

Molecular Phylogeny of a Circum-Global, Diverse Gastropod Superfamily (Cerithioidea: Mollusca: Caenogastropoda): Pushing the Deepest Phylogenetic Limits of Mitochondrial LSU rDNA Sequences

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The Cerithioidea is a very diverse group of gastropods with ca. 14 extant families and more than 200 genera occupying, and often dominating, marine, estuarine, and freshwater habitats. While the composition of Cerithioidea is now better understood due to recent anatomical and ultrastructural studies, the phylogenetic relationships among families remain chaotic. Morphology-based studies have provided conflicting views of relationships among families. We generated a phylogeny of cerithioideans based on mitochondrial large subunit rRNA and flanking tRNA gene sequences (total aligned data set 1873 bp). Nucleotide evidence and the presence of a unique pair of tRNA genes (i.e., threonine + glycine) between valine-mtLSU and the mtSSU rRNA gene support conclusions based on ultrastructural data that Vermetidae and Campanilidae are not Cerithioidea, certain anatomical similarities being due to convergent evolution. The molecular phylogeny shows support for the monophyly of the marine families Cerithiidae, Turritellidae, Batillariidae, Potamididae, and Scaliolidae as currently recognized. The phylogenetic data reveal that freshwater taxa evolved on three separate occasions; however, all three recognized freshwater families (Pleuroceridae, Melanopsidae, and Thiaridae) are polyphyletic. Mitochondrial rDNA sequences provide valuable data for testing the monophyly of cerithiidae families and relationships within families, but fail to provide strong evidence for resolving relationships among families. It appears that the deepest phylogenetic limits for resolving caenogastropod relationships is less than about 245–241 mya, based on estimates of divergence derived from the fossil record. © 2002 Elsevier Science (USA)

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INTRODUCTION

Cerithioidean gastropods presumably radiated extensively during the Cretaceous (Houbrick, 1988; Healy and Wells, 1998), but their fossil record can be traced back at least to the early Triassic (Tracey *et al.*, 1993). Members of the superfamily are particularly diverse in the tropics and subtropics and occupy a variety of marine, estuarine, and freshwater habitats including mangrove forests (potamidids), estuarine mudflats (batillariids), seagrass beds (modulids, cerithiids), rocky intertidal shores (cerithiids, planaxids), algal fronds (litiopids), fast-flowing streams and rivers (pleurocerids, melanopsids, thiarids), and quiet lakes (pleurocerids, thiarids) (Houbrick, 1988; Healy and Wells, 1998). Cerithioideans are presumed to be monophyletic, representative basal members of the major gastropod clade Caenogastropoda and to play a fundamental role in evaluating higher-order phylogenetic studies of gastropods (Haszprunar, 1988; Ponder and Lindberg, 1997; Harasewych *et al.*, 1998; Colgan *et al.*, 2000).

Although numerous morphological, anatomical, and ecological studies have been conducted on various cerithioidean taxa (reviews in Houbrick, 1988; Healy and Wells, 1998), many phylogenetic issues remain unresolved or uncertain. Three morphology-based cladistic analyses of extant cerithioidean families have been published (Houbrick, 1988, Ponder, 1991; Glaubrecht, 1996), but all of them differ considerably in detail.

The composition of the Cerithioidea has changed over the years with the removal of several families, which were originally placed in the superfamily based on similar shell morphology or similar anatomical traits, notably the presence of open pallial genital ducts and the lack of a penis. The family Campanilidae, which is composed of a single relictual living species, *Campanile symbolicum*, was originally placed

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within the family Cerithiidae. Based on subsequent anatomical studies including sperm (Healy, 1986) and osphradial ultrastructure (Haszprunar, 1988), it was recognized as a separate family (Campanilidae) within the Cerithioidea (Houbrick, 1988; Healy, 1986, 1988a; Ponder and Warén, 1988), its own superfamily outside the Caenogastropoda (Haszprunar, 1988), and its own superfamily within the Caenogastropoda, but outside Cerithioidea (Houbrick, 1989; Haszprunar, 1992). Morphology-based (Ponder and Lindberg, 1997) and molecular studies (Harasewych *et al.*, 1998; Colgan *et al.*, 2000) support the placement within Caenogastropoda; however, taxonomic sampling in these studies was limited to one or a few cerithioideans.

Another problematic group whose placement has been debated in recent years is the family Vermetidae (i.e., tube mollusks). Vermetids were traditionally placed in the family Cerithioidea and indeed in the first morphological analysis of the group were shown to be sister to the filter-feeding family Turritellidae (Houbrick, 1988). Subsequent studies of sperm ultrastructure (Healy, 1988a,b) showed that they differ from Cerithioidea and were consequently placed in their own superfamily with closer affinities to more derived caenogastropods (Ponder and Warén, 1988). Independent data corroborating these anatomy-based studies are needed to further test the placement of the Vermetidae.

Freshwater cerithioideans are a very diverse assemblage of snails found on all continents except Antarctica. They are the dominant members of rivers and streams in the southeastern United States, African lakes, and many freshwater ecosystems of Asia and the Indo-Pacific (Holznagel and Lydeard, 2000; Glaubrecht, 1996, 1999). Initially, all freshwater taxa were treated as a single family, the nomenclaturally invalid Melaniidae (see Glaubrecht, 1999 for review). An alternative hypothesis proposed that there were three freshwater families, each closely related to three separate marine families (Morrison, 1954). The Pleuroceridae of the Americas, Asia, and possibly Africa was closely related to the marine family Cerithiidae, the Melanopsidae in Europe, New Zealand, and New Caledonia was closely related to the Modulidae, and the pantropical Thiaridae was closely related to the Planaxidae. Morrison's study was based on similarity of reproductive systems and was not widely accepted (e.g., Mandahl-Barth, 1967; Brown, 1994). The analyses of Houbrick (1988) and Ponder (1991) found evidence for two origins of freshwater taxa, the Melanopsidae + Pleuroceridae and the Thiaridae. A phylogenetic analysis of 13 cerithioidean families using 48 anatomical and ultrastructural characters found evidence for four separate origins of freshwater taxa (Glaubrecht, 1996); however, relationships of 7 of the families (including three of the freshwater taxa) remained in an unresolved basal polytomy. In addition,

no data matrix was provided and details of the narrative Hennigian method were not addressed, making it difficult to assess the robustness of the presented analysis (Bieler, 1998). It is evident that more data are needed to better resolve the evolution of freshwater cerithioideans.

The uncertainty that remains in our understanding of evolutionary relationships and composition of the Cerithioidea hinders research efforts to adequately address evolutionary, biogeographic, and ecological hypotheses. To this end, we present a molecular phylogeny of Cerithioidea based on near-complete mitochondrial (mt) large subunit (LSU) rRNA and upstream, flanking tRNA gene sequences (1873 aligned bp in length). Our objective is to test the monophyly of the Cerithioidea and specifically reassess the placement of Campanilidae and Vermetidae. In addition, we examine the evolution of freshwater taxa and the phylogenetic limits of the mt LSU rRNA gene.

MATERIALS AND METHODS

Specimens and Outgroups

We obtained specimens representing 11 and 30 cerithioidean families and genera, respectively. Regrettably, although we had obtained *Diala* (sole genus of the Dialidae; Ponder, 1991), we were unable to obtain quality DNA from ethanol-preserved specimens. In addition, we were unable to secure any specimens of Diastomatidae, a relict group, now represented by a single living species from Western Australia (Houbrick, 1981), or specimens of Siliquariidae, a marine family composed of a single genus. Although not examined phylogenetically, siliquariids are presumably closely related to the Turritellidae (Morton, 1953), a family from which we obtained 2 genera. Specimens were collected and preserved either by being frozen in an ultralow freezer (-70°C) or stored in 75–95% ethyl alcohol (etoh). Specimens used in the present study including traditional classification scheme, locality data, and/or source are shown in Table 1.

To test the monophyly of Cerithioidea, determine its sister taxon, and address several aforementioned phylogenetic hypotheses, *Campanile symbolicum* (Campaniloidea), *Hydrobia* sp. (Rissooidea) *Serpulorbis squamigerus* (Vermetoidea), *Littorina saxatilis* and *Nodilittorina unifasciata* (Littorinioidea), and *Busycotypus spiratum* (Neogastropoda) were included as ingroups (see Table 1). Two Architaenioglossan species (*Pomacea paludosa* and *Cyclophorus hirasei*) were selected as outgroup taxa based on results from other phylogenetic studies examining higher relationships within the Gastropoda (Ponder and Lindberg, 1997; Colgan *et al.*, 2000; C. Lydeard *et al.*, unpublished data).

TABLE 1

Caenogastropod Taxa Included in This Study Following Current Views of Classification (Gastropoda—Ponder and Lindberg (1997); Cerithioidea—Houbrick (1988), Ponder (1991); freshwater families—Morrison (1954), Houbrick (1988))

Taxon	GenBank Accession No.	Locality/source
Architaenioglossa		
Ampullarioidea		
<i>Cyclophorus hirasei</i>	AY010505	Amami-O-Shima, Japan J. Haresewych, USNM.
<i>Pomacea paludosa</i>	AY010506	Florida, USA, F. Thompson, FLMNH.
Sorbeoconcha		
Campaniloidae		
Campanilidae		
<i>Campanile symbolicum</i>	AY010507	Rottneest Island off Perth, Australia; F. Wells, AMS.
Cerithioidea		
Scaliolidae		
<i>Scaliola</i> sp.	AY010508	Kings Beach Bowen, Queensland, Australia, W. Ponder, AMS.
<i>Finella</i> sp.	AY010509	Shelly Beach, Queensland, Australia, W. Ponder, AMS.
Litiopidae		
<i>Alaba opinosa</i>	AY010510	Long Reef, Sydney, Australia, P. H. Colman, AMS.
Batillariidae		
<i>Batillaria australis</i>	AY010511	Narrabeen Lagoon, Sydney, Australia, W. F. Ponder, AMS.
<i>Pyrazus ebeninus</i>	AY010512	Careel Bay, Australia; W. F. Ponder, AMS.
Cerithiidae		
<i>Cacozeliana lacertina</i>	AF101007	Long Reef, Sydney, Australia, P. H. Colman, AMS.
<i>Cerithium eburneum</i>	AY010513	FK111; P. Mikkelsen, AMNH.
<i>Cerithium coralium</i>	AY010514	Donovan Bay, Queensland, Australia, M. Glaubrecht, AMS.
<i>Clypeomorus</i> sp.	AY010515	Kings Beach Bowen, Queensland, Australia, W. Ponder, AMS.
Thiaridae (sensu lato)		
<i>Hemisinus cubanianus</i>	AY010516	Cuba, Pinar del Rio, Arroyo la Sierra, leg. J.-P. Pointier.
<i>Melanoides tuberculata</i>	AY010517	Florida, USA, F. Thompson, FLMNH.
<i>Paracrostoma paludiformis</i>	AF101008	Thailand, FMNH 15706.
<i>Stenomelania</i> sp.	AY010518	Lombok, Indonesia, M. Glaubrecht.
<i>Tarebia granifera</i>	AY010519	Florida, USA, F. Thompson, FLMNH.
<i>Thiara amarula</i>	AY010520	Mowbray River, Queensland, Australia, M. Glaubrecht, AMS.
Pleuroceridae		
<i>Elimia interrupta</i>	AY010521	Tennessee, USA, A. Bogan, NCSM.
<i>Hua calculus</i>	AY010522	Primorye Territory, Russia, L. Prozorova.
<i>Juga nigrina</i>	AY010523	Oregon, USA, T. Frest.
<i>Pachychilus</i> sp.	AY010524	Mexico, F. Thompson, FLMNH.
<i>Pleurocera canaliculatum</i>	AF100991	Tennessee, USA, C. Lydeard, NCSM.
<i>Semisulcospira libertina</i>	AY010525	Ginowan City, Okinawa, Japan, N. Okura.
Melanopsidae		
<i>Faunus ater</i>	AY010526	Sulawesi, Indonesia, M. Glaubrecht.
<i>Holandria holandri</i>	AY010314	Lake Skoddar, Albania, leg. S. Bequiraj.
<i>Melanopsis praemorsa</i>	AY010315	Nahal Tanninim, Israel, leg. J. Heller.
Potamididae		
<i>Cerithidea anticipata</i>	AY010316	Cockle Bay, Queensland, Australia, W. Ponder, AMS.
<i>Cerithidea moerchii</i>	AY010317	Amami-O-Shima, Japan; J. Harasewych, USNM.
<i>Telescopium telescopium</i>	AY010318	Cairns, Queensland, Australia, W. F. Ponder, AMS.
<i>Terebralia palustris</i>	AY010319	Funa-uva Bay, Okinawa, Japan, Shin-Ichi Satou.
Planaxidae		
<i>Planaxis sulcatus</i>	AY010320	Cairns, Australia, M. Glaubrecht.
Modulidae		
<i>Modulus modulus</i>	AY010321	Florida, USA, J. Harasewych, USNM.
Turritellidae		
<i>Maoricolpus roseus</i>	AY010322	Tasmania, Australia, K. Gowlett-Holmes.
<i>Protomella capensis</i>	AY010323	N Cape Town, South Africa, G. Branch.
Rissooidea		
Hydrobia sp.	AY010324	M. Mulvey, College of William & Mary.
Vermetoidea		
Vermetidae		
<i>Serpulorbis squamigerus</i>	AY010325	Barbara Island, California, S. Gardner.
Littorinioidae		
<i>Littorina saxatilis</i>	AJ132137	Wilding <i>et al.</i> (1999).
<i>Nodilittorina unifasciata</i>	AY010326	Kurnell, Sydney, Australia, W. F. Ponder, AMS.
Neogastropoda		
<i>Busycotypus spiratum</i>	AY010327	Florida, J. Harasewych, USNM.

Note. AMNH, American Museum of Natural History; AMS, Australian Museum, Sydney; FLMNH, Florida Museum of Natural History; NCSM, North Carolina State Museum of Natural Sciences; USNM, United States Museum of Natural History. Voucher material for specimens collected from Australia are housed at the Australian Museum; specimens actually sequenced are housed at the University of Alabama.

DNA Extraction, PCR, and Sequencing

DNA was extracted from 40 frozen or etoh-preserved taxa using standard phenol–chloroform procedures. DNA was precipitated with absolute ethyl alcohol, washed with 70% ethanol, and resuspended in water. The polymerase chain reaction (PCR) amplifications were performed using primers from the mtSSU rRNA gene through the 3' end of Domain V of the mtLSU rRNA gene following the methods described previously for caenogastropods (Holznagel and Lydeard, 2000; Lydeard *et al.*, 2000). The mtLSU rRNA gene was selected because it exhibits conservative evolution useful for estimating phylogenetic relationships among divergent molluscan taxa (e.g., Holznagel and Lydeard, 2000; Lydeard *et al.* 1996, 1997) and because we were interested in estimating the utility of the gene for inferring relationships among cerithioidean gastropods. In addition, comparative molecular studies have been conducted for the entire gene for mollusks (Lydeard *et al.*, 2000). PCR products were purified by centrifugal filtration (Millipore Ultra-free-MC 30,000) and sequenced using either manual sequencing protocols (Holznagel and Lydeard, 2000) or automated sequencing using an Applied Biosystems 373A, 377, or 3100 DNA sequencer using *Taq* DyeDeoxy terminator chemistry according to the manufacturer's protocol. To ensure accuracy and to resolve ambiguities, both DNA strands were sequenced.

Data Analyses

Data were entered into the software DCSE (De Rijk and De Wachter, 1993) and alignment of the mtLSU rDNA sequences was based on recently published secondary structure models of gastropods as a guide, particularly the caenogastropod *Cacozeliana lacertina* (Lydeard *et al.*, 2000). The aligned data matrix is available electronically on the World Wide Web (<http://www.bama.ua.edu/~clydeard>) and individual sequences have been submitted to GenBank (see Table 1 for accession codes).

Parsimony analysis was performed by using version 4.0b5 of PAUP* (Swofford, 2001) with ACCTRAN, MULPARS, and TBR options. Heuristic searches were conducted by using 25 random addition sequences. Gaps in aligned sequences were treated as missing with the exception of the presence or absence of two tRNAs threonine + glycine, which were treated as a separate binary character (see details under Results). Previous analyses of mtLSU rDNA sequences among cerithioidean pleurocerid species revealed a transition:transversion ratio >2:1; however, the ratio declined to 1:1 or less among more distantly related taxa due to site saturation (Holznagel and Lydeard, 2000; Lydeard *et al.*, 2000). Therefore, we conducted both unweighted and weighted phylogenetic analyses to reduce the influence from site saturation. In the weighted analyses, we used a step matrix to weight transversions twice as

much as transitions. In addition, we also conducted analyses downweighting stems to one-half single-stranded regions to examine the sensitivity of the topology to the effects of compensatory mutations. Bootstrap values (1000 replicates) using the FAST stepwise addition option of PAUP* (Felsenstein, 1985) and decay indices/Bremer support values (Bremer, 1988, 1994; Donoghue *et al.*, 1992) using the Decay Index option of MacClade 4.0 (Maddison and Maddison, 2000) in conjunction with PAUP* (Swofford, 2001) were calculated to assess support for the individual nodes of the resulting phylogenetic hypotheses.

RESULTS

Sequence Variation

The mtLSU rRNA and tRNA gene data set comprises 40 nearly full-length sequences; the aligned sequences were 1873 bp in length. A few areas of ambiguous alignment (totaling 188 bp) were identified and omitted from the phylogenetic analyses. Identifying areas of ambiguous alignment to include or exclude remains somewhat subjective; however, we tend to exclude areas that seem to have many equally plausible alignments. Although identifying ambiguous areas of alignment remains somewhat subjective, we consider that the nucleotide frequencies, transition:transversion ratios, and general features of the mtLSU rRNA genes follow those previously described for caenogastropods (Lydeard *et al.*, 2000). PCR products were larger for cerithioidean taxa than for all other caenogastropods, and upon further investigation, we discovered that all cerithioideans have a unique gene order, mtSSU-thr-gly-val-mtLSU, instead of mtSSU-val-mtLSU found for noncerithioidean caenogastropods. We treated the presence or absence of the tRNAs as a separate binary character. Although this particular tRNA gene order has not been documented elsewhere, phylogenetically informative variation in mitochondrial gene order useful for resolving the deepest nodes among gastropods has been noted (Wilding *et al.*, 1999; Kurabayashi and Ueshima, 2000).

Phylogenetic Analyses

Of the 1685 aligned nucleotide positions and the single binary character representing presence or absence of threonine + glycine included in the analysis, 432 characters were constant, 227 were variable, and 1027 were parsimony informative. Heuristic searches using equal weighting of all characters resulted in four equally parsimonious trees of 8318 steps (CI = 0.2742, HI = 0.7258, RI = 0.3594). A strict consensus tree of the four equally parsimonious trees is shown in Fig. 1. Using the two architaenioglossids as outgroups results in a monophyletic Sorbeoconcha (Ponder and Lindberg, 1997) with relationships altering among the basal

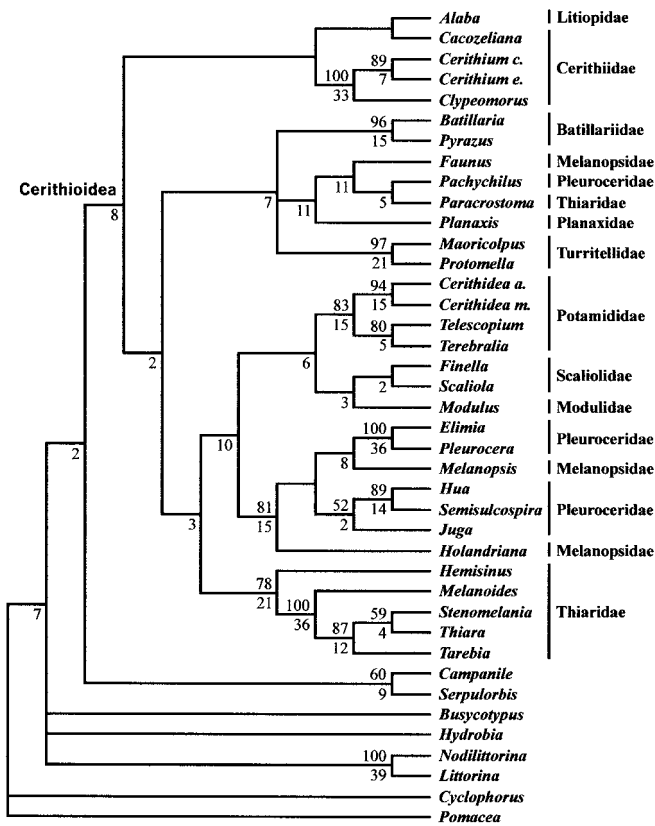


FIG. 1. A strict consensus tree of four equally parsimonious trees based on a parsimony analysis of 1685 aligned nucleotide positions of the mtLSU rDNA sequences and a single binary character representing the presence or absence of threonine + glycine (presence = synapomorphy of Cerithioidea). All characters unordered, equal weight (TL = 8318 steps, CI = 0.2742, HI = 0.7258, RI = 0.3594). Bootstrap values (>50%) and Bremer support values are shown above and below the nodes, respectively.

caenogastropods *Busycotypus* (Neogastropoda), *Nodilittorina* + *Littorina* (Littorinoidea), and *Hydrobia* (Rissooidea). *Serpulorbis* (Vermetoidea) + *Campanile* (Campanilidae) are sister to a monophyletic Cerithioidea. None of the aforementioned clades have bootstrap values greater than 50%. Within the Cerithioidea support is found for the monophyly of the marine families Turritellidae, Batillariidae, Potamididae, and Scaliolidae. The marine family Cerithiidae is paraphyletic with *Cacozeliana* sister to *Alaba* (Litiopidae). All three freshwater families (sensu Morrison, 1954; see Table 1) are polyphyletic with one clade including *Faunus* (Melanopsidae) + *Pachychilus* (Pleuroceridae) and *Paracrostoma* (Thiariidae) and a second clade containing *Elimia*, *Pleurocera*, *Hua*, *Semisulcospira*, and *Juga* (Pleuroceridae) with *Melanopsis* and *Holandriana* (Melanopsidae). Bootstrap values are high (>70%) for many of the family- and intrafamily-level clades.

Parsimony analyses weighting transversions twice transitions resulted in a similar tree (Fig. 2; TL = 12308) with the primary exception being that cerithiids

are now monophyletic with *Alaba opinosa* (Litiopidae), being moved to the basal-most position within the Cerithioidea.

Phylogenetic analyses downweighting stems to 0.5 versus 1 for all other characters (all characters unordered) resulted in a single most parsimonious tree (TL = 6994.5) similar to the four equally parsimonious trees based on equal weighting (all unordered) shown in Fig. 1, with the exception that *Campanile symbolicum* + *Serpulorbis squamigerus* are the most basal members of the Sorbeoconcha. Downweighting stems and weighting transversions twice transitions resulted in a single most parsimonious tree (TL = 10309.0), which is identical to the tree shown in Fig. 2 without downweighting stems.

DISCUSSION

Phylogenetic Placement of Vermetidae and Campanile

Although there is generally weak support (as measured by bootstraps being below 50% and low Bremer support values) for the deepest nodes, the molecular

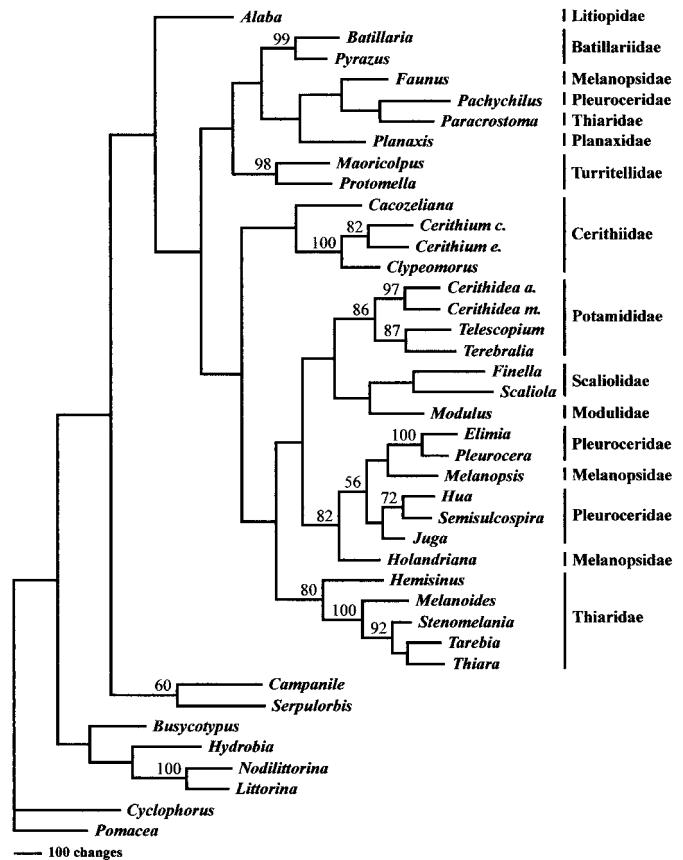


FIG. 2. The single most-parsimonious phylogram based on weighted parsimony (transversions 2x transitions) analysis of mtLSU rDNA sequences (total length = 12,308). Bootstrap values (>50%) are shown above the nodes.

phylogeny does not support the placement of the family Vermetidae as sister to the Turritellidae, but rather supports its placement outside of the clade currently recognized as the Cerithioidea. The molecular phylogeny is also supported by findings based on sperm ultrastructure (Healy, 1988a,b). The most probable explanation for the sister group relationship of vermetids + turritellids obtained in a previous morphology-based cladistic analysis (Houbrick, 1988) is anatomical convergence due to both families being filter feeders. Turritellids are a filter-feeding family in Cerithioidea and one of the three nonhomoplastic anatomical characters putatively uniting turritellids and vermetids is associated with filter feeding and a sessile lifestyle in the analysis by Houbrick (1988).

The enigmatic *Campanile* (Campanilidae) is sister to the Vermetidae, which in turn is sister to Cerithioidea. The placement of *Campanile* outside the Cerithioidea as currently construed is supported by sperm and osphradial ultrastructure data (Healy, 1986; Haszprunar, 1992) and partial 18S nuclear cytoplasmic sequence data (Harasewych *et al.*, 1998). Although one interpretation of the cladogram would be to unite the three superfamilies (Cerithioidea (Vermetoidea + Campaniloidea)) into a single larger superfamily, we believe such a recommendation would be premature and unwarranted without denser taxonomic sampling among all caenogastropods and particularly Sorbeoconcha.

Evolution of Freshwater Cerithioidea

Several different views of the classification of freshwater cerithioideans have been published (see Glaubrecht, 1999). The molecular phylogeny reveals that freshwater taxa evolved on at least three separate occasions. The clade (Figs. 1 and 2) containing *Elimia*, *Pleurocera*, *Melanopsis*, *Hua*, *Semisulcospira*, *Juga*, and *Holandriana* corresponds to a Laurasian distribution of taxa currently assigned to the Melanopsidae and Pleuroceridae. Eastern North American taxa (*Elimia* + *Pleurocera*) are sister to European (*Melanopsis*) and, in turn, sister to a clade containing western North American (*Juga*) + eastern Asian *Hua* and *Semisulcospira*. A morphology-based cladistic study (Houbrick, 1988) supported the sister relationships of Melanopsidae + Pleuroceridae, but the taxonomic sampling was too limited to reveal the patterns that we obtained (i.e., only *Elimia*, *Pleurocera*, and *Melanopsis* were examined). The second molecular-based freshwater clade includes two taxa (*Pachychilus* and *Paracrotoma*), which were initially assigned to the subfamily Melanatriinae of Thiele's Melaniidae (Thiele, 1928, 1929) and the brackish water *Faunus* included in the Melanopsidae by some workers (Houbrick, 1988; Ponder, 1991; Glaubrecht, 1996). Recent phylogenetic analyses of unpublished (E. Strong, M. Glaubrecht, W. F. Ponder) morphological data support the recogni-

tion of this clade and that it should be referred to as a Gondwanan family Pachychilidae (Glaubrecht, 1999). The final freshwater clade includes pantropical thiarids corresponding roughly to the Melaniinae of Thiele (1928, 1929) or Thiaridae *sensu stricto* (Glaubrecht, 1996, 1999), rendering earlier traditional systematic views of thiarids as unnatural. The multiple origins and distribution of freshwater taxa and the minimal fossil evidence dating back to at least the Cretaceous suggest that the separation of Pangaea into Laurasia and Gondwana (separated by the Tethys Sea) about 200 mya may have played a critical role in the distribution and phylogeny of freshwater cerithioideans, but this hypothesis awaits denser taxonomic sampling within each clade.

Phylogeny of Cerithioidean Families

The topologies obtained among the earlier morphology-based analyses of cerithioideans is in part due to taxonomic sampling, missing data, use of different outgroups (Ponder, 1991 versus Houbrick, 1988), and different methodological approaches (e.g., all unordered (Ponder, 1991) versus ordered and character state trees (Houbrick, 1988) and intuitive approaches (Glaubrecht, 1996). Despite the lack of congruence among morphology-based studies, certain aspects are shared with the molecular-based phylogeny. For example, the sister group status between the freshwater families Melanopsidae and Pleuroceridae is supported (Houbrick, 1988; Ponder, 1991), and it is possible that further detailed taxonomic sampling may indicate that only one family name is warranted. In addition, the close relationship between the families Modulidae and Potamididae is supported (Houbrick, 1988; Glaubrecht, 1996), but Scaliolidae is deemed sister to Modulidae and together they are sister to Potamididae in the molecular phylogeny (scaliolids were not included in the published morphology-based studies). Furthermore, the close relationship of Litiopidae and Cerithiidae, to which litiopids were once assigned (Houbrick, 1987), is supported by one molecular phylogenetic hypothesis (i.e., characters are unordered and equal weight). However, the basal position of Litiopidae within the Cerithioidea, after weighting transversions twice as much as transitions, is congruent with the morphology-based cladogram of Houbrick (1988). The sister status of the two clades *Cerithidea anticipata* + *C. moerchii*, formerly assigned to the family Cerithiidae (Houbrick, 1988), and *Telescopium telescopium* + *Terebralia palustris* of the family Potamididae is congruent with the morphology-based phylogeny of Houbrick (1988). Houbrick (1991) subsequently chose to synonymize Cerithiidae with Potamididae. Efforts are now on-going to generate a morphological database with similar taxonomic sampling as used herein to further examine phylogeny of cerithioideans in a total-evidence framework.

It is apparent that mtLSU rDNA sequences offer valuable phylogenetic information within and up to what corresponds to the family level within Cerithioidea. Sensitivity of the topology to weighting and low bootstrap values for the deeper nodes indicates that the deepest phylogenetic limits of the mtLSU rRNA gene may have been surpassed. Previous estimates based on an analysis of 10 molluscan mt LSU rDNA sequences was greater than 80 mya to fewer than 400 mya (Lydeard *et al.*, 2000); however, the present study places the deepest limit within Caenogastropoda below about 245–241 mya using the earliest known fossil evidence of Cerithioidea as a rough gauge (Tracey *et al.*, 1993). Additional questions that need to be considered are whether the weakly supported deeper nodes are a reflection of reaching the deepest phylogenetic limits of the gene (soft polytomy), a function of the rapid radiation of the living cerithioidean families (hard polytomy), or largely spurious, not indicating phylogenetic relationships at all. This question can be examined further by examining more conservative nuclear genes and morphological data in a more rigorous manner.

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