J. Moll. Stud. (1997), 63, 301–309

PROCEEDINGS OF AN INTERNATIONAL MEETING

RIBOSOMAL RNA PHYLOGENY OF SELECTED MAJOR CLADES IN THE MOLLUSCA

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ABSTRACT

New sequence data from the D6 region of 28S rRNA and rDNA are presented for 28 species of molluscs and two annelids. These sequences were analyzed along with previously published molluscan sequences. Five of the molluscan classes were represented: Gastropoda, Cephalopoda, Bivalvia, Scaphopoda, and Polyplacophora, the last two by only one species each. Allowing for presumed long branch effects of Helicinidae, Patellidae, and the annelids, cladistic analysis supported monophyly of the following groups: Cephalopoda, Nautiloida, Coleoida, Gastropoda, Apogastropoda, Caenogastropoda, Neogastropoda, Rissooidea, Pulmonata, Stylommatophora, and a subset of Heterodonta including Sphaeriidae, Dreissenidae, and Veneridae but not Cardiidae. No resolution of relationships among molluscan classes was obtained.

INTRODUCTION

This paper results from a collaboration of three research groups, Rosenberg and Williams; Tillier and Tillier, and Hanlon and Kuncio. Each group had obtained up to a dozen sequences from the D6 region of 28S ribosomal RNA, but the sets of sequences in isolation were not particularly informative. By pooling our data, we have been able to supplement the 43 species analyzed by Rosenberg *et al.* (1994) with sequences from 28 molluscan species and two annelids. These sequences include representatives of three additional molluscan classes, Polyplacophora, Scaphopoda and Cephalopoda. The annelids represent Oligochaeta (*Eisenia*) and Polychaeta (*Sthenelais*). Our goals were 1) to analyze relationships within and between molluscan classes and 2) to examine whether adding outgroups and taxa intersecting long branches (Felsenstein, 1978) provided better congruence with patterns expected on the basis of morphology. Added taxa could show that characters interpreted as synapomorphic were plesiomorphic or convergent, and that characters scored as autapomorphies in divergent taxa were basal synapomorphies.

MATERIALS AND METHODS

Sequences were obtained in three laboratories by a variety of methods: Hanlon and Kuncio used direct sequencing of rRNA as described by Emberton, Kuncio, Davis, Phillips, Monderewicz, & Guo (1990; see also Kuncio & Hanlon, 1990); Tillier and Tillier also used direct sequencing of rRNA as described by Tillier, Masselot & Tillier (1996), with the primer from Emberton *et al.* (1990); Rosenberg and Williams used PCR of rRNA and rDNA as described below, generating rRNA and rDNA sequences for each species. Table 1 lists the taxa sequenced by each laboratory, along with locality data and voucher information. The current classification of the taxa is given in Table 2, along with GenBank accession numbers. Accession numbers were obtained for sequences of Emberton *et al.* (1990) [U82243–U82252], Rosenberg *et al.* (1994) [U82332–U82364] as well as for the new sequences presented herein [U82404–U82431]. Some of the submitted sequences extend beyond the region reported here. In preparing the sequences from Rosenberg *et al.* (1994) for submission, two errors were found in their figure 1: *Truncatella subcylindrica* has an A not a G at position 84 and the pulmonates have a G not a C at position 95.

Table 1. Materials studied. Collector is indicated by 'I'. The laboratory contributing the sequence is indicated in square brackets: HK = Hanlon and Kuncio; RW = Rosenberg and Williams; TTM = Tillier, Tillier and Masselot. ANSP = Academy of Natural Sciences of Philadelphia, in catalogue numbers of voucher specimens.

Annelida

Eisenia fetida (Savigny, 1826). Worm farm near Dijon, Cote d'Or, France, 1989. [TTM].
Sthenelais boa (Johnston, 1839). Roscoff, Finistère, France, 1988 [TTM]

Bivalvia

Mytilus edulis (Linné, 1758). Roscoff, Finistère, France [TTM].
Colletopterum sp. Desna River at junction with Dnieper River, north of Kiev, Ukraine, M. Ludyanskiy! 23 August 1993, ANSP 399812 [RW].
Cerastoderma edule (Linné, 1758). Sables d'Olonnes, Vendée, France, M. Masselot! 1990 [TTM].
Sphaerium nitidum Westerlund, 1876. Desna River at junction with Dnieper River, north of Kiev, Ukraine, M. Ludyanskiy! 23 August 1993, ANSP 399813 [RW].
Mytilopsis leucophaeata (Conrad, 1831). Sanibel Island, Florida, USA, G. Rosenberg! 6 March 1994, ANSP A18824 [RW].
Mercenaria mercenaria (Linné, 1758). Bought in market, Philadelphia, Pennsuylvania, USA [RW].
Venus verrucosa Linné, 1758. Bought in market, Atlantic coast of France, 1989 [TTM].

Scaphopoda

Antalis vulgare (da Costa, 1778). Roscoff, Finistère, France [TTM].

Polyplacophora

Acanthochitona fascicularis (Linné, 1767). Roscoff, Finistère, France [TTM].

Cephalopoda

Loligo forbesi Steenstrup, 1856. Azores, 1985. Hanlon! [HK].
Loligo opalescens Berry, 1911. Hopkins Marine Station, Pacific Grove, California, USA. W. Gilly! [HK].
Loligo pealii Lesueur, 1821. Woods Hole, Massachusetts, USA (WH-13), A. Kuzirian! [HK].
Loligo pleii de Blainville, 1823. Galveston, Texas, USA, J. Forsythe! [HK].
Loliolus uyii Wakiya & Ishikawa, 1921. Japan, R. Hanlon! [HK].
Uroteuthis edulis Hoyle, 1885. Japan, R. Hanlon! [HK].
Sepia officinalis Linné, 1758. Laboratory reared in Galveston, Texas; stock from England, R. Hanlon! [HK].
Nautilus pompilius Linné, 1758. Imported from Indo Pacific (probably the Philippines), lab held in Galveston [HK].
Nautilus macromphalus Sowerby, 1849. Nouméa, New Caledonia, P. Joannot! November 1988 [TTM].

Gastropoda

Patella vulgata Linné, 1758. Roscoff; Finistère, France, 1988 [TTM].
Monodonta lineata (da Costa, 1778). Saint-Malo, Rothéneuf, France, A. Tillier! November 1990 [TTM].
Viviparus viviparus (Linné, 1758). Desna River at junction with Dnieper River, north of Kiev, Ukraine, M. Ludyanskiy! 23 August 1993, ANSP 399814 and Shosha River at Ivankov Reservoir, north of Moscow, Russia, G. Rosenberg! 7 September 1993, ANSP 399816 [RW].
Cerithidea costata (da Costa, 1778). Sanibel Island, Florida, USA, G. Rosenberg! 6 March 1994, ANSP A18825 [RW].
Cerithidea scalariformis (Say, 1825). Sanibel Island, Florida, USA, G. Rosenberg! 6 March 1994, ANSP A18826 [RW].
Melanoides tuberculata (Müller, 1774). Sanibel Island, Florida, USA, G. Rosenberg! 6 March 1994, ANSP A18827 [RW].
Truncatella pulchella Pfeiffer, 1839. Bay side, Mile 57, Grassy Key, Florida Keys, G. Rosenberg! 28 August 1988, ANSP 397274 [RW].
Heleobops sp. Sanibel Island, Florida, USA, G. Rosenberg! 6 March 1994, ANSP A18828 [RW].
Lymnaea stagnalis (Linné, 1758). Shosha River at Ivankov Reservoir, north of Moscow, Russia, G. Rosenberg! 7 September 1993, ANSP 399818 [RW].
Planorbarius corneus (Linné, 1758). Shosha River at Ivankov Reservoir, north of Moscow, Russia, G. Rosenberg! 7 September 1993, ANSP 399817 [RW]..
Helix aspersa Müller, 1774. Around Besançon, Doubs, France, Bride! 1989 France [TTM].

Table 2. Current classification of taxa studied, with GenBank accession numbers for sequences.

Annelida
Oligochaeta
Lumbricidae
U82405 *Eisenia fetida*
Polychaeta
Polyodontidae
U82404 *Sthenelais boa*
Bivalvia
Pteriomorphia
Mytiloida
Mytilidae
U82408 *Mytilus edulis*
Paleoheterodonta
Unionoidea
Unionidae
U82409 *Colletopterum* sp.
U82348 *Gonidea angulata*
'*Anodonta* group'
U82332 *Anodonta grandis*
U82333 *Anodonta imbecilis*
U82334 *Anodonta cataracta*
U82335 *Lampsilis claibornensis*
U82336 *Lampsilis teres*
U82337 *Megalonaias boykiniana*
U82338 *Obliquaria reflexa*
U82339 *Quadrula cylindrica*
U82340 *Quadrula quadrula*
U82341 *Uniomerus tetralasmus*
'*Elliptio* group'
U82342 *Amblema plicata*
U82343 *Elliptio complanata*
U82344 *Fusconaia cerina*
U82345 *Plectomerus dombeyanus*
U82346 *Pleurobema cordatum*
U82347 *Unio pictorum*
Margaritiferidae
U82349 *Margaritifera falcata*
U82350 *Margaritifera margaritifera*
Heterodonta
Veneroida
Cardiidae
U82410 *Cerastoderma edule*
Dreissenidae
U82412 *Mytilopsis leucophaeata*
Sphaeriidae
U82411 *Sphaerium nitidum*
Veneridae
U82413 *Mercenaria mercenaria*
U82414 *Venus verrucosa*
Scaphopoda
Dentaliidae
U82407 *Antalis vulgare*
Polyplacophora
Acanthochitonidae
U82406 *Acanthochitona fascicularis*
Cephalopoda
Nautiloida
Nautilidae
U82419 *Nautilus pompilius*
U82420 *Nautilus macromphalus*
Coleoida
Sepiida
Sepiidae
U82418 *Sepia officinalis*
Teuthoida
Loliginidae
U82415 *Loligo spp.*
U82416 *Loliolus uyii*
U82417 *Uroteuthis edulis*
Gastropoda
Patellogastropoda
Patellidae
U82421 *Patella vulgata*
Neritopsina
Neritoidea
Helicinidae
U82245 *Helicina orbiculata*
Vetigastropoda
Pleurotomariidae
U82352 *Perotrochus amabilis*
Trochidae
U82422 *Monodonta lineata*
Caenogastropoda
Architaenioglossa
Viviparidae
U82423 *Viviparus viviparus*
Cerithiimorpha
Potamididae
U82424 *Cerithidea costata*
U82425 *Cerithidea scalariformis*
Thiaridae
U82426 *Melanoides tuberculata*
Neotaenioglossa
Rissooidea
Hydrobiidae
U82427 *Heleobops* sp.
Pomatiopsidae
U82250 *Oncomelania hupensis*
Truncatellidae
U82354 *Geomelania typica*
U82353 *Geomelania* sp.
U82357 *Truncatella caribaeensis*
U82361 *Truncatella clathrus*
U82359 *Truncatella pulchella*
U82428 *Truncatella pulchella*
U82356 *Truncatella reclusa*
U82360 *Truncatella scalaris*
U82358 *Truncatella subcylindrica*
U82355 *Truncatella* sp.
Neogastropoda
Muricidae
U82363 *Mancinella deltoidea*
Melongenidae
U82362 *Busycon carica*
Cancellariidae
U82364 *Progabbia cooperi*
Pulmonata
Basommatophora
Lymnaeoidea
Lymnaeidae
U82429 *Lymnaea stagnalis*

Table 2. (*Cont.*)

Planorboidea	U82248 *Mesodon normalis*
Planorbidae	U82249 *Neohelix albolabris*
U82243 *Biomphalaria glabrata*	U82251 *Triodopsis hopetonensis*
U82430 *Planorbarius corneus*	Holopodopes
Stylommatophora	Rhytidoidea
Holopoda	Haplotrematidae
Helicoidea	U82244 *Haplotrema concavum*
Helicidae	Aulacopoda
U82431 *Helix aspersa*	Zonitidae
Polygyroidea	U82246 *Mesomphix latior*
Polygyridae	U82252 *Ventridens cerinoideus*
U82247 *Mesodon inflectus*	

PCR and Automated Sequencing

Genomic DNA was isolated using the QIAamp tissue kit (Qiagen), starting from a 2 × 2 mm piece of tissue, taken from the foot whenever possible; the whole animal was used for the hydrobiid. DNA was quantitated by ultraviolet spectroscopy at 260 nm and 50 ng was used for PCR. Total RNA was isolated using the RNeasy Total RNA kit (Qiagen), with starting material as described above. The nucleic acid resulting from the first round of purification was subjected to extensive DNAase treatment and repurified prior to amplification via PCR.

Primers to amplify a 190 bp region of the D6 loop of 28S rRNA were derived from the conserved stem regions (Gutell & Fox, 1988). Primer sequences were as follows.

CCR-1: 5′ TATAGACAGCAGGACGGTGG 3′ (sense)
CCR-2: 5′ AGGCTTCAAGGCTCACCGCA 3′ (antisense)
CCR-5: 5′ AGGACGGTGGCCATGGAAGT 3′ (nested sense)

For amplification of genomic DNA, 50 ng of template DNA was amplified in a 100 μl reaction volume containing 10 pmoles of each primer (CCR-1 and CRR-2) using standard PCR reagents as described by the manufacturer (Perkin Elmer Cetus). A second, nested PCR reaction used 5 μl from the first reaction and 10 pmoles each of CRR-5 and CRR-2 in a 100 μl reaction volume, again with reagents as recommended by the manufacturer. Thermocycling conditions over 30 cycles were: 94°C for 30 sec, 58°C for 30 sec, 72°C for 1 min.

For amplification of total RNA, RNA was reverse transcribed for 30 min at 42°C using 50 pmoles of the downstream primer (CRR-2) to prime the reaction. This mixture was then amplified by PCR using 50 pmoles of the upstream primer (CRR-1) and PCR buffers as specified by the manufacturer. Thermocycling conditions over 30 cycles were: 94°C for 30 sec, 58°C for 30 sec, 72°C for 2 min. A second round of nested amplifications was not necessary.

For RT-PCR reactions, controls with no RT reaction were included to assess the extent of DNA contamination in the RT-PCR reaction. For all PCRs, a no-template control was included to guard against the possibility of contamination. All PCR reactions were purified on a QIAquick spin purification column (Qiagen) prior to automated cycle sequencing.

PCR products were analyzed on 2% agarose gels and subjected to automated sequencing using PCR primers to prime the cycle sequencing reactions in both the sense and antisense directions. Automated cycle sequencing was performed using dideoxy terminator reaction chemistry for sequence analysis in a model 373A or model 377 DNA sequencing system (Applied Biosystems Inc., Foster City, CA).

To check the results of automated sequencing, some samples were also subject to manual dideoxynucleotide sequencing. This was performed on the single stranded templates using Sequenase Version 2.0 (United States Biochemical Co., Cleveland, OH), the nucleotide analog 7-deaza-dGTP, and ^{35}S=dATP as the labelled nucleotide. Sequencing reactions were electrophoretically analyzed on a 6% polyacrylamide/ urea gel. A sequence for *Truncatella pulchella* obtained by PCR and automated sequencing of rDNA exactly matched that reported by Rosenberg *et al.* (1994) from direct sequencing of rRNA, but resolved the two ambiguous nucleotides shown in their figure 1.

Alignment and phylogenetic analysis

Sequences were added to the alignment presented by Rosenberg *et al.* (1994) and aligned manually, in the manner described by Tillier *et al.* (1996). In the case of ambiguities, alignments that rendered positions uninformative to parsimony analysis without adding gaps were preferred. Informative nucleotide positions, indicated by 'i' in Figure 1, were analyzed using Hennig86. The 'ie' command, which guarantees finding minimal length trees could not be completed because of the large number of taxa, so 'mhennig*; bb*;' was used instead. All characters were equally weighted and unordered. Single gaps were scored as a fifth nucleotide; multiple gaps were scored as unknown. Taxa with no differences in sequence were combined for the purpose of the phylogenetic analysis. The partial *Perotrochus* (Pleurotomariidae) sequence of Rosenberg *et al.* (1994) was excluded.

The mouse and annelid sequences were used as outgroups.

RESULTS

Aligned sequences are shown in Figure 1. Only partial sequences were obtained for *Loliolus*, *Uroteuthis* and *Sepia*. The 71 mollusc species were represented by 50 different sequences. Identical sequences occurred only in cases where the species were congeneric or confamilial; in this study, the four species of *Loligo* did not differ in sequence. The 5′ flanking region and stem (positions 1–57) and the 3′ flank and stem (positions 96–173) are conservative, and only a few gaps were inserted to align the sequences. The D6 loop shows considerable variation in length, and was too variable to be globally aligned, particularly from positions 60 to 85. The alignment shown has the D6 loop aligned flush right, with gaps added to align sequences locally. Minor changes in alignment produced major changes in topology in the cladistic analysis, so the D6 loop was excluded from further analysis.

Exclusion of the D6 loop and consideration only of informative sites rendered several sets of taxa identical: *Nautilus macromphalus* and *N. pompilius*; *Margaritifera falcata* and *M. margaritifera*; *Mercenaria* and *Venus*; *Loligo*, *Uroteuthis* and *Loliolus*; *Geomelania sp.*, *G. typica*, *Truncatella pulchella*, *T. reclusa*, *T. sp.*, *T. subcylindrica* and *T. caribaeensis*; *Truncatella clathrus* and *T. scalaris*; and *Ventridens* and *Haplotrema*. These taxa were combined in the cladistic analysis, leaving 40 distinct terminal taxa.

The cladistic analysis yielded 792 most parsimonious trees of length 231 (ci 42, ri 66). In the strict consensus of these (Figure 2A), the two annelids (*Sthenelais* and *Eisenia*) are pulled into the ingroup. An analysis excluding the annelids generated 120 most parsimonious trees of length 216 (ci 45, ri 68); the strict consensus of these shown in Figure 2B. The most notable difference between the consensus trees is that Cephalopoda becomes monophyletic when the annelids are excluded. Neither tree provides resolution of the relationships of the molluscan classes. Several clades (numbered in Figure 2) are seen in both consensus trees: Unionidae, Heterodonta (less Cardiidae), Gastropoda (less Helicinidae), Apogastropoda and Caenogastropoda (both including *Patella*), Rissooidea, Neogastropoda, Pulmonata and Stylommatophora.

DISCUSSION

The prediction of Rosenberg *et al.* (1994) that addition of outgroups and taxa intersecting long branches would provide better congruence with patterns expected on the basis of morphology is not supported. In Figure A, the annelids are pulled into the ingroup, Scaphopoda groups with Cardiidae, and Polyplacophora with the Unionidae. Helinidae (Neritopsina) still does not group with the Gastropoda, despite addition of the vetigastropod *Monodonta* (Trochidae), the patellogastropod *Patella*, and the architaenioglossan *Viviparus*. *Patella* groups with the caenogastropods and *Viviparus* (in Figure 2B) groups with the pulmonates, whereas *Monodonta* falls at the base of the gastropods.

Part of the explanation might be that many of these taxa still have long branches, and that better resolution would be obtained if additional representatives of each of the major lineages Neritopsina, Vetigastropoda, and Patellogastropoda were included in the analysis. Another factor might be the effect of homoplasy overwhelming phylogenetic signal due to saturation of substitutions. Tillier *et al.* (1996) showed that the D2 region of 28S rRNA is saturated for substitutions within the Gastropoda and so restricted their analysis to the Pulmonata. The D6 region used here is somewhat less variable than the D2 region, but may prove useful only for studying relationships within molluscan classes, not between them. Even if the molecule is not saturated for substitutions, the early radiation of the Mollusca might have been so rapid that the short sequences used here would not have sufficient resolving power (see Philippe, Chenuil and Aduotte 1994; Lecointre, Philippe, Lê and Le Guyard, 1994). Tillier *et al.* (1996) postulate a similar problem in resolving stylommatophoran phylogeny.

Rosenberg *et al.* (1994) discussed a number of factors affecting their analyses of ribosomal sequences: reliability of alignments, bias in nucleotide composition, ratio of transitions to transversions, effects of complementary mutations in base-paired regions, and weighting data. Their discussion applies with equal force to the analysis herein. Choice of taxa must also be considered. The analysis presented here still omits important taxa, even within the Gastropoda: Neomphalina, Cocculiniformia, Opisthobranchia, Systellommatophora and others are not represented. Other major taxa are represented by only a single species, yet choice of species can have a strong effect on phylogenetic

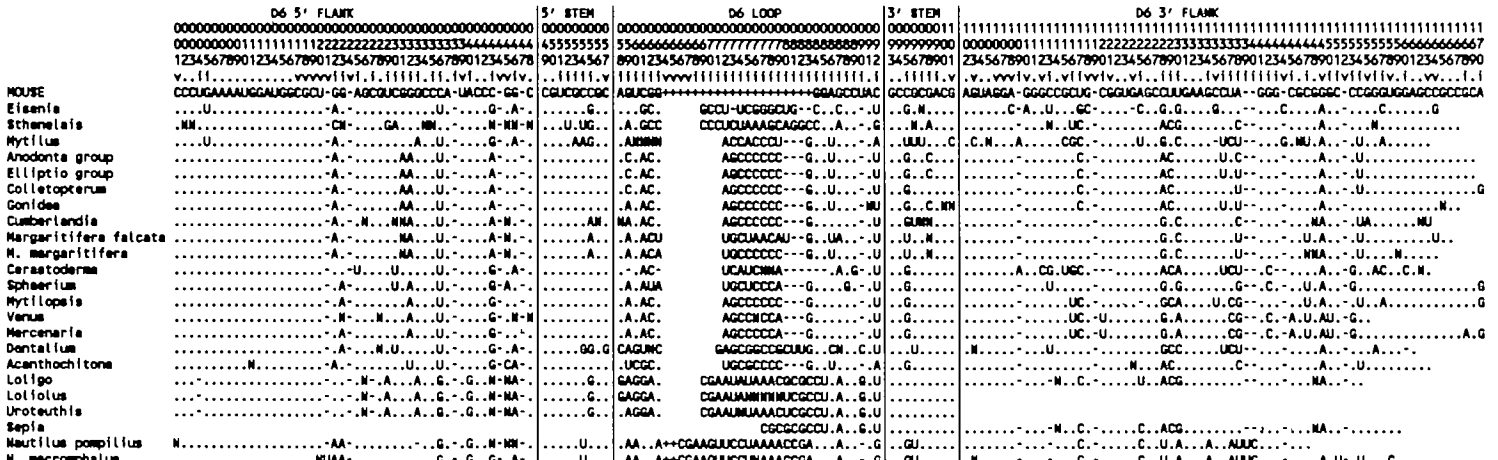

Figure 1. Aligned sequences. Numbers across the top in vertical triplets refer to the nucleotide position. The 5′ end of the sequences corresponds to mouse position 1836 of Hassouna *et al.* (1984). Nucleotides unchanged from mouse are represented by a dot (.); gaps by a dash (–). Nucleotides that could not be scored are marked 'N'. Pluses (+) in the D6 loop of mouse and the *Nautilus* species are an ellipsis where those sequences are longer than those of the other taxa and could not be aligned. Above the mouse sequence, positions variable in mollusks are marked 'v'; those potentially informative for cladistic analysis are marked 'i'. The vertical lines delimit the 5′ and 3′ stems, where the ribosomal molecule folds back and base pairs with itself.

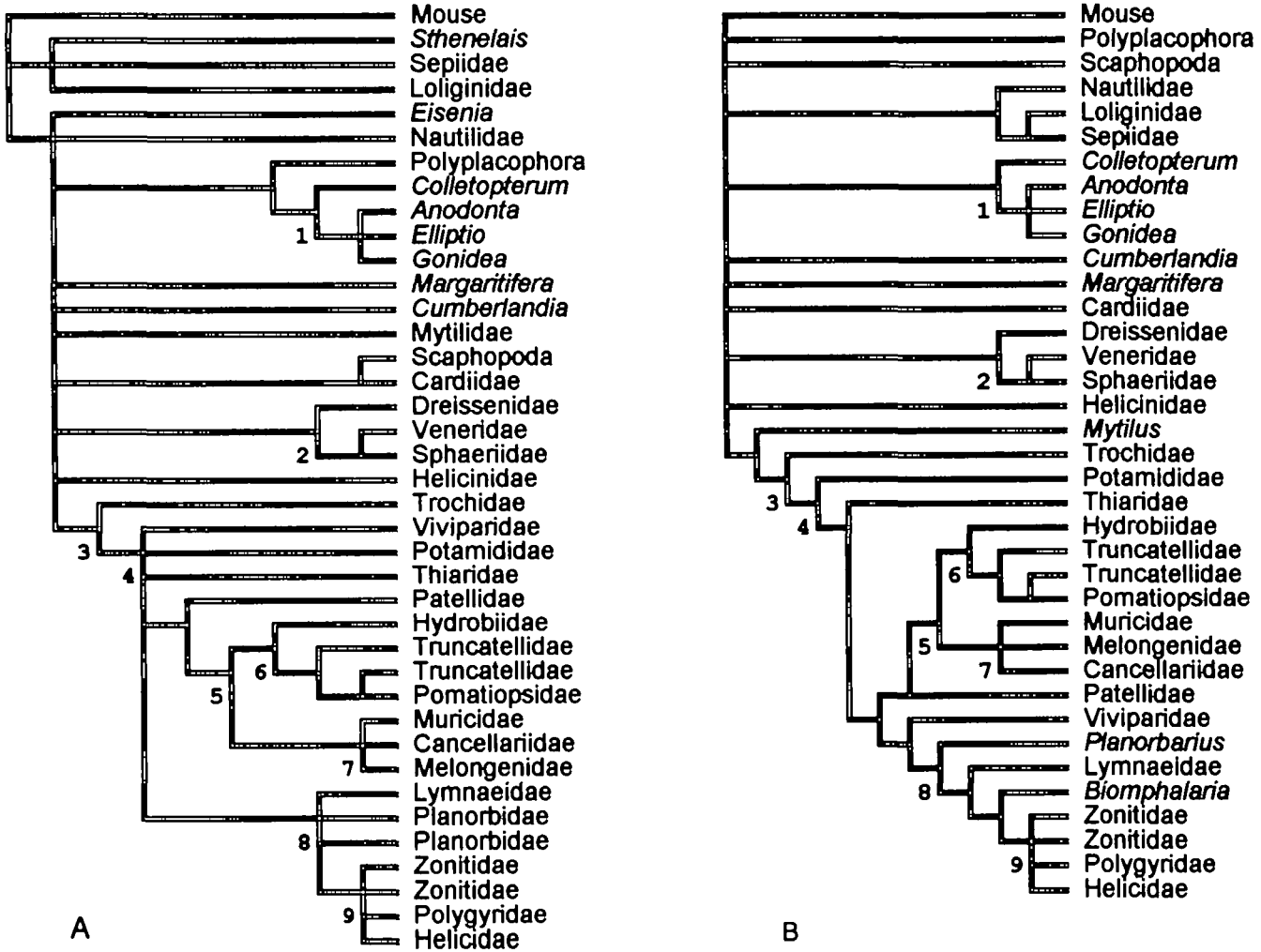

Figure 2. Strict consensus trees from cladistic analysis of informative characters. **A.** Consensus of 792 trees, lenth 231, ci 42, ri 66, with both mouse and annelids as outgroups. **B.** Consensus of 120 trees, length 216, ci 45, ri 68, excluding annelids. Numbered clades appear in both trees: 1) Unionidae, 2) Heterodonta less Cardiidae, 3) Gastropoda less Helicinidae, 4) Apogastropoda, 5) Caenogastropoda, 6) Rissooidea, 7) Neogastropoda, 8) Pulmonata, 9) Stylommatophora.

inference (Lecointre, Philippe, Lê and Le Guyard, 1993).

Comparisons to the golden standard of morphology are also important. When J. E. Gray published his classification of the Mollusca, he considered the anatomy of more than 5,000 species (Gray, 1847, p. 132), and vast additional amounts of information on morphology have accumulated since. Although there are still major gaps in anatomical knowledge of the Mollusca and much synthesis yet to be done (Ponder & Lindberg, 1996), available morphological data on molluscs far exceeds molecular data. We are not faced merely with a question of appropriate choice of exemplars and genes in molecular studies of molluscs. We are faced with compiling a database of sequences for thousands of species across dozens if not hundreds of genes to generate reliable molecular phylogenies across the Mollusca. In that context, the discussion of the relationships of various taxa below should be considered preliminary. Nonetheless, the data presented here indicate that 28S rRNA sequences, coupled with those from other genes, will be powerful tools for inferring caenogastropod and heterobranch phylogeny, even if less useful in resolving the early radiation of the molluscan classes.

Bivalvia

Little resolution was obtained among the Bivalvia. Perhaps most interesting is the clade containing *Mytilopsis* (Dreissenidae), *Sphaerium* (Sphaeriidae), and *Venus* and *Mercenaria* (Veneridae), which includes all of the Heterodonta in the analysis except *Cerastoderma* (Cardiidae). These four taxa are placed near each other in traditional classifications (e.g., Moore, 1969), although there does not appear to be a name for the clade, since Veneroida includes Cardiidae. This clade does not support the classification of Starobogatov (1992), which places Sphaeriidae in a separate order (Luciniformes) from Dreissenidae and Veneridae (Cardiiformes). Broader sampling of major lineages is needed to draw further conclusions about higher classification of bivalves.

Monophyly of the Unionidae is supported by the analysis, but that of Unionoidea and Margaritiferidae is not. This might be due to convergence in the D6 stem, where margaritiferids share nucleotides with some gastropods. Use of longer 28S sequences might provide characters that would unify Unionoidea. Within Unionidae, *Colletopterum* does not group with *Anodonta* although it is usually considered synonymous. This supports Russian classifications recognizing it as a full genus (e.g., Stadnichenko, 1984).

Cephalopoda

In the analysis with annelids excluded, Cephalopoda is monophyletic, as are Nautiloida and Coleoida (Figure 2B). Finding monophyly of the Nautiloida and Coleoida is not surprising since both the groups are well supported on the basis of morphological characters. Monophyly of the Cephalopoda is interesting since the Coleoida and Nautiloida probably diverged in the early Ordovician (Moore, 1964) and thus represent long branches. Monophyly of Loliginidae is also supported, with 4 species of *Loligo* and one each of *Loliolus* and *Uroteuthis* have almost identical sequences. Addition of sequences from other cephalopod lineages is necessary to test these relationships.

Gastropoda

Except for the position of Helicinidae, both consensus trees support monophyly of the Gastropoda. Within the Gastropoda, Patellidae is anomalous in grouping with the Caenogastropoda, which are otherwise monophyletic. If the positions of *Helicina* and *Patella* are disregarded as representing long branch effects, the cladogram in Figure 2B is compatible with that of Ponder and Lindberg (1996, fig. 5), with Vetigastropoda (in the form of Trochidae) as sister group to the clade Caenogastropoda + Pulmonata [Heterobranchia]. Tillier, Masselot, Guerdoux & Tillier (1994) also show monophyly of Caenogastropoda and Pulmonata, a group that corresponds to Apogastropoda of Hazprunar (1988). Ponder & Lindberg (1996) included Architaenioglossa in Caenogastropoda, whereas Haszprunar (1988) and Tillier *et al.* (1994) show it as sister group to the Apogastropoda. Our data are equivocal about the position of Architaenioglossa: in Figure 2A the position of Viviparidae is unresolved and in 2B it groups with the pulmonates.

Within Caenogastropoda, Neogastropoda and Neotaenioglossa (in the form of Rissooidea) are sister groups. The monophyly of Stylommatophora is supported, as already found by Tiller *et al.* (1996), but that of Basommatophora (=Hygrophila) is not. Additional taxa must be added to test these relationships. Neotaenioglossa includes a number of

former mesogastropod superfamilies, such as Vermetoidea, Littorinoidea, Stromboidea, Calyptraeoidea, Cypraeoidea, Heteropoda, Naticoidea and Tonnoidea (Haszprunar, 1988) which have not yet been studied. Sequences from various heterobranch and pulmonate groups such as Valvatoidea, Architectonicoidea, Opisthobranchia, Archaepulmonata and Systellommatophora must be included to test the monophyly of Basommatophora and Stylommatophora.

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