Molecular phylogeny of Vetigastropoda reveals non-monophyletic Scissurellidae, Trochoidea, and Fissurelloidea

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Abstract

A molecular phylogeny using cytochrome *c* oxidase subunit 1, Histone H3, and 18S rRNA, comprising 3038 aligned characters, was developed for 38 ingroup vetigastropods and two neritimorph outgroups. Vetigastropoda is diagnosed by the presence of bursicles and their presence and absence in putative vetigastropod groups is discussed. The molecular data support the monophyly of Fissurellidae, Haliotidae, Lepetodrilidae, Pleurotomariidae, and Peltospiridae; single exemplar taxa were available for Clypeosectidae, Cocculinidae, Neomphalidae, and Anatomidae. The monophyly of Scissurellidae s.l. (Scissurellinae plus Anatominae) is rejected and diagnosing radular characters of the family s.l. are identified as symplesiomorphies for Vetigastropoda. *Sukashitrochus* is part of Scissurellinae and not Anatominae. Neither Fissurelloidea (Fissurellidae, Clypeosectidae) nor Trochoidea (Trochidae, Turbinidae, Stomatellidae) is monophyletic. Previously cited trochoidean synapomorphies are identified as either being symplesiomorphies or affected by reductive heterochrony (paedomorphosis followed by hypermorphosis). Change in body size between major lineages is likely accomplished by peramorphy from a small ancestor and not by paedomorphy from a large ancestor. The colonization of the hydrothermal vent environment has occurred at least three times independently with some vent lineages not yet included in this study (Temnocinclinae, Sutilizoninae); vent colonization seems stochastic and not restricted to one particular geological time interval.

Key words: deep-sea, hydrothermal vent, heterochrony, paedomorphy, peramorphy, evolution, bursicles

Introduction

Understanding of the phylogeny of gastropods is still in considerable flux. The uncertainty of the placement and membership of various groups extends throughout the class (Lindberg *et al*. 2004). One of the clades generally recognized is Vetigastropoda, though membership of Vetigastropoda is still unsettled. There is a broad consensus that Haliotidae (abalone), Scissurellidae (little slit shells), Fissurellidae (key hole limpets), Pleurotomariidae (slit shells) and Trochoidea (top snails, turban snails) belong in Vetigastropoda. Some of the hydrothermal vent groups (Lepetodrilidae, Peltospiridae, Neomphalidae) and Seguenziidae have variously been included in Vetigastropoda or have been recognized at a higher level as distinct lineages (e.g., Salvini-Plawen and Haszprunar 1987, Hedegaard 1997, Ponder 1998, Sasaki 1998). One outstanding question relates to the diagnostic character used to define the taxon. The most appropriate diagnostic character is the presence of sensory structures on the gills, the bursicles (e.g., Haszprunar 1987, Salvini-Plawen and Steiner 1996, Sasaki 1998). Table 1 summarizes the distribution of bursicles among putative vetigastropod families. Bursicles are found in all traditional vetigastropod families (Haliotidae, Scissurellidae, Trochoidea, Fissurellidae) with some discussion about their presence in Pleurotomariidae (Haszprunar 1987, Sasaki 1998, Harasewych 2002). Bursicles have been confirmed in some hydrothermal vent gastropods (Peltospiridae, Clypeosectidae, Temnocinclinae) as well as in Seguenziidae,

with the notable exception of Lepetodrilidae and Neomphalidae (Table 1). *Cocculina* lacks a true gill and the condition of the bursicle is not applicable to them (Sasaki 1998). The systematics of 'Cocculiniformia'/'Cocculinida' is still far from settled and has recently been discussed (Strong *et al*. 2003); some recent molecular studies (Colgan *et al*. 2000, 2003) placed some cocculinids (*Notocrater*) in a derived position within traditional vetigastropods, which has also been suggested for *Caymanabyssia* (Pseudococculinidae) by Hedegaard (1997) based on shell mineralogy. Other molecular studies have found that *Cocculina* plus *Notocrater* is more closely related to patellogastropods than vetigastropods (Harasewych & McArthur 2000).

A number of phylogenies have been generated for Vetigastropoda both with morphological characters (Haszprunar 1988, Ponder & Lindberg 1996, Hickman 1996, Batten 1975, Sasaki 1998) as well as molecular data (Harasewych *et al*. 1997, Tillier *et al*. 1994, Colgan *et al*. 2000, 2003, Giribet & Wheeler 2002). The most striking feature of these phylogenies is their fundamental disagreement with one another (see Geiger & Poppe 2000: fig. 3). The disagreement transcends the problem of morphology versus molecules and encompasses all studies. The cause for such lack of commonality may lie in three factors: limited taxon sampling and exclusion of important lineages such as Scissurellidae; focus on hydrothermal vent taxa to the exclusion of shallow water representatives (Schwarzpaul 2002); and the limited number of characters sampled.

TABLE 1. Taxonomic distribution of bursicles in putative vetigastropod lineages.

Taxon	Bursicles	Reference
Fissurellidae		
Emarginula elongata	present	Haszprunar 1987
Diodora italica	present	Haszprunar 1987
Scutus sinensis	present	Sasaki 1998
Macroschisma dilatatum	present	Sasaki 1998
Pseudorimula marianae	present	Haszprunar 1989a
Fissurella volcano	present	Szal 1971†
Megathura crenulata	present	Szal 1971†
Puncturella cooperi	present	Geiger, pers. obs.
Clypeosectidae		
Clypeosectus delectus	present	Haszprunar 1989a
Clypeosectus curvus	present	Haszprunar 1989a
Haliotidae		
Haliotis lamellosa	present	Hasprunar 1987
Haliotis aquatilis	present	Sasaki 1998
Trochidae		
Gibbula varia	present	Haszprunar 1987
Tegula eiseni	present	Szal 1971
Calliostoma sp.	present	Szal 1971
Umbonium sp.	present	McLean 1986
Lirularia lirularia	present	McLean 1986
Turbinidae		
Astraea rugosa	present	Hasprunar 1987
Turbo stenogyrum	present	Sasaki 1998
Chlorostoma lischkei	present	Sasaki 1998
Broderipia iridescens	present	Sasaki 1998
Homalopoma sp.	present	Szal 1971
Stomatellidae		
Stomatia phymotis	present	Sasaki 1998
Phasianellidae		
Tricolia pullus	present	Haszprunar 1987
Pleurotomariidae		
Perotrochus caledonicus	present	Haszprunar 1987
Mikadotrochus beyrichii	**absent**	Sasaki 1998
Scissurellidae		
Incisura lytteltonensis	present	Bourne 1910
Sinezona rimuloides	present	Geiger 2004
Thieleella reticulata*	present	Sasaki 1998
Temnocinclininae		
Temnocinclis euripes	present	Haszprunar 1989a
Temnozaga parilis	present	Haszprunar 1989a
Seguenziidae		
Seguenzia sp.	present	Sasaki 1998, Haszprunar 1993
Peltospiridae		
Melanodrymia aurantiaca	present	Haszprunar 1989b
Neomphalidae		
Neomphalus fretterae	**absent**	cf. Sasaki 1998, Haszprunar 1988
Lepetodrilidae		
Lepetodrilus nux	**absent**	Sasaki 1998
Lepetelloidea		
Bathyphytophilus caribaeus	present	Haszprunar and McLean 1996
Bathyphytophilus diegensis	present	Haszprunar and McLean 1996
Cocculinidae		
Cocculina nipponica	inapplicable	Sasaki 1998

..... *continued*

TABLE 1 continued

Taxon	Bursicles	Reference
Outgroups		
Neritidae		
Nerita albicilla	**absent**	Sasaki 1998
Phenacolepadidae		
Cinnalepeta pulchella	**absent**	Sasaki 1998

* As *Anatoma* sp., see Bandel (1998) for description of species. † Szal (1971) only identified genera; the species are extrapolated for monotypic genera or genera with single species found in the geographic area studied by Szal (California).

We have attempted here to address some of these points by broad taxon sampling including both shallow water as well as hydrothermal vent taxa and through the use of three loci that are intended to help resolve the tree at various levels: cytochrome oxidase *c* subunit I for genus and species level resolution and Histone H3 plus 18S rRNA for family and ordinal level separation. We assumed the monophyly of Vetigastropoda in the sense outlined above to be correct, which has been recovered in the vast majority of studies. While we do not claim to resolve all matters of vetigastropod systematics, we can confirm here the validity of the approach (dense taxon sampling, multi-locus study) and highlight some areas that require additional attention. The latter includes issues with respect to more restricted within-family studies that presume the monophyly of the respective taxon.

Materials and Methods

Ethanol-preserved tissues for DNA sequencing were obtained from several sources (Table 2). DNA was extracted with Qiagen DNeasy kit (QIAGEN Inc. Chatsworth, CA). The PCR was performed with a profile of 94°C for 5 min, followed by 40–45 cycles of 94°C/30 s denaturation, 45–52°C/20 s annealing, and 70°C/45 s extension, and a terminal hold at 4°C. The primers of Colgan *et al.* (2000) for Histone H3, of Folmer *et al.* (1994: most taxa) and Burton (1990: for Haliotidae) for cytochrome oxidase *c* subunit I (COI), and those of Giribet *et al.* (1996) and Whiting *et al.* (1997) for 18S ribosomal RNA (18S) were used. PCR was carried out with AmpliTaq DNA polymerase (Perkin-Elmer, Foster City, CA), or Platinum *Taq* DNA Polymerase (Invitrogen Corp., Carlsbad, CA) for difficult templates. The reactions were electrophoresed on a 1.5% low melting point agarose gel and stained with ethidium bromide. Amplified bands were visualized and photographed under UV light, cut from the gel and DNA purified from the bands using the QIAquick gel extraction kit (QIAGEN Inc., Chatsworth, CA).

Using the same primers (1 µM rather than 10 µM solution) the short PCR fragments were cycle sequenced using Big Dye terminator ready reaction kits (Perkin-Elmer, Foster City, CA) and run on an ABI 377XL automated sequencer. Both strands were sequenced separately for each PCR fragment. The resultant chromatograms for both strands were reconciled in Sequencher (version 4.1.2. Gene Codes Corp., Ann Arbor, MI) to check basecalling. Histone H3 and

COI were aligned by eye and no ambiguities or gaps in the alignment were found; 18S was automatically aligned with Sequencher. Missing data were coded as ? and gaps were treated as new state (fifth base). Aligned nucleotide sequences were exported from Sequencher as NEXUS files. All parsimony analyses were performed using PAUP*, version 4.0b4a (Swofford 1998). One thousand replications of a heuristic search were run, using TBR branch swapping. The data were designated as equally weighted, following Källersjö et al., (1999) and Broughton et al., (2000). Decay indices (Bremer 1988) were calculated with PAUP* and TreeRot v.2 (Sorenson 1999). Two neritimorphs, Nerita polita (Neritidae) and Phenacolepas osculans (Phenacolepadidae: identification kindly confirmed by Takenori Sasaki), were designated outgroup taxa to root the tree and for character polarization. Consistency index (ci), retention index (ri), and rescaled consistency index (rc) were calculated without un-informative characters.

TABLE 2. Repository of voucher specimens used in this project, and fragments available for each taxon. SBMNH: Santa Barbara Museum of Natural History, California, USA. ZSM: Zoologische Staatssammlung München, Germany. LACM: Los Angeles County Museum of Natural History, California, USA. GenBank: sequences downloaded from GenBank. H3: Histone 3. COI: Cytochrome c oxidase subunit 1. 18S-1: aligned 1214 bp from 5' end of 18S rRNA gene. 18S-2: aligned 766 bp from 3' end of 18S rRNA gene.

Genus species Author, date	Family	Voucher	Locality	H3	COI	18S-1	18S-2
Nerita polita Linnaeus, 1758	Neritidae	SBMNH	Tahiti, French Polynesia	•	•	•	•
Phenacolepas osculans (Adams, 1852)	Phenacolepadidae	SBMNH	Moorea, French Polynesia	•	•		•
Clypeosectus sp.	Clypeosectidae	SBMNH 348725	Costa Rica	•	•		•
Cocculina messingi McLean & Harasewych, 1995	Cocculinidae	GenBank	—		•	•	•
Emarginula sp.	Fissurellidae	LACM	Louisiana, USA	•		•	•
Montfortula rugosa (Quoy & Gaimard, 1834)	Fissurellidae	SBMNH	Sydney, NSW, Australia	•		•	•
Fissurella virescens Sowerby I, 1835	Fissurellidae	SBMNH	Cocos Islands	•	•	•	•
Scutus unguis (Linnaeus, 1758)	Fissurellidae	SBMNH	Tathra, NSW, Australia	•	•	•	•
Diodora cayenensis (Lamarck, 1822)	Fissurellidae	GenBank	—		•	•	•
Diodora graeca (Linnaeus, 1758)	Fissurellidae	GenBank	—		•	•	•
Haliotis jacnensis Reeve, 1846	Haliotidae	SBMNH	Tonga	•	•		•
Haliotis asinina Linnaeus, 1758	Haliotidae	SBMNH	QLD, Australia	•	•	•	•
Haliotis pustulata Reeve, 1846	Haliotidae	SBMNH	Sinai, Egypt	•	•		•
Haliotis virginea Gmelin, 1791	Haliotidae	DNA extract	—	•	•	•	•
Haliotis kamtschatkana Jonas, 1845	Haliotidae	SBMNH (tissue sample)	British Columbia	•	•	•	•
Haliotis midae Linnaeus, 1758	Haliotidae	SBMNH	Cape Town, RSA	•	•	•	•
Haliotis varia Linnaeus, 1758	Haliotidae	SBMNH	—		•	•	•
Lepetodrilus elevatus McLean, 1988	Lepetodrilidae	SBMNH	13°N vent field	•	•	•	•
Lepetodrilus pustulosa McLean, 1988	Lepetodrilidae	SBMMH	13°N vent field	•	•	•	•
Lepetodrilus ovalis McLean, 1988	Lepetodrilidae	SBMNH	13°N vent field	•	•	•	•
Cyathermia naticoides Warén & Bouchet, 1989	Neomphalidae	SBMNH	13°N vent field	•	•	•	•
Perotrochus amabilis (Bayer, 1963)	Pleurotomariidae	LACM 152930 (animal) E. Garcia (shell)	Louisiana, USA	•	•		•
Perotrochus midas Bayer, 1965	Pleurotomariidae	GenBank	—		•	•	•
Peltospira delicata McLean, 1989	Peltospiridae	SBMNH	13°N vent field	•	•	•	•
Rhynchopelta concentrica McLean, 1989	Peltospiridae	SBMNH	13°N vent field	•	•	•	•
Depressigyra globulus (Bouchet & Warén, 1989)	Peltospiridae	GenBank	—		•	•	•
Scissurella "coronata-group-1"*	Scissurellidae	SBMNH	Huahine, French Polynesia	•	•	•	•
Scissurella "coronata-group-2"*	Scissurellidae	SBMNH	Moorea, French Polynesia	•	•		•
Scissurella "coronata-group-3"*	Scissurellidae	SBMNH	Moorea, French Polynesia	•	•		•
Sukashitrochus atkinsoni Tenison Woods, 1876	Scissurellidae	SBMNH	Sydney, NSW, Australia	•			•
Anatoma euglypta Pelseneer, 1903	Anatomidae	ZSM 20021328	Antarctica	•	•	•	•
Stomatellidae	Stomatellidae	SBMNH	Moorea, French Polynesia	•	•	•	•
Stomatella sp.	Stomatellidae	SBMNH	Sydney, NSW, Australia	•	•	•	•
Trochus niloticus Linnaeus, 1758	Trochidae	SBMNH	Moorea, French Polynesia	•	•	•	•
Gibbula zonata Woods, 1828	Trochidae	SBMHH	Cape Town, South Africa	•		•	•
Tegula eiseni (Jordan, 1936)	Trochidae	SBMNH	Los Angeles, CA, USA	•	•	•	•
Astraea undosa (Wood, 1828).	Turbinidae	SBMNH	Los Angeles, CA, USA	•	•	•	•
Turbo setosus Gmelin, 1791	Turbinidae	SBMNH	Huahine, French Polynesia	•	•	•	•
Homalopoma maculosa (Pease, 1868)	Turbinidae	SBMNH	Moorea, French Polynesia	•	•		•
Batillus cornutus (Lightfoot in Solander, 1786)	Turbinidae	GenBank	—		•		•

* The Scissurella coronata-group contains at least three species based on discrete differences in protoconch morphology. The species was described by Watson in 1886 with no mention of protoconch sculpture. The type material in the BMNH has decayed beyond recognition. Additional potential type material may be found in the National Museum of Wales at Cardiff, which may reveal which of the morphs is the true Sci. coronata and which are so far un-described species.

The molecular data were not partitioned into separate genes for analysis; all data were combined in a total evidence analysis, such that the resultant hypothesis best explains all the data (Barrett *et al.* 1991, Eernisse and Kluge 1993, Brower 1996, Nixon and Carpenter 1996, Kluge 1998) and because it has been shown that homoplasy or misleading signal takes a more complicated pattern than can be represented in process partitions such as separate genes (DeSalle and Brower 1997, Siddall 1997).

Voucher specimens with repository collections and available sequence fragments are listed in Table 2. Sequences have been deposited in GenBank under accession numbers AY923870–AY923982.

Results and Discussion

The data matrix with 40 taxa including two designated outgroups had 3038 characters (Histone H3: 376, COI: 682, 18S: 1980 aligned), of which 1119 were informative. Analyses of the individual fragments did not indicate any problems with contamination or paralogs (not shown). Two most parsimonious trees of 6853 steps ($ci = 0.489$, $ri = 0.579$, $rc = 0.283$) were found. All 1000 replications rapidly found those two trees, indicating a strong signal in the data, which is concordant with the rather high ci and ri values given the size of the data matrix. One of the two equally parsimonious trees is shown in Figure 1 as a phylogram. The only difference between the two topology was the placement of *Rhynchopelta* within Peltospiridae; the star symbol on the figure marks the node not found in the alternate topology.

Decay index values were generally high for nodes at the family-level and above (Vetigastropoda: 61, Peltospiridae: 32. Pleurotomariidae: 44, Fissurellidae: 19, Haliotidae: 18, Lepetodrilidae: 15, Scissurellidae s.s.: 12) with some notable exceptions (Neomphalidae-Peltospiridae: 1, Cocculinidae-Pleurotomariidae: 1). While no cut-off values or probability-equivalent values have been proposed for the decay index, we consider values >10 as providing strong support for the particular node.

Taxon sampling

Vetigastropoda is a sizable group with an estimated 2000 species (Scissurellidae s.l. 165 described, 50–100 undescribed [Geiger 2003]; 606 Fissurellidae [Geiger unpubl. data], 55 Haliotidae [Geiger and Poppe 2000], 1000 Trochoidea [estimate], 26 Pleurotomariidae [Anseeuw and Goto 1996], 12 Lepetodrilidae, 25 Peltospiridae, 3 Clypeosectidae [McLean 1989, 1992], 130 Cocculiniformia [Haszprunar 1998], 12 Neomphalidae, 139 Seguenzioidea [Hickman 1998]). We have included exemplar taxa from most major lineages, yet some were not available for study. The most significant gaps are the Seguenziidae, as well as the vent scissurellids Temnocinclidae and Sutilizonidae. Greater sampling density for Fissurellidae, Scissurellidae, and trochoidean lineages will also be desirable in the future. Despite these weak spots in taxon sampling, support indices indicate that the phylogeny is robust.

Selection of markers

We have chosen here two coding markers (Histone H3, COI) in order to minimize issues with alignment; these genes may be aligned through comparison of their translated amino acid sequences. COI is used for between species to between genera studies. For deeper divergences we used 18S rRNA, as it is most widely used locus for deep divergences.

Sistergroup relationships

Agreements with traditional classification. The ingroup Vetigastropoda, defined as the most inclusive group of bursicle-bearing gastropods, is strongly supported with a decay index value of 61. Two vent groups, Neomphalidae and Peltospiridae, are recovered, though their relative placement is poorly supported with a decay value of 1. The relationships within Peltospiridae are currently unresolved due to data conflict. Although the only neomphalid investigated (*Neomphalus fretterae*) lacks bursicles, we recommend retention of Neomphalidae in Vetigastropoda, as it may quite likely be sistergroup to Peltospiridae, in which all investigated species possess bursicles. Lindberg and Ponder (2001) discussed the problematic position of Neomphaloidea and also suspected that they are vetigastropods. Assessment of the condition of bursicles in other neomphalids and peltospirids is required to fully address this issue.

The remainder of Vetigastropoda is strongly supported with a decay index value of 31. The placement of *Cocculina* here is surprising. It was included to function as a more distant outgroup, but was placed in the ingroup. *Cocculina* and the two *Perotrochus* species (Pleurotomariidae) are poorly separated and may constitute a basal clade. The systematics of 'Cocculiniformia' is still unsettled (Strong *et al.* 2003). From a morphological perspective, the situation is complicated by the modified respiratory structures. 'Cocculinformia' is subdivided into Cocculinoidea (Cocculinidae and Bathysciadiidae) and Lepetelloidea (Lepetellidae, Cocculinellidae, Choristellidae, Pseudococculinidae, Osteopeltidae, and Pyropeltidae). Cocculinoidea do not have true gills, but instead possess a variety of respiratory structures ranging from ciliary tracts to pseudoplicate gills (Strong *et al.* 2003), which are not homologous with the bursicle-bearing gills in other Vetigastropoda, hence, the bursicle condition is inapplicable to them (Sasaki 1998: but see Ponder and Lindberg 1996, 1997, for alternative view and coding). Members of Lepetelloidea have been shown to have bursicles (Haszprunar and McLean 1996). Strong *et al.* (2003) discussed other systematic uncertainties including the monophyly of 'Cocculiniformia' and the various proposed affinities of cocculinid groups. Only a single member of 'Cocculiniformia' was included in this study, which fell within Vetigastropoda, not outside as would be expected if *Cocculina* would have an affinity with either Neritimorpha (Ponder and Lindberg 1996, 1997) or Patellogastropoda (Harasewych *et al.* 1997, Harasewych and McArthur 2000). There is strong support for the placement of *Cocculina* within Vetigastropoda (decay index value of 93). The

pseudococculinid *Notocrater* was also found in a derived position within traditional Vetigastropoda by Colgan *et al.* (2000, 2003), supporting the conclusion reached here that 'Cocculiniformia' are part of Vetigastropoda. Most authors thus far have viewed 'Cocculiniformia' as distinct from Vetigastropoda (e.g., Haszprunar 1998, Lindberg *et al.* 2004). The findings reported here only further highlight the problematic status of cocculiniforms. It is beyond the scope of this study to fully address either the monophyly or the placement of cocculiniform taxa.

The bursicle condition of Pleurotomariidae is also unsettled. Haszprunar (1987) noted the presence of bursicles in a *Perotrochus* species, while Sasaki (1998) could not find bursicles in a *Mikadotrochus*. In the most recent review of Pleurotomariidae, Harasewych (2002) noted the discrepancy without adding any new data. Inspection of two further *Perotrochus* species using a dissecting scope did not reveal bursicles, which in animals of that size are quite conspicuous (Geiger, pers. obs.); a detailed histological examination remains to be carried out. It is possible that bursicles should be added to the list of vetigastropod characters secondarily lost in Pleurotomariidae (such as fecal groove, epipodial sense organs, sensory papillae) as discussed by Harasewych (2002).

Haliotidae is monophyletic with a decay index value of 18, and despite limited taxon sampling as compared to other recent studies (Geiger 2000, Ozawa and Mouri 2004, Degnan *et al.* 2004), the geographic patterns recovered by all studies is also encountered here: An Indo-Pacific clade (((*H. jacnensis, H. varia*) *H. asinina*) *H. pustulata*) and deeper splits of the New Zealand (*H. virginea*), North Pacific (*H. kamtschatkana*), and South African (*H. midae*) species.

The three species of Lepetodrilidae are strongly supported as a monophyletic group (decay index value of 15), as well as Scissurellidae s.s. (decay index value of 12). Fissurellidae is also shown to be monophyletic, with a decay index value of 19. The subfamily Emarginulinae (*Emarginula, Montfortula, Scutus*) is monophyletic, but Fissurellinae (*Fissurella, Diodora*) is paraphyletic with respect to Emarginulinae. The latter condition is surprising as Emarginulinae, which possess an open slit, is generally accepted to be basal to Fissurellinae with an apical hole (e.g., McLean and Geiger 1998). The placement of Emarginulinae basal to Fissurellinae is further supported by the fossil record (Bandel 1998). There are approximately 40 genera in Fissurellidae, hence we have only sampled a small portion of the overall diversity and additional taxa may alter our present conclusion.

New insights. Some of our results do not agree with the classifications based on shell and animal morphology. We discuss these groups.

The systematics of Scissurellidae s.l. is currently being re-evaluated; Geiger (2003) provided a summary of the current knowledge and highlighted some of the problems. Recent studies have questioned the monophyly of Scissurellidae. Warén and Bouchet (2001) considered the vent subfamilies Temnocinclinae and Sutilizoninae more closely related to Lepetodrilidae, though they did not elaborate on their reasons. Schwarzpaul (2002), on the other hand, found Temnocinclinae and Sutilizoninae more closely related to Fissurelloidea (Fissurellidae and Clypeosectidae) than to Lepetodrilidae using morphological data on a larger array of hydrothermal vent "archaeogastropods" to the exclusion of shallow water representatives.

The position of genera has also been disputed. A phylogenetic analysis of shell characters by Geiger (2003) suggested that *Sukashitrochus* has no affinity with Anatomidae as had previously been suggested (McLean 1989), but is part of Scissurellidae s.s. This assessment is here confirmed with molecular data.

Rather surprisingly, *Anatoma euglypta* did not group anywhere near the remaining Scissurellidae. The placement cannot be explained by missing data, as all four fragments were available for this species. We ran an analysis in which *Scissurella*, *Sukashitrochus*, and *Anatoma* were constrained to be monophyletic, which resulted in a tree that was 40 steps longer than the most parsimonious one. The main differences between the constrained and the overall most-parsimonious tree in addition to the constrained monophyly of Scissurellidae, was the placement of *Clypeosectus* as sistergroup to Lepetodrilidae, along with minor changes (Neomphalidae- Peltospiridae monophyletic; placement of *Homalopoma* with remainder of Turbinidae). All indications point to the fact that Anatomidae plus Scissurellidae is not monophyletic. Furthermore, neither Anatomidae nor Scissurellidae forms a clade with Pleurotomariidae, hence the superfamily Pleurotomaroidea comprising Pleurotomariidae and its supposed miniature version, Scissurellidae (e.g., Brookes Knight *et al.* 1960), is not recovered here; Harasewych (2002) reviewed the various proposed affinities of Pleurotomariidae and membership of Pleurotomarioidea.

Scissurellidae is composed of six subfamilies: Scissurellinae, Anatominae, Depressizoninae, Larocheinae, Sutilizoninae, and Temnocinclinae. These subfamilies are distinguished based on overall shape and radular patterns (see Geiger 2003). The two that are most similar both in terms of shell as well as radular morphology are Scissurellinae and Anatominae, and these two in particular are shown here not to form a clade. It is highly questionable where the other scissurellid lineages belong; it is best to treat them as Vetigastropoda incertae sedis. Changes in rank for the subfamily taxa are premature, as it is not clear where the slit-less Larocheinae may be placed. The phylogenetic placement and inclusiveness with respect to their monophyly of the various vent slit limpets (Temnocinclinae, Sutilizoninae, Clypeosectinae) is unresolved. The monotypic *Depressizona* (Depressizoninae) is currently known from only three shells (Geiger 2004).

It is illustrative to reexamine with the benefit of hindsight the morphological characters that were used to unite the various scissurellid subfamilies, particularly the radular characteristics. Since Thiele (1931), common radular patterns are viewed as indicative for family level memberships in many gastropods. The unique characteristics of the radulae found in the various subfamilies of

Scissurellidae have in the past been regarded as minor modifications of the ground pattern of Scissurellidae (Rachidian tooth + lateral teeth + marginal teeth): Scissurellinae R + 5 + ∞ with fifth lateral tooth broadened; Anatominae R + 5 + ∞ with fifth lateral tooth elongated; Larocheinae, Sutilizoninae Temnocinclinae R + 3–4 + ∞

(Sasaki 1998, Geiger 2003). However, the radula with a serrated rachidian, supposedly diagnostic for Scissurellidae, is of questionable value as it is likely a common ancestral condition for vetigastropods based on an ontogenetic argument.
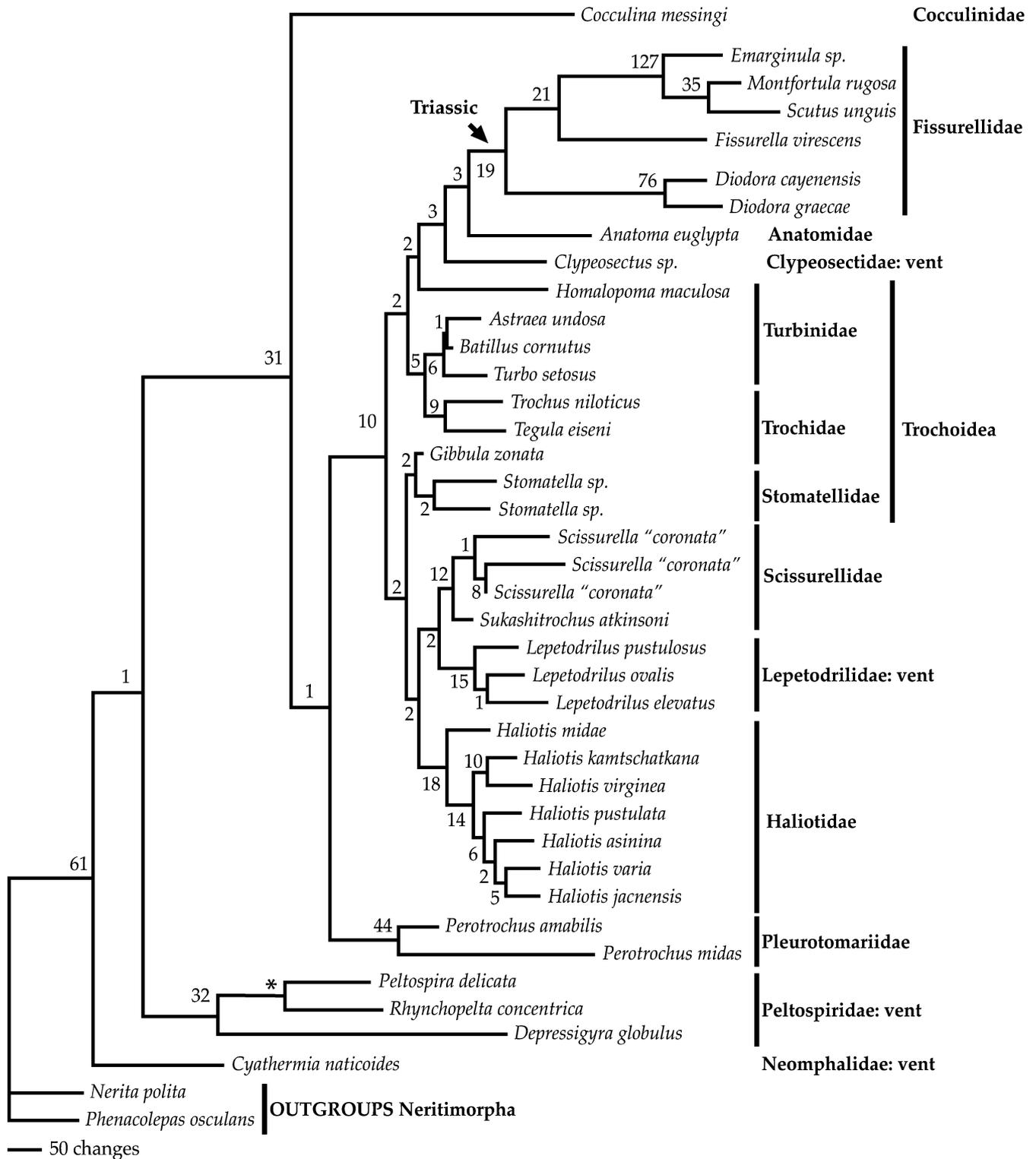
FIGURE 1. One of the two most parsimonious topologies, shown as a phylogram. The alternate topology differed in the placement of *Rhynchopelta* within Peltospiridae. That particular node is labeled with a star; it would collapse in a strict consensus tree. Tree length = 6853 steps, ci = 0.489, ri = 0.579, rc = 0.283. Numbers on nodes are Bremer indices.

The following discussion relies on the concepts of heterochrony; we use the terms as in Fink (1982) and McKinney and McNamara (1991). Radulae of juvenile trochoideans (Warén 1990) and abalone (Dinamani and McRae 1986), are very similar to scissurellid radulae. Accordingly, it is possible that ancestral vetigastropods had a radula very similar to those found in juvenile trochoideans and abalone, as well as adult scissurellids, and that the distinct radulae of large bodied forms (adult trochids, abalone) are peramorphically derived. This interpretation is diametrically opposed to Batten's (1975) argument of paedomorphic Scissurellidae. Recognizing that Scissurellidae is polyphyletic changes the interpretation of its radular characteristics to an overall constraint imposed by small size and minor lineage specific modification from an ancestral vetigastropod ground pattern: lateral tooth 5 broadened in Scissurellidae, elongated in Anatomidae; poor separation of lateral and marginal teeth in Larocheinae, Temnocinclinae, and Sutilizoninae. In this new light, the modifications of the lateromarginal field become paramount in diagnosing micro-vetigastropod families. The common serrated rachidian is recognized as a symplesiomorphy in Vetigastropoda, which is only retained in juveniles of large bodied forms due to peramorphic heterochrony. This hypothesis is testable by producing early juveniles of other vetigastropod lineages.

Trochoidea is not recovered as a monophyletic group, although it has been treated until recently as such (Hickman and McLean 1990, Hickman 1996). The characters used by Hickman and McLean (1990: 31) to diagnose Trochoidea can be grouped into two categories. General vetigastropod characters include papillate cephalic and epipodial tentacles, neck lobes, lateromarginal plate (shared with Fissurellidae) and shafts of the marginal teeth with a curved profile. Second, the organs of the pallial cavity complex are affected by heterochrony (paedomorphosis followed by varying degrees of hypermorphosis): lack of slit, emargination, or hole(s) and the ontogenetically un-developed right gill (Crofts 1937, evolutionarily reduced right gill of Hickman and McLean 1990). In typical vetigastropods the left gill forms before the right one and is always larger (e.g., Crofts 1929). The radula, on the other hand, is affected by peramorphy as demonstrated ontogenetically by Warén (1990). Trochoideans are therefore a good example that demonstrate decoupling of major organ systems (mantle cavity, radula) with respect to heterochronic processes.

In our results, the three large-bodied turbinids (*Astraea, Batillus, Turbo*) form a clade with the exclusion of the fourth traditional turbinid (*Homalopoma*). Two of the three trochids (*Trochus, Tegula*) form the sisterclade to the large-bodied turbinids, while the last trochid (*Gibbula*) is basal to two stomatellids (*Stomatella*). Our results should, however, be viewed with caution, as sampling of this large and diverse group has been limited and support indices at some of the nodes are as low as 2. Nevertheless, the broad implication of non-monophyletic Trochoidea is very clear. A similar conclusion has recently been reached by Williams and Ozawa (2004). Previous analysis with fewer molecular markers also placed the phasianellid *Tricolia* outside the trochoideans. *Tricolia* has long been suspected not to be a member of Trochoidea: Turbinidae, because *Tricolia* retains two shell muscles as opposed to one in the remainder of Trochoidea.

Fissurellidae and Clypeosectidae have been classified in the same superfamily Fissurelloidea by McLean (1989). The sistergroup relationship of the two families has not been recovered. Warén and Bouchet (2001) suggested a closer relationship of Clypeosectidae with Lepetodrilidae, a conclusion that is not supported here, unless monophyly of Scissurellidae plus Anatomidae is constrained.

Colonization of hydrothermal vents

The hydrothermal vent habitat has attracted much interest, including the origin of its fauna. By mapping the habitat of the Recent taxa sampled in this study, we can reconstruct the past colonization pattern of this habitat. We assumed that the vent snail lineages retained their habitat preference, i.e., did not switch their habitat over evolutionary time, and the lineage split was accompanied by shift in habitat preference. We can then place a minimum age on these vent taxa, using minimum ages of sistergroups supported by the fossil record.

Hydrothermal vents were colonized by at least four different vetigastropod lineages (Neomphalidae, Peltospiridae, Lepetodrilidae, Clypeosectidae) and at least at three different time periods. Our count is limited by some vent groups (Temnocinclinae, Sutilizoninae, Trochoidea) that could not be included in the present study. The absence of a vent-gastropod clade shows that the vent habitat was colonized repeatedly within vetigastropods.

Little & Vrijenhoek (2003) recently suggested that vents were only colonized fairly recently in the Eocene, following a global anoxic event. The Eocene diversification may be correct for lepetodrilids, but is highly doubtful for the other taxa sampled in this study. *Clypeosectus* as well as the two vent families Neomphalidae (*Cyathermia*) and Peltospiridae (*Peltospira, Rhynchopelta, Depressigyra*) are basal to the non-vent Fissurellidae; the latter has a fossil record reaching back to at least the Triassic (cf. Brookes Knight *et al.* 1960; Bandel 1998, Fig. 1). Given that these vent taxa are basal to Fissurellidae, the they must have originated at least in the Triassic (245–208 mya). The age of *Clypeosectus*, Neomphalidae and Peltospiridae, implied by their relative phylogenetic position with respect to Fissurellidae, makes a global anoxic event in the Eocene (55–34 mya) very unlikely, unless a radical habitat shift of the Recent vent families is added as an ad-hoc assumption. Even assuming some fairly significant rearrangements on the phylogeny presented here, a Mesozoic colonization of vents is extremely likely.

Acknowledgments

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