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Molecular phylogenetics of Caenogastropoda (Gastropoda: Mollusca)

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Abstract

Caenogastropoda is the dominant group of marine gastropods in terms of species numbers, diversity of habit and habitat and ecological importance. This paper reports the first comprehensive multi-gene phylogenetic study of the group. Data were collected from up to six genes comprising parts of 18S rRNA, 28S rRNA (five segments), 12S rRNA, cytochrome *c* oxidase subunit I, histone H3 and elongation factor 1α. The alignment has a combined length of 3995 base positions for 36 taxa, comprising 29 Caenogastropoda representing all of its major lineages and seven outgroups. Maximum parsimony, maximum likelihood and Bayesian analyses were conducted. The results generally support monophyly of Caenogastropoda and Hypsogastropoda (Caenogastropoda excepting Architaenioglossa, Cerithioidea and Campanilioidea). Within Hypsogastropoda, maximum likelihood and Bayesian analyses identified a near basal clade of nine or 10 families lacking an anterior inhalant siphon, and Cerithiopsidae *s.l.* (representing Triphoroidea), where the siphon is probably derived independently from other Hypsogastropoda. The asiphonate family Eatoniellidae was usually included in the clade but was removed in one Bayesian analysis. Of the two other studied families lacking a siphon, the limpet-shaped Calyptraeidae was associated with this group in some analyses, but the tent-shaped Xenophoridae was generally associated with the siphonate Strombidae. The other studied hypsogastropods with an anterior inhalant siphon include nine families, six of which are Neogastropoda, the only traditional caenogastropod group above the superfamily-level with strong morphological support. The hypotheses that Neogastropoda are monophyletic and that the group occupies a derived position within Hypsogastropoda are both contradicted, but weakly, by the molecular analyses. Despite the addition of large amounts of new molecular data, many caenogastropod lineages remain poorly resolved or unresolved in the present analyses, possibly due to a rapid radiation of the Hypsogastropoda following the Permian–Triassic extinction during the early Mesozoic.

Keywords: Architaenioglossa; Bayesian analysis; Cladistics; Heteropoda; Hypsogastropoda; Maximum likelihood; Multi-gene phylogeny; Neogastropoda; Ptenoglossa

1. Introduction

Among the living snails, the largest and most diverse group is Caenogastropoda. The group includes a large number of ecologically and commercially important marine families, as well as several groups that have independently achieved major freshwater and terrestrial radiations. Its members display a wide array of often-convergent shell morphologies (coiled, uncoiled, elongate, globose, limpet-shaped, etc.), and some species have the shell reduced or (rarely) lost. They occupy a wide range of habitats and have diverse habits (benthic epifaunal or burrowers, pelagic drifters or active swimmers; detritus or sedentary suspension feeders, herbivores or grazing or active carnivores, ectoparasites or shell-less internal parasites).

Caenogastropoda consists of about 136 extant and 65 extinct families and thousands of genera currently arranged in 41 superfamilies (Bouchet and Rocroi, 2005). The relationships between the families and superfamilies remain largely unresolved phylogenetically with most named higher taxa probably paraphyletic or even polyphyletic. Available hypotheses about the relationships are largely based on a few key shell characters and anatomical details

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(particularly from radulae). The first explicitly cladistic analysis that focussed on the whole group was undertaken by Strong (2003), as discussed below, and Simone (2001, 2004, 2005) has made phylogenetic analyses of several of its substantial components.

The taxon Caenogastropoda was introduced by Cox (1960) to include many elements of the "Prosobranchia" recognized by Thiele (1929–31) but shown to be paraphyletic by Haszprunar (1988) and Ponder and Lindberg (1997). These were (1) Architaenioglossa, containing Cyclophoroidea, the major group of operculate land snails, and the freshwater families Ampullariidae and Viviparidae, sometimes considered to belong to one superfamily but now each recognized as a separate superfamily; (2) the remaining "mesogastropods" of Thiele (1929–31) including predominantly marine groups such as the Littorinidae (periwinkles), Cypraeidae (cowries), Cerithiidae (creepers), Calyptraeidae (slipper limpets), Tonnidae (tun shells), Cassidae (helmet shells), Ranellidae (tritons), Strombidae (strombs), Naticidae (moon snails) and Heteropoda (=Pterotracheoidea) and (3) Stenoglossa (=Neogastropoda) an almost exclusively marine and carnivorous group that contains such well-known, diverse and ecologically significant families as Muricidae (rock shells, oyster drills, etc.), Volutidae (balers, etc.), Mitridae (mitres), Buccinidae (whelks), Turridae (turrids) and Conidae (cones). Thiele's concept of Mesogastropoda is polyphyletic including several groups now known to be members of the Heterobranchia, the major gastropod clade that also includes the opisthobranchs and pulmonates and that is currently recognised as the sister group of Caenogastropoda (Haszprunar, 1985, 1988; Ponder and Lindberg, 1997).

The family-group taxa studied in this paper are shown in Fig. 1 with many of the higher taxon names used herein indicated. For a comprehensive listing of higher taxa used in Gastropoda see Bouchet and Rocroi (2005). Names other than Architaenioglossa and Neogastropoda in current or recent use for major groups within Caenogastropoda include:

Sorbeoconcha introduced by Ponder and Lindberg (1997), includes all caenogastropods other than the Architaenioglossa. Basal members are Cerithioidea and Campaniloidea.

Hypsogastropoda also named by Ponder and Lindberg (1997) includes the great majority of extant caenogastropods, and is defined as all caenogastropods other than Architaenioglossa, Cerithioidea or Campaniloidea.

Neotaenioglossa has been used for the non-architaenioglossan "mesogastropods" (Haszprunar, 1988; Ponder and Warén, 1988). This group is equivalent to Sorbeoconcha excluding Neogastropoda. It is now generally acknowledged that Neotaenioglossa is either paraphyletic or polyphyletic (Ponder and Lindberg, 1997; Strong, 2003).

Cerithiimorpha and *Littorinimorpha* have been used as groupings within Neotaenioglossa. Most recently Littorinimorpha was used to encompass the taenioglossate Hypsogastropoda (Bouchet and Rocroi, 2005).

Heteropoda comprises only the pelagic Pterotracheoidea (=Carinarioidea) and has been sometimes used as a high-rank taxon (e.g., Bandel and Hemleben, 1987; Ponder and Warén, 1988). Pterotracheoidea is included in the Littorinimorpha by Bouchet and Rocroi (2005) and is given no higher rank, agreeing with Thiele (1929–31) and Wenz (1938–44).

Ptenoglossa (=Ctenoglossa), a probably polyphyletic (Ponder and Lindberg, 1997) grouping of Eulimoidea, Janthinoidea and Triphoroidea.

Several new higher taxon names have been introduced by Bandel and his colleagues (e.g., Bandel, 1991, 1993) largely to accommodate fossils. These include: Palaeo-Caenogastropoda—taxa with their first occurrence in the Palaeozoic; Meta-Mesogastropoda—taxa with Mesozoic origins; Neo-Mesogastropoda—late Mesozoic taxa that have an "expanded ontogeny" (does not include Neogastropoda); and Scaphoconchoidea—taxa with modified veliger stages known as echinospira or limacosphaera larvae. Bandel and Riedel (1994) and Riedel (2000) introduced Latrogastropoda to include Neomesogastropoda and Neogastropoda. Within this grouping, Pleurembolica includes Troschelina (Laubierinioidea and Calyptraeoidea) and Vermivora (Ficoidea, Tonnoidea and Neogastropoda).

Current understanding of the systematics of Caenogastropoda broadly follows the findings of the morphological study of overall gastropod phylogeny by Ponder and Lindberg (1997). This study resolved Architaenioglossa, Sorbeoconcha and Hypsogastropoda but revealed little structure within the latter group. Ponder and Lindberg (1997) included 11 species of Hypsogastropoda but only one group, the Neogastropoda, was resolved. Bouchet and Rocroi (2005) differ from Ponder and Lindberg (1997) mainly in the recognition of Littorinimorpha (see above).

The membership of Caenogastropoda has not recently been widely questioned except for Architaenioglossa and Campanilidae (Haszprunar, 1988). Architaenioglossa is an enigmatic, entirely non-marine group. While generally regarded as a member of "Mesogastropoda" (Thiele, 1929–31; Wenz, 1938–44) or a caenogastropod group (Ponder and Warén, 1988), Haszprunar (1988) considered it to be the sister group of a clade comprising Caenogastropoda and Heterobranchia. Ponder and Lindberg (1997) found Architaenioglossa (represented by Cyclophoridae and Ampullariidae in their analysis) to be included within Caenogastropoda. They also found it to be monophyletic, albeit with support provided only by four forward homoplasies. Robust support was found for the sister pairing of the two Architaenioglossa (Cyclophoridae and Ampullariidae) included in Strong's (2003) analysis. Monophyly of the group has, however, been questioned by other morphological studies (Haszprunar, 1988; Simone, 2004) and is contradicted by analyses of DNA sequences including Viviparidae, Ampullariidae and Cyclophoridae (Harasewych et al., 1998) or only Ampullariidae and Cyclophoridae (Colgan et al., 2000, 2003).

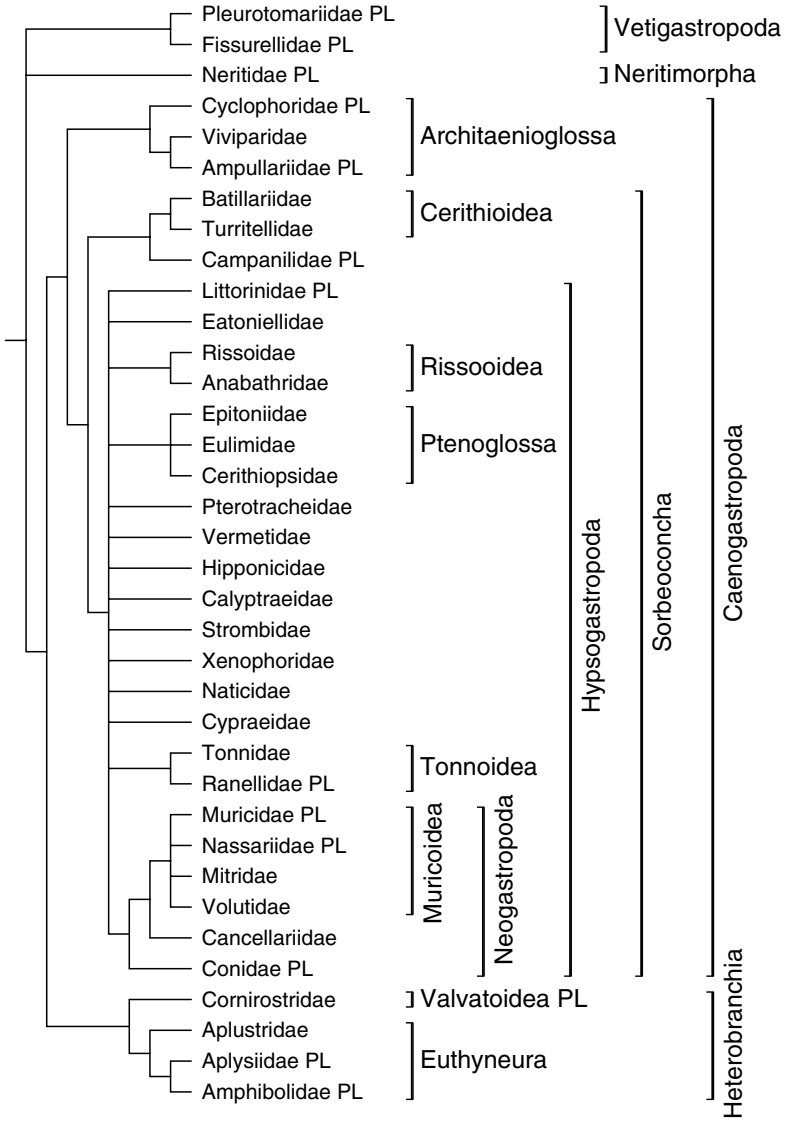

Fig. 1. Named groups within the Caenogastropoda studied here based on Ponder and Lindberg's (1997) topology. Taxa included in Ponder and Lindberg's (1997) analysis are indicated with "PL". Taxa not included in Ponder and Lindberg (1997) are placed on the tree in unresolved positions within clades according to current understanding of their affinities. For example Viviparidae although not included in Ponder and Lindberg (1997) is placed as the sister group of Ampullariidae.

Campaniloidea (then comprising only *Campanile*) was regarded by Haszprunar (1988) as the sister group to Heterobranchia. The single living species of Campanilidae, *C. symbolicum*, has a distinctive anatomy (Houbrick, 1981, 1989) and sperm morphology (Healy, 1986). Subsequently, Healy (1993) transferred the family Plesiotrochidae to the Campaniloidea on the basis of similarity in sperm ultrastructure. The morphological analyses of Ponder and Lindberg (1997) placed Campaniloidea within Caenogastropoda, as anticipated by Ponder and Warén (1988). Molecular studies have also consistently included Campaniloidea in Caenogastropoda (Harasewych et al., 1998; Colgan et al., 2000, 2003; McArthur and Harasewych, 2003).

Only one large-scale cladistic analysis of caenogastropod morphology has been published (Strong, 2003). That study included the two architaenioglossans mentioned earlier, a cerithioidean and 14 hypsogastropods (including six neogastropods) and utilized 64 characters from a wide range of organ systems such as the alimentary, renal/pericardial, nervous, reproductive and the mantle cavity. Unlike Ponder and Lindberg (1997); Strong (2003) did not include ultrastructural characters. Caenogastropoda, Architaenioglossa, Sorbeoconcha and Neogastropoda were monophyletic in Strong's analyses (2003), but Hypsogastropoda included the studied cerithioidean (family Batillariidae).

This investigation of DNA sequence data was conducted to identify the major lineages within Caenogastropoda and their relationships. We investigate molecular support for monophyly of groups such as Sorbeoconcha, Hypsogastropoda and Neogastropoda that have some morphological support, as well as named groups such as Architaenioglossa,

Neotaenioglossa and Ptenoglossa whose status is doubtful. The data collected for this paper also permitted limited testing, based on two taxa in each case, of the monophyly of some superfamilies that have not previously been tested with a large number of outgroups, although some analyses of those groups have been conducted. These include the Rissooidea (Ponder, 1988; Wilke et al., 2001), Cerithioidea (Houbrick, 1988; Ponder, 1991; Simone, 2001; Lydeard et al., 2002), Tonnoidea (Riedel, 1995), Neogastropoda (Kantor, 1996) and Triphoroidea + Janthinoidea (Nützel, 1998).

To date there has not been a comprehensive molecular study of caenogastropod evolution. Some papers discussed below have included a substantial number of caenogastropod taxa. Most have focussed on a particular subgroup such as a family or group of families (e.g., Harasewych et al., 1997; Oliverio and Mariottini, 2001; Oliverio et al., 2002; Collin, 2003; Meyer, 2003, 2004; Williams et al., 2003; Hayashi, 2005). The few studies directed at larger groups within Caenogastropoda have concentrated on the non-hypsogastropod Caenogastropoda (Harasewych et al., 1998; Lydeard et al., 2002), the "higher" Caenogastropoda, principally Neogastropoda and associated families (Harasewych et al., 1997; Riedel, 2000) or on overall gastropod phylogeny (Colgan et al., 2000, 2003; McArthur and Harasewych, 2003). There is a notable lack of resolution within Caenogastropoda in these overall gastropod phylogenies.

Data for this study were already available for a number of taxa used in our previous studies of gastropod phylogeny. Seventeen caenogastropod taxa were scored for two segments of 28S rRNA (abbreviated as 28S rRNA A and 28S rRNA B) and histone H3 (H3) in Colgan et al. (2000). In this context and below, the term "segment" indicates the product of a single PCR amplification, although most of the multiple segments from 28S rRNA were originally designed for the investigations of particular expansion regions. Three extra sequences were studied for 16 taxa in Colgan et al. (2003). These were an additional segment of 28S ribosomal RNA (abbreviated as 28S rRNA D1), small nuclear RNA U2 (U2 snRNA) and part of cytochrome *c* oxidase subunit 1 (COI). These extra segments were sequenced here for the taxon (Eulimidae) included in Colgan et al. (2000) but not in Colgan et al. (2003). Data were not collected for U2 snRNA as this sequence is quite short (less than 150 bases).

For this paper, data were collected from five additional gene segments, these being part of the 12S rRNA domain III (abbreviated as 12S rRNA), two segments of the 28S rRNA containing identified expansion regions (abbreviated as 28S rRNA D6 and 28S rRNA D9-10), part of the 18S ribosomal RNA (18S rRNA) and elongation factor 1 alpha (EF1-α). As well as collecting sequences from more segments, data were collected from 12 additional caenogastropods for as many of the 10 studied segments as could be amplified from each specimen.

The nuclear ribosomal genes are regularly used in molecular phylogenetic surveys. Among the coding regions, H3 has been widely used in higher-level phylogenetics (Colgan et al., 1998; Brown et al., 1999; Whiting et al., 2003; Thollesson and Norenburg, 2003; Okusu et al., 2003). EF1-α is also becoming widely used in the phylogenetics of higher level taxa, for example in Polychaeta (McHugh, 1997) and Arthropoda (Regier and Shultz, 1997; Giribet et al., 2001). COI sequences are too variable to resolve major groups in higher-level phylogenetic analyses by themselves (Nylander et al., 1999) but have proven useful in combination with other data for recovering some high rank taxa, e.g. Clitellata in Jördens et al. (2004).

Outgroups were chosen from a range of major gastropod groups. The Heterobranchia is usually found to be the sister group to Caenogastropoda in morphological and molecular analyses, the two clades together comprising the Apogastropoda (*sensu* Ponder and Lindberg, 1997). More distant outgroups were selected from the Neritimorpha and the Vetigastropoda.

2. Materials and methods

2.1. DNA extraction and sequencing

Specimens were frozen at −70 °C or stored in 70–100% ethanol. Species examined, classification and voucher details are listed in Table 1. DNA was isolated from up to 1 g of foot tissue or from the entire animal, if it were small (body size <5 mm length). Ethanol-preserved samples were re-hydrated in sterile double filtered H_2O for at least 2 h before extraction. DNA was extracted by the CTAB method of Saghai-Maroof et al. (1984) or the AMRESCO RapidGene™ Genomic DNA Purification Kit (Solon, OH, USA) according to manufacturer's instructions. Extractions of mRNA were carried out using TRIZOL (Life Technologies, Rockville, MD, USA) and cDNA synthesis performed using the Superscript™ preamplification system (Life Technologies) according to the manufacturer's protocol Table 2.

DNA dilutions up to 1 in 100 were made to obtain single-banded PCR products. PCRs were generally performed using 0.2–1.0 Units of Red Hot™ thermostable DNA polymerase in a 10-fold dilution of Buffer IV (Advanced Biotechnologies, Columbia, MD, USA: 20 mM $(NH_4)_2SO_4$, 750 mM Tris–HCL pH 9.0, 0.1% (w/v) Tween), with a final concentration of 0.05 mM dNTPs, 3.5–4.5 mM $MgCl_2$ and 12.5–25 pmol of each primer in a total reaction volume of 50 µl. Negative controls were included in each reaction array. Other DNA polymerases were occasionally used in the particular manufacturer's buffer, with similar concentrations of other reagents. To optimise PCR products, annealing temperatures and times, and $MgCl_2$ concentration were varied. The basic cycling profile was as follows: (95 °C for 5 min, annealing for 45 s, 72 °C for 1 min) for one cycle, (95 °C for 30 s, annealing for 45 s, 72 °C for 1 min) for 30–34 cycles and (95 °C for 30 s, annealing for 45 s, 72 °C for 5 min) for the

Table 1
Classification, provenance and museum registration of the studied specimens

Higher taxon	Family	Species	Source	Reg No./Voucher No.
Vetigastropoda				
Fissurelloidea	Fissurellidae	*Montfortula rugosa*	Edwards Beach, Balmoral, NSW	C335476
Pleurotomarioidea	Pleurotomariidae	*Bayerotrochus midas*	Johnson Sea Link, Bahamas, 2100ft Depth	USNM888645
Neritimorpha (= Neritopsina)				
Neritoidea	Neritidae	*Nerita atramentosa*	Edwards Beach, Balmoral, NSW	C335471
Caenogastropoda				
"Architaenioglossa"				
Cyclophoroidea	Cyclophoridae	*Leptopoma perlucida*	Coonya Beach, Qld	QM M03142
Ampullarioidea	Ampullariidae	*Pomacea bridgesii*	Brookfield, W of Brisbane, Qld	C333043
Viviparoidea	Viviparidae	*Bellamya heudi guangdungensis*	Halfway Pt, Lane Cove River, NSW	C203209
Sorbeoconcha				
Cerithioidea	Batillariidae	*Zeacumantus subcarinatus*	Edwards Beach, Balmoral, NSW	EBU30432
	Turritellidae	*Maoricolpus roseus*	Pirates Bay, Tasmania	EBU30403
Campaniloidea	Campanilidae	*Campanile symbolicum*	Rottnest Island, WA.	C203211
Hypsogastropoda				
Littorinoidea	Littorinidae	*Austrolittorina unifasciata*	Edwards Beach, Balmoral, NSW	C335438
Cingulopsoidea	Eatoniellidae	*Crassitoniella flammea*	Edwards Beach, Balmoral, NSW	C335411
Rissooidea	Anabathridae	*Pisinna albizona*	Edwards Beach, Balmoral, NSW	C335405
	Rissoidae	*Rissoina fasciata*	Long Reef, Collaroy, NSW,	C335432
Stromboidea	Strombidae	*Strombus luhuanhus*	Heron Island, Qld	C203214
Vanikoroidea	Hipponicidae	*Antisabia foliacea*	Long Reef, Collaroy, NSW,	C335477
Calyptraeoidea	Calyptraeidae	*Bostrycapulus pritzkeri*	Edwards Beach, Balmoral, NSW	C335468
Xenophoroidea	Xenophoridae	*Xenophora indica*	"Kapala" cruise K951805/09 E of Clarence R., NSW	C454048
Vermetoidea	Vermetidae	*Serpulorbis* sp.	Heron Island, Qld	C203213
Cypraeoidea	Cypraeidae	*Cypraea annulus*	Heron Island, Qld	C203215
Naticoidea	Naticidae	*Conuber melanostoma*	Careel Bay, Pittwater, NSW	EBU30442
Tonnoidea	Ranellidae	*Cabestana spengleri*	Long Reef, Collaroy, NSW	C203216
	Tonnidae	*Tonna cerevisina*	"Kapala" cruise K951828 off Brooms Head, NSW	C453719
Pterotracheoidea ("Heteropoda")	Pterotracheidae	*Pterotrachea coronata*	San Pedro Basin,, trawled, USA	EBU30445
"Ptenoglossa"				
Triphoroidea	Cerithiopsidae[a]	*Ataxocerithium* sp.	Off Cronulla, NSW	C203217
Epitonioidea	Epitoniidae	*Epitonium jukesianum*	Edwards Beach, Balmoral, NSW	C335429
Eulimoidea	Eulimidae	*Stilifer* sp.	Heron Island, Qld	DNA only
Neogastropoda				
Buccinoidea	Nassariidae	*Nassarius burchardi*	The Entrance, NSW	C203219
Muricoidea	Muricidae	*Dicathais orbita*	Edwards Beach, Balmoral, NSW	C335420
	Mitridae	*Mitra carbonaria*	Long Reef, Collaroy, NSW,	C335424
	Volutidae	*Cymbiolista hunteri*	"Kapala" cruise K960401 off Newcastle	C453717
Cancellarioidea	Cancellariidae	*Cancellaria undulata*	Woolgoolga, NSW	C203222
Conoidea	Conidae	*Conus miles*	Heron Island, Qld	C203223
Heterobranchia				
Valvatoidea	Cornirostridae	*Cornirostra pellucida*	Fingal Head, Port Stephens, NSW	C203224
Opisthobranchia				
Acteonoidea	Aplustridae	*Bullina lineata*	Long Reef, Collaroy, NSW	C203226
Aplysioidea	Aplysiidae	*Aplysia juliana*	Long Reef, Collaroy, NSW	C203227
Pulmonata				
Amphiboloidea	Amphibolidae	*Salinator solida*	Tilligerry Ck, Port Stephens, NSW	C203229

The higher classification follows Bouchet and Rocroi (2005). Registration numbers with the prefix C denote specimens in the Malacology collection of the Australian Museum. EBU numbers denote DNA or whole specimens in the frozen tissue collection of the Australian Museum, QM M numbers indicate specimens from the Queensland Museum and USNM numbers indicate specimens from the National Museum of Natural History.

[a] Bouchet and Rocroi (2005) use Newtoniellidae as the family name for *Ataxocerithium* but as the systematics of this group are poorly understood, we choose to use the better known family name Cerithiopsidae (in the broad sense).

final cycle. Annealing temperatures are indicated below. To obtain PCR products from difficult samples, 20 μl of GeneReleaser™ (Bioventures, Murfreesboro, TN, USA) was added to the DNA template and microwaved for 6 min. The remaining PCR mix (with reduced H_2O) was immediately added and cycling commenced.

Table 2
Primers used in this investigation

Gene	Primer	Sequence	Reference
18S rRNA	18S1F	TACCTGGTTGATCCTGCCAGTAG	Giribet et al. (1996)
	18S4R	GAATTACCGCGGCTGCTGG	Giribet et al. (1996)
28S rRNA	28S D1F	ACCCSCTGAAYTTAAGCAT (19)	McArthur and Koop (1999)
	28S D1R	AACTCTCTCMTTCARAGTTC (356)	Colgan et al. (2003)
	28SAF	GACCCGAAAGATGGTGAACTAT (1003)	Colgan et al. (2000)
	28SAR	AGCGCCAGTTCTGCTTACCAAAA (1305)	Colgan et al. (2000)
	28S D6F	CAACTAGCCCTTAAAATGGATGG (1495)	McArthur and Koop (1999)
	28S D6R	AMAGAAAAGARAACTCTYCC (1913)	Colgan et al. (2003)
	28S D62F	GTGAACAGCAGTTGAACATGG (1719)	Present study
	28S D62R	ACGGACTTCTCCTATCTCTTAGG (1730)	Present study
	28SBF	GGGAGTTTGACTGGGGCGGTACA (2912)	Colgan et al. (2000)
	28SBR	TGGGTGAACAATCCAACGCTTGG (3192)	Colgan et al. (2000)
	28S VI	AAGGTAGCCAAATGCCTCATC (2565)	Hillis and Dixon (1991)
	28S X	GTGAATTCTGCTTCATCAATGTAGGAAGAGCC (3161)	Hillis and Dixon (1991)
12S rRNA	12S F	AAAGCTTCAAACTGGGATTAGATACCCCACTAT	Kocher et al. (1989)
	12S R	TGACTGCAGAGGGTGACGGGCGGTGTGT	Kocher et al. (1989)
	12S2F	TTAAAACTYAAAGGRCWTGGCGG	Present study
	12S2R	TTACTTTYAAGTCCWCCTTC	Present study
EF1-α	EF F	TCYGTCAAGGATATYCGCCGTGG (33)	Present study
	EF R	GAAGGYCTCYACGCACATTGGCTT (287)	Present study
Histone H3	H3F	ATGGCTCGTACCAAGCAGACVGC (41)	Colgan et al. (2000)
	H3R	ATATCCTTRGGCATRATRGTGAC (372)	Colgan et al. (2000)
	H3NF	ATGGCTCGTACCAAGCAGAC (41)	Colgan et al. (2000)
	H3NR	ATRTCCTTGGGCATGATTGTTAC (372)	Colgan et al. (2000)
COI	COX AF	CWAATCAYAAAGATATTGGAAC (41)	ACOX1AF in Colgan et al. (2001)
	COX AR	AATATAWACTTCWGGGTGACC (725)	ACOX1AR in Colgan et al. (2001)
	COX BF	CMCGWATAAATAATATARGATTYTG (299)	ACO351F in Colgan et al. (2001)
	COX BR	AAYAATTCCKGTTARWCCTCC (1062)	ACO1140R in Colgan et al. (2001)
	COX 623R	GGTAARTYTATTGTAATAGCWCC (623)	ACO602R Colgan et al. (2001)
	COX917R	TGRGCYCAMACAATRAAMCC (831)	Present study
	JMCO1BF	GCWGGWGCWATTACRATRYT (617)	Present study
	JMCO1BR	CCRTGAATYGTRGCAAGTCA (958)	Present study

Positions for 28S rDNA primers are given for the 3′ end of the oligonucleotide in the *Ilyanassa obsoleta* 28S rDNA sequence (GenBank AY145411; Passamaneck et al., 2004). Positions for the CO1 primers are given for the 3′ end of the oligonucleotide in the GenBank oligochaete cytochrome *c* oxidase I sequence (GenBank LTU24570; Boore and Brown, 1995) Positions for the EF primers are given for the 3′ end of the oligonucleotide in the GenBank *Alvinoconcha hessleri* elongation factor sequence (GenBank D14975: Kojima et al., 1993). Positions for H3 primers are given for the 3′ end of the oligonucleotide in the histone H3 sequence of the bivalve *Spisula solidissima* (GenBank SIUHIS3A: Swenson et al., 1987).

Primer sequences are listed in Table 1. Several primer pairs (Cox AF and AR, Cox BF and BR, etc.) were used for cytochrome *c* oxidase subunit I. Annealing temperatures were usually 45 °C for all primer combinations but were reduced to as low as 40 °C if this were necessary to obtain products or as high as 52 °C to eliminate secondary bands. Generally, 12SF and 12SR (the universal primers of Kocher et al. (1989) were used for 12S rRNA. For taxa not successfully amplified with this pair, the nested primers 12S2F and 12S2R were used. These were designed from sequences generated using the first primer pair. Annealing temperatures were usually 50 °C for all primer combinations but were reduced to 46 °C if necessary. For the 28S rRNA D1 expansion region, D1F and D1R were used with the addition of 5% DMSO to the PCR reaction. Annealing temperatures were between 47 °C and 50 °C. Generally, primers D6F and D6R were used for the 28S rRNA D6 expansion region. D62F and D62R, designed from sequences collected here, were used with D6R and D6F respectively to amplify the 28S rRNA D6 region in two sections. Annealing temperatures were between 47 and 50 °C for all primer combinations. Annealing temperatures for 28S rRNA A, 28S rRNA B and histone H3 ranged from 48–53 °C. The 28S 9-10 region overlaps 28SB at the 3′ end of the former segment. There were no differences in the sequences in the overlap region. EF-1α was amplified with primers EFF and EFB, designed from the gastropod *Alvinoconcha hessleri* (D14975) (Kojima et al., 1993) with reference to *Drosophila melanogaster* sequences. Initial amplifications of genomic DNA were generally unsuccessful. Subsequent amplifications were performed using RT-PCR as described above using an annealing temperature of 60 °C.

Reaction products were resolved on 2% agarose gels containing ethidium bromide. Single band products were purified using the QIAquick™ PCR Purification Kit (Qiagen, Venlo, The Netherlands) or by AMPURE magnetic beads (Agencourt, Beverly, MA, USA) processed by a

liquid handling system (CAS-3800, Corbett Engineering, Mortlake, Australia). Where single products were not obtained, the correct sized band was excised from 2% low melting point agarose in TAE buffer and purified using the QIAquick kit. Products were sequenced in both directions with an ABI® 310 DNA Automatic Capillary Sequencer (Applied Biosystems, Foster City CA, USA) using the Dye-Deoxy™ Terminator sequencing method (Big Dye™, version 1.0 or 2.0) according to the manufacturer's protocol except that the amount of Big Dye was generally reduced to 2 μl. Sequencing primers (1 μL) were used at a concentration of 3.2 pM/μL. Reactions were purified by ethanol precipitation or using CleanSeq magnetic beads (Agencourt) on the Corbett liquid handling system.

2.2. Data analysed

Electropherograms of the two sequence directions were checked using Sequence Navigator (Applied Biosystems 1994) and a consensus sequence was generated. Accession numbers are listed in Table 3. Data collected for this paper have accession numbers of DQ916496–DQ916508 for COI, DQ916585–DQ916605 for EF1-α, DQ916445–DQ916454 for H3, DQ916414–DQ916444 for 12S rRNA, DQ916572–DQ916584 for 28S rDNA D1, DQ916509–DQ916518 for 28S rRNA A, DQ916467–DQ916495 for 28S rRNA D6, DQ916543–DQ916571 for 28S rRNA D9-10, DQ916455–DQ916466 for 28S rRNA B and DQ916519–DQ916542 for 18S rRNA. The data include published sequences from this laboratory (Colgan et al., 2000, 2003, 2006): AF033716–AF033794 for 28S rRNA A and 28S rRNA B, AF033675–AF033715 for H3, AY296815–AY996850 for CO1, and AY296873–AY296909 for 28S rRNA D1. Additional published sequence data from the following GenBank accessions were included: 28S rRNA D1, Viviparidae (U75863: *Viviparus viviparus* McArthur, 1996) and Valvatidae (U75862: *Valvata* sp. McArthur, 1996); 28S rRNA D6, Viviparidae (U82423: *Viviparus* sp. Tillier et al., 1994), Valvatidae (U78672: *Valvata* sp., McArthur and Koop (1999), Aplysiidae (U78644: *Aplysia californica*, McArthur and Koop, 1999), Ampullariidae (U78643: *Ampullaria* sp., McArthur and Koop, 1999); 18S rRNA, Pleurotomariidae (L78893: *Bayerotrochus midas*, Harasewych et al., 1997). A representative range of the 16S data available in GenBank was downloaded and analysed as a separate dataset. The 16S sequences were not included in the overall compilation as there was too little overlap in family representation in the two datasets.

McCLADE (Maddison and Maddison, 1992) was used for data manipulations such as joining files for the individual segments and specifying character sets. Tree figures were drawn with TGF (Müller and Müller, 2004).

2.3. Phylogenetic analysis

Sequences were aligned using the default values for parameters in CLUSTAL X (Thompson et al., 1997). The "slow-accurate" algorithm was used for pairwise alignment with costs of 10.0 for gap opening and 0.10 for gap extension. For multiple alignments, the cost for gap opening was set at 10.0 and gap extension at 0.20, with a DNA transition weight of 0.50 and a "delay divergent sequences" percentage of 30. Areas of uncertain alignment (found only in the segments from non-coding genes) were omitted from all reported analyses. In reporting the results, we adopt the conventions that (a) a comma separates monophyletic groups within clades specified by parentheses; (b) a + sign indicates that the group before the sign is paraphyletic with respect to the group following the sign; and (c) that the bootstrap or posterior probability is given immediately after the closing parenthesis. Posterior probabilities are written as "support" levels with the actual probability being multiplied by 100 to give an integer value.

Maximum parsimony analyses were conducted using heuristic searches in PAUP* 4.0 version beta 10 (Swofford, 2001). Analyses were performed using the tree-bisection-reconnection (TBR) branch-swapping algorithm for multiple replications of random stepwise addition of taxa. MULPARS was in effect. All characters were unordered and unweighted except where specified. The steepest descent option was not enforced. Zero length branches were collapsed to give polytomies. Gaps were treated as unknown in most analyses but as a fifth state in one series of the "sensitivity" analyses (see below).

Analyses were conducted separately for the combined data and for 16S rRNA using 1000 random sequence addition replicates. For bootstrap pseudo-sampling, heuristic searches were conducted for 200 bootstrap replicates, each with 20 random addition iterations. The searches using the combined data were repeated with the individual imposition of constraints in separate analyses that enforced monophyly of: Sorbeoconcha (Viviparidae plus Ampullariidae); Architaenioglossa (Ampullariidae, Viviparidae and Cyclophoridae); Rissooidea (Rissoidae and Anabathridae); Ptenoglossa (Cerithiopsidae, Eulimidae, Epitoniidae); or Neogastropoda (Muricidae, Volutidae, Nassariidae, Mitridae, Cancellariidae, Conidae).

Analyses were performed for various subsets of the data including the individual segments (and sub-segments within 28S rRNA D6 and CO1 when these were amplified in multiple reactions) to search for possible PCR artefacts. BLAST searches were made for taxa with long branch lengths in these analyses to confirm that the sequences are caenogastropod in origin. Analyses were performed using 200 replications of random stepwise addition of taxa. To prevent filling of the tree buffer by large numbers of equally long trees from a single replicate, no more than 200 trees (PAUP command nchuck = 200) longer than 50 steps (command chucklen = 50) were kept. For bootstrap pseudo-sampling for individual segments, heuristic searches were conducted for 200 bootstrap replicates, each with 20 random addition iterations where no more than 200 trees longer than 50 steps were kept.

Table 3
GenBank Accession Numbers for the dataset compiled here

Family	Species	COI	EF1-α	H3	12S rRNA	28S rRNA D1	28S rRNA A	28S rRNA D6	28S rRNA D9-10	28S rRNA B	18S rRNA
Fissurellidae	*Montfortula rugosa*	AY296819		AF033698		AY296879	AF033762	DQ916493	DQ916569	AF033763	DQ916541
Pleurotomariidae	*Bayerotrochus midas*	AY296820		AF033709	DQ916443	AY296880	AF033782	DQ916494	DQ916570	AF033783	L78893
Neritidae	*Nerita atramentosa*	AY296824		AF033701	DQ916444	AY296885	AF033764	DQ916495	DQ916571	AF033765	DQ916542
Cyclophoridae	*Leptopoma perlucida*	AY296826	DQ916585	AF033696	DQ916414	AY296887	AF033758	DQ916467	DQ916543	AF033759	DQ916519
Ampullariidae	*Pomacea bridgesii*	DQ916496		DQ916445	DQ916416	DQ916572	DQ916509	U78643	DQ916545	DQ916455	DQ916521
Viviparidae	*Bellamya heudi guangdungensis*	AY296827	DQ916587	AF033679	DQ916415	U75863	AF033724	U82423	DQ916544	AF033725	DQ916520
Batillariidae	*Zeacumantus subcarinatus*	AY296834	DQ916587	AF033715	DQ916417	AY296894	AF033794	DQ916468	DQ916546	AF033795	DQ916522
Turritellidae	*Maoricolpus roseus*	DQ916497	DQ916588	DQ916446	DQ916418	DQ916573	DQ916510	DQ916469	DQ916547	DQ916456	DQ916523
Campanilidae	*Campanile symbolicum*	AY296828		AF033683	DQ916419	AY296888	AF033732	DQ916470	DQ916548	AF033733	DQ916524
Littorinidae	*Austrolittorina unifasciata*	AY296829	DQ916589	AF033705	DQ916420	AY296889	AF033774	DQ916471	DQ916549	AF033775	DQ916525
Eatoniellidae	*Crassitoniella flammea*	DQ916498		DQ916447		DQ916574		DQ916472		DQ916457	DQ916526
Anabathridae	*Pisinna albizona*	DQ916499		DQ916448	DQ916421	DQ916575	DQ916511	DQ916473	DQ916550	DQ916458	DQ916527
Rissoidae	*Rissoina fasciata*	DQ916500			DQ916422	DQ916576	DQ916512	DQ916474	DQ916551	DQ916459	DQ916528
Strombidae	*Strombus luhuanhus*	AY296831	DQ916590	AF033711	DQ916425		AF033786			AF033787	
Hipponicidae	*Antisabia foliacea*	DQ916502		DQ916460		DQ916578	DQ916512	DQ916477		DQ916461	DQ916530
Calyptraeidae	*Bostrycapulus pritzkeri*	DQ916503	DQ916592	DQ916451	DQ916426	DQ916579	DQ916514	DQ916478	DQ916553	DQ916462	DQ916531
Xenophoridae	*Xenophora indica*	DQ916501		DQ916445	DQ916424	DQ916577		DQ916476	DQ916552	DQ916460	DQ916529
Vermetidae	*Serpulorbis sp.*	AY296830	DQ916590	AF033710	DQ916423	AY296890	AF033784	DQ916475		AF033785	
Cypraeidae	*Cypraea annulus*	AY296832		AF033681	DQ916427	AY296892	AF033728	DQ916479	DQ916554	AF033729	DQ916532
Naticidae	*Conuber melanostoma*	DQ916504	DQ916593	DQ916452	DQ916428	DQ916580	DQ916515	DQ916480	DQ916555	DQ916463	DQ916533
Ranellidae	*Cabestana spengleri*	AY296833	DQ916595	AF033686	DQ916430	AY296893	AF033738	DQ916482	DQ916557	AF033739	
Tonnidae	*Tonna cerevisina*	DQ916506	DQ916596	DQ916453	DQ916431	DQ916582	DQ916517	DQ916483	DQ916558	DQ916465	DQ916534
Pterotracheidae	*Pterotrachea coronata*	DQ916505	DQ916594		DQ916429	DQ916581	DQ916516	DQ916481	DQ916556	DQ916464	
Cerithiopsidae	*Ataxocerithium sp.*	AY296835	DQ916597	AF033678	DQ916432	AY296895	AF033722	DQ916484	DQ916559	AF033723	DQ916535
Epitoniidae	*Epitonium jukesianum*	AY296836	DQ916598	AF033691		AY296896	AF033748	DQ916485	AY583716	AF033749	AY583724
Eulimidae	*Stilifer* sp.	DQ916507		AF033692		DQ916583	AF033750		DQ916560	AF033751	
Nassariidae	*Nassarius burchardi*	AY296837	DQ916599	AF033702	DQ916433	AY296897	AF033766	DQ916486		AF033767	DQ916536
Muricidae	*Dicathais orbita*	AY296838	DQ916600	AF033690	DQ916434	AY296898	AF033746	DQ916487	DQ916561	AF033747	DQ916537
Mitridae	*Mitra carbonaria*	AY296839		AF033697	DQ916435	AY296899	AF033760	DQ916488		AF033761	
Volutidae	*Cymbiolista hunteri*	DQ916508	DQ916601	DQ916454	DQ916436	DQ916584	DQ916518		DQ916562	DQ916466	
Cancellariidae	*Cancellaria undulata*	AY296841		AF033688	DQ916437	AY296901	AF033742	DQ916489	DQ916563	AF033743	
Conidae	*Conus miles*	AY296840	DQ916602	AF033684	DQ916438	AY296900	AF033734	DQ916490	DQ916564	AF033735	DQ916538
Cornirostridae	*Cornirostra pellucida*	AY296842	DQ916603	AF033685	DQ916439	U75862	AF033736	U78672	DQ916565	AF033737	
Aplustridae	*Bullina lineata*	AY296847	DQ916604	AF033680	DQ916440	AY296906	AF033726	DQ916491	DQ916566	AF033727	
Aplysiidae	*Aplysia juliana*	AY296846	DQ916605	AF033675	DQ916441	AY296905	AF033716	U78644	DQ916567	AF033717	DQ916539
Amphibolidae	*Salinator solida*	AY296845		AF033712	DQ916442	AY296904	AF033788	DQ916492	DQ916568	AF033789	DQ916540

Gene segments not included in the data are indicated by blank cells. Accession numbers with the prefix DQ were collected for this study. All other data are from publications from this laboratory except the seven sequences specified in Section 2.2.

An Incongruence Length Difference Test (Farris et al., 1994) was conducted with PAUP (Swofford, 2001) to assess conflict between the character partitions defined by gene segments using maximum parsimony heuristic searches with 100 replicates, with 20 random additions of sequences in each and keeping no more than 200 trees greater than 20 steps in each replicate.

Two series of "sensitivity" analyses *sensu* Wheeler (1995) were conducted to ascertain if the relationships inferred from parsimony results were robust against changes in the assumed transformation matrix. All consistent combinations assuming that the costs of transitions, transversions and gaps could be 1, 2 or 4 were tested for the case where gaps were treated as a fifth state using 200 random taxon addition replicates and keeping no more than 100 trees greater than 400 steps in each. A similar series using the same search strategy was used for the case where gaps were treated as missing data, allowing the costs of transitions and transversions to take the values 1, 2 or 4.

Maximum likelihood analyses were performed with PAUP* using the parameters suggested by the Akaike Information Criterion test in MODELTEST (Posada and Crandall, 1998) for 30 replicates with random addition sequences for the overall data and 50 for other analyses. For the overall data, the model selected by MODELTEST was GTR + G + I. The parameter settings for the model were as follows. Initial base frequencies were A = 0.2809, C = 0.1594, G = 0.2419 and T = 0.3178). The number of substitution types was 6 with substitution rate matrix (A–C: 3.2339, A–G: 6.6870, A–T: 3.2945; C–G: 4.0560; C–T: 18.6100; and G–T: 1.0000). A discrete gamma distribution with four rate categories and an α parameter of 0.3550 was assumed. The proportion of invariant sites was set at 0.4137. When the third base positions were excluded, the model selected was GTR + G + I with the following settings. Initial base frequencies were A: 0.2440, C: 0.2320, G: 0.2619, and T: 0.2621. The number of substitution types was 6 with substitution rate matrix (A–C: 1.4467, A–G: 3.4592; A–T: 2.5336; C–G: 1.3119; C–T: 6.7463; and G–T: 1.000). A discrete gamma distribution with four rate categories and an α parameter of 0.3849 was assumed. The proportion of invariant sites was 0.4604). For the 16S rDNA data, the model selected was GTR + G + I with the following settings. Initial base frequencies were A: 0.4109, C: 0.0833, G: 0.1172, and T: 0.3886. The number of substitution types was 6 with substitution rate matrix (A–C: 2.1885, A–G: 10.4245; A–T: 1.8106; C–G: 2.0122; C–T: 19.0461; and G–T: 1.000). A discrete gamma distribution with four rate categories and an α parameter of 0.4518 was assumed. The proportion of invariant sites was estimated as 0.1751.

Bayesian analyses were conducted using MrBayes (Version 3.01) (Huelsenbeck and Ronquist (2001). Metropolis coupled, Monte Carlo Markov Chains were run for 1,000,000 steps and a number of generations (varying with the particular analysis) were discarded to allow for convergence. The discard cut-off point was determined by the rule that all trees remaining had likelihoods less than 0.2% worse than the final asymptote of the sample. Four differentially heated chains were run simultaneously. Topologies were sampled every 100 generations. Likelihood settings were determined during the run. Base frequencies were estimated, as were the independent rates of the six substitution types. A discrete gamma distribution was assumed for variation in the rate of substitution between nucleotide positions in the alignment and the shape parameter of this distribution was estimated. The estimation of the various likelihood parameters was conducted separately for each gene segment (and each codon position within coding sequences) using a character partition and the "unlink" command in MrBayes.

Abbreviations for the analyses principally discussed in this paper are given in the following list. As noted above, regions of uncertain alignment were excluded from all analyses.

AMP: Maximum parsimony, combined data;
AML: Maximum likelihood, combined data;
ABY: Bayesian, combined data;
AMP-P3: Maximum parsimony, combined data, excluding third codon positions;
AML-P3: Maximum likelihood, combined data, excluding third codon positions;
ABY-P3: Bayesian: combined data, excluding third codon positions;
16SMP: Maximum parsimony, 16S data;
16SML: Maximum likelihood, 16S data;
16SBY: Bayesian, 16S data.

3. Results

The alignments used in these analyses are available from the first author or from an accession in TREEBASE. In total, the alignment contains 3995 bases, of which 344 were excluded as belonging to regions of uncertain alignment. Of the remaining bases, 2164 showed no variation in the present sample of species, 1119 were parsimony-informative and 368 were variable but not parsimony-informative. χ-squared tests of the homogeneity of base composition in the various taxa gave a probability close to 1 for the 16S rRNA dataset and for all gene segments except CO1. When the base position data for the coding regions are considered individually, inhomogeneity was detectable for all three sets of third base position data but not for any other codon positions. The ILD test returned a probability of 0.91 for AMP and 0.65 for AMP-P3, thereby not rejecting the hypothesis of no character conflict between the individual segments.

3.1. Analyses of the combined dataset

The topologies for the AML, AML-P3, ABY and ABY-P3 analyses are shown in Figs. 2–5 respectively. The first 110,000 generations of the simulation were discarded for the calculation of posterior probabilities for ABY and the

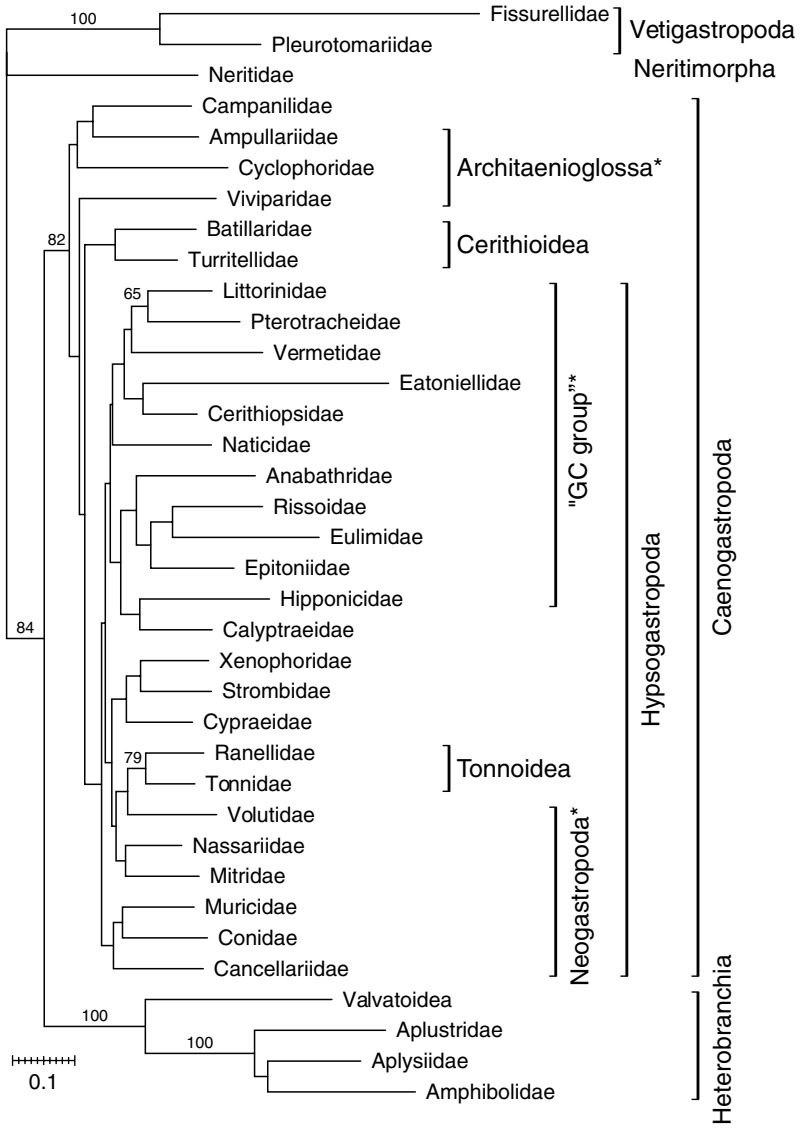

Fig. 2. The maximum likelihood tree for the combined data (AML). Figures above branches are bootstrap support percentages over 50 in the maximum parsimony analysis. The scale bar is graduated in units of 0.01 substitutions per site. The sister pairings of (Cyclophoridae, Batillariidae) and (Aplysiidae, Aplustridae) respectively received MP bootstrap supports of 50% and 86% but are not shown in the AML topology. Named higher level taxa are indicated by bars to the right of the topology. Asterisks indicate that the named clade is not monophyletic.

first 80,000 generations for ABY-P3. Both Caenogastropoda and Hypsogastropoda were monophyletic in all of these topologies. Sorbeoconcha was contradicted in all, owing to the inclusion of Campanilidae in a clade containing otherwise only Ampullariidae or Cyclophoridae. Cerithioidea (Batillariidae and Turritellidae) and Tonnoidea (Ranellidae and Tonnidae) were well–supported monophyletic clades in all of these analyses. Neogastropoda was never monophyletic in any analyses of the compiled data although there was no strong evidence against its monophyly.

In the AML topology (Fig. 2), a clade of three neogastropods was the sister group to all other Hypsogastropoda. The two clades formed by the second division within Hypsogastropoda were (1) a group comprising the three other Neogastropoda, the Tonnoidea, Cypraeidae, Strombidae and Xenophoridae and (2) a group comprising Littorinidae, Anabathridae, Rissoidae, Eatoniellidae, Vermetidae, Hipponicidae, Naticidae, Pterotracheidae, Cerithiopsidae, Eulimidae, Epitoniidae and Calyptraeidae. These families except Calyptraeidae are referred to informally as the "GC group", the name deriving from a shared sequence motif detailed below. The "GC group" was seen in the AML-P3 topology where Calyptraeidae was its sister group and in ABY-P3 where it received posterior probability support of 84. The group excepting Eatoniellidae was recognisable in ABY with posterior probability support of 73. In ABY, Calyptraeidae was unresolved within Hypsogastropoda and in ABY-P3, it was the sister of the neogastropod family Cancellariidae with posterior probability support of 74.

One maximum parsimony tree was found for AMP (length 8251; consistency index (CI) 0.303). The bootstrap

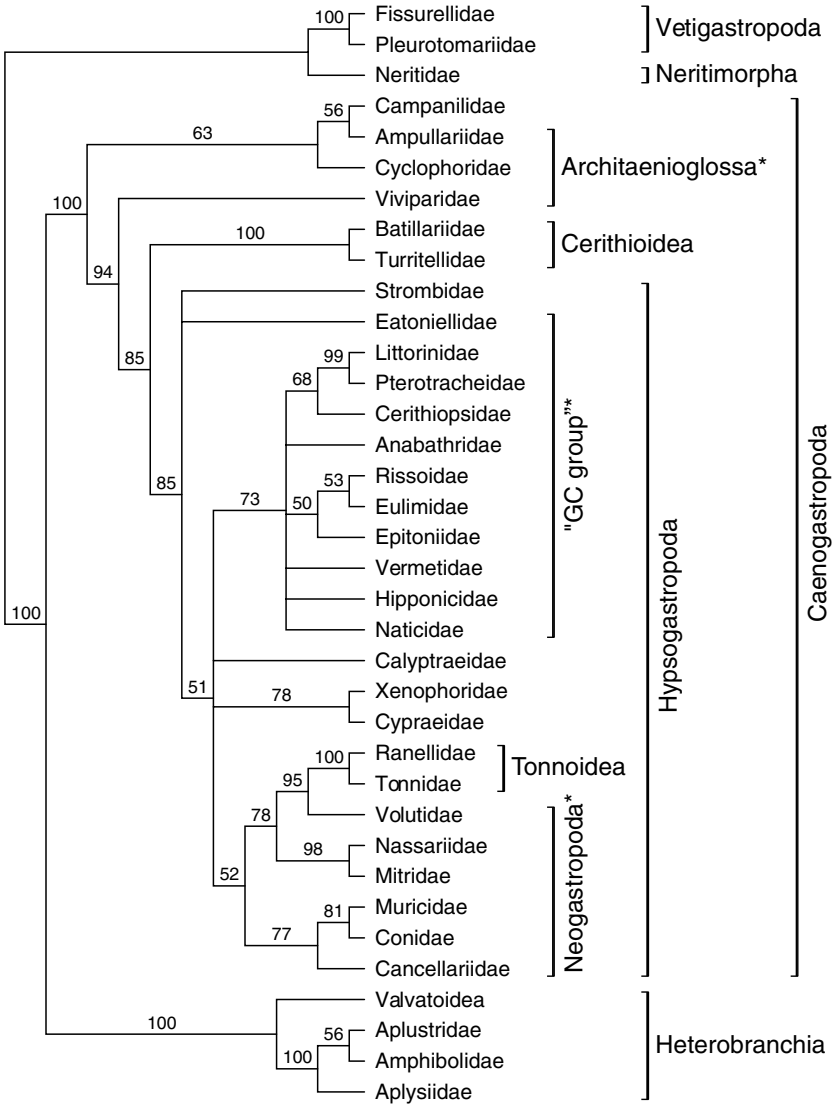

Fig. 3. The majority rule consensus of the trees sampled during the ABY analysis of the combined data. Posterior probability support levels are given above branches. Named higher level taxa are indicated by bars to the right of the topology. Asterisks indicate that the named clade is not monophyletic.

supported clades in this analysis are shown in Table 4 and Fig. 2. The supported clades for the assumption that gaps are a fifth state are included for comparison with their treatment as missing data. In the AMP maximum parsimony tree, Turritellidae was included in a clade of five neogastropods that formed one of two branches forming the basal division within Hypsogastropoda, thereby also contradicting Cerithioidea. The other neogastropod (Volutidae) was the sister group to Naticidae in a clade deriving from the third division in Hypsogastropoda. Trees constrained to show the "GC group" as monophyletic had a length of 8270.

The lengths of AMP trees constrained to show previously named groups as monophyletic were: (Viviparidae, Ampullariidae): two trees, of 8267 steps with a consistency index (CI) of 0.303; Architaenioglossa: one tree, 8271 steps, CI = 0.303; Rissooidea: two trees, 8252 steps CI = 0.303; Sorbeoconcha: one tree, 8284 steps, CI = 0.302; Ptenoglossa: four trees, 8276 steps, CI = 0.303; and Neogastropoda: one tree, 8263 steps, CI = 0.303. None of the constraints produced trees that were significantly longer than the unconstrained trees using either the Kishino–Hasegawa Test (KH Test) (Kishino and Hasegawa, 1989) or the non-parametric Templeton test (Templeton, 1983).

Two trees were found for the AMP-P3 analysis (length 3854; CI 0.411). The bootstrap supported clades found in this analysis are listed in Table 4. In both these trees, the inclusion of Viviparidae as the sister group to Eatoniellidae and the sister group pairing of Strombidae and Cerithioidea at the base of the clade including all Hypsogastropoda contradicted monophyly of this group.

Pterotracheidae were generally closely associated with Littorinidae (Figs. 2–5). The families formed a sister group in all analyses, the pairing having bootstrap support of 65% in AMP and 56 in AMP-P3 and posterior probability support of 99 in ABY and 95 in ABY-P3. The two Rissooidea

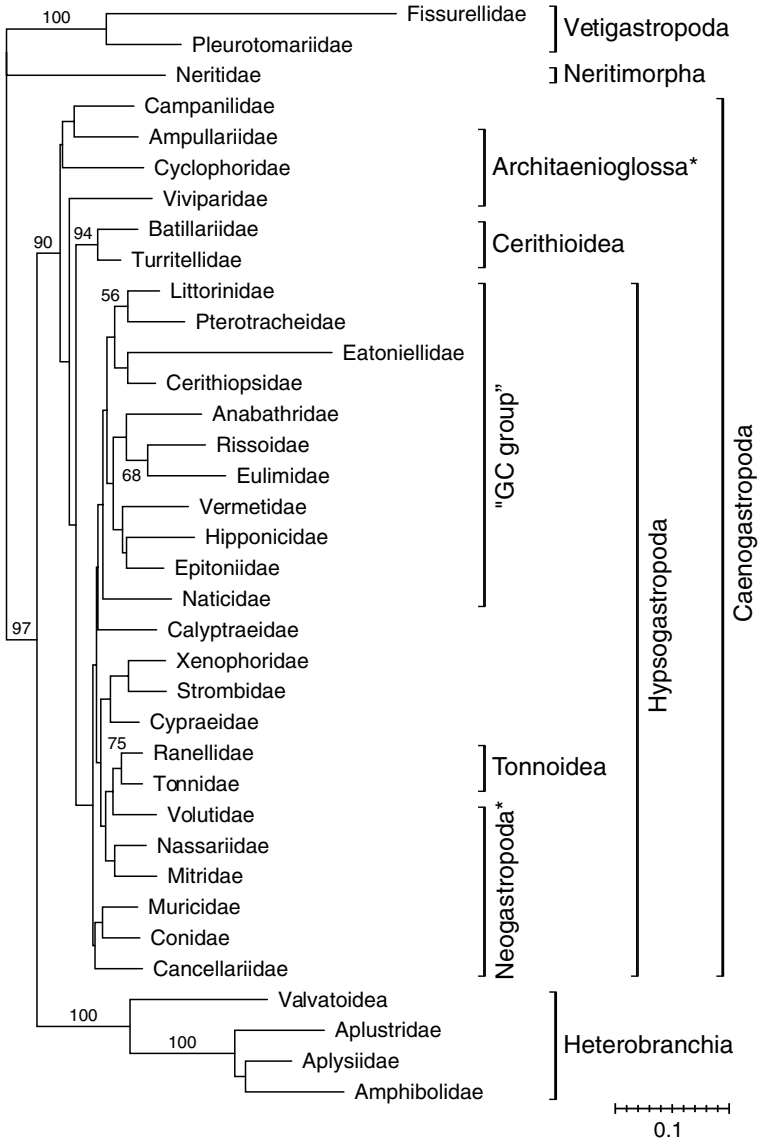

Fig. 4. The maximum likelihood tree for the combined data excluding third codon positions (AML-P3). Figures near nodes are the maximum likelihood bootstrap support percentages and those below are MP bootstrap support percentages greater than 50. The scale bar is graduated in units of 0.01 substitutions per site. Aplustridae is sister to Aplysiidae in AMP-P3 (bootstrap 52). Named higher level taxa are indicated by bars to the right of the topology. Asterisks indicate that the named clade is not monophyletic.

(Rissoidae and Anabathridae) were never monophyletic in the combined analyses. Often Rissoidae (AML, AML-P3, ABY-P3, AMP-P3) formed an unsupported clade with Eulimidae (Figs. 2, 4, 5), sometimes with Epitoniidae as the sister group to this pair (AML, ABY, Figs. 2 and 3). The two tonnoidean families formed a generally well-supported clade in all combined analyses including AMP and AMP-P3 (Figs. 2–5) as did Strombidae and Xenophoridae, although with less support (Figs. 2–5) and excepting AMP and AMP-P3. Ptenoglossa was not monophyletic in any of the combined analyses. Architaenioglossa was never monophyletic. In all combined analyses except AMP where it was the sister group to Batillariidae and AMP-P3 where it was the sister group to Campanilidae, Cyclophoridae was the sister group to (Campanilidae, Ampullariidae). This clade was sister group to the rest of the caenogastropods in all likelihood and Bayesian analyses. Viviparidae was the next branching taxon in these analyses, having a sister group comprised of two monophyletic clades, Cerithioidea and Hypsogastropoda (Figs. 2–5).

A total of 18 non-redundant transformation matrices among those with parameter variation as specified in Section 2 were found to be consistent during PAUP processing. These are specified in the form [a, b, c] where "a" refers to the assumed cost of transitions, "b" to the cost of transversions and "c" to the cost of inserting a gap. Topologies resulting from the searches always recovered monophyly of Caenogastropoda. Tonnoidea and (Littorinidae, Pterotracheidae) were monophyletic for all matrices. The sister pair (Xenophoridae, Strombidae) was sometimes observed ([1 4 0], [2 4 0], [2 2 1], [1 2 2] and [1 4 2] as was Cerithioidea ([1 4 0], [1 2 0], [1 1 2], [1 2 2], [1 4 2],

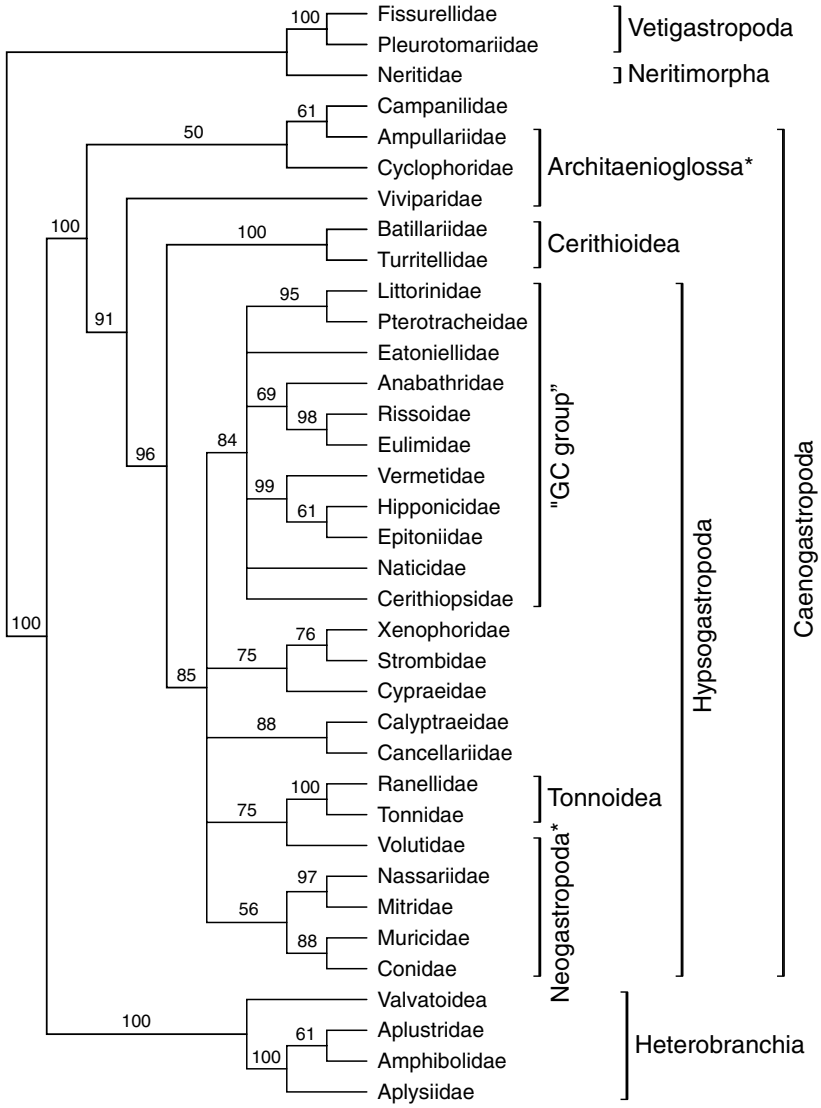

Fig. 5. The majority rule consensus of the trees sampled during ABY-P3 analysis of the combined data excluding third codon positions. Posterior probability support levels are written above the branches. Named higher level taxa are indicated by bars to the right of the topology. Asterisks indicate that the named clade is not monophyletic.

[1 1 4], [1 2 4] and [1 1 4]). Hypsogastropoda was never monophyletic, being shown to include Viviparidae or Turritellidae or to exclude Eatoniellidae and/or Eulimidae. Monophyly of Architaenioglossa, Sorbeoconcha, the "GC group" or Neogastropoda was never recovered. Notably, however, as many as five of the six neogastropods were often found in a clade. For two matrices ([1 1 2] and [1 4 2]) this did not include other taxa.

3.2. Individual segment analyses

Details of the various datasets and the trees from maximum parsimony analyses of them are given in Table 5. Clades receiving bootstrap support of more than 50% in analyses of individual gene segments are specified in Table 4. Individual gene maximum parsimony analyses rarely recovered named higher taxa. Caenogastropoda was seen in the CO1-P3 (excepting Eulimidae), 28S rRNA D6 and 18S rRNA (excepting Eatoniellidae) MP analyses. Sorbeoconcha was not seen in any individual gene maximum parsimony analysis. Hypsogastropoda was monophyletic only in 28S rRNA D6 but was supported in 18S rRNA except for the exclusion of Eatoniellidae.

There were only three positions in the 28S rRNA B segment where two or more bases were observed in two or more taxa within Caenogastropoda. These were at position 2985 in the 28S rRNA sequence in GenBank accession AY145411 from *Ilyanassa obsoleta* (Passamaneck et al., 2004) where thymine was found in Turritellidae and all Hypsogastropoda except Eatoniellidae, Strombidae and Nassariidae. Cytosine was found in these exceptions and in Architaenioglossa, Campanilidae, Batillariidae and outgroup taxa. Membership of the informal "GC group" was defined by the bases present at the other two variable positions. At position 3026 in the *I. obsoleta* sequence in

Table 4
Bootstrap supported clades in maximum parsimony analyses

Analysis	Bootstrap supported clades
AMP	(Cyclophoridae, Batillariidae)-50; (Littorinidae, Pterotracheidae)-65; (Ranellidae, Tonnidae)-79; (((Aplustridae, Aplysiidae)-86, Amphibolidae)-100, Valvatoidea)-100; (Fissurellidae, Pleurotomariidae)-100; Caenogastropoda-82; Apogastropoda-84
AMP-P3	(Cyclophoridae, Campanilidae)-62; (Batillariidae, Turritellidae)-94; (Littorinidae, Pterotracheidae)-54; (Rissoidae, Eulimidae)-68; (Ranellidae, Tonnidae)-75; (((Aplustridae, Aplysiidae)-52, Amphibolidae)-100, Valvatoidea)-100; (Fissurellidae, Pleurotomariidae)-100; Caenogastropoda-90; Apogastropoda-97
AMP gap = fifth state	(Cyclophoridae, Batillariidae)-55; (Ampullariidae, Campanilidae)-59; (Littorinidae, Pterotracheidae)-60; (Ranellidae, Tonnidae)-83; (((Aplustridae, Aplysiidae)-78, Amphibolidae)-100, Valvatoidea)-99; (Fissurellidae, Pleurotomariidae)-100; Caenogastropoda-86; Apogastropoda-88
AMP-P3 gap = fifth state	(Cyclophoridae, Campanilidae)-62; (Batillariidae, Turritellidae)-93; (Littorinidae, Pterotracheidae)-57; (Rissoidae, Eulimidae)-64; (Ranellidae, Tonnidae)-80; (((Aplustridae, Aplysiidae)-51, Amphibolidae)-100, Valvatoidea)-100; (Fissurellidae, Pleurotomariidae)-100; Caenogastropoda-90; Apogastropoda-94
18S rRNA	(Batillariidae, Turritellidae)-82; (Xenophoridae, Conidae)-60; (Caenogastropoda excluding Eatoniellidae)-88; (Caenogastropoda excluding Eatoniellidae and Ampullariidae)-58; (Aplysiidae, Amphibolidae)-87
28S rRNA D1	(Batillariidae, Turritellidae)-70; (((Aplysiidae, Amphibolidae)-79, Valvatoidea)-55, Aplustridae)-80; Apogastropoda – 52
28S rRNA A	(Batillariidae, Turritellidae)-65; (Aplysiidae, Amphibolidae)-57; (all taxa excluding Fissurellidae and Pleurotomariidae)-99
28S rRNA D6	((Aplustridae, Aplysiidae, Amphibolidae)-73, Valvatoidea)-69; Caenogastropoda-83
28S rRNA D9-10	(Ampullariidae, Campanilidae)-69; (Xenophoridae, Cypraeidae)-55; (((Aplustridae, Aplysiidae)-54, Amphibolidae)-72, Neritidae)-70; (all taxa except Fissurellidae and Pleurotomariidae)-71
28S rRNA D9-10 and 28S rRNA B	(Ampullariidae, Campanilidae)-61; (Xenophoridae, Cypraeidae)-55; (Aplustridae, Aplysiidae, Amphibolidae, Neritidae)-59; (all taxa except Fissurellidae and Pleurotomariidae)-66;
28S rRNA B	(Littorinidae, Anabathridae, Rissoidae, Eatoniellidae, Vermetidae, Hipponicidae, Naticidae, Heteropoda, Cerithiopsidae, Eulimidae, Epitoniidae)-69; (all taxa excluding Fissurellidae, Pleurotomariidae and Valvatoidea)-64; (Apogastropoda, Neritidae)-97
12S rRNA	(Turritellidae, Batillariidae)-89; (Ranellidae, Tonnidae)-51; (((Aplustridae, Aplysiidae)-90; Amphibolidae)-100, Valvatoidea)-100; Apogastropoda-55
EF	None
EF-P3	None
H3	(Batillariidae, Cyclophoridae)-99; (Ranellidae, Tonnidae)-64; (all taxa except Fissurellidae, Pleurotomariidae and Hipponicidae)-96; (Apogastropoda, Neritidae)-95
H3-P3	(Batillariidae, Cyclophoridae)-84; (Apogastropoda + Neritidae)-76; (Fissurellidae, Hipponicidae)-83; Heterostropha – 52
COI	(Batillariidae, Mitridae)-56; (Aplustridae, Aplysiidae, Amphibolidae)-100
COI-P3	(((Aplustridae, Amphibolidae)-64, Aplysiidae)-100, Valvatoidea)– 84

GenBank accession AY145411, cytosine is the most common form, but is replaced by guanine in Littorinidae, Anabathridae, Rissoidae, Eatoniellidae, Vermetidae, Hipponicidae, Naticidae, Pterotracheidae, Cerithiopsidae, Eulimidae and Epitoniidae. At position 3035 in the *Ilyanassa obsoleta* sequence, the situation is reversed with guanine, the common form being replaced by cytosine in the families just listed. These are apparently compensatory changes in the stems of a loop system.

Clades with bootstrap or posterior probability support greater than 50 observed in the 16SMP or 16SBY analyses are shown in Fig. 6 that illustrates the optimal 16SML tree. The first 120,000 generations of the simulation were discarded for the calculation of Bayesian posterior probabilities. In the 16SML tree, Caenogastropoda and Hypsogastropoda were recovered but Cerithioidea are paraphyletic. A clade containing all Neogastropoda was recognisable but was paraphyletic as it included a subclade comprised of Triviidae and Velutinidae. The three families of the "GC group" represented in this dataset were found in two monophyletic clades one of which (including Littorinidae and a number of other asiphonate families) was associated by short branches with clades containing predominantly siphonate taxa (Fig. 6), but also including Xenophoridae, Calyptraeidae, Velutinidae and Capulidae. The other clade, including only Rissoidae and Hipponicidae was shown as one of the sister groups formed by the basal division in Hypsogastropoda. Members of the Rissooidea were seen in both of these clades rendering this superfamily diphyletic.

Table 5
Numbers of aligned base positions of various types and tree statistics for individual segment datasets

Dataset	Invariant	Variable uninformative	Informative	Uncertain	Total bases	Trees	CI	Length
18S rRNA	290	71	71	99	531	9000	0.663	288
28S D1	221	39	83		343	734	0.450	460
28S rRNA A	190	23	28	8	249	6400	0.708	96
28S r RNA D6	232	60	81	69	442	267	0.504	407
28S rRNA D9-10 plus 28 S rRNA B	448	56	49	62	615	9000	0.653	216
12S rRNA	77	44	197	106	424	6	0.383	1129
CO1	428	41	395		864	1	0.219	3873
H3	161	12	101		274	2	0.269	676
EF	117	22	114		253	5	0.404	550
Total	2164	368	1119	344	3995	1	0.303	8251
28S rRNA D9-10	439	55	49	62	605	30	0.690	186
28S rRNA B	245	14	15		274	84	0.850	40
EF-P3	114	15	40		169	395	0.482	166
CO1-P3	425	37	114		576	96	0.330	678
H3-P3	154	8	20		182	9000	0.558	52
16S rRNA	158	56	206	178	598	24	0.250	1726

The columns give, in order, the number of invariant bases, the number of variable but parsimony uninformative bases, the number of parsimony informative bases, the number of bases excluded owing to uncertain alignment, the number of maximum parsimony trees found in heuristic searches, the consistency index of these trees and their length. Figures for the segments included in the compiled data are written in the "Total" row. Rows below this give figures for subsets of the data or the 16S rRNA data.

4. Discussion

Most analyses reported here, using the combined data, individual segments or the independent 16S rRNA dataset, support monophyly of Caenogastropoda, often with substantial statistical support. Caenogastropod monophyly has generally been supported, or only weakly contradicted, in studies using DNA sequences from a significant number of species (Harasewych et al., 1998; McArthur and Harasewych, 2003; Colgan et al., 2003). Monophyly is strongly supported by morphology (Ponder and Lindberg, 1997). Consequently the following discussion will focus on relationships within the group.

4.1. Sorbeoconcha and Architaenioglossa

Sorbeoconcha, as defined by Ponder and Lindberg (1997), included Campanilidae and Cerithioidea. This group is not observed in any of our analyses. Sorbeoconcha has never previously been supported by a large molecular dataset, generally owing to the exclusion of Campanilidae or to the inclusion of architaenioglossans within the smallest monophyletic clade including all of its supposed members (Harasewych et al., 1998; Colgan et al., 2003).

Morphologically, monophyly of Architaenioglossa has been considered doubtful (Haszprunar, 1988; Ponder and Lindberg, 1997). However, Strong (2003) found two unambiguous synapomorphies and six forward homoplasies supporting the monophyly of the two studied Architaenioglossa (Cyclophoridae and Ampullariidae), which was shown as the sister group of Sorbeoconcha, also monophyletic. Campanilidae and Viviparidae were not included in Strong's (2003) study. In the most recent morphological analysis of architaenioglossans, Simone (2004) found no support for the sister pairing of any architaenioglossan families. While the architaenioglossans sampled here (Ampullariidae, Viviparidae and Cyclophoridae) are generally included with a cluster of non-hypsogastropods, they did not ever form a monophyletic group. Instead, the architaenioglossans were paraphyletic, with one or more of its members basal in Caenogastropoda in all combined analyses.

Harasewych et al. (1998) conducted the first study of relationships at the base of the caenogastropods that included a substantial number of non-hypsogastropods. They sequenced parts of the 18S rRNA gene in 19 caenogastropods including five architaenioglossans (two Cyclophoridae, two Ampullariidae and one Viviparidae), *Campanile* and three cerithioideans. Cerithioidea, Ampullariidae and Cyclophoridae were monophyletic in several of their analyses (Harasewych et al., 1998), however the frequent exclusion of Viviparidae and the inclusion of Campanilidae contradicted monophyly of Architaenioglossa. Harasewych et al. (1998) found the non-hypsogastropod Caenogastropoda comprised a monophyletic group in maximum likelihood analyses and in parsimony analyses using only transversions. This intriguing clade was not observed in their other analyses. Nor was it obtained in later analyses of 18S rRNA data (McArthur and Harasewych, 2003; herein). Monophyly of this grouping as a derived clade within Hypsogastropoda was observed for two of 18 transformation matrices in the sensitivity analyses (this study). Monophyly requires a non-significant increase of 15 steps when compared to the unconstrained topology in AMP analyses (results not illustrated).

All molecular analyses containing *Campanile*, with the exception of the present 16S rRNA analyses, suggest that an architaenioglossan is the sister group of this family, a

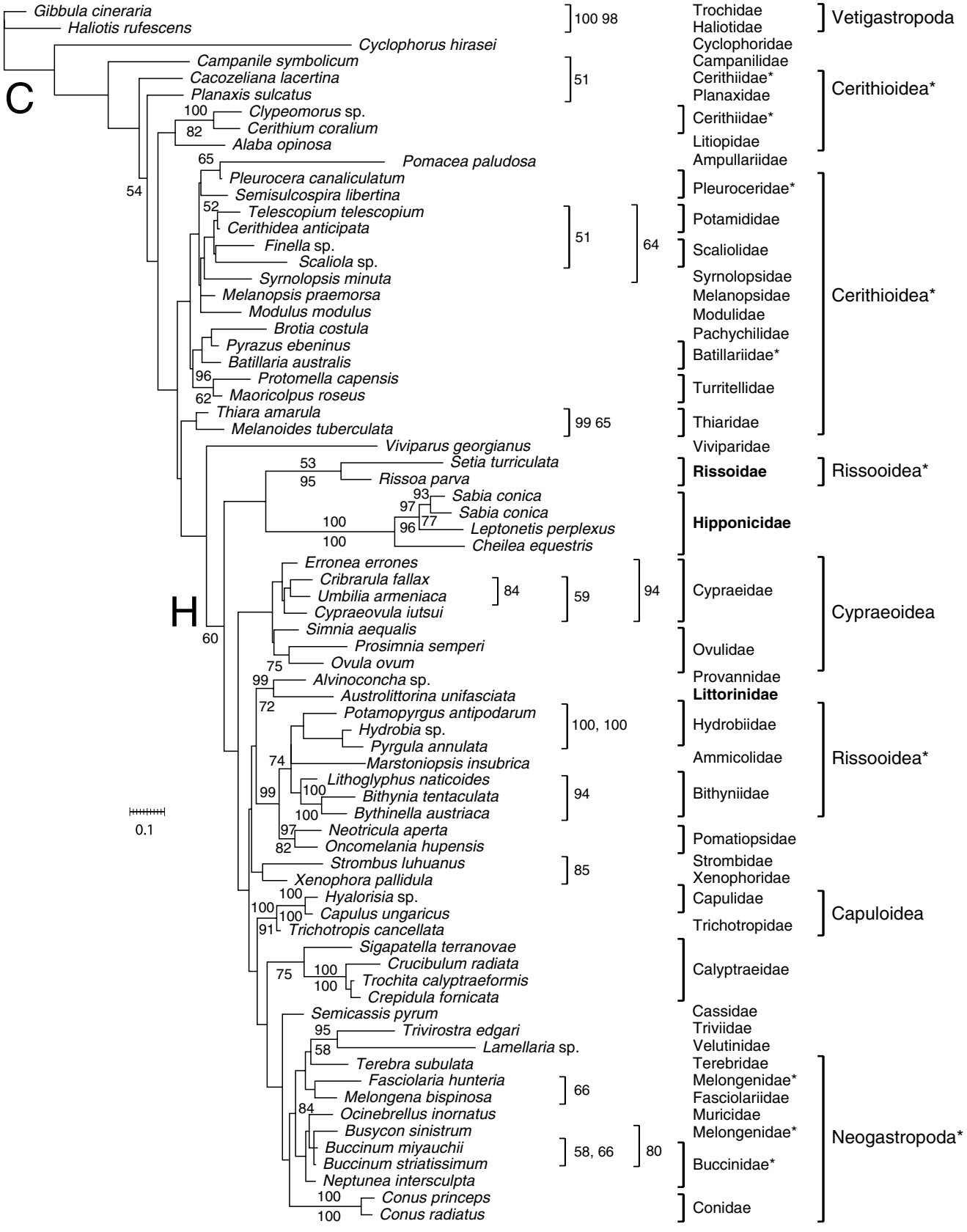

Fig. 6. The optimal tree found in 16SML analysis of the 16S rRNA data. Brackets to the right of taxon names define taxonomic groups or statistically supported relationships. Taxa are monophyletic except where contradiction is indicated by an asterisk beside the group name. The node linking Caenogastropoda is identified by "C" and that linking Hyspogastropoda by "H". The three families of the "GC group" represented in this analysis are written in bold. The scale bar is graduated in units of 0.01 substitutions per site. Posterior probability support levels greater than 60 and maximum parsimony bootstrap support values greater than 50 are written near nodes or to the right of linking brackets. Posterior probabilities are written first. Where only one figure is shown, this is a posterior probability support.

possibility first raised by Harasewych et al. (1998). The relationship they observed was with Cyclophoroidea, as also seen in the present analyses only in AMP-P3, rather than with Ampullariidae as suggested by the support for a sister-group pairing of these families in the likelihood and Bayesian analyses. A possible explanation for these affiliations is that the taxon sampling of Cerithioidea is better for the 16S rRNA dataset than for the other sets used in this paper or in Harasewych et al. (1998).

4.2. Hypsogastropoda

Sorbeoconcha was divided into two major clades in Strong's (2003) morphological analysis. Hypsogastropod monophyly was contradicted by the inclusion of Batillariidae (Cerithioidea) in the first major clade. This also included Vermetidae, Strombidae, Crepidulidae and Bithyniidae. The second major clade included only hypsogastropods (Littorinidae, Naticidae, Cypraeidae, Epitoniidae and Neogastropoda). Both of these major clades were supported by only two homoplasous characters (Strong, 2003, Fig. 26). In contrast, hypsogastropod monophyly has often been observed in molecular analyses. It was recovered in all analyses in Harasewych et al. (1998) and McArthur and Harasewych (2003) and in all current analyses of the combined data excepting AMP and AMP-P3, with posterior probability support of 85 in both ABY and ABY-P3. In AMP, Turritellidae is the sister group to Conidae and in AMP-P3, Cerithioidea is a monophyletic sister group of Strombidae, this group of three taxa being sister to the remainder of Hypsogastropoda plus Viviparidae. Where monophyly is contradicted in Colgan et al. (2003) and in the present AMP and AMP-P3 analyses, a clade containing all Hypsogastropoda is usually seen although including one or both cerithioideans (Batillariidae and Turritellidae) in agreement with Strong (2003) and sometimes Viviparidae.

When topologies from analyses of the 16S rRNA dataset were rooted on Vetigastropoda, the included heterobranchs were placed in the clade containing the hypsogastropods but with such a long branch length that this placement was considered an artefact. With the removal of the heterobranchs from the analysis, monophyly of Hypsogastropoda was recovered in 16SML and 16SMP but not 16SBY where (Hypsogastropoda + Cyclophoridae) had posterior probability support of 60.

4.3. The "GC Group" and inhalant siphons

Only one major group of taxa within Hypsogastropoda received support in a notable proportion of the present analyses. Further work, including the investigation of other families is required to establish whether this group is robustly monophyletic so it was here named only for convenience of reference. The name "GC group" recognises a shared sequence motif in the 28S rRNA B segment. The group comprises Littorinidae, Eatoniellidae, Rissoidae, Anabathridae, Vermetidae, Hipponicidae, Pterotracheidae, Cerithiopsidae, Epitoniidae, Eulimidae and Naticidae. The group includes most studied Hypsogastropoda that lack an anterior siphon and the siphonate Cerithiopsidae. The other asiphonate taxa included in these studies were the limpet-shaped Calyptraeidae that was sometimes associated with the "GC group" and the tent-shaped Xenophoridae that was always excluded. The group was seen in its entirety in ABY-P3 (with posterior probability support of 84) and AML-P3. The group excepting Eatoniellidae was seen in ABY (with posterior probability support of 73) and is also discernible in AML, but with Calyptraeidae included as the sister group of the limpet-shaped Hipponicidae. The inclusion of the Cerithiopsidae in the "GC group" raises the possibility that the inhalant siphon in cerithiopsids (and the related Triphoridae that are not included in this analysis) may not be homologous with that in other hypsogastropods, a possibility supported by the putative stem group, Pseudozygopleuridae, having lacked a siphonal notch (Nützel, 1998).

Although Calyptraeidae are included in, or closely related to, the "GC group" in some analyses, they are not included in the group as hypothesised here as the sequenced representative does not share the characteristic 28S rRNA B motif. The lack of an inhalant siphon in this family may be convergent owing to the limpet form of its shell. Analogous situations are seen in other families that have at least some taxa with limpet-like shells. For example, considering *Trichotropis* and *Capulus*, two taxa sometimes included in a single family (Capulidae), the coiled taxon (*Trichotropis*) has a siphon while the limpet-shaped *Capulus* does not.

The presence of the siphon is indicated in the shell by the presence of an anterior apertural notch or siphonal canal. The siphon itself is an anterior extension of the mantle edge and is used to direct the inhalant water current. Anterior apertural notches have, however, been developed in a few groups to accommodate other organs; for example some Rissoidae (e.g. *Rissoina*) have a notch for a pallial tentacle and stromboideans have, in addition to their small siphonal canal, a notch for a stalked eye-bearing tentacle. A great variety of mantle/siphon and anterior shell notch character combinations is seen in Cerithioidea but in those that do bear a mantle edge siphon, it is formed differently from that in hypsogastropods (Simone, 2001). Ponder and Lindberg (1997) proposed that the Sorbeoconcha were differentiated from the architaenioglossan grade in that they switched from the plesiomorphic exhalant control of mantle cavity water currents to inhalant control and that the formation of an inhalant siphon was a consequence of this switch. This change conferred significant evolutionary advantages which were enhanced by the, probably multiple, development of a siphon. In particular, it maximized the potential of the chemosensory facilities of the osphradium enabling more efficient detection of predators, mates and prey (Lindberg and Ponder, 2001).

4.4. Neogastropoda

Neogastropoda was one of the few traditional clades maintained during the revolution in the understanding of

gastropod phylogeny in the 1980s and 1990s. The group is morphologically defined by multiple synapomorphies (Ponder, 1974; Taylor and Morris, 1988; Ponder and Lindberg, 1996, 1997; Kantor, 1996; Strong, 2003). For example, in Strong's (2003) analysis, seven non-homoplasous synapomorphies and seven homoplasous synapomorphies supported monophyly and the group had a Bremer support of five.

Neogastropoda has usually been contradicted, albeit weakly, in molecular analyses (Harasewych et al., 1997; Colgan et al., 2000, 2003; Riedel, 2000; McArthur and Harasewych, 2003; this study). Harasewych et al. (1997) included only two other hypsogastropods and two architaenioglossans but Neogastropoda was not resolved as monophyletic in their analyses of partial 18S rRNA sequences. There was little structure in their topologies, and even some putative neogastropod genera lacked support. Harasewych et al. (1998) recovered Neogastropoda (3 taxa) in a few of their analyses (MP, ML), but not all. In Colgan et al. (2003), at most two of the five studied neogastropods were included in a monophyletic clade exclusive of other taxa. Riedel's (2000) analyses of several neogastropod families and a few other caenogastropods using 16S rRNA and 18S rRNA data also failed to recover monophyly with Marginellidae and, in the 16S rRNA dataset, *Vexillum* (Costellariidae) consistently falling within lower hypsogastropods. *Ficus* (Ficidae) and *Bufonaria* (Bursidae) were nested within the other neogastropods in 18S rRNA analyses (Riedel, 2000).

The two main hypotheses that have been advanced regarding the origin of neogastropods are that they arose from an "archaeogastropod" or primitive "mesogastropod" (Ponder, 1974), or alternatively that they arose from a "higher" mesogastropod, usually considered to be a tonnoidean or sharing a common ancestor with that group (e.g., Fretter and Graham, 1962; Taylor et al., 1980; Taylor and Morris, 1988; Riedel, 2000). The latter hypothesis has received some support from morphological (Ponder and Lindberg, 1996, 1997) and ultrastructural data (e.g. Haszprunar, 1985; Healy, 1988, 1996). However, Strong's analyses (2003) suggest other possible affinities for the group, the nearest relatives of Neogastropoda being Epitoniidae, Cypraeidae and Naticidae (Tonnoidea were not represented in these analyses). Our analyses find relationships between neogastropod families (either singly or as groups) and Turritellidae, Tonnoidea, Stromboidea or Cypraeidae. The analyses do not suggest that the Neogastropoda are derived Hypsogastropoda although there is a close relationship with Tonnoidea. In all analyses of the multi-gene data (except AMP-P3), one of the sister groups in the first division of Hypsogastropoda is predominantly or solely composed of neogastropods.

In the fossil record, Neogastropoda are first recognized during the Cretaceous (Tracey et al., 1993; Bandel, 1993) and Kollmann (1982) and Taylor and Morris (1988) suggested that they originated during the early stages of that period. Numerous proposals for the stem neogastropod lineage have been made. These have included the Palaeozoic siphonate Subulitidae (Cox, 1960; Ponder, 1974), the Purpurinidae from the Triassic/Jurassic (Taylor et al., 1980; Kaim, 2004) and *Maturifusus* (Maturifusidae) from the early Jurassic to Cretaceous (Szabó, 1983; Schröder, 1995; Bandel, 1993; Riedel, 2000; Kaim, 2004). A possible Late Triassic maturifusid has also been reported (Nützel and Erwin, 2004). The lack of support for a late origin of Neogastropoda here emphasises the need for further work in determining the fossil history of the group.

4.5. Other clades within Hypsogastropoda

With the exception of Tonnoidea (Riedel, 1995, as Cassoidea), few previously named clades within Hypsogastropoda are supported in the present analyses. In agreement with Simone's (2005) morphological analysis as well as earlier placements, Xenophoridae and Strombidae form a clade in likelihood and Bayesian analyses (Figs. 2–6). Our results strongly indicate the non-monophyly of the Rissooidea, a very large group of mainly small-sized, families that may have little in common other than superficial similarity. The two families (Anabathridae and Rissoidae) included in the main analyses form a clade with Eulimidae in AMP-P3, AML-P3 (Fig. 4) and ABY-P3 with posterior probability support of 69 (Fig. 5). The two Rissoidae included in the 16S analysis are separated from other rissooidean families (Fig. 6) being more closely associated, albeit with weak support, to the Hipponicidae and Cyclophoridae. The remaining rissooideans including Hydrobiidae *s.l.*, Pomatiopsidae and Bithyniidae are predominantly freshwater taxa. They have a clade formed by Littorinidae and Provannidae as their sister group (Fig. 6).

The sister group pair of Rissoidae with Eulimidae that contradicts Ptenoglossa has low posterior probability support of 53 in ABY (Fig. 3) and a moderate bootstrap support of 68 in AMP-P3 but a high posterior probability of 98 in ABY-P3. Epitoniidae was generally closely associated with (Rissoidae, Eulimidae) in overall analyses but not when third codon positions were excluded. 16S data were not available for ptenoglossans. Eulimidae differ from other ptenoglossan families in having a concentrated nervous system and a penis and its members lack the distinctive parasperm seen in other ptenoglossans (Healy, 1988). The traditional concept of Ptenoglossa has not been tested in morphological analyses, although Nützel (1998), using fossils, argues for a sister relationship of Janthinoidea (including Epitoniidae) and Triphoroidea. The only support for this suggestion among the many explorations of the present data was that (Epitoniidae, Cerithiopsidae) was shown in maximum parsimony analyses of all data including the areas of uncertain alignment (results not reported).

One further clade within Hypsogastropoda, the sister pairing of Littorinidae and Pterotracheidae (representing "Heteropoda"), is consistently supported by all analyses of the combined data. This clade received bootstrap support of 65% in AMP and 56% in AMP-P3, and posterior

probability support of 99 in ABY and 95 in ABY-P3. The taxa are clearly closely related as also suggested by Strong and Harasewych (2004) and clearly, as suggested in Bouchet and Rocroi (2005), "Heteropoda" does not warrant "subordinal" status.

4.6. Why are relationships within Hypsogastropoda difficult to resolve?

The general pattern of caenogastropod evolution suggested by the present analyses, in the context of the current understanding of morphology-based phylogenies and classifications, is that several basal clades, including Cyclophoroidea, Ampullarioidea, Viviparoidea, Campaniloidea and Cerithioidea were early offshoots from the lineage leading to the pre-eminently successful Hypsogastropoda. As the known members of the architaenioglossan groups are all non-marine, their marine ancestors are assumed to be extinct. The hypsogastropods diversified greatly to form multiple major lineages including the morphologically well-supported Neogastropoda and a group proposed here that includes most of its asiphonate members and Cerithiopsidae representing Triphoroidea.

Despite their diversification in the Mesozoic, few hypsogastropod lineages are definitely known to be present in the Palaeozoic. Pseudozygopleuroidea, while not monophyletic (Nützel, 1998), includes Palaeozoic members similar in shell features to some ptenoglossans (Bandel, 2002). At least two families that may belong to this group, Palaeozygopleuridae (questionably caenogastropods) and Pseudozygopleuridae, survived across the Permian–Triassic boundary (Bandel, 2002). Two recent families, Abyssochrysidae and Provannidae, are included in the "zygopleuroid group" along with the Pseudozygopleuridae by Bouchet and Rocroi (2005) but outside the hypsogastropods. However, the anatomy and, particularly, the sperm morphology (see Healy, 2000 for references and discussion) of both these recent taxa indicate that they are probably hypsogastropods. Although molecular data from Abyssochrysidae are not available, Provannidae are shown as the sister group to Littorinidae in 16S rRNA analyses (Fig. 6). If these families are indeed "zygopleuroid", Palaeozoic members of that group are presumably all or part Hypsogastropoda. There appear to be few other possible Palaeozoic hypsogastropods (Bandel, 1993, 2002; Nützel and Mapes, 2001), suggesting that the group's dominance of the marine gastropod fauna began after the Permian–Triassic extinction (Nützel, 2005). At least three extant hypsogastropod families (Rissoidae, Carinariidae and Aporrhaidae) were definitely present in the mid-Jurassic (Tracey et al., 1993; Bandel, 2002; Gründel, 1999), with a few others such as "Hydrobiidae", Lamelliphoridae and Epitoniidae appearing later in the Jurassic (Tracey et al., 1993).

The difficulty of resolving relationships within Hypsogastropoda could be due to a rapid early radiation of the group. This idea is consistent with the lengths of basal hypsogastropod branches in analyses of the combined data and 16S (particularly MP). These branches are short, as would be expected if the initial divisions within the group occurred quickly. The ecological vacuum following the Permian–Triassic extinction may have presented the opportunity for an explosive radiation of the Hypsogastropoda, although this is not yet supported by the available fossil record.

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