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TESTING RELATIONSHIPS AMONG THE VETIGASTROPOD TAXA: A MOLECULAR APPROACH

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ABSTRACT

Identifying the unambiguous members of Vetigastropoda and understanding the relationships among its families has been challenging. This study investigates the internal relationships among putative members of Vetigastropoda *sensu lato* (Fissurelloidea, Haliotoidea, Lepetelloidea, Lepetodriloidea, Pleurotomarioidea, Scissurelloidea, Seguenzioidea, Trochoidea, Angarioidea, Phasianelloidea, Neomphaloidea and Cocculinoidea) in a molecular phylogeny utilizing nearly 6 kb of molecular data from up to five nuclear and mitochondrial genes. Single-step parsimony-based and two-step maximum-likelihood analyses are employed as phylogenetic methods to analyse the data. Sequence data from all vetigastropod groups are included and in order to overcome shortfalls of previous vetigastropod analyses resulting from the under-sampling of outgroups, this study also includes broad outgroup representation. Fissurelloidea, Haliotoidea, Lepetodriloidea, Scissurelloidea, Seguenzioidea, Trochoidea, Angarioidea and Phasianelloidea formed a clade identified as Vetigastropoda *sensu stricto* united by morphological synapomorphies such as the presence of bursicles and epipodial sense organs. In contrast, Neomphalina, Cocculinoidea and Pleurotomarioidea fell outside Vetigastropoda *s. s.*, indicating a need to reexamine the classification of these clades as vetigastropods.

INTRODUCTION

Vetigastropoda, a clade of marine snails first recognized by Salvini-Plawen (1980), has been redefined multiple times in the 30 years since its introduction (see Fig. 1). Commonly accepted members of this clade are Fissurelloidea, Haliotoidea, Lepetelloidea, Lepetodriloidea, Pleurotomarioidea, Scissurelloidea, Seguenzioidea, Trochoidea, Angarioidea and Phasianelloidea, with Neomphaloidea and Cocculinoidea also included by some (Geiger & Thacker, 2005; Geiger, Nützel & Sasaki, 2008). Approximately 3,700 vetigastropod species are found exclusively in marine habitats, ranging from the shallow rocky intertidal to the deep sea. They display a variety of shell morphologies including limpet, conical and trochiform types and also may possess slits, fissures or holes in the shells. Internal morphology is also highly variable; vetigastropods possess both symmetrical and asymmetrical pallial structures such as ctenidia, osphradia and hypobranchial glands. Internal features including ctenidial anatomy, epipodial sensory structures and oesophageal characters have historically been used to define members of Vetigastropoda, but the characters utilized vary depending on the classification. Some authors consider the presence of bursicles as an unambiguous synapomorphy for Vetigastropoda *s. l.* (Geiger & Thacker, 2005; Geiger *et al.*, 2008) while others (Salvini-Plawen & Haszprunar, 1987; Ponder & Lindberg, 1997; Sasaki, 1998) identify epipodial tentacles with basal epipodial sense organs (ESO) as a synapomorphy for a more restricted Vetigastropoda (excluding Neomphalina and Cocculinidae). Further complicating matters, the presence of these characters in some groups such as Pleurotomariidae is uncertain and these characters are absent or reduced in some vetigastropod taxa, namely some fissurellids (ESO) and lepetodrilids (bursicles) (Salvini-Plawen, 1980; Haszprunar, 1987b, 1988c; Salvini-Plawen & Haszprunar, 1987; Salvini-Plawen & Steiner, 1996; Sasaki, 1998; Geiger & Thacker, 2005; Geiger *et al.*, 2008). Identifying the unambiguous members of this clade and understanding the internal relationships existing between putative vetigastropod groups, therefore, have been challenging.

Relationships within Vetigastropoda

The internal relationships of the major vetigastropod clades still varies significantly across analyses. Traditionally, vetigastropods with paired ctenidia (Pleurotomarioidea, Haliotoidea, Fissurelloidea and Scissurelloidea) were united as Zeugobranchia, but this relationship is rarely observed in phylogenetic analyses. Sasaki (1998) recovered this clade as sister to Trochoidea using morphological data, but other morphological and molecular phylogenetic analyses (Giribet *et al.*, 2006;

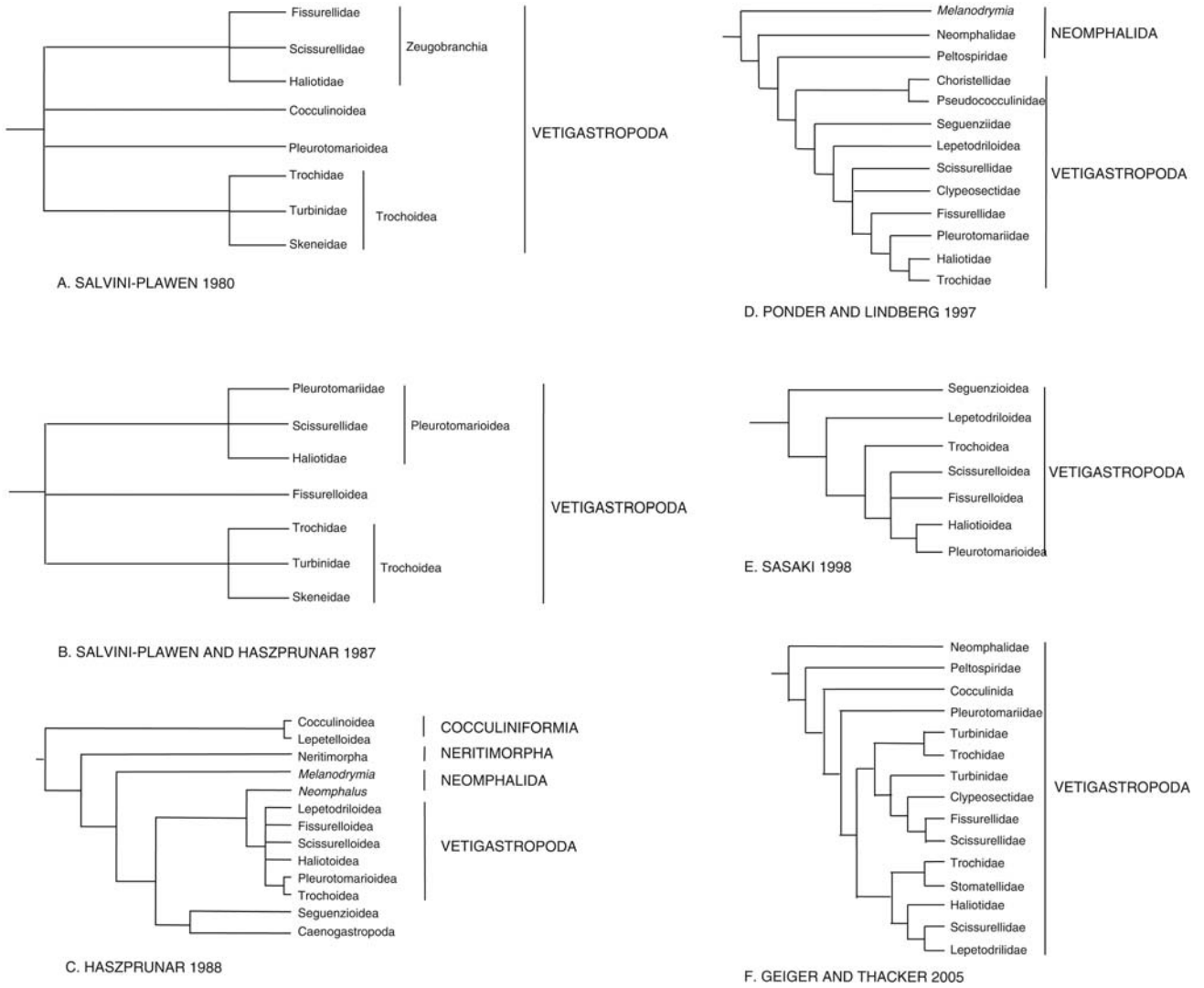

Figure 1. Phylogenetic hypotheses of vetigastropod relationships. Terminal taxa and classifications to the right follow the authors' original treatment. **A.** Tree reconstructed following Salvini-Plawen's (1980) discussion of molluscan phylogeny. **B.** Tree reconstructed from Salvini-Plawen & Haszprunar's (1987) discussion of Vetigastropoda and the systematics of streptoneurous Gastropoda (Mollusca). **C.** Tree redrawn from Haszprunar (1988a: fig. 5). **D.** Ponder & Lindberg (1997). **E.** Sasaki (1998). **F.** Geiger & Thacker (2005). **C–F** were originally published as cladograms. **F** is a molecular analysis, the rest are based on morphology.

Aktipis *et al*., 2008; Aktipis & Giribet, 2010) have not recovered Zeugobranchia. Pleurotomarioidea instead fall as sister to all other vetigastropods, with the remaining zeugobranch clades falling in different areas of the tree. The placement of other vetigastropod clades has varied according to the analysis used (see Geiger *et al*., 2008: fig. 12.3 for an overview), but one interesting clade repeatedly recovered in analyses is the sister relationship between Scissurellidae and Lepetodriloidea (Giribet *et al*., 2006; Aktipis, Boehm & Giribet, 2011). Overall, additional phylogenetic analyses are required before the internal topology of Vetigastropoda is understood.

Although recovering a stable vetigastropod internal phylogeny has been difficult, there have been many studies focusing on specific vetigastropod clades. The best-studied groups are Trochoidea (Hickman & McLean, 1990; Hickman, 1996; Hellberg & Vacquier, 1999; Williams & Ozawa, 2006; Williams, Karube & Ozawa, 2008; Williams *et al*., 2010) and Haliotidae, a monophyletic group comprised of a variety of ocean-basin-specific clades (Brown, 1993; Lee & Vacquier, 1995; Geiger, 2000; Estes, Lindberg & Wray, 2005; Streit, Geiger & Lieb, 2006). Although seguenzioids, skeneimorphs and other small vetigastropods are often underrepresented in phylogenetic analyses due to their small size and their habitation in deep-sea environments, a recent molecular phylogeny of vetigastropods included a large number of seguenzioids and skeneimorphs (Kano, 2008). This study highlighted the placement of Seguenzioidea within Vetigastropoda and indicated that morphological features such as the presence of the penis and seminal receptacle are derived conditions connected with small size and isolation in deep-sea habitats. Morphological characters utilized in a phylogenetic analysis of cocculinoids supported a monophyletic Cocculinoidea and Cocculinidae (Strong, Harasewych & Haszprunar, 2003). A molecular phylogeny of Pleurotomarioidea recovered the monophyly of this group, confirmed the three traditional pleutomariid genera (*Entemnotrochus*, *Perotrochus* and *Mikadotrochus*) and established a new genus, *Bayerotrochus* (Harasewych, 2002). In contrast, Fissurellidae are one of the least studied vetigastropod clades, since to date only one phylogenetic analysis using 22 morphological characters from 11 genera has been published (McLean & Geiger, 1998), and only a single detailed molecular analysis exists (31 species; Aktipis, Boehm & Giribet, 2011). In general,

although some large vetigastropod groups have been well studied, many will benefit from further investigation.

Phylogenetic obstacles

Vetigastropods have a geologic history dating back to the Cambrian/Ordovician boundary (Knight *et al.*, 1960; Frýda, Nützel & Wagner, 2008), but many stem group vetigastropods became extinct during the Permian/Triassic extinction event (Frýda *et al.*, 2008). Fossils that can be placed confidently within modern vetigastropod clades are first seen in Jurassic fossil assemblages (Geiger *et al.*, 2008). As noted by Rokas, Krüger & Carroll (2005), however, rapid extinction/radiation events occurring over the evolutionary history of a clade make the recovery of robust molecular phylogenies difficult. This problem is significant for vetigastropod phylogenetic reconstruction. Another major impediment to a better understanding of evolution within Vetigastropoda is limited sampling. Taxon sampling in most vetigastropod analyses focuses on mostly medium- and large-bodied, shallow-water taxa, leaving the many microgastropods and deep-sea vetigastropods undersampled. The study with the most diverse sampling to date sequenced up to three genes from 75 vetigastropods and included many minute taxa such as seguenziids, skeneimorphs and scissurellids (Kano, 2008). Aktipis & Giribet (2010) investigated the deep relationships between 'archaeogastropods' using increased genetic sampling and vetigastropods were naturally represented in that study. Taxon sampling for Vetigastropoda, however, was not comprehensive, as they focused on samples available for RNA extraction. Other recent vetigastropod analyses have failed to include a large number of taxa or otherwise focus on a particular group of vetigastropods in their sampling (Geiger & Thacker, 2005; Yoon & Kim, 2005; Williams *et al.*, 2008).

In addition to limited ingroup sampling, recent phylogenetic analyses of vetigastropods using molecular data do not include well-sampled outgroups. Although one study (Geiger & Thacker, 2005) using only two neritimorphs as outgroups identified Neomphalina and Cocculinidae as vetigastropods, other studies have used neomphalines and cocculinids as the only outgroup taxa (Kano, 2008; Williams *et al.*, 2008). The lack of additional outgroups makes determining the relationship of Neomphalina and Cocculinidae to vetigastropods difficult. Furthermore, when choosing outgroup taxa, including more than just the putative sister taxon of the ingroup through enhanced sampling of related taxa increases stability of the results (Nixon & Carpenter, 1993; Giribet & Ribera, 1998). In groups such as Vetigastropoda where the identity of the sister group is uncertain, it is especially necessary to have broad outgroup representation.

This phylogenetic study uses nearly 6 kb of molecular data from up to five nuclear and mitochondrial genes in order to elucidate evolutionary relationships between all putative vetigastropod clades. Sequence data were obtained for 82 vetigastropod, neomphaloid and cocculinoid ingroup species and 38 outgroup taxa representing Neritimorpha, Patellogastropoda, Apogastropoda and three additional molluscan classes. The variety of outgroup taxa utilized in this analysis allows the monophyly of Vetigastropoda to be thoroughly tested and informs the placement of problematic gastropod groups such as Neomphalina, Cocculinoidea and Pleurotomariidae. The five genes utilized in these analyses have been frequently used alone or in combination in many gastropod phylogenies: the complete 18S rRNA, partial 28S rRNA, the protein-encoding nuclear gene histone H3, the mitochondrial ribosomal 16S rRNA and the mitochondrial protein-encoding cytochrome *c* oxidase subunit I (e.g. Harasewych *et al.*, 1997, 1998; Colgan, Ponder & Eggler, 2000; Yoon & Kim, 2000; Colgan *et al.*, 2003, 2007; Geiger & Thacker, 2005; Yoon & Kim, 2005; Giribet *et al.*, 2006; Aktipis *et al.*, 2008; Kano, 2008; Williams *et al.*, 2008).

MATERIAL AND METHODS

Taxon selection and identification

Supplementary material, Appendix 1, contains locality information, collection details and museum voucher numbers for the specimens utilized in this study. Vetigastropoda were represented by 69 terminals from the superfamilies Pleurotomarioidea (two species), Fissurelloidea (11 species), Haliotoidea (four species), Scissurelloidea (two species), Seguenzioidea (six species), Lepetelloidea (nine species), Lepetodriloidea (four species), Trochoidea (23 species), Angarioidea (one species), Phasianelloidea (six species) and the family Areneidae (three species). In order to test the placement of suggested vetigastropod groups Neomphalina and Cocculinoidea, this study includes six and seven specimens representing each clade, respectively. Thirty-eight outgroup taxa representing the putative vetigastropod sister groups Patellogastropoda (12 species), Neritimorpha (10 species) as well as Apogastropoda (eight species) and the molluscan classes Scaphopoda (two species), Polyplacophora (two species) and Bivalvia (four species) are utilized in the analyses. For most specimens, sequences for all five genes were represented, but all taxa have sequences for ribosomal genes 18S rRNA and at least one other gene. In order to include specimens from some undersampled groups, sequences from 22 ingroup taxa were obtained from GenBank. Between two and four genes are represented for these terminals as these sequences were generated for other vetigastropod phylogenetic studies (Geiger & Thacker, 2005; Kano, 2008; Williams *et al.*, 2008). All remaining sequences were obtained from preserved tissues available to the authors, although some sequences had been generated for previous analyses (Giribet *et al.*, 2006; Aktipis & Giribet, 2010). In total, 196 novel sequences were generated for this study. Table 1 lists the species included in the phylogenetic analysis along with GenBank accession numbers for appropriate molecular loci sequenced. Specimen identification was conducted by the authors or with the assistance of Anders Warén (Swedish Museum of Natural History, Stockholm) and David Lindberg (University of California, Berkeley, CA, USA). Family and superfamily level classification follows Bouchet *et al.* (2005) and Williams *et al.* (2008).

Data collection

Genomic DNA was extracted from specimens preserved in 96% ethanol (EtOH), RNA*later* or frozen at −80°C, using the DNeasy™ Tissue Kit from QIAGEN. Following the same techniques and protocols described in Aktipis & Giribet (2010), five molecular loci were PCR-amplified from the genomic DNA, cleaned and directly sequenced using an automated ABI Prism® 3730 Genetic Analyzer in the Harvard University Bauer Center for Genomic Research: the complete 18S rRNA gene and portions of the 28S rRNA, histone H3 (H3), 16S rRNA and cytochrome *c* oxidase subunit I (COI). Chromatograms obtained from the automatic sequencer were viewed and 'contigs' assembled using the sequence editing software Sequencer™4.8. The assembled sequences were then edited in Se-Al Sequence Alignment Editor v2.0a11 for Mac OS X (Rambaut, 1996–2002), where external primer regions were removed from these edited sequences.

Phylogenetic reconstruction

Molecular data were analysed using a single-step phylogenetic approach using parsimony under the direct optimization

Table 1. List of genes sequenced with GenBank accession numbers and specimen voucher numbers.

Superfamily	Family	Terminal	DNA#	18S	28S	16S	H3	COI
Lepetelloidea	Lepetellidae	*Lepetella* sp.	DNA103283	GQ160778	GQ160626	GQ160673	GQ160712	GQ160746
Lepetelloidea	Lepetellidae	*Lepetella* sp.	DNA103284	GQ160779	GQ160627	GQ160674	GQ160713	
Lepetelloidea	Lepetellidae	*Lepetella* sp.	DNA103285	GQ160780	GQ160628	GQ160675	GQ160714	
Lepetelloidea	Lepetellidae	*Lepetella* sp.	DNA103286	GQ160781	GQ160629	GQ160676	GQ160715	
Lepetelloidea	Lepetellidae	Lepetellidae sp.	DNA103282	GQ160782	GQ160630	GQ160677	GQ160716	GQ160747
Lepetelloidea	Caymanabyssiidae	*Caymanabyssia* sp.	DNA103287	GQ160783	GQ160631	GQ160678	GQ160717	GQ160748
Lepetelloidea	Pseudococculinidae	*Notocrater* sp.	DNA103277	GQ160784	GQ160632	GQ160679	GQ160718	GQ160749
Lepetelloidea	Pyropeltidae	*Pyropelta* sp.	DNA102429	GQ160785	GQ160633	GQ160680	GQ160719	GQ160750
Lepetelloidea	Pyropeltidae	*Pyropelta* sp.	DNA102472	FJ977636	FJ977666	FJ977700	FJ977729	FJ977753
Pleurotomarioidea	Pleurotomariidae	*Bayerotrochus midas**	DNA100666 & (DNA102482)	AF120510	(FJ977668)	DQ093474	DQ093500	AY296820
Pleurotomarioidea	Pleurotomariidae	*Entemnotrochus adansonianus*	DNA100665	AF120509	FJ977667	AY377621	AY377774	L78910
Scissurelloidea	Scissurellidae	*Scissurella coronata*	GenBank	AM048637	AM048696			
Scissurelloidea	Scissurellidae	*Sinezona confusa*	Not available	AF120512	DQ279981	AY377620	AY377773	AF120631
Haliotoidea	Haliotidae	*Haliotis asinine*	DNA100432	GQ160786	GQ160634	GQ160681	GQ160720	
Haliotoidea	Haliotidae	*Haliotis tuberculata**	DNA101959 & (DNA100110)	GQ160787	GQ160635	GQ160682	GQ160721	(AY377729)
Haliotoidea	Haliotidae	*Haliotis corrugata*	DNA102585	FJ977644	FJ977675	FJ977707	FJ977736	FJ977760
Haliotoidea	Haliotidae	*Haliotis discus*	GenBank	AM048640	AM048700	AM048891		AM049335
Fissurelloidea	Fissurellidae	*Diodora dysoni*	DNA102140	FJ977638	FJ977669	FJ977701	FJ977730	FJ977754
Fissurelloidea	Fissurellidae	*Diodora cayenensis*	DNA101963	GQ160788	GQ160636	GQ160683	GQ160722	GQ160751
Fissurelloidea	Fissurellidae	*Diodora gibberula*	DNA101961	GQ160789	GQ160637	GQ160684	GQ160723	GQ160752
Fissurelloidea	Fissurellidae	*Tugali parmophoidea*	DNA101187	GQ160790	GQ160638	GQ160685	GQ160724	GQ160753
Fissurelloidea	Fissurellidae	*Emarginula octaviana*	DNA101219	GQ160791	GQ160639	GQ160686	GQ160725	
Fissurelloidea	Fissurellidae	*Emarginula variegata*	DNA103295	GQ160792	GQ160640	GQ160687	GQ160726	GQ160754
Fissurelloidea	Fissurellidae	*Puncturella* sp.	DNA102473	FJ977641	FJ977672	FJ977704	FJ977733	FJ977757
Fissurelloidea	Fissurellidae	*Cranopsis cucullata*	DNA102464	GQ160793	GQ160641	GQ160688	GQ160727	GQ160755
Fissurelloidea	Fissurellidae	*Fissurella barbadensis*	DNA102128	FJ977639	FJ977670	FJ977702	FJ977731	FJ977755
Fissurelloidea	Fissurellidae	*Lucapina suffusa*	DNA102017	FJ977642	FJ977673	FJ977705	FJ977734	FJ977758
Fissurelloidea	Fissurellidae	*Hemitoma octoradiata*	DNA102469	FJ977643	FJ977674	FJ977706	FJ977735	FJ977759
Trochoidea	Trochidae (Trochinae)	*Clanculus cruciatus**	DNA101960 & (DNA100664)	(AF120514)	GQ160642	GQ160689	GQ160728	(DQ093522)
Trochoidea	Trochidae (Trochinae)	Trochidae nov. gen.	DNA102413	GQ160794	GQ160643	GQ160690	GQ160729	GQ160756
Trochoidea	Trochidae (Cantharadinae)	*Gibbula cineraria*	DNA102440	FJ977645	FJ977676	FJ977708	FJ977737	AM049339
Trochoidea	Trochidae (Monodontinae)	*Monodonta australis*	GenBank	EU530075	EU530026			EU530127
Trochoidea	Trochidae (Stomatellinae)	*Pseudostomatella erythrocoma*	DNA102148	FJ977647	FJ977678	FJ977710	FJ977739	
Trochoidea	Trochidae (Umboniinae)	*Umbonium costatum*	GenBank	AM048646	AM048706			AM049341
Trochoidea	Turbinidae (Turbininae)	*Turbo castanea*	DNA102131	FJ977650	FJ977681	FJ977713	FJ977742	FJ977763
Trochoidea	Turbinidae (Turbininae)	*Lithopoma milloni*	DNA102403	GQ160798	GQ160647	GQ160693	GQ160733	GQ160758
Trochoidea	Turbinidae (Turbininae)	*Lithopoma phoebium*	DNA102144	FJ977649	FJ977680	FJ977712	FJ977741	FJ977762
Trochoidea	Turbinidae (Margaritinae)	*Margarites helicinus*	DNA102408	GQ160795	GQ160644		GQ160730	
Trochoidea	Turbinidae (Skeneindae)	*Dillwynella vitrea*	GenBank	AM048641	AM048701	AY163406		AM049336
Trochoidea	Turbinidae (Skeneinae)	*Protolira* sp.	DNA102432	GQ160803	GQ160652	GQ160698	GQ160738	
Trochoidea	Turbinidae (Tegulinae)	*Cittarium pica*	DNA102127	FJ977646	FJ977677	FJ977709	FJ977738	FJ977761
Trochoidea	Turbinidae (Tegulinae)	*Tegula fasciata*	DNA102139	GQ160801	GQ160650	GQ160696	GQ160736	GQ160761
Trochoidea	Calliostomidae	*Calliostoma antonii*	DNA102415	GQ160796	GQ160645	GQ160691	GQ160731	GQ160757
Trochoidea	Solariellidae	*Microgaza* sp.	DNA102418	GQ160797	GQ160646	GQ160692	GQ160732	
Trochoidea	Solariellidae	*Microgaza fulgens*	GenBank	EU530089	EU530040			EU530141
Trochoidea	Liotiidae (Liotiinae)	*Liotina semiclathratula*	GenBank	AB365305			AB365268	AB365220
Phasianelloidea	Collonidae (??)	*Cantrainea macleani*	DNA102474	FJ977648	FJ977679	FJ977711	FJ977740	
Phasianelloidea	Colloniidae (Colloniinae)	*Homalopoma picta*	DNA102419	GQ160799	GQ160648	GQ160694	GQ160734	GQ160759
Phasianelloidea	Colloniidae (Colloniinae)	*Collonia* sp.	DNA102406	GQ160800	GQ160649	GQ160695	GQ160735	GQ160760
Phasianelloidea	Colloniidae (Colloniinae)	*Collonista costulosa*	GenBank	AM048652	AM048713			AM049346

Continued

Table 1. *Continued*

Superfamily	Family	Terminal	DNA#	18S	28S	16S	H3	COI
Phasianelloidea	Phasianellidae (Phasianellinae)	*Phasianella ventricosa*	GenBank	AM048659	AM048720			AM049355
Phasianelloidea	Phasianellidae (Tricolinae)	*Tricolia pullus*	GenBank	AM048661	AM048722			AM049358
Angarioidea	Angariidae (Angariinae)	*Angaria formosa*	GenBank	AM048648	AM048708			AM049342
Angarioidea	Areneidae	*Arene cruentata*	GenBank	EU530060	EU530005			EU530110
Angarioidea	Areneidae	Areneidae sp.	DNA102414	GQ160802	GQ160651	GQ160697	GQ160737	GQ160762
Angarioidea	Areneidae	*Marevalvata* sp.	DNA102467	FJ977651	FJ977682	FJ977714	FJ977743	
??	??	*Bathyxylophila* sp. A	GenBank	AB365309			AB365281	AB365236
??	??	*Munditiella ammonoceras*	GenBank	AM048642	AM048702			AM049337
Seguenzioidea	Seguenziidae	*Ventsia tricarinata*	GenBank	AB365311			AB365290	AB365248
Seguenzioidea	Seguenziidae	*Fluxinella* sp.	GenBank	AB365312			AB365292	AB365250
Seguenzioidea	Chilodontidae (Chilodontinae)	*Bathymargarites symplector*	DNA101220	DQ093433	GQ160653	DQ093477	DQ093503	DQ093521
Seguenzioidea	Chilodontidae (Chilodontinae)	*Granata lyrata*	GenBank	EU530064	EU530010			EU530114
Seguenzioidea	Chilodontidae (Chilodontinae)	*Lischkeia alwinea*	GenBank	EU530066	EU530012			EU530115
Seguenzioidea	Calliotropidae (Calliotropinae)	*Calliotropis pagodiformis*	GenBank	AB365307			AB365275	AB365229
Seguenzioidea	Cataegidae (Cataeginae)	*Cataegis* sp.	GenBank	AB365308			AB365280	AB365235
Lepetodriloidea	Lepetodrilidae	*Lepetodrilus elevatus*	DNA100930	DQ093432	GQ160654	DQ093475	DQ093501	DQ093520
Lepetodriloidea	Lepetodrilidae	*Lepetodrilus pustulosus*	DNA101606	FJ977652	FJ977683	FJ977715	FJ977744	
Lepetodriloidea	Lepetodrilidae	*Gorgoleptis spiralis*	DNA102426	GQ160804	GQ160655	GQ160699	GQ160739	
Lepetodriloidea	Clypeosectidae	*Clypeosectus* sp.	GenBank	AY923874			AY923949	AY923913
Neomphaloidea	Peltospiridae	*Peltospira smaragdina*	DNA102425	GQ160806	GQ160657	GQ160701	GQ160741	GQ160764
Neomphaloidea	Peltospiridae	*Peltospira delicata*	DNA102420	FJ977653	FJ977684	FJ977716	FJ977745	FJ977764
Neomphaloidea	Peltospiridae	*Depressigyra globulus*	DNA101123	DQ093431	GQ160658	AF033689	DQ093499	DQ093519
Neomphaloidea	Melanodrymiidae	*Melanodrymia auratiaca*	DNA102421	GQ160805	GQ160656	GQ160700	GQ160740	GQ160763
Neomphaloidea	Melanodrymiidae	*Leptogyropsis inflata*	GenBank	AB365313			AB365300	AB365258
Neomphaloidea	Neomphalinae	*Cyathermia naticoides*	DNA100855 & DNA101607	DQ093430	FJ977685	DQ093472	DQ093498	DQ093518
Cocculinoidea	Cocculinidae	*Cocculina messingi*	DNA100663	AF120508	AY377696	AY377624	AY377777	AY377731
Cocculinoidea	Cocculinidae	*Cocculina* sp.	DNA101540	GQ160772	GQ160620	GQ160668		GQ160743
Cocculinoidea	Cocculinidae	*Cocculina subcompressa*	DNA102398	GQ160773	GQ160621	GQ160669	GQ160708	GQ160744
Cocculinoidea	Cocculinidae	*Cocculina* sp.	DNA103275	GQ160774	GQ160622	GQ160670	GQ160709	GQ160745
Cocculinoidea	Cocculinidae	*Cocculina* sp.	DNA103276	GQ160775	GQ160623	GQ160671		
Cocculinoidea	Cocculinidae	*Cocculina* sp.	DNA103281	GQ160776	GQ160624		GQ160710	
Cocculinoidea	Bathysciadiidae	*Bathysciadium* sp.	DNA102400	GQ160777	GQ160625	GQ160672	GQ160711	
Outgroups								
Patelloidea	Patellidae	*Patella laticostata*	DNA101186	GQ160768	GQ160614	GQ160664	GQ160704	
Nacelloidea	Nacellidae	*Cellana nigrolineata*	DNA100662	DQ093425	GQ160615	DQ093467		DQ093515
Lottioidea	Lottiidae (Lottiinae)	*Lottia asmi*	DNA102020	FJ977634	FJ977664	FJ977698	FJ977727	
Lottioidea	Lottiidae (Lottiinae)	*Lottia scabra*	DNA101969	GQ160769	GQ160616	GQ160665	GQ160705	
Lottioidea	Lottiidae (Lottiinae)	*Tectura fenestrata*	DNA102022	FJ977631	FJ977661	FJ977695	FJ977724	FJ977749
Lottioidea	Lottiidae (Lottiinae)	*Tectura testudinalis*	DNA101952	FJ977630	FJ977660	FJ977694	FJ977723	FJ977748
Lottioidea	Lottiidae (Lottiinae)	*Lottia gigantea*	DNA101968	FJ977632	FJ977662	FJ977696	FJ977725	FJ977750
Lottioidea	Lottiidae (Lottiinae)	*Lottia jamaicensis*	DNA102130	FJ977633	FJ977663	FJ977697	FJ977726	FJ977751
Lottioidea	Lottiidae(Patelloidinae)	*Patelloida pustulata*	DNA102143	GQ160770	GQ160617	GQ160666	GQ160706	GQ160742
Lottioidea	Acmaeidae (Pectiondontinae)	*Pectinodonta* sp.	DNA102399	GQ160771	GQ160618	GQ160667	GQ160707	
Neolepetopsoidea	Neolepetopsidae	*Eulepetopsis vitrea**	DNA100846 & (DNA101029)	DQ093427	(GQ160619)	DQ093468	DQ093495	DQ093516

Continued

Table 1. *Continued*

Superfamily	Family	Terminal	DNA#	18S	28S	16S	H3	COI
Neolepetopsoidea	Neolepetopsidae	*Paralepetopsis* sp.	DNA102471	FJ977635	FJ977665	FJ977699	FJ977728	FJ977752
Neritoidea	Neritidae	*Theodoxus fluviatilis*	DNA100668	AF120515	GQ160659	DQ093470		AF120633
Neritoidea	Neritidae	*Nerita funiculata**	DNA101206 & (DNA100938)	DQ093429	(GQ160660)	DQ093471	DQ093497	DQ093517
Neritoidea	Neritidae	*Nerita versicolor*	DNA102126	GQ160807	GQ160661	GQ160702		GQ160765
Neritoidea	Neritidae	*Nerita peloronta*	DNA102129	GQ160808	GQ160662	GQ160703		GQ160766
Neritoidea	Neritidae	*Nerita tessellata*	DNA102135	FJ977654	FJ977686	FJ977717		FJ977765
Neritoidea	Neritidae	*Neritina viriginea*	DNA102465	FJ977655	FJ977687	FJ977718		FJ977766
Neritoidea	Phenacolepadidae	*Bathynerita naticoidea*	DNA102209	FJ977658	FJ977690	FJ977721	FJ977747	FJ977768
Neritoidea	Neritidae	*Smaragdia viridis*	DNA102162	FJ977657	FJ977689	FJ977720	FJ977746	
Neritoidea	Neritidae	*Puperita pupa*	DNA102136	FJ977656	FJ977688	FJ977719		FJ977767
Helicinoidea	Helicinidae	*Helicina dysonia*	DNA101386	DQ093428	GQ160663	DQ093469	DQ093496	
Apogastropoda	Megalomastomidae	*Aperostoma palmeri*	MCZ DNA101457	DQ093435	DQ279983	DQ093479	DQ093505	DQ093523
Apogastropoda	Littorinidae	*Littorina littorea*	MCZ DNA101389	DQ093437	FJ977692	DQ093481	DQ093507	DQ095325
Apogastropoda	Philinidae	*Philine aperta**	DNA101268 & (DNA101778)	DQ093438	DQ279988	DQ093482	DQ093508	(GQ160767)
Apogastropoda	Ampullariidae	*Pomacea bridgesi*	DNA obtained from D. Colgan	DQ093436	FJ977693	DQ093480	DQ093506	DQ093524
Apogastropoda	Onchidiidae	*Onchidella* sp.	MCZ DNA101393	DQ093441	DQ279992	DQ093485	DQ093511	DQ093529
Apogastropoda	Amphibolidae	*Salinator solida*	DNA obtained from D. Colgan	DQ093440	DQ279991	DQ093484	DQ093510	DQ093528
Apogastropoda	Siphonariidae	*Siphonaria pectinata*	MCZ DNA100660	X91973	DQ279993	AY377627	AY377780	AF120638
Apogastropoda	Ellobiidae	*Ophicardelus ornatus*	DNA obtained from D. Colgan	DQ093442	DQ279994	DQ093486	DQ093512	DQ093530
Polyplacophora	Chitonidae	*Chiton olivaceus*	MCZ100157	AY377651	DQ279955	AY377605	AY377755	AY377716
Polyplacophora	Leptochitonidae	*Leptochiton asellus*	AToL000071/ 000316	AY377631	AY145414	AY377586	AY377734	
Scaphopoda	Dentaliidae	*Dentalium inaequicostatum*	DNA101022	DQ279935	DQ279959	DQ280026	DQ279999	DQ280015
Scaphopoda	Dentaliidae	*Antalis entalis*	AToL000061	DQ279936	AY145388	DQ280027	DQ280000	DQ280016
Bivalvia	Trigoniidae	*Neotrigonia margaritacea*	AToL000073	AF411690	DQ279963	DQ280034	AY070155	U56850
Bivalvia	Myidae	*Mya arenaria*	AToL000002	AF120560	AB126332	AY377618	AY377770	AY070140
Bivalvia	Nuculidae	*Nucula sulcata*	GenBank	DQ279937	DQ279960	DQ280029	DQ280001	DQ280017
Bivalvia	Nuculanidae	*Nuculana minuta*	GenBank	DQ279938	DQ279961	DQ280030	DQ280002	DQ280018

*Multiple specimens were used for the terminal taxon, and sequences and voucher numbers in parentheses are the alternative specimens.

method (Wheeler, 1996) implemented in the computer program POY v 4.0.2911 (Varón *et al*., 2008a) and v 4.1 (Varón *et al*., 2008b; Varón, Sy Vinh & Wheeler, 2010). Prior to phylogenetic analyses in POY and in order to increase analysis efficiency, long sequences were separated according to internal primer regions and secondary structure features following Giribet (2001). 18S rRNA was partitioned into 23 fragments, 28S rRNA into eight, 16S rRNA into seven and COI into five. Histone H3 was not partitioned and was utilized in the phylogenetic analysis as prealigned data due to lack of sequence-length variability. Six datasets were analysed independently, including each of the five molecular loci individually and all of the molecular data combined. Although two loci are protein encoding (COI and H3), all molecular data were examined on a DNA level. The POY analyses were run in a Linux cluster using 20 processors at Harvard University (odyssey.fas.harvard.edu). Processes were executed in parallel and preliminary tree space searched with random addition replicates. All analyses utilized subtree pruning and regrafting, and tree bisection and reconnection branch swapping followed by multiple rounds of tree fusing (Goloboff, 2002).

A parameter space of two variables was explored (Wheeler, 1995; Giribet, 2003) for each partition. A total of 10 parameter sets were analysed per partition; gap/change ratio values of 1, 2, 3 and 4, as well as transversion/transition ratios of 1 (transversions and transitions of equal weight), 2 (transversions twice the weight of transitions) and 4 (transversions four times the weight of transitions). To summarize, the 10 parameter sets utilized in this analysis were 111 (all transformations receive equal weights), 121, 141, 211, 221, 241, 411, 421, 441 and 3221. Under the 3221 parameter set, indel extensions were down-weighed in comparison to indel opening costs (indel opening three times the weight of extensions) with transversions and transitions given an equal cost of two (Varón & Wheeler, 2008). Congruence was used as an optimality criterion and we chose the parameter set that maximized the overall congruence among all molecular partitions (Wheeler, 1995), by employing a modified version of the incongruence length difference (ILD) metric (Mickevich & Farris, 1981; Farris *et al*., 1995).

Following this preliminary search and the identification of the most congruent parameter set, the shortest trees from all

Table 2. Tree lengths for the individual and combined datasets analysed under parsimony direct optimization at different parameter sets, with ILD values.

	18S	28S	16S	H3	COI	5-gene	ILD
111	7,467	14,215	7,457	1,959	8,610	41,474	0.04258
121	11,929	23,678	11,684	2,759	12,739	65,726	0.04469
141	20,462	41,752	19,616	4,287	20,590	112,126	0.04833
211	9,648	19,687	9,071	1,959	8,732	51,763	0.05150
221	16,072	33,983	14,622	2,759	12,915	84,899	0.05357
241	28,577	61,864	25,274	4,287	20,946	149,521	0.05734
411	13,347	29,008	11,475	1,959	8,810	68,816	0.06128
421	23,301	51,823	19,269	2,759	13,048	118,170	0.06745
441	42,837	96,404	34,422	4,287	21,209	215,061	0.07394
3221	**14,751**	**27,187**	**15,171**	**3,918**	**17,419**	**81,479**	**0.03722**

Individual datasets: 18S, 18S rRNA; 28S, 28S rRNA; 16S, 16S rRNA; H3, histone H3; COI: cytochrome *c* oxidase subunit. Combined dataset: five-gene = (18S + 28S + 16S + H3 + COI). Bold ILD and rows reflect the parameter set that minimizes incongruence among datasets. Single gene trees found under the optimal 3221 parameter set can be found in Supplementary material.

initial searches were pooled in a sensitivity analysis tree-fusing (SATF) search in order to more thoroughly search tree space (Giribet, 2007). Tree lengths for all analyses are summarized in Table 2. Nodal stability (Giribet, 2003) under the 10 different parameter sets was also explored (Fig. 2) using the program Cladescan (Sanders, 2010). Nodal support was measured for the combined dataset under the 'best-fit' parameter set (3221) measured using 500 bootstrap replicates.

A two-step phylogenetic approach was also followed, where the sequenced data were aligned with MUSCLE 3.7 (Edgar, 2004) using the EMBL-EBI online interface (http://www.ebi.ac.uk/Tools/muscle/). The resulting multiple sequence alignments (static homology) were concatenated using Phyutility (Smith & Dunn, 2008) with no ambiguously aligned regions removed. A model-based approach using maximum likelihood as an optimality criterion was performed in the program RAxML v. 7.04 using multiple partitions and gamma estimation (Stamatakis, Ludwig & Meier, 2005) on the CIPRES web portal v. 1.14 (http://www.phylo.org/). This program utilizes GTR, the 'best-fit' model for the combined dataset and all individual genes as selected by the Akaike Information Criterion in Modeltest v. 3.7 (Posada & Crandall 1998). In the RAxML analysis the data were partitioned according to the five genes utilized to incorporate rate heterogeneity among the multiple loci and a gamma distribution (Γ) was used to estimate the rate of variation among sites. The proportion of invariable sites (θ), however, was not estimated in the analysis as it is documented that there is a high correlation between the two parameters (Γ and θ) which can negatively affect the accuracy of the likelihood estimation (Sullivan, Swofford & Naylor, 1999). Nodal support for the resulting phylogenetic hypothesis was measured using 1,000 bootstrap replicates (Stamatakis, Hoover & Rougemont, 2008). All data files used in this analysis as well as output and standard error files can be obtained by request from SWA.

RESULTS AND DISCUSSION

Gastropoda and outgroup taxa

Combined analysis of all five genes – 18S, 28S, H3, 16S and COI – in POY under the parsimony optimal parameter set (3221; ILD 0.3722) yielded a single shortest tree of 81,479 steps found seven times over 495 independent replicates. When rooted with Polyplacophora, Gastropoda were monophyletic, although this topology was not recovered in a majority of bootstrap replicates (Fig. 2). The presumed vetigastropod outgroups Apogastropoda, Neritimorpha and Patellogastropoda were each monophyletic in this analysis with bootstrap support values of 55%, 86% and 100%, respectively, but Patellogastropoda appeared nested within Vetigastropoda. In the optimal parameter set, Apogastropoda and Neritimorpha fell outside a clade comprised of Neomphalina + Cocculinoidea and Vetigastropoda, including Patellogastropoda, but this topology did not receive high bootstrap support and was only recovered under two weighting schemes (Fig. 2).

The optimal tree recovered under the RAxML maximum-likelihood analysis for the 5,487 positions aligned with MUSCLE had a $-\log L$ score of 170,159.1065 (Fig. 3) and similar topology to the single shortest tree under the optimal parameter set in the parsimony direct optimization analysis (Fig. 2), except for the internal relationship of Vetigastropoda *s. s.* (as defined in this study). Gastropoda were monophyletic in 100% of bootstrap replicates and the clades Neritimorpha, Apogastropoda and Patellogastropoda were found in all bootstrap replicates. Neritimorpha and Apogastropoda fell outside a clade comprised of Neomphalina + Cocculinoidea and Vetigastropoda, including Patellogastropoda (89% bootstrap support).

Vetigastropoda sensu stricto

Vetigastropoda *s. l.* were not recovered as monophyletic in any analysis performed in this study. The historically recognized clade Zeugobranchia composed of vetigastropods possessing paired ctenidia (Pleurotomarioidea, Haliotoidea, Fissurelloidea and Scissurelloidea) was also not supported as all of these clades fell in different regions of the phylogenetic trees. Instead, the optimal parsimony and maximum-likelihood trees have a topology similar to that described in Aktipis & Giribet (2010): Neomphalina, Cocculinoidea and Pleurotomariidae fell outside a clade of Lepetelloidea, Patellogastropoda, plus all remaining vetigastropod groups. This clade of vetigastropods sister to Lepetelloidea + Patellogastropoda is identified as Vetigastropoda *s. s.* and includes the clades Fissurelloidea, Haliotoidea, Lepetodriloidea, Scissurellidae, Seguenzioidea, Trochoidea, Angarioidea and Phasianelloidea (76% bootstrap support in likelihood analysis; Fig. 3), but not Pleurotomarioidea. These taxa are united by recognized synapomorphies such as the presence of bursicles and ESO (Geiger *et al.*, 2008).

Although these results differ from those of other morphological and molecular analyses (Ponder & Lindberg, 1997; Sasaki, 1998; Geiger & Thacker, 2005; Kano, 2008; Williams *et al.*, 2008), many of those studies had limited outgroup sampling which prevents them from thoroughly testing the monophyly of Vetigastropoda. The topologies recovered in these analyses are similar to the ones shown in a recent molecular study using only 18S rRNA data (Yoon & Kim, 2005), although that study also had limited outgroup sampling. In studies using molecular or combined morphological and molecular data with increased outgroup sampling (Giribet *et al.*, 2006; Aktipis *et al.*, 2008), topologies similar to the one recovered in this study have been obtained, but these studies relied largely on the markers and methods utilized here. A more recent analysis, exploring additional nuclear protein-encoding genes is also highly congruent with the topology here presented (Aktipis & Giribet, 2010). The presence of bursicles and ESO in pleurotomariids, lepetellids and neomphalines varies depending on species examined and it is hypothesized that secondary loss of these characters is common, especially in small-sized species (Woodward, 1901; Fretter, 1964; Haszprunar, 1989a, b; Hickman, 1996; Harasewych, 2002; Geiger *et al.*, 2008). In contrast, these traits are well accepted for the members of Vetigastropoda *s. s.* (other than the need to confirm the presence of ESO in *Seguenzia*)

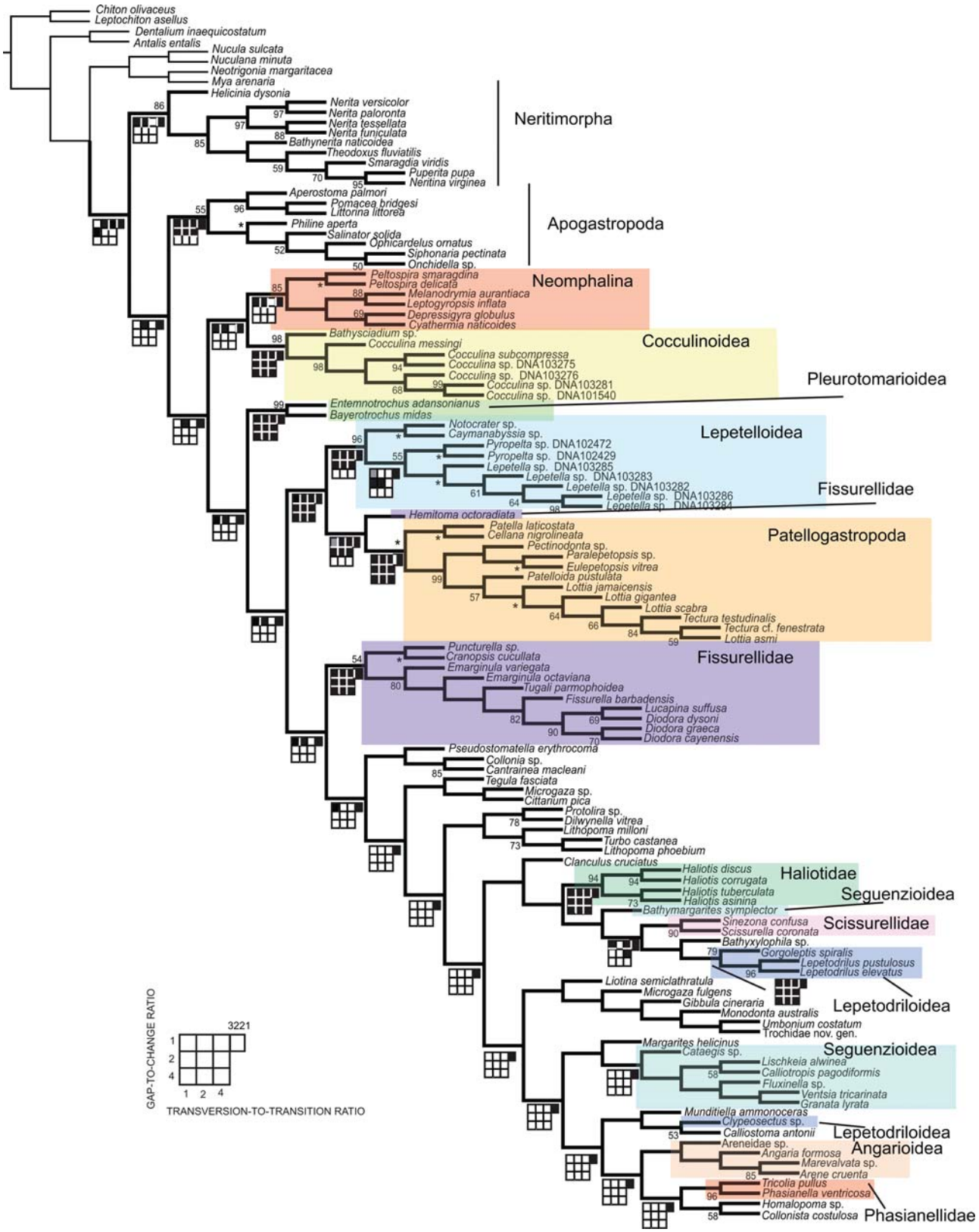

Figure 2. Cladogram based on the analyses of the five-gene combined dataset. Cladogram is single shortest tree (81,479 weighted steps) under the optimal parameter set (3221). See text for further details and Table 1 for family designations. Numbers on branches indicate bootstrap support values above 50% and asterisks indicate values of 100%. Graphic plots of sensitivity analyses (Navajo Rugs) indicate monophyly (black square) or nonmonophyly (white square) of nodes at different parameter sets (see legend in lower left corner). Bold branches indicate gastropod taxa. Labelled boxes around terminal taxa indicate clade designations: Neomphalina, Cocculinoidea, Pleurotomarioidea, Lepetelloidea, Fissurellidae, Patellogastropoda, Haliotidae, Seguenzioidea, Scissurellidae, Lepetodriloidea, Phasianellidae and Angarioidea. Trochidae, Turbinidae and Solariellidae are not highlighted. Independent gene trees available in Supplementary material.

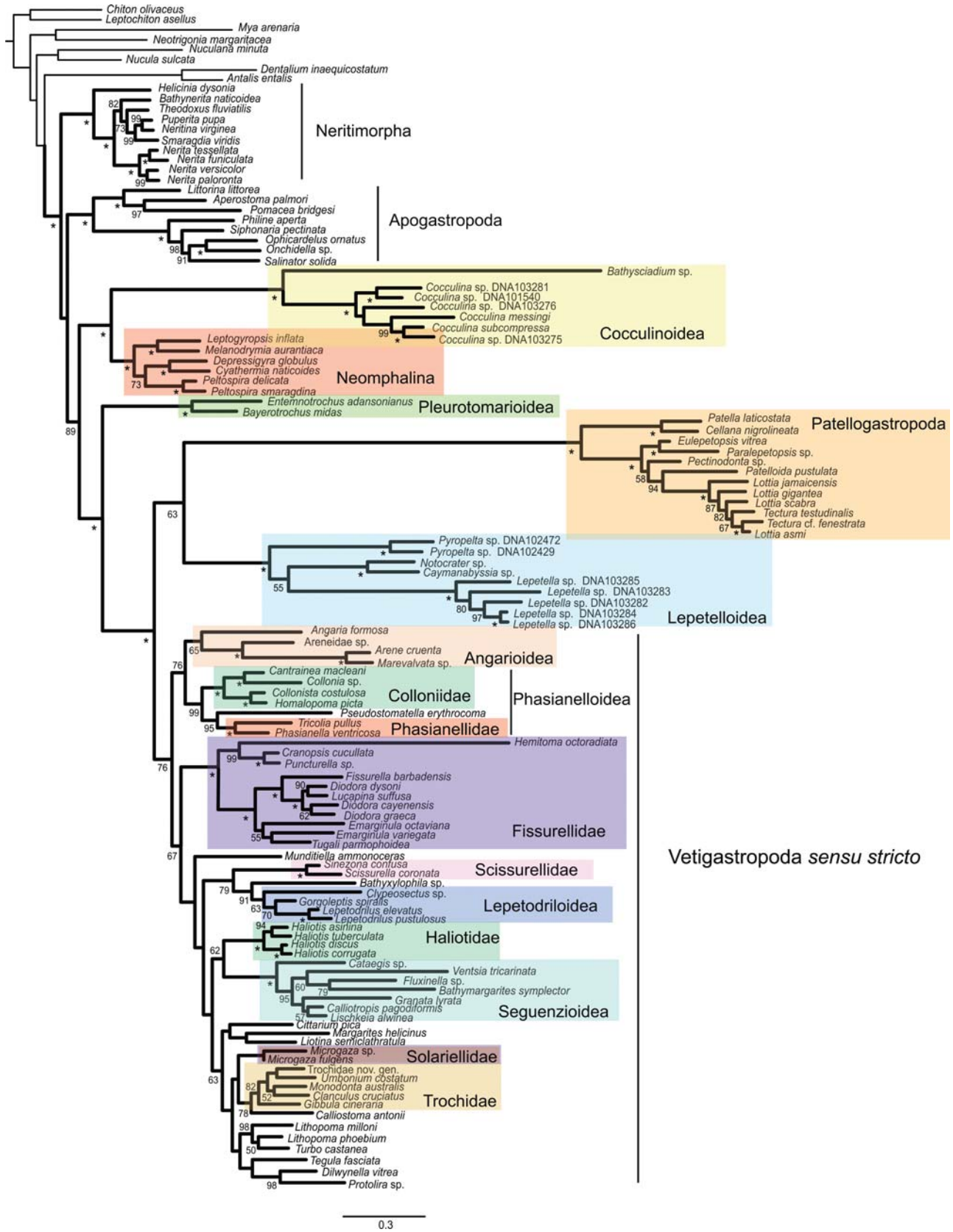

Figure 3. Maximum-likelihood tree based on five-gene combined molecular data ($-\log L = 170{,}159.1065$). Numbers on branches indicate bootstrap support values above 50% and asterisks indicate a bootstrap support value of 100%. Bold branches indicate gastropod taxa. Labelled boxes around terminal taxa indicate clade designations: Cocculinoidea, Neomphalina, Pleurotomarioidea, Patellogastropoda, Lepetelloidea, Angarioidea, Colloniidae, Phasianellidae, Fissurellidae, Scissurellidae, Lepetodriloidea, Haliotidae, Seguenzioidea, Solariellidae and Trochidae.

(Geiger & Thacker, 2005; Geiger *et al*., 2008). The internal relationships among many of the clades in Vetigastropoda *s. s*., however, varied depending on the analytical parameters (Figs 2, 3). These relationships will be addressed in the following sections along with the phylogenetic placement of previously proposed vetigastropod clades, Neomphalina and Lepetelloidea. As the placement of Pleurotomarioidea outside of Vetigastropoda *s. s*. was discussed by Giribet & Aktipis (2010), it will not be discussed further here.

Neomphalina

The placement of Neomphalina outside Vetigastropoda in this study corroborates the results presented by Aktipis & Giribet (2010). Additionally, morphological features shared with cocculinids, neritimorphs and other rhipidoglossate clades (Heß *et al*., 2008) reinforce the independence of Neomphalina from Vetigastropoda. The precise position of Neomphalina within the Gastropoda, however, remains uncertain. In this study, Neomphalina were monophyletic in the maximum-likelihood analysis (100% bootstrap support) and under the optimal (85% bootstrap support) and two other parameter sets in the parsimony direct-optimization analyses. In the seven other weighing schemes, *Peltospira delicata*, *P. smaragdina*, *Melanodrymia aurantiaca* and *Cyathermia naticoides* formed a clade separate from *Depressigyra* and/or *Leptogyropsis*.

Both the monophyletic Neomphalina and the reduced group of neomphalines fell sister to Cocculinoidea. Cocculinids, a group of small, white-shelled deep-sea limpets with a penis typically located close to the right cephalic tentacle, also lack ESO and bursicles, diagnostic features for Vetigastropoda that some neomphalines do not possess (Sasaki, 1998; Geiger *et al*., 2008; Heß *et al*., 2008). This clade possibly indicates a shared ancestor among some gastropods found in deep-sea reducing habitats; some cocculinids are found in reducing habitats such as decaying squid beaks, whale bone and wood falls in the deep sea (Haszprunar, 1998; Lindberg, 2008) while neomphalines are found exclusively in hydrothermal vents, hydrocarbon seeps and in sunken-wood habitats (Heß *et al*., 2008). Although more supporting evidence for this sister relationship is needed, the relationship between these two deep-sea groups provides some evidence for the hypothesis that wood and whale falls may serve as 'stepping stones' to sulphide-rich hydrothermal vent and hydrocarbon seeps for at least some gastropod species, as has similarly been suggested for other deep-sea molluscs (Smith *et al*., 1989; Gage & Tyler, 1991; Heß *et al*., 2008).

There has been much debate regarding the internal relationships among the hydrothermal-vent taxa since the discovery of these unusual deep-sea gastropods. Fretter, Graham & McLean (1981) noted the unusual anatomy of *Neomphalus*, but she was unable to identify its place among other extant gastropod clades. Warén & Bouchet (1989) united Neomphalidae and Peltospiridae in the superfamily Neomphaloidea, based on shared anatomical characters such as a ventricle uninterrupted by the rectum, the presence of a transverse pallial vein, smooth tentacles, the lack of nacre and the type of radula. Others, however, separated these two clades into the superfamilies Neomphaloidea and Peltospiroidea (Haszprunar, 1988a, c; Fretter, 1989; Sasaki, 1998) based on reproductive features. Members of Neomphaloidea have the left cephalic tentacles of males modified for copulation while most members of Peltospiroidea do not show sexual dimorphism (Heß *et al*., 2008). Males of *Melanodrymia* species, although generally placed within Peltospiroidea, have both cephalic tentacles modified for copulation (Haszprunar, 1989b) and Melanodrymiidae were therefore recognized as an independent family by Salvini-Plawen & Steiner (1996).

Furthermore, some morphological analyses have rendered Neomphalina polyphyletic (Haszprunar, 1988c; Ponder & Lindberg, 1997), while others using molecular, morphological or combined data have recovered a monophyletic Neomphalina (McArthur & Koop, 1999; Warén *et al*., 2003; Aktipis *et al*., 2008). A recent analysis has recognized three clades as families within a monophyletic Neomphalina: Neomphalidae, Melanodrymiidae and Peltospiridae (Heß *et al*., 2008). The results of our study support the monophyly of Neomphalina, but not that of these three families. Instead, only Melanodrymiidae (*Melanodrymia* + *Leptogyropsis*) is monophyletic, with *Peltospira delicata* + *P. smaragdina* and *Cyathermia naticoides* + *Depressigyra globulus* forming the other two groups. These three clades form a monophyletic Neomphalina in many analyses in this study, but the internal relationship among them varies under different analytical methods. Although the monophyly of Neomphalina is, therefore, increasingly supported, further studies incorporating additional neomphaline taxa must be performed in order to achieve a better understanding of the relationships among these extraordinary gastropods.

Lepetelloidea

Lepetelloidea, a group of small, white deep-sea limpets, were once recognized as sister to Cocculinoidea and placed within Cocculiniformia (Haszprunar, 1987a, 1988b; Salvini-Plawen & Haszprunar, 1987). However, some authors subsequently acknowledged that Cocculiniformia may not be monophyletic (Haszprunar, 1993; Salvini-Plawen & Steiner, 1996). Further phylogenetic analyses supported this suspicion (Ponder & Lindberg, 1997; Geiger & Thacker, 2005; Kano, 2008; Lindberg, 2008) and placed Lepetelloidea within Vetigastropoda *s. l*. Furthermore, members of the lepetelloid families Bathyphytophilidae, Pyropeltidae, Lepetellidae and Pseudococculinidae have bursicles, and other lepetelloids (Addisoniidae and Choristellidae) have internal skeletal rods in their ctenidia, a character also present in many vetigastropods (Haszprunar, 1993; Geiger & Thacker, 2005; Geiger *et al*., 2008). It now is accepted that lepetelloids are vetigastropods and that there were two independent radiations of small, white deep-sea limpets.

Lepetelloidea did not fall within Vetigastropoda *s. s*. in this study. Instead, Lepetelloidea were sister to Patellogastropoda in all analyses, and this clade was sister to Vetigastropoda *s. s*. (Figs 2, 3). While this relationship only received bootstrap support in the maximum-likelihood analysis (63%), it was monophyletic in a majority of parsimony analyses and therefore stable to parameter-set variation (Figs 2, 3). Patellogastropods and lepetelloids share morphological characters that are generally considered to be convergent such as limpet shell shape and a flat mantle cavity (Ponder & Lindberg, 1997; Lindberg, 2008). The close relationship between these groups, however, indicates that these morphological traits should be reexplored as possible synapomorphies.

Lepetelloidea were monophyletic in all but three parameter sets and received 96% bootstrap support in the parsimony analysis and 100% bootstrap support in the likelihood analysis. Four lepetelloid families were included in this study and they formed three main clades: Pyropeltidae, Lepetellidae and Pseudococculinidae + Caymanabyssiidae. The placement of Pyropeltidae and (Pseudococculinidae + Caymanabyssiidae) in relation to Lepetellidae varied depending on the analytical parameter used. Haszprunar (1988b) identified Lepetellidae as the most primitive lepetelloid lineage, while McLean & Haszprunar (1987) noted the similarities between Pyropeltidae and Pseudococculinidae. A later phylogenetic analysis of Lepetelloidea using morphological characters recovered three

main lineages: (Pyropeltidae (Lepetellidae, Bathyphytophilidae)); (Pseudococculinidae, Caymanabyssiidae); and ((Osteopeltidae, (Cocculinellidae, (Addisoniidae, Choristellidae)))) (Haszprunar & McLean, 1996). Of those lineages, the results of this study corroborate only the sister relationship between Pseudococculinidae and Caymanabyssiidae and cannot test the other relationships due to a lack of representative taxa. Furthermore, no molecular phylogenetic analysis to date has included species from more than two lepetelloid families, making it difficult to determine intrafamilial relationships among Lepetelloidea using molecular characters. In general, our understanding of Lepetelloidea will benefit from future analyses incorporating greater taxon representation among all families.

Vetigastropoda sensu stricto

Fissurelloidea: The fissurellid *Hemitoma* fell outside of all remaining fissurellids under all parameter sets in the parsimony analysis; this placement may be due to some sort of systematic error, such as long-branch attraction. Fissurellidae were, however, monophyletic in the maximum-likelihood analysis (100% bootstrap support) and Fissurellidae minus *Hemitoma* was one of the most stable clades among Vetigastropoda *s. s.* in the parsimony analyses (54% bootstrap support) (Figs 2, 3). In the maximum-likelihood analysis, the emarginulids *Hemitoma*, *Puncturella* and *Cranopsis* fell sister to the remaining fissurellids and, in the parsimony analysis, (*Puncturella* sp. + *Cranopsis cucullata*) was again sister to a clade of all remaining fissurellids. The remaining emarginulines *Tugali parmophoidea*, *Emarginula variegata* and *E. octaviana* fell outside a clade of a fissurellines and diodorines in all analyses, following Aktipis *et al.* (2011). Furthermore, Diodorinae was not monophyletic due to the placement of the fissurellid *Lucapina suffusa* sister to *Diodora dysoni*. As results from this study correspond to results published in Aktipis *et al.* (2011), we refer readers to that larger phylogenetic analysis of Fissurellidae for further discussion.

Lepetodriloidea: Clypeosectidae and Lepetodrilidae: Upon the discovery of a hydrothermal vent limpet with a slit in its shell, McLean (1989) erected the family Clypeosectidae and placed it sister to Fissurellidae within the superfamily Fissurelloidea based on shared excretory, reproductive and digestive system features (Haszprunar, 1989a). The authors, however, acknowledged that clypeosectids lack many key fissurellids features including the characteristic shell pits or pores (Haszprunar, 1989a; McLean, 1989). Furthermore, clypeosectids are not sister to fissurellids in phylogenetic studies (Ponder & Lindberg, 1997; Schwarzpaul & Beck, 2002; Geiger & Thacker, 2005; Aktipis *et al.*, 2008; Kano, 2008). Instead, they have frequently fallen sister to or in a clade with Lepetodrilidae (Schwarzpaul & Beck, 2002; Aktipis *et al.*, 2008; Kano, 2008) and other researchers have placed them within Lepetodriloidea based on morphological characters (Warén & Bouchet, 2001; Bouchet *et al.*, 2005). Both groups of hydrothermal vent limpets show external sexual dimorphism, with males having a penis and females having a genital groove and sperm receptaculum (Fretter, 1988; Haszprunar, 1989a). In this study, *Clypeosectus* was sister to the hydrothermal vent Lepetodriloidea in the maximum-likelihood analysis (63% bootstrap support), but its placement varied among trochiform taxa in the parsimony analyses.

Lepetodriloidea were initially described as comprised of the families Lepetodrilidae and Gorgoleptidae (Hickman, 1983; McLean, 1985). These families have similar shell characters and internal anatomy, but differ in the development of the shell muscles, the existence of an operculum and the composition and placement of the penis (Fretter, 1988; McLean, 1988). Although Bouchet *et al.* (2005) synonymized Gorgoleptidae with Lepetodrilidae, no Gorgoleptidae have been included in previous molecular phylogenetic analyses. In this study *Gorgoleptis spiralis* fell sister to *Lepetodrilus* in all analyses (79% bootstrap support in parsimony, 70% bootstrap support in maximum likelihood), but inclusion of additional gorgoleptid species is necessary for testing the synonymy of Gorgoleptidae with Lepetodrilidae suggested by Bouchet *et al.* (2005).

Scissurellidae: Scissurellids are either limpet-like or coiled vetigastropods with a slit or foramen in their shells, a shell lacking nacre and a symmetrical rhipidoglossate-type radula (Haszprunar, 1989a; McLean, 1989; Hickman, 1998a). Although six subfamilies have been placed within this clade (Scissurellinae, Anatominae, Depressizoninae, Larocheinae, Sutilizoninae and Temnocinclinae), only species from the subfamily Scissurellinae are included in our analyses. This study therefore cannot test the monophyly of Scissurellidae, but instead tests the placement of Scissurellidae within Vetigastropoda.

Scissurellidae were monophyletic in all analyses in this study (90% bootstrap support in parsimony analysis, 100% in maximum likelihood). Scissurellidae also formed a clade with *Bathyxylophila* and Lepetodriloidea in four different weighting schemes in the parsimony analysis (Fig. 2). In the maximum-likelihood analysis, Scissurellinae, *Bathyxylophila*, *Clypeosectus* and Lepetodriloidea formed a clade (79% bootstrap support). Based on morphological data, scissurellids are hypothesized to be derived from other taxa with slit shells such as fissurellids (Batten, 1975). Nevertheless, the affinity between scissurellids and lepetodrilids has been noted in other molecular analyses (Yoon & Kim, 2005; Giribet *et al.*, 2006) despite their differing morphological features. For example, most scissurellids possess symmetrically paired ctenidia and a slit or hole in their shell, while lepetodrilids lack a slit or hole and have a single left gill (Kano, 2008). This close relationship contradicts the traditional hypothesis of gastropod evolution that bases relationships on shared symmetry (or asymmetry) of pallial characters, and instead provides evidence for the plasticity of pallial characters (Sasaki, 1998; Kano, 2008).

In contrast, one similar feature observed in some lepetodrilids, scissurellids and skeneimorphs is the presence of modified spermatozoa for internal or semi-internal fertilization in the mantle cavity as well as modified penes in lepetodrilids and some skeneimorphs (Hodgson, 1995; Kano, 2008). Since most vetigastropods reproduce through external fertilization, the modifications of spermatozoa and copulatory structures are unique to some members of these groups. These reproductive features, however, may not be homologous due to the multiple origins of internal and semi-internal fertilization, along with associated reproductive structures, among vetigastropods. Instead, the modifications of these copulatory organs may represent convergent evolution among some groups of deep-sea and small vetigastropods (Kano, 2008).

Kano (2008) also recovered the skeneimorph *Bathyxylophila* in a clade with scissurellids and lepetodrilids. He argued that this species may be incorrectly classified as a skeneid and may, instead, be a member of the scissurellid subfamily Larocheinae. Similar to *Bathyxylophila*, larocheine scissurellids have slit-less shells and lack the right ctenidium; these two species also have similar shells and radula (Marshall, 1988, 1993; Kano, 2008). The results of this study corroborate this suggestion, with *Bathyxylophila* falling within a clade composed of scissurellids and lepetodrilids in both the likelihood and parsimony analyses.

Haliotoidea: Haliotoidea, commonly known as abalones, are one of the best-studied vetigastropod clades and this study, like others, supports the monophyly of Haliotidae (Brown, 1993;

Lee & Vacquier, 1995; Geiger, 2000; Estes *et al*., 2005; Streit *et al*., 2006). Its placement within Vetigastropoda *s. s*., however, varied depending on weighting schemes and optimality criteria (Figs 2, 3). Under the optimal parameter set in the parsimony analysis, Haliotidae were sister to a clade comprised of *Bathymargarites*, Scissurellidae, *Bathyxylophila* and Lepetodriloidea, but lacking significant bootstrap support. In the maximum-likelihood analysis, Haliotidae were sister to seguenziids (64% bootstrap support). The inconsistent placement makes it difficult to assess confidently the placement of Haliotidae within Vetigastropoda, but the family was always recovered amongst the clade of nonfissurellid vetigastropods.

The placement of Haliotidae among vetigastropods also varies in other phylogenetic analyses. In one analysis using morphological characters, Haliotidae were sister to Pleurotomariidae (Sasaki, 1998). In contrast, some molecular and morphological analyses have recovered Haliotidae as sister to Trochidae (Tillier *et al*., 1994; Ponder & Lindberg, 1997; McArthur & Harasewych, 2003; Yoon & Kim, 2005). Other molecular analyses have found Haliotidae to be closely related to Scissurellidae and Lepetodrilidae, with Fissurellidae and the chilodontid seguenzioids sometimes falling within this clade (Geiger & Thacker, 2005; Williams & Ozawa, 2006; Aktipis *et al*., 2008; Williams *et al*., 2008). Furthermore, different morphological features have been suggested linking Haliotidae with various vetigastropod clades. Haszprunar (1985) observed similar osphradia in haliotids and trochid, turbinid and phasianellid species, while Salvini-Plawen (1980) grouped haliotids with fissurellids and scissurellids due to similar shell structure, paired pallial organs and paired dorsoventral retractor muscles. Haliotids have also been grouped with pleurotomariids and scissurellids (Salvini-Plawen & Haszprunar, 1987), but Hickman (1984) rejected this relationship based on radular characters. It is clear that although Haliotidae belong within Vetigastropoda *s. s*., there remains much work to be done before the precise relationship of this clade to other vetigastropods can be conclusively determined.

Previous haliotid phylogenies have recovered internal clades corresponding to geographic areas, with major clades including a European–Australasian clade, as well as one including Indo-Pacific, New Zealand, North Pacific and tropical New World species (Lee & Vacquier, 1995; Geiger, 2000; Estes *et al*., 2005; Streit *et al*., 2006). The internal relationships obtained in this study reveal similar patterns; *H. discus* (Japan) + *H. corrugata* (California) represent a Pacific clade, while *H. asinina* (Thailand) + *H. tuberculata* (Mediterranean) form a Mediterranean and Indo-Pacific clade.

The 'trochiform' groups: Trochoidea, Angarioidea, Phasianelloidea and Areneidae: Trochoidea, Angarioidea, Phasianelloidea and the family Areneidae are vetigastropod groups of globally distributed marine species. Traditionally, these groups constituted the superfamily Trochoidea, based on morphological features such as radula, ctenidia, operculum, epipodium and characters of the foot and shell (Hickman & McLean, 1990). Bouchet *et al*. (2005) split the group into the two superfamilies Turbinoidea and Trochoidea, while a molecular analysis using three genes by Williams & Ozawa (2006) testing the monophyly of Trochoidea as defined by Hickman & McLean (1990) recovered a polyphyletic Trochoidea and Turbinidae. In a more recent molecular analysis, Williams *et al*. (2008) redefined Trochoidea, Trochidae and Turbinidae, establishing the vetigastropod superfamilies Angarioidea and Phasianelloidea. Trochoidea were redefined as composed of the families Trochidae, Turbinidae, Calliostomatidae, Liotiidae and Solariellidae (see Williams *et al*., 2008: table 1 for a summary of recent Trochoidea classifications). Williams *et al*. (2008) also noted that Areneidae may belong to Angarioidea, but that this needed further testing with additional taxa.

With the exception of the placement of *Pseudostomatella erythrocoma* (likely due to sequencing contamination or error and therefore disregarded in this discussion), results of the likelihood analysis in this study closely correspond with those of Williams *et al*. (2008, 2010). The relationships between the trochiform taxa in the parsimony analyses, however, varied across all analyses, with most trochiform species forming a large polytomy in the strict consensus of all the parsimony trees.

In the likelihood analysis, the trochiform species were located in two different sections of the tree. The monophyletic superfamilies Angarioidea (Areneidae + Angariidae) and Phasianelloidea formed a clade (76% bootstrap support) sister to all remaining vetigastropods (76% bootstrap support). Trochoidea were sister to the Haliotidae + Seguenzioidea clade, although without bootstrap support above 50%. Within Trochoidea, Solariellidae and Trochidae were monophyletic, with Trochidae receiving 78% bootstrap support. Turbinidae were not monophyletic.

This study supports the tentative placement of Williams *et al*. (2008) for Areneidae within Angarioidea. Similar to the results in Williams *et al*. (2008) and Williams & Ozawa (2006), Angarioidea and Phasianelloidea were found at the base of the vetigastropod *s. s*. clade. Unlike the result of Williams and Ozawa (2006) and Williams *et al*. (2008), however, in this study Angarioidea and Phasianelloidea were sister clades. Future analyses with increased sampling from Angariidae, Areneidae, Colloniidea and Phasianellidae should clarify the relationship between these groups. Complete discussion of Trochidae, Turbinidae, Calliostomidae and Sollaridae is beyond the reach of this study, as complete subfamily representation is lacking and few relationships were recovered with bootstrap support above 50%.

Seguenzioidea: Seguenziidae are a clade of small deep-sea vetigastropods with a single monopectinate ctenidium (with the exception of *Bathymargarites*, which has a bipectinate gill), a monotocardian heart and sexually dimorphic reproductive features (Ponder & Lindberg, 1997; Hickman, 1998b; Sasaki, 1998; Kano, 2008). As many morphological characters are similar to those found in caenogastropods, the placement of this clade was uncertain in early studies (Ponder & Lindberg, 1997). However, the presence of bursicles and ESO in some species and phylogenetic position in molecular analyses confirm their placement within Vetigastropoda (Ponder & Lindberg, 1997; Sasaki, 1998; Kano, 2008; Williams *et al*., 2008). In addition, Bouchet *et al*. (2005) proposed that chilodontids be placed with seguenziids in the superfamily Seguenzioidea and Kano (2008) recovered this affinity using molecular characters, noting that the penis in males may be the determining character for identifying seguenziids. Kano, Chikyu & Warén (2009) identified six monophyletic groups in Seguenzioidea: Seguenziidae, Chilodontidae, Calliotropidae, Cataegidae, *Spinicalliotropis* and the skeneimorph seguenzioids.

In the maximum-likelihood and optimal parameter set for the parsimony analysis, *Fluxinella*, the single confirmed Seguenziidae included in this study, fell among a clade of chilodontids, cataegids and the deep-sea skeneimorph *Ventsia* (Figs 2, 3). A similar clade was recovered by Kano (2008). Furthermore, Bouchet *et al*. (2005) tentatively identified *Cataegis* within Seguenzioidea, a classification later supported by Kano (2008). In both optimal trees in this study *Cataegis* is sister to the remaining group of *Fluxinella*, *Ventsia tricarinata* and *Granata lyrata* (Figs 2, 3), but this placement is sensitive to parsimony parameter set variation. Kano (2008) and Kano *et al*. (2009) also suggested that *Bathymargarites* may be a seguenziid

due to the presence of a penis. In this study, *Bathymargarites* was sister to *Fluxinella* in the maximum-likelihood analysis, but separate from *Fluxinella* in the parsimony analyses. Consequently, results regarding Kano's (2008) and Kano *et al.*'s (2009) proposed classification are inconclusive. However, the results from this study confirm that *Cataegis*, chilodontids and some skeneimorph species may actually belong to Seguenzioidea, as proposed earlier (Kano, 2008; Kano *et al.*, 2009).

CONCLUSION

Gastropoda were monophyletic in the maximum-likelihood analysis and under the optimal parameter set in the direct-optimization parsimony analysis, with Neritimorpha and Apogastropoda forming the sister group to Vetigastropoda and Patellogastropoda. However, Vetigastropoda *s. l.* were not monophyletic in any of the analyses. Instead, species from Fissurelloidea, Haliotoidea, Lepetodriloidea, Scissurellidae, Seguenzioidea, Trochoidea, Angarioidea and Phasianelloidea formed a clade identified as Vetigastropoda *s. s.* Gastropods in these groups are united by recognized vetigastropod synapomorphies such as the presence of bursicles and ESO. Of these vetigastropod clades, however, only Fissurelloidea, Haliotoidea and Lepetodriloidea received significant bootstrap support in both the parsimony and maximum-likelihood analyses, and were relatively stable to parameter-set variation. The vetigastropod clade Seguenzioidea, as defined by Kano (2008) and Kano *et al.* (2009), was recovered in some analyses and received high support in the maximum-likelihood analysis. Angarioidea, Phasianelloidea, Solariellidae, Trochidae and Trochoidea were also monophyletic in the maximum-likelihood analysis, with Angarioidea and Phasianelloidea forming a clade basal to the other vetigastropods, and Trochoidea falling sister to Haliotidae and Seguenzioidea.

Outside Vetigastropoda *s. s.*, Neomphalina and Cocculinoidea were recovered as sister groups in most analyses and the close relationship between these two groups of deep-sea taxa provides evidence for theories suggesting common ancestry between some gastropods populating deep-sea reducing habitats. Pleurotomarioidea also fell outside of Vetigastropoda *s. s.* Additionally, Lepetelloidea and Patellogastropoda were sister clades in all analyses and are placed as sister to Vetigastropoda *s. s.*

This study highlights the complicated relationships existing among groups classified as Vetigastropoda *s. l.* Although some results, such as the sister relationship between Lepetelloidea and Patellogastropoda and the exclusion of Pleurotomarioidea from Vetigastropoda *s. s.*, contradict those suggested by morphological analyses, their recovery in multiple analyses in this study as well as in other published molecular studies calls attention to the need for further investigation of these placements. Additionally, results of a recent multigene phylogenetic analysis of Heterobranchia (Dinapoli & Klussman-Kolb, 2010) also contradict traditional hypotheses of deep gastropod relationships. These results provide further evidence that some hypotheses of gastropod deep relationships may not be as robust as previously thought. Increased utilization of multiple molecular markers may help to clarify such relationships across many gastropod groups.

Furthermore, previous studies have shown that incorporation of morphological characters with molecular data leads to the stabilization of clades in phylogenetic analyses. Such characters should also be utilized in combination with molecular character in future vetigastropod phylogenies. Ultimately, although this study utilizes the greatest overall variety of vetigastropods and closely related species among existing molecular studies, it still lacks full representation of many understudied or obscure groups such as Cocculinoidea, Lepetelloidea, Pleurotomarioidea, Scissurelloidea, Seguenzioidea and Neomphaloidea and therefore cannot fully elucidate the composition of Vetigastropoda *s. s.* and related clades. Including more species from these groups in future analyses is likely further to clarify relationships among the members of these important gastropod groups.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Molluscan Studies* online.

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