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Gastropod phylogeny based on six segments from four genes representing coding or non-coding and mitochondrial or nuclear DNA

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Abstract

Significant differences remain between gastropod phylogenetic hypotheses based on morphological and molecular datasets. We collected additional data from three gene segments (*28S* rDNA expansion region *D1* (36 taxa plus two from GenBank), cytochrome *c* oxidase subunit 1 (35 species plus one from Genbank) and small nuclear RNA *U2* (24 species)). These were combined with data available for the same species for histone *H3* and two other segments of *28S* rDNA. Analyses of these data using cladistic, maximum likelihood or Bayesian methodologies were conducted in an attempt to resolve some of the differences between current hypotheses of gastropod relationships based on morphological and molecular data. The results were of particular interest in four areas. (*1*) Patellogastropoda in most analyses are included in a derived clade with some Vetigastropoda. In an analysis with *Nautilus* as the sole outgroup, transversions weighted threefold as costly as transitions and, with third codon position data ignored, Patellogastropoda are excluded from an otherwise monophyletic Gastropoda. (*2*) Cocculiniformia was never monophyletic in our analyses, although this possibility is not statistically rejected. (*3*) *Nerita*, the only representative of Neritopsina in this dataset, is placed anomalously in most analyses, but is, in a few cases, shown as a sister-group to the Apogastropoda, in accord with some morphological hypotheses. (*4*) Heterobranchia is rarely monophyletic in our analyses owing to the variable placement of the architectonicoid *Philippea*. This genus, even judged by the high levels of divergence within Heterobranchia, has undergone extreme rates of substitution. The Euthyneura is invariably monophyletic and nearly always included in a clade with the valvatoidean *Cornirostra* as its sister-group.

Additional keywords: DNA sequence, heterobranch, multiple gene, Neritopsina, patellogastropod.

Introduction

Recent phylogenetic investigations of gastropods have used a variety of different datasets, ranging from shell morphology (including fossils, protoconch morphology and shell structure; Bandel 1997; Frýda, 1999; Wagner 2002; for a review, see Wagner 2001), ultrastructure (Ponder and Lindberg 1997; Künz and Haszprunar 2001), development (Freeman and Lundelius 1992; van den Bigelaar 1996; van den Bigelaar and Haszprunar 1996; Lindberg and Guralnick 2001), pallial cavity structures (Haszprunar 1988*a*; Ponder and Lindberg 1996, 1997; Lindberg and Ponder 2001), overall morphology (Haszprunar 1988*a*; Ponder and Lindberg 1996, 1997; Barker 2001), mitochondrial gene order (Kurabayashi and Ueshima 2000*a*, 2000*b*) and molecular sequences.

These studies show general agreement for the recognition of five major groups within Recent gastropods: (*1*) the Patellogastropoda (or Docoglossa), being the true limpets; (*2*) the Vetigastropoda (trochids, haliotids, fissurellids etc.); (*3*) the Neritopsina (or Neritimorpha), the nerites and relatives; (*4*) the Caenogastropoda (most of the 'mesogastropods', including the architaenioglossan taxa, and the neogastropods); and (*5*) the Heterobranchia (or Heterostropha as used by some palaeontologists (Bandel 1997),

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whereas others (Ponder and Warén 1988) use Heterostropha as a paraphyletic subgroup within the Heterobranchia). The Caenogastropoda and Heterobranchia form the Apogastropoda in Ponder and Lindberg's (1997) sense. This taxon was originally introduced by Salvini-Plawen and Haszprunar (1987) to include the caenogastropods and only the basal heterobranchs, or 'allogastropods', in their usage, making it paraphyletic. An additional extant group, the Cocculiniformia (for a review, see Haszprunar 1998), is sometimes recognised and placed near the base of the gastropods.

The first three groups and the Cocculiniformia comprise the paraphyletic 'archaeogastropods', whereas the first four groups comprise the paraphyletic 'prosobranchs'. The Heterobranchia, as first recognised by Haszprunar (1985*a*), contains the euthyneurans, a grouping of two taxa previously given subclass rank (the opisthobranchs and the pulmonates), as well as a number of assumed more 'primitive' members, all of which were included within the 'prosobranchs' at some time. These latter groups comprise the paraphyletic 'Heterostropha' (*sensu* Ponder and Warén 1988) or 'Allogastropoda' (Haszprunar 1985*b*; Bandel 1997).

Hickman (1988) advocated restricting the usage of the name Archaeogastropoda to the Vetigastropoda, whereas some (Bandel 1982, 1997; Bandel and Geldmacher 1996) formally use the same name for a group encompassing the patellogastropods together with the vetigastropods and the Cocculiniformia (i.e. excluding the Neritopsina), based primarily on their possession of a similar larval shell. However, the artificial nature of such a grouping is recognised (Frýda 1999). In stark contrast with this view is the idea that the patellogastropods represent the sister-taxon to the rest of the gastropods, a result suggested by most recent morphological studies (Golikov and Starobogatov 1975; Graham 1985; Haszprunar 1988*a*; Ponder and Lindberg 1996, 1997; Sasaki 1998; Barker 2001). It was on this basis that Ponder and Lindberg (1997) introduced the Eogastropoda, to encompass the Patellogastropods and their assumed coiled ancestors, and Orthogastropoda for the remainder of the gastropods.

Gastropods date from the early Cambrian, although there is considerable speculation over which of the small univalve fossils in that period represent torted gastropods or monoplacophorans. For example, Parkhaev (2001) assigns Helcionelloidea *s.s.* to Gastropoda, whereas members of this group are often considered to be monoplacophorans (Wen 1990; Peel 1991).

Only recently have individual molecular investigations of gastropod phylogeny included examples from nearly all critical taxa in the class (McArthur and Koop 1999; Colgan *et al.* 2000). The genes now available for a broadly representative set of taxa include histone *H3* (Colgan *et al.* 2000) and four regions of the *28S* rDNA gene. These are the *D1* (Tillier *et al.* 1992, 1994; McArthur and Koop 1999), *D6* (Rosenberg *et al.* 1997; McArthur and Koop 1999) and *D4–5* ('28SA' in Colgan *et al.* 2000) expansion regions and a section near the *D7* area including a new expansion region ('28SB' in Colgan *et al.* 2000). With some notable exceptions, major groups are supported or weakly contradicted in molecular investigations. Nevertheless, the most comprehensive analysis (Colgan *et al.* 2000) is based on relatively short sequences (less than 900 aligned base positions), so it is not surprising that there are still disagreements between molecular and morphological (Haszprunar 1988*a*; Ponder and Lindberg 1997) assessments of gastropod relationships (Fig. 1). The collection of more molecular and morphological data offers the best chance of resolving such disagreements, although data from fossils are also being examined rigorously and may well assist in further elucidating gastropod phylogeny (Wagner 2001).

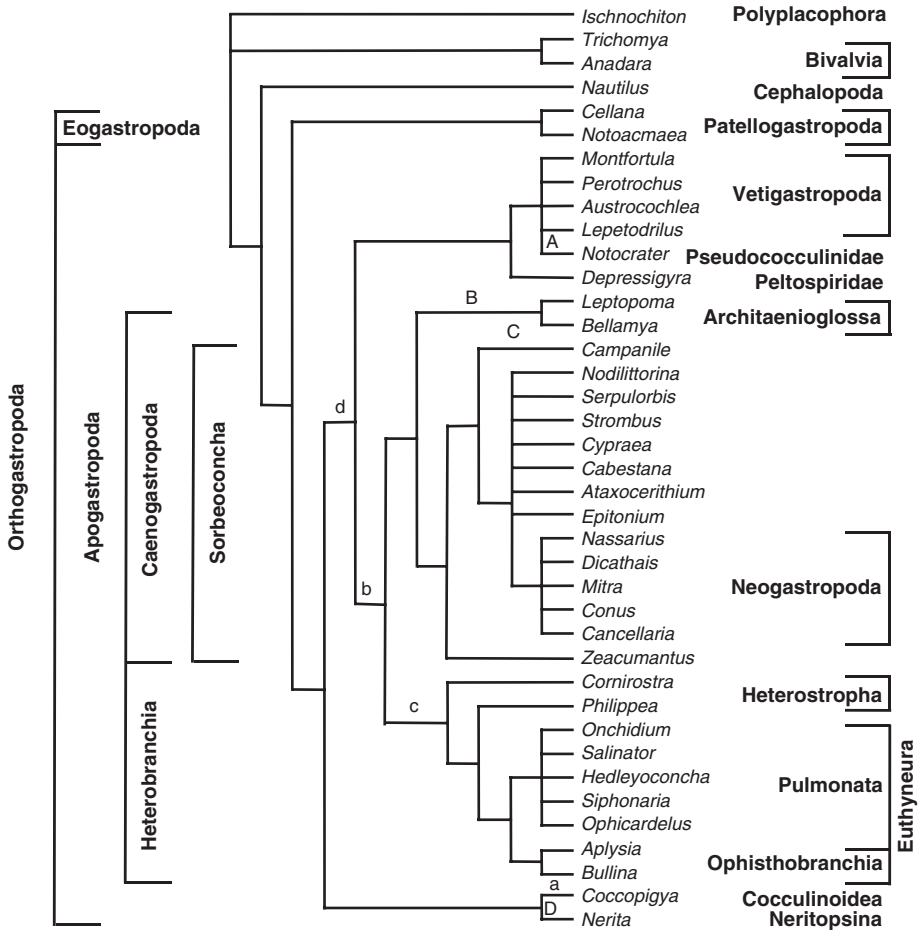

Fig. 1. Gastropod phylogeny based on the morphological analyses of Ponder and Lindberg (1997). Families not included in their analyses are placed according to their general taxonomic classification (i.e. Cancellariidae and Mitridae in Neogastropoda, Onchidiidae, Siphonariidae, Ellobiidae and Charopidae in the Pulmonata). Taxa studied by Ponder and Lindberg (1997) but not here are pruned from the tree. Names of higher categories follow Ponder and Lindberg (1997). Differences of this topology from the Haszprunar (1988) topology are indicated. An upper case letter on a clade in the Ponder and Lindberg (1997) tree indicates that it is shown at the point specified by the corresponding lower case letter in the Haszprunar tree.

In the present paper, we report analyses of an enlarged molecular dataset principally addressed to four areas of disagreement with morphologically based hypotheses, namely: (*1*) the position of the Patellogastropoda; (*2*) the monophyly and relationships of Cocculiniformia; (*3*) the relationships of Neritopsina; and (*4*) the monophyly of the Heterobranchia. Parts of two genes not previously used in overall gastropod phylogeny (cytochrome *c* oxidase subunit 1 (*CO1*) and small nuclear *U2* RNA (*snU2* RNA)) have been sequenced and new sequences from the *D1 28S* rDNA expansion region have been collected for most species studied in Colgan *et al.* (2000). The new genes extend the range of gene types used in gastropod phylogeny because they are respectively mitochondrial coding (*CO1*) and nuclear non-coding (*snU2* RNA). The *snU2* RNA is a component of the

spliceosome (Guthrie and Patterson 1988) that has previously provided useful characters for higher-level phylogenetic studies of Arthropoda (Colgan *et al.* 1998) and Polychaeta (Brown *et al.* 1999).

The division of Gastropoda into Eogastropoda and Orthogastropoda has not been supported in comprehensive molecular studies to date. In Colgan *et al.* (2000), Patellogastropoda plus a cocculiniform limpet (Cocculinoidea : *Coccopigya*) was a sister-group to the remainder of the studied species. A similar topology is seen in analyses of the approximately 450 (aligned) base pairs of *18S* rDNA in the compiled dataset of Harasewych and McArthur (2000), where Patellogastropoda plus a monophyletic Cocculiniformia is a sister-group to all other gastropods. In McArthur and Koop (1999), where Cocculiformia are not represented, Patellogastropoda is a sister-group to Apogastropoda. Statistically, no placement of Patellogastropoda as a sister-group to another major clade has a significantly higher likelihood than any other in the McArthur and Koop (1999) analyses.

Monophyly of 'cocculiniform' limpets is one of the major areas of disagreement between the Haszprunar (1988*b*) and Ponder and Lindberg (1996, 1997) morphological topologies. The two main constituent groups, Cocculinoidea and Lepetelloidea, comprise a monophyletic Cocculiniformia (Haszprunar 1988*a*; 1998) or are diphyletic (Ponder and Lindberg 1996, 1997). The molecular datasets also disagree. Cocculiniformia are diphyletic in Colgan *et al.*(2000), *Notocrater houbricki* (Lepetelloidea) being placed in Vetigastropoda and *Coccopigya hispida* (Cocculinoidea) with Neritopsina in accordance with Ponder and Lindberg (1997). Cocculiniformia represented by *Cocculina messingi* (Cocculinoidea) and *N. houbricki* are monophyletic (albeit with weak support) in Harasewych and McArthur (2000), using partial *18S* rDNA data, and are a sister-group to Patellogastropoda.

The relationship of the Neritopsina (or Neritimorpha) to the rest of the gastropods is unresolved with two main scenarios: they are either a sister-group to the vetigastropods and the apogastropods or a sister-group to the apogastropods. Frýda (1999), Bandel and Frýda (1999) and Bandel (2000) have discussed the fossil evidence for the evolution of this group since its first undoubted appearance in the Late Silurian–Devonian (428–374 million years ago; Frýda and Blodgett 2001), although earlier origins have been argued.

Within the Heterobranchia, Euthyneura, comprising two of the three classes (Opisthobranchia and Pulmonata) in Thiele's (1925, 1929–31) classification, are monophyletic in most recent molecular analyses where few taxa are used, but not in studies with larger taxonomic samples (Thollesson 1999; Dayrat *et al.* 2001). In morphological analyses involving Recent taxa, Heterobranchia is the sister-clade to Caenogastropoda (Haszprunar 1988*a*; Ponder and Lindberg 1996, 1997). In McArthur and Koop (1999), the only included heterostrophan (*Valvata* sp.) is a sister-taxon to Euthyneura. In Colgan *et al.* (2000), two representatives of the group are included. They are monophyletic but not a sister-group to Euthyneura, possibly owing to the high degree of autapomorphy in their sequences, this being particularly notable for the architectonicoid species *Philippea lutea* (see below).

Materials and methods

Materials and molecular methods

The taxa used, collection and registration data of specimens, methods and tissue types used for DNA extraction are given in Colgan *et al.* (2000). Our naming of higher groups follows Ponder and Lindberg (1997). Specimen voucher numbers are given in Table 1.

Primer pairs for *U2* are given in Colgan *et al.* (1998). The primers for *CO1* were as follows: Cox AF, CWAATCAYAAAGATATTGGAAC (41); Cox AR, AATATAWACTTCWGGGTGACC (725); and Cox 623R, GGTAARTYTATTGTAATAGCWCC (623). The figures in parentheses refer to the 3′ end of the oligonucleotide in the sequence of *Lumbricus terrestris* (Boore and Brown 1995; GenBank accession LTU24570). Primers Cox AF and AR were used together to produce a 690 bp product. Where a product was not obtained using this pair, Cox 623R was used with Cox AF to give a 626 bp product.

The primers for the *D1* expansion region were as follows: 28S D1F, ACCCSCTGAAYTTAAGCAT (43); 28S D1R, AACTCTCTCMTTCARAGTTC (406). Figures in parentheses refer to the 3 end of the oligonucleotide in the mouse *28S* rDNA sequence (X00525; Hassouna *et al.* 1984). Primer D1F was taken from Macarthur and Koop (1999) and D1R was designed from an alignment of *Ascaris lumbricoides* (U94751), *Drosophila melanogaster* (M21017, M29800) and *Mus musculus* (X00525) sequences.

The basic polymerase chain reaction (PCR) profile was as follows: 95°C for 5 min, 43–54°C for 45 s, 72°C for 1 min for one cycle; 95°C for 30 s, 43–54°C for 45 s, 72°C for 1 min for 30–34 cycles; and 95°C for 30 s, 45–60°C for 45 s, 72°C for 5 min for the final cycle. The annealing temperatures varied according to the primers' specificity for the different taxa and were usually 50–52°C for *U2*, 52–54°C for *D1 28S* rDNA and 43–45°C for *CO1*. To obtain PCR products from difficult samples, 20 μL GeneReleaser™ (Bioventures, Murfreesboro, TN, USA) was added to the DNA template and microwaved for 6 min. The remaining PCR mix was immediately added and cycling commenced. Reaction products were resolved on 2% agarose gels containing ethidium bromide. All single band products were purified using the QIAquick™ PCR Purification Kit (Qiagen, Venlo, The Netherlands) and, where multiple band products were obtained, the correct sized band was excised from 2% low-melt agarose in TAE buffer (0.04 M Tris , 0.001 M EDTA (pH 8.0), 0.02 M glacial acetic acid) and purified using the same kit. Products were sequenced in both directions by the Applied Biosystems (ABI®; Norwalk, CT, USA) 310 DNA Sequencing System using the DyeDeoxy™ Terminator sequencing method (Big Dye™ version 1 or 2; ABI), according to the manufacturer's instructions. The consensus sequence for each individual was obtained by reconciling forward and reverse sequences using Sequence Navigator (ABI).

The GenBank accession numbers of sequences used in Colgan *et al.* (2000) are AF033716–AF033794 for *28SA* rRNA and *28SB* rRNA and AF033675–AF033715 for *H3*. *Nautilus pompilius CO1* data are from AF000054 (Carlini and Graves 1999). Sequences for Viviparidae (U75863) and Cornirostridae (U75862) were obtained from GenBank (McArthur and Koop 1999). The GenBank accession numbers for the new sequences are AY296815–AY296850 for *CO1*, AY296873–AY296909 for *28S D1* and AY296851–AY296872 for *snU2* RNA.

Phylogenetic analysis

Sequences were aligned using the default values in CLUSTAL W (Thompson *et al.* 1994). The CLUSTAL output was inspected and, where required, indels were edited by hand. The *CO1* and *U2* sequences required no modification, but some regions of the *D1* segment were altered. These were specified as regions of uncertain alignment and were not used for analyses. All bases in the *28SA* and *H3* alignments from Colgan *et al.* (2000) were used. The region of uncertain alignment in *28S* rDNA B in Colgan *et al.* (2000) was not used. The alignments are available from the authors and as Accessory Material from the journal's website.

PAUP* 4.0b10 (Swofford 2000) was used for maximum parsimony analysis by conducting heuristic searches (100 iterations with random input order) with the default options (unless otherwise stated below). All characters were assumed unordered and indels treated as unknown in all reported analyses. One hundred bootstrap pseudoreplicates (Felsenstein 1985) were conducted with 20 random input replicates at each replicate to estimate support for nodes. Bremer decay indices were calculated using AutoDecay version 3.03 (Eriksson and Wikström 1996)

Maximum likelihood analyses of a reduced taxon set were performed using 10 random addition sequences for heuristic searches with the following settings. The number of substitution types was two, with the transition/transversion ratio estimated by maximum likelihood. Empirical nucleotide frequencies were assumed. The assumed proportion of invariable sites was zero, with a gamma discrete approximation (with shape parameter 0.5) using four rate categories. Another ML analysis was conducted with the same assumptions except that the maximum parsimony trees were used to start the analysis, branch swapping was by subtree pruning and reconnection, and five replicates were used. Quartet puzzling (100 000 steps) was performed using PAUP with the same likelihood settings, entering the transition/transversion ratio estimated during the likelihood searches by hand.

A Bayesian analysis was conducted with the program MrBayes (Huelsenbeck and Ronquist 2001) using the same likelihood parameters as the maximum likelihood analysis and sampling a tree every 100 steps

Table 1. Classification, species and voucher details

Higher-order grouping	Species	Specimen no.	Missing data
POLYPLACOPHORA Neoloricata			
Ischnochitonina, Ischnochitonidae	*Ischnochiton* (*Ischnoradsia*) *australis* (Sowerby, 1840)	C.203201	
BIVALVIA Pteriomorpha			
Arcoidea, Arcidae	*Anadara trapezia* (Deshayes, 1839)	C.203202	
Mytiloidea, Mytilidae	*Trichomya hirsuta* (Lamarck, 1819)	C.203203	
CEPHALOPODA Nautiloida			
Nautilida, Nautiloidea	*Nautilus scrobiculatus* Lightfoot, 1786	USNM 885678	
GASTROPODA			
EOGASTROPODA Patellogastropoda			
Patellina, Patelloidea, Patellidae	*Cellana tramoserica* (Holten, 1802)	C.203204	
Nacellina, Acmaeoidae, Lottiidae	*Notoacmaea petterdi* (T. Woods, 1876)	C.203205	*CO1*
ORTHOGASTROPODA			
Neomphaloidea, Peltospiridae	*Depressigyra globulus* Warén & Bouchet, 1989	T. HYS-202	
Cocculiniformia			
Cocculinoidea, Cocculinidae	*Coccopigya hispida* Marshall, 1986	NMNZ M.75188	
Pseudococculinoidea, Pseudococculinidae	*Notocrater houbricki* McLean & Harasewych, 1995	USNM 888656	*U2*
Vetigastropoda			
Fissurelloidea, Fissurellidae	*Montfortula rugosa* (Quoy & Gaimard, 1834)	C.203206	
Lepetodriloidea, Lepetodrilidae	*Lepetodrilus fucensis* McLean, 1988	T. F20-A2413	*U2*, *CO1*
Pleurotomaroidea, Pleurotomariidae	*Perotrochus midas* Bayer, 1965	USNM 888645	
Trochoidea, Trochidae	*Austrocochlea porcata* (A. Adams, 1851)	C.203207	
Neritopsina			
Neritoidea, Neritidae	*Nerita atramentosa* Reeve, 1855	C.203208	*U2*
Caenogastropoda			
'Architaenioglossa'			
Cyclophoroidea, Cyclophoridae	*Leptopoma perlucida* (Grateloup, 1840)	QM MO31142	
Ampullarioidea, Viviparidae	*Bellamya heudi guangdungensis* (Kobelt, 1906)	C.203209	*U2*
Sorbeoconcha 'Neotaenioglossa'			
Cerithioidea, Batillariidae	*Zeacumantus subcarinatus* (Sowerby, 1855)	C.203210	
Campaniloidea, Campanilidae	*Campanile symbolicum* Iredale, 1917	C.203211	

Hypsogastropoda			
Littorinoidea, Littorinidae	*Nodilittorina unifasciata* (Gray, 1826)	C.203212	
Vermetoidea, Vermetidae	*Serpulorbis* sp.	C.203213	*U2*
Stromboidea, Strombidae	*Strombus* (*Conomurexi*) *luhuanus* Linnaeus, 1758	C.203214	*U2*
Cypraeidae, Cypraeoidea	*Cypraea* (*Monetaria*) *annulus* Linneaus, 1758	C.203215	*U2*
Tonnoidea, Ranellidae	*Cabestana spengleri* Perry, 1811	C.203216	*U2*
Sorbeoconcha *'Ptenoglossa'*			
Triphoroidea, Cerithiopsidae	*Ataxocerithium* sp.	C.203217	*U2*
Janthinoidea, Epitoniidae	*Epitonium* cf. *jukesianum* (Forbes, 1852)	C.203218	
Sorbeoconcha Neogastropoda			
Buccinidae	*Nassarius* (*Plicarcularia*) *burchardi* (Dunker *in* Philippi, 1849)	C.203219	*U2*
Muricidae	*Dicathais orbita* (Gmelin, 1791)	C.203220	
Mitridae	*Mitra cucumerina* Lamarck, 1811	C.203221	*U2*
Cancellarioidea, Cancellariidae	*Cancellaria* (*Sydaphera*) *undulata* Sowerby, 1849	C.203222	*U2*
Conoidea, Conidae	*Conus miles* Linnaeus, 1758	C.203223	
Heterobranchia 'Heterostropha'			
Valvatoidea, Cornirostridae	*Cornirostra pellucida* (Laseron, 1954)	C.203224	
Architectonicoidea, Architectonicidae	*Philippea lutea* (Lamarck, 1822)	C.203225	
Heterobranchia Euthyneura			
Ophisthobranchia			
Cephalaspidea, Hydatinidae	*Bullina lineata* (Gray, 1825)	C.203226	*U2*
Notaspidea, Aplysiidae	*Aplysia* cf. *juliana* Quoy & Gaimard, 1832	C.203227	
Pulmonata			
Onchidiidae	*Onchidium* sp.	C.203228	
Amphiboloidea, Amphibolidae	*Salinator solida* (Schacko, 1878)	C.203229	*U2*
Siphonarioidea, Siphonariidae	*Siphonaria zelandica* (Quoy & Gaimard, 1833)	C.203230	
Ellobioidea, Ellobiidae	*Ophicardelus ornatus* (Férussac, 1821)	C.203231	
Stylommatophora, Charopidae	*Hedleyoconcha delta* (Pfeiffer, 1857)	QM MO6068	

Unless otherwise specified, samples are identified by their registration in the malacological collection of the Australian Museum. Other abbreviations are as follows: NMNZ, National Museum of New Zealand, Wellington; QM, Queensland Museum, Brisbane; USNM, National Museum of Natural History, Washington, DC; T, Tunnicliffe Collection).

An entry in the 'Missing data' column indicates that the specified gene was not scored for that taxon.

Sequences from GenBank are specified by accession number and referenced in the text.

along a 100 000 step Markov chain. Only two simultaneous chains were run, owing to computer memory restrictions, and the first 43 300 steps were discarded because convergence to an area of stable likelihood did not occur until this time.

All analyses listed below, except (*ix*)–(*xi*), were conducted with maximum parsimony.

(*i*) All data, excluding areas of uncertain alignment in the *28S* rDNA.
(*ii*) All data, excluding areas of uncertain alignment in the *28S* rDNA and third codon positions.
(*iii*) All data, excluding areas of uncertain alignment in the *28S* rDNA, transversions weighted threefold as costly as transitions.
(*iv*) All data, excluding areas of uncertain alignment in the *28S* rDNA and third position data, transversions weighted threefold as costly as transitions.
(*v*) All data, excluding areas of uncertain alignment in the *28S* rDNA, transversions weighted threefold as costly as transitions; *Nautilus* was used as the only outgroup.
(*vi*) All data, excluding areas of uncertain alignment in the *28S* rDNA and third codon position data, transversions weighted threefold as costly as transitions; *Nautilus* was used as the only outgroup.
(*vii*) All data, excluding areas of uncertain alignment in the *28S* rDNA, and excluding *Philippea lutea*.
(*viii*) All data, excluding areas of uncertain alignment in the *28S* rDNA and third codon position data, and excluding *Philippea lutea*.
(*ix*) A maximum likelihood analysis of a reduced dataset using random taxon addition and excluding areas of uncertain alignment in the *28S* rDNA.
(*x*) A maximum likelihood analysis using maximum parsimony starting trees excluding areas of uncertain alignment in the *28S* rDNA.
(*xi*) A Bayesian analysis of all data excluding areas of uncertain alignment.

Although analyses of individual genes are not reported in detail, they were conducted to confirm that there was no cross-contamination between sequences within this study or the inclusion of sequences from other material treated in this laboratory.

MacCLADE (Maddison and Maddison 1992) was used to set character types and substitution weights and to compare trees with the 'winning-sites test' (Prager and Wilson 1988) using the 'compare two tree files' option. Files containing all trees from each analysis were used. One tree (or set of trees) was preferred to another if the number of characters for which it had less steps than the alternative tree(s) was significantly greater than the number of such characters for the alternative. Probabilities were estimated using a two-tailed normal approximation.

Results

The number of aligned bases excluding the areas of uncertain alignment, the number of variable characters and the number of parsimony informative characters (in order for the individual gene segments) were: *D1 28S* rDNA, 317, 154 and 113 respectively; *28SA*, 255, 111 and 71 respectively; *28SB*, 256, 73 and 47 respectively; *CO1*, 567, 400 and 318 respectively; histone *H3* 274, 121 and 107 respectively; and *snU2* RNA 131, 56 and 41 respectively.

The mean GC content of the studied species for *snU2* RNA was lower (46.11%) than the other non-coding RNAs and notable differences were observed between the three *28S* rDNA segments: 57.35% for the *D1* region, 50.67% for *28SA* and 48.67% for the *28SB* region. The GC content was 58.42% for histone *H3*. The percentage of GC in *CO1* was very low (38.36%), but increased to 45.37% with the exclusion of third-position bases, consistent with the usual pattern for animal mitochondrial coding DNA (Wirth *et al.* 1999). Chi-square tests suggest that the percentage nucleotide composition was homogeneous within the studied species for *H3* ($P = 0.571$), *U2* ($P \approx 1$) and all *28S* segments ($P \approx 1$ for *D1 28S* rDNA, *28SA* and *28SB*), but was significantly inhomogeneous for *CO1* ($P < 0.001$). This inhomogeneity was due to third codon position data; when these data were omitted, the hypothesis of compositional homogeneity was not rejected ($P \approx 0.999$).

The average transition to transversion ratios for the six gene segments based on the Kimura two-parameter distance, and ignoring the areas of uncertain alignment and pairwise

comparisons without transversions, were: 1.792 ± 1.560 for *U2* (range range 0–13.31); 1.260 ± 0.600 for *H3* (range 0.348–5.260); 1.276 ± 0.874 for *D1 28S* rDNA (range 0–12.036); 3.017 ± 2.790 for *28SA* (range 0–26.065); 2.555 ± 3.602 for *28SB* (range 0–18.622); and 1.063 ± 0.306 for *CO1* (range 0.456–2.345). Ratios were also examined omitting the third position of *H3* and *CO1*. For *H3*, the average was 1.980 ± 1.028 (range 0.279–9.793). For *CO1*, the average was 1.372 ± 1.376 (range 0.279–21.186).

Incongruence length difference analysis for the data set for analysis (*i*) with 100 replicates returned a probability of 0.27 that the phylogenetic implications of the various gene segments do not differ. For other analyses, the probabilities are: (*ii*) 0.99; (*iii*) 0.99; (*iv*) 0.01; (*v*) 0.99; (*vi*) 0.99; (*vii*) 0.01; and (*viii*) 0.62. Summaries of maximum parsimony analyses for individual genes are given in Table 2. Generally, few clades receive bootstrap support greater than 50% in these analyses, so the results are not discussed in detail.

Details of the various analyses, including the number of maximum parsimony trees, the consistency index, the length of the trees and the minimum length of trees satisfying the Ponder and Lindberg (1997) topology, are presented in Table 3. The maximum parsimony trees for analyses (*i*), (*ii*), (*iii*), (*vi*) and (*ix*) are presented in Figs 2–6. The results for other analyses are compared with these figures below. Their bootstrap supported clades are indicated in Table 2.

Figure 2 shows one of two maximum parsimony trees for analysis (*i*). The topology of the second differs only above point A on the figure (with bolded branches found in both trees). The topology with *Philippea* removed (analysis (*vii*)) is the same as for analysis (*i*) (omitting *Philippea*) at nodes below the arrows on Fig. 2. The topologies differ above the arrowheads, notably in that Opisthobranchia and Pulmonata are monophyletic sister-groups in analysis (*vii*). Bootstrap supported clades are the same as for analysis (*i*) and have similar percentages.

The topology for analysis (*viii*) is the same as for analysis (*ii*) (with the removal of *Philippea*) at nodes below the arrow on Fig. 3, except that Patellogastropoda is a sister-group to the clade comprising (*Montfortula*, *Austrocochlea*, *Notocrater* and *Lepetodrilus*). Branches above the arrow seen in the strict consensus for analysis (*ii*) and for analysis (*viii*) are indicated in bold.

Figure 4 shows one of the two maximum parsimony trees for analysis (*iii*). The second tree differed in the placement of some taxa in the area between *Nodilittorina* and *Bellamya*, with *Nerita* shown in the equivalent position to *Bellamya* in the first tree. In analysis (*v*) *Nautilus*, *Coccopigya* and the other gastropods form a basal trichotomy. The gastropods are then divided into: (a) Euthyneura; and (b) the remaining taxa. Group (b) is further divided: (b.1) *Leptopoma* and *Campanile*; and (b.2) other Caenogastropoda, Vetigastropoda, Patellogastropoda and *Philippea*.

The topology for analysis (*iv*) is similar to that for analysis (*vi*). Addition of the other outgroup taxa in analysis (*iv*) places the root of the tree at the position marked by A in Fig. 5.

The maximum likelihood topology of the reduced taxon set is shown in Fig. 6. The estimated transition/transversion ratio on which this is based was 1.64. The estimated ratio for analysis (*x*) was 1.62. Analysis (*x*) produced two trees differing only in some placements within Caenogastropoda. The primary dichotomy lay between a group comprising Patellogastropoda, *Notocrater* and the Vetigastropoda except *Perotrochus* and the other gastropods. Caenogastropoda was monophyletic except that it anomalously included *Nerita* and that *Leptopoma* was grouped with *Nautilus* plus *Philippea* as a sister-group to a clade comprising *Coccopigya*, *Pleurotomaria* and *Depressigyra*. Euthyneura was monophyletic

Table 2. Summary of results for individual segments

Analysis	Bootstrap-supported clades	No. trees	*CI*	Length
28S D1	(*Strombus*, *Conus*) 90%; Eut plus *Cornirostra* and *Coccopigya* 79%; Eut plus *Coccopigya* 55%; (*Onchidium*, *Ophicardelus*) 82%; (*Bullina*, *Coccopigya*) 56%	1001	0.497	531
28SA	Biv 70%; Pat 100%; Eut 53%; (*Serpulorbis*, *Epitonium*, *Mitra*) 58%; (*Onchidium*, *Salinator*) 55	600	0.645	262
28SB	Pat 100%; (*Nodilittorina*, *Serpulorbis*, *Epitonium*, *Ataxocerithium*) 90%	8	0.690	142
28S (combined)	Gastropoda plus *Nautilus* 52%; Pat 100%; (*Montfortula*, *Austrocochlea*, *Lepetodrilus*) 62%; (*Austrocochlea*, *Lepetodrilus*) 51%; (*Nodilittorina*, *Serpulorbis*, *Epitonium*, *Ataxocerithium*) 73%; (*Serpulorbis*, *Epitonium*) 82%; (*Strombus*, *Conus*) 64%; (*Onchidium*, *Ophicardelus*) 73%; (*Hedleyoconcha*, *Onchidium*, *Ophicardelus*) 53%	5	0.525	1027
H3	Pat 56%	2	0.236	905
H3 (no third position)	(*Leptopoma*, *Strombus*) 52%; (*Montfortula*, *Austrocochlea*, *Notocrater*, *Lepetodrilus*) 72	320	0.500	70
U2	Pat 68%; (*Montfortula*, *Austrocochlea*) 86%; (*Leptopoma*, *Campanile*) 53%; Het except *Hedleyoconcha* 51%; (*Salinator*, *Onchidium*) 51%	20	0.503	183
CO1	Biv plus *Philippea* 91%; (*Nautilus*, *Leptopoma*) 54%; (*Coccopigya*, *Depressigyra*) 60%; Eut 71%; Eut except *Siphonaria* 78%; Pul except *Siphonaria* 57%	3	0.253	3175
CO1 (no third position)	(*Coccopigya*, *Depressigyra*) 53%; (*Trichomya*, *Philippea*) 54%; Biv plus *Philippea* 91%; Eut plus Biv plus *Philippea* 69%; Eut 73%; Eut except *Siphonaria* 81%	16	0.411	852

The columns show the number of maximum parsimony trees, their consistency index and length. The grouping of taxa by 'plus' does not necessarily indicate reciprocal monophyly.
Biv, Bivalvia; Pat, Patellogastropoda; Het, Heterobranchia; Eut, Euthyneura; Pul, Pulmonata.

Table 3. Statistics for individual analyses

Analysis	Trees	*CI*	Length	Ponder/Lindberg length	Winning sites		Normal deviate
(*i*)	2	0.298	5533	5603	179	92	5.28***
(*ii*)	4	0.447	2273	2347	68	25	4.46***
(*iii*)	1	0.277	10559	10842	217	127	4.85***
(*iv*)	12	0.455	4132	4273	87	26	5.74***
(v)	1	0.285	9280	9443	177	152	1.38
(*vi*)	28	0.440	2307	2383	73	27	4.60***
(*vii*)	4	0.298	5275	5411	164	81	5.30***
(*viii*)	10	0.453	2096	2143	65	31	2.14*
Eogastropoda/Orthogastropoda	4	0.298	5565		95	74	1.61
Heterobranchia	6	0.297	5556		119	101	1.21
Cocculiniformia	1	0.297	5551		140	114	1.63

The winning-sites test column shows the number of characters shorter in all trees of the specified analysis than in all trees satisfying the Ponder and Lindberg topology for that dataset, followed by the number of characters shorter in all Ponder and Lindberg trees.
In the 'Normal deviate' column, $*P < 0.05$ and $***P < 0.001$.
Refer to text for details of the analyses conducted.

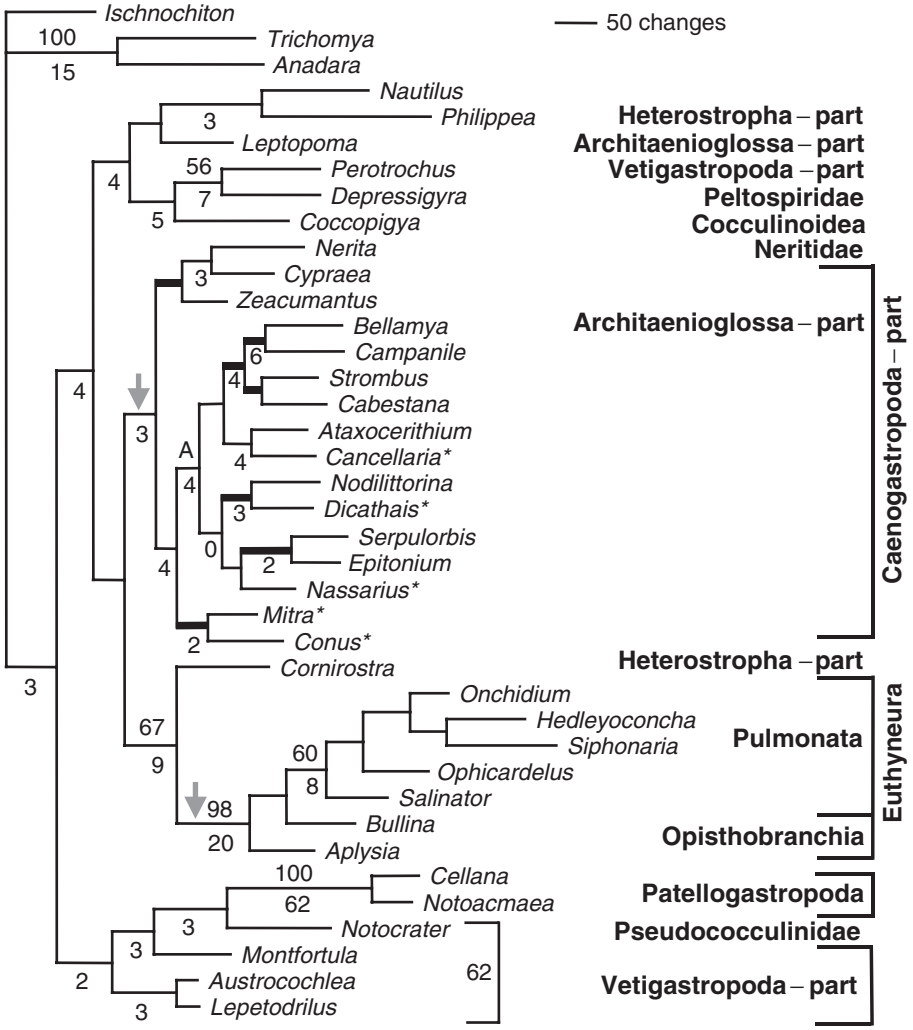

Fig. 2. One of two maximum parsimony trees for all data, excluding areas of uncertain alignment (analysis (*i*)). The topology of both is identical below point A. The arrowheads indicate topological similarities with analysis (*vii*) detailed in the text. Bold branches above A are also seen in the strict consensus of analysis (*i*) and analysis (*vii*). Bootstrap percentages above 50% are shown above a branch. Bremer decay indices are shown below the branch. Branches without figures have indices of 1. Asterisks on genus names indicate membership of Neogastropoda. Note that the brackets on the right-hand side do not necessarily show monophyletic clades.

with *Cornirostra* its sister-group. The clades with puzzling support more than 50% were: (*Nautilus*, *Philippea*) 55%; ((*Trichomya*, *Anadara*) 84%; (*Cellana*, *Notoacmaea*) 51%; ((*Austrococchlea*, *Lepetodrilus*) 68%; *Montfortula*) 61%; ((*Perotrochus*, *Depressigyra*) 65%; *Coccopigya*) 56%; (*Ataxocerithium*, *Cancellaria*) 60%; Pulmonata 52%; Ophisthobranchia (here *Aplysia*, *Bullina*) 69%; (Euthyneura plus *Cornirostra*) 60%.

The topology of the Bayesian analysis (*xi*) with *Ischnochiton* as the outgroup is broadly similar to the results of other analyses, albeit with many instances of high 'posterior probabilities' of clades that are unexpected on morphological grounds but that are shown

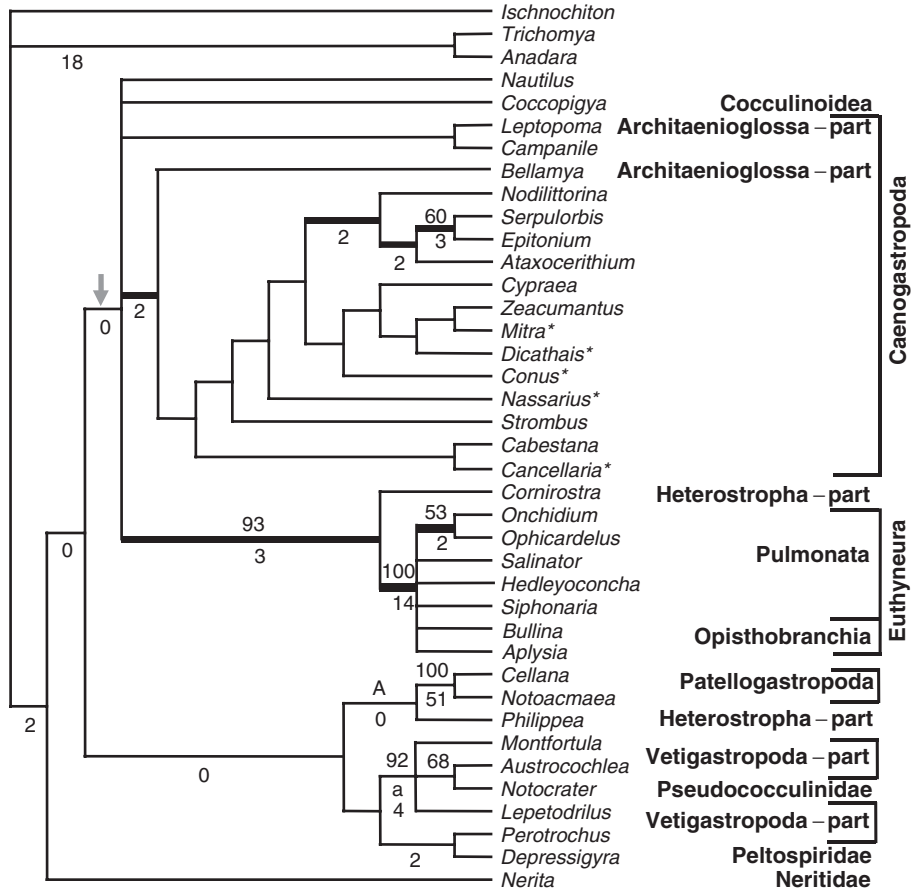

Fig. 3. The strict consensus tree for analysis (*ii*), all data excluding areas of uncertain alignment and third positions. Bootstrap percentages above 50% are shown above a branch. Bremer decay indices are shown below the branch. Branches without figures have indices of 1. Moving branch A to branch a makes the topology of this analysis identical to that for analysis (*viii*) at points below the arrowhead. All Euthyneura except *Siphomaria* are included in a clade with bootstrap support of 54%, although not found in the maximum parsimony tree. Asterisks on genus names indicate membership of Neogastropoda. Note that the brackets on the righ-hand side do not necessarily show monophyletic clades.

in parsimony or maximum likelihood analyses without great support. For instance, the anomalous pairing of *Nerita* with *Cypraea* has a posterior probability of 0.99; the pairing of *Strombus* with *Nassarius* in a group including most Neogastropods has a probability of 0.70 and the pairing of the remaining neogastropod *Cancellaria* with *Ataxocerithium* has a probability of 0.99.

Patellogastropoda was monophyletic with high bootstrap support in all parsimony analyses, but was shown in a basal position only in analysis (*vi*) (Fig. 5). In other analyses, the group was included in a clade with some vetigastropods and *Notocrater* (analyses (*i*), (*vii*); Fig. 2), with this group plus *Depressigyra* (analysis (*x*)), paired with *Philippea* (analysis (*iii*)) as a sister-group to a clade comprising other heterobranchs, caenogastropods, *Coccopigya*, *Nerita* and *Nautilus* (Fig. 4); or paired as sisters with *Philippea* (analyses (*ii*), (*v*); Fig. 3), *Nautilus* (analysis (*iv*)) or *Notocrater*, *Montfortula*,

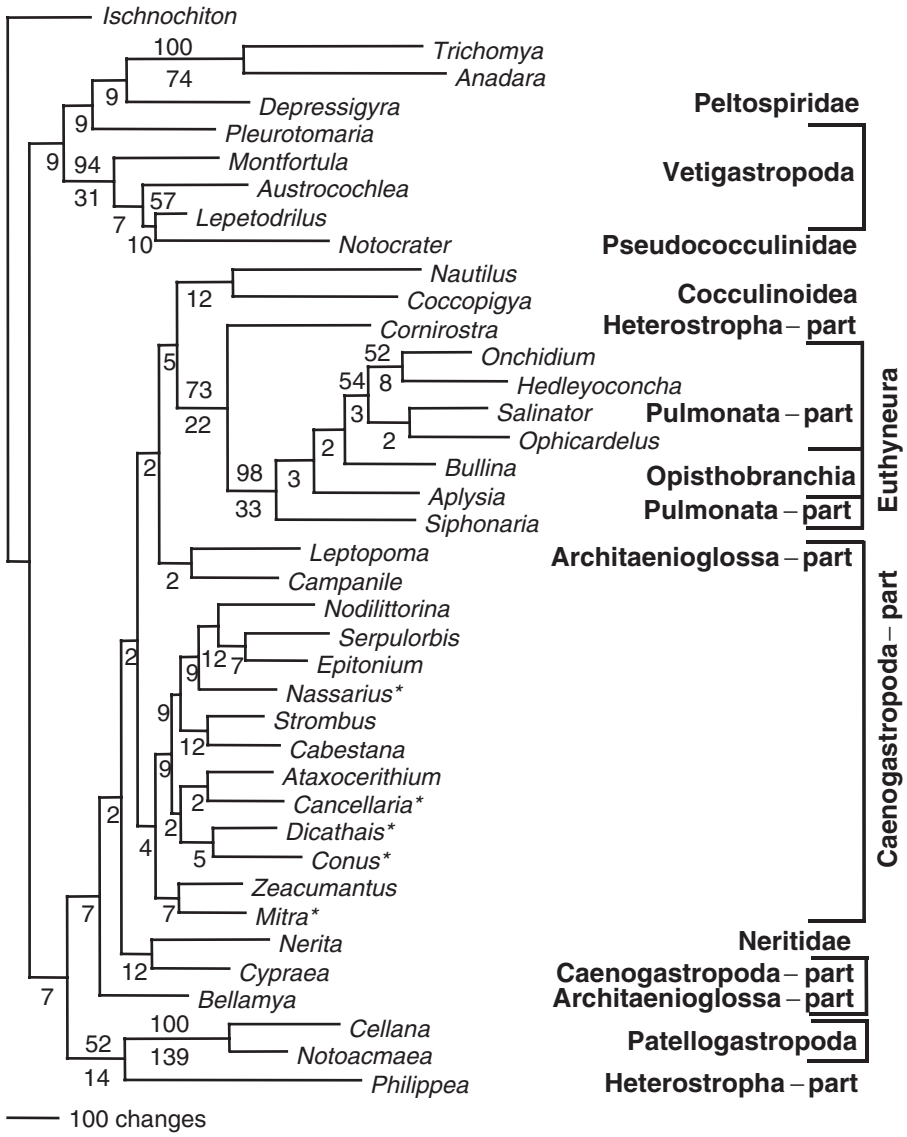

Fig. 4. One of two maximum parsimony trees for all data, excluding areas of uncertain alignment with transition/transversion weighting (analysis (*iii*)). Bootstrap percentages above 50% are shown above a branch. Asterisks on genus names indicate membership of Neogastropoda. Note that the brackets on the right-hand side do not necessarily show monophyletic clades.

Austrocochlea and *Lepetodrilus* (analysis (*viii*)) within a grouping of all vetigastropods and hot-vent taxa except *Coccopigya*. In no case did the pairing of Patellogastropoda as sister-groups with any other taxon receive bootstrap support greater than 50%. In analysis (*xi*), Patellogastropoda is a sister-group to the grouping of all vetigastropods and hot-vent taxa except *Coccopigya* with a posterior probability of 0.52. Imposing the constraint that Eogastropoda and Orthogastropoda were monophyletic sister-groups required 32 more steps ($P \approx 0.11$ using the winning-sites test).

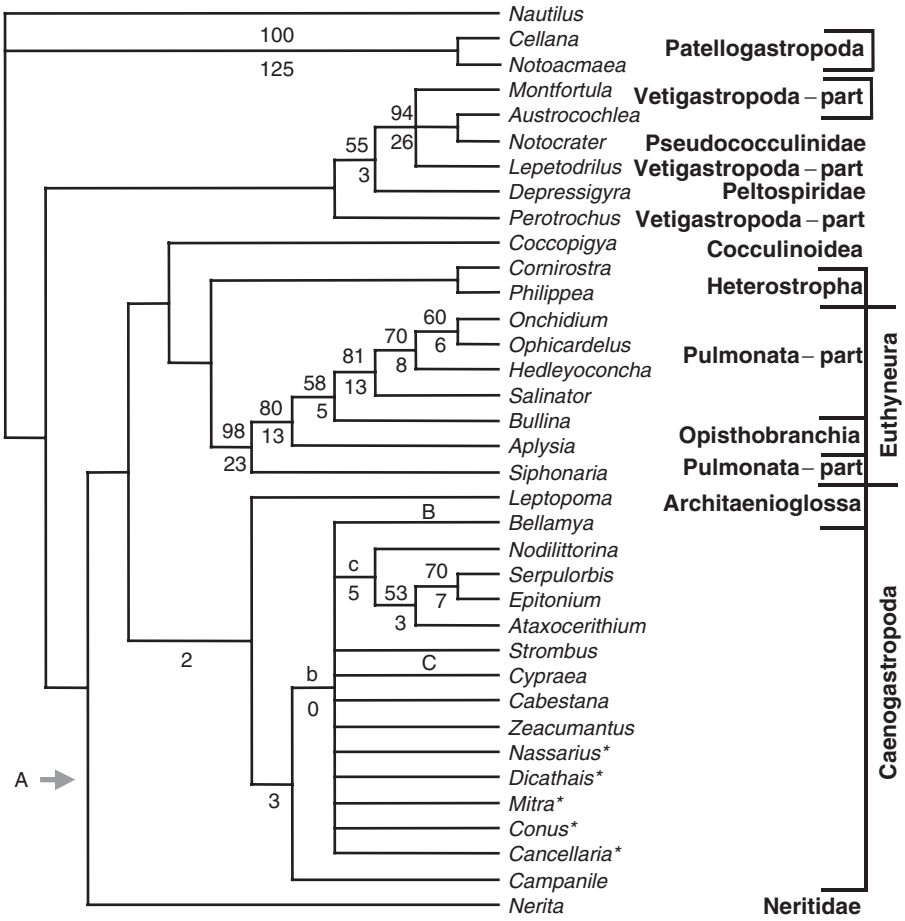

Fig. 5. The strict consensus tree for analysis (*vi*), all data excluding areas of uncertain alignment and third positions, with *Nautilus* as the only outgroup and transition/transversion weighting. Bootstrap percentages above 50% are shown above a branch. Bremer decay indices are shown below the branch. Branches without figures have indices of 1. 'A' indicates the root when the other outgroup taxa are added (analysis (*iv*)). The other differences between the strict consensus trees of these two analyses is that branch B moves to b and branch C to c in analysis (*iv*). Asterisks on genus names indicate membership of Neogastropoda. Note that the brackets on the right-hand side do not necessarily show monophyletic clades.

Cocculiniformia was never monophyletic in our analyses. *Coccopigya* was a sister-group to Euthyneura plus *Cornirostra* in analyses (*ii*), (*iv*), (*vi*), (*viii*), (*ix*), (*x*) and (*xi*), with significant bootstrap support in analyses (*ii*) and (*viii*) and a posterior probability of 100 in analysis (*xi*). It was a sister-group to *Nautilus* in analysis (*iii*) and *Perotrochus* plus *Depressigyra* in analysis (*i*) and analysis (*vii*) and formed one member of a basal trichotomy with *Nautilus* in analysis (*v*). *Notocrater* was always closely associated with a group of Vetigastropoda (*Montfortula*, *Austrocochlea* and *Lepetodrilus*). This group of four taxa was monophyletic with high support in all analyses except analyses (*i*) and (*vii*), where it also included the Patellogastropoda (as a sister-group to *Notocrater*). Even in analyses (*i*) and (*vii*), although the group of four was not shown in maximum parsimony trees, it received

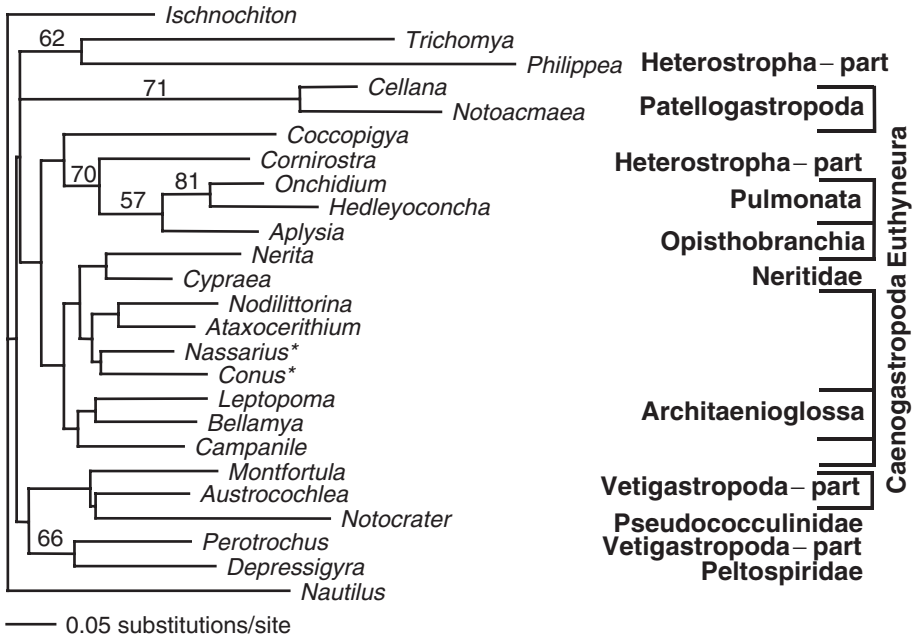

Fig. 6. Maximum likelihood tree of a reduced taxon set, including all data, excluding areas of uncertain alignment. Asterisks on species names indicate membership of Neogastropoda. The figures above branches indicate puzzling support of more than 50%: *Nodilittorina* plus *Nassarius* (54%), *Ataxocerithium* plus *Conus* (60%) and *Montfortula* plus *Austrocochlea* (64%) received puzzling support more than 50%, despite not appearing as clades in the likelihood tree. Note that the brackets on the right-hand side do not necessarily show monophyletic clades.

bootstrap support of more than 60%. Relationships within the group varied, with *Notocrater* being found as a sister-group to each of the three other members in at least one analysis. Imposing the constraint that Cocculiniformia is monophyletic required 18 more steps ($P \approx 0.10$ using the winning-sites test).

Euthyneura was monophyletic in all analyses with high bootstrap support (Figs 2–6; Table 2). Pulmonata and Opisthobranchia were both monophyletic only in analyses (*vii*), (*x*) and (*xi*), here with high posterior probabilities for each clade. In some other analyses (*i* and *ii*), Opisthobranchia was paraphyletic with respect to Pulmonata, but the groups were intermingled in analyses (*iii*), (*iv*), (*v*), (*vi*) and (*vii*), with bootstrap support for a clade of all Euthyneura except *Siphonaria* in analysis (*iv*) (85%) and analysis (*vi*) (80%).

In all analyses, *Cornirostra* was closely associated with Euthyneura, being shown with high bootstrap support or posterior probability as the sister-group to this clade except for analyses (*iv*) and (*vi*). In these analyses, *Cornirostra* was paired with *Philippea* as a sister-group to Euthyneura to form a monophyletic Heterobranchia, with bootstrap support of 67% in analysis (*iv*). Generally, Heterobranchia was disrupted by the association (not bootstrap supported) of *Philippea* with other taxa: *Nautilus* in analyses (*i*) and (*x*); and Patellogastropoda in analyses (*ii*), (*iii*) and (*v*). Imposing the constraint that Heterobranchia is monophyletic required 23 more steps ($P \approx 0.22$ using the winning-sites test).

The genetic divergence of the Heterobranchia as measured by the distance of terminals from the root in maximum parsimony analyses is striking, although less pronounced in likelihood analysis.

In each analysis except analyses (*v*) and (*vii*), the great majority of the Caenogastropoda and Heterobranchia grouped to form a recognisable but weakly supported 'apogastropodan clade'. Examples of the exclusion of *Philippea* from this are listed above. *Lepotopoma* was excluded in analyses (*i*) and (*vii*). Unexpectedly included taxa are *Nerita* in analysis (*i*), (*iv*), (*viii*), (*ix*) and (*x*), *Nautilus* and *Coccopigya* in analyses (*ii*) and (*iii*), and *Coccopigya* in analysis (*vi*). In analysis (*v*), Euthyneura plus *Cornirostra* was a sister-group to all other gastropods except *Coccopigya*. In analysis (*vii*), five unexpected taxa (*Nautilus*, *Nerita*, *Pterotrochus*, *Depressigyra* and *Coccopigya*) disrupt the 'apogastropod' lineage. None of the unexpected inclusions or exclusions had bootstrap support greater than 50%.

Discussion

The present analyses used data from six gene segments from four loci to address four of the major differences between morphological (Haszprunar 1988*a*; Ponder and Lindberg 1997) and molecular (Tillier *et al.* 1992, 1994; Rosenberg *et al.* 1997; McArthur and Koop 1999; Colgan *et al.* 2000; Harasewych and McArthur 2000) understanding of gastropod relationships. These were: (*1*) the position of Patellogastropoda; (*2*) the relationships of members of Cocculiniformia; (*3*) the relationships of members of Neritopsina; and (*4*) the monophyly of Heterobranchia.

From the molecular perspective, the division of Gastropoda into Eogastropoda and Orthogastropoda remains an open question, despite the addition of more data in the present paper. The position of Patellogastropoda varies in present analyses as in previous molecular investigations (Rosenberg *et al.* 1997; McArthur and Koop 1999; Colgan *et al.* 2000). Long-branch length attraction (Felsenstein 1978; Lyons-Weiler and Hoelzer 1997; Siddall and Whiting 1999; Stiller and Hall 1999; Philippe and Germot 2000) may be a possible explanation for the pairing of Patellogastropoda with groups that would be unexpected on morphological grounds. Morphological support for the eogastropod/orthogastropod division is strong, but not incontestable. Character states supporting the division of Gastropoda into Eogastropoda and Orthogastropoda should be synapomorphic in the latter group and plesiomorphic or having an autapomorphy not derived from the state in Orthogastropoda in Patellogastropoda. The potential number of such characteristics is impressive. Fifteen changed state between the nodes uniting all gastropods and all orthogastropods in the Ponder and Lindberg (1997) topology. However, five have consistency indices less than 0.4 and one (adult operculum) is not applicable to Patellogastropoda, although they possess a larval operculum. Among the other nine characteristics, only three (18, the presence of a hypobranchial gland; 60, the bending plane of the radula; and 98, statocyst position) have possibly derived states in all major orthogastropod clades. In the remaining six characteristics, some or most Vetigastropoda share the same derived state as Patellogastropoda or have the symplesiomorphic state for Gastropoda.

The situation with some characteristics is ambiguous; for example, with the hypobranchial gland. Sasaki (1998; his character 25) confirmed that a hypobranchial gland is absent in patellogastropods and noted its absence in *Nautilus* (although the nidamental glands may be homologous; Salvini-Plawen 1990). Sasaki (1998) also stated that the gland is absent in Fissurellidae on the basis of his own observations and Neomphalidae (*fide* McLean 1981), although it was recorded as present in these taxa by Ponder and Lindberg (1997). Fretter and Graham (1962) recorded a hypobranchial gland in *Diodora* and implied its presence in *Emarginula* (both fissurellids). Haszprunar (1989*b*) observed a small hypobranchial gland in males of *Pseudorimula* (a fissurellid) but not in females. Israelsson

(1998) recorded a hypobranchial gland in *Pachydermia* (related to *Neomphalus*) and one has also been recorded in *Melanodrymia* (Haszprunar 1989*a*), but the presence or absence of one is not noted in *Neomphalus* by Fretter *et al.* (1981). Sasaki (1998) bases his statement regarding the absence of a hypobranchial gland on McLean (1981), who says that a thick folded gland, as seen in haliotids and trochids, is absent but that there are '…scattered subepithelial gland cells … comparable to …. the Fissurellidae in which gland cells are present in the mantle skirt but do not form a discrete organ with a folded surface'. Given the presence of an undisputed gland in closely related taxa, what is present in *Neomphalus* is certainly a reduced hypobranchial gland, similar reductions being seen in many other gastropods, even within genera. These observations do not discount the possibility that the hypobranchial glands are secondarily absent in patellogastropods. They are absent in Scaphopoda, and possibly Cephalopoda, and a possibly homologous gland is present in Monoplacophora (Lemche and Wingstrand 1959; Wingstrand 1985; Haszprunar 1997).

One of the characteristics supporting the monophyly of the orthogastropods, the flexoglossage condition of the radula (Haszprunar 1988*a*; Salvini-Plawen 1988; Ponder and Lindberg 1997), is now thought to be plesiomorphic, owing to some lateral bending of the radula being found in chitons (Guralnick and Smith 1999). Guralnick and Smith (1999) suggest that the stereoglossate condition of the radula in patellogastropods is secondary. The radular stroke of living monoplacophorans has not been examined, so the condition of their radula can only be inferred. However, Guralnick and Smith (1999) argue that it is also probably flexoglossate with the structure of the radula most like that of lepetid patellogastropods. Available data also suggest a flexoglossate condition in cephalopods, scaphopods and in 'aplacophorans' (Guralnick and Smith 1999). Thus, on the basis of these findings, the stereoglossate condition appears to be an autapomorphy of the patellogastropods. There are, however, some plesiomorphic states retained in the patellogastropod radula that are not found in other gastropods (Guralnick and Smith 1999).

Since the publication of Ponder and Lindberg (1997), two new datasets add additional weight to the basal position of the patellogastropods. These relate to the buccal cartilages and the fine structure of the cephalic tentacles.

Only the number of buccal cartilages present in the odontophore was scored by Ponder and Lindberg (1997). Sasaki (1998) and Guralnick and Smith (1999) have attempted to homologise the cartilages. Guralnick and Smith (1999) used position and shape as a primary means of tracking the evolution of the buccal cartilages. They argue that a medial pair of cartilages is plesiomorphic for Mollusca, as also (probably, therefore being secondarily absent in 'aplacophorans') are the dorsolateral (= anterolateral of Sasaki (1998)) cartilages. More likely, assuming 'aplacophorans' are basal (e.g. Haszprunar 2000), the cartilages are probably synapomorphic of Testaria (*sensu* Haszprunar 2000). Whereas Sasaki (1998) considered these latter cartilages to be autapomorphies of patellogastropods, Guralnick and Smith (1999) argued that they were homologues of the dorsolateral cartilages of chitons and monoplacophorans. In these latter taxa, the two pairs of cartilages are attached by a connective tissue sheath, the space between being the hollow vesicles seen in those groups. Dorsolateral cartilages are absent in all Apogastropoda. A pair of dorsal cartilages is found in chitons and these are absent in modern Monoplacophora, but present in some patellogastropods. In addition, there are two pairs of posterior cartilages in chitons and the patellid patellogastropods (absent, presumably lost, in some of the more modified patellogastropods and in living Monoplacophora; Guralnick and Smith 1999). A single posterior pair is found in some vetigastropods and neritopsines. In patellogastropods, the subradular membrane is not associated with the medial cartilages

as it is in other gastropods, but is, instead, associated with the plesiomorphic dorsolateral (and dorsal cartilages when present), lying well above the medial cartilages.

Künz and Haszprunar (2001) showed that the fine structure of the cephalic tentacles of patellogastropods differs significantly from that of vetigastropods and neritopsines and that they share ciliary features observed in bivalves and 'aplacophorans'. A similar configuration (stiff cilia with a more or less homogeneous pattern of microtubules) is unknown in most other gastropods, although somewhat similar cilia are known from the tentacles of the pulmonate *Lymnaea* (Emery 1992). In addition, the ciliary tufts of patellogastropods have several ciliary types, whereas in the other two groups the ciliary morphology is much more uniform. Further, patellogastropods, vetigastropods and neritopsines all show differences in their sensory elements, supporting and mucous cells.

Other recent datasets that are less well resolved, but also appear to show that the patellogastropods are distinct from the vetigastropods and other gastropods, include larval musculature and the development of adult muscles (Wanninger *et al.* 1999) and sperm ultrastructure (e.g. Hodgson and Morton 1998). In other characteristics (e.g. cleavage pattern (van den Bigelaar and Haszprunar 1996), larval morphology and ciliation (Hadfield *et al.* 1997)), the patellogastropods and vetigastropods share assumed plesiomorphic conditions.

A significant problem for the patellogastropod ancestors being the sister-group to the orthogastropods is the lack of undoubted patellogastropods or obvious coiled ancestors in the early fossil record. The oldest undoubted patellogastropod has been confirmed recently (on the basis of shell structure) from the Triassic (Hedegaard *et al.* 1997). Recognition of such, probably coiled, ancestors will be difficult, but Wagner (2002) very tentatively suggests 'euomphalinaes' as candidates. If this was the case, the split in the two main gastropod lineages occurred in the Late Cambrian, around 510 million years ago.

Cocculiniformia are not monophyletic in our analyses. *Notocrater* (Pseudocculinidae) is strongly associated with Vetigastropoda, as found by Ponder and Lindberg (1997). *Coccopigya* is variously associated in derived positions (e.g. with Heterobranchia or *Depressigyra* and *Perotrochus*). The pairing with *Nerita* at the base of Orthogastropoda observed by Ponder and Lindberg (1997) is not found in any of our analyses. There are no morphological characteristics directly suggesting that Cocculinidae and Heterobranchia are sister-groups, although the sinistral protoconch coiling found in all members of the latter group has its analogue in at least some Cocculinidae. The pairing of *Coccopigya* with two other deep-sea taxa (*Depressigyra* and *Perotrochus*) appears to be coincidental because *Notocrater* and *Lepetodrilus* are also found in this environment.

Heterobranchia is rarely monophyletic in our analyses, owing to the variable placement of *Philippea*. Euthyneura is monophyletic in all analyses. Within Euthyneura, Opisthobranchia and Pulmonata are rarely monophyletic, concurring with Dayrat *et al.* (2001). They found that Opisthobranchia is paraphyletic with respect to Pulmonata, albeit that the nodes suggesting this observation had low bootstrap support.

The clade comprising Euthyneura plus *Cornirostra* is strongly supported in our analyses, supporting the suggestion of Haszprunar (1988*a*) that Valvatoidea is closer to Euthyneura than is Architectonicoidea. Confirmation of this will require data from other members of the family because *Philippea* is undoubtedly highly autapomorphic and its placement appears to depend on long-branch attraction. Questions of monophyly or paraphyly of Heterostropha, which includes the well-established families Omalogyridae, Pyramidellidae, Valvatoidea, Architectonicoidea and Rissoellidae, as well as a number of recently created Recent and fossil families, will be a fruitful area for further research.

The relatively large genetic differentiation of Heterobranchia in maximum parsimony trees suggests that the clade has a long evolutionary history, particularly if substitution rates are even remotely clocklike. The genetic distinction of Heterobranchia is emphasised by mitochondrial DNA genome organisation. In studied Euthyneura (for references, see Kurabayashi and Ueshima 2000*a*), this is radically different to the arrangement in the caenogastropod *Littorina* (Widling *et al.* 1999) but similar to that of *Omalogyra* (Kurabayashi and Ueshima 2000*b*). Other gene order work on opisthobranchs (Grande 2001; Medina *et al.* 2001) has yet to be reported in full. To date, the gene order data are based on an extremely small sampling and whether or not *Littorina* is typical of caenogastropods is unknown. For example, Collins *et al.* (2001) and Rawlings *et al.* (2001) report a major gene order rearrangement within the caenogastropod Vermetidae. Sperm structure (Healy 1993) also supports the monophyly of heterobranchs as a whole and Euthyneura. The earliest undoubted heterobranch fossils date from the early Devonian (390–408 million years ago; Frýda and Blodgett 2001), although some taxa included in the subulitoideans are likely heterobranchs and this grouping extends into the Ordovician (Nützel *et al.* 2000).

The affinities of the Heterobranchia (excepting *Philippea*) are with the Caenogastropoda in a recognisably 'apogastropodan' group (Salvini-Plawen and Haszprunar 1987; Haszprunar 1988*a*; as extended by Ponder and Lindberg 1997), although some taxa are anomalously included or excluded in some analyses. This contrasts with Colgan *et al.* (2000), where the 'apogastropod' group also had unexpected inclusions (*Nerita* and *Nautilus*) and Caenogastropoda and Heterobranchia were intermixed. Apogastropoda are monophyletic and comprised of monophyletic Caenogastropoda and Heterobranchia in McArthur and Koop (1999) and Harasewych and McArthur (2000), although these studies include fewer taxa from these latter groups.

Caenogastropoda is monophyletic with the exception of the anomalous inclusion of *Nerita* and/or *Nautilus* in some analyses and the exclusion of *Leptopoma* and *Cypraea* in analyses (*i*) and (*vii*). Relationships within Caenogastropoda are not well resolved in the present analyses. In particular, although various sets of two of the five genera of Neogastropoda included in the dataset are found in monophyletic clades in some analyses and four of the five are grouped in the Bayesian analysis (*xi*), this morphologically strongly supported group is not otherwise shown as closely related, as also found by Harasewych *et al.* (1997*b*).

Architaenioglossa comprise a number of superfamilies (previously two, now three) not considered close relatives by Ponder and Lindberg (1997). The taxa included here, *Bellamya* representing Viviparioidea (previously included within what is now considered to be a separate superfamily Ampullarioidea) and *Leptopoma* representing Cyclophoroidea, are monophyletic only in analysis (*ix*) based on maximum likelihood and analysis (*xi*) based on Bayesian likelihood. *Bellamya* is a member of the Caenogastropoda in all our analyses, but the position of *Leptopoma* varies widely although remaining within an apogastropodan group, except when all data are considered (analysis (*i*)), where it is a sister-group to an heterogeneous taxon (Fig. 2). *Campanile* is always associated with *Bellamya* or *Leptopoma*. McArthur and Koop (1999), using partial *28S* rDNA sequences, also found that the architaenioglossans (*Ampullaria* and *Viviparus*) were not monophyletic and, unlike our result, that *Ampullaria* and *Campanile* were sister-taxa. In their analyses, and with most of our analyses, the architaenioglossans lie within the caenogastropods, as suggested by Ponder and Warén (1988) and demonstrated in the morphological analyses of Ponder and Lindberg (1996, 1997). Alternative hypotheses have been produced on the basis of

morphological analyses, notably suggesting that the architaenioglossans are the sister-group to the apogastropods and part of a paraphyletic 'Archaeogastropoda' (Haszprunar 1988*a*) or that they belong to a clade (together with Neritopsina and Neomphaloidea), which is sister-group to the caenogastropods (Barker 2001).

The placement of the Neritopsina (or Neritimorpha) remains uncertain. This group, plus the Cocculiniformia (among our studied taxa), formed a sister-clade to all other Orthogastropoda in Ponder and Lindberg's (1997) preferred topology and in Rosenberg *et al.* (1997), although in this latter analysis the patellogastropod was included within the apogastropods. In the morphological analysis undertaken by Sasaki (1998), the patellogastropods formed the base of the gastropod clade and *Cocculina* appeared within a clade containing *Neomphalus* and the vetigastropods. Neritopsines formed one of four branches in an unresolved Gastropoda in the analysis of Harasewych *et al.* (1997*a*) and one branch of a basal trichotomy in McArthur and Koop (1999: fig. 3) and our analysis (vi). As in analyses (*ii*), (*iv*) and (*viii*), the Neritopsina was a sister-group to all other gastropods in maximum parsimony analyses (excluding one 25 bp insert) of the *18S* rDNA data of Harasewych and McArthur (2000: fig. 2*A*) and their maximum likelihood analysis (fig. 2*C*). When two longer inserts were excluded, Neritopsina was a sister-group to Vetigastropoda, this pair being a sister-group to Apogastropoda (Harasewych and McArthur 2000: fig. 2*B*). *Nerita* was placed within Caenogastropoda in Colgan *et al.* (2000), in accordance with our analyses (*i*), (*v*), (*vii*), (*ix*) and (*xi*) but in contrast with all recent morphological assessments (cf. Bieler 1992). In Barker's (2001) morphological analysis, Neritopsina was the sister-group to a clade consisting of Neomphaloidea + Architaenioglossa. This combination was a sister-group taxon to the caenogastropods. In our analyses (*iii*) and (*vi*), *Nerita* is a sister-group to Apogastropoda, allowing the possibility that larval planktotrophy arose once only in gastropods (cf. Ponder 1991; see also discussion in Ponder and Lindberg 1997: 209–213; and Frýda 2001).

The strict consensus of the maximum parsimony trees from analysis (*vi*) has notable similarities to recent morphological hypotheses. Agreeing with Ponder and Lindberg (1997), Caenogastropoda and Heterobranchia are monophyletic, as is Vetigastropoda, with the predicted inclusion of *Notocrater*. Patellogastropoda is basal, although excluded from Gastropoda.

Although the results of all analyses should be included in discussions of the phylogenetic implications of our data, we give a little more weight, when results differ, to those of analysis (*vi*), where, with *Nautilus* as the only outgroup, third-position data are excluded and transitions and transversions are weighted differently (Fig. 5). Comparison of consistency indices supports the exclusion of data because they are higher in trees including third positions than those excluding them. As judged by the consistency indices, excluding these data reduces the amount of phylogenetic noise. Arguing for differential weighting is the low transition to transversion ratio in the overall data for coding genes *CO1* and *H3*. This ratio increases when third positions are excluded, indicating a substantial degree of saturation. This analysis (as well as some others) has a high probability of homogeneity of phylogenetic inferences from the separate gene data.

When the chiton and bivalves are included, the outgroups are not monophyletic in any analysis. The use of *Nautilus* as the sole outgroup is suggested by the consensus on morphological grounds that Cephalopoda or Monoplacophora are the sister-taxon to Gastropoda (reviewed by Ponder and Lindberg 1997). Although not included in our analysis, scaphopods have recently been shown to be the sister-taxon to the cephalopods (Waller 1998; Haszprunar 2000; Giribet and Wheeler 2002; Wanninger and Haszprunar

2002), in contrast with earlier hypotheses that linked them to the bivalves. This relationship is, however, not apparent in the analysis of Rosenberg *et al.* (1997).

Unfortunately, despite considerable advancement in our knowledge of Palaeozoic fossils in the past decade, the origins of the major gastropod groups remain obscure, although all should have differentiated by the early Ordovician shortly after gastropods evolved (Wagner 2001, 2002). Whereas the considerable extinctions that have occurred during gastropod evolution may account for some of the long branch attraction issues encountered (especially between the patellogastropods and the remainder of the gastropods), breaking down some of the long branches encountered in this dataset by the addition of more taxa (Graybeal 1998) may be possible.

Despite more molecular data having been incorporated in these present analyses, some major aspects of gastropod phylogeny remain equivocal. Additional genes, gene order data and more refined morphological data will be required to resolve many of these issues, as well as better data on Palaeozoic gastropods.

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