouchers
Subclass Pteriomorpha			
Mytilus edulis Linnaeus, 1758	–	Z29550	
Subclass Heterodonta			
Astarte castanea (Say, 1822)	–	AF131001	
Subclass Palaeoheterodonta			
Order Trigonioida			
Neotrigonia margaritacea (Lamarck, 1804)	U56850	AF400695	ANSP 411352
Order Unionoida			
Family Etheriidae			
Etheria elliptica Lamarck, 1807	AF231742	–	UMMZ 112671
Acostaea rivolii (Deshayes, 1827)	AF231739	–	UMMZ 23485
Pseudomulleria dalyi (E.A. Smith, 1898)	AF231750	–	UMMZ 112653
Family Iridinidae			
Mutela rostrata (Rang, 1835)	U56849	–	ANSP 41819
Mutela dubia (Gmelin, 1791)	AF231737	–	ANSP 248788
Chambardia wahlbergi (Krauss, 1848)	–	–	ANSP 41812
Aspatharia rugifera (Dunker, 1858)	–	–	ANSP 125638
Family Mycetopodidae			
Anodontites trigonus (Spix, 1827)	AF231738	–	UMMZ 112400
Anodontites guanarensis Marshall, 1927	AF231741	–	INHS 16994
Monocondylaea minuana (d'Orbigny, 1835)	AF231745	–	UMMZ 248906
Family Hyriidae			
Hyridella depressa (Lamarck, 1819)	AF156496	AF305375	ANSP 334436, ANSP 65249
Hyridella australis (Lamarck, 1819)	AF305367	AF305373	ANSP 125846
Hyridella menziesi (Gray in Dieffenbach, 1843)	AF305370	AF305377	ANSP 41801
H. menziesi	AF231747	–	
Velesunio angasi (Sowerby, 1867)	AF231743	–	ANSP 71739
Velesunio ambiguous (Philippi, 1847)	AF305371	AF305378	ANSP 41802
Lortiella rugata (Sowerby, 1868)	AF231746	–	
Lortiella froggattii Iredale, 1934	–	–	INHS 16213
Diplodon chilensis (Gray, 1828)	DQ191410	AF305380	ANSP 125828
Diplodon deceptus (Simpson, 1914)	AF231744	–	
Castalia stevensi (H.B. Baker, 1930)	AF231736	–	INHS 14890
Castalia sp.	–	AF305381	
Family Margaritiferidae			
Margaritifera margaritifera (Linnaeus, 1758)	U56847	–	UMMZ 107585
Cumberlandia monodonta (Say, 1829)	AF156498	AF305382	UMMZ 107650
Family Unionidae			
Coelatura aegyptiaca (Cailliaud, 1827)	AF231735	–	ANSP 366277
Pilsbryoconcha exilis (Lea, 1838)	–	AF400693	ANSP 125614, ANSP A3655
Pseudodon vondembuschianus (Lea, 1840)	–	AF400694	ANSP 69923, ANSP A3650
Contradens contradens (Lea, 1838)	DQ191411	AF400692	ANSP 389059
Gonidea angulata (Lea, 1838)	DQ191412	AF400691	UMMZ 107895
Subfamily Unioninae			
Tribe Unionini			
Unio pictorum (Linnaeus, 1758)	AF156499	AF305383	ANSP 350622, ANSP 41466
Cafferia caffra (Krauss, 1848)	AF156501	AF400687	ANSP 189005
Tribe Anodontini			
Lasmigona compressa (Lea, 1829)	AF156503	DQ191414	UMMZ 104085
Strophitus undulatus (Say, 1817)	AF156505	DQ191415	UMMZ 58222
Pyganodon grandis (Say, 1829)	AF156504	AF305384	UMMZ 230451
Alasmidonta marginata Say, 1818	AF156502	AF400688	ANSP 373804, ANSP 103977

Table 2. *Continued*

Taxon	COI	28S	Morphology vouchers
Subfamily Ambleminae			
Tribe Amblemini			
Amblema plicata (Say, 1817)	AF156512	AF305385	ANSP 366019
Tribe Quadrulini			
Tritogonia verrucosa (Rafinesque, 1820)	DQ191413	DQ191416	UMMZ 62984
Quadrula quadrula (Rafinesque, 1820)	AF156511	DQ191417	UMMZ 129175
Tribe Pleurobemini			
Elliptio dilatata (Rafinesque, 1820)	AF156507	AF400690	UMMZ 171460
Pleurobema coccineum (Lea, 1838)	AF156509	DQ191418	UMMZ 156154
Fusconaia flava (Rafinesque, 1820)	AF231733	–	UMMZ 60313
Tribe Lampsilini			
Obliquaria reflexa Rafinesque, 1820	AF385114	AF400689	UMMZ 150651
Truncilla truncata Rafinesque, 1820	AF156513	DQ191419	UMMZ 153998
Actinonaias carinata (Barnes, 1823)	AF156517	DQ191420	ANSP 170825 ANSP 397257, ANSP 397371
Ptychobranchus fasciolaris (Rafinesque, 1820)	AF156514	DQ191421	UMMZ 205681
Villosa iris (Lea, 1829)	AF156524	DQ191422	UMMZ 85324
Ligumia recta (Lamarck, 1819)	AF156516	DQ191423	UMMZ 98325
Lampsilis cardium Rafinesque, 1820	AF156519	AF305386	UMMZ 89483
Epioblasma triquetra (Rafinesque, 1820)	AF156528	DQ191424	UMMZ 91330

Literature for sequence data: Littlewood (1994), Hoeh *et al*. (1998), Bogan & Hoeh (2000), Graf & Ó Foighil (2000a, b), Park & Ó Foighil, 2000), Roe *et al*. (2001), Graf (2002a).

Literature for morphological characters: Woodward (1898), Ortmann (1910a, b, 1911b, 1912a, b, c, 1913–16, 1916, 1917, 1918a, b, 1921), Percival (1931), Bloomer (1932, 1946, 1949), Bonetto (1951, 1961a, b, 1962), McMichael & Hiscock (1958), Fryer (1959, 1961), Bonetto & Ezcurra (1962, 1965), Saleuddin (1965), Yonge (1962, 1976, 1978), Hebling & Penteado (1974), Gould & Jones (1974), Bayne, Thompson & Widdows (1976), Heard & Vail (1976a, b), Smith (1979, 1980, 1983), Jones *et al*. (1986), Morton (1987, 1992), Arteaga (1994), Panha & Eongrakornkeaw (1995), Jupiter & Byrne (1997), Darragh (1998), Graf (2000a), Ó Foighil & Graf (2000), Giribet & Wheeler (2002), Ponder & Bayer (2004).

geneity test (ILD; Farris *et al*., 1995), as implemented in PAUP* (Swofford, 2002; 1000 ILD replicates; heuristic tree searching with starting trees obtained by 100 random stepwise additions; MaxTrees = 500).

Phylogenetic analyses were performed using PAUP*4b10 (Swofford, 2002). Each of the three partitions was analysed separately under the optimality criterion of maximum parsimony (MP); combined Molecular Only and Molecular + Morphology ('Combined evidence', CE) analyses were also performed. For all analyses, a first-pass heuristic search for trees was performed with starting trees generated by 100 random stepwise additions (branch swapping by tree bisection–reconnection, gaps handled as missing data, and MULTREES in effect). To circumvent a known PAUP* bug, wherein more trees than just the optimal set were retained during heuristic searches with random stepwise addition, a second-pass search was performed by branch swapping on the set of trees recovered in the first pass.

On the basis of their performance in previous analyses, three COI sequences were considered to be potentially problematic: *Pseudomulleria dalyi* (Bogan & Hoeh, 2000; GenBank number AF231750), *Coelatura aegyptiaca* (Bogan & Hoeh, 2000; AF231735), and *Obliquaria reflexa* (sequences amplified from UAUC 19, the same individual from which Lydeard *et al*., 1996 obtained 16S; AF385114). We treated these sequences as potentially problematic because of their inconsistency (relative to other available data) and their importance to family-level hypotheses of monophyly. All relevant analyses were performed both with and without these problematic sequences to examine their effects on the resolution, robustness, and logical consistency of the resultant phylogenetic hypotheses. Kishino–Hasegawa (Kishino & Hasegawa, 1989) and nonparametric tests, as implemented in PAUP*, were used to evaluate differences in topology between sets of most parsimonious trees from the various analyses and the trees recovered from the CE analysis; taxa with problematic sequences were not included. The robustness of the individual clades in all analyses was evaluated in PAUP* using bootstrap resampling (1000 bootstrap replicates; heuristic tree searching with

Table 3. Morphological character matrix. Characters and coding correspond to Appendix 1

Mytilus edulis	1 1 - - 1 0 0 0 - ?	0 0 0 0 - 0 0 0 0 0	- 0 0 0 0 0 0 1 - 0	- 1 1 0 ? 1 0 0 0 0	? 0 0 - - - - - - -	0 0 1 0 - - - - - -
Astarte castanea	0 2 0 - 0 0 0 0 - ?	0 0 0 0 - 0 1 0 0 1	- 0 0 1 0 0 0 1 - 0	- 0 0 0 0 0 0 1 0 1	? 0 0 - - - - - - -	0 0 0 0 - - - - - -
Neotrigonia margaritacea	0 0 1 0 0 0 0 0 0 1	1 1 1 0 - 0 0 0 0 0	- 1 0 0 0 0 0 0 - 0	- 1 1 0 1 0 0 0 0 1	0 0 0 - - - - - - -	0 1 ? 0 - - ? - -
Mutela rostrata	0 0 1 1 0 0 0 0 1 1	0 0 0 0 - 0 1 0 2 2	0 1 1 1 1 1 1 1 0 2	- 2 0 0 0 0 0 0 ? 1	? 1 1 1 ? - ? ? 1 0	? ? ? 1 1 1 - - 1
Mutela dubia	0 0 1 2 0 0 0 0 1 0	0 0 0 0 - 0 1 0 2 2	0 1 1 1 1 1 1 1 0 2	- 2 0 0 0 0 0 0 ? 1	? 1 1 1 ? - ? ? 1 0	? ? ? 1 1 1 - - 1
Chambardia wahlbergi	0 0 1 1 0 1 0 1 1 1	0 0 0 0 - 0 1 0 2 2	0 1 1 0 1 1 0 1 0 2	- 0 0 0 0 0 0 0 ? 1	? 1 1 1 ? - ? 2 1 ?	? ? ? ? ? ? ? - ?
Aspatharia rugifera	0 0 1 1 0 1 0 0 1 1	0 2 0 0 - 0 1 0 2 2	0 1 1 0 1 1 0 1 0 2	- 0 0 0 0 0 0 0 ? 1	? 1 1 1 ? - 0 2 1 0	? ? ? ? ? ? ? - ?
Anodontites trigonus	0 0 1 1 0 1 0 0 2 0	0 0 0 0 - 0 1 0 2 2	0 1 1 1 1 1 0 1 0 0	- 0 0 0 0 0 0 0 0 1	1 1 1 1 ? - 0 2 1 0	? ? ? 1 1 0 - - 0
Anodontites guanarensis	0 0 1 1 0 1 0 0 2 0	0 0 0 0 - 0 1 0 2 2	0 1 1 1 1 1 0 1 0 0	- 0 0 0 0 0 0 0 0 1	1 1 1 1 ? - 0 2 1 0	? ? ? 1 1 0 - - 0
Monocondylaea minuana	0 0 1 1 0 1 0 0 2 1	0 0 0 0 - 0 1 0 2 2	0 1 1 1 1 1 0 1 0 0	- 0 0 0 0 0 0 0 ? 1	1 1 1 1 ? - 0 ? 1 0	? ? ? 1 1 0 - - 0
Etheria elliptica	0 1 1 1 2 1 0 1 - 0	0 ? ? 1 0 0 1 1 1 2	1 1 1 1 1 1 0 1 0 0	- 0 0 0 2 0 0 0 0 1	? 1 1 1 ? - 0 2 1 0	? ? ? ? ? ? ? - ?
Acostaea rivolii	0 1 1 1 0 1 1 - - 0	0 ? ? 1 1 0 1 1 ? ?	? 1 1 1 1 1 0 1 0 0	- 0 0 0 2 0 1 0 0 1	1 1 1 ? ? - ? ? ? ?	? ? ? 1 1 0 - - 0
Pseudomulleria dalyi	0 ? 1 1 0 1 1 - - 0	0 ? ? 1 1 0 1 1 ? ?	? 1 1 1 ? 1 0 1 0 0	- 0 0 0 2 0 1 0 0 1	? 1 1 ? ? - ? ? ? ?	? ? ? ? ? ? ? - ?
Hyridella depressa	0 0 1 0 0 0 0 0 0 0	0 1 0 0 - 0 1 0 1 2	0 1 1 1 1 0 0 1 1 2	- 0 0 0 0 0 0 0 ? 1	? 1 1 1 ? - 1 0 0 0	? ? ? 1 0 0 1 1 -
Hyridella australis	0 0 1 0 0 0 0 0 0 0	0 1 0 0 - 0 1 0 1 2	0 1 1 1 1 0 0 1 1 2	- 0 0 0 0 0 0 0 ? 1	? 1 1 1 ? - 1 0 0 0	? ? ? 1 0 0 1 1 -
Hyridella menziesi	0 0 1 0 0 0 0 0 0 0	0 1 0 0 - 0 1 0 1 2	0 1 1 1 1 0 0 1 0 2	- 0 0 0 0 0 0 0 ? 1	? 1 1 1 ? - 1 0 0 0	? ? ? 1 0 0 1 1 -
Velesunio angasi	0 0 1 0 0 0 0 1 0 ?	0 0 0 0 - 0 1 0 1 2	0 1 1 1 1 0 0 1 1 2	- 0 0 0 0 0 0 0 ? 1	? 1 1 1 ? - 1 0 0 0	? ? ? 1 0 0 1 1 -
Velesunio ambiguus	0 0 1 0 0 0 0 0 0 0	0 0 0 0 - 0 1 0 1 2	0 1 1 1 1 ? 0 1 1 2	- 0 0 0 0 0 0 0 ? 1	? 1 1 1 ? - 1 0 0 0	? 1 ? 1 0 0 1 ? -
Lortiella rugata	0 0 1 1 0 0 0 0 0 0	0 0 0 0 - 0 1 0 1 2	0 1 1 ? 1 0 0 1 1 2	- 0 0 0 0 0 0 0 ? 1	? 1 1 1 ? - 1 0 0 0	? ? ? 1 0 0 1 1 -
Diplodon chilensis	0 0 1 0 0 0 0 0 0 0	0 1 0 0 - 0 1 0 1 2	0 1 1 1 1 0 0 1 0 2	- 0 0 0 0 0 0 0 0 1	0 1 1 1 ? - 1 0 0 0	? 1 ? 1 0 0 1 ? -
Diplodon deceptus	0 0 1 0 0 0 0 0 0 0	0 1 0 0 - 0 1 0 1 2	0 1 1 1 1 0 0 1 0 2	- 0 0 0 0 0 0 0 0 1	0 1 1 1 ? - 1 0 0 0	? ? ? 1 0 0 1 ? -
Castalia sp.	0 0 1 0 0 0 0 0 0 0	0 1 1 0 - 0 1 0 1 2	0 1 1 1 1 0 0 1 0 2	- 0 ? 0 0 0 0 0 0 1	0 1 1 1 ? - 1 0 0 0	? ? ? 1 0 0 1 ? -
Margaritifera margaritifera	0 0 1 0 0 0 0 0 2 0	1 0 0 0 - 0 1 0 0 2	0 1 0 0 0 2 0 0 - 0	- 0 0 0 0 0 0 0 1 1	0 1 1 0 ? 0 0 - - 0	? ? 1 1 0 0 0 ? -
Cumberlandia monodonta	0 0 1 1 0 0 0 0 2 0	1 0 0 0 - 0 1 0 0 2	0 1 0 0 0 2 0 0 - 0	- 0 0 0 0 0 0 0 1 1	0 1 1 0 ? 0 0 - - 0	? ? ? 1 0 0 0 0 -
Coelatura aegyptiaca	0 0 1 0 0 0 0 0 0 0	0 2 3 0 - 0 1 0 1 2	0 1 1 1 0 2 0 0 - 1	0 0 0 0 0 0 0 0 0 1	0 1 1 0 ? 0 0 0 0 0	? ? ? 1 0 0 0 ? -
Pilsbryoconcha exilis	0 0 1 1 0 0 0 0 0 ?	0 0 0 0 - 0 1 0 1 2	0 1 1 0 0 2 0 0 - 1	0 0 0 0 0 0 0 0 0 1	? 1 1 0 ? 0 0 0 ? 0	? ? ? 1 0 0 0 ? -
Pseudodon vondembuschianus	0 0 1 1 0 0 0 0 0 0	0 ? 0 0 - 0 1 0 1 2	0 1 1 0 0 2 0 0 - 1	0 0 0 0 0 0 0 0 0 1	? 1 1 0 ? 0 0 0 ? 0	? ? ? 1 0 0 0 ? -
Contradens contradens	0 0 1 0 0 0 0 0 0 0	0 2 0 0 - 0 1 0 1 2	0 1 1 0 0 2 0 0 - 1	0 0 0 0 0 0 0 0 ? 1	? 1 1 2 ? 0 - 0 0 0	? ? ? 1 0 0 0 ? -
Gonidea angulata	0 0 1 1 0 0 0 0 0 0	0 0 0 0 - 0 1 0 1 2	0 1 1 0 0 2 0 0 - 1	0 0 0 0 0 0 0 0 ? 1	0 1 1 0 0 0 0 0 0 0	? ? ? 1 0 0 0 0 -
Tritogonia verrucosa	0 0 1 0 0 0 0 0 0 0	0 ? 4 0 - 0 1 0 2 2	0 1 1 0 0 ? 0 0 - 1	1 0 1 0 0 0 0 0 ? 1	? 1 1 0 0 0 0 0 0 0	? ? ? 1 0 0 0 0 -
Quadrula quadrula	0 0 1 0 0 0 0 0 0 0	0 0 4 0 - 0 1 0 2 2	0 1 1 0 0 2 0 0 - 1	1 0 0 0 0 0 0 0 0 1	0 1 1 0 0 0 0 0 0 0	? ? ? 1 0 0 0 0 -
Amblema plicata	0 0 1 0 0 0 0 0 0 0	0 0 2 0 - 0 1 0 2 2	0 1 1 0 0 2 0 0 - 1	1 0 1 0 0 0 0 0 0 1	0 1 1 0 0 0 0 0 0 0	? ? ? 1 0 0 0 0 -
Elliptio dilatata	0 0 1 0 0 0 0 0 0 0	0 0 0 0 - 0 1 0 2 2	0 1 1 0 0 2 0 0 - 1	1 0 1 0 0 0 0 0 0 1	0 1 1 2 0 0 - 0 0 0	? ? ? 1 0 0 0 1 -
Pleurobema coccineum	0 0 1 0 0 0 0 0 0 0	0 0 0 0 - 0 1 0 2 2	0 1 1 0 0 2 0 0 - 1	1 0 1 0 0 0 0 0 0 1	0 1 1 2 0 0 - 0 0 0	? ? ? 1 0 0 0 1 -
Fusconaia flava	0 0 1 0 0 0 0 0 0 1	0 0 0 0 - 0 1 0 2 2	0 1 1 0 0 2 0 0 - 1	1 0 1 0 0 0 0 0 ? 1	? 1 1 0 0 0 0 0 0 0	? ? 1 1 0 0 0 1 -
Obliquaria reflexa	0 0 1 0 0 0 0 0 0 1	0 0 4 0 - 0 1 0 2 2	0 1 1 0 0 2 0 0 - 1	0 0 0 0 0 0 0 0 ? 1	? 1 1 2 0 1 - 0 1 2	? ? ? 1 0 0 0 1 -
Truncilla truncata	0 0 1 0 0 0 0 0 0 0	0 0 0 0 - 0 1 0 2 2	0 1 1 1 0 2 0 0 - 1	0 0 0 0 0 0 0 0 ? 1	? 1 1 2 1 1 - 0 1 2	? ? ? 1 0 0 0 ? -
Actinonaias carinata	0 0 1 0 0 0 0 0 0 0	0 2 0 0 - 0 1 0 2 2	0 1 1 1 0 2 0 0 - 1	0 0 0 0 0 0 0 0 0 1	0 1 1 2 1 1 - 0 1 2	? ? 1 1 0 0 0 0 -
Ptychobranchus fasciolaris	0 0 1 0 0 0 0 0 0 1	0 0 0 0 - 0 1 0 2 2	0 1 1 0 0 2 0 0 - 1	0 0 0 0 0 0 0 0 ? 1	? 1 1 2 1 1 - 0 0 2	? ? ? 1 0 0 0 1 -
Villosa iris	0 0 1 0 0 0 0 0 0 0	0 2 0 0 - 1 1 0 2 2	0 1 1 1 0 2 0 0 - 1	0 0 0 1 0 0 0 0 ? 1	? 1 1 2 1 1 - 0 1 2	? ? ? 1 0 0 0 ? -
Ligumia recta	0 0 1 0 0 0 0 0 0 0	0 2 0 0 - 1 1 0 2 2	0 1 1 1 0 2 0 0 - 1	0 0 0 1 0 0 0 0 0 1	0 1 1 2 1 1 - 0 1 2	? 1 1 1 0 0 0 ? -
Lampsilis cardium	0 0 1 0 0 0 0 0 0 0	0 2 0 0 - 1 1 0 2 2	0 1 1 1 0 2 0 0 - 1	0 0 0 1 0 0 0 0 ? 1	? 1 1 2 1 1 - 0 1 2	1 ? ? 1 0 0 0 1 -
Epioblasma triquetra	0 0 1 0 0 0 0 0 0 1	0 0 0 0 - 1 1 0 2 2	0 1 1 1 0 2 0 0 - 1	0 0 0 1 0 0 0 0 ? 1	? 1 1 2 1 1 - 0 1 2	? ? ? 1 0 0 0 ? -
Lasmigona compressa	0 0 1 1 0 0 0 0 2 0	0 2 0 0 - 0 1 0 2 2	0 1 1 0 0 2 0 0 - 1	0 0 0 0 0 0 0 0 ? 1	? 1 1 2 1 0 - 1 0 1	1 ? ? 1 0 0 2 0 -
Strophitus undulatus	0 0 1 1 0 0 0 0 0 0	0 0 0 0 - 0 1 0 2 2	0 1 1 1 0 2 0 0 - 1	0 0 0 0 0 0 0 0 0 1	0 1 1 2 1 0 - 1 0 1	? ? ? 1 0 0 2 1 -
Pyganodon grandis	0 0 1 1 0 0 0 0 0 ?	0 2 0 0 - 0 1 0 2 2	0 1 1 0 0 2 0 0 - 1	0 0 0 0 0 0 0 0 0 1	0 1 1 2 1 0 - 1 0 1	1 ? 1 1 0 0 2 0 -
Alasmidonta marginata	0 0 1 1 0 0 0 0 2 0	0 0 0 0 - 0 1 0 2 2	0 1 1 1 0 2 0 0 - 1	0 0 0 0 0 0 0 0 ? 1	? 1 1 2 1 0 - 1 0 1	? ? ? 1 0 0 2 0 -
Unio pictorum	0 0 1 0 0 0 0 0 2 0	0 2 0 0 - 0 1 0 1 2	0 1 1 0 0 2 0 0 - 1	0 0 0 0 0 0 0 0 0 1	0 1 1 2 0 0 - 0 0 0	? 1 ? 1 0 0 2 ? -
Cafferia caffra	0 0 1 0 0 0 0 0 2 0	0 2 0 0 - 0 1 0 1 2	0 1 1 0 0 2 0 0 - 1	0 0 0 0 0 0 0 0 ? 1	? 1 1 2 0 0 - 0 0 0	? ? ? 1 0 0 2 ? -

-, inapplicable characters; ?, missing data.

starting trees by ten random stepwise additions; MaxTrees = 5000). Character support was also determined via Bremer support (aka decay index), as facilitated by TreeRot version 2 (Sorenson, 1999). For each node, the Bremer decay index (BDI) indicates the difference in length of the next shortest tree without that node. The larger the BDI, the better the support (Bremer, 1995).

The 'Describe Trees' function of PAUP* was used to determine the most parsimonious reconstruction of the synapomorphies on the CE phylogeny. Characters were traced on an arbitrary tree (Tree 1) from this analysis to provide a fully resolved cladogram. Morphological characters were optimized in PAUP* under both 'accelerated transformation' (ACCTRAN) and 'delayed transformation' (DELTRAN) models to explore alternative equally parsimonious reconstructions and make apparent ambiguous synapomorphies.

The NEXUS file used for the analyses described above, as well as the matrices of other published studies, are available on our website (http://www.mussel-project.net/).

RESULTS

Forty-four COI sequences were aligned to 650 nucleotide (nt) positions, although the mean sequence length was 638 nt (median, 649 nt). There were no gaps attributed to insertion–deletion events (in-dels); missing data occurred only at the ends of the sequences. Large nuclear ribosomal subunit (28S) sequences were available for fewer taxa: 33 aligned to 473 nt, with an average length of 424.42 nt (median, 429 nt). In-dels were common in the alignment of ribosomal sequences. Morphological data (albeit sometimes incomplete) were available for all taxa considered, including two species, *Chambardia wahlbergi* and *Aspatharia rugifera*, for which there were no sequence data. ILD tests (Farris *et al*., 1995) detected no significant (95%) heterogeneity in pairwise examinations of the three data partitions: COI vs. 28S ($P = 0.409$), COI vs. morphology ($P = 0.363$), and 28S vs. morphology ($P = 0.091$). The supermatrix of the three data sets (COI, 28S, and morphology) was combined for a total of 50 taxa and 1182 characters, 484 of which were parsimony informative.

Phylogenetic analyses of the COI, 28S, and morphology partitions individually behaved as expected, given the results of previous studies (COI: Bogan & Hoeh, 2000; Graf & Ó Foighil, 2000a; Hoeh *et al*., 2001, 2002a; Roe & Hoeh, 2003; 28S: Graf & Ó Foighil, 2000b; Graf, 2002a; morphology: Graf, 2000a; Hoeh *et al*., 2001; Roe & Hoeh, 2003). The trees from our analyses of the separate partitions (not shown; archived at http://www.mussel-project.net/) were not qualitatively different from those of previous studies, accounting for different terminal taxa in the analyses. The analysis of morphology alone recovered more than 30 000 MP trees, each of 126 steps; 28S recovered 904 trees of 544 steps. The COI analysis with the three potentially problematic sequences included resulted in 69 trees of 2307 steps; with those sequences excluded, the set was reduced to 27 trees of 2137 steps.

For both of the separate analyses of COI and 28S, the trees recovered were not statistically different from the 'best' (as determined by the analysis) CE topology when only the individual respective character partition was considered (Kishino–Hasegawa and nonparametric tests implemented in PAUP*); with all characters included, at least some of the recovered topologies from these analyses were significantly different from the CE topology. The most parsimonious topologies recovered from the analysis of morphological characters only were significantly different from the 'best' CE topology regardless of whether the character set was restricted to the morphology partition or the whole supermatrix was considered. However, basal relationships were largely unresolved in the morphology consensus tree (not shown), and the topology was not qualitatively different from those of previous analyses (cited above).

The consensus trees from the combined COI + 28S analyses (i.e. Molecular Only), both with and without the problematic COI sequences included, are shown in Figure 2 (top). With the problematic COI sequences included, the resolution was better than with them excluded. In the former analysis, 15 MP trees were recovered, all of which were 2869 steps in length. With the three problematic sequences excluded, there were 394 MP trees of 2699 steps each (Fig. 2). The CE phylogenies, based on all three character sets, provided estimates of palaeoheterodont phylogeny that were more consistent with the various published phylogenies (cited above). With the three problematic COI sequences included, two trees of 3028 steps were recovered; the strict consensus tree is shown in Figure 2 (bottom). When these sequences were excluded, the resultant set of ten MP trees were each 2841 steps in length (Fig. 2). Although more equally parsimonious trees were saved in the latter analysis, the unresolved areas occurred only within the family Unionidae, and the phylogeny was generally better supported, as judged by bootstrap and Bremer support. On the basis of the Kishino–Hasegawa and nonparametric tests with all the characters included, none of the trees from either combined evidence analysis differed significantly from the 'best' tree. Thus, with the exception of the phylogenetic placement of the species with problematic COI sequences, the analyses yielded compatible results.

The problematic species (*Pseudomulleria dalyi*, *Coelatura aegyptiaca*, and *Obliquaria reflexa*) bear

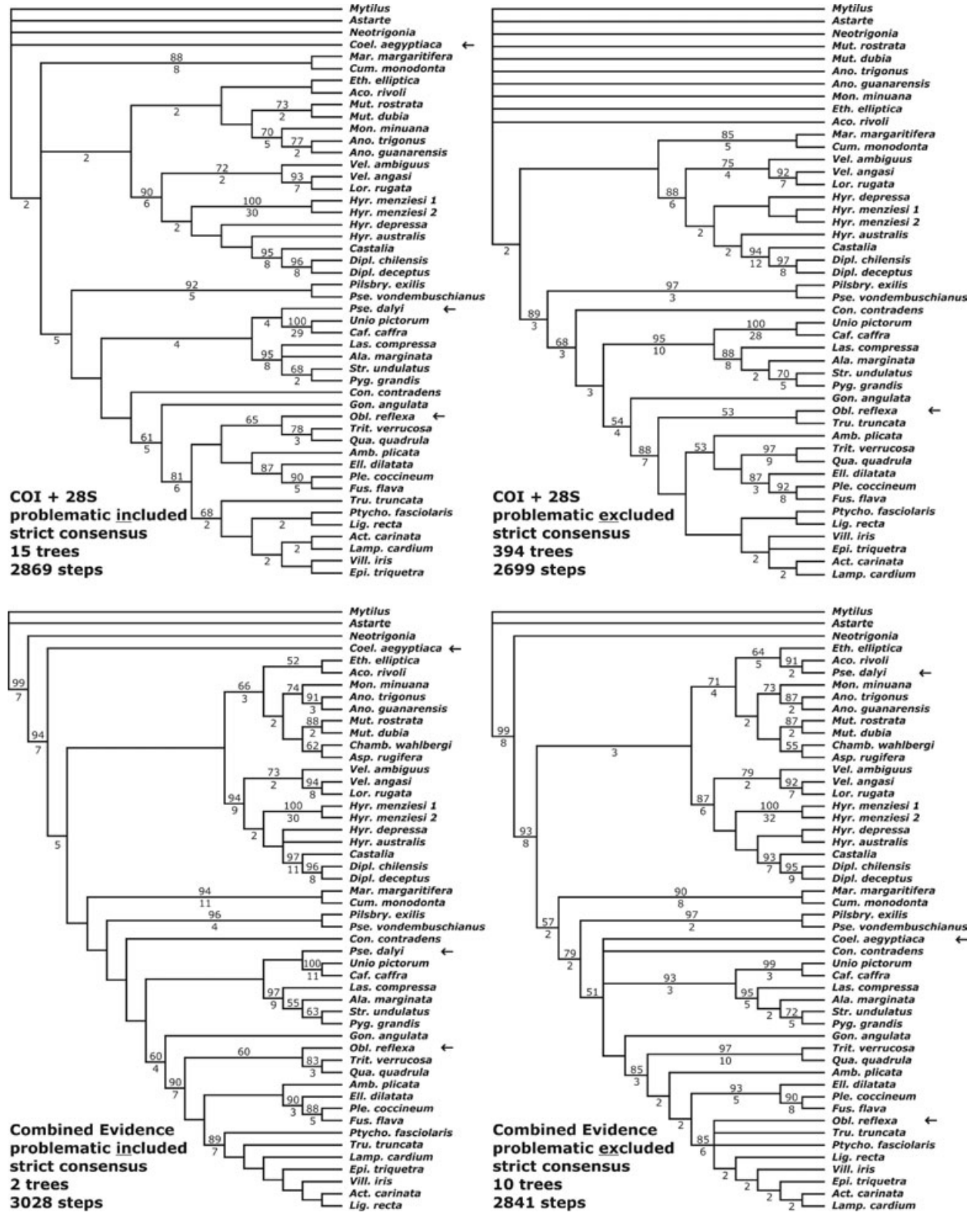

Figure 2. Strict consensus cladograms derived from phylogenetic analysis of molecular and combined evidence data. Numbers above the branches are bootstrap percentages; those below are Bremer decay index values (≥ 2). Arrows indicate taxa with problematic cytochrome oxidase subunit I sequences (as discussed in the text), including cases in which these sequences have been excluded.

most greatly on the problem of the monophyly of the Unionidae (or various subtaxa of that clade; Fig. 2). When COI was analysed alone (not shown), *C. aegyptiaca* and *P. dalyi* were placed as part of an unresolved basal polytomy in the Unionoidea (= Unionidae + Margaritiferidae); this result agreed well with the published phylogeny of Bogan & Hoeh (2000: fig. 1). When we added 28S and morphology for a simultaneous analysis of the three character sets, *C. aegyptiaca* dropped to become the basal member of the Unionoida, and *P. dalyi* moved up the tree to a position sister to the Unionini. For these two problematic taxa, no 28S data were available, although both were coded for morphology (Table 3). When the problematic COI sequences were excluded, *C. aegyptiaca* was recovered as part of a polytomy within the Unionidae, and *Pseudomulleria* was found to be part of a monophyletic Etheriidae (the families to which they had been traditionally assigned; Haas, 1969a).

For *O. reflexa*, all three character sets were available. With the problematic COI sequence included, *O. reflexa* was recovered as sister to the Quadrulini (Fig. 2), as in similar analyses (Lydeard *et al*., 1996). However, on the basis of 28S and morphology only in the CE analysis, *Obliquaria* was placed robustly as part of the Lampsilini (Fig. 2, bottom).

Figure 3 depicts a fully resolved phylogram of one of the ten equally most parsimonious trees (arbitrarily chosen) recovered by CE analysis with the three problematic COI sequences excluded. The branch lengths indicate the sum of the characters of the three partitions that change along these limbs. Those branches not resolved in the strict consensus (Fig. 2, bottom right) are shown with broken lines. Each of the seven traditional families was supported as monophyletic: Trigoniidae, Etheriidae, Mycetopodidae, Iridinidae, Hyriidae, Margaritiferidae, and Unionidae.

The pattern of character transformation for all three character partitions (and various interesting subsets thereof) on this fully resolved tree is shown in Table 4. Of the three main partitions, COI showed the greatest number of informative characters (275); that is, characters that varied in ≥ 2 taxa. However, most of the COI transformations corresponded to third codon positions (73.8%) and were highly homoplastic [consistency index (CI), 0.230]. Overall, the dataset with the highest consistency was 28S [CI, 0.617; retention index (RI), 0.800]. The morphological transformations tended to be synapomorphies, with only 28.6% overall occurring on terminal branches. For COI, on the other hand, the majority of the observed transformations traced to terminal branches amongst palaeoheterodonts (56.9%).

DISCUSSION

The CE phylogeny (Figs 2, 3) serves as the basis for the following discussion of freshwater mussel evolution. At this time, we focus on what the best-corroborated phylogeny indicates about the family-level taxonomy of the Palaeoheterodonta and the supporting evidence (i.e. synapomorphies). By dwelling as we do on morphological characters, we can provide a framework for placing new and heretofore unconsidered freshwater mussels into a natural classification. Molecular data will be crucial as freshwater malacology moves forward, but they are meaningless for species that have not been sequenced. Given the rarity of suitable material for many of the evolutionarily most interesting freshwater mussels, a system that relies strictly on nucleic acid characters will be of limited utility. Figure 4 shows the clades of interest: the families of the Palaeoheterodonta and their interrelationships. The nodes under consideration are indicated by circled letters; our discussion is organized around these nodes. Figure 4 also shows the morphological synapomorphies of the taxa that form the basis of our family group-level classification. A complete diagnosis of the apomorphies along all branches of the MP tree shown in Figure 3 can be found in Appendix 2. Appendix 3 shows our revised classification of the family group-level taxa of the Palaeoheterodonta, including a complete synonymy.

Our analyses incorporate the data from much of the cladistic work published to date on the higher relationships of the Palaeoheterodonta. Our efforts to pull together what we know also serve to highlight the considerable lacuna in our knowledge of freshwater mussel evolution. These areas of future research promise are addressed together with each clade.

THE ARCHETYPICAL HETEROCONCH

The phylogenetic position of the Palaeoheterodonta amongst the Bivalvia has recently been addressed through the comprehensive analysis of Giribet & Wheeler (2002). This study adroitly incorporated the data and results of previous cladistic analyses of bivalve relationships (e.g. Salvini-Plawen & Steiner, 1996; Adamkewicz *et al*., 1997; Hoeh *et al*., 1998; Waller, 1998; Giribet & Distel, 2003; and references cited therein) using a variety of character sets, both morphological and molecular. Although the study of Giribet & Wheeler (2002) leaves some room for future refinement, it is an excellent landmark in the field of bivalve systematics, and provides the necessary backdrop for our discussion of palaeoheterodont evolutionary patterns. Their phylogeny of bivalve orders is summarized in Figure 5.

Amongst the extant Bivalvia, *Neotrigonia*, freshwater mussels, Anomalodesmata, and those

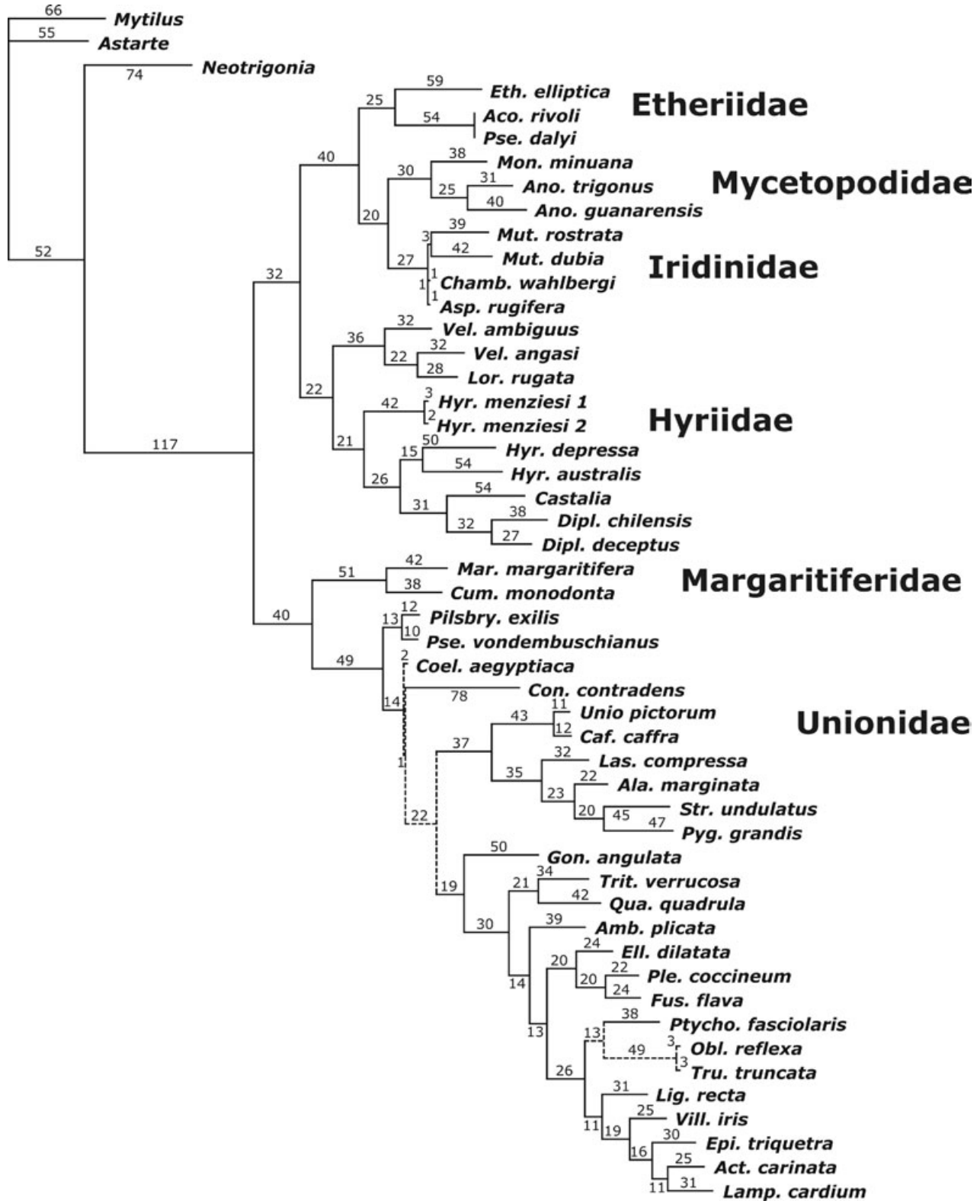

Figure 3. Phylogram of one of the ten equally most parsimonious trees recovered by combined evidence analysis. Numbers associated with the branches are lengths, summed across all character partitions. Branches that were not resolved in the strict consensus (Fig. 2) are shown as broken lines. Problematic cytochrome oxidase subunit I sequences were excluded from the analysis.

Table 4. Character set statistics. Data on the total number of characters (*N*), informative characters (inf.), consistency index (CI, informative characters only), retention index (RI), maximum parsimony steps (S), and the percentage of transformations occurring along terminal branches (ingroup only) for each character set, as traced on the phylogeny in Figure 3

Set/subset	*N*	inf.	CI	RI	S	Terminal (%)
COI	650	275	0.248	0.442	2154	56.9
First codon position	216	59	0.335	0.591	269	56.9
Second codon position	217	13	0.621	0.833	50	62.0
Third codon position	217	203	0.230	0.406	1835	56.8
28S	473	159	0.617	0.800	548	35.2
Morphology	59	50	0.519	0.825	139	28.6
Shell	16	12	0.388	0.600	54	46.0
Ctenidia and labial palps	10	9	0.500	0.865	25	13.6
Mantle	8	8	0.500	0.846	20	22.2
Other anatomy	7	4	1.000	1.000	8	20.0
Life history	12	11	0.667	0.920	22	14.3
Larvae	6	6	0.700	0.903	10	20.0
Overall	1182	484	0.319	0.548	2841	52.1

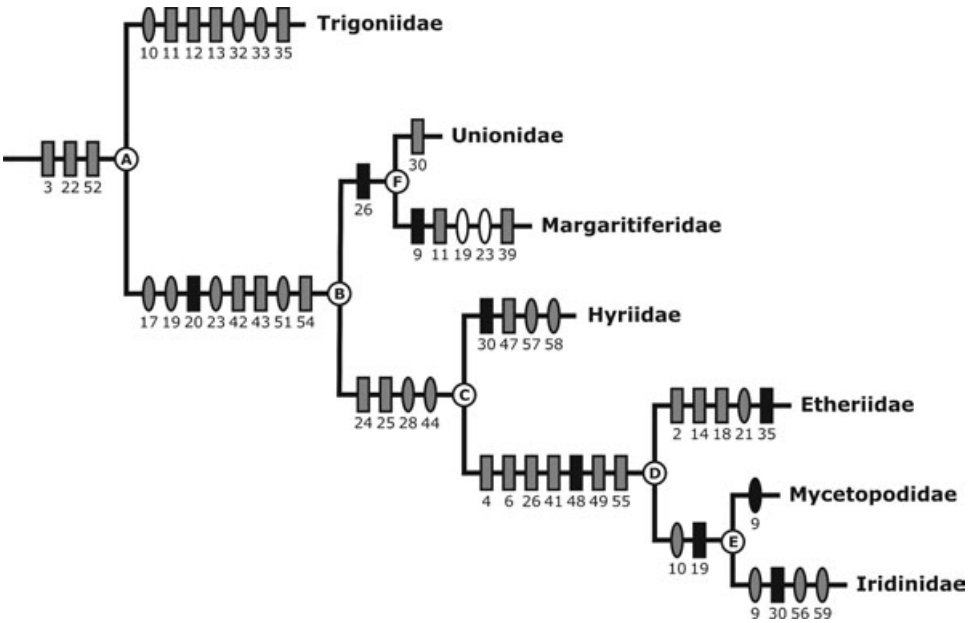

Figure 4. Combined evidence phylogeny of palaeoheterodont families. Internal nodes are labelled with letters (A–F) and are the basis for the organization of the text. Synapomorphies, reconstructed from our analysis (Fig. 3), are marked along the branches. Rectangles indicate unambiguous transformations; ovals indicate transformations that have equally parsimonious alternative optimizations (Appendix 2). Character numbers are listed below each mark; shading denotes character state: white, 0; grey, 1; black, >1 (Appendix 1).

traditional (and apparently nonmonophyletic) taxa known as 'veneroid' and 'myoid' clams comprise the Heteroconchia, which is in turn sister to a monophyletic Pteriomorpha (Fig. 5; Giribet & Wheeler, 2002). Before we discuss our way up the unionoid tree, it is instructive to momentarily digress to describe the 'hypothetical archetypical heteroconch' (HAH). The HAH, given its ancestral position on the phylogeny (Fig. 5), was a chimera of primitive bivalve characters and the derived characters diagnostic of its descendents. Of course, it is unnecessary (as well as intractable and meaningless) to list all the plesiomorphies of the HAH. Nevertheless, it is useful to establish the ancestral condition of the HAH to polarize character change within the ingroup. The character numbers in

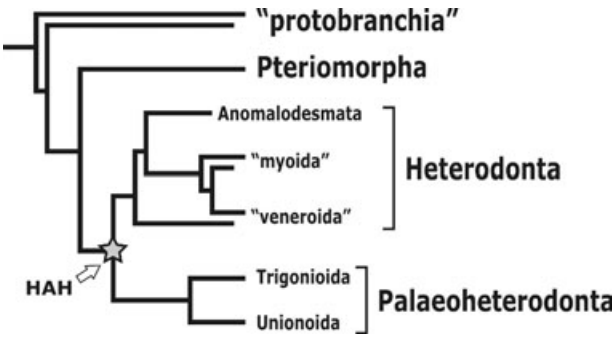

Figure 5. Phylogeny of bivalve orders. Drawn from Giribet & Wheeler (2002: Fig. 11). The star indicates the position of the hypothetical archetypical heteroconch (HAH). The traditional taxa 'protobranchia', 'myoida', and 'veneroida' are depicted as nonmonophyletic.

square brackets refer to those listed in Appendix 1.

The HAH had an equivalved shell [character 14] with an external, opisthodetic ligament [5]. Presumably, the HAH had an aragonitic shell composed of three layers: an outer prismatic layer, with middle and inner layers of nacre [1, 2]. Interestingly, Giribet & Wheeler (2002) listed these last two shell microstructural characters as synapomorphies of the Palaeoheterodonta. It has traditionally been argued that, given the distribution of these characters amongst the basal members of the major bivalve lineages (and, indeed, amongst the Mollusca at large), an aragonitic shell composed of three layers must be representative of 'primitive' conditions (Taylor, Kennedy & Hall, 1969; 1973; Taylor, 1973).

The mantle margins of the HAH lacked fusion [27–31] (except at the mantle isthmus underlying the hinge). Its stomach was Type IV (Purcheon, 1987) [40], and it had Type B, filibranch ctenidia [17, 20] (Atkins, 1938), with a Type I association of the ctenidia and labial palps [25] (Stasek, 1963). The ancestor of the Heterodonta and Palaeoheterodonta had a byssus only as a juvenile [36] and presumably also had an abdominal sense organ [38], although the latter has been lost amongst heterodonts (Giribet & Wheeler, 2002).

Filibranch ctenidia, which lack the tissue-grade fusion of the gill filaments seen in eulamellibranch ctenidia, have, instead, their filaments bound only through the association of ciliary tufts (Cox, 1969; Brusca & Brusca, 1990). As coded by Giribet & Wheeler (2002) and Salvini-Plawen & Steiner (1996), filibranch ctenidia are an intermediate step in the transformation from the unspecialized ctenidia of protobranchs to the derived, filter-feeding organs seen in more derived bivalves (e.g. Ostreoida, Unionoida, and Heterodonta; Boss, 1982). *Neotrigonia*, the only extant genus of the Trigonioida, has Atkins Type B filibranch ctenidia, and, contrary to Morton (1987), this is a symplesiomorphy shared with pteriomorphs, retained from the progenitor of the Autobranchia (Fig. 5; Salvini-Plawen & Steiner, 1996; Waller, 1998).

The HAH also had a rather primitive life history strategy. It was a marine mollusc [42], with separate sexes and freely spawned gametes (nonbrooding) [43]. The larvae were probably veligers [54], similar to those described for pteriomorphs and the Heterodonta (Cragg, 1996).

In addition to the primitive characters enumerated above, Giribet & Wheeler (2002) listed two synapomorphies of the Heteroconchia, characters passed on to the extant heterodonts and palaeoheterodonts from the HAH: a provinculum with differentiated hinge dentition and reduction of the dorsoventral muscles ('DVM') to two or fewer pairs (their character 105). The first of these two traits is perhaps associated with the ancestral 'actinodont' hinge teeth [3] that have been modified in the descendants of the HAH (Scarlato & Starobogatov, 1979). The heteroconch synapomorphy 'reduction of the DVM' was introduced as a character by Salvini-Plawen & Steiner (1996: 50, character 5). We are not precisely sure which muscles present in protobranchs and pteriomorphs are absent in the heterodonts and palaeoheterodonts (Cox, 1969: fig. 31).

Clade A

Subclass Palaeoheterodonta (= Unionoida + Trigonioida)

It was from this ancestral archetypical heteroconch that the Palaeoheterodonta originated. The extant Palaeoheterodonta has been recovered as monophyletic in most cladistic studies, including the present one (Fig. 4; Hoeh *et al*., 1998; Graf & Ó Foighil, 2000a; Giribet & Wheeler, 2002; Graf, 2002a; but see Salvini-Plawen & Steiner, 1996). Giribet & Wheeler (2002) listed three morphological synapomorphies of the Palaeoheterodonta: aragonitic shell composed of three layers [1], three duct orifices of the digestive diverticula, and sperm with multiple acrosomal vesicles [52]. The first of these was discussed above as a likely plesiomorphic condition in the Mollusca. The last two, however, are questionable as synapomorphies of the Palaeoheterodonta.

Healy (1989: 83) reported that, 'spermatozoa of *Neotrigonia* spp. and unionoids (*Velesunio*) – alone among the Bivalvia (and possibly the Mollusca) – possess an acrosomal complex composed of multiple acrosomal vesicles'; however, as subsequently noted by Healy (1996), other studies have found freshwater mussels to have either a single, minute acrosome (*Ligumia*) or none at all (*Diplodon*, *Unio*, and *Hyriop-*

sis; Peredo, Garrido & Parada, 1990). Rocha & Azevedo (1990) reported that, in *Anodonta*, there are multiple proacrosomal vesicles that fuse to become a single acrosome. In Healy's (1989, 1996) figures, these electron-dense vesicles are referred to as 'proacrosomal vesicles'. The generality of this potential synapomorphy needs to be further examined in the Unionoida.

Traditionally, the traits that affiliated the Trigonioida and Unionoida were a prismatonacreous shell [2], an unfused mantle [27–31], and schizodont dentition [3] (Newell, 1965, 1969). The first two of these are symplesiomorphies, but, despite the protest by Cox (1969: N52) of their being of 'little value', we recover schizodont teeth as a synapomorphy uniting *Neotrigonia* and freshwater mussels (Fig. 4). Schizodont dentition, as it occurs in extant palaeoheterodonts (Thiele, 1934), is characterized by posterior, interlocking lamellar hinge teeth running parallel to the external ligament; these are the 'pseudolaterals'. In *Neotrigonia* and unionoids with primitive hinges (e.g. *Velesunio*, *Coelatura*, and *Contradens* in the present analysis), the anterior teeth resemble shorter versions of the lamellar laterals, but in front of the umbos (Fig. 6A, B). Amongst the more derived members of the Unionidae (Ambleminae), these anterior teeth become more peg-like and robust, and are generally referred to as 'pseudocardinal teeth' (Fig. 6C). Other lineages have secondarily become edentulous, as described below.

In addition to nucleic acid characters, schizodont dentition, and (perhaps) a unique sperm morphology, the Palaeoheterodonta also appear to share calcification of the chitonous rods supporting the gill filaments (Taylor *et al.*, 1973; shown in Atkins, 1938) [22], although Morton (1987) (also Giribet & Wheeler, 2002) questioned whether the condition seen in *Neotrigonia* could be distinguished from that of nonpalaeoheterodonts.

Order Trigonioida (= Trigoniidae = Neotrigonia) (Fig. 7)

The most parsimonious reconstruction of morphological evolution on the phylogeny in Figure 4 suggests four apomorphies of *Neotrigonia*: lateral muscle scars (Smith, 1983) [11], radial postlarval and adult shell sculpture [12, 13], and an anchor-like foot with a well-defined 'toe' and 'heel' (Newell & Boyd, 1975; Morton, 1987) [35]. In addition to these, there are three characters equivocally traced to the *Neotrigonia* branch: the posterior retractor scar is distinct from the posterior adductor [10], and both the incurrent and excurrent apertures are distinctly papillate [32, 33]. Given that the historical Trigonioida was much more diverse than we are able to represent here, it is unclear

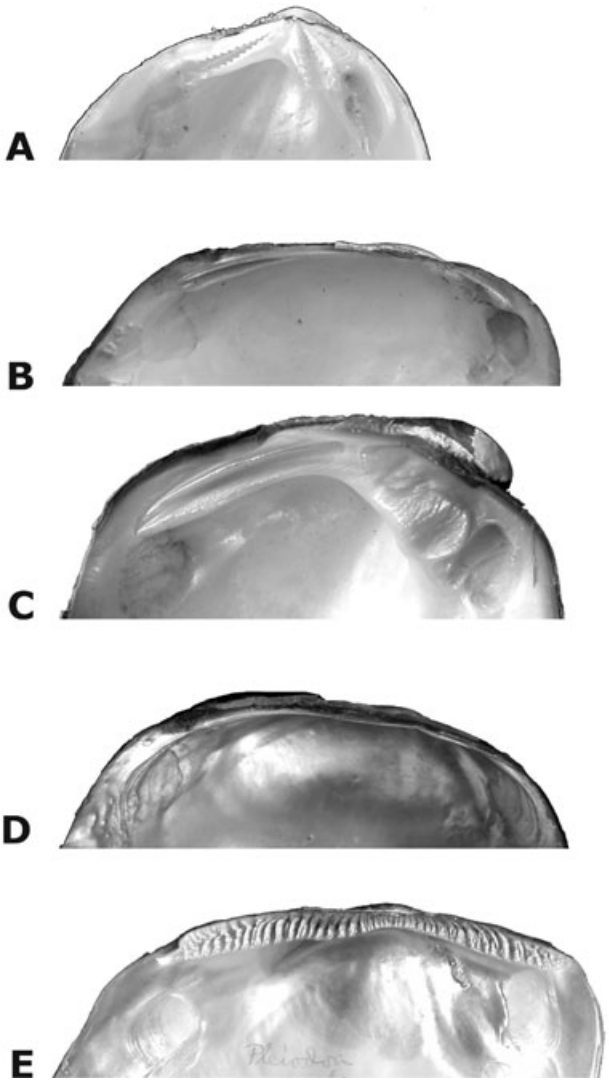

Figure 6. Palaeoheterodont hinges. A, *Neotrigonia pectinata* (Lamarck, 1819) ANSP 71515. B, *Lamellidens marginalis* (Lamarck, 1819) ANSP 41775. C, *Fusconaia ebena* (Lea, 1831) ANSP 188259. D, *Aspatharia chaiziana* (Rang, 1835) ANSP 41813. E, *Pleiodon ovata* (Swainson, 1823) UMMZ 112006.

Figure 7. A representative of the Trigoniidae. *Neotrigonia margaritacea* UMMZ 253004.

whether these synapomorphies will hold up when fossil evidence is considered. What we can say is that *Neotrigonia* is a 'living fossil', retaining many of the characteristics of the no longer hypothetical, ancestral heteroconch. As described above, *Neotrigonia* is the only genus in the Heteroconchia to possess filibranch ctenidia. The presence of a veliger has not been confirmed but is presumed (Tevesz, 1975; Prezant, 1998; Ó Foighil & Graf, 2000).

Watters (1994a) argued that the external shell sculpture [12, 13] seen in both *Neotrigonia* and certain freshwater mussels was inherited from their common ancestor. Our phylogeny, however, supports shell sculpture arising multiple times amongst the palaeoheterodonts (Appendix 2).

Clade B

Order Unionoida, the freshwater mussels (= Etherioidea + Unionoidea)

The monophyly of the Unionoida has been supported by all relevant cladistic studies (Hoeh *et al*., 1998, 2001, 2002a; Bogan & Hoeh, 2000; Graf, 2000a; Roe & Hoeh, 2003). Our analysis supports eight morphological synapomorphies of the Unionoida (Fig. 4). Four of these characters are associated with the morphology of the ctenidia: eulamellibranch ctenidia with tissue-grade fusion of the gill filaments [17], discontinuous (perforate) septa dividing the interlamellar spaces into water-tubes (at least in the brooding demibranchs) [19], ctenidia with Atkins Type D ciliary currents [20], and the ctenidia fused to the adjacent mantle forming a complete (or 'slightly incomplete') diaphragm [23]. The remaining synapomorphies refer to life history and reproductive traits: restriction to freshwater [42], parental care (brooding) [43], spermatozuegmata [51], and larvae that are obligate parasites of aquatic vertebrates [54]. As the interpretation of some of these characters as synapomorphies at this level requires certain assumptions to be made explicit, and will influence how some transformations are treated further up the tree, we discuss them here in some detail.

Two circumstances contribute to equivocal character transformation polarizations along the Unionoida branch. The first is that the Margaritiferidae shares the deficiency of a number of derived characters with *Neotrigonia*, including the lack of vertical septa dividing the interlamellar spaces of the demibranchs and the absence of fusion of the ascending lamellae of the outer demibranchs to the adjacent mantle along their entire length. To put a cladistic spin on the traditional interpretation, most authorities have regarded these similarities as symplesiomorphic – that is, margaritiferid morphology is 'primitive' (Ortmann, 1912b; Heard, 1974; Davis & Fuller, 1981). However, recent molecular phylogenetic studies have favoured the hypothesis that the apparently simple anatomy of the Margaritiferidae may actually be derived (Hoeh *et al*., 2001, 2002a; Graf, 2002a). These apparent character 'reductions' are discussed below with the Margaritiferidae.

The second complicating circumstance is that characters dealing with brooding and parasitic larvae are inapplicable to the basal members of the nonbrooding, strictly free-living outgroup taxa: *Neotrigonia* and the Heterodonta. Although restriction to freshwater, parental care (brooding), and larval parasitism can be invoked to diagnose freshwater mussels, specific larval morphologies and brooding strategies have been traditionally applied to denote subgroups (Parodiz & Bonetto, 1963; Heard & Guckert, 1970). At the base of the unionoid tree, multiple equally parsimonious optimizations of character transformation are possible for these states, and the result is a situation that is more complicated than the traditional authoritarian story of freshwater mussel classification. It is therefore important to critically appraise the evolution of these brooding and larval morphologies within the Unionoida.

As their common name suggests, freshwater mussels are restricted to freshwater. Other bivalve families have independently invaded freshwaters, most notably Corbiculidae, Sphaeriidae, and Dreissenidae (McMahon, 1991; Park & Ó Foighil, 2000), but none as successfully as the Unionoida. As the Palaeoheterodonta is without terrestrial representatives with overland vagility, the initial invasion of freshwater by their marine progenitor must have occurred via estuaries, up rivers from the ocean. As such, two important aspects of unionoid life history – parental care and parasitism – apparently adapt freshwater mussels to reproduction in flowing water.

The plesiomorphic veliger of other bivalves provides an appropriate contrast. Marine bivalves can freely spawn their gametes and can reasonably expect their fusion in the water column. Prevailing currents facilitate the dispersion of the developing zygotes and veligers to colonize hospitable habitats. However, in a freshwater stream, reliance on the prevailing current for dispersal leads inevitably to inhospitable habitats. Freshwater mussels (freshwater bivalves, in general) have evolved traits to counteract this unidirectional fall back to the ocean.

One of these traits is parental care by brooding. Larval brooding has evolved independently in the freshwater Corbiculidae, Sphaeriidae (both Heterodonta), and Unionoida (Park & Ó Foighil, 2000). Amongst freshwater mussels, the larvae are brooded in the interlamellar spaces of the females' demibranchs, and these spaces are divided by vertical septa formed of interlamellar junctions into a series of compartments known as 'water-tubes' (Ortmann, 1911b). The plesio-

morphic condition amongst the Unionoida is for the interlamellar septa to be perforated (i.e. discontinuous) (Graf, 2000a, 2002a), although complete septa have arisen convergently within the Unionidae and the Etherioidea (Fig. 4; Clade C), and the Margaritiferidae lack vertical septa all together (see below).

It has traditionally been assumed that the characteristic of brooding larvae in all four demibranchs (tetrageny) is 'primitive' amongst freshwater mussels (Ortmann, 1912b; Heard & Guckert, 1970; Davis & Fuller, 1981); this conclusion has been supported in certain morphology-based cladistic studies (Graf, 2000a; Hoeh *et al*., 2001: Fig. 14.2; Roe & Hoeh, 2003). A strict cladistic interpretation of the CE phylogeny, however, shows that the most parsimonious ancestral brooding morphology is equivocal (Fig. 8). The plesiomorphic brooding condition of the Unionoidea (= Unionidae + Margaritiferidae) is tetrageny, whereas endobranchy (brooding only in the inner demibranchs) is the rule amongst etherioideans. On the basis of the taxonomic distribution of these characters amongst extant groups and the topology in Figure 4, it is equivocal whether brooding in all four demibranchs or only in the inner pair is the plesiomorphic condition for the order. Thus, the inapplicability of brooding morphology to nonbrooding outgroup taxa (i.e. *Neotrigonia* and the Heterodonta) provides no basis for polarizing the hypothesized character transformations.

Some recent phylogenetic analyses have maintained tetrageny as a synapomorphy of the Unionoida, but this conclusion generally follows from weakly supported topologies recovering the tetragenous Margaritiferidae as basal to the rest of the Unionoida (Graf, 2000a; Hoeh *et al*., 2001: Fig. 14.2; Roe & Hoeh, 2003). These results were based on homoplastic morphological characters (see Discussion under Margaritiferidae below), and molecular analyses have generally placed margaritiferids as sister to the Unionidae (Graf & Ó Foighil, 2000a; Graf, 2002a) or nested within this family (Bogan & Hoeh, 2000; Hoeh *et al*., 2001, 2002a), in either case supporting the Unionoidea as we use it here (Fig. 4; Clade F). Graf & Ó Foighil (2000a) and Hoeh *et al*. (2001, 2002a) favoured endobranchy as the plesiomorphic brooding condition amongst the Unionoida. This was based in all cases on weakly supported mtDNA phylogenies suggesting etherioidean paraphyly. However, other analyses (including that in this study) have supported the monophyly of the Etherioidea (Fig. 4; Graf, 2000a; Hoeh *et al*., 2001: Fig. 14.2; Roe & Hoeh, 2003).

Hoeh *et al*. (2001: 175–176) (see also Ihering, 1901) provided a structural explanation for ancestral endobranchy:

'The ancestral nature of endobranchous brooding for the Unionoida is most likely the result of functional constraints.

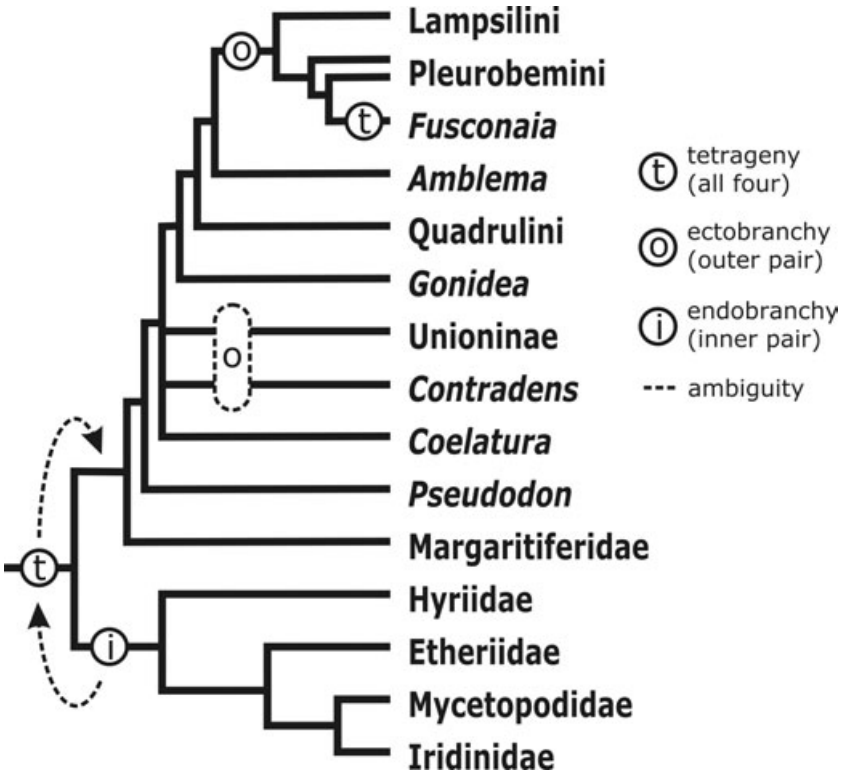

Figure 8. Evolution of brooding morphology in the Unionoida. See text for discussion.

The paired unionoid gonopores open into the inner suprabranchial canals above the inner demibranchs. Ciliary tracts are most likely used to facilitate the transport of oocytes from the inner suprabranchial canals to the outer suprabranchial canals (and then into the outer demibranchs) in tetragenous and ectobranchous taxa. Therefore, the initial transition to brooding in the ancestral unionoid lineage may have been aided by the use of only the inner demibranchs.'

We can provide no objective, phylogenetic criterion for favouring tetrageny over endobranchy as the plesiomorphic brooding morphology of the Unionoida, and Hoeh *et al*.'s structural argument remains an unfalsified hypothesis for ancestral endobranchy.

Another character of unionoids that facilitates their habitation of flowing freshwaters is their parasitic larval stage. Almost all freshwater mussel larvae are obligate parasites of freshwater fishes, with few reported exceptions (Howard & Anson, 1923; Allen, 1924; Howard, 1951; Parodiz & Bonetto, 1963; Kondo, 1990; Watters, 1997; Watters & O'Dee, 1998). Whereas adult unionoids cannot vigorously work against the current, their active larval host can, dispersing freshwater mussels upstream. In addition, as reviewed by Kat (1984) and Wächtler *et al*. (2001), the mussels do gain some nutrition from their hosts (i.e. the relationship is truly parasitic rather than just strictly phoretic). It has also been hypothesized that the host provides an 'osmotic medium favourable for larval development' (Ziuganov *et al*., 1994: 23).

For all parasitic unionoids, it is during their infectious period that they complete their metamorphosis (Kat, 1984). That being said, there is enormous variation in larval morphology, mode of attachment, and degree of specialization to particular host fishes. This suite of characters has proven to be both useful and contentious throughout the history of freshwater mussel classification (Parodiz & Bonetto, 1963; Modell, 1964).

Amongst freshwater mussels, there are two basic larval types: 'glochidium' and 'lasidium'. Glochidia are small (70–350 μm) with calcified, bivalved larval shells and a single adductor muscle (Fig. 9A–D). Lasidia are also small (85–150 μm, not including the 'larval thread'), but with an uncalcified, univalved larval shell and a trilobed body (Fig. 9E, F). At least one mussel, *Mutela bourguignati* (Bourguignat 1885), has a modified lasidium known as a 'haustorium' (Fig. 9F). Whereas both glochidia and lasidia attach to their host by encysting in either gill or fin epithelium, haustoria attach via tubular appendages (Fryer, 1959, 1961). The latter process has recently been documented by Wächtler *et al*. (2001).

As reviewed by Graf (2000a), Parodiz & Bonetto (1963: 185) proposed that the extant Unionoida could be divided into two superfamilies based solely on larval type.

'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.'

The mussels with glochidia were considered to comprise the 'Unionacea' (= Unionidae + Margaritiferidae + Hyriidae), and the 'Mutelacea' was made up of the lasidium-bearing families (Mycetopodidae + Muteliidae [= Iridinidae] + Etheriidae). The classification of Parodiz & Bonetto (1963) is still prevalent in the freshwater mussel literature. The most parsimonious explanation, however, suggests that the original parasitic larval condition of the Unionoida was a glochidium, and it was from this condition that lasidia evolved (Fig. 4; Graf, 2000a).

The ancestral unionoid glochidial type remains equivocal. Glochidial morphology is as variable as the hundreds of species that have glochidia (Hoggarth, 1999), but they have traditionally been grouped into only three morphological types, hooked, unhooked, and axe-head (Fig. 9), and this classification continues in common usage (Lefevre & Curtis, 1910; Wächtler *et al*., 2001). The confusion over the ancestral larval morphology is a result of both the imprecision of the traditional larval categories and competing phylogenies with their implicit hypotheses of larval character evolution. We can dispel some of this ambiguity given the results of our analyses (Fig. 10).

Hooked glochidia, as traditionally described, are found in two separate clades of freshwater mussels, the Unioninae (including the anodontines, *Unio*, and related genera; Fig. 3) and the Hyriidae (Wächtler *et al*., 2001). The glochidia of these groups tend to be larger (> 250 μm in length, but see Jones, Simpson & Humphry, 1986), subtriangular in shape, and possess the medioventral hooks that merit such attention (Fig. 9A, B). However, as highlighted over 80 years ago by Ortmann (1921: 468), the hooked glochidia of the Unioninae and Hyriidae are analogous rather than homologous.

'The [Hyriidae] hook differs entirely from the [Unioninae] hook. The latter is triangular, attached by a broad base to the point of the lower margin, and carries upon its upper surface a number of fine spinules. The [Hyriidae] hook is long and narrow, spiniform, with very narrow base, articulated to the point of the lower margin, and without any spinules on the upper surface, and furthermore has a peculiar S-shaped curve.'

Ortmann (1921) was specifically discussing the Neotropical hyriids. Jones *et al*. (1986) and Smith (1998) confirmed that the Hyriidae of Australia also lack the broad-based hook and proximal spinules of the Unioninae. Unhooked glochidia, as their name suggests, lack ventral hooks. They tend to be subcircular to subelliptical or spatulate in shape, and vary amongst genera in length (from less than 50 to 250 μm; Hoggarth, 1999). Glochidia without hooks are known from the

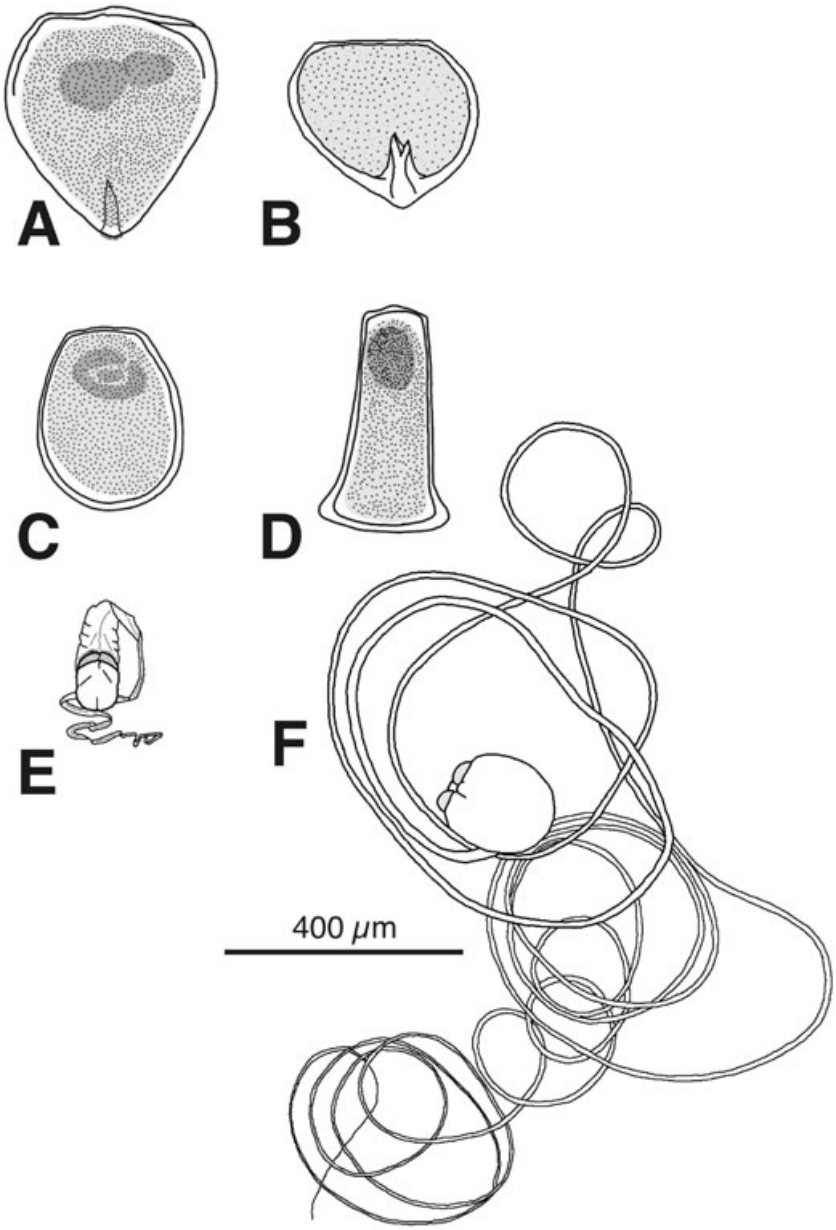

Figure 9. Unionoida parasitic larval types. A, Hooked-type glochidium of *Alasmidonta marginata* (Unioninae). B, Hooked-type glochidium of *Triplodon corrugatus* (Lamarck, 1819) (Hyriidae). C, Unhooked-type glochidium of *Villosa iris* (Lampsilini). D, Axe-head-type glochidium of *Potamilus alatus* (Say, 1817) (Lampsilini). E, Lasidium of *Monocondylaea paraguayana* (d'Orbigny, 1835) (Mycetopodidae). F, Haustorium-type lasidium of *Mutela bourguignati* (Bourguignat, 1885) (Iridinidae). A, C, D, redrawn from Baker (1928); B, E, redrawn from Bonetto & Ezcurra (1963); F, re-drawn from Fryer (1961).

Margaritiferidae and the Unionidae (excluding the Unioninae and *Potamilus*). Thus, in the Unionoida, there are properly four glochidial types: (1) subcircular unhooked; (2) subtriangular hooked with spinules; (3) subtriangular hooked without spinules; and (4) axe-head, which are limited to the lampsiline genus *Potamilus* (Roe, Simons & Hartfield, 1997; Roe & Lydeard, 1998). Our coding for character 57 reflects these previous hypotheses (Appendix 1).

Some of the historical confusion over the polarity of transitions in unionoid larval evolution can be attributed to investigator bias. For example, in the recent review of freshwater mussel larvae by Wächtler *et al*. (2001: 101), they erred when stating, 'From the com-

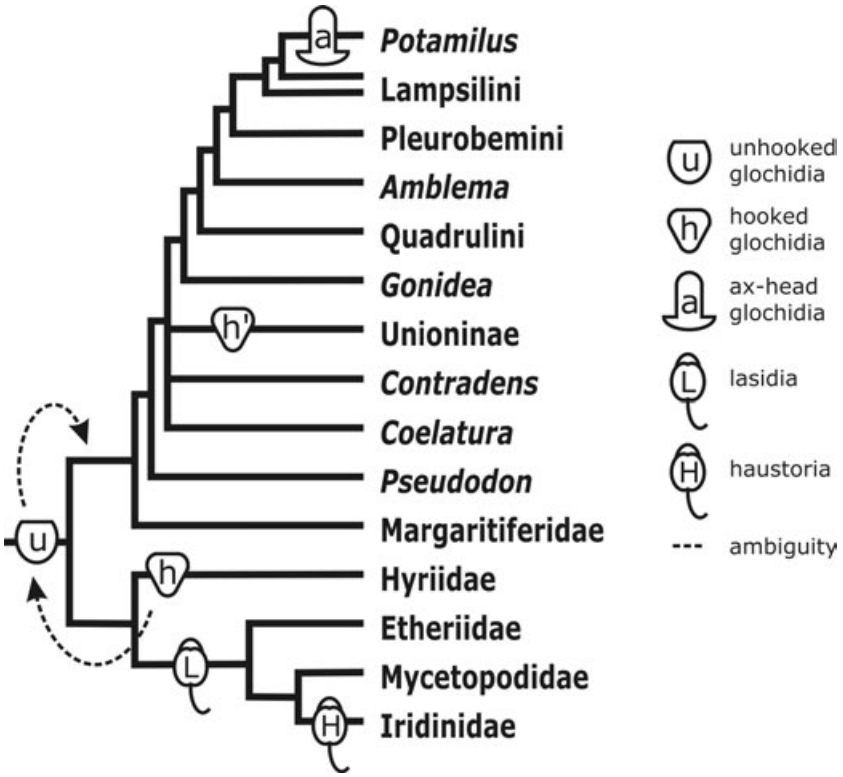

Figure 10. Evolution of larval morphologies in the Unionoida. *Potamilus* was not included in the present analysis, but is well supported amongst the Lampsilini (Roe & Lydeard, 1998). See text for discussion.

parative observations available, one can conclude that the larger glochidia with hooks are the more general and widely distributed type, whereas the smaller and hookless ones represent the more specialized type with restricted occurrence'. Their conclusion was based on the sample of freshwater mussels on which they chose to focus (mostly hyriids and unionines) and their lack of a phylogeny. Even when glochidia have been treated in cladistic studies, the authors have invariably ignored the distinction between the two types of glochidia bearing ventral hooks in their character coding (Graf, 2000a; Hoeh *et al*., 2001; Roe & Hoeh, 2003).

There is wide agreement amongst recent analyses that hooked glochidia with spinules and axe-head-type glochidia each evolved from unhooked ancestors within the Unionidae (Graf, 2000a, 2002a; Hoeh *et al*., 2001, 2002a; Roe & Hoeh, 2003). That is, the most parsimonious conclusion is that unhooked glochidia are plesiomorphic amongst the Unionoidea (Fig. 4; Clade F). However, in the end, we reach a logical impasse analogous to that described above for the plesiomorphic brooding morphology. Because the outgroups of the Unionoida do not have parasitic larvae, there is no objective criterion to decide whether unhooked glochidia were the primitive larval morphology and were subsequently modified to hooked glochidia in the Hyriidae, or hooked glochidia are plesiomorphic and the hooks were lost in the ancestor of the Unionoidea. We somewhat arbitrarily favour the hypothesis that both varieties of hooked glochidia were independently derived from hookless forms (Fig. 10).

Salvini-Plawen & Steiner (1996: 50–51, character 10) and Giribet & Wheeler (2002: 300, character 55) attributed posterior siphons to the Unionoida, both hypothesizing homology (implicitly through their coding) with the conditions found amongst the 'veneroids', 'myoids', and Anomalodesmata (Fig. 5). However, neither study explicitly defined what they considered to constitute a 'siphon'. On Giribet & Wheeler's (2002: Fig. 6) optimal topology, the presence of siphons cannot be unambiguously optimized. It is either a synapomorphy of their Nuculanoidea + Autolamellibranchia clade with numerous independent losses, or homoplastic with convergent origins throughout the Bivalvia. Amongst the phylogenetic analyses of the freshwater mussels, determinations of the plesiomorphic extent of posterior aperture development have been biased by the topology of the phylogeny under consideration and alternative coding schemes (Graf, 2000a; Hoeh *et al*., 2001; Roe & Hoeh, 2003).

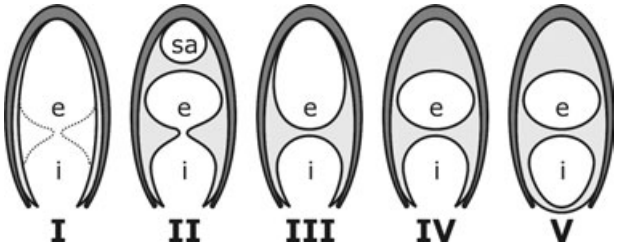

Figure 11. Diagram of patterns of posterior mantle fusion types in the Palaeoheterodonta. In the diagrams, the darker, outer layer represents the outer fold of the mantle, and the inner layer is the inner fold. The middle sensory fold is greatly reduced in the Unionoida. See text for discussion. e, excurrent aperture; i, incurrent aperture; sa, supra-anal aperture.

Based on our analysis, the plesiomorphic posterior mantle condition observed amongst unionoids would be better characterized by the absence of mantle fusion, with varying degrees of aperture development appearing independently in various lineages. We recognize five categories of posterior mantle fusion, simplified as Types I–V (Fig. 11). Type I is the simplest condition, present in *Neotrigonia* and the Margaritiferidae. The posterior mantle is broadly unfused, and the diaphragm dividing the infra- from the suprabranchial chamber is grossly incomplete. Division of the mantle cavity is functionally achieved via 'pallial ridges' (Fig. 12A; Gould & Jones, 1974; Smith, 1980). Type II occurs only in the Unionidae. There is no mantle fusion between the incurrent and excurrent apertures, but the ctenidia are fused to the adjacent mantle along their length, forming a 'slightly incomplete' diaphragm (Davis & Fuller, 1981). The inner folds of the mantle are fused for a short distance dorsal to the excurrent aperture and then re-open to form a third, supra-anal aperture (Fig. 12B).

Type III is the plesiomorphic condition amongst etherioideans. The inner folds of the mantle are fused between the incurrent and excurrent apertures; this type occurs in both the Etheriidae and Mycetopodidae (Fig. 12C). Type IV has fusion between the apertures, but there is also fusion of the inner folds of the mantle dorsal to the excurrent aperture, often forming an excurrent siphon. The principal distinction between Types III and IV is that, in the former, the inner folds are not joined independent of the outer folds of the mantle above the excurrent aperture. Type IV posterior mantle fusion occurs in the Hyriidae (Fig. 12D) and Aspathariinae (Iridinidae).

In certain genera with Type IV posterior pallial fusion, there is often a short association of the inner folds ventral to the incurrent aperture, e.g. *Castalia* (Ortmann, 1921) and *Chambardia* (+ *Spathopsis*) (Ortmann, 1918a; Mandahl-Barth, 1988). The presence of these short attachments appears to vary within species and is never associated with a pallial sinus (Fig. 12E). Type V is characterized by retractable incurrent and excurrent siphons and a pallial sinus, present only in *Mutela*, *Pleiodon*, *Chelidonopsis*, and related genera (Fig. 12F). The full range of palaeoheterodont aperture morphology falls under Yonge's (1957, 1982) Type A siphons; further details of these posterior mantle conditions are discussed below under each of the families.

A previously unconsidered potential synapomorphy of the Unionoida, or perhaps the Palaeoheterodonta, is the presence of doubly uniparental inheritance (DUI) of mitochondria [53]. The normal course for mitochondrial inheritance in metazoans is via the maternal lineage. However, in both marine mussels (Mytilidae; Zouros *et al.*, 1994a, b) and freshwater mussels (Hoeh *et al*., 1996; Liu, Mitton & Wu, 1996; Curole & Kocher, 2002), separate male and female mitochondrial lineages are maintained. With DUI, the mitochondrial contribution to a male zygote via the sperm is sequestered into the germ line. Thus, although female mussels are homoplasmic (i.e. their entire mitochondrial complement is maternal), males are heteroplasmic. Male somatic tissue contains mainly maternal mitochondria (F-type), with varying proportions of M-type; male gonads and testes, however, harbour (almost exclusively) M-type mitochondria inherited from the paternal lineage. DUI of mtDNA has not been demonstrated in other groups of bivalves (Hoeh, Stewart & Guttman, 2002b).

In the Unionoida, both M- and F-type mitochondria have been reported from both basal lineages of freshwater mussels (Etherioidea: *Hyridella menziesi*; Unionoidea: several species; Hoeh *et al*., 2002b). DUI has not been confirmed for *Neotrigonia*. However, the phylogeny of mitotypes (Hoeh *et al*., 2002b: fig. 1) suggests that the historical split between the M-type and F-type mitochondrial lineages in palaeoheterodonts predates the divergence between trigonioids and the freshwater mussels. More data are needed from other bivalve groups to determine the generality of DUI.

CLADE C

SUPERFAMILY ETHERIOIDEA (= HYRIIDAE + ETHERIIDAE + MYCETOPODIDAE + IRIDINIDAE)

The Etherioidea *s.l.* has not recently been considered a valid taxon (but see Ortmann, 1921; reviewed in Graf, 2000a). However, most cladistic analyses of morphological characters (Graf, 2000a; Hoeh *et al*., 2001: Fig. 14.2; Roe & Hoeh, 2003; this study: Fig. 4) and our CE treatment (Fig. 2) have recovered a clade that includes the Hyriidae as sister to the lasidium-bearing mussels (= Mutelacea *sensu* Parodiz & Bonetto, 1963).

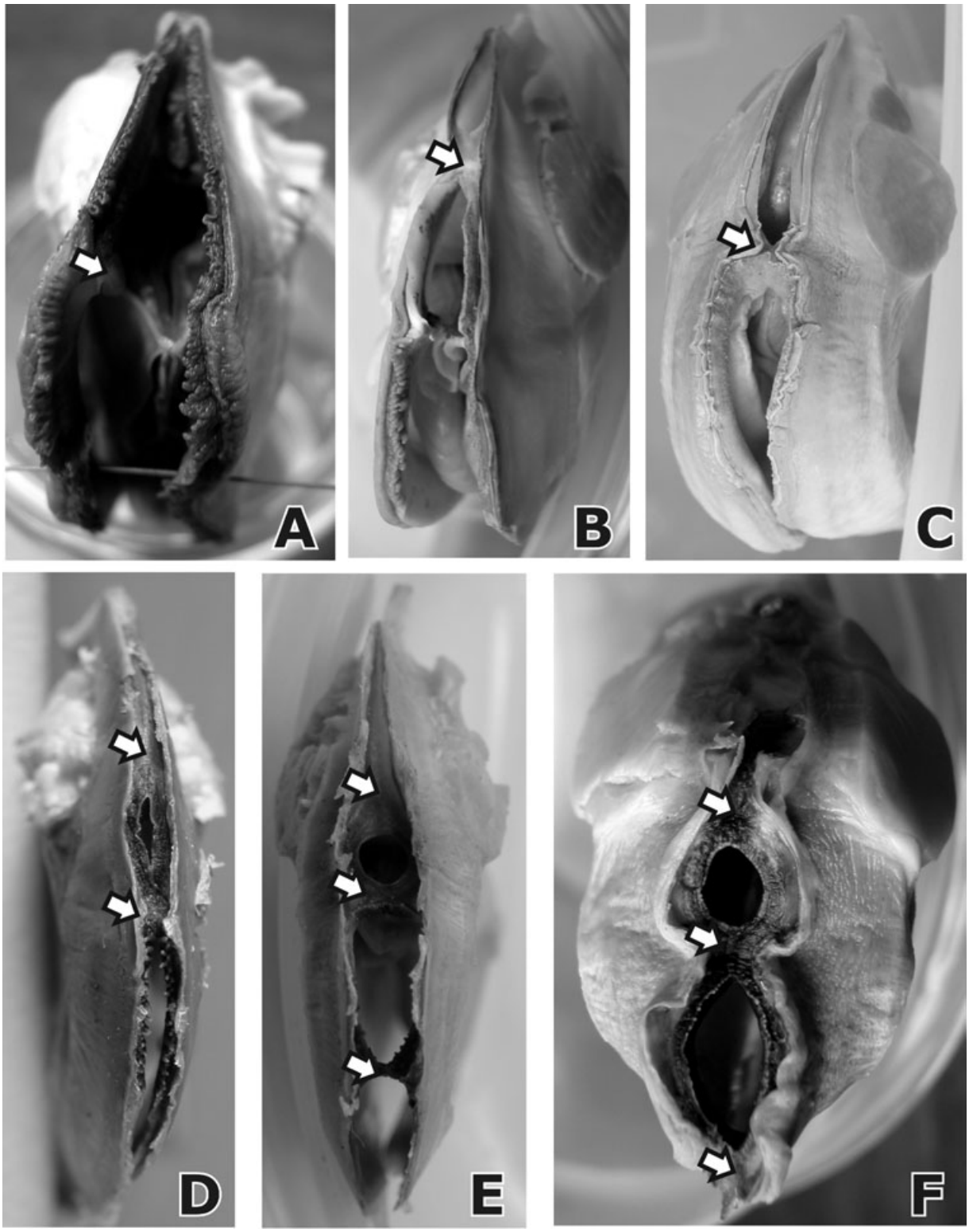

Figure 12. Photographs of palaeoheterodont apertures. A, Type I, *Margaritifera margaritifera* ANSP A7659 (Margaritiferidae). B, Type II, *Actinonaias carinata* ANSP A11149 (Unionidae). C, Type III, *Anodontites trapesialis* (Brug., 1797) INHS 17028 (Mycetopodidae). D, Type IV, *Hyridella menziesi* ANSP 413054 (Hyriidae). E, Type IV, *Chambardia nyassaensis* (Lea, 1864) ANSP A17036 (Iridinidae). F, Type V, *Pleiodon spekii* (Woodward, 1859) ANSP 413055 (Iridinidae). Arrows indicate mantle structures: either 'pallial ridges' (A) or mantle fusion (B–F).

Graf (2000a) advocated removing the Hyriidae from their traditional placement amongst the Unionoidea to the Etherioidea *s.l.*, and we concur with revising the classification of the Unionoida rather than clinging to a traditional arrangement supported by neither data nor modern phylogenetic theory (Appendix 3).

In our analysis, Etherioidea is supported by four morphological synapomorphies: the inner demibranchs are fused to the visceral mass [24], the anterior ends of the inner demibranchs attach to the visceral mass adjacent to the labial palps [25], the mantle is fused between the incurrent and excurrent apertures [28], and the larvae are brooded only in the inner demibranchs (endobranchy) [44]. In our topology (Fig. 4), the first two of these are unambiguous in their support of a monophyletic Etherioidea; however, the last two are somewhat equivocal, as the discussion above about the evolutionary polarity of aperture and brooding morphological transformations indicates.

Family Hyriidae (Fig. 13)

All previous phylogenetic analyses have recovered the Hyriidae as monophyletic. However, in those studies, there has been disagreement over the phylogenetic position of this family. Although morphologically based studies have placed hyriids as sister to the lasidium-bearing mussels (= Etheriidae + Mycetopodidae + Iridinidae; Clade D) (Graf, 2000a; Hoeh *et al.*, 2001: fig. 14.2; Roe & Hoeh, 2003), some analyses of rapidly evolving mtDNA have recovered the Hyriidae as the basal members of the Unionoida, sister to a clade composed of all other freshwater mussels (Bogan & Hoeh, 2000; Hoeh *et al.*, 2001, 2002a). Our re-analysis recovered the Hyriidae as monophyletic and sister to the lasidium-bearing mussels (Fig. 4), and this is based on at least four synapomorphies: fusion of the mantle dorsal to the excurrent aperture, forming a siphon (Fig. 11; Type IV) [30], larvae brooded only in a middle portion of the inner demibranch [47], subtriangular glochidia with medioventral hooks (but lacking spines) [57], and glochidia packaged into conglutinates (Walker *et al.*, 2001) [58].

Hyriids have an excurrent siphon with a slit-like aperture formed by fusion of the inner folds of the mantle (Fig. 12D); there is generally no fusion ventral to the simple incurrent aperture. The exception is *Castalia* (Castaliini), which occasionally has a short fusion ventral to the incurrent aperture, although its presence may vary within species, and it is never associated with a pallial sinus (Ortmann, 1921). Dorsal

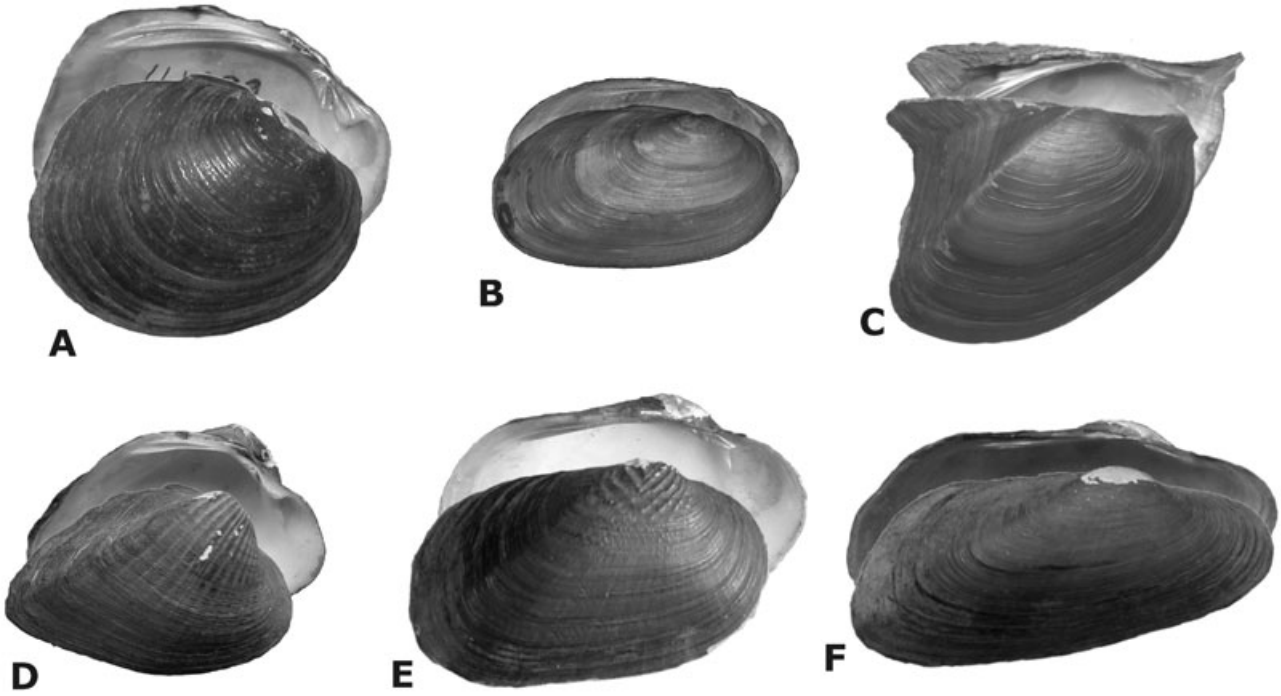

Figure 13. Representatives of the Hyriidae. A, *Diplodon rotundus* (Spix & Wagner, 1827) (+ *D. deceptus fide* Parodiz, 1968) UMMZ 111283. B, *Diplodon chilensis* BMNH uncat. C, *Prisodon obliquus* Schumacher, 1817 UMMZ 110938. D, *Castalia ambigua* (Lamarck, 1819) FMNH 67901. E, *Hyridella australis* MCZ 89361. F, *Lortiella froggattii* Iredale, 1934 FMNH 115329.

fusion of the mantle forming an excurrent siphon occurs in the Iridinidae as well, and Graf (2000a) hypothesized that this character was a synapomorphy of the Etherioidea, secondarily lost in a '(Mycetopodidae + Etheriidae) clade'. However, in the present analysis, mycetopodids are recovered as sister to the Iridinidae, and the most parsimonious reconstruction is that excurrent siphons evolved independently in the two lineages (Fig. 4).

The Hyriidae has been divided into two subfamilies on the basis of their geography: Hyridellinae in Australasia (McMichael & Hiscock, 1958; Graf & Ó Foighil, 2000b) and Hyriinae in South America (Parodiz & Bonetto, 1963). In our analyses, Hyridellinae was represented by *Velesunio*, *Lortiella*, and *Hyridella*; *Diplodon* and *Castalia* have been classified as Hyriinae. The traditional hypothesis of reciprocal monophyly was supported (albeit weakly) by recent analyses of mtDNA (Bogan & Hoeh, 2000; Hoeh *et al*., 2001, 2002a; Roe & Hoeh, 2003). However, Graf & Ó Foighil's (2000b) analysis of nuclear ribosomal DNA robustly recovered a monophyletic Hyriinae nested within a paraphyletic 'hyridellinae'. Our CE analysis favours the latter topology (Fig. 3).

The paraphyletic basal position of the Australasian hyriids suggests another potential synapomorphy for the family: there is a minute gap between the posterior ends of the ctenidia and mantle bridge between the apertures, resulting in a 'perforated' diaphragm dividing the mantle cavity [29]. McMichael & Hiscock (1958: fig. 5) regarded a perforated diaphragm to be diagnostic of the 'hyridellinae', and this hypothesis has persisted into modern analyses of the Hyriidae (Graf, 2000a; Hoeh *et al*., 2001; Roe & Hoeh, 2003). Amongst the taxa included in our analysis, a perforated (but otherwise complete) diaphragm is present in *Velesunio*, *Lortiella*, *Hyridella depressa*, and *H. australis*. According to McMichael & Hiscock (1958: 463), the gill diaphragm of *H. menziesi* is 'apparently imperforate', and the same condition is known from *Castalia*, *Diplodon*, and the other Neotropical hyriids. Intuitively, this pattern suggests that a perforated diaphragm may be a synapomorphy of the Hyriidae that has been subsequently reversed in certain lineages. However, in the present topology (Figs 2, 3), two independent acquisitions – in the Velesunioninae and the (*H. depressa* + *australis*) clade – are more parsimonious than a gain and two losses.

Our results (see also Graf & Ó Foighil, 2000b) support a revision of the classification of the Hyriidae, with a basal, strictly Australasian Velesunioninae (+ Lortiellinae) sister to a much more diverse Hyriinae (+ Hyridellinae) ranging over both Australasia and South America. The Hyriinae *s.l.* is supported by a single morphological synapomorphy in our analysis (the presence of radial umbonal sculpture [12]), whereas the Velesunioninae retains the plesiomorphic smooth umbos shared by other etherioideans.

Despite the fact that the hyriids have received more phylogenetic attention in recent years than some other freshwater mussel families (except the Unionidae), hyriid diversity has been sampled only sparsely in a wider phylogenetic context. It is possible that, as the data matrix expands, the paraphyly of the 'hyridellinae' may not seem so tidy. For example, many of the other genera traditionally included in the Velesunioninae (e.g. *Westralunio*, *Alathyria*; McMichael & Hiscock, 1958), the cucumerunionines (= *Cucumerunio* + *Virgus*), or the several unconsidered genera from South America (*Prisodon*, *Triplodon*, and *Callonaia*, to name a few) could weaken the utility of the proposed two-subfamily system for the Hyriidae. Nevertheless, we have revised our family-level classification according to the available data (Appendix 3), and expect that our bold hypothesis will receive further scrutiny in the near future.

Clade D

The lasidium-bearing mussels (= Etheriidae + Mycetopodidae + Iridinidae)

The clade composed of the families Etheriidae, Mycetopodidae, and Iridinidae has been traditionally recognized (Parodiz & Bonetto, 1963), and it has been supported by phylogenetic analyses (Graf, 2000a; Hoeh *et al*., 2001, 2002a; Roe & Hoeh, 2003; but see Bogan & Hoeh, 2000). Over the years, these mussels have been given various names, e.g. 'Mutelinae' (Ortmann, 1921), 'Mutelacea' (Parodiz & Bonetto, 1963), and Etherioidea (Kabat, 1997). What unites these taxa is the presence of a lasidium larval stage (discussed above). Unfortunately, the family group-level Linnaean hierarchy for the Unionoida is dense, and there is no room for a formal name for this clade; that is, not without a radical revision of the nomenclature. Frame shifting the entire system of the Unionoida to accommodate a formal name for every branch might perhaps be semantically more satisfying, but would add an unnecessary layer of complexity for students of bivalve evolution.

In addition to parasitic lasidia [55], these mussels share at least six other morphological synapomorphies: an edentulous hinge [4], with robust ligamental nymphae (Waller, 1990) and a conspicuous, V-shaped ligamental fossette [6], the labial palps are semicircular to reniform [26], and the intestine is complex (Hoeh *et al*., 2001; Roe & Hoeh, 2003) [41]. Furthermore, the relative number of interlamellar septa in brooding vs. nonbrooding portions of the demibranchs is the same. That is, the brooding septa are not more densely arrayed as with the Hyriidae and Unionidae [49].

The marsupial septa of brooding females also bears what Ortmann (1921: pl. 48, fig. 7b) and Heard & Vail (1976a: figs 7, 8) referred to as a 'marked swelling' [48]. This swelling, produced by a blood vessel, functionally divides each marsupial water-tube, producing one chamber for brooding ova and larvae, and a second that remains open. This character is a crude analogy of the 'tripartite' water-tubes seen in anodontine unionid mussels (Ortmann, 1911b: pl. 86, figs 9–10b), and presumably serves to facilitate the flow of the respiration/feeding current through the brooding demibranchs. Heard & Dougherty (1980) reported that *Pleiodon spekii* (Woodward, 1859) lacked these swellings and that, indeed, they were often absent (perhaps seasonally) from the other iridinids they had examined. However, Ortmann (1910a, 1918a) reported 'marked swellings', observable with a hand lens, in both *Chambardia wahlbergi* and *Aspatharia rugifera*. Although reported from *Etheria* (Heard & Vail, 1976a) and numerous mycetopodids as well (Ortmann, 1921), the generality of this character amongst the lasidium-bearing mussels requires further study.

Graf's (2000a: character 29) study of etherioidean relationships and our own morphology only analysis (not shown) supported complete (imperforate) septa dividing the interlamellar spaces [19] as a synapomorphy of the lasidium-bearing mussels. This interpretation was based on a topology with the Iridinidae as the basal member of the clade of lasidium-bearing freshwater mussels, i.e. (Iridinidae + (Mycetopodidae + Etheriidae)) (Graf, 2000a: fig. 1). Our CE analysis, however, recovered the Etheriidae in the basal position (Fig. 4). Heard & Vail (1976a) reported perforated septa in the brooding demibranchs of *Etheria elliptica*. Although the states of the interlamellar septa of *Acostaea* and *Pseudomulleria* have not been examined in sufficient detail for us to code them (Woodward, 1898; Yonge, 1978), the presence of perforated septa in *Etheria* suggests that the ancestral etheriid retained the plesiomorphic condition, and the most parsimonious conclusion is that the presence of complete interlamellar septa is a synapomorphy of the (Mycetopodidae + Iridinidae) clade (Fig. 4; Clade E).

Family Etheriidae, the freshwater oysters (Fig. 14)

Amongst the lasidium-bearing etherioideans, the Etheriidae, also known as freshwater oysters, are characterized by cementation and a trend towards 'oysterization'. The Etheriidae is composed of three or four monotypic genera: *Etheria* (widespread in Africa and Madagascar), *Acostaea* (Colombia, South America), *Pseudomulleria* (India), and *Bartlettia* (South America). Some authorities have argued that *Bartlettia*, which is strictly a 'wedger' rather than a cementer, actually belongs in the Mycetopodidae (Parodiz & Bonetto, 1963); others disagree (Dreher-Mansur & da Silva, 1990). *Bartlettia* has never been included in a cladistic analysis.

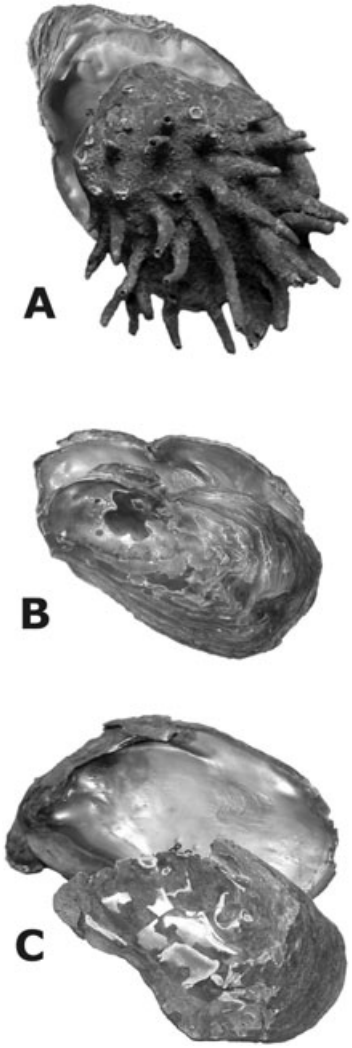

Figure 14. Representatives of the Etheriidae. A, *Etheria elliptica* MCZ 293466. B, *Acostaea rivolii* UMMZ 23485. C, *Pseudomulleria dalyi* UMMZ 112658.

Graf (2000a) found *Etheria* and *Acostaea* to be sister taxa (supporting a monophyletic Etheriidae) based strictly on morphological characters; this phylogeny also supported the contention of Heard & Vail (1976a) that etheriids are merely cementing mycetopodids. The only other analysis to test the monophyly and sister relationship of the Etheriidae was the mtDNA phylogeny of Bogan & Hoeh (2000). This study included COI sequences from *Etheria*, *Acostaea*, and *Pseudomulleria*, but it did not support a monophyletic Etheriidae. Instead, Bogan & Hoeh (2000) recovered *Etheria* and *Acostaea* as a paraphyletic grade at the base of the mycetopodids, and *Pseudomulleria* was concluded to be a unionoidean. From this, Bogan & Hoeh (2000)

echoing Morrison (1973) predicted that, when the larval morphology of *Pseudomulleria* was discovered, it would prove to be a glochidium. Other etheriids are known to have lasidia (Arteaga, 1994).

What is known of the anatomy of *Pseudomulleria* suggests that it is similar to *Acostaea* (Woodward, 1898; Pain & Woodward, 1961a; Yonge, 1978), and its placement in the glochidium-bearing Unionoidea in a strictly molecular analysis (Bogan & Hoeh, 2000) contradicted the hierarchy of morphological synapomorphies supported by previous and subsequent analyses (Graf, 2000a, 2002a; Roe & Hoeh, 2003). For these reasons, in our re-analysis of these sequences, we treated the COI sequence of *Pseudomulleria* indicated by Bogan & Hoeh (2000) as potentially problematic (see above).

In our CE analysis, the Etheriidae is monophyletic and sister to the (Mycetopodidae, Iridinidae) clade. Monophyly is supported by four unambiguous synapomorphies (Fig. 4): the valves are asymmetrical due to a cementing habit [14], the middle shell layer of lenticular nacre is lost [2], the ctenidia are heterorhabdic and plicate [18], and the foot is largely reduced or absent [35]. Moreover, as pointed out by Yonge (1962), the ctenidial ciliary currents remain the typical, unionoid Atkins Type D, but a second marginal furrow is added on the outer demibranch [21]. *Pseudomulleria* forms a clade with *Acostaea* on the basis of three synapomorphies: loss of the anterior adductor in the adult (i.e. monomyarian) [7], the shell bears a 'claw' that is the remnant of the equivalved, postlarval shell [15], and reduction of the anterior pedal retractor [37]; these three characters occur nowhere in the Unionoidea. The present result, based largely on the available morphological data (Table 3; Appendix 1), suggests that the published sequence for *Pseudomulleria dalyi* (GenBank AF231750) may not be reliable for estimating etheriid relationships. This is discussed further under the Unionidae below.

Family Iridinidae (Fig. 15)

The African Iridinidae (+ Mutelidae) have only been represented in previous cladistic analyses by a pair of closely related genera: *Mutela* and *Pleiodon* (Bogan & Hoeh, 2000; Graf, 2000a; Graf & Ó Foighil, 2000a; Hoeh *et al*., 2001; 2002a; Roe & Hoeh, 2003). Our study has added representatives of two other iridinid genera to better represent the known range of morphological diversity in the family. Based on this study, *Mutela* is shown to actually be quite derived relative to other iridinids, with well-developed, Type V (Figs 11, 12F) incurrent and excurrent siphons [27–30] and pseudotaxodont dentition [4] (Fig. 6E).

The Iridinidae is supported by four synapomorphies: well-developed pedal elevator scars (usually as

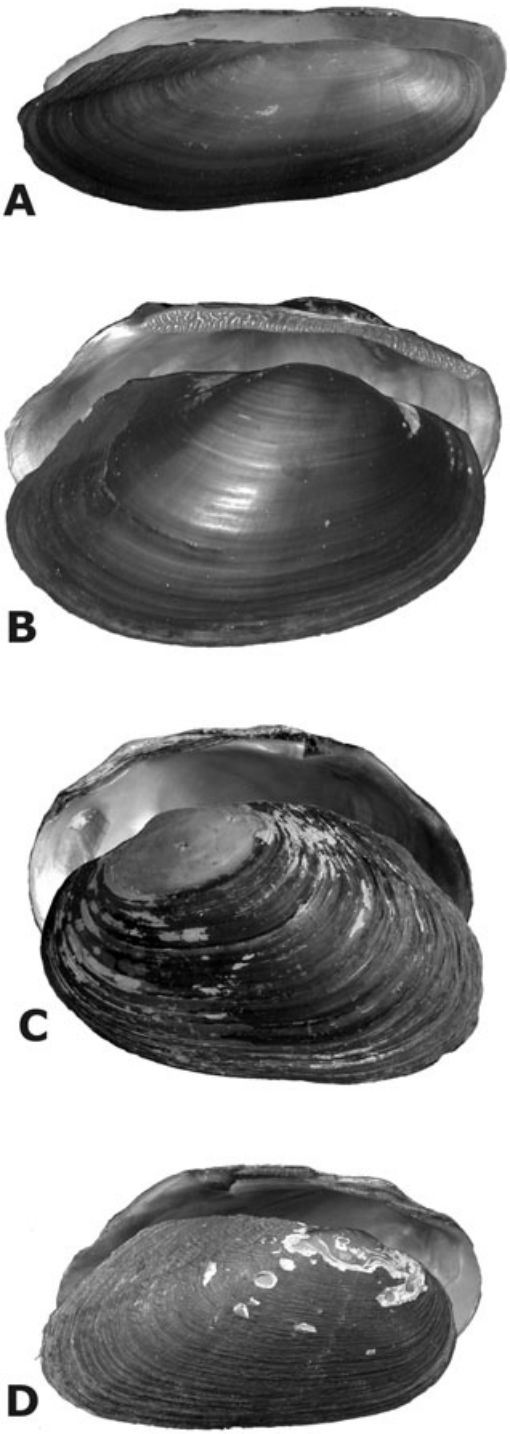

Figure 15. Representatives of the Iridinidae. A, *Mutela rostrata* MCZ 172817. B, *Pleiodon ovata* (Swainson, 1823) MCZ 30613. C, *Chambardia rubens* (Lamarck, 1819) FMNH 2588. D, *Aspatharia rugifera* UMMZ 111952.

a single, robust impression under the umbo) [9], closure of the mantle dorsal to the excurrent aperture forming a tubular excurrent siphon (Fig. 11; Type IV) [30], the parasitic lasidium attaches via tubular appendages, forming a haustorium [56], and larvae are released still encased in a durable egg capsule (Wächtler *et al*., 2001) [59]. Unfortunately, these striking synapomorphies hypothesized to support the monophyly of the Iridinidae are based on rather sparse published accounts and will probably be refined as our knowledge base improves.

As far as is known, all iridinids have distinct excurrent siphons, formed by fusion of the posterior mantle both above and below the excurrent aperture [28, 30]. However, a wide range of states are presented for the incurrent apertures, and some authorities consider them to be diagnostic of genera (e.g. Mandahl-Barth, 1988). *Mutela* has a distinct incurrent siphon (Fig. 11; Type V), with extensive ventral fusion of the mantle and a distinct pallial sinus [27], whereas *Aspatharia* always has the ventral mantle unfused (Fig. 11; Type IV). *Chambardia* (+ *Spathopsis*) has a short fusion closing the ventral margin of the incurrent aperture that is not associated with a pallial sinus (Fig. 12E; Ortmann, 1918a). Unfortunately, although the adult anatomy of various *Mutela* species has been described, anatomical observations of the Iridinidae are few (Daget, 1998), and the distinction between *Aspatharia* and *Chambardia* is generally made on the basis of shell characters (Daget, 1961, 1962).

The lasidium larvae of the Iridinidae attach to their host via tubular appendages, forming a haustorium. This in contrast with other lasidia (and glochidia) that encyst in their host. However, the generality of the haustorium as an iridinid synapomorphy is equivocal. We have been able to locate published reports of larval morphology for only two species of iridinids: *Mutela bourguignati* (Fryer, 1959, 1961; Wächtler *et al*., 2001) and *Moncetia lavigeriana* (Kondo, 1984). Further complicating the equivocal status of a haustorium as a synapomorphy of the Iridinidae, rather than just a synapomorphy of *Mutela* or even autapomorphic for *M. bourguignati*, is Kondo's (1984: fig. 3) observation of a glochidium in *M. lavigeriana*. From the position of the Iridinidae on the CE phylogeny (Fig. 4), the presence of an unhooked-type glochidium would be considered a nonhomologous, convergent derivation of that larval type from a lasidium. Kondo's report has not received much scrutiny in the literature, and we eagerly await confirmation of his conclusions.

Family Mycetopodidae (Fig. 16)

Mycetopodids are a relatively diverse but conchologically disparate assemblage of Neotropical freshwater mussels. The Mycetopodidae has only been repre-

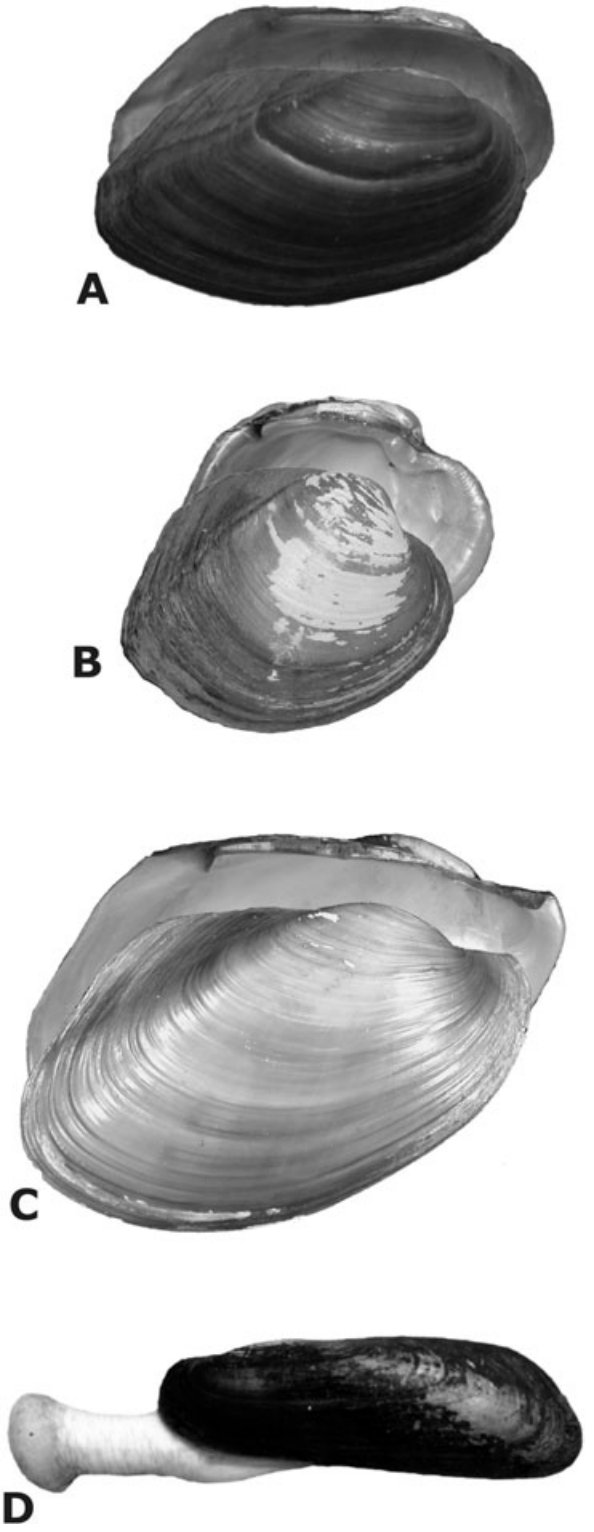

Figure 16. Representatives of the Mycetopodidae. A, *Anodontites trigonus* FMNH 21479. B, *Monocondylaea minuana* UMMZ 248904. C, *Leila blainvilliana* (Lea, 1834) ANSP 41827. D, *Mycetopoda pittieri* Marshall, 1927 INHS 14870.

sented in phylogenetic analyses by three genera: *Mycetopoda*, *Monocondylaea*, and *Anodontites* (Bogan & Hoeh, 2000; Graf, 2000a; Hoeh *et al*., 2001, 2002a; Roe & Hoeh, 2003). Most of these studies recovered the included mycetopodids as a clade, but Graf (2000a) could not resolve any morphological synapomorphies for the group. Some recent work had hypothesized a close affinity between mycetopodids and etheriids: the former are simply noncementing varieties of the latter (Heard & Vail, 1976a; Bogan & Hoeh, 2000; Graf, 2000a). However, the characters on which this conclusion was based are actually symplesiomorphies of the lasidium-bearing mussels rather than synapomorphies (Fig. 4).

Our CE analysis supports a monophyletic Mycetopodidae, sister to the Iridinidae (Fig. 4). Our character set provided only a single morphological synapomorphy of the family, inconspicuous pedal elevator scars [9], which are otherwise quite common in the Unionoida and *Neotrigonia* (Cox, 1969). Extensive pedal elevator muscles are present in species of the mycetopodid genus *Leila*, and this, together with the presence of tubular posterior apertures, has been the basis for arguing a close relationship between this genus and the Iridinidae (Bonetto, 1963; Veitenheimer, 1973; Heard & Vail, 1976a; Bogan & Hoeh, 2000; Graf, 2000a). Mycetopodids have Type III posterior aperture development, with mantle fusion only between the otherwise simple incurrent and excurrent apertures (Figs 11, 12C).

Traditionally, subfamilies have been recognized for the four morphologically discrete groups of mycetopodid genera: Mycetopodinae, Anodontitinae, Leilinae, and Monocondylaeinae. The monophyly and interrelationships of these subtaxa have yet to be tested in a phylogenetic context.

Clade F

Superfamily Unionoidea (= Margaritiferidae + Unionidae)

It is difficult to determine a consensus about the traditional view of the Unionoidea. The Margaritiferidae has been simultaneously regarded as 'primitive', but also part of the family group taxon that includes the Unionidae (Davis & Fuller, 1981). Although most morphology-based cladistic studies have indeed recovered the Margaritiferidae as the basal member of the order (i.e. sister to all other freshwater mussels; Graf, 2000a; Hoeh *et al*., 2001: fig. 14.2; Roe & Hoeh, 2003), molecular phylogenetic analyses routinely support a unionoidean clade composed of the Margaritiferidae and Unionidae (Bogan & Hoeh, 2000; Graf & Ó Foighil, 2000a; Hoeh *et al*., 2001, 2002a; Graf, 2002a).

Although our analyses of 28S only (not shown) and molecular data (Fig. 2, top) recover the Margaritiferidae as sister to the Hyriidae or part of a polytomy at the base of the order, the CE phylogeny supports a monophyletic Unionoidea [Figs 2 (bottom), 4]. As discussed above, morphological synapomorphies of the Unionoidea are difficult to determine and depend on the characters deemed to be plesiomorphic for the order. Our analysis indicates only a single derived morphological character to diagnose the clade: falciform labial palps [26], as opposed to the triangular palps seen in the Hyriidae and *Neotrigonia* (Hoeh *et al*., 2001; Roe & Hoeh, 2003). If endobranchy, as discussed above, is indeed considered as the plesiomorphic brooding morphology of the Unionoida, then tetrageny [44] would be considered a synapomorphy of the Unionoidea as well.

The dearth of quality morphological synapomorphies for the Unionoidea may weaken the hypothesis that the Unionidae and Margaritiferidae are indeed sister taxa, but molecular characters, from both the mitochondrial and nuclear genomes (cited above), generally point in this direction (but see Fig. 2, top; Roe & Hoeh, 2003). This is discussed further below in the context of the oxymoronically derived primitive morphology of the Margaritiferidae.

Family Margaritiferidae (Fig. 17)

Margaritiferids have long been regarded as the most 'primitive' freshwater mussels. This view has persisted into the modern era, precipitated from the observations that they lack certain of the characters possessed by the other 'higher' unionoids: ctenidia fused to the mantle along their entire length, posterior fusion of the mantle dorsal to the excurrent aperture, and interlamellar junctions of the demibranchs arranged into vertical septa (Ortmann, 1912b). The 'plesiomorphic' nature of margaritiferid anatomy has also been supported by some recent cladistic studies, i.e. *Neotrigonia* also lacks the characters of 'higher'

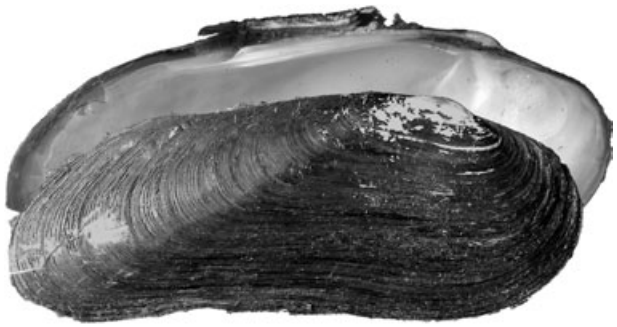

Figure 17. A representative of the Margaritiferidae. *Cumberlandia monodonta* (Say, 1829) ANSP 358640.

unionoids, and trigoniids and margaritiferids have been recovered as a grade at the base of the palaeoheterodont tree (Graf, 2000a; Hoeh *et al*., 2001: fig. 14.2; Roe & Hoeh, 2003). However, molecular studies, in general, tend to place margaritiferids as sister to (or nested within) the Unionidae (Bogan & Hoeh, 2000; Graf & Ó Foighil, 2000a; Hoeh *et al*., 2001, 2002a; Graf, 2002a; Huff *et al*., 2004). Our CE phylogeny [Figs 2 (bottom), 4] shows the same result (Margaritiferidae sister to the Unionidae), but the 28S data on their own (not shown) and as part of the molecular analysis (Fig. 2, top) connect margaritiferids to the Etherioidea branch. The weight of the available evidence suggests that the Margaritiferidae is monophyletic and, indeed, the sister group of the Unionidae (Fig. 4); however, as more evidence is accumulated, this conclusion may shift.

The Margaritiferidae branch is supported by five morphological synapomorphies in our analysis: the pedal elevators are inconspicuous, the muscle attachment apparently obscured behind the hinge [9], lateral mantle-muscle scars are present (Smith, 1983) [11], the interlamellar septa are reduced to scattered connections [19], the diaphragm dividing the infrabranchial from the suprabranchial chamber is incomplete [23], and the anus is located on the dorsal edge of the posterior adductor muscle (Hoeh *et al*., 2001; Roe & Hoeh, 2003) [39]. From our reconstruction, it would seem that the Margaritiferidae is characterized by reduction (Graf, 2002a), but, as we suggested above, there are equally parsimonious interpretations.

The conundrum of margaritiferid 'reduction' was discussed in detail by Graf (2002a): for characters such as the nature of the interlamellar junctions and the extent of development of the diaphragm, there are two equally parsimonious ways to interpret the history of evolutionary transformations. For example, let us consider the diaphragm dividing the mantle cavity. If fusion of the ascending lamellae of the outer demibranchs along their entire length [19] is homologous in both the Etherioidea and Unionidae, a complete (or 'slightly incomplete', *sensu* Davis & Fuller, 1981) diaphragm is a synapomorphy of the Unionoida – it was derived in these mussels from a common ancestor that shared this trait. The diaphragm was subsequently reduced in the Margaritiferidae to a secondary condition analogous to that seen in *Neotrigonia* (and the outgroup taxa in our analyses). In trigoniids and margaritiferids, the posterior portions of the ctenidia are free from the mantle, and the grossly incomplete diaphragm is somewhat improved by mantle ridges that functionally divide the mantle cavity (Gould & Jones, 1974; Smith, 1980).

The alternative is that the similarities amongst the diaphragms of *Neotrigonia* and margaritiferids are homologous symplesiomorphies, and the conditions seen in the Etherioidea and Unionidae are homoplastic analogues. Either way, two evolutionary steps are required: a gain (Unionoida) and a loss (Margaritiferidae), or a gain (Unionidae) and another gain (Etherioidea). The criterion of MP is unable to distinguish between these two alternatives, and the same logic applies to the 'negative gain' (Mikkelsen, 1998) of interlamellar septa in the Margaritiferidae.

Graf (2002a) specifically discussed the issue of the 'loss' of posterior mantle fusion in the margaritiferids, but this problem is solved in our topology (Fig. 4). Most published phylogenies support the hypothesis that mantle fusion dorsal to the excurrent aperture is plesiomorphic in etherioideans. Thus, we reach the same philosophical problem: it is equally parsimonious for the absence of posterior mantle fusion in margaritiferids to be either derived or ancestral. In the phylogeny in Figure 4, the ambiguity is resolved; the Margaritiferidae retains the simple, unfused mantle margins from the ancestral palaeoheterodont. That posterior mantle fusion was derived separately in the unionids and etherioideans is perhaps not too surprising, especially given the differences between the two groups: unionids have Type II apertures, whereas the plesiomorphic etherioidean condition is Type III (Figs 11, 12).

Family Unionidae (Fig. 18)

Up to this point, we have discussed the relationships and synapomorphies of six of the seven palaeoheterodont families (Fig. 4), and yet we have classified only one-fifth of the species (Fig. 1A). This tally of mussel diversity is necessarily derived from a century's worth of regional treatments (cited above); no single reference (or even the union of a few) provides a modern, comprehensive view of global freshwater mussel diversity. Rather, the best that is available is an often conflicting, jury-rigged system of geographical monographs. The same could be said for the higher level taxonomy of the subclass and for the Unionidae, which represents the bulk of the order.

The Unionidae, especially those of eastern North America, have been the object of numerous family group-level phylogenetic studies, using a variety of taxa and character sets (e.g. Lydeard *et al*., 1996; Bogan & Hoeh, 2000; Graf & Ó Foighil, 2000a; Hoeh *et al*., 2001, 2002a; Roe & Hoeh, 2003; Campbell *et al*., 2005); many of these unionids are shown in Cummings & Mayer (1992) and Parmalee & Bogan (1998). Unfortunately, only a handful of studies have dwelt on the relationships of Old World genera (Graf, 2000a, 2002a; Huang *et al*., 2002). Furthermore, we were unable to incorporate 16S mtDNA sequences into our supermatrix because of limited taxon overlap, and so

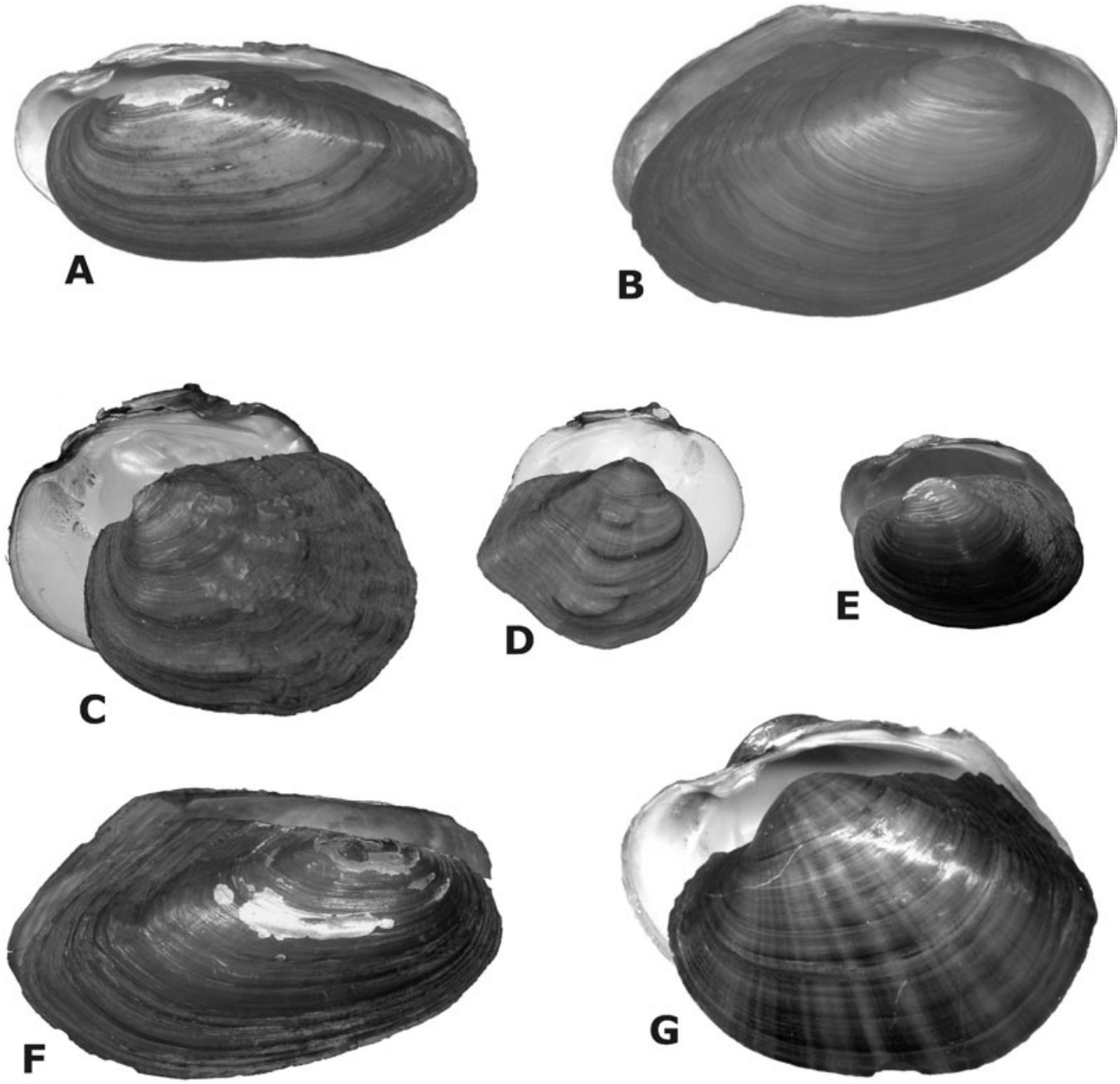

Figure 18. Representatives of the Unionidae. A, *Unio pictorum* UMMZ 9320. B, *Pyganodon grandis* UMMZ 205535. C, *Amblema plicata* INHS 12149. D, *Obliquaria reflexa* INHS 5892. E, *Coelatura aegyptiaca* FMNH 11597. F, *Pilsbryoconcha exilis* ANSP 48270. G, *Lampsilis cardium* UMMZ 130005.

our analyses have not benefited directly from the sample of South-East Asian freshwater mussels studied by Huang *et al*. (2002). A detailed examination of unionid subfamily relationships is well beyond the scope of this study; indeed, it is beyond the sum of the phylogenetic work performed to date. Nevertheless, our analysis does offer some insights.

In previous analyses, the task of determining unionid synapomorphies has not been a priority; many phylogenetic analyses have not supported this family as monophyletic (Bogan & Hoeh, 2000; Graf, 2000a; Hoeh *et al*., 2001, 2002a) or have not included sufficient outgroups to provide a meaningful test (Lydeard *et al*., 1996; Graf & Ó Foighil, 2000b; Huang *et al*., 2002). The weight of available evidence, including our CE phylogeny, supports a monophyletic Unionidae sister to the Margaritiferidae (Fig. 4). However, from this conclusion, the question is raised: why have the very data that we combined and used for our analyses not always come to the same conclusion when studied in

isolation? We can provide two answers to this question: morphological homoplasy and incongruent sequences.

Strictly morphological analyses have had trouble with the Unionidae. The analyses of Graf (2000a) and Hoeh *et al*. (2001: fig. 14.2), and our own morphology only analysis (not shown), did not recover the Unionidae as monophyletic, but nor did these studies find strong support for an alternative topology that could reject unionid monophyly. The cladogram shown by Roe & Hoeh (2003), derived from their analysis of a matrix of strictly binary morphological characters, supported unionid monophyly (their fig. 4.2). However, their experimental approach to phylogenetic analysis makes it difficult to interpret their results from a cladistic perspective.

Roe & Hoeh's (2003) fundamental deviation from standard cladistic methods (as applied earlier, for example, by Hoeh *et al*., 2001 on the same data) was their 'presence/absence' coding of 57 morphological characters derived from their original matrix of 28 binary and multistate characters. 'Presence/absence' coding as binary characters is common to all phylogenetic analyses of morphology, including that in this paper (Table 3, Appendix 1). A character may have only two states across an entire taxon set; either that character is present or it is not. However, following a strictly cladistic philosophy implies that one of the states (i.e. the presence or absence of a character) is hypothesized to be ancestral and the other derived (Wiley, 1980; Wiley *et al*., 1991).

Roe & Hoeh (2003) applied 'presence/absence' coding differently. For example, in the original morphological matrix, Hoeh *et al*. (2001) identified their character 7 as a binary character:

'7. Attachment of the dorsal margin of the outer lamella of the outer demibranchs to the inner surface of the mantle. 0 = except at the posterior ends of those demibranchs, 1 = for the entire length of those demibranchs.'

This corresponds to character 23 in our character set (Appendix 1). Roe & Hoeh (2003) re-coded this character as follows:

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

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

What was one character in the original analysis became duplicated in the 'presence/absence' re-analysis: the presence of their character 14 is the same as the absence of 15, and vice versa. This has the effect of weighting this particular trait in a parsimony analysis, and we have diagnosed more than a dozen such inappropriate pairs (identified in Appendix 1). Thus, Roe & Hoeh's (2003) analysis supported unionid monophyly by effectively weighting, whereas previous analyses were unweighted.

Rosenberg *et al*. (1994, 1997), Lydeard *et al*. (1996), Hoeh *et al*. (1998), and Graf & Ó Foighil (2000a, b) published the first nucleotide-based studies of unionid phylogeny. Unfortunately, each of these studies lacked sufficient ingroup or outgroup sampling (or both) to test the monophyly of the global Unionidae. Rather, they focused on the intergeneric relationships of the eastern North American assemblage, or were focused on other questions in which unionids were only included incidentally. These studies presented topologies consistent with the monophyly of the Unionidae.

Hoeh's several analyses of mtDNA (Bogan & Hoeh, 2000; Hoeh *et al*., 2001, 2002a) were the first with sufficient taxon sampling to reasonably test unionid monophyly; these phylogenies weakly supported a paraphyletic Unionidae, with the margaritiferids nestling between *Coelatura* (representing one of two Old World unionid genera in their analyses) and the rest of the family. Bogan & Hoeh's (2000: fig. 1) phylogeny recovered *Pseudomulleria* (traditionally a cementing, monomyarian etheriid) as part of the unionid–margaritiferid clade as well. Graf's (2002a) phylogeny, which was derived from nuclear ribosomal DNA and included five Old World genera, found robust support for a monophyletic Unionidae. Roe & Hoeh's (2003) re-analysis of the same COI data that previously supported unionid paraphyly recovered that family as monophyletic. Furthermore, Roe & Hoeh (2003) claimed (p. 112) that their 'well-supported analysis represents the best-resolved, best-supported hypothesis of unionoid bivalve higher-order relationships produced to date.' Our own CE re-analysis also supports unionid monophyly (Figs 2, 3). Unfortunately, these discrepancies raise more questions than they answer.

If the Unionidae is indeed monophyletic, why did the earlier studies of Bogan & Hoeh (2000) and Hoeh *et al*. (2001, 2002a) support the paraphyly of this group? We propose that their analyses may have been twice compromised: (1) protein coding mtDNA evolves too rapidly to reasonably estimate such ancient divergences; and (2) they insufficiently considered the implications of potentially problematic sequences. As evidenced by our own analysis (Table 4), COI is a highly homoplastic dataset amongst these taxa. Consistency and retention indices are low (CI = 0.248, RI = 0.442), and most of the transformations traced to the terminal branches (57%). These patterns are characteristic of 'saturation'; character support for internal branches is eroded by subsequent 'over-printing' at the same nucleotide position. Graf & Ó Foighil's (2000a: fig. 1) saturation plot confirmed this, demonstrating

the drop in the transition : transversion ratio even within the Unionidae. Bogan & Hoeh (2000), Hoeh *et al*. (2001, 2002a), and Roe & Hoeh (2003) transformed their data to reduce this saturation bias by down-weighting (a priori) to zero all third codon position transitions (i.e. purine-to-purine or pyrimidine-to-pyrimidine transformations). In the phylogeny shown in Figure 3, transforming the COI data in this way would eliminate 53.2% of COI's influence on the tree (1262 of 2372 steps, with problematic COI sequences included). Most of the information in the COI matrix so violates the assumptions of cladistic analysis that it does not merit inclusion.

The monophyly of the Unionidae in these mtDNA studies was actually only challenged by two sequences: GenBank AF231735 *Coelatura aegyptiaca* and GenBank AF231750 *Pseudomulleria dalyi*. *Coelatura*, traditionally a unionid (Mandahl-Barth, 1988), was not recovered as part of a unionid clade. Morphologically, *Pseudomulleria* is an etheriid (Pain & Woodward, 1961a; Yonge, 1978), but this sequence is always found as part of a unionoidean clade.

What makes these two sequences problematic is not their conflict with the traditional classification. Rather, it is their inconsistent behaviour. In our COI Only analysis (not shown), these two sequences were placed in the strict consensus in a polytomy at the base of a (Unionidae + Margaritiferidae) clade that had no bootstrap support; Bogan & Hoeh (2000: fig. 1) resolved *Pseudomulleria* and *Coelatura* as the basal members of this clade. With the addition of 28S and morphology, *Pseudomulleria* migrated to a nonsensical position sister to the (*Unio* + *Cafferia*) clade, and *Coelatura* fell to near the base of the Palaeoheterodonta – and in at least a small number of MP trees, it was even placed outside of that clade (Fig. 2). When these two problematic sequences were excluded from the CE analysis, *Coelatura* and *Pseudomulleria* were recovered in their traditional families, the Unionidae and Etheriidae, respectively (Figs 2, 3). Scientifically, we are in no position to say that any of these various results is 'incorrect'. However, it is our opinion that the available evidence does not warrant these two DNA sequences rejecting the null hypotheses that the Etheriidae and Unionidae are monophyletic.

If COI mtDNA evolves too rapidly to convincingly test hypotheses of palaeoheterodont family-level relationships, and if a small number of these often used sequences consistently produce inconsistent results, what led Roe & Hoeh (2003) to such a robust phylogeny in support of unionid monophyly? After re-analysing their matrix (available on our website, http://www.mussel-project.net/), we can cite three factors that precipitated both bootstrap and jackknife support greater than 90% for the Unionidae node. The first was that they did not consider any etheriids, including *Pseudomulleria*. The second was the combination of the mtDNA with their inadvertently weighted 'presence/absence' characters (discussed above). Finally, the use of successive approximations weighting before the resampling analyses inflated these measures of support.

Successive approximations weighting was proposed by Farris (1969, 1989) as an a posteriori weighting method to reduce the number of equally most parsimonious trees following a standard parsimony analysis. As implemented in PAUP* (Swofford, 2002), the consistency index or rescaled consistency index of each character is used to re-weight evolutionary transformations for a subsequent round of tree searching. Thus, each character is weighted according to its consistency with a resultant topology or set of topologies. The cycle of re-weighting and tree searching is continued until the process reaches equilibrium. The result of successive approximations re-weighting is one tree (or a few, relative to the original set) and a matrix of characters weighted in favour of that topology.

It was with this weighted matrix that Roe & Hoeh (2003) performed their bootstrap and jackknife analyses. During resampling, the characters most consistent with the original analysis were weighted higher than those favouring competing topologies. Graf & Ó Foighil (2000a: Fig. 2) depicted phylogenies derived from unweighted and re-weighted COI analyses, and the inflation in jackknife support was evident. It is also significant that the single 'most parsimonious' tree recovered by Roe & Hoeh (2003: Fig. 4.3) from successive approximations, which so robustly supported unionid monophyly, matched neither of the two equally parsimonious unweighted trees. In our re-analysis of their matrix, the strict consensus of these two cladograms (not shown) did not support unionid monophyly: *Coelatura*, the Margaritiferidae, and the rest of the Unionidae formed a tricotomy.

Our CE analysis, in which COI was combined with morphology and the 28S data, and three potentially problematic COI sequences were excluded, supported a monophyletic Unionidae sister to the Margaritiferidae (Figs 3, 4). The sole morphological synapomorphy recovered for this family was the presence of a short mantle fusion dorsal to the excurrent aperture, resulting in a third, supra-anal aperture [30]. Thus, a Type II pattern of posterior mantle fusion is diagnostic for the Unionidae (Figs 11, 12).

A detailed discussion of the infra-familial relationships of the Unionidae is beyond the scope of this paper. Indeed, only a fraction of the apparent diversity has ever been considered in a phylogenetic context. Despite the size of the gaps in our knowledge (as reflected in the number of *incertae sedis* subfamilies in our classification; Appendix 3), certain patterns have been recovered in repeated analyses of the Unionidae,

including this one, and circumscribing what we 'know' will delimit the areas in which additional work is most needed.

Traditionally, the Western School of Malacology has divided the Unionidae into two subfamilies corresponding to the modern Unioninae and Ambleminae (Heard & Guckert, 1970; Davis & Fuller, 1981; but see Modell, 1942, 1949, 1964; Starobogatov, 1970); these (and other) authoritarian treatments were reviewed by Roe & Hoeh (2003) (see also Campbell *et al*., 2005). Our analyses (Figs 2, 3) support the subfamilies and tribes reported in previous analyses. Graf (2002a) corrected the classification of the Nearctic mussels, and we maintain that system here (Appendix 3). The two subfamilies Unioninae and Ambleminae are both represented in North America. Unioninae is composed of the strictly Old World Unionini and the Holarctic Anodontini (Figs 2, 3). The North American Ambleminae is composed of five tribes: the monogeneric Gonideini west of the Rocky Mountains, and the Amblemini, Quadrulini, Pleurobemini, and Lampsilini in the Interior Basin and eastward. Graf (2001) coined the informal taxon 'Amblemini Tribe Group' for the clade of the eastern tribes. In general, these New World and European unionid subtaxa have been repeatedly supported in the cladistic literature (Lydeard *et al*., 1996; Bogan & Hoeh, 2000; Graf & Ó Foighil, 2000a, b; Hoeh *et al*., 2001, 2002a; Huang *et al*., 2002; Roe & Hoeh, 2003).

The Lampsilini is the best-recognized and most diverse (more than 140 species) taxon of the eastern 'Amblemini Tribe Group' (Ihering, 1901; Ortmann, 1912b; Heard & Guckert, 1970). Its distinction is based on a suite of anatomical and life history characters, including long-term brooding [45] and a well-developed marsupium restricted to a limited portion of the outer demibranchs and capable of great expansion (Graf & Ó Foighil, 2000a) [46, 49, 50]. Amongst certain genera, the shells exhibit distinct sexual dimorphism [16] and the mantle ventral to the incurrent aperture is often adorned with host lures of various morphologies (Kraemer, 1970; see also Roe *et al*., 2001). Despite the historical identification of the lampsilines as a natural taxon, some molecular studies have been confounded by *Obliquaria reflexa*. On the basis of the characters listed above, *Obliquaria* is clearly a lampsiline (Ortmann, 1912b), and this has been supported by a phylogeny based on nuclear DNA (Graf, 2002a). However, mtDNA studies of 16S, COI, and ND1 (Lydeard *et al*., 1996; Serb *et al*., 2003; this study: Fig. 2) have suggested an affinity between *Obliquaria* and the Quadrulini. For this reason, we have treated the COI sequence of *Obliquaria reflexa* (GenBank AF385114) as potentially problematic. These mtDNA results using different markers are still based on extractions from the same collection (UAUC 19) and need to be further corroborated before the classification of the Lampsilini can be prudently revised. On the basis of a conservative appraisal of the available evidence, we maintain the Lampsilini (including *Obliquaria*) as valid (Appendix 3).

Only a handful of the unionid species from Central America, Asia, and Africa have been considered in phylogenetic studies, and those that have do not support the classic two-subfamily system for the Unionidae (Graf, 2002a). South-East Asian and African genera are represented in our CE analysis by *Pseudodon vondembuschianus*, *Pilsbryoconcha exilis*, *Contradens contradens* (all from Thailand), and *Coelatura aegyptiaca* (Africa). There is no support that any of these taxa belong to either of the traditional Unioninae or Ambleminae (Figs 2, 3). These Asian species consistently form a paraphyletic grade at the base of the Unionidae (Graf, 2002a).

A recent analysis of 16S mtDNA by Huang *et al*. (2002) included 13 Chinese freshwater mussels together with several sequences from the study of Lydeard *et al*. (1996). Contrary to our result, their analyses recovered all of these genera as members of the two traditional subfamilies. That is, no taxa were placed as part of a grade basal to the rest of the Unionidae. For the Asian Unioninae, Huang *et al*.'s (2002: Fig. 1) results can be incorporated easily: *Nodularia douglasiae* (Griffith & Pidgeon, 1834), *Acuticosta ovata* (Simpson, 1914), *Cuneopsis pisciculus* (Heude, 1875), *Lepidodesma languilata* (Heude, 1874), *Lanceolaria grayana* (Lea, 1834), *Schistodesmus lampreyanus* (Baird & Adams, 1867), and *Arconaia lanceolata* (Lea, 1856) all belong to the Unionini; *Sinanodonta woodiana* (Lea, 1834) and *Cristaria plicata* (Leach, 1815) represent the Asian Anodontini.

Amongst the Ambleminae, Huang *et al*.'s (2002: fig. 1) results cannot unequivocally be reconciled with ours because of insufficient taxon overlap. Specifically, their study did not include *Gonidea*, and therefore the relative positions of *Hyriopsis cumingii* (Lea, 1852), *Lamprotula leai* (Gray, 1834), *Ptychorhynchus pfisteri* (Heude, 1874), *Solenaia oleivora* (Heude, 1877), and the Amblemini Tribe Group cannot be determined. Is *Gonidea* the basal member of the Nearctic Ambleminae? Or, is it part of a grade that includes Asian genera at the base of that subfamily? The hypothesized affinity of *Gonidea* with certain East Asian genera was reviewed in Watters (2001) (see also Campbell *et al*., 2005). Family group-level nomina have been introduced for some of these Asian 'amblemines', but we treat them as *incertae sedis* subfamilies until more evidence of their phylogenetic position becomes available (Appendix 3).

It should be evident from the preceding discussion that this system of the Palaeoheterodonta should be regarded as only a small step towards a natural, log-

ically consistent classification of the extant Palaeoheterodonta. Through our analyses, we have endeavoured to combine the various available datasets comprehensive enough to test hypotheses of family group-level relationships. Although the resultant most parsimonious patterns largely resembed the previous analyses – which is to be expected, given that we used the same data! – some new patterns were recovered: for example, the position of the Etheriidae. This work also highlights the weak areas in our understanding. These are not a result of flaws in the cladistic methodology, but rather an indication of the inadequacy of the data thus far available. By looking back to the Confucian quote at the head of this article, one should be reminded that it is only by owning the inconsistencies of our system that malacology can move forward.

It goes without saying that more taxa need to be included in phylogenetic analyses. Only a few species and genera (relative to the global diversity) have actually been studied in a phylogenetic context. Especially amongst Old World taxa, more DNA sequences are necessary, and the loci employed should take into consideration previous molecular censuses and the results of their analyses. To relate new work to that already performed, there needs to be some overlap between datasets. To improve our understanding of family-level relationships amongst freshwater mussels, we can recommend domain 2 of 28S (protocol available in Graf & Ó Foighil, 2000b; Graf, 2002a). Other sequences, such as COI, 16S, and other mtDNA markers, may be appropriate for shallower relationships, but they are unable to provide the necessary resolution at the family level. That being said, new markers also need to be explored (Giribet & Distel, 2003).

Given the often pronounced failure of morphological characters for recovering bivalve relationships, it is surprising that, for many lineages, the data are often too sparse to reach any conclusions. For numerous palaeoheterodont taxa, soft anatomy and larval morphology have never been reported, and for more species these character sets have not been fully explored. The problem of iridinid haustorium larvae is striking, but the scarcity of basic anatomical and reproductive data is a systemic problem for the entire group that needs to be solved before we can explore the now veiled portions of the phylogeny of the Palaeoheterodonta.

One particular line of evidence that has not been pursued in an evolutionary context is the phylogeny of the mussels' host fishes. A few excellent reviews of freshwater mussel host affinities are available (Hoggarth, 1992; Watters, 1994b; and references cited therein), but data are sparse for most mussel species, and no reference is made to the phylogeny of the hosts. Although it is clear that new host colonization by mussels has happened frequently and across phylogenetic boundaries (Graf, 1997c), other taxa show some inherited host preferences (e.g. *Potamilus*; Cummings & Mayer, 1993; Roe *et al*., 1997).

If nothing else, our re-analysis and review have laid bare much of the data heretofore employed to explain the phylogeny of the Palaeoheterodonta. By presenting the classification of freshwater mussel relationships and the supporting synapomorphies as a falsifiable 'bold hypothesis' (*sensu* Popper, 1968), we hope that this work will invigorate systematic malacologists to focus their attention and critical thought on this very interesting group of bivalves.

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APPENDIX 1

MORPHOLOGICAL CHARACTERS AND STATES

Descriptions of the morphological characters and their states used to construct our matrix of morphological characters (Table 3) are given below. Characters are divided into six categories for convenience: 'Shell', 'Ctenidia and labial palps', 'Mantle', 'Other soft anatomical', 'Life history', and 'Larval'. These characters have been drawn largely from those employed in published studies. Previously used characters are coded with the following abbreviations: G, Graf (2000a); GO, Graf & Ó Foighil (2000a); GW, Giribet & Wheeler (2002); HBH, Hoeh *et al.* (2001); LMD, Lydeard *et al.* (1996); RH, Roe & Hoeh (2003); such citations follow the character description in square brackets. Character state 'X' refers to a condition that does not occur amongst the sample of taxa in our study but has been treated as a synapomorphy elsewhere.

SHELL CHARACTERS

1. Shell microstructure: 0, shell composed of two or three aragonitic layers; 1, shell composed of both aragonite and calcite. All palaeoheterodonts are coded as having an aragonitic shell (Taylor *et al*., 1969; Taylor, 1973). [GW/3]

2. Nacre: 0, shell composed of three layers, an outer prismatic layer, a middle layer of lenticular nacre, and an inner layer of sheet nacre; 1, middle nacreous layer absent; 2, both nacreous layers absent. All palaeoheterodonts are coded as having a three-layered, prismato-nacreous shell except *Etheria* and *Acostaea*, which are both missing the middle layer lenticular nacre (Taylor *et al*., 1969; Taylor, 1973). [GW/4, 5, 8, 9, 12]

3. Hinge dentition type: 0, differentiated hinge teeth, of heterodont type with true cardinal teeth; 1, dentition schizodont, typically with both posterior lateral and anterior pseudocardinal teeth, although either may be secondarily reduced or modified. Schizodont dentition is discussed in the text as a synapomorphy of the Palaeoheterodonta (Fig. 6). *Mytilus* is coded as 'inapplicable' because it lacks differentiated hinge teeth. [G/1; GW/38, 39]

4. Secondary modifications to schizodont dentition: 0, present and unmodified (Fig. 6A–C); 1, dentition secondarily reduced or edentulous (Fig. 6D); 2, pseudotaxodont (Fig. 6E). Coded as inapplicable in outgroup taxa lacking schizodont dentition. [G/2–6; HBH/21, 22; RH/40–44]

5. Hinge ligament: 0, external, opisthodetic, and parivincular; 1, alivincular or irregularly multivincular; 2, amphidetic. *Etheria elliptica* has an amphidetic ligament (Yonge, 1962: fig. 8). [GW/30–32]

6. Lamellar ligament fossette: 0, fossette shallow, not V-shaped; 1, robust hinge nymphae, resulting in a deep V-shaped fossette at the posterior end of the hinge (Fig. 15C; Waller, 1990: fig. 1). A V-shaped ligamental fossette occurs in the families Etheriidae, Iridinidae, and Mycetopodidae. [HBH/23; RH/45]

7. Number of adult adductor scars: 0, dimyarian; 1, monomyarian, with only the posterior adductor remaining in the adult (Fig. 14C). All palaeoheterodonts have two adductors except *Acostaea* and *Pseudo-*

mulleria (Woodward, 1898: fig. 1; Yonge, 1978: fig. 7). [G/8; GW/21]

8. Anterior adductor scar shape: 0, round; 1, elongate. Coded as 'inapplicable' in *Acostaea* and *Pseudomulleria* where the anterior adductor is absent. Many taxa are coded differently than indicated by Hoeh *et al*. (2001). [HBH/27; RH/54, 55]

9. Pedal elevator scars: 0, present but weakly developed, usually as a series of impressions in the umbo cavity or along the buttress of the anterior teeth, if present; 1, well developed, a robust impression under the umbo; 2, inconspicuous, apparently hidden behind the hinge. Inapplicable in those taxa in which the foot is highly reduced. Coded as 'inapplicable' in *Mytilus* and *Astarte* (Cox, 1969: N38). This character has not been used in previous phylogenetic analyses of the Palaeoheterodonta.

10. Position of posterior pedal retractor scar: 0, retractor scar not distinct from and generally dorsal to the posterior adductor; 1, retractor scar distinct from posterior adductor and generally anterior (Daget, 1961: fig. 1). Distinctly separate posterior pedal retractors occur widely amongst palaeoheterodont families. Coded as 'missing' in *Mytilus*, *Astarte*, *Velesunio angasi*, and *Pilsbryoconcha exilis*. [GW/23]

11. Lateral muscle scars: 0, absent; 1, present. Coding follows Smith (1983); lateral muscle scars occur only in *Neotrigonia* and margaritiferids amongst the ingroup. [G/11; HBH/25; RH/50]

12. Beak/postlarval shell sculpture: 0, simple concentric or absent; 1, radial (Fig. 13D, E); 2, double-looped or zigzag (Modell, 1942: pl. 6). See Ó Foighil & Graf (2000) for electron micrographs of *Neotrigonia* postlarval sculpture. [G/10; HBH/24; RH/46–49]

13. Adult shell sculpture: 0, shell more or less smooth; 1, radial; 2, plications; 3, zigzag; 4, pustules. Palaeoheterodont external sculpture was discussed by Watters (1994a). Coded as 'missing' amongst the asymmetrical etheriids. [LMD/14; G/9; HBH/20; RH/39]

14. Valve symmetry: 0, equivalved; 1, asymmetrical, usually due to cementation. *Etheria*, *Acostaea*, and *Pseudomulleria* attach to hard substrates by cementing (Fig. 14; Yonge, 1962, 1978). [G/7; GW/53]

15. Asymmetrical 'talon' (Anthony, 1907): 0, absent; 1, a 'claw' or 'spur' formed by the remnant of the precemented, postlarval shell (Fig. 14C; Yonge, 1978: fig. 1). Known only from *Acostaea* and *Pseudomulleria* (Yonge, 1962, 1978). Coded as 'inapplicable' amongst noncementing taxa. This character has not been used in previous phylogenetic analyses of the Palaeoheterodonta.

16. Shell sexually dimorphic: 0, shells of males and females alike; 1, shells of males and females externally distinct. Male shells usually less inflated, with a pointed posterior; female shells generally with a more squared posterior and much inflated. Compare the male and female *Lampsilis siliquoidea* (Barnes, 1823) shells shown by Parmalee & Bogan (1998: pl. 52) and others. This character has not been used in previous phylogenetic analyses of the Palaeoheterodonta.

Ctenidia and labial palp characters

17. Ctenidial morphology: 0, filibranch, with adjacent filaments connected by cilia only; 1, eulamellibranch, with tissue-grade fusion (Cox, 1969: N17, figs 14, 17; Brusca & Brusca, 1990: 736, fig. 31). All Unionoida coded as having tissue-grade fusion. [G/12; GW/68]

18. Ctenidial filament morphology: 0, homorhabdic (ctenidia smooth); 1, heterorhabdic (ctenidia plicate) (Heard & Vail, 1976a: figs 8, 9). All etheriids coded as having heterorhabdic ctenidia (Yonge, 1962). [G/21; GW/65, 74]

19. Interlamellar connections: 0, none or scattered; not arranged into vertical septa; 1, perforated septa in at least the brooding demibranchs; 2, continuous (i.e. imperforate) septa. Ortmann (1921: pl. 47) shows the contrasting perforate and imperforate septa of various hyriids and mycetopodids, respectively. Coded as 'missing' for both *Acostaea* and *Pseudomulleria*. [LMD/3; G/28, 29; GO/5, 6; HBH/12, 14; RH/25, 28, 29]

20. Ctenidial ciliary currents (gill type): 0, Type B (Atkins, 1937: figs 2–4); 1, Type C (Atkins, 1937: figs 6–13); 2, Type D (Atkins, 1937: fig. 14). All unionoids coded as Type D (Atkins, 1938). *Neotrigonia* coded as Type B (Morton, 1987). [GW/70, 71]

21. Type D ctenidial feeding groove: 0, marginal furrow present only along the ventral edge of the inner demibranch (Ortmann, 1912b); 1, marginal furrow present along the ventral edges of both demibranchs. Known only in *Etheria* (Yonge, 1962: fig. 6). Coded as inapplicable in taxa without Type D ctenidial ciliary currents. This character has not been used in previous phylogenetic analyses of the Palaeoheterodonta.

22. Chitonous rods of the ctenidial filaments (gill spicules): 0, not calcified; 1, calcified (Atkins, 1938: text fig. 1C). All palaeoheterodonts coded as calcified (Taylor *et al*., 1969, 1973; Taylor, 1973). [GW/72, 73]

23. Fusion of the ascending lamella of the outer demibranchs: 0, not fused to the mantle along their entire length; posterior integrity of the infra- and suprabranchial chambers may be completed via 'pallial ridges' (Gould & Jones, 1974; Smith, 1980); 1, fused to the mantle along its entire length or nearly so. Hoeh *et al*. (2001) and Roe & Hoeh (2003) coded *Neotrigonia* for the presence of 'attachment' of the ascending lamella of the outer demibranch to the mantle. However, in that genus, the ctenidia are only weakly attached via cilia, not a tissue-grade fusion as found in the Unionoida (Morton, 1987). [LMD/6; G/13; HBH/7, 9(2); RH/14, 15, 20]

24. Fusion of the ascending lamella of the inner demibranchs: 0, tending not to be fused to the visceral mass except at their anterior extremity; 1, tending to be fused to the visceral mass. Amongst palaeoheterodonts, the ctenidia are generally free of the visceral mass except in etheriids (Heard & Vail, 1976a), hyriids (McMichael & Hiscock, 1958), mycetopodids (Ortmann, 1921), *Mutela* (Bloomer, 1932), and certain anodontine and lampsiline (Unionidae) genera (Ortmann, 1912b). [G/14; HBH/8; RH/16, 17]

25. Attachment of the anterior end of the inner demibranchs: 0, attach to the visceral mass distant from the labial palps (Stasek Type I); 1, attach close to or in contact with the labial palps (Stasek Type III; Stasek, 1963). Ortmann (1911a: pl. 6) showed *Margaritifera*, *Elliptio*, *Fusconaia*, and *Parreysia* having inner demibranchs that attach distant from the labial palps (figs 1–4), and *Aspatharia*, *Prisodon*, and *Castalia* with them attaching close to or in contact with the labial palps (figs 5–7). Coded as 'missing' in *Pseudomulleria* because Woodward's (1898: fig. 1) and Yonge's (1962: fig. 17) figures are insufficient to distinguish the state. [G/15; GW/78]

26. Labial palp shape: 0, triangular; 1, semicircular to kidney-shaped; 2, falciform. Coding generally follows Hoeh *et al*. (2001) and Roe & Hoeh (2003). [HBH/26; RH/51–53]

Mantle characters

The complex of characters that comprise the posterior incurrent and excurrent apertures of palaeoheterodonts can be subdivided in a number of ways, and there is no clear correspondence between the homology hypotheses of Graf (2000a), Hoeh *et al*. (2001) and Roe & Hoeh (2003). Whereas Graf (2000a) coded the individual mantle fusions ventral, between, and dorsal to the apertures, Hoeh *et al*. (2001) treated the presence of individual siphons vs. simple openings unbounded by pallial fusion. Characters 27–31 below follow Graf (2000a).

27. Pallial fusion ventral to the incurrent aperture: 0, none, or occasionally with a short connection; 1, extensive fusion of the inner folds of the mantle, with pedal gape; generally associated with siphons and a pallial sinus (Type V) (Figs 11, 12F). Incurrent siphons are only found in *Mutela* (Bloomer, 1932), *Pleiodon* (Pain & Woodward, 1961b; Heard & Dougherty, 1980), and related genera. [G/16; HBH/2(2); RH/4]

28. Pallial fusion betwixt the incurrent and excurrent apertures: 0, none; integrity of the incurrent and excurrent apertures is accomplished by fusion of the ctenidia to the mantle ('slightly incomplete' diaphragm) or via 'pallial ridges' (incomplete diaphragm); 1, fusion of the inner folds of the mantle present (complete diaphragm) (Type III–V) (Figs 11, 12C–F). A complete diaphragm is found in hyriids (McMichael & Hiscock, 1958), mycetopodids (Ortmann, 1921), etheriids (Yonge, 1962; Heard & Vail, 1976a), and iridinids (Ortmann, 1910a, 1918a; Bloomer, 1932), as well as the two outgroup species (Boss, 1982). [G/17; HBH/2, 9(0); RH/18; GW/55]

29. Complete diaphragm perforated: 0, not perforated; 1, perforated (McMichael & Hiscock, 1958: fig. 5). Perforated diaphragms are known only from certain Australasian hyriids. Taxa without a complete diaphragm (i.e. without mantle fusion between the apertures) coded as 'inapplicable'. [G/20; HBH/9(1); RH/19]

30. Pallial fusion dorsal to the excurrent aperture: 0, none; the folds of the mantle come together at the dorsal mantle isthmus coincident with the outer folds; 1, short fusion of the inner folds of the mantle, forming a third, supra-anal aperture (Type II) (Figs 11, 12B); 2, fusion of the inner folds of the mantle continuous to the mantle isthmus, without supra-anal aperture (Types IV, V) (Figs 11, 12D–F). Complete closure of the mantle dorsal to the excurrent aperture is known from hyriids and iridinids (Ortmann, 1910a, 1918a, 1921); only unionids have a supra-anal aperture (Ortmann, 1912b). [LMD/5; G/18; HBH/1, 2; RH/1, 3, 4]

31. Length of the pallial fusion betwixt the supra-anal and excurrent apertures: 0, not distinctly shorter than the excurrent aperture; 1, distinctly shorter than the excurrent aperture. Coded as inapplicable in taxa without a supra-anal aperture (Ortmann, 1912b). [G/19]

32. Edge of the incurrent aperture: 0, bearing simple papillae on the inner fold of the mantle; middle fold may be highly reduced; 1, elaborate papillae, with middle fold present; 2, smooth. Coding simplified from Hoeh *et al*. (2001) and Roe & Hoeh (2003). Almost all unionoids with a simple incurrent aperture have variously developed incurrent papillae (Ortmann, 1912b, 1921). [HBH/3; GW/131, 132; RH/5–7]

33. Edge of the excurrent aperture: 0, without papillae, or only crenulated; 1, bearing distinct papillae. Distinct excurrent papillae are known from *Neotrigonia* and some North American species (Ortmann, 1912b; Morton, 1987). This character has not been used in previous phylogenetic analyses of the Palaeoheterodonta.

34. Mantle elaborations ventral to the incurrent aperture: 0, elaborations lacking; 1, posteroventral mantle elaborations with conspicuous papillae or a ribbon-like flap. Certain lampsiline (Unionidae) species have mantle 'lures' (Ortmann, 1912b). [LMD/13; G/22; GO/13; HBH/4; RH/8]

Other soft anatomical characters

35. Foot: 0, laterally compressed foot for burrowing; 1, anchor-like foot with 'toe' and 'heel'; 2, foot reduced or absent; X, mushroom-shaped foot (Fig. 16D). The anchor-like foot of *Neotrigonia* was shown by Morton (1987: fig. 4). The foot is reduced in *Etheria*, *Acostaea*, and *Pseudomulleria* (Yonge, 1962, 1978). [GW/109, 112, 116]

36. Byssus: 0, present only in juveniles; 1, present in both juveniles and adults (Brusca & Brusca, 1990: 727, fig. 21b). In our taxon set, an adult byssus is known only from *Mytilus.* All palaeoheterodonts are coded as lacking an adult byssus (Gould, 1969). [GW/115]

37. Pedal retractor muscles: 0, both anterior and posterior retractors present in adults; 1, anterior retractor absent in adults. Both *Acostaea* and *Pseudomulleria* have lost the anterior muscle complex, including the associated pedal retractor (Yonge, 1978); *Etheria* retains its vestigial anterior pedal retractor (Yonge, 1962: fig. 3) [GW/83]

38. Abdominal sense organ: 0, present; 1, absent. All palaeoheterodonts are coded as having an abdominal sense organ, following Giribet & Wheeler (2002). [GW/137]

39. Position of the anus on the posterior adductor muscle: 0, posterior edge of the posterior adductor muscle; 1, dorsal edge of the posterior adductor muscle. Coding generally follows Hoeh *et al*. (2001), except that 'posterior' and 'posteroventral' placement of the anus are combined into a single state; many taxa coded as 'missing'. Only *Margaritifera* and *Cumberlandia* have the anus located on the dorsal edge of the posterior adductor muscle (Hoeh *et al*., 2001; Roe & Hoeh, 2003). [HBH/5; RH/9–11]

40. Stomach type: 0, Type III; 1, Type IV. Coding follows Purcheon (1987). All palaeoheterodonts coded as Type IV. [GW/94]

41. Intestine complexity: 0, intestine simple, undifferentiated; 1, intestine complex with three compartments. Coding generally follows Hoeh *et al*. (2001); many taxa coded as 'missing'. [HBH/6; RH/12, 13]

Life history characters

42. Habitat: 0, marine; 1, freshwater. [G/23; GO/1]

43. Parental care: 0, no parental care, with gametes of both sexes spawned freely; 1, ova fertilized and larvae brooded in the interlamellar spaces of the ctenidia. All Unionoida coded as brooding. [G/24; GO/2; GW/152, 153]

44. Demibranchs occupied by marsupium: 0, tetragenous (all four demibranchs); 1, endobranchous (inner pair of demibranchs only); 2, ectobranchous (outer pair of demibranchs only). Endobranchy is known from hyriids (McMichael & Hiscock, 1958), mycetopodids (Ortmann, 1921), iridinids (Ortmann, 1910a, 1918a; Bloomer, 1932), and etheriids (Heard & Vail, 1976a). Tetrageny and ectobranchy occur in various lineages of the Unionoidea (Ortmann, 1912b). Coded as 'inapplicable' in nonbrooding taxa. [LMD/1; G/25; GO/3; HBH/10; RH/21–23]

45. Brooding period: 0, tachtictia; 1, bradytictia. Coded only for the Unioninae and Ambleminae, for which there are reliable data (Graf, 1997a); coded as 'inapplicable' in nonbrooding taxa. Bradytictia occurs in the anodontine and lampsiline Unionidae (Graf & Ó Foighil, 2000a). [LMD/2; GO/12]

46. Portion of the outer demibranch that is marsupial: 0, entire outer demibranch; 1, only a central or posterior portion is utilized for brooding. The marsupium is restricted in lampsiline genera to only a portion of the outer demibranch (Ortmann, 1912b). Coded as 'inapplicable' in taxa that do not brood in the outer demibranchs. [LMD/12; G/26; GO/4; HBH/28(2); RH/56]

47. Portion of the inner demibranch that is marsupial: 0, entire inner demibranch; 1, only a central portion is utilized for brooding (Ortmann, 1921: pl. 46). Among hyriids, the interlamellar septa are more dense in the central section of the marsupial demibranch where brooding occurs (McMichael & Hiscock, 1958). Coded as 'inapplicable' in taxa that do not brood in the inner demibranchs. [G/27; HBH/28(1); RH/57]

48. Subdivision of marsupial water-tubes: 0, not subdivided; 1, subdivided by secondary, lateral septa (i.e. tripartite) (Ortmann, 1911b: pl. 86, figs 9–10b); 2, interlamellar septa effectively separated by a 'marked swelling' (Ortmann, 1921: pl. 48, Fig. 7b; Heard & Vail, 1976a: figs 7, 8). Discussed in the text. Coded as 'inapplicable' in taxa in which the interlamellar space of the marsupium is not divided into water-tubes. [LMD/4; G/30, 31; GO/7, 8; HBH/15, 16; RH/30, 31]

49. Relative number of transverse (primary) septa: 0, greater in marsupial than in nonmarsupial regions of marsupial and in nonmarsupial demibranchs; 1, similar in marsupial and nonmarsupial regions of marsupial and in nonmarsupial demibranchs. Coding generally follows Hoeh *et al*. (2001) and Roe & Hoeh (2003); coded as 'inapplicable' in taxa in which the interlamellar space of the marsupium is not divided into water-tubes. [HBH/13; RH/26, 27]

50. 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 (Ortmann, 1911b: pl. 88, fig. 3a, b); 2, mantle capable of expansion ventrally as well as laterally; tissue may allow larvae to be released via the ventral margin (Ortmann, 1911b: pl. 88, figs 18–20). Amongst anodontine (Unionidae) mussels, the gravid demibranchs distend laterally, taking on the appearance of an inflated air mattress. The lampsiline marsupium is restricted to a portion of the demibranch. It is easily identifiable by its ventral

extension and inflation. Ortmann (1910b, 1912b) observed that glochidia were forcibly expelled through the ventral margin of the lampsiline marsupium. [LMD/7; G/32, 33, 35; GO/9–11; HBH/11; RH/22]

51. Spermatozuegmata: 0, spermatozoa released separately; 1, spermatozoa released as part of a motile, acellular sphere (Edgar, 1965: fig. 1; Lynn, 1994: fig. 1; Waller & Lasee, 1997). Only palaeoheterodonts for which observations exist were coded. All outgroup taxa were coded as releasing spermatozoa separately. This character has not been used in previous phylogenetic analyses of the Palaeoheterodonta.

52. Spermatozoa with multiple pro-acrosomal vesicles: 0, absent; 1, present. Only palaeoheterodonts for which there are observations are coded as having spermatozoa with multiple pro-acrosomal vesicles, although these may fuse (or disappear) in the mature gamete (Healy, 1989, 1996; Peredo *et al*., 1990; Rocha & Azevedo, 1990). Discussed in the text. [GW/155]

53. Doubly uniparental inheritance of mitochondria: 0, absent, mitochondrial inheritance strictly maternal; 1, present. Most palaeoheterodont species are coded as 'missing data'. Observations come from Curole & Kocher (2002) and Hoeh *et al*. (2002b). Discussed in the text. This character has not been used in previous phylogenetic analyses of the Palaeoheterodonta.

Larval characters

As with the characters described above for palaeoheterodont apertures, there is no simple one-to-one correspondence between the codings employed in the previous analyses of Graf (2000a), Hoeh *et al*. (2001), and Roe & Hoeh (2003).

54. Parasitic larvae: 0, free-living veliger; 1, larvae an obligate parasite on aquatic vertebrates; X, direct development of larvae. All unionoids in the analysis coded as parasitic. Although direct-developing species are known, none that are completely free-living [e.g. *Grandidieria burtoni* (Woodward); Kondo, 1990] were included in the present study. [G/35; HBH/17; RH/32, 33; GW/177, 181]

55. Parasitic larval morphology: 0, bivalved glochidium, with a calcareous shell and a single adductor muscle (Fig. 9A–D); 1, trilobed lasidium, with a univalved, noncalcareous larval shell (Fig. 9E, F). Discussed in the text. Coded as 'inapplicable' in nonparasitic taxa. [G/36; HBH/17; RH/32, 33]

56. Parasitic larval attachment: 0, larva attaches by encysting in host epithelium; 1, larva attaches by tubular appendages, forming a 'haustorium'. Glochidia and most lasidia attach to a host fish by forming a cyst (Wächtler *et al*., 2001: fig 6.7), but the lasidium of *Mutela bourguignati* (Bourguignat) attaches via tubular appendages (Wächtler *et al*., 2001: figs 6.13–6.15). A haustorium has only been observed for this species; following Hoeh *et al*. (2001), we have coded both congeners in our analysis similarly. Coded as 'inapplicable' in nonparasitic taxa. [G/38; HBH/17; RH/34]

57. Glochidium morphological type: 0, subcircular or subovate, without medioventral hooks (Fig. 9C); 1, subtriangular, with a medioventral hook lacking spines (Fig. 9B); 2, subtriangular, with a medioventral hook bearing numerous basal spines (Fig. 8A); X, celtiform, 'axe-head'-shaped (Fig. 9D). Discussed in the text. Coded as 'inapplicable' in taxa without glochidia. [LMD/8–11; G/37; HBH/18, 19; RH/35–38]

58. Glochidial 'placentae': 0, glochidia released as loose masses more or less associated by mucus; 1, glochidia released as part of persistent 'placentae' or conglutinates (Ortmann, 1912b; Kat, 1984: fig. 2). In some Nearctic freshwater mussels (for which data are available), glochidia are released as part of mucus structures that may be adapted for attracting a host fish (e.g. Hartfield & Hartfield, 1996; Watters, 1999, 2002). Walker *et al*. (2001: 15) reported for hyriids that 'the glochidia generally are released in straw-coloured, worm-like conglutinates that must attract fish'. However, for most species, the persistence of these mucus conglutinates after glochidial release has not been thoroughly investigated (C. Barnhart, Missouri State University, Springfield, MO, pers. comm.). Coded as 'inapplicable' in taxa without glochidia. This character has not been used in previous phylogenetic analyses of the Palaeoheterodonta.

59. Release of lasidia: 0, lasidia do not remain in the egg membrane after discharge from the marsupium; 1, lasidia 'remain enclosed in the egg membrane for a few hours or even a day before infection' (Wächtler *et al*., 2001: 106). This character has not been used in previous phylogenetic analyses of the Palaeoheterodonta.

APPENDIX 2

MORPHOLOGICAL CHARACTER DIAGNOSES

The following is a diagnosis of the hypothesized most parsimonious transformations of the morphological characters (listed in Appendix 1) on the fully resolved arbitrary tree from our CE analysis (problematic sequences excluded). For each character, the consistency and retention indices and number of steps on the tree are given. In the case of ambiguous transformations, both the ACCTRAN and DELTRAN optimizations are listed; some transformations are ambiguously optimized because they transform along unrooted basal branches. The taxonomy follows that used in Table 2.

CH	CI	RI	S	Optimization	Transformation
Shell characters					
1	1.000	8	1	UNAMB	*0* → *1*: *Mytilus*
2	0.667	0.500	3	AMB	*0* → *1*: *Mytilus*
				AMB	*0* → *2*: *Astarte*
				UNAMB	*0* → *1*: Etheriidae
3	1.000	8	1	UNAMB	*0* → *1*: Palaeoheterodonta
4	0.286	0.706	7	UNAMB	*0* → *1*: (Etheriidae + Mycetopodidae + Iridinidae)
				UNAMB	*1* → *2*: *Mutela dubia*
				UNAMB	*0* → *1*: *Lortiella*
				UNAMB	*0* → *1*: *Cumberlandia*
				UNAMB	*0* → *1*: Anodontini
				UNAMB	*0* → *1*: *Gonidea*
				UNAMB	*0* → *1*: (*Pseudodon* + *Pilsbryoconcha*)
5	1.000	8	2	UNAMB	*0* → *1*: *Mytilus*
				UNAMB	*0* → *2*: *Etheria*
6	0.500	0.857	2	UNAMB	*0* → *1*: (Etheriidae + Mycetopodidae + Iridinidae)
				UNAMB	*1* → *0*: *Mutela*
7	1.000	1.000	1	UNAMB	*0* → *1*: (*Acostaea* + *Pseudomulleria*)
8	0.333	0.000	2	UNAMB	*0* → *1*: *Chambardia*
				UNAMB	*0* → *1*: *Velesunio angasi*
			1	DELTRAN	*0* → *1*: *Etheria*
				ACCTRAN	*0* → *1*: Etheriidae
9	0.400	0.727	2	DELTRAN	*0* → *1*: Iridinidae
				DELTRAN	*0* → *2*: Mycetopodidae
				ACCTRAN	*0* → *1*: (Etheriidae + Mycetopodidae + Iridinidae)
				ACCTRAN	*1* → *2*: Mycetopodidae
			3	UNAMB	*0* → *2*: Margaritiferidae
				UNAMB	*0* → *2*: Unioninae
				UNAMB	*2* → *0*: (*Strophitus* + *Pyganodon*)
10	0.125	0.125	3	ACCTRAN	*0* → *1*: (Mycetopodidae + Iridinidae)
				ACCTRAN	*1* → *0*: *Mutela dubia*
				ACCTRAN	*1* → *0*: Anodontites
				DELTRAN	*0* → *1*: *Mutela rostrata*
				DELTRAN	*0* → *1*: (*Chambardia* + *Aspatharia*)
				DELTRAN	*0* → *1*: *Monocondylaea*
			2	DELTRAN	*0* → *1*: *Obliquaria*
				DELTRAN	*0* → *1*: *Ptychobranchus*
				ACCTRAN	*0* → *1*: (*Ptychobranchus* + *Obliquaria* + *Truncilla*)*
				ACCTRAN	*1* → *0*: *Truncilla*
			3	AMB	*0* → *1*: *Neotrigonia*
				UNAMB	*0* → *1*: *Fusconaia*
				UNAMB	*0* → *1*: *Epioblasma*
11	0.500	0.500	2	UNAMB	*0* → *1*: *Neotrigonia*
				UNAMB	*0* → *1*: Margaritiferidae
12	0.222	0.588	7	UNAMB	*0* → *1*: *Neotrigonia*
				UNAMB	*0* → *2*: *Aspatharia*

APPENDIX 2. *Continued*

CH	CI	RI	S	Optimization	Transformation
				UNAMB	*0* → *1*: (*Hyridella* + *Castalia* + *Diplodon*)
				UNAMB	*0* → *2*: (*Coelatura* + *Contradens* + *Gonidea* + *Unioninae* + *Ambleminae*)
				UNAMB	*2* → *0*: (*Gonidea* + Ambleminae)
				UNAMB	*0* → *2*: (*Ligumia* + *Villosa* + *Epioblasma* + *Actinonaias* + *Lampsilis*)
				UNAMB	*2* → *0*: *Epioblasma*
			2	DELTRAN	*2* → *0*: *Strophitus*
				DELTRAN	*2* → *0*: *Alasmidonta*
				ACCTRAN	*2* → *0*: (*Alasmidonta* + *Strophitus* + *Pyganodon*)
				ACCTRAN	*0* → *2*: *Pyganodon*
13	0.667	0.333	6	UNAMB	*0* → *1*: *Neotrigonia*
				UNAMB	*0* → *1*: *Castalia*
				UNAMB	*0* → *3*: *Coelatura*
				UNAMB	*0* → *4*: (*Tritogonia* + *Quadrula*)
				UNAMB	*0* → *2*: *Amblema*
				UNAMB	*0* → *4*: *Obliquaria*
14	1.000	1.000	1	UNAMB	*0* → *1*: Etheriidae
15	1.000	8	1	AMB	*0* → *1*: (*Acostaea* + *Pseudomulleria*)
16	0.500	0.667	2	UNAMB	*0* → *1*: (*Ligumia* + *Villosa* + *Epioblasma* + *Actinonaias* + *Lampsilis*)
				UNAMB	*1* → *0*: *Actinonaias*
Ctenidia and labial palp characters					
17	0.500	0.000	2	AMB	*0* → *1*: *Astarte*
				AMB	*0* → *1*: Unionoida
18	1.000	1.000	1	UNAMB	*0* → *1*: Etheriidae
19	0.400	0.857	2	ACCTRAN	*0* → *1*: Unionoida
				ACCTRAN	*1* → *0*: Margaritiferidae
				DELTRAN	*0* → *1*: (Hyriidae + Etheriidae + Iridinidae + Mycetopodidae)
				DELTRAN	*0* → *1*: Unionidae
			3	UNAMB	*1* → *2*: (Mycetopodidae + Iridinidae)
				UNAMB	*1* → *2*: Anodontini
				UNAMB	*1* → *2*: (Quadrulini + Amblemini + Pleurobemini + Lampsilini)
20	1.000	1.000	2	UNAMB	*0* → *1*: *Astarte*
				UNAMB	*0* → *2*: Unionoida
21	1.000	8	1	ACCTRAN	*0* → *1*: Etheriidae
				DELTRAN	*0* → *1*: *Etheria*
22	1.000	1.000	1	UNAMB	*0* → *1*: Palaeoheterodonta
23	0.500	0.750	2	ACCTRAN	*0* → *1*: Unionoida
				ACCTRAN	*1* → *0*: Margaritiferidae
				DELTRAN	*0* → *1*: (Hyriidae + Etheriidae + Iridinidae + Mycetopodidae)
				DELTRAN	*0* → *1*: Unionidae
24	0.125	0.667	6	UNAMB	*0* → *1*: *Astarte*
				UNAMB	*0* → *1*: (Hyriidae + Etheriidae + Iridinidae + Mycetopodidae)
				UNAMB	*1* → *0*: (*Chambardia* + *Aspatharia*)
				UNAMB	*0* → *1*: *Coelatura*
				UNAMB	*0* → *1*: *Truncilla*
				UNAMB	*0* → *1*: (*Ligumia* + *Villosa* + *Epioblasma* + *Actinonaias* + *Lampsilis*)
			2	ACCTRAN	*0* → *1*: (*Alasmidonta* + *Strophitus* + *Pyganodon*)
				ACCTRAN	*1* → *0*: *Pyganodon*
				DELTRAN	*0* → *1*: *Strophitus*
				DELTRAN	*0* → *1*: *Alasmidonta*
25	1.000	1.000	1	UNAMB	*0* → *1*: (Hyriidae + Etheriidae + Iridinidae + Mycetopodidae)
26	1.000	1.000	2	UNAMB	*0* → *1*: (Etheriidae + Iridinidae + Mycetopodidae)
				UNAMB	*0* → *2*: (Margaritiferidae + Unionidae)

APPENDIX 2. *Continued*

CH	CI	RI	S	Optimization	Transformation
Mantle characters					
27	1.000	1.000	2	UNAMB	*0* → *1*: *Mutela*
28	0.500	0.952	2	ACCTRAN	*1* → *0*: Palaeoheterodonta
				ACCTRAN	*0* → *1*: (Hyriidae + Etheriidae + Iridinidae + Mycetopodidae)
				DELTRAN	*1* → *0*: *Neotrigonia*
				DELTRAN	*1* → *0*: (Margaritiferidae + Unionidae)
29	0.500	0.750	2	UNAMB	*0* → *1*: (*Velesunio ambigua* + *V. angasi* + *Lortiella*)
				UNAMB	*0* → *1*: (*Hyridella depressa* + *H. australis*)
30	0.667	0.957	3	UNAMB	*0* → *2*: Iridinidae
				UNAMB	*0* → *2*: Hyriidae
				UNAMB	*0* → *1*: Unionidae
31	0.500	0.800	2	UNAMB	*0* → *1*: (Quadrulini + Amblemini + Pleurobemini + Lampsilini)
				UNAMB	*1* → *0*: Lampsilini
32	0.667	0.500	3	AMB	*0* → *1*: *Mytilus*
				AMB	*0* → *1*: *Neotrigonia*
				UNAMB	*0* → *2*: *Mutela*
33	0.200	0.333	2	AMB	*0* → *1*: *Mytilus*
				AMB	*0* → *1*: *Neotrigonia*
			3	ACCTRAN	*0* → *1*: (Quadrulini + Amblemini + Pleurobemini + Lampsilini)
				ACCTRAN	*1* → *0*: *Quadrula*
				ACCTRAN	*1* → *0*: Lampsilini
				DELTRAN	*0* → *1*: *Tritogonia*
				DELTRAN	*0* → *1*: *Amblema*
				DELTRAN	*0* → *1*: Pleurobemini
34	0.500	0.667	2	UNAMB	*0* → *1*: (*Ligumia* + *Villosa* + *Epioblasma* + *Actinonaias* + *Lampsilis*)
				UNAMB	*1* → *0*: *Actinonaias*
Other anatomical characters					
35	1.000	1.000	2	UNAMB	*0* → *1*: *Neotrigonia*
				UNAMB	*0* → *2*: Etheriidae
36	1.000	8	1	UNAMB	*0* → *1*: *Mytilus*
37	1.000	1.000	1	UNAMB	*0* → *1*: (*Acostaea* + *Pseudomulleria*)
38	1.000	8	1	UNAMB	*0* → *1*: *Astarte*
39	1.000	1.000	1	UNAMB	*0* → *1*: Margaritiferidae
40	1.000	8	1	UNAMB	*1* → *0*: *Mytilus*
41	1.000	1.000	1	UNAMB	*0* → *1*: (Etheriidae + Iridinidae + Mycetopodidae)
Life history characters					
42	1.000	1.000	1	UNAMB	*0* → *1*: Unionoida
43	1.000	1.000	1	UNAMB	*0* → *1*: Unionoida
44	0.400	0.880	1	AMB	*0* → *1*: (Hyriidae + Etheriidae + Iridinidae + Mycetopodidae)
			2	DELTRAN	*0* → *2*: *Contradens*
				DELTRAN	*0* → *2*: Unioninae
				ACCTRAN	*0* → *2*: (*Contradens* + *Gonidea* + Unioninae + Ambleminae)*
				ACCTRAN	*2* → *0*: (*Gonidea* + Ambleminae)
			2	UNAMB	*0* → *2*: (Pleurobemini + Lampsilini)
				UNAMB	*2* → *0*: *Fusconaia*
45	0.333	0.778	3	UNAMB	*0* → *1*: Anodontini
				UNAMB	*0* → *1*: Lampsilini
				UNAMB	*1* → *0*: *Obliquaria*
46	1.000	1.000	1	UNAMB	*0* → *1*: Lampsilini
47	1.000	1.000	1	UNAMB	*0* → *1*: Hyriidae
48	1.000	1.000	2	UNAMB	*0* → *2*: (Etheriidae + Iridinidae + Mycetopodidae)
				UNAMB	*0* → *1*: Anodontini
49	0.333	0.857	1	UNAMB	*0* → *1*: (Etheriidae + Iridinidae + Mycetopodidae)
			2	ACCTRAN	*0* → *1*: Lampsilini

APPENDIX 2. *Continued*

CH	CI	RI	S	Optimization	Transformation
				ACCTRAN	*1 → 0*: *Ptychobranchus*
				DELTRAN	*0 → 1*: (*Obliquaria* + *Truncilla*)
				DELTRAN	*0 → 1*: (*Ligumia* + *Villosa* + *Epioblasma* + *Actinonaias* + *Lampsilis*)
50	1.000	1.000	2	UNAMB	*0 → 1*: Anodontini
				UNAMB	*0 → 2*: Lampsilini
51	1.000	1.000	1	ACCTRAN	*0 → 1*: Unionoida
				DELTRAN	*0 → 1*: (*Gonidea* + Unioninae + Ambleminae)*
52	1.000	1.000	1	UNAMB	*0 → 1*: Palaeoheterodonta
53	1.000	8	1	UNAMB	*1 → 0*: *Astarte*
Larval characters					
54	1.000	1.000	1	UNAMB	*0 → 1*: Unionoida
55	1.000	1.000	1	UNAMB	*0 → 1*: (Etheriidae + Iridinidae + Mycetopodidae)
56	1.000	1.000	1	ACCTRAN	*0 → 1*: Iridinidae
				DELTRAN	*0 → 1*: *Mutela*
57	1.000	1.000	1	DELTRAN	*0 → 1*: Hyriidae
				ACCTRAN	*0 → 1*: (Hyriidae + Etheriidae + Iridinidae + Mycetopodidae)
			1	UNAMB	*0 → 2*: Unioninae
58	0.250	0.625	1	DELTRAN	*0 → 1*: Hyriidae
				ACCTRAN	*0 → 1*: (Hyriidae + Etheriidae + Iridinidae + Mycetopodidae)
			3	UNAMB	*0 → 1*: *Strophitus*
				UNAMB	*0 → 1*: (Pleurobemini + Lampsilini)
				UNAMB	*1 → 0*: *Actinonaias*
59	1.000	1.000	1	ACCTRAN	*0 → 1*: Iridinidae
				DELTRAN	*0 → 1*: *Mutela*

ACCTRAN, accelerated transformation; AMB, ambiguous; CH, character number; CI, consistency index; DELTRAN, delayed transformation; RI, retention index; S, maximum parsimony steps; UNAMB, unambiguous.
*Clades not present in the strict consensus (Fig. 2).

APPENDIX 3

Classification of the palaeoheterodonta

The family group-level classification of the Palaeoheterodonta follows, including an exhaustive synonymy of family group nomina introduced for nonfossil genera. Synonyms are listed with the suffix appropriate to the level to which they are applied here (Art. 36, Principle of Coordination). For each nomen, the type genus is listed. In the case of *Hemisolasma* Rafinesque (type of HEMISOLASMINAE Starobogatov), we chose *Diplasma vitrea* Rafinesque as the type species to fix its synonymy with *Parreysia* Conrad (*fide* Frierson, 1927).

The arrangements proposed by Modell (1942, 1949, 1964), Starobogatov (1970), and others introduced numerous nominal family group taxa, especially for subtaxa within the Unionidae. Many of the type genera for these Old World lineages have not been included in phylogenetic analyses, and their position in the family is uncertain. We list these as *incertae sedis* subfamilies.

PHYLUM MOLLUSCA
CLASS BIVALVIA
SUBCLASS PALAEOHETERODONTA
ORDER TRIGONIOIDA

Family TRIGONIIDAE Lamarck, 1819

TRIGONIIDAE (‘Les trigonées’). Lamarck, 1819, *Anim. Sans. Vert.* **6**: 60. Properly latinized in Herrmannsen, 1849, *Ind. Gen. Malac.* **2**: 598, *fide* Cox (1969). Type genus: *Trigonia* Bruguière 1789 (Valid, ICZN O.327).

ORDER UNIONOIDA
SUPERFAMILY UNIONOIDEA RAFINESQUE, 1820

UNIODIA (‘Les Uniodés’). Rafinesque, 1820, *Ann. Gèn. Sci. Phys.* **5**: 290. Properly latinized in Fleming, 1828, *Hist. Brit. Anim.*: 408, 415. Type genus: *Unio* Philipsson in Retzius 1788. Valid (ICZN O.495); updated in Melville & Smith (1987).

Family UNIONIDAE s.s.

Subfamily UNIONINAE s.s.

Tribe UNIONINI s.s.
+ CAFFERIINI. Modell, 1942, *Arch. Moll.* **74**: 188. Type genus: *Cafferia* Simpson, 1900.
+ CUNEOPSINI. Mongin, 1963, *Mém. Soc. Géol. France, Paléont.* **96**: 19. Type genus: *Cuneopsis* Simpson, 1900.
+ ACUTICOSTINI. Starobogatov, 1967, *Trudy sess. vses. Palaeont. Obshch.* **9**: 178. Type genus: *Acuticosta* Simpson, 1900.
+ NODULARIINI. Zatravkin & Bogatov, 1987, *[Bivalves of the Far East]*: 25. Type genus: *Nodularia* Conrad, 1853.

Tribe ANODONTINI Rafinesque 1820
ANODONTIDIA ('Les Anodontides'). Rafinesque, 1820, *Ann. Gén. Sci. Phys.* **5**: 316. Properly latinized by Swainson, 1840, *Treatise Malac.*: 286, 381. Type genus: *Anodonta* Lamarck, 1799.
+ ALASMIDIA ('Les Alasmides'). Rafinesque, 1820, *Ann. Gén. Sci. Phys.* 5(13): 317. Properly latinized in Frierson, 1927, *Check List*: 8–9, 18. Type genus: *Alasmidonta* Say, 1818.
+ ALASMODONTINI. Swainson, 1840, *Treatise Malac.*: 264, 268, 275, 287–288, 290, 381. Type genus: *Alasmodonta* Say, 1819 and 'Of Authors', an unjustified emendation of *Alasmidonta* Say.
+ PSEUDANODONTINI. ('Jaeckel, 1962') Stadnichenko, 1984, *Fauna Ukrayiny* **29**: 112. Type genus: *Pseudanodonta* Bourguignat, 1876.
+ STROPHITINI. Starobogatov, 1970, *[Fauna Moll. Contin.]*: 69, 287. Type genus: *Strophitus* Rafinesque, 1820.

Subfamily AMBLEMINAE Rafinesque, 1820
AMBLEMIDIA ('Les Amblémides'). Rafinesque, 1820, *Ann. Gén. Sci. Phys.* **5**: 310. Properly latinized in Modell, 1942, *Arch. Moll.* **74**: 180. Type genus: *Amblema* Rafinesque, 1820.

Tribe GONIDEINI Ortmann, 1916
GONIDEINI. Ortmann, 1916, *Nautilus* **30**: 53. Type genus: *Gonidea* Conrad, 1857.

(The Amblemini Tribe group)

Tribe AMBLEMINI s.s.

Tribe QUADRULINI von Ihering, 1901
QUADRULINI. von Ihering, 1901, *Nautilus* **15**: 53. Type genus: *Quadrula* Rafinesque, 1820.
+ MEGALONAIADINI. Heard & Guckert, 1970, *Malacologia***10**: 338. Type genus: *Megalonaias* Utterback, 1915.

Tribe PLEUROBEMINI Hannibal, 1912
PLEUROBEMINI. Hannibal, 1912, *Proc. Malac. Soc. London* **10**: 118–119. Type genus: *Pleurobema* Rafinesque, 1819.
+ ELLIPTIONINI. Modell, 1942, *Arch. Moll.* **74**: 178, 180. Type genus: *Elliptio* Rafinesque, 1819.
+ POPENAIADINI. Heard & Guckert, 1970, *Malacologia* **10**: 339. Type genus: *Popenaias* Frierson, 1927.

Tribe LAMPSILINI von Ihering, 1901
LAMPSILINI. von Ihering, 1901, *Nautilus* **15**: 53. Type genus: *Lampsilis* Rafinesque, 1820.
+ PROPTERINI. Hannibal, 1912, *Proc. Malac. Soc. London* **10**: 118–119. Type genus: *Proptera* Rafinesque, 1819 (= *Potamilus* Rafinesque, 1818).
+ CYPROGENIINI. Starobogatov, 1970, *[Fauna Moll. Contin.]*: 70, 287. Type genus: *Cyprogenia* Agassiz, 1852.
+ DROMINI. Starobogatov, 1970, *[Fauna Moll. Contin.]*: 70, 287. Type genus: *Dromus* Simpson, 1900.
+ FRIERSONIINI. Starobogatov, 1970, *[Fauna Moll. Contin.]*: 70, 287. Type genus: *Friersonia* Ortmann, 1912.
+ GLEBULINI. Starobogatov, 1970, *[Fauna Moll. Contin.]*: 70, 287. Type genus: *Glebula* Conrad, 1853.
+ MEDIONIDINI. Starobogatov, 1970, *[Fauna Moll. Contin.]*: 70, 287. Type genus: *Medionidus* Simpson, 1900.
+ PILEINI. Starobogatov, 1970, *[Fauna Moll. Contin.]*: 70, 287 (incorrectly as 'PILAEINI'). Type genus: *Pilea* Simpson, 1900 (= *Epioblasma* Rafinesque, 1831).
+ PTYCHOBRANCHINI. Starobogatov, 1970, *[Fauna Moll. Contin.]*: 70, 287. Type genus: *Ptychobranchus* Simpson, 1900.

Incertae sedis
LAMPROTULINAE. Modell, 1942, *Arch. Moll.* **74**: 187. Type genus: *Lamprotula* Simpson, 1900.

NANNONAIINAE. Modell, 1942, *Arch. Moll.* **74**: 190. Type genus: *Nannonaia* Haas, 1913 (= *Elongaria* Haas, 1913).

OXYNAIINAE. Starobogatov, 1970, *[Fauna Moll. Contin.]*: 65, 284. Type genus: *Oxynaia* Haas, 1913.

PSEUDODONTINAE. Frierson, 1927, *Check List*: 67. Type genus: *Pseudodon* Gould, 1844.

HEUDEANINAE. Modell, 1942, *Arch. Moll.* **74**: 184. Type genus: *Heudeana* Frierson, 1922 (= *Ptychorhynchus* Simpson, 1900).

ARCIDOPSINAE. Starobogatov, 1970, *[Fauna Moll. Contin.]*: 61, 283. Type genus: *Arcidopsis* Simpson, 1900.

RECTIDENTINAE. Modell, 1942, *Arch. Moll.* **74**: 189. Type genus: *Rectidens* Simpson, 1900.
+ CONTRADENTINAE. Modell, 1942, *Arch. Moll.* **74**: 189. Type genus: *Contradens* Haas, 1913. [Family nomina synonymized by Brandt (1974: 287).]

HYRIOPSINAE. Modell, 1942, *Arch. Moll.* **74**: 188. Type genus: *Hyriopsis* Conrad, 1853.
+ LIMNOSCAPHINAE. Lindholm, 1932, *Trans. United Geol. Prosp. Serv. USSR* **238**: 12, 29. Type genus: *Limnoscapha* Lindholm, 1932 (= *Hyriopsis*).

MODELLNAIINAE. Brandt, 1974, *Arch. Moll.* **105**: 301. Type genus: *Modellnaia* Brandt, 1974.

PARREYSIINAE. Henderson, 1935, *GSA, Spec. Pap.* **3**: 69 (*nomen nudum*, unavailable); Modell, 1942, *Arch. Moll.* **74**: 186. Type genus: *Parreysia* Conrad, 1853.
+ HEMISOLASMINAE. Starobogatov, 1970, *[Fauna Moll. Contin.]*: 73. Type genus: *Hemisolasma* Rafinesque, 1831 [type species, *Diplasma vitrea* Rafineque (= *Parreysia olivaria* Lea, 1831 *fide* Frierson, 1927: 99), here selected].

LAMELLIDENTINAE. Modell, 1942, *Arch. Moll.* **74**: 188. Type genus: *Lamellidens* Simpson, 1900.
+ DIPLASMINAE. Modell, 1942, *Arch. Moll.* **74**: 177. Type genus: *Diplasma* Rafinesque, 1831 (= *Lamellidens fide* Frierson, 1914: 7).

PHYSUNIONINAE. Starobogatov, 1970, *[Fauna Moll. Contin.]*: 68. Type genus: *Physunio* Simpson, 1900.

BRAZZAEAINAE. Leloup, 1950, *Res. Sci. Explor. Hydrob. Lac Tanganyika (1946–47)* **3**: 73; Pain & Woodward, 1968, *Rev. Zool. Bot. Afric.* **77**: 192–193. Type genus: *Brazzaea* Bourguignat, 1885. [The proper spelling for this family group nomen should be 'Brazzaeinae', but as Pain & Woodward maintained Leloup's original usage, we retain it here (ICZN, Art. 29.5); Pain & Woodward's citation validates Leloup's pre-1960 nude usage (ICZN, Art. 13.2.1).]

COELATURINAE. Modell, 1942, *Arch. Moll.* **74**: 190 (incorrectly as 'Caelaturinae'). Type genus: *Coelatura* Conrad, 1853. [According to ICZN Art. 32.5.3.3, this family group nomen should be corrected because it is based on a misspelling of the type genus. See Rosenberg, Bogan & Spamer (1990).]

MWERUELLINAE. Pain & Woodward, 1968, *Rev. Zool. Botan. Afr.* **77**: 193, 200. Type genus: *Mweruella* Haas, 1936.

PSEUDAVICULINAE. Modell, 1942, *Arch. Moll.* **74**: 176. Type genus: *Pseudavicula* Simpson, 1900 non Etheridge, 1892 (= *Prisodontopsis* Tomlin, 1928).
+ DENTASPATHARIINAE. Modell, 1964, *Arch. Moll.* **93**: 83. Type genus: *Dentaspatharia* Modell, 1964 (fossil).
+ PRISODONTOPSINAE. Pain & Woodward, 1968, *Rev. Zool. Botan. Afr.* **77**: 193, 206. Type genus: *Prisodontopsis* Tomlin, 1928.

PSEUDOSPATHINAE. Leloup, 1950, *Res. Sci. Explor. Hydrob. Lac Tanganyika (1946–47)* **3**: 111; Pain & Woodward, 1968, *Rev. Zool. Bot. Afr.* **77**: 193, 212. Type genus: *Pseudospatha* Simpson, 1900. [Pain & Woodward's citation validates Leloup's pre-1960 nude usage (ICZN, Art. 13.2.1).]

LEGUMINAIINAE. Starobogatov, 1970, *[Fauna Moll. Contin.]*: 61, 283. Type genus: *Leguminaia* Conrad, 1865.

PSILUNIONINAE. Starobogatov, 1970, *[Fauna Moll. Contin.]*: 65, 284. Type genus: *Psilunio* Sabba Stefanescu, 1896.

Family MARGARITIFERIDAE Haas, 1940
+ MARGARITANIDAE. Ortmann, 1910, *Nautilus* **23**: 114. Type genus: *Margaritana* Schumacher, 1817. [Suppressed, ICZN O.495.]
MARGARITIFERIDAE. Haas, 1940, *Zool. Ser. Field Mus. Nat. Hist.* **24**: 119. Type genus: *Margaritifera* Schumacher, 1816. [Valid, ICZN O.495.]
+ CUMBERLANDIIDAE. Heard & Guckert, 1970, *Malacologia* **10**: 338 (incorrectly as 'Cumberlandinae'). Type genus: *Cumberlandia* Ortmann, 1912.

SUPERFAMILY ETHERIOIDEA DESHAYES, 1830

ETHERIOIDEA ('Ethéries'). Deshayes, 1830, *Enc. Méth.* **2**: fold-out, fam. no. 20. Properly latinized by Swainson, 1840, *Treatise Malac.*: 257, 390. Type genus: *Etheria* Lamarck, 1807.
+ AETHERIOIDEA ('Aethéries'). 'Deshayes, 1830' Herrmannsen, 1846, *Ind. Gen. Malac.* **1**: 24. Type genus: *Aetheria* Oken, 1818, an unnecessary emendation of *Etheria* Lamarck, 1807.
+ MULLERIOIDEA ('Mullerie'). Deshayes, 1830, *Enc. Méth.* **2**: fold-out, fam. no. 23. Properly latinized by Starobogatov, 1970, *[Fauna Moll. Contin.]*: 73–74. Type genus: *Mulleria* Férussac, 1823.

Family HYRIIDAE Swainson, 1840
HYRIANAE. Swainson, 1840, *Treatise Malac.*: 268, 282, 287, 379 (also as 'Hyrinae'). Suffix emended by Ortmann, 1910, *Nautilus* **23**: 115. Type genus: *Hyria* Lamarck, 1819 (= *Prisodon* Schumacher, 1817).

Subfamily VELESUNIONINAE Iredale, 1934
VELESUNIONAE. Iredale, 1934, *Austral. Zool.* **8**: 58, 76. Suffix emended by Modell, 1942, *Arch. Moll*. **74**: 178. Type genus: *Velesunio* Iredale, 1934.
+ LORTIELLINAE. Iredale, 1934, *Austral. Zool.* **8**: 58, 77. Type genus: *Lortiella* Iredale, 1934.

Subfamily HYRIINAE s.s.
+ PRISODONTINAE. Modell, 1942, *Arch. Moll.* **74**: 174. Type genus: *Prisodon* Schumacher, 1817.

Tribe HYRIDELLINI McMichael, 1956 (1934)
+ PROPEHYRIDELLINI. Iredale, 1934, *Austral. Zool.* **8**: 58, 76–77. Type genus: *Propehyridella* Cotton & Gabriel, 1932 (= *Hyridella* Swainson, 1840).
HYRIDELLINI. McMichael, 1956, *Nautilus* **70**: 42. Type genus: *Hyridella* Swainson, 1840. [The priority of Hyridellini over Propehyridellini due to priority of the type genus is valid because the replacement occurred before 1961 (ICZN, Art. 40.2).]
+ CUCUMERUNIONI. Iredale, 1934, *Austral. Zool.* **8**: 58, 77. Suffix emended by Modell, 1942, *Arch. Moll*. **74**: 184. Type genus: *Cucumerunio* Iredale, 1934.

Tribe RHIPIDODONTINI Starobogatov, 1970
+ DIPLODONTINI. von Ihering, 1901 (non Carpenter, 1861), *Nautilus***15**: 52–53. Type genus: *Diplodon* Spix & Wagner, 1827.
RHIPIDODONTINI. Starobogatov, 1970, *[Fauna Moll. Contin.]*: 72. Type genus: *Rhipidodonta* Mörch, 1853 (= *Diplodon*).

Tribe CASTALIINI Parodiz & Bonetto 1963
CASTALIINI. Lange de Morretes, 1949, *Arqu. Mus. Paranaense* **7**: 21 (*nomen nudum*, unavailable); Parodiz & Bonetto, 1963, *Malacologia* **1**: 201. Type genus: *Castalia* Lamarck, 1819.

Tribe HYRIINI s.s.

(The lasidium-bearing mussels)

Family ETHERIIDAE s.s.
+ BARTLETTIIDAE. Modell, 1942, *Arch. Moll.* **74**: 176. Type genus: *Bartlettia* Adams, 1866.
+ ACOSTAEIDAE. Morrison, 1973, *Bull. Am. Mala. Union (1972)*: 45. Type genus: *Acostaea* d'Orbigny, 1851.
+ PSEUDOMULLERIIDAE. Starobogatov, 1970, *[Fauna Moll. Contin.]*: 75, 288. Type genus: *Pseudomulleria* Anthony, 1907.

Family MYCETOPODIDAE Gray, 1840
MYCETOPODIDAE. Gray, 1840, *Synopt. Cat. Shells Brit. Mus.*: 142, 155. Type genus: *Mycetopoda* d'Orbigny, 1835.
+ MYCETOPIDAE. Adams & Adams, 1857, *Gen. Rec. Moll.* **2**: 504. Type genus: *Mycetopus* d'Orbigny, 1840 (= *Mycetopoda*).

Subfamily ANODONTITINAE Modell, 1942
ANODONTITINAE. Modell, 1942, *Arch. Moll.* **74**: 175. Type genus: *Anodontites* Bruguière, 1792.
+ GLABARINAE. Modell, 1942, *Arch. Moll.* **74**: 175. Type genus: *Glabaris* Gray, 1847.

Subfamily MYCETOPODINAE s.s.

Subfamily MONOCONDYLAEINAE Modell, 1942
MONOCONDYLAEINAE. Modell, 1942, *Arch. Moll.* **74**: 175. Type genus: *Monocondylaea* d'Orbigny, 1835.
+ FOSSULINAE. Bonetto, 1966, *Arch. Moll.* **95**: 3, 5. Type genus: *Fossula* Lea, 1870.

Subfamily LEILINAE Lange de Morretes, 1949
LEILINAE. Lange de Morretes, 1949, *Arqu. Mus. Paranaense* **7**: 28; Starobogatov, 1970, *[Fauna Moll. Contin.]*: 74. Type genus: *Leila* Gray, 1840. [Starobogatov's citation validates Lange de Morretes's pre-1960 nude usage (ICZN, Art. 13.2.1).]

Family IRIDINIDAE Swainson, 1840
IRIDINIDAE. Swainson, 1840, *Treatise Malac.*: 261, 286–287, 380. Type genus: *Iridina* Lamarck, 1819 (= *Mutela* Scopoli, 1777).
+ MUTELIDAE. Gray, 1847, *Proc. Zool. Soc. London* **15**: 197. Type genus: *Mutela* Scopoli, 1777.

Subfamily IRIDININAE s.s.
+ PLEIODONTINAE. Rochebrune, 1914, *Bull. Mus. d'Hist. Nat.* **10**: 342 (incorrectly as 'Pliodontidae'). Type genus: *Pliodon* Agassiz, 1846 and 'Of Authors', an unjustified emendation of *Pleiodon* Conrad, 1834. [According to ICZN Art. 32.5.3.2, this family name should be corrected.]
+ PLEIODONINAE. Pain & Woodward, 1964, *Annals Mus. R. Afr. Cent.***8**: 5. Type genus: *Pleiodon* Conrad, 1834.

Subfamily ASPATHARIINAE Modell, 1942
ASPATHARIINAE. Modell, 1942, *Arch. Moll.* **74**: 176. Type genus: *Aspatharia* Bourguignat, 1885.
+ SPATHOPSINAE. Modell, 1942, *Arch. Moll.* **74**: 176. Type genus: *Spathopsis* Simpson, 1900 (= *Chambardia* Servain 1890).