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Animal phylogeny and the ancestry of bilaterians: inferences from morphology and 18S rDNA gene sequences

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SUMMARY Insight into the origin and early evolution of the animal phyla requires an understanding of how animal groups are related to one another. Thus, we set out to explore animal phylogeny by analyzing with maximum parsimony 138 morphological characters from 40 metazoan groups, and 304 18S rDNA sequences, both separately and together. Both types of data agree that arthropods are not closely related to annelids: the former group with nematodes and other molting animals (Ecdysozoa), and the latter group with molluscs and other taxa with spiral cleavage. Furthermore, neither brachiopods nor chaetognaths group with deuterostomes; brachiopods are allied with the molluscs and annelids (Lophotrochozoa), whereas chaetognaths are allied with the ecdysozoans. The major discordance between the two types of data concerns the rooting of the bilaterians, and the bilaterian sister-taxon. Morphology suggests that the root is between deuterostomes and protostomes, with ctenophores the bilaterian sister-group, whereas 18S rDNA suggests that the root is within the Lophotrochozoa with acoel flatworms and gnathostomulids as basal bilaterians, and with cnidarians the bilaterian sister-group. We suggest that this basal position of acoels and gnathostomulids is artifactal because for 1000 replicate phylogenetic analyses with one random sequence as outgroup, the majority root with an acoel flatworm or gnathostomulid as the basal ingroup lineage. When these problematic taxa are eliminated from the matrix, the combined analysis suggests that the root lies between the deuterostomes and protostomes, and Ctenophora is the bilaterian sister-group. We suggest that because chaetognaths and lophophorates, taxa traditionally allied with deuterostomes, occupy basal positions within their respective protostomian clades, deuterostomy most likely represents a suite of characters plesiomorphic for bilaterians.

INTRODUCTION

With the explosion of new developmental, paleontological, and phylogenetic tools and data over the last 10 years, the question of the origin and early evolution of animals has never been more tractable, nor generated as much interest from as many different fields of inquiry. However, a proper understanding of animal evolution is predicated upon a proper phylogenetic framework. As but one example taken from a survey of Distal-less (Dll) expression among bilaterians, Panganiban et al. (1997) argued that the latest common ancestor of coelomate bilaterians utilized Dll in the development of both its nervous system and in the developmental pathway of some kind of "appendage." This was inferred because all of the coelomate bilaterians analyzed (chordates, echinoderms, onychophorans, arthropods, and annelids) expressed Dll in both places. Nematodes, however, lack a true coelom and those studied to date lack appendages and express Dll only in the nervous system. Panganiban et al. (1997) thus hypothesized that recruitment of Dll into appendage development is a coelomate apomorphy and expression in the nervous system a coelomate plesiomorphy, assuming that nematodes branched off the bilaterian line of evolution before this latest common ancestor of coelomates. If nematodes are, instead, members of a clade of molting animals along with arthropods (Aguinaldo et al. 1997), the evolutionary process hypothesized by Panganiban et al. (1997) must be reexamined. Did the latest common ancestor of bilaterians (with or without a coelom) express Dll in both nervous system and body wall outgrowths, and this was later lost in the nematode lineage, or is the expression of Dll in various bilaterian "appendages" convergent? Hence, a proper understanding of animal evolutionary developmental biology clearly requires knowledge of animal phylogeny (see also Jenner 1999; Adoutte et al. 2000).

Many evolutionary scenarios (e.g., the trochaea theory of Nielsen 1979, 1995) put forth to explain various aspects of animal evolution rely on relationships derived from traditional morphological analyses. These analyses (e.g., Brusca and Brusca 1990; Ax 1996; Nielsen et al. 1996; Schram 1997) usually find support for the following five hypotheses: (1) annelids are closely related to arthropods; (2) brachio-

pods and phoronids (and sometimes ectoprocts) are closely related to deuterostomes; (3) hemichordates (or enteropneusts if "Hemichordata" is paraphyletic) are the sister-taxon of chordates; (4) pseudocoelomates are monophyletic and are either basal to the coelomate clade, or basal to the protostome clade; and (5) ctenophores are the sister-taxon of bilaterians.

Commencing with the pioneering study of Field et al. (1988; see Adoutte et al. 2000 for recent review), phylogenetic analyses using 18S rDNA (hereafter abbreviated 18S) have revolutionized our understanding of how animal groups are related to one another. Curiously, 18S supports entirely different phylogenetic hypotheses than those discussed above. Likewise, all but the ctenophore case have recently received support from other types of molecular data, including Hox genes (de Rosa et al. 1999) and mitochondrial DNA (e.g., Cohen et al. 1998b; Stechmann and Schlegel 1999; Noguchi et al. 2000; Castresana et al. 1998a,b; Blanchette et al. 1999; Boore and Brown 2000). First, 18S strongly suggests that arthropods and annelids are not closely related (Aguinaldo et al. 1997; Eernisse, 1997; Aleshin et al. 1998; Giribet and Wheeler 1999; Giribet et al. 2000; as did the morphological analyses of Eernisse et al. 1992, and Zrzavý et al. 1998). Instead, arthropods group with nematodes, priapulids, and other "aschelminthes" into the monophyletic clade Ecdysozoa (see Table 1 for taxonomic nomenclature); annelids group with molluscs, brachiopods, and nemerteans and many other spiral-cleaving taxa into the Lophotrochozoa. Second, 18S supports a close relationship between lophophorates and spiralian protostomes (Lophotrochozoa of Halanych et al. 1995; see also Cohen et al. 1998a). Third, 18S supports a close relationship between hemichordates and echinoderms with Hemichordata monophyletic (Wada and Satoh 1994; Halanych 1995; Bromham and Degnan 1999; Cameron et al. 2000). Fourth, 18S supports the polyphyly of pseudocoelomates (Winnepenninckx et al. 1995a; Aguinaldo et al. 1997; Eernisse, 1997). Finally, 18S has been claimed to support a basal position of ctenophores relative to cnidarians, often supporting a sister grouping with calcareous sponges (Cavalier-Smith et al. 1996; Collins 1998; Kim et al. 1999; reviewed in Borchiellini et al. 2000).

Our goals here are twofold. First, we ask whether morphology is as incongruent with 18S as the above discussion would indicate. We reexamine what morphology alone indicates about metazoan relationships using a new compilation of characters (including absence/presence of molecular characters, such as mitochondrial codon usage, and Hox genes). Then, we run an extensive analysis of over 300 18S sequences both alone and then in combination with the morphology data set to generate a "total evidence" tree. We next show that the primary difference between the two types of data concerns the rooting of the bilaterians, as well as which taxon is the bilaterian sister-group. We argue that the placement of the bilaterian root according to 18S is likely artifactual, and the elimination of potential problematic taxa results in a tree whose rooting is consistent with the morphological data alone. We use this tree as our best estimate of metazoan phylogeny to address several significant evolutionary issues, including what inferences can now be made about the developmental biology of the latest common ancestor of the bilaterians.

MATERIALS AND METHODS

Taxa

The 40 taxa selected for morphological analysis included Fungi and Choanoflagellata as outgroups. Many studies (most recently by Herr et al. 1999; Atkins et al. 2000; Baldauf et al. 2000) suggest that Fungi is the closest multicellular relative to Metazoa, and choanoflagellates are among the nearest protist relatives. For metazoans, we generally assumed that each conventional "phylum" was monophyletic, with the following exceptions split into presumed monophyletic subgroups when the corresponding "phylum" has been recently claimed to be para- or even polyphyletic: Porifera (reviewed in Borchiellini et al. 2000); Brachiopoda (Cohen et al. 1998a; Cohen 2000); Platyhelminthes (most recently by Ruiz-Trillo et al. 1999); Hemichordata (e.g., Cripps 1991; Peterson 1995; Nielsen et al. 1996; Schram 1997); and Enteropneusta (Halanych 1995; Cameron et al. 2000).

We provisionally followed recent authors in treating several taxa as highly modified members of particular terminal taxa: Myxozoa as parasitic cnidarians (Siddall et al. 1995, Siddall and Whiting 1999; Cavalier-Smith et al. 1996; Zrzavý et al. 1998; but see e.g., Anderson et al. 1998; Kim et al. 1999); "Mesozoa" as parasitic flatworms (Cavalier-Smith et al. 1996; Van de Peer and De Wachter 1997; Kobayashi et al. 1999); acanthocephalans as parasitic rotifers (Lorenzen 1985; Garey et al. 1996, 1998; Zrzavý et al. 1998; García-Varela et al. 2000; Mark Welch 2000); pogonophorans (including vestimentiferans) as polychaete annelids (e.g., Bartolomaeus 1995; Young et al. 1996; Black et al. 1997; McHugh 1997; Rouse and Fauchald 1997; Kojima 1998; Halanych et al. 1998; Boore and Brown 2000; we do, however, include them in our molecular analyses, and confirm their annelid affinity, see below); pentastomids as crustacean arthropods (e.g., Wingstrand 1972; Abele et al. 1989; Storch and Jamieson 1992; Giribet et al. 1996; Zrzavý et al. 1997; but see Min et al. 1998); and *Xenoturbella* as a highly derived bivalve mollusc (Norén and Jondelius 1997; Israelsson 1997, 1998; but see Ehlers and Sopott-Ehlers 1997). *Buddenbrockia*, Lobatocerebromorpha, and Myzostomida were not considered.

For the 18S analyses, 304 sequences (Appendix 2) were selected for analysis from a large alignment (see below for methods) of over 600 metazoan 18S sequences, which itself is an extension of the alignment used by Eernisse (1997). These represented most (i.e., without including all of the heavily sampled insect and vertebrate 18S sequences) near full-length 18S sequences available from GenBank by January 2000, as well as some unpublished sequences made available to D. J. E. The latter included (see Appendix 2) two

Table 1. Taxonomic nomenclature[1] and abbreviations

Opisthokonta[2] = Fungi (Fun) + Choanoflagellata (Cho) + Mesomycetozoa (Mes) + Metazoa
Metazoa = Porifera + Epitheliozoa
Porifera[3] = Silicea (Sil) + Calcarea (Cal)
Silicea[3] = Hexactinellida (Hex) + Demospongia (Dem)
Epitheliozoa[4] = Placozoa (Pla) + Eumetazoa
Eumetazoa[4] = Cnidaria (Cni) + Ctenophora (Cte) + Bilateria
Cnidaria = Anthozoa (Ant) + Tesserazoa (Tes)[5]
Acrosomata[4] = Ctenophora + Bilateria
Bilateria = Deuterostomia (Deu) + Protostomia
Deuterostomia = Ambulacraria (Amb) + Chordata (Chd)
Ambulacraria[6] = Echinodermata (Ecm) + Hemichordata (Hem)
Hemichordata = Ptychoderidae (Pty) + Spengelidae (Spe) + Harrimaniidae (Har) + Pterobrancha (Pte)
Protostomia[7] = Ecdysozoa (Ecd) + Lophotrochozoa (Ltz) + Gastrotricha (Gas)
Lophotrochzoa[8] = Lophophorata (Lop) + Spiralia
Lophophorata[9] = Phoronida (Pho) + Brachiopoda (Bra)
Brachiopoda[10] = Linguliformea (Lig) + Craniiformea (Cra) + Rhynchonelliformea (Rhy)
Linguliformea = Lingulidae (Lin) + Discinidae (Dis)
Spiralia = Ectoprocta (Ect) + Platyzoa (Pla) + Trochozoa (Tro)
Platyzoa[11] = Rotifera (Rot) + Cycliophora (Cyc) + Gnathostomulida (Gna) + Platyhelminthes
Platyhelminthes = Catenulida (Cat) + Euplathelminthes
Euplathelminthes[4] = Rhabditophora (Rha) + Acoelomorpha
Acoelomorpha[4] = Acoela (Aco) + Nematodermatida (Ned)
Trochozoa[12] = Entoprocta (Ent) + Eutrochozoa (Eut)
Eutrochozoa[12] = Nemertea (Net) + Neotrochozoa (Neo)
Neotrochozoa[13] = Annelida (Ann) + Mollusca (Mol) + Echiura (Ech) + Sipuncula (Sip)
Annelida[14] = Pogonophora (Pog) + Phyllodocidae (Phy) + Terrebellidae (Ter) + Sabellidae (Sab) + Clitellata (Cli)
Mollusca[14] = Gastropoda (Gpd) + Polyplacophora (Ppl)
Ecdysozoa[15] = Chaetognatha (Cha) + Panarthropoda (Par) + Cycloneuralia (Cyn)
Cycloneuralia[16] = Scalidophora (Sca) + Nematoida
Scalidophora[17] = Priapulida (Pri) + Kinorhyncha (Kin) + Loricifera (Lor)
Nematoida[18] = Nematoda (Nem) + Nematomorpha (Nep)
Panarthropoda[19] = Tardigrada (Tar) + Onychophora (Ony) + Arthropoda (Art)
Arthropoda = Myriapoda (Myr) + Chelicerata (Che) + Pancrustacea (Pcr)
Pancrustacea[14,20] = Ostracoda (Ost) + Branchiopoda (Brp) + Ichthyostraca (Ich)[20]+ Onychophora (Ony) + Insecta (Ins) + Cirripedia[21] (Cir) + Malacostraca (Mal)

[1]Monophyly is not necessarily assumed for any specific clade. References are given for possibly unfamilar or relatively new taxonomic clades.
[2]Cavalier-Smith (1987), modified here to include Mesomycetozoa (Herr et al. 1999).
[3]Böger (1988).
[4]Ax (1996).
[5]Salvini-Plawen (1978).
[6]Because the original spelling is as given, and "Ambulacralia" was used by later authors for uncertain reasons (Hyman 1955) we prefer to use the original spelling (contra e.g., Zrzavý et al. 1998).
[7]Because the phylogenetic position of gastrotrichs is unclear it is assumed that they are protostomes, but exactly where they lie is unclear (see text).
[8]Halanych et al. (1995); also proposed as Eutrochozoa Ghiselin (1988) based on Field et al. (1988), but this earlier analysis lacked an ectoproct sequence.
[9]Because only phoronids and brachiopods possess a lophophore (Character 58) we feel that this is the appropriate taxon name for this clade.
[10]Williams et al. (1996).
[11]Ax (1987).
[12]Ghiselin (1988), modified herein; see also Eernisse et al. (1992), Eernisse (1997), and Zrzavý et al. (1998).
[13]Proposed herein.
[14]This describes the taxa analyzed herein and their abbreviations; it is not meant to be a comprehensive list of all of the taxa traditionally included in the group.
[15]Modified from Aguinaldo et al. (1997) to include chaetognaths.
[16]Ahlrichs (1995).
[17]Lemburg (1995); see also Schmidt-Rhaesa et al. (1998), contra Nielsen (1995).
[18]Schmidt-Rhaesa (1996).
[19]Nielsen (1995).
[20]Zrzavý et al. (1997).
[21]Cirripedia includes "cirripeds" + copepods.

nematodes (J. Vanleteren and P. De Lay, pers. comm., unreferenced), two centipedes, two tardigrades, and an onychophoran (G.-S. Min, pers. comm., unreferenced). The complete alignment of all 297 published 18S sequences is available upon request, but only the informative sites of these still unpublished sequences will be distributed with our data matrix.

In selection of sequences, we sought to be as inclusive as we could without sacrificing the feasibility of phylogenetic analysis, including our ability to at least partially estimate node robustness. Sequences were eliminated as objectively as possible for one or more of the following reasons: (1) most were pruned as taxonomically redundant (i.e., a subanalysis revealed clades of incompletely resolved clades at a low taxonomic level, in which case sequences were selected for pruning, often by those taxa also pruned from agreement subtree subanalyses as calculated with PAUP*); (2) they were partial or had unusual length (i.e., their alignment was problematic); (3) they had unusual base composition (i.e., GC/AT bias); (4) they had more autapomorphies than other retained representative sequences, as computed with software by Eernisse (2000); (5) relatively few were noted as highly unstable in their topological position as taxonomic composition in the analysis varied, so were pruned to permit better resolution of the remaining sequences. In some cases, we had to relax these criteria in order to represent our morphological terminal taxa as completely as possible. Particular sequences considered "long branch" sequences by previous authors (e.g., acoels, chaetognaths, nematodes) were still included because of their central importance to questions of bilaterian phylogeny, and because most of those conclusions were based on data sets with far fewer taxa.

We largely followed Eernisse and Kluge (1993) in their "total evidence" methodology for combining the morphological and molecular data sets for simultaneous analysis. As in that study, we did not attempt to score morphology for every species included in the 18S analysis. Instead, we assigned all sequences derived from members of a "morphology" terminal taxon with identical morphology scores. This is effectively similar to imposing a topological constraint that favors the monophyly of all of our morphology terminal taxa, but is not expected to unduly affect our attempts to estimate the interrelationships of these terminal taxa. Mesomycetozoa, which are a poorly known group of parasitic protists supported in the parsimony (but not distance) result of Herr et al. (1999) as the most proximal sister-taxon of Metazoa, were given "Choanoflagellata" morphology scores except for Character 1 (choanocytes with contracile microvilli, Appendix 1). This has not been reported for these species.

Complete 18S rDNA sequences were unavailable for three taxa (Loricifera, Pterobranchia, and Spengelidae). The available Nemertodermatida sequences (AF051328 and U70083) were not included because their preliminary analysis yielded highly dubious results, suggesting that further sampling of this taxon is in order (see Giribet et al. 2000 for the point that U70083 is a sequence artifact). For the combined analysis, these four "morphology only" taxa were added to the 304 18S matrix with the 18S characters coded as unknown.

Characters

Of all the characters we explored for possible inclusion in the morphological analysis, we selected 138 characters without regard to *a priori* assumptions of convergence or evolutionary scenarios (Appendix 1, Table 2) for analysis. Some of these characters are, in fact, not strictly morphological (e.g., Hox sequence or expression attributes), but are referred to as such hereafter. We chose to use the absent/present coding system for each character (Pleijel 1995), unless there was a compelling reason to use multistate coding. Such was the case if there are clearly two different states of a character (e.g., Character 119), or where one or more taxa lack a component of the character definition (e.g., Character 41). In a few cases, we intentionally scored groups of two characters with additive binary coding (equivalent to ordered multistate coding), but only if we were willing to assume a linear transformation series. This is manifest in a "00" rather than "0?" coding for a taxon that lacked both derived states. We acknowledge that these coding issues are contentious but feel that at the moment this is the most conservative coding scheme available.

Selection of characters for the 18S analysis was tied to the alignment procedure employed. One of us (D. J. E.) expanded the alignment employed by Eernisse (1997) to 626 18S sequences with the aid of software for manually editing, checking errors, and viewing colored sequence alignments (Eernisse 2000). Automated alignment of this many sequences is not currently feasible without a substantial sacrifice in alignment quality. The most ambiguously aligned sites were determined visually and these were eliminated from all analyses reported yielding a relatively robust data set. All 844 parsimony-informative sites included in the 18S data set were also included in the combined analyses, along with the addition of the 138 morphological characters.

Phylogenetic analysis

Our searches primarily employed the parsimony criterion using PAUP (Phylogenetic Analysis Using Parsimony, version 4.0b3; Sinauer Associates, Inc., Sunderland, MA, USA; Swofford 2000)*. Morphological analyses were performed with a TBR heuristic search employing 100 random addition sequence searches, and otherwise default settings in PAUP*. The 18S and combined analyses required a more aggressive two-part search strategy, which we found to consistently outperform a more standard PAUP* heuristic search. The first part involved a 100 random addition sequence TBR search with no more than 10 trees saved per replicate search (NCHUCK=10). The second part then started with all unique minimum length trees already in memory, and swapped more extensively with the maximum number of trees (MAXTREES) reset to either 1000 or 2500. The latter value corresponded to an estimated limit before PAUP* would exhaust available RAM (up to 40 MB) with our data set. In our experience, the strict consensus tree from the first 1000 trees found was always identical to that from the first 2500 trees found, and we took this as an indication that additional unsampled minimum length trees that existed might be unlikely to change our estimated strict consensus. This assumption was additionally tested by analysis of our data set with a test version of a new parsimony search program, TNT (Goloboff 1999; Nixon 1999; Goloboff et al. 2000), as performed on our data set by P. Goloboff (pers. comm., unreferenced). This program outperformed PAUP* by one or more orders of magnitude in speed of finding islands of minimum length trees, so that it was feasible to find our minimum-length island(s) of trees thousands of times. Our TNT strict consensus estimates for this particular data set were never observed to differ from those estimated with PAUP* (see Results).

For estimating node robustness, Bremer support indices (bsi) (Bremer 1988; also known as "support" or "decay" indices) were calculated for all nodes of our morphological analysis and for selected nodes of our 18S and combined analyses, using PAUP* converse constraint searches for each node supported in our strict consensus. These used software by Eernisse (1992, 2000; see also Eernisse and Kluge 1993) to automate generation of the appropriate PAUP* search blocks. Selected clades supported by our morphological analysis, but not supported in our other analyses, were addi-

Table 2. Morphological data matrix

	1	11	21	31	41	51	61	71	81	91	101	111	121	131
Fun	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000020000	0000000000	00000000
Cho	1000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000020???	????????	????????
Dem	1110011100	0000013101	0000001000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000?20	0001101000	00000???
Hex	1110011100	0000011?1??	?00??01?00	0100000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000020???	????????	????????
Cal	1110011010	0000013101	0000001000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000020???	????????	????????
Pla	0111000010	1010011???	??0?001?00	0?00000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000020???	??????1000	00000???
Cni	0111100010	1110011101	0100101000	0100000000	0000100000	0000000000	0000000000	0000000000	0000000000	0100000010	0000000000	0000000170	0011101000	00000010
Cte	0101100010	1120011102	1100101100	0100100000	0000100000	0000000000	0000000000	0000000000	0000000000	0102000011	0000000000	0000000???	??211?1010	00?00???
Cha	0111100010	1120012102	1102111100	0101110000	0000100000	0000000000	0000000100	0000001000	0000001000	0101000011	1011000000	0000000???	????????	????????
Gas	0111100010	1120012102	1102111100	01?1100000	1000000000	0000000000	0000001000	0000000010	0000001000	0111000011	1010000000	0000020???	????????	????????
Nem	0111100010	1100012200	0100111100	0111100000	2000000000	0000000000	0000002000	0000000111	1010001000	0111000011	1110100000	0000020120	1111111110	00100110
Nep	0111100010	1100012202	1102111200	0101?000000	2000000000	0000000000	0000000000	0000000111	1010001100	0111000011	1110100000	0070020?22	???????111	00100???
Pri	0111100010	1100012102	1102111?00	01?1?0???0	10?0000000	0000000000	0000001000	0000000111	0110001100	0111000011	1110000010	1010120???	???????111	00100???
Kin	0111100010	1100012100	0102112???	?2?1?0???0	1?20000000	0000000000	0000001000	0000000111	0110001110	0111000012	1110000010	1110020???	????????	????????
Lor	0111100010	1100012102	?102112???	???1?0???0	10?0000000	0000000000	0000001000	0000000111	012000110	0111000012	1110000010	1110020???	????????	????????
Tar	0111100010	1100012102	1102111?00	0?01??0?20	1?000000000	0000000000	0000000000	0000000111	0010011000	1111000011	1010000000	0000020???	????????	????????
Ony	0111100010	1100012102	0102111100	0111100000	1100000000	0000000000	0000001000	0010000111	0010010000	1111000011	1011000000	0000020???	??????1111	00100???
Ath	0111100010	1100012102	1101111000	0111100000	1100000000	0000000000	0000001000	0010000111	0010010000	1111000011	1011000000	0000000120	1111111111	00100110
Pho	0111100010	1110012102	?102111100	0111110000	1000100000	0010101100	0000001000	0100000000	0000000000	0101000011	1000010000	0000020???	????????	????????
Lin	0111100010	1110012102	1102111100	0111??0000	1000100000	0000?01111	1111101000	0101010000	0001000000	0101100011	1000?21000	0000100???2	??????1110	10110???
Cra	0111100010	1110012102	1102111100	0101110000	1000?00000	0000?01111	1111101000	0101111000	0001000000	0101000011	1000021000	0000020???	????????	????????
Art	0111100010	1110012102	1101111100	0111110000	1000100000	0000?01111	0111101000	0101101000	0001000000	0100100011	1000021000	0000020???	????????	????????
Ect	0111100010	1120012102	?102111100	0111??0000	1000110100	0000000000	0000001100	0000000000	0001000000	0101000011	1000?20000	0000000???	????????	????????
Rot	0111100010	1120012112	1102111?20	0111??1000	1000000111	0000000000	0000001100	0000000000	0000000001	0101000011	1010000000	0000020???	????????	????????
Gna	0111100010	1110012110	0102111112	?1?1??2?00	1000000000	0000000000	0000001100	0000000000	0000000001	0111000011	1010000000	0000020???	????????	????????
Cat	0111100010	1120012211	0102?11?1?	?1?1?0?200	1001000000	0000000000	0000001000	0000000000	0000000000	0100000012	1010000000	0000020???	????????	????????
Aco	0211100011	1121112111	0102?11100	0111100000	1001000000	0000000000	0000000000	0000000000	0000000000	0210000012	1010000100	0000020???	????????	????????
Ned	0111100011	1121112101	0102?12???	??2?1??0?0	1001000000	0000000000	0000001000	0000000000	0000000000	0110000012	1000000100	0000020???	????????	????????
Rha	0111100010	1121012111	0102?111?0	0111101100	1001110000	0000000000	0000001000	0000000000	0000000000	0110000011	1010000100	0000000020	0111111110	101?01??
Cyc	0111100010	1120012?12	?20?11????	??2?1??0?0	10?2000110	1000000000	0000001000	0000000010	0000000000	0101000012	1010000?00	0000?20???	????????	????????
Ent	0111100010	1120012100	0102?111?0	0111110100	0000110111	0100000000	0000001000	0000000100	0000000000	0101000011	1000000002	0000020???	????????	????????
Net	0111100010	1120012102	1102111110	0111101111	1000110100	?000000000	0000001000	0000000000	0000000000	0111000011	1010000001	0000000?2?	??2?111110	101?0???
Ann	0111100010	1120012102	1111111111	0111101111	1101000000	0000001000	0000001000	0000000000	0001100000	0101000011	1011000001	0001100?20	1111111110	10110111
Ech	0111100010	1120012102	1112111111	0111101111	1101000000	0000001000	0000000000	0000000000	0001100000	0101000011	1011000000	0001020?2?	????????	????????
Mol	0111100010	1120012102	1111111110	1111101111	1010110111	1000000000	0000001000	0000000100	0001000000	0101000011	1011000001	0000000?20	1111111110	101?0???
Sip	0111100010	1120012102	1112111110	1111101111	1010111110	0001000000	0000001000	0000000000	0000000000	0101000011	1011000000	0000100?22	???????2??	????????
Chd	0111100010	1120012102	1102111100	0101110000	1000100002	0000000000	0000001002	0000000100	0000000000	0101011011	1000000001	0000011110	1101111110	01001110
Bcm	0111100010	1110012102	1102111100	0101110000	1000100002	0001010000	0000001011	0000000000	0000000000	0101?30011	1000000001	0000010121	0111111110	01001111
Pty	0111100010	1120012102	1101111100	0101110000	1000100002	1010110000	0000001011	0000000000	0000000000	0101011111	1000010001	0000011?21	0117?21110	01001?11
Spe	0111100010	1120012102	1102111?00	0101110000	1000100002	1010110000	0000001011	0000000000	0000000000	010101011?	1000010001	0000021???	????????	????????
Har	0111100010	1120012102	1101111100	0101110000	1000100002	0000110000	0000011011	1000000000	0000000000	0101010111	1000010001	0000011???	??2??21110	01001???
Pte	0111100010	1110012102	?102111?00	0?01??0000	1000100002	0000111000	0000011011	1000000000	0000000000	0101010111	1000010001	0000010???	????????	????????

tionally considered for those latter data sets by performing "noconverse" constraint analyses in PAUP* (i.e., to find the shortest trees containing these particular clades). For both the converse and noconverse constraint searches for the large 18S and combined data sets, the two-part search strategy described above was employed, except that we swapped on only the first 1000 trees (MAXTREES=1000) in the second part of each search. As in the unconstrained searches, each search took about 12 h to complete on a Power Macintosh G3, rendering the calculation of bsi values for each node of our supported consensus infeasible. We could not use TNT to speed these estimates because it did not support constraint searches.

We additionally used PAUP* to calculate bootstrap proportions (Felsenstein 1985) for all nodes of our morphological result, based on 1000 bootstrap replicates. Attempts to employ "fast heuristic" methods (e.g., employing more limited branch swapping) to estimate bootstrap proportions for our larger 18S data set proved unsatisfactory. When these faster search methods were employed, generally fewer than 5% of the replicates found trees as short as could be estimated with the slower two-part method described above. Therefore, any bootstrap consensus estimate would be lacking in value.

Except for the morphological analysis, we chose the "phylogram" output of PAUP* (horizontal branch length proportional to the number of changes as optimized by PAUP*) for the display of our results. This format was preferred because it conveys useful information about differences in the relative rate of change across all taxa; however, such branch length estimates lose relevance when applied to a consensus topology (R. Olmsted, pers. comm., unreferenced). For this reason, we arbitrarily selected the first of all minimum length trees found for each figure. In order to partially display the range of minimum length trees found, we added black boxes to those nodes that were not supported in the strict consensus.

We also performed a series of "random outgroup" analyses (Wheeler 1990; Stiller and Hall 1999) in order to assess the possibility that a portion of our ingroup, Bilateria, might be artifactually attracted to available outgroups. The analysis is conducted by performing replicate phylogenetic analyses of the ingroup plus one pseudo-random sequence declared as outgroup. If a high percentage of such replicate random outgroup analyses (with a different random outgroup in each case) are rooted to a position at or close to the observed rooting (i.e., with available outgroups), then it is reasonable to suspect that the available outgroups are behaving as if they were effectively random sequences. An ideal outgroup would instead be one that has retained plesiomorphic similarity to the last common ancestor of the ingroup, so that rooting is based on historical resemblance. An outgroup could lose its value if it has diverged substantially since it last shared a common ancestor with the ingroup, or if it is too distantly related, with no closer outgroups having survived extinction. There has been little study of why completely random sequences should be attracted to particular portions of the ingroup network, but empirically these factors appear to involve more than just the branch length, although this is clearly important.

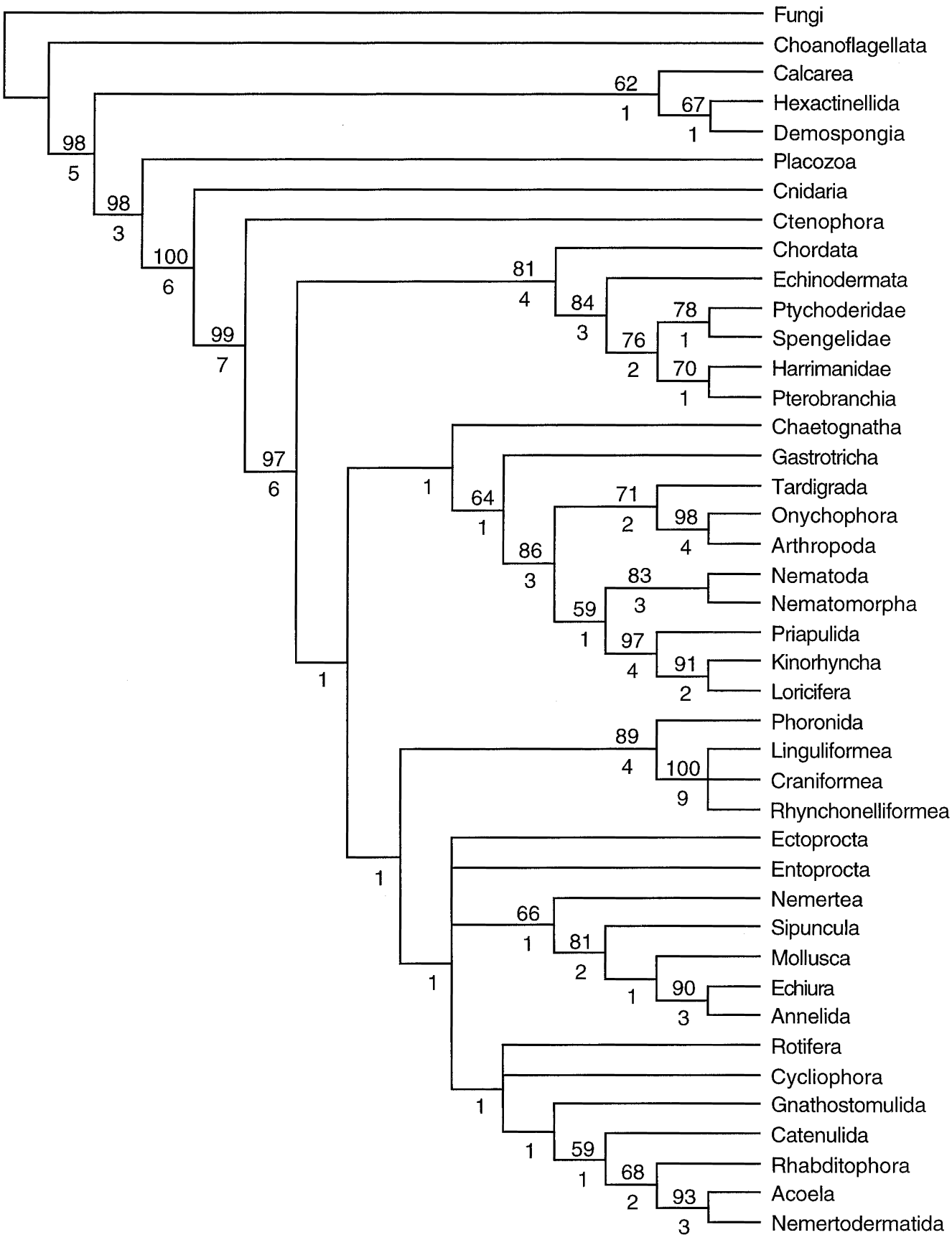
To perform random outgroup analyses with all outgroup (i.e., nonbilaterian) sequences deleted, we used software by one of us (D. J. E., unpubl.) to generate three sets of 1000 random sequences, and to automate the searches and parsing of search output in order to make these analyses practical. These three sets of random sequences differed in their average base composition of the random sequences. One set had, on average, equal base composition (GC/AT = 1.0). The other two had GC- or AT-biased composition, on average equal to the maximum observed bias across the 304 sequences in our data set, as calculated with software by Eernisse (2000; see also Fig. 5). These average GC/AT biases were 1.13 and 0.88, respectively. Each set of 1000 random sequences was appended to the data matrix, and 1000 search blocks were constructed to differ only in which of the random sequences was included. Because the neighbor-joining (NJ) tree ("distance = HKW85") for our 18S data set was observed to approximately correspond to our parsimony-based estimate and because the NJ tree was so much faster to calculate, this was employed for all random outgroup analyses reported. After the 1000 separate NJ trees had been computed and appended to a single tree file (with "outroot=para"), this tree file was parsed using software by Eernisse (2000) to compile, by percentage, which ingroup lineages of one or more sequences was basal. This entire procedure was then repeated for the other two sets of random outgroup sequences with biased base composition.

RESULTS

Morphological analysis

Maximum parsimony analysis of 138 characters (Table 2; Appendix 1) in 42 taxa produces the strict consensus tree shown in Fig. 1. We found 14 shortest trees at 245 steps with a C. I. of 0.60, a R. I. of 0.82, and an R. C. of 0.50. A most striking result of this analysis is the relatively strong support for the topology of (Outgroup taxa (Porifera (Placozoa (Cnidaria (Ctenophora, Bilateria))))), in agreement with other morphological analyses (Ax 1996; Nielsen et al. 1996; Zrzavý et al. 1998). In fact, constraining ctenophores to be basal epitheliozoans (see Table 1 for taxonomic nomenclature) required 13 additional steps (analysis not shown). Our results also suggest that Porifera and Silicea are monophyletic, although a constraint analysis (not shown) revealed only one additional step is required for Calcarea to be the sister-taxon of Epitheliozoa, in accordance with many molecular studies (see Introduction and below).

Within the bilaterians, deuterostomes are monophyletic with echinoderms and hemichordates sister-taxa. This is contrary to all previous morphological analyses, which have suggested that hemichordates (or enteropneusts if "Hemichordata" was paraphyletic) are allied with chordates and not to echinoderms. Furthermore, there is the surprising result that Enteropneusta is paraphyletic with harrimaniids, the sister-taxon of pterobranchs. Although the paraphyly of both Hemichordata (e.g., Schram and Ellis 1994; Peterson 1995; Nielsen et al. 1996) and Pterobranchia (Cripps 1991) has been suggested, we are unaware of anyone having previously suggested that Enteropneusta is paraphyletic based on morphological considerations alone (but see Cameron et al. 2000 for a similar result based on 18S data).


Fungi
Choanoflagellata
Calcarea
Hexactinellida
Demospongia
Placozoa
Cnidaria
Ctenophora
Chordata
Echinodermata
Ptychoderidae
Spengelidae
Harrimanidae
Pterobranchia
Chaetognatha
Gastrotricha
Tardigrada
Onychophora
Arthropoda
Nematoda
Nematomorpha
Priapulida
Kinorhyncha
Loricifera
Phoronida
Linguliformea
Craniformea
Rhynchonelliformea
Ectoprocta
Entoprocta
Nemertea
Sipuncula
Mollusca
Echiura
Annelida
Rotifera
Cycliophora
Gnathostomulida
Catenulida
Rhabditophora
Acoela
Nemertodermatida

Contrary to previous morphological analyses, phoronids and brachiopods do not group with deuterostomes, but within the protostomes. Although both the monophyly of Protostomia and the basal position of lophophorates within the lophotrochozoans are weakly supported, we note that a separate analysis constraining lophophorates to be in a clade with deuterostomes required two additional steps (analysis not shown). Moreover, our analysis never groups ectoprocts with the lophophorates, consistent with Nielsen's arguments (summarized in Nielsen 1995; Nielsen et al. 1996). Within the brachiopods, although there is strong support for brachiopod monophyly, there is no resolution amongst the three main brachiopod lineages, thus little can be said based solely on our morphological analysis regarding the mono- or paraphyly of Inarticulata.

As in Eernisse et al. (1992) and Zrzavý et al. (1998), we found the conventional grouping "Articulata" to be polyphyletic, such that Annelida was nested within Eutrochozoa and Arthropoda was nested within Ecdysozoa. Constraining annelids to be in a clade with the panarthropods required 28 additional steps (not shown). Within Eutrochozoa, Nemertea is the sister-taxon of Neotrochozoa (Table 1), and within Neotrochozoa there is relatively strong support for an Annelida + Echiura sister grouping. However, our selection of "Annelida" as a terminal taxon does not allow for the possibility that echiurans could be derived polychaete annelids as argued by Nielsen (1995) and McHugh (1997). Eutrochozoa, Entoprocta, Ectoprocta, and Platyzoa comprise the weakly supported Spiralia. Although there is little resolution within this clade, the analysis does suggest that flatworms are monophyletic with both Euplathelminthes and Acoelomorpha monophyletic subgroups. Constraining acoels to be basal bilaterians required nine additional steps (not shown). Flatworms are then weakly supported as the sister-taxon of gnathostomulids, and this clade groups with rotifers and cycliophorans in an unresolved polytomy.

This analysis provides relatively strong support for the monophyly of Cycloneuralia, Scalidophora, Nematoida, and Panarthropoda. Within the Scalidophora kinorhynchs are the sister-taxon of the loriciferans, and within the panarthropods onychophorans are the sister-taxon of arthropods. As for the annelid/echiuran situation above, our choice of "Arthropoda" as a terminal taxon precluded testing the possibility that onychophorans are actually nested within the arthropods, as has been suggested by some molecular studies (e.g., Ballard et al. 1992). Our results place gastrotrichs as the sister-taxon of Panarthropoda + Cycloneuralia, and weakly suggest that chaetognaths are then basal to this clade.

Thus, unlike most previous morphological analyses, our analysis does not support a deuterostome affinity of lophophorates, nor does it support an annelid affinity with arthropods. Furthermore, we find considerable support for the monophyly of Ambulacraria with Enteropneusta paraphyletic, and for the monophyly of Acrosomata. These results are independent of the "molecular characters" (Characters 117–138, Appendix 1) considered, although the lophophorates are no longer lophotrochozoans, but part of a large basal bilaterian polytomy (results not shown). Based on the complete matrix, there is some suggestion that chaetognaths are basal ecdysozoans, that lophophorates are basal lophotrochozoans, and that acoel flatworms are members of Platyhelminthes. Finally, we found weak but notable support for the monophyly of Spiralia, corresponding to those taxa with some members having typical quartet spiral cleavage.

18S rDNA sequence analysis

Figure 2 depicts one of the 960 trees supported by our heuristic parsimony analysis of 304 18S sequences. Bremer support indices for selected nodes (abbreviations listed in Table 1) are given in Table 3. Contrary to the morphological analysis, but in accord with other 18S studies (see Introduction), 18S supports a basal position of ctenophores relative to cnidarians and placozoans. In fact, some of the shortest trees, including the one in Figure 2, support calcareous sponges as more closely related to placozoans and cnidarians than are ctenophores, making both Acrosomata and Porifera polyphyletic, whereas other minimum length trees at least support Eumetazoa as monophyletic (see also Fig. 2 legend and Table 3). Furthermore, "Silicea" is paraphyletic with demosponges more closely related to the calcareans + epitheliozoans than to hexactinellids, although the Silicea hypothesis requires only one additional step (Table 3).

As expected from all previous 18S analyses, there is strong and unambiguous support for the monophyly of Bilateria. Within Bilateria both Deuterostomia and Ecdysozoa are supported, and within deuterostomes "enteropneusts" are the sister-taxon of echinoderms (no complete pterobranch sequences were available at that time for inclusion, but see Cameron et al. 2000 for analysis of a complete sequence). Within ecdysozoans, the scalidophorans are basal, and chaetognaths are the sister-taxon of nematomorphs. Furthermore, this clade is then the sister-taxon of tardigrades, and finally this clade is the sister-group of nematodes, thus (Nematoda (Tardigrada (Nematomorpha, Chaetognatha))). Therefore, the monophyly of Cycloneuralia, Nematoida, and Panarthropoda is not found with 18S. Finally, the gastrotrich is not supported as an ecdysozoan, but as a basal bilaterian.

Fig. 1. Morphology analysis. Strict consensus of the 14 most parsimonious trees (length = 245; CI = 0.60; RI = 0.82; RC = 0.75) based on 138 morphological characters (Table 2, Appendix 1). Numbers above the nodes are bootstrap values (1000 replications); numbers below the nodes are Bremer support indices (bsi). See text for discussion.

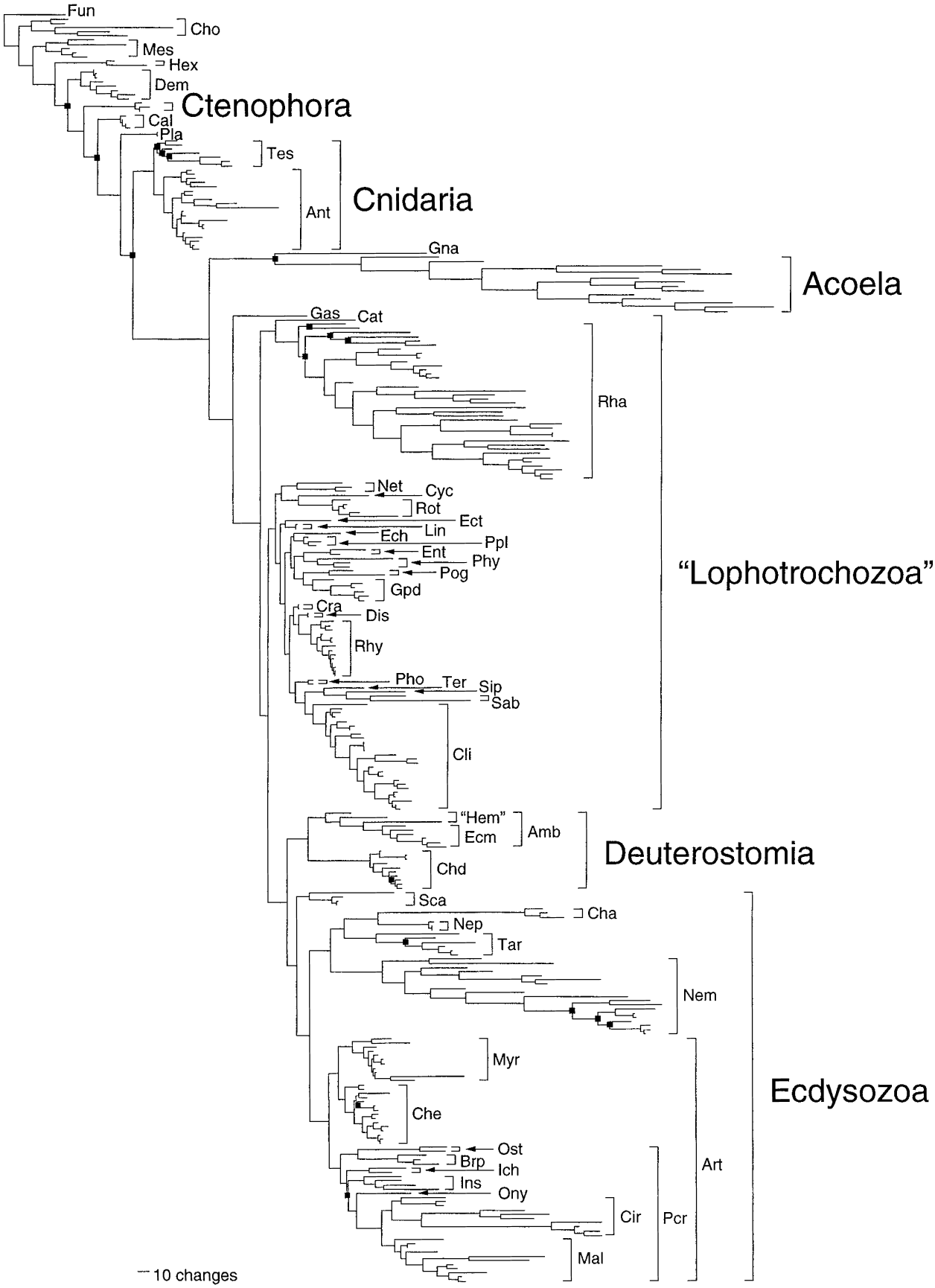

Fun
Cho
Mes
Hex
Dem
Ctenophora
Cal
Pla
Tes
Ant
Cnidaria
Gna
Acoela
Gas
Cat
Rha
Net
Cyc
Rot
Ect
Lin
Ech
Ppl
Ent
Phy
Pog
Gpd
Cra
Dis
Rhy
Pho
Ter
Sip
Sab
Cli
"Lophotrochozoa"
"Hem"
Ecm
Amb
Chd
Deuterostomia
Sca
Cha
Nep
Tar
Nem
Myr
Che
Ost
Brp
Ich
Ins
Ony
Art
Cir
Pcr
Mal
Ecdysozoa
10 changes

Table 3. Constraint Analyses

Test Clade	Morphology	18S rDNA	Combined	NoAGG
Unconstrained Length	245	10556[1]	10862	9304
Metazoa	5[2]	7	16	15
Porifera	1	−5[3]	−2	−3
Silicea	1	−1	0[4]	1
Epitheliozoa	3	0	5	5
Eumetazoa	6	−1	4	1
Acrosomata	7	−14	−4	1
Bilateria	6	24	10	19
Deuterostomia	4	5	3	4
Ambulacraria	3	3	5	6
Protostomia	1	−8	−8	4
Lophotrochozoa	1	−14	−8	6
Lophophorata	4	−1	4	5
Brachiopoda	9	−2	6	9
Spiralia	1	−18	−13	−4
Platyzoa	1	−11	−11	−5
Trochozoa	0	−1	1	1
Eutrochozoa	1	−5	1	1
Neotrochozoa	2	−6	3	3
Cyn + Par	3	−6	−3	−4
Cyn + Par + Cha	−1	1	3	4
Cyn + Par + Cha + Gas	1	−10	−8	NA
Cycloneuralia	1	−8	−4	−5
Scalidophora	4	5	7	7
Nematoida	3	−5	−3	−4
Panarthropoda	2	−2	−1	1

[1]For the larger data sets with 18S rDNA included (304 or 308 taxa), length for the corresponding constraint or converse constraint analyses was determined by the same two-part heuristic search procedure employed for the unconstrained searches (see text). The first part, which involved 100 replicate random addition sequence searches keeping no more than 10 trees per replicate, found an island of minimum length trees on average only 1.0, 3.6, or 6.1 replicates out of 100, for the 18S, Combined, and No AGG constraint searches listed, respectively. This difference in search effectiveness was significant for all three possible pairwise comparisons, 18S versus combined ($p < 0.01$), 18S versus No AGG ($p << 0.001$), and combined versus No AGG ($p < 0.05$); paired one-tailed t-test of means). The second part of the search started by swapping on all minimum length trees from the first part, swapping on only the first 1000 trees found. This was effective at finding shorter trees than any of the first part replicate searches in 21%, 25%, and 4% of these searches for 18S, combined, and No AGG constraint searches, respectively. These results indicate that the search strategy employed was more effective than normal default searches. Still, values may be overestimates in some of the cases (i.e., more exhaustive searches could lead to a reduction in the values estimated).

[2]A positive value indicates that the taxon was supported by the analysis, and this value is equivalent to the Bremer support index for the corresponding node, searched for with a converse constraint in PAUP*.

[3]A negative value indicates the number of additional steps required in order to satisfy the specificed constraint, searched for with a normal (noconverse) constraint in PAUP*.

[4]Zero indicates that the taxon is one of multiple resolutions of minimal length supported by the data set.

Although Arthropoda was a terminal taxon in our morphology analysis, this was not the case for the 18S analysis. The monophyly of arthropods is found except that the sequence purported to be from an onychophoran (G.-S. Min, pers. comm., unreferenced) lies within Pancrustacea. Within arthropods, separate constraint analyses (not shown) of our 18S data set likewise did not support Pancrustacea (see Zrzavý et al. 1998; Shultz and Regier 2000), but only because of the tendency for the purported onychophoran to fall within this group. If this sequence was considered a pancrustacean, then the support for the monophyly of Pancrustacea was substantial (bsi = 6). In contrast, Atelocerata (see Edgecombe et al. 2000) required 11 extra steps (with or without the onychophoran) for our data set, which is also the number

Fig. 2. 18S rDNA analysis. Phylogram of one of the 960 shortest trees found with parsimony criterion (length = 10556; CI = 0.17; RI = 0.69; RC = 0.12). The heuristic two-part PAUP* search (see text) was based on inclusion of all 844 parsimony-informative sites remaining after exclusion of ambiguously aligned regions, with equal character weighting, for 304 selected 18S rDNA sequences (Appendix 2). Black boxes are placed at nodes that collapse in the strict consensus tree (not shown). For example, the strict consensus would collapse the node in this particular tree supporting Calcarea (Cal) as sister-taxon to Eumetazoa (unlabeled) because 48 of the other 959 trees favor Ctenophora as sister-taxon to Eumetazoa instead. Abbreviations are listed in Table 1. See text for discussion.

of extra steps required for a myriapod + crustacean clade. The shortest trees with a monophyletic Crustacea were three steps longer than the shortest unconstrained trees.

A major difference between the morphological (Fig. 1) and 18S analyses (Fig. 2) concerns Lophotrochozoa. Although both analyses agree for the most part on its taxonomic composition, the 18S data set suggests that Lophotrochozoa is paraphyletic with acoel flatworms and gnathostomulids the most basal bilaterian clades. Furthermore, even the monophyly of several lophotrochozoan phyla is not found including not only "Platyhelminthes," but also "Brachiopoda," "Mollusca," and "Annelida." Surprisingly, the echiuran groups with the polyplacophorans. Likewise, the sipunculan groups with two members of the polychaete taxon Sabellida, whereas the pogonophorans do not, against the morphological considerations of Bartolomaeus (1995) and Rouse and Fauchald (1997) who argue that pogonophorans and sabellids are closely related. Finally, the rotifers group with the cycliophoran.

The morphological and molecular analyses are congruent in several important respects. Both support: (1) the monophyly of Bilateria; (2) the groupings of annelids with other eutrochozoans and arthropods with other ecdysozoans; (3) the alliance between lophophorates and eutrochozoans; (4) the monophyly of Deuterostomia; (5) the monophyly of Ambulacraria; (6) the close relationship between kinorhynchs and priapulids; and (7) the close relationship between onychophorans and arthropods. There are three major discrepancies between these two analyses. First, morphology strongly suggests that ctenophores are the sister-taxon of bilaterians, whereas 18S suggests that cnidarians are the sister-taxon of bilaterians. Second, morphology suggests that Porifera is monophyletic, whereas 18S suggests that Porifera is para- or polyphyletic. Third, morphology suggests that Lophotrochozoa is monophyletic with acoel flatworms grouping with the other platyhelminth taxa and the bilaterian root lying between deuterostomes and protostomes, whereas the 18S root renders Lophotrochozoa as paraphyletic with acoel flatworms and gnathostomulids as basal bilaterians.

Combined analysis

Zrzavý et al. (1998; see also Eernisse and Kluge 1993; Littlewood and Smith 1995; Lafay et al. 1995; Nixon and Carpenter 1996; Littlewood et al. 1997) argued that a combined data set analysis has advantages as an overall estimate of phylogeny in the context of character congruence testing. Among its reported advantages, there is no *a priori* decision regarding the priority of one class of characters over another. Thus, this approach allows for the maximum possible test of congruence among all available data, not just subsets of the data. In this vein, we decided to combine the two data sets, with all characters weighted equally. This minimized our decisions (and explorations) of weighting, but we recognize that, consequently, complex characters such as the acquisition spiral cleavage is given equal consideration as a single hypothesized "event" as for a change from one nucleotide state to another. We maintain this is a valid first approach because the alternative of increasing the weight of such a complex character *a priori* will ultimately allow us to conclude less *a posteriori* about its congruence (or lack of it) with other evidence.

Employing this "total evidence" approach, we present our combined analysis in Figure 3. The bsi's for selected test nodes are presented in Table 3. The addition of morphology has a profound effect upon several parts of the 18S tree, while having little apparent effect upon other regions where we detected conflict between morphology and 18S (see Discussion section for consideration of the considerable phylogenetic congruence between the two data sets). First, the addition of morphological characters results in the monophyly of Hemichordata and several of the major lophotrochozoan taxa. In contrast to the 18S analysis, the combined analysis suggests that Brachiopoda, Mollusca, and Annelida (including Echiura and Sipuncula) are all monophyletic. Furthermore, the topology of the lophotrochozoan taxa resembles the morphological analysis alone: (Brachiopoda (Nemertea (Mollusca, Annelida))) with echiurans and sipunculans nested within the annelids proper. Like most of the shortest morphological trees, entoprocts are the sister-group of the Eutrochozoa (i.e., Trochozoa is monophyletic). Finally, as in Figure 1, phoronids are the sister-taxon of the brachiopods, but here Inarticulata is monophyletic and "Linguliformea" is paraphyletic.

The second significant change is that ctenophores are now the sister-taxon of cnidarians, placozoans, and bilaterians. Hence, Epitheliozoa is now monophyletic (bsi = 5). Calcarea is the sister-taxon of Epitheliozoa, and a monophyletic Silicea is the sister-taxon of Calcarea + Epitheliozoa.

Despite the addition of morphology, which when analyzed separately suggests otherwise, the combined analysis still suggests that acoelomorphs are basal bilaterians and that "Lophotrochozoa" is paraphyletic. The combined result also still has bilaterians grouping with placozoans plus cnidarians and not with the ctenophores. Thus, the inclusion of morphological data did little to change these particular results, although this is hardly surprising given that Lophotrochoza is only weakly supported for the morphological data set (bsi = 1). Therefore, the most apparent conflict between morphological and 18S data is not the position of arthropods with respect to annelids, nor the phylogenetic position of lophophorates, but where Bilateria is rooted and which taxon is its closest outgroup.

Rooting the bilaterians

Figure 4 considers an unrooted network of bilaterians (our ingroup) derived from Figure 2, with each bilaterian clade color coded to show that each could be monophyletic so long as the root is not located within any one of the subgroups.

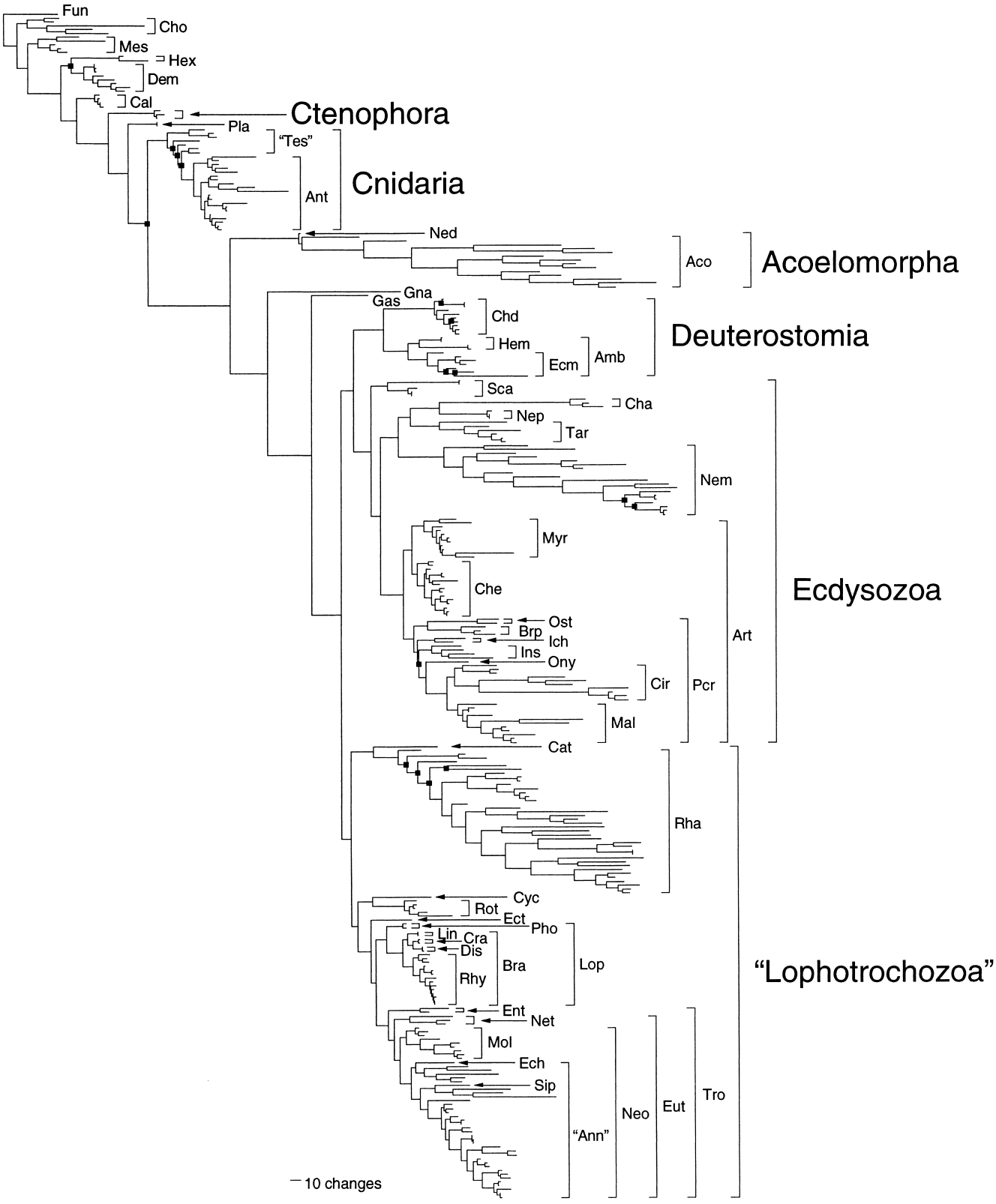


Fig. 3. Combined parsimony analysis of morphology and 18S rDNA. Phylogram (length = 10862; CI = 0.18; HI = 0.82; RI = 0.74; RC = 0.14) of one of the first 2500 minimum length trees found with heuristic two-part PAUP* search (see text). Data sets described in legends for Figures 1 and 2 are combined here, for a total of 982 informative characters. The taxa are those 304 sequences analyzed for 18S rDNA (Fig. 2), with morphology characters appended (see text), and four additional taxa with morphology characters only, for 308 total taxa. The strict consensus of first 1000 trees was identical to that of first 2500 trees found in PAUP*, as well as that of a more extensive search accomplished with TNT. As in Fig. 2, black boxes are placed at nodes that collapse in this estimated consensus. Abbreviations as in Figure 2. See text for discussion.

This result is remarkable, a striking confirmation of the hierarchical pattern in the 18 data set, considering how unlikely these associations that are congruent with morphology might occur by chance alone. With this network, there are four possible rooted topologies (three resolved and one unresoved) rendering each subgroup monophyletic. Our morphology-only result (Fig. 1) corresponds to one of these four possible roots, specifically the one with deuterostomes and protostomes as sister-taxa (labeled "M" on Fig. 4). Also labeled on Figure 4 are the 18S-only ("18S") (Fig. 2) and combined data set ("M + 18S") (Fig. 3) supported roots, each of which falls within "Lophotrochozoa," thus rendering it paraphyletic. It is immediately obvious that the placement of the 18S root is among some of the longest-branched taxa considered (gnathostomulid and acoel flatworms). On the other hand, the position of the morphology-based root is nested deep inside the network on a relatively short internal branch.

The placement of the 18S root at the base of these long-branch sequences is immediately suspicious. Many workers have demonstrated the unreliability of the basal position of long-branched taxa (e.g., Aguinaldo et al. 1997; Stiller and Hall 1999; Philippe et al. 2000). Such long- branch attraction remains a strong possibility in this case despite (or perhaps explaining) the apparent statistical support for this rooting (Ruiz-Trillo et al. 1999). Hence, we decided to examine this rooting further by determining where, on average, a replicate random sequence roots the bilaterian network (see Materials and Methods). The results are summarized in Table 4. It is clear that, irrespective of the average GC/AT ratio of the random outgroup, a vast majority of the random outgroups will attach to an acoel or gnathostomulid. If both of these taxa are deleted from the analysis, the majority of the random outgroups will then group with particular nematodes, and if nematodes are deleted they will then group with particular

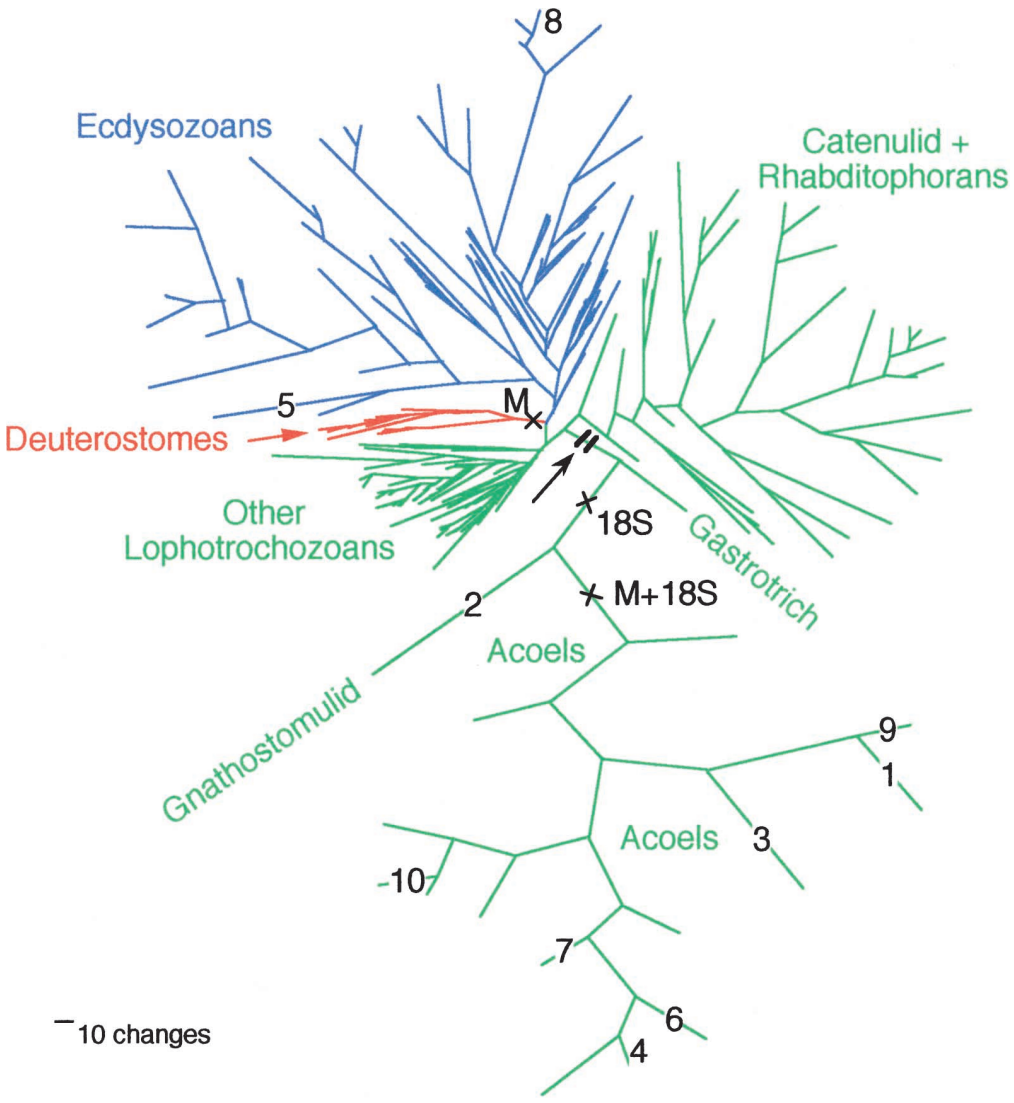


Fig. 4. Unrooted phylogram network from parsimony analysis of 248 ingroup bilaterian 18S rDNA sequences only (L = 9331). This is one of first 2500 minimum trees found in PAUP*. The three major bilaterian groups are color coded: blue = Ecdysozoa; green = Lophotrochozoa; and red = Deuterostomia. Our three respective observed roots based on included outgroups (compare Figs. 1–3) are indicated on the figure: morphology (M) between deuterostomes and protostomes; 18S rDNA (18S) at the base of the acoel branch; and the combined analysis (M + 18S) at the node connecting acoels and the gnathostomulid. The arrow indicates the branch that is deleted in the combined-No AGG analysis (Fig. 6). Also shown on the figure are the top 10 attractors to 1000 random sequences with on average equal base composition, employed separately as outgroups in replicate searches. Higher-level group percentages are summarized in Table 4. The top 10 attachment points for the random outgroups were all terminal branches, numbered here from top (1) to tenth (10) most commonly observed.

Table 4. Random outgroup placement within bilaterians[1]

GC/AT	1.0	1.13	0.88	1.0	1.0	1.0
Acoels	58.5[2]	65.2	54.1	X[3]	X	X
Gnathostomulan	12.8	12.2	12.9	31.6	X	X
Nematodes	10.4	6.7	10.8	30.2	54.8	X
Crustaceans	6.5	6.4	10.3	17.3	22.7	41.6
Rhabditophorans	6.5	5.2	7.0	12.7	14.0	43.1
Chaetognaths	1.6	1.0	1.8	3.0	3.1	6.0
All others	3.7	3.3	3.1	5.2	5.4	9.3

[1]Note that, in a typical replicate, the random outgroup was attracted to a single terminal branch, not as sister-taxon to the group reported above. Summaries reflect monophyletic groups supported by our analyses. Certain members within each group typically accounted for the majority of attractions tallied for the group (see Fig. 4).

[2]Percentage of 1000 replicate searches that an acoel (in this case) grouped with the random outgroup.

[3]Pruned from analysis.

crustaceans or rhabditophorans (Table 4). The specific taxon of each of these groups which attracts the random outgroup is indicated with a number on Figure 4 ranging from 1 (the taxon which attracts the random outgroup the most) to 10 (the lowest indicated on the figure). It is of interest that most of these taxa have been hypothesized to be basal bilaterians based on 18S analyses (e.g., acoels, Ruiz-Trillo et al. 1999; nematodes, Winnepenninckx et al. 1995a; arthropods, Lake 1989; rhabditophorans, Turbeville et al. 1992; Riutort et al. 1993; chaetognaths, Telford and Holland 1993). We would suggest that because acoels and gnathostomulids have a very high propensity to attract a random DNA sequence, and because the nearest outgroups are separated from Bilateria by a long internal branch, we cannot ignore the possibility that acoels/gnathostomulids are resolved as basal bilaterians for spurious reasons.

Another potential problem is explored in Figure 5. On the top of the figure is a rotated and re-sized version of the same 18S phylogram depicted in Figure 2. Below this tree are three sequence parameters arranged in a corresponding order, with sponges on the left and ecdysozoans on the right. Each of the three parameters can be compared against other taxa by lining up the histogram bars directly beneath a terminal branch on the tree. Acoels and gnathostomulids are "long branch," both as apparent from their phylogram branch lengths and also as estimated from their tallies of unique sites (see also Fig. 4). It is also striking how similar their GC/AT ratio is to the non-bilaterian taxa in general, in stark contrast to most other bilaterians (a subset of rhabditophoran flatworms are the exception). Thus, not only do acoels and gnathostomulids attract random sequences in an inordinate percentage of the time and also display clear indications of being "long-branch" taxa, they also have a GC/AT ratio that strongly resembles that of non-bilaterians. Although this latter similarity could be historical (i.e., representing the plesiomorphic condition for bilaterians), it could also be a homoplastic similarity that is largely responsible for their basal position, in conflict with our morphological result. Interestingly, acoels and gnathostomulids also attracted a high percentage (combined 67%) of our AT-biased random outgroups (Table 4), suggesting that their GC-biased tendencies cannot entirely explain their disproportionate attraction to random outgroups.

Combined analysis without acoels, gnathostomulids, and gastrotrichs

Because of the strong possibility that the basal position of acoels and gnathostomulids within the bilaterians according to 18S data is artifactual, we decided to reanalyze the combined data set after pruning these two taxa. The only sampled gastrotrich sequence also joined this branch in the unrooted tree shown in Figure 4, but was a different case because the sequence was not attracted to random outgroups. We thus reanalyzed our combined data set with acoelomorphs and gnathostomulids excluded, with and without the exclusion of gastrotrichs. These newly excluded taxa (including gastrotrichs) are all those below the arrow in Figure 4. The results of this analysis are shown in Figure 6. The deletion of acoels, the gnathostomulid, and the gastrotrich, has two immediate effects upon the arrangement of the taxa. First, Lophotrochozoa (minus the excluded taxa) is now monophyletic, and is the sister-taxon of Ecdysozoa. Together, as Protostomia, they are the sister-taxon of Deuterostomia. Second, cnidarians are no longer the sister-taxon of the bilaterians. In fact, this analysis weakly supports (Table 3) the monophyly of Acrosomata (Table 1). One final and interesting difference is that tardigrades are now the sister-taxon of Arthropoda + Onychophora, thus Panarthropoda is now monophyletic. Inclusion of the gastrotrich resulted in the same topology amongst bilaterians as removal of all three taxa, but ctenophores are again supported as basal epitheliozoans as in Figure 3 (analysis not shown).

DISCUSSION

We have inferred the interrelationships among animal phyla using not only a new morphological data matrix but also a very large 18S data set. Both of these data subsets were analyzed separately, and then simultaneously generating a combined-data-set tree. A summary of these results is presented in Figure 7. Here, we discuss the congruence and conflict between these two data sets and the broader implications of this study. In particular, we emphasize the extent to which the evolutionary developmental biology of the latest common ancestor of bilaterians can be inferred, given our phylogenetic conclusions.

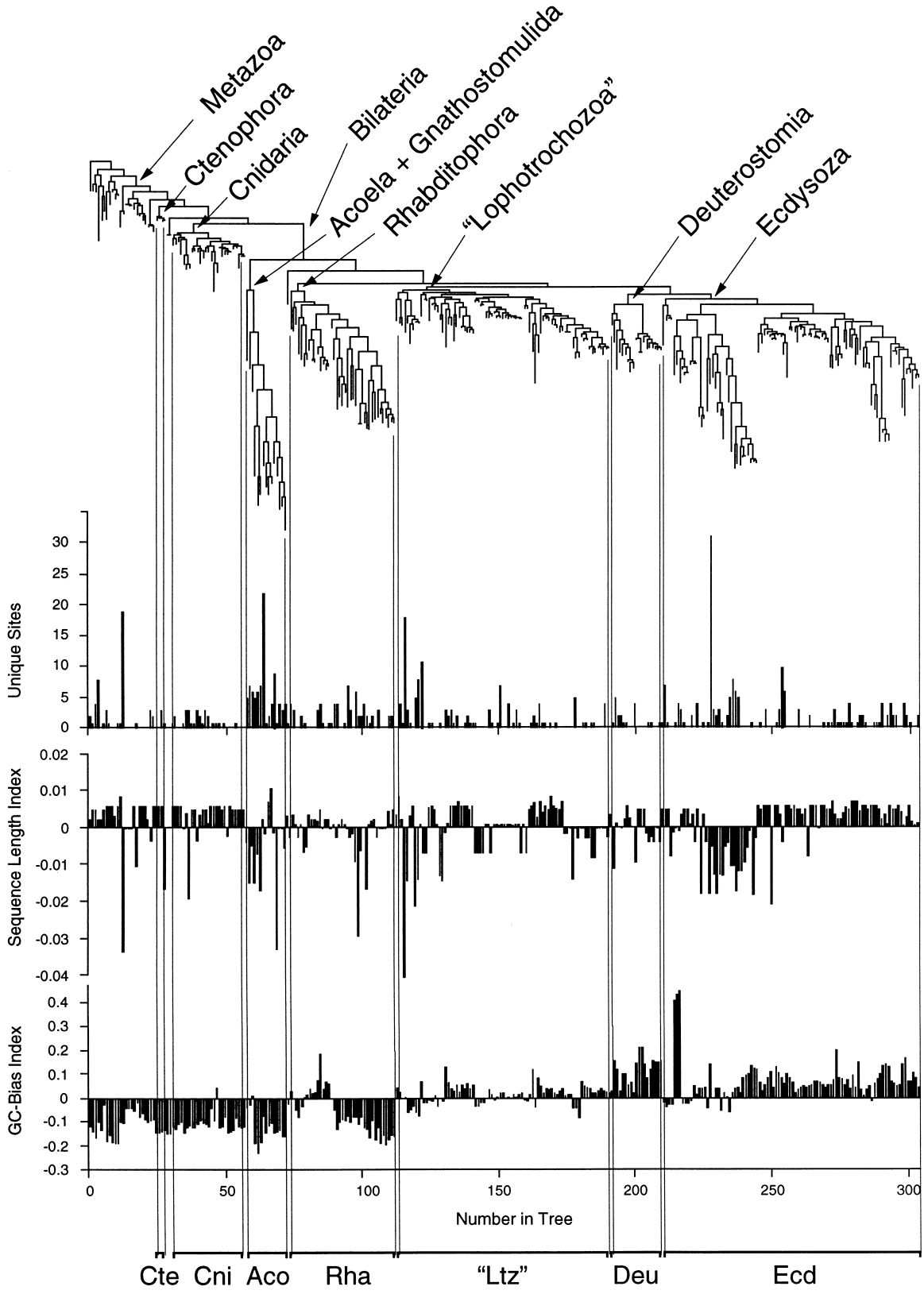


Fig. 5. Sequence attributes of 304 18S rDNA sequences. Phylogram at top corresponds to a rotated version of Figure 2. Directly beneath each sequence are values plotted for the following, as calculated with software by Eernisse (2000): (1) Number of autapomorphies compared to all other sequences; (2) Sequence length index for included sites only, excluding terminal or large internal unknown regions, with values normalized to the mean sequence length for all sequences; and (3) GC-bias index (GC/AT) of included sites only. Abbreviations are as in Table 1.

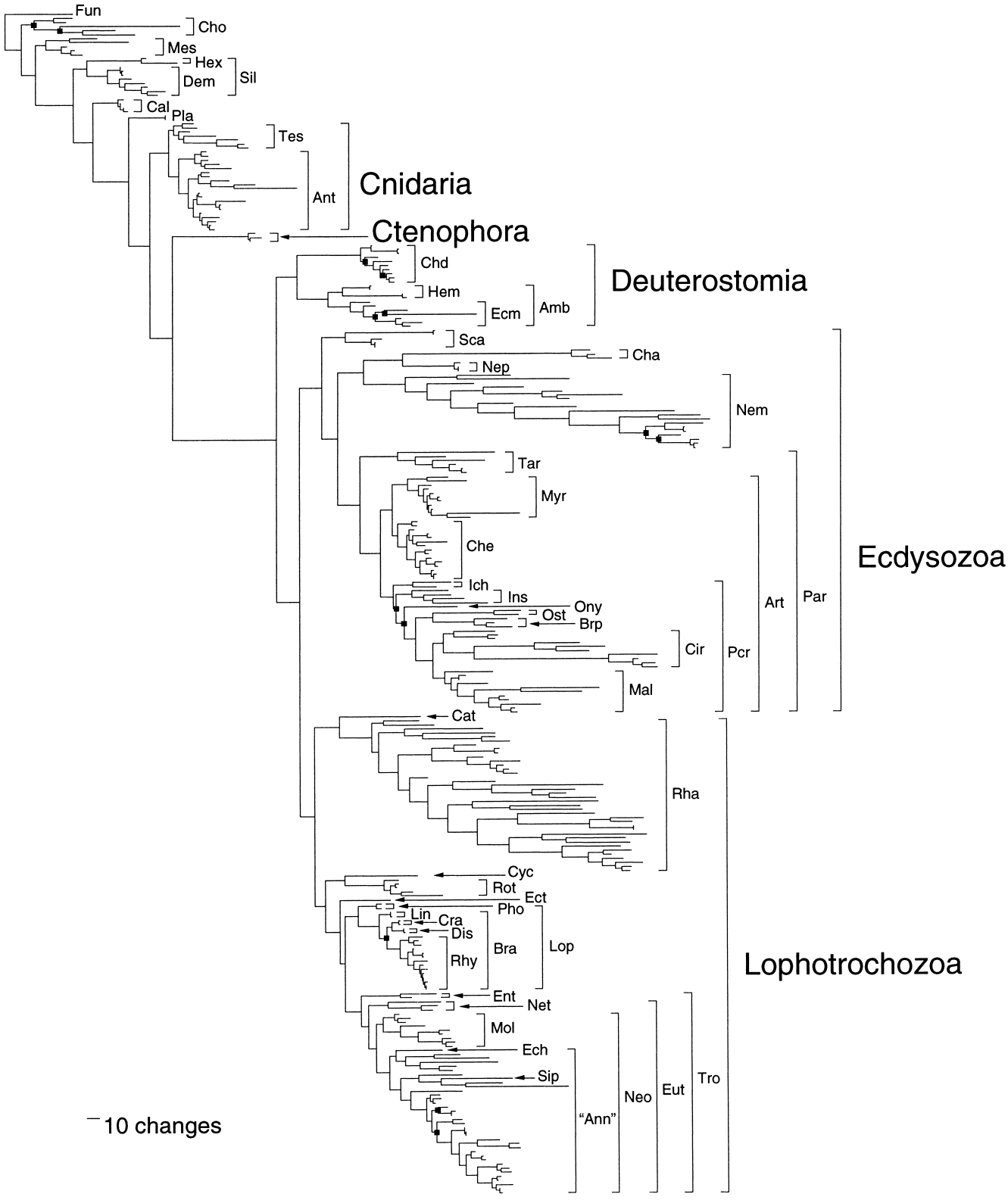


Fig. 6. Combined parsimony analysis of morphology and 18S rDNA, with 291 of the 308 taxa used for the analysis described in the legend for Figure 3, but without the 15 acoel flatworms, the gnathostomulid, or the gastrotrich (Combined-No AGG). Phylogram of one of the first 2500 minimum length trees found (length = 9304; CI = 0.20; HI = 0.80; RI = 0.75; RC = 0.15). Black boxes and abbreviations as in Figures 2 and 3. See text for discussion.

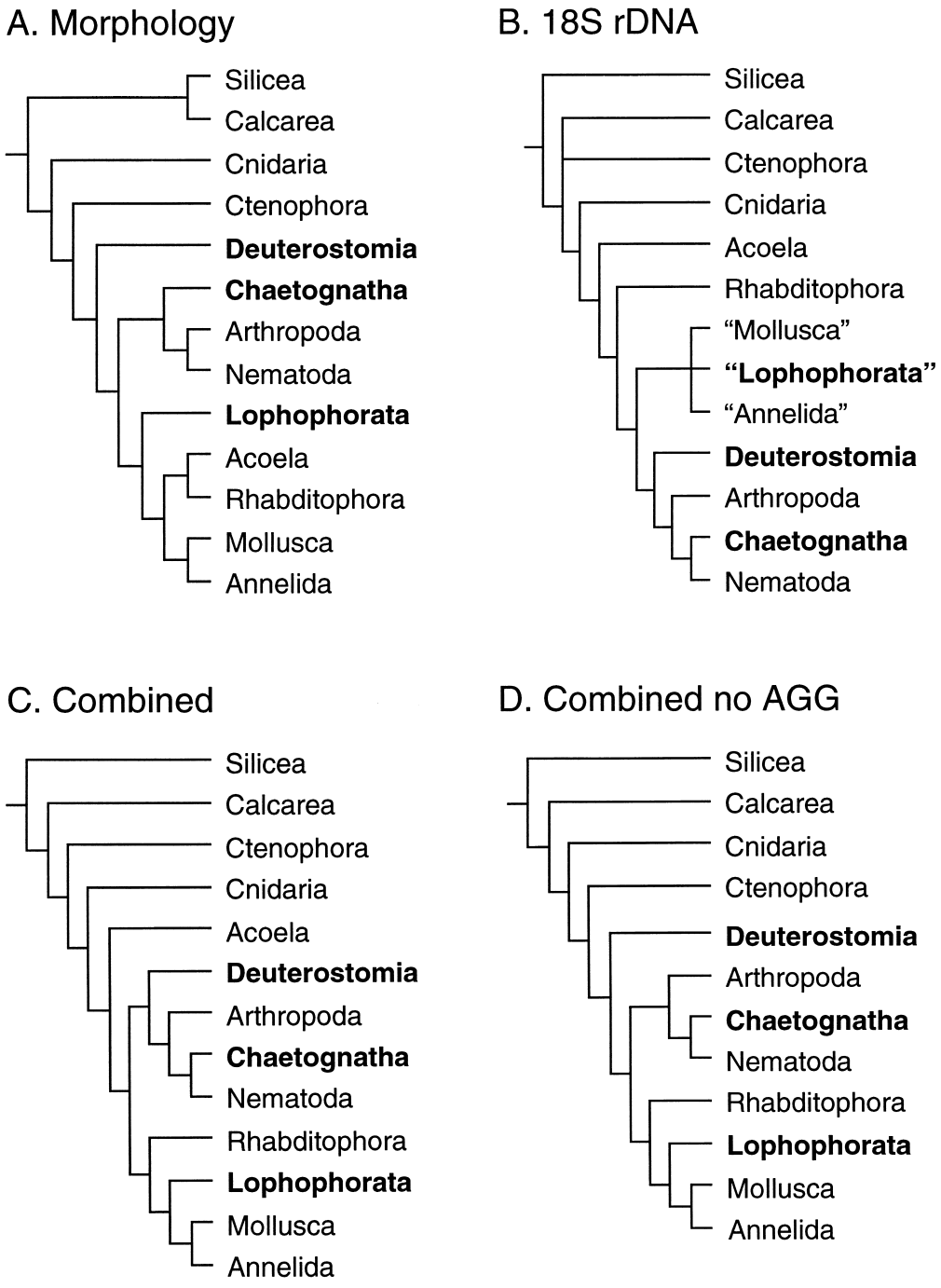


Fig. 7. Summary diagram for all four analyses. A. Morphology alone. B. 18S rDNA alone. C. Combined analysis. D. Combined analysis without acoels, gnathostomulid, or gastrotrich (No AGG). Taxa in bold are groups traditionally allied with one another because of their possession of a suite of deuterostome-like characters.

Morphology + 18S rDNA: congruence and minor conflict

A very interesting and potentially significant result of our analysis is that Porifera is not monophyletic. Although this has been suggested elsewhere based on molecular analyses (see Introduction for references), the unresolved position of ctenophores has confounded attempts to understand the basal portion of the metazoan tree. With ctenophores supported as epitheliozoans and sponges as paraphyletic, it becomes much clearer that the latest common ancestor of Calcarea + Epitheliozoa must have been constructed like a sponge complete with a water-canal system. Although it was most parsimonious, based on our morphology-alone matrix, to conclude that Porifera is monophyletic, it is worth pointing out that only calcareans and epithelializoans have cross-striated rootlets (Appendix 1, Character 9), which is optimized

as a synapomorphy of this taxon in the combined analyses. Furthermore, sponge paraphyly with Calcarea + Epitheliozoa is only one step longer for our morphology matrix than poriferan monophyly (see Results). This same topology, namely (Silicea (Calcarea (Placozoa (Cnidaria, Acrosomoata)))) was also found by Zrzavý et al. (1998).

The position of ctenophores has remained controversial because both types of data strongly support their respective positions. Morphology would suggest they are the sister-taxon of bilaterians (Fig. 1; see also Nielsen et al. 1996; Ax 1996; Schram 1997), whereas 18S has strongly supported Placozoa + Cnidaria + Bilateria. Previous 18S analyses have grouped ctenophores either with the calcareous sponges (e.g., Cavalier-Smith et al. 1996; Collins 1998; Kim et al. 1999) or at an unresolved position as in our own analysis (Fig. 2, Table 3). The former affiliation with calcareous sponges has even prompted some authors to reinterpret certain aspects of early animal evolution. For example, based on their 18S results, Cavalier-Smith et al. (1996) concluded that the origin of nervous systems must have been diphyletic. Instead, we found that the types of morphological characters supporting Acrosomata are numerous and compelling. These include the possession of the namesake, the acrosome with perforatorium (Characters 20 and 21), cleavage pattern and embryonic specification mechanisms (Character 28), endomesodermal muscle cells (Character 35), acetylcholine used as a neurotransmitter (Character 100), and a central Hox gene (Character 129). Given that the combined analysis without AGG results in a monophyletic Acrosomata (Fig. 6, Table 3), we feel that conclusions regarding the polyphyly of Acrosomata (and the resulting morphological implications) derived solely on 18S should be viewed with caution. Interestingly, Zrzavý et al. (1998) found a monophyletic Acrosomata even with acoels included in their combined analysis.

The subdivision of bilaterians, the monophyly of which is well supported in all analyses (Table 3), into two major groups, the deuterostomes and protostomes, has long been recognized. Nonetheless, the taxonomic composition of, and relationships within, these classical groupings have been radically revised based on a variety of molecular analyses as discussed in the Introduction (see above). Unlike most previous morphological investigations, both Ecdysozoa and Lophotrochozoa are realized with our morphology-alone analysis, albeit the latter is weakly supported. Ecdysozoa and Lophotrochozoa were both supported in our 18S analysis alone (Fig. 2) and in combination with morphology (Fig. 3). Hence, we found no conflict between 18S and morphology with respect to these aspects. Neither supports the monophyly of "Articulata" or the monophyly of Lophophorata + Deuterostomia.

Recently, Hausdorf (2000) and Wägele et al. (1999) have argued that the results of Aguinaldo et al. (1997) (and in essence the results obtained here and by others employing 18S comparisons including Eernisse 1997) are flawed such that Ecdysozoa is not a natural group. Their own molecular analyses led them to each repropose the monophyly of Articulata, and each uses the presumption that morphology is much more consistent with the monophyly of Articulata than it is with Ecdysozoa. However, here we show using morphological data that the monophyly of Ecdysozoa is much more parsimonious than the monophyly of Articulata. In fact, constraining the analysis so that Articulata is monophylytic results in a tree 28 steps longer than the most parsimonious trees (see above). Moreover, removal of the "molecular" characters (117–138, Appendix 1) did nothing to change this result (analysis not shown). Most of the characters discussed by Wägele et al. (1999) are considered here, but it is worth pointing out that even though teloblastic segmentation is coded as present here for both arthropods and annelids (Character 42), new evidence from polychaetes suggests that teloblasts are absent and cell division occurs throughout the germ bands (E. Seaver, pers. comm., unreferenced), reminiscent of what Newby (1940) described for echiurans (see also Minelli and Bortoletto 1988). Moreover, Akam (2000) proposed that a fixed number of segments, like that seen in kinorhynchs, may be the plesiomorphic condition of arthropods. Hence, the teloblastic growth seen in clitellate annelids and some arthropods may not be the plesiomorphic state for either taxon. The reanalysis of Zrzavý et al.'s morphological data by Giribet et al. (2000) also supported the monophyly of Ecdysozoa. Finally, the sister grouping of Nematoda + Arthropoda with respect to Annelida, Mollusca and Deuterostomia, is also realized with other molecules, specifically β-thymosin (Manuel et al. 2000).

Except for gastrotrichs (see below), both morphology and 18S agree on the membership of Ecdysozoa, the monophyly of Kinorhyncha + Priapulida, and on the monophyly of Onychophora + Arthropoda with Tardigrada lying outside of the onychophoran + arthropod clade. Our molecular tree gave the surprising result that the onychophoran sequence analyzed was nested within the pancrustaceans. The inclusion of Onychophora within Arthropoda was not tested with our morphological analysis as Arthropoda was treated as a terminal taxon. Other morphological analyses with Arthropoda subdivided into smaller units have generally found that Arthropoda is monophyletic with onychophorans as their immediate sister-group (e.g., Eernisse et al. 1992; Wheeler et al. 1993; see also Budd 1996). Because only one putative onychophoran sequence was analyzed here (Appendix 2), we feel that it is premature to conclude that onychophorans are nested within arthropods proper. Moreover, given that Zrzavý et al. (1998) found Onychophora to be the sister-group of Tardigrada + Arthropoda, and Giribet et al. (2000) found Arthropoda the sister-group of Onychophora + Tardigrada, it is apparent the interrelationships within Panarthropoda remain unresolved.

One interesting difference between the morphological and molecular analyses concerns the interrelationship among panarthropods, nematoidans, and scalidophorans. The morphological analysis (Fig. 1) weakly suggests that Cycloneuralia is monophyletic, united by the possession of a circumpharyngeal brain (Character 102) and an introvert (Character 88). The molecular analyses supported a sister grouping with nematoidans and panarthropods with scalidophorans basal ecdysozoans. This is the same topology found by Zrzavý et al. (1998), whereas the combined analysis of Giribet et al. (2000) supported the monophyly of Cycloneuralia. Given that there are no morphological characters known to us that support Nematoida + Panarthropoda, we would tenatively suggest that the relationships as shown in Figure 1 are most congruent with the current data.

A second minor discordance concerns the phylogenetic position of chaetognaths, a taxon that may be of some importance for a proper understanding of the biology of the latest common ancestor of bilaterians (see below). Morphology suggests that chaetognaths are basal to the remaining ecdysozoans because they lack a trilaminate epicuticle (Character 79), trilayered cuticle (Character 80), and ecdysis (Character 83). Perhaps related to the presence of ecdysis is the absence of epidermal cilia (Character 13) (Valentine and Collins 2000), and chaetognaths possess multiciliated epidermal cells. Our 18S analysis places chaetognaths as the sister-taxon of nematomorphs. A placement within the Nematoida was also found by Halanych (1996b) who suggested that chaetognaths are the sister clade to nematodes, and that this topology was not the result of long-branch attraction. Aguinaldo et al. 1997 demonstrated that the substitution rate of *Sagitta* is very high, but we did not attempt to further test branch length attractions with our data set. We did observe that chaetognath 18S sequences were quite unstable in their position, depending on the taxonomic composition of the analysis, and that they exhibit the most extreme GC bias of any of our included bilaterian sequences (Fig. 5). For these reasons, we suspect the placement within Nematoida is potentially artifactual, and are unaware of any morphological synapomorphies shared exclusively by chaetognaths and either nematodes or nematomorphs. Furthermore, inclusion within Nematoida would imply that either ecdysis evolved independently multiple times within Ecdysozoa (assuming chaetognaths do not molt, which has never been observed), or that chaetognaths lost molting (and the other cuticle characters mentioned above) and reevolved epidermal cilia. Again, this seems unlikely. Given that chaetognaths have other bilaterian plesiomorphies not found in other ecdysozoans, we suggest that they are more likely basal to the other ecdysozoan clades (see below).

Interrelationships among lophotrochozoans remain enigmatic both from a morphological and molecular perspective. It has long been recognized that 18S has very little resolving power within this particular portion of the metazoan tree (e.g., Winnepenninckx et al. 1995b; Eernisse 1997; Halanych 1998; reviewed in Adoutte et al. 2000). Likewise, we found several well-established taxa to be para- or polyphyletic in our 18S results (Fig. 2). Our morphological results (Fig. 1) support albeit weakly the monophyly of Lophotrochozoa and Spiralia, and strongly support the monophyly of Lophophorata (the name restricted here to Brachiopoda + Phoronida, see Table 1). Within Lophophorata, our analysis suggests that brachiopods are monophyletic. This conclusion differs from the recent 18S results of Cohen and colleagues (Cohen et al. 1998a; Cohen 2000) who argued that phoronids should be considered an inarticulate brachiopod lineage. Cohen (2000) even proposed to formally include phoronids as one of four basal subdivisions within Brachiopoda (a classification somewhat at odds with their phylogenetic conclusions), and this proposal has already been adopted for GenBank's taxonomy. In contrast, our morphological analysis strongly supported the monophyly of Brachiopoda with no less than eight synapomorphies (Fig. 1, Table 3). Most of these characters involve the morphology of the lophophore (e.g., Characters 59–65), but others include the presence of the mantle (Character 74) and the subenteric ganglion (Character 107). Monophyly with relatively strong support is also found in the combined analyses (Figs. 3 and 6; Table 3), but the interrelationships between the three subgroups, "Linguliformea," Craniiformea, and Rhynchonelliformea, are not resolved (note that this node collapses in the consensus tree, see Fig. 6). The disparity between our analysis and Cohen et al.'s (or Cohen 2000) is not easily resolved, but could involve our much more complete representation of close nonlophophorate sequences analyzed here or, conversely, Cohen et al.'s more complete representation of lophophorate sequences and/or alignment sites. We have also not ignored morphology, and the fact that Giribet et al. (2000) found the same topology as we did is at least reason to challenge the formal inclusion of phoronids within the brachiopods in favor of the more conventional arrangement as sister-taxa.

Along with Lophophorata, the second grouping consistently found within Lophotrochozoa is Eutrochozoa. Within the eutrochozoans, we found that nemerteans were basal to the annelid/mollusc clade, a group we herein christen Neotrochozoa. In our results, this taxon includes the latest common ancestor of annelids and mollusc plus all of its descendants. Synapomorphies supporting this node are the gonads present with gametes passing through coelom and metanephridium (Character 23), and a somatoblast (Character 43). There is conflict between the two analyses with respect to the interrelationships amongst the neotrochozoans in that the morphological analysis weakly suggests that molluscs are the sister-taxon of Echiura + Annelida, whereas the combined analysis suggests that both sipunculans and echiurans are derived annelids. From a molecular perspective, the inclusion of Echiura within Annelida has precedent (McHugh 1997) but

Siddall et al. (1998) has criticised this result and Black et al. (1997) have found molecular support for the monophyly of Annelida exclusive of Echiura or Sipuncula. From a morphological perspective, the monophyly of Annelida (including Pogonophora) is usually recognized (Rouse and Fauchald 1997). Prior combined data set analyses (Zrzavý et al. 1998; Giribet et al. 2000) also recover a monophyletic Eutrochozoa, although without Sipuncula nested within the annelids as in our result. Our analysis is limited with respect to this finding because we do not test the monophyly of Annelida with our morphological analysis and only a single sequence of both Sipuncula and Echiura was included for analysis (Appendix 2). As for the onychophoran situation discussed above, the inclusion of these two taxa within Annelida awaits at least additional detailed morphological studies and more 18S sequences. Until such time, the interrelationships amongst neotrochozoans, aside from the close relationships between annelids and echiurans, remain obscure.

Our combined analysis and most of the shortest morphology-alone trees suggest that Entoprocta is the sister-taxon of Eutrochozoa, rendering Trochozoa monophyletic (a conclusion that agrees with the recent combined analysis of Giribet et al. 2000). This topology would have one very significant implication. It appears more parsimonious to us that, unlike the conclusions of Rouse (1999), the pilidium larva of nemerteans is also a derived trochophore larval type, not a unique larval form specific to heteronemerteans (or even holopnemerterans as well, Maslakova et al. 1999). This conclusion is supported by the unequivocal existence of a trochophore larva in the annelid/mollusc clade and also in entoprocts, and also by a similar cell lineage of the specialized ciliary bands in the larvae of annelids/molluscs and the pilidium (Klerkx, 2001; not coded herein). Moreover, Eeckhaut et al. (2000) employed 18S and elongation factor-1 (EF1α) sequence comparisons to infer that myzostomids are more closely related to flatworms than to annelids. If further supported, then because the larva of myzostomids is unquestionably a trochophore as well (Eeckhaut and Jangoux 1993), this would further imply that the Müller's larva of polyclads is also derived from a trochophore larva. Thus, it remains at least plausible and probably most parsimonious that the latest common ancestor of all spiralians had a trochophore larval stage (Peterson et al. 2000a).

An important discordance between the morphological and combined analyses concerns the relationship between lophophorates and trochozoans. Morphology suggests that lophophorates are more basal, and that Spiralia is monophyletic. Our combined analysis supported the finding of Giribet et al.'s (2000) combined analysis, suggesting that lophophorates are the sister-group of Trochozoa. This latter result implies that spiral cleavage (Character 29) and trochophore characters, such as the prototroch (Character 48), are either drastically misinterpreted or that these characters were lost in lophophorates and replaced with deuterostome-type characters (see below). Neither seems plausible, and like the chaetognath situation discussed above, it seems to us that the inclusion of lophophorates within Spiralia is dubious, with insufficient data to resolve this question at present. Nonetheless, it is worth pointing out that the use of setae as a putative synapomorphy for brachiopods + annelids (e.g., Conway Morris and Peel 1995) is weakened by the results of Eeckhaut et al. (2000). The affinity of myzostomids and flatworms would suggest instead that setae are plesiomorphic for Lophotrochozoa in general, and are not synapomorphic for any subset of lophotrochzoan taxa in particular, except for possibly Echiura + Annelida where the setae are protrusible and retractable (Character 85).

Further congruence between the morphological and molecular analyses was found within the deuterostomes. Not only do both types of data support the monophyly of Deuterostomia, but both also support the monophyly of Ambulacraria (see also Zrzavý et al. 1998; Giribet et al. 2000). This has the important implication that pharyngotremy (Character 96) was present in the latest common ancestor of deuterostomes, and has been lost in Recent echinoderms (Bromham and Degnan 1999). Interestingly, the morphological analysis supports the paraphyly of "Enteropneusta" such that harrimaniid enteropneusts are the sister-taxon of pterobranchs. Characters supporting this node include the presence of a ventral post-anal stalk (Character 66) and the presence in pterobranchs and some harrimaniid enteropneusts of two hydropores (Character 71) (see also Cameron et al. 2000). Recent 18S analyses support this result such that harrimaniid enteropneusts group with pterobranchs to the exclusion of the ptychoderid enteropneusts (Halanych 1995; Cameron et al. 2000), and the implications of this result with respect to deuterostome evolution and the origin of chordates are nicely discussed in Cameron et al. (2000). The placement of the spengelids, however, remains somewhat enigmatic. Our morphological analysis supports a sister grouping between the ptychoderids and spengelids. There are, however, lingering doubts given that the character supporting this relationship (telotroch, Character 51) is also found in other taxa (Table 2) and that spengelids share with harrimaniids + pterobranchs the absence of synapticules (Character 97; a potential plesiomorphy of deuterostomes). Spengelidae could instead be allied with the Harrimaniidae + Pterobranchia, as suggested by Peterson et al. (2000a). Further data including, especially 18S sequences from spengelids, should help resolve this issue.

Morphology vs. 18S rDNA: major conflict

By far the biggest discrepancy between the two data sets concerns how acoel flatworms, gnathostomulids, and gastrotrichs are related to other bilaterians. The morphologi-

cal analysis suggests that Platyhelminthes is monophyletic (unique synapomorphy is possession of neoblasts, Character 44). Within Platyhelminthes, the analysis agrees with Ax (1996) that catenulids are basal, and strongly suggests that acoels are the sister-group of nemertodermatids (Fig. 1; Table 3). As discussed at some length above, 18S strongly supports the polyphyly of Platyhelminthes with acoels (with or without Gnathostomulida) as the basal-most bilaterian clade (see also Ruiz-Trillo et al. 1999). The molecular data do suggest that catenulids are related to rhabditophorans (Figs. 2, 3, and 6), and Ruiz-Trillo et al. (1999) suggested that nemertodermatids are included within the catenulid/rhabditophoran clade; hence the focus here concerns only the phylogenetic position of acoels.

We have shown that there is a striking tendency for the 18S sequences of acoel flatworms to be attracted to random outgroup DNA sequences (Fig. 4; Table 4). This implies that any non-bilaterian group of organisms that had diverged substantially in sequence similarity could be attracted to the acoel flatworm sequences and hence would pull the acoels to the basal portion of the bilaterian tree. Peterson et al. (2000a) summarized the available morphological and molecular data and argued that the results obtained by Ruiz-Trillo et al. (1999), and by inference the results presented in our Figure 2, are flawed such that acoels are not basal bilaterians, but are closely related to the other platyhelminth groups. Berney et al. (2000), using EF1α DNA sequence data, tested the results of Ruiz-Trillo et al. (1999) and found that acoels do in fact cluster with the other flatworm taxa analyzed and are not basal bilaterians. Moreover, both acoel EF1α sequences examined share with tricad flatworms a particular sequence signature in the central region of the sequence. Adoutte et al. (2000) further review this problem and report that the Hox fragments from an acoel show clear lophotrochozoan signatures (see Characters 132 and 134). Finally, Giribet et al. (2000) find support with their combined 18S and morphology analysis for inclusion of acoels within Platyhelmithes. We thus predict that the inclusion of acoels within the flatworms, as depicted in Figure 1, will be the best supported phylogenetic position of acoels once these new sources of data have been incorporated. This has the important implication that duet cleavage, even though distinct from the quartet spiral cleavage found in polyclads (among others), is most likely derived from quartet spiral cleavage (see Henry et al. 2000; and Character 29). Moreover, like the case of amitochondriate eukaryotes (Philippe et al. 2000), the absence of protonephridia (Character 67) and ectomesenchyme (Character 37), rather than indicating their primitiveness amongst bilaterians (e.g., Ruiz-Trillo et al. 1999), suggests their highly derived nature within flatworms.

The phylogenetic position of gnathostomulids seems to be somewhat coupled with the phylogenetic position of acoels. Morphology weakly suggests that gnathostomulids are the sister-taxon of flatworms, consistent with Ax (1996), whereas 18S alone suggests that they are the sister-taxon of acoels at the base of the bilaterian tree (Fig. 2). Our combined analysis (Fig. 3) supported acoels are the sister-group of gnathostomulids plus all remaining bilaterians. Based on 18S, Littlewood et al. (1998) argued that gnathostomulids are allied with a chaetognath + nematode group. Given the results of our random outgroup sequence experiment (Table 4), this result might not be surprising if acoel flatworms are not included in the analysis, as they were not in Littlewood et al. (1998). Gnathostomulids and nematodes + chaetognaths could simply be the "next longest branches" attracted to the distant outgroups available when acoels are pruned, as we found when random outgroups were employed (Table 4). Moreover, the types of characters suggesting an affinity between chaetognaths and nematodes given by Littlewood et al. (1998) (active benthic, vermiform creatures with sclerotized cuticular jaws) are not compelling. The jaw structure is much more reminiscent of rotifers (Character 90), which themselves are arguably also allied with flatworms (Garey et al. 1998, and Fig. 1). As for acoels, it seems much more plausible that gnathostomulids are spiralian lophotrochozoans, specifically platyzoans (see also Giribet et al. 2000), and not basal bilaterians. Surely, more sequence data from gnathostomulids, including Hox gene sequences, will decide between these two (or other) positions on the metazoan tree.

The phylogenetic position of gastrotrichs is also enigmatic. The morphological analysis suggests that they are allies with ecdysozoans, consistent with the analysis of Schmidt-Rhaesa et al. (1998) (Fig. 1). The 18S analysis suggests that the lone gastrotrich sequence included is the sister-taxon of Bilateria minus acoels and gnathostomulids (see Figs. 2 and 3). Although not attracted to random DNA sequence (Table 4), its basal position could be due to strong 18S support for a lophotrochozoan, specifically a platyzoan, affinity. We suspect that a platyzoan affinity is the most likely taxonomic position of gastrotrichs (Garey et al. 1998; Giribet et al. 2000) and eagerly await other types of data (e.g., Hox genes, de Rosa et al. 1999) to test this proposition.

The biology of LCB

The most interesting thing about a tree is what is says or does not say about the evolutionary history of a group in general. Our results do have some interesting (but speculative) implications with respect to the biology of the latest common ancestor of bilaterians (LCB). These optimizations of ancestral states are speculative, even if the exact phylogeny were somehow known (Strathmann and Eernisse, 1994), but their greatest value is that they generate testable hypotheses.

In Figure 7, several taxa are shown in bold: Deuterostomia, Chaetognatha, and Lophophorata. All of these taxa have been allied with one another on morphological grounds, and

this association is usually based on the following characteristics (e.g., Brusca and Brusca 1990; Lüter and Bartolomaeus 1997): (1) complete gut with mouth not arising from blastopore [Character 33; note that this character is polymorphic across lophophorates]; (2) mesoderm derived directly from archenteron [Character 36]; (3) coelom tripartite and derived by enterocoely [Character 55]; (4) sheets of subepidermal muscles derived, in part, from archenteric mesoderm [equivalent to Character 36]; and (5) longitudinal nerve cords not ladder-like in arrangement and not emphasized ventrally [Character 103; note that chaetognaths have a ventral nervous system]. Other traditional character states uniting lophophorates and deuterostomes include: (1) the neotroch [Character 51]; (2) ciliated extension of the mesocoels [Character 59]; (3) heterogeneous metanephridium [Characters 69 and 72]; and (4) a dorsal ganglion associated with the mesosome [Character 106]. This rather impressive list of characters has forced many authors to conclude that at least lophophorates must be allied with the deuterostomes despite the evidence derived from 18S studies (e.g., Carlson 1993; Lüter and Bartolomaeus 1997). On the other hand, molecular workers have argued that because lophophorates are allied with the spiralians these characters must either be seriously misinterpreted or are the result of convergence (e.g., Halanych 1996a; Cohen 2000).

There is a third possibility. We advocate it here based primarily on consideration of our morphological results alone, namely that these characters are plesiomorphies of Bilateria. Valentine (1997) first made this suggestion, and the tree he presented is very similar to our Figure 1 with lophophorates as basal lophotrochozoans (chaetognaths were not considered). We would like to take this one step further and propose that chaetognaths, supported here as ecdysozoans, are also sharing plesiomorphies with lophophorates and deuterostomes. Furthermore, the morphological tree suggests that neither group has the apomorphies found in their sister-taxa: ecdysis and associated characters in the remaining Ecdysozoa, and spiral cleavage and associated characters in Spiralia. This interpretation is at least largely consistent with the combined analysis result, only lophophorates and chaetognaths group within their sister clade of the morphology result rather than group elsewhere among bilaterians.

If characters originally ascribed to deuterostomes are actually primitive for bilaterians, and if ctenophores are the sister-group to bilaterians, then it follows that the latest common ancestor of bilaterians would have developed very much like recent deuterostomes. This implies it would have a primary larval stage derived from Type 1 embryogenesis, and some sort of adult stage derived from set-aside cells (Peterson et al. 1997) patterned, in part, with Hox genes. (See Davidson et al. 1995; Peterson and Davidson 2000; and Peterson et al. 2000a,b for arguments concerning the development of LCB.) This view of the latest common ancestor of bilaterians contrasts sharply with many others, including Valentine and Collins (2000) who postulated that this ancestor would have been a direct developing non-coelomate animal. They observed that many of the basal clades of both ecdysozoans and lophotrochozoans, according to 18S studies, are taxa formerly classified as "aschelminthes" (priapulids and allies within the ecdysozoan, not considering chaetognaths; rotifers and allies within the lophotrochozoans). They also noted that the fossil record is more consistent with the primitiveness of direct development due to the presence of large possibly arthropodian, embryos from the latest Neoproterozic (Xiao et al. 1998; Zhang et al. 1998). Hence, Valentine and Collins concluded that the primary larval form would have evolved at least three times independently among bilaterians.

Valentine and Collins (2000) note that two sources of data would be most informative regarding the developmental mode of the latest common ancestor: Hox expression studies from protostomes, and fossil larval assemblages from the Neoproterozoic. Both of these points have been recently addressed. First, Peterson et al. (2000b) showed that like the sea urchin (Arenas-Mena et al. 1998, 2000), the polychaete annelid *Chaetopterus* does not use its Hox complex during embryogenesis, but uses it instead in a temporally colinear fashion during adult body plan formation. Moreover, Giusti et al. (2000) have shown that *Hox5* in the gastropod is not used during embryogenesis, but expression is detected in the branchial ganglia. Second, Chen et al. (2000; but see Xiao et al. 2000) describe fossils that appear to be remarkably similar to modern indirect-developing bilaterians. Furthermore, the embryos described by Xiao et al. (1998) are poriferan, not arthropodian, because they contain clear sponge spicules (Chen et al. 2000; unpublished observations). Therefore, among extant bilaterians, we postulate the LCB was not like acoel flatworms or non-coelomate protostomes but perhaps instead like ptychoderid enteropneusts, which in our analysis had generally retained plesiomorphic features of "deuterostomes" and suggest these are the best proxy for a "living fossil" among bilaterians. The fact that relevant morphological, developmental, and paleontological discoveries are accelerating gives us optimism that these problems of inference will be subject to ever more rigorous testing in the near future.

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Appendix 1

Description of characters. Most characters can be found in Brusca & Brusca (1990); Ruppert & Barnes (1994), the Microscopic Anatomy of Invertebrates series (Harrison, ed.), Ax (1996), and Nielsen (1995; Nielsen et al. 1996). Some characters were found to have mistakes, or new data were published bearing on a specific character, after we analyzed the data, and these are given in bold brackets below. Future analyses should take these changes into account. All characters are 0 = absent; 1 = present, unless otherwise stated.

1. Choanocytes. Although many taxa have cells similar in structure to choanocytes (e.g., collar cells), only choanocytes lack contractile microvilli (Nielsen 1995).
2. Multicellularity with extracellular matrix: 0 = absent; 1 = present; 2 = present but with reduced or absent ECM (Morris 1993, Müller 1995; Varner 1996). Note that the presence of collagen alone is not apomorphic for metazoans (Celerin et al. 1996).
3. Septate junctions.
4. Gap junctions. Note that among cnidarians only hydrozoans possess gap junctions (Mackie et al. 1984). **[Onychophorans are miscoded; contra Nielsen et al. (1996) they appear to lack gap junctions (Lane et al. 1994).]**
5. Hemidesmosomes.
6. Flagellar vanes (Mehl and Reiswig 1991.
7. Water-canal system.
8. Siliceous spicules.
9. Cross-striated rootlets. See Woollacott and Pinto (1995) for calcareous sponges.
10. "Acoelomorph" type of ciliary rootlet.
11. Belt desmosomes.
12. Basal lamina.
13. Ciliated epidermis: 0 = absent; 1 = present with monociliated cells; 2 = present with multi- or multi- + monociliated cells.
14. Densely multiciliated epidermis.
15. Distinct "step" in cilia. In acoelomorphs four of the nine peripheral double tubules in the locomotory cilia end a considerable distance below the tip of the cilium (see Ax 1996).
16. Egg with four polar bodies.
17. Position of polar bodies: 0 = absent; 1 = present and vegetal; 2 = present and animal; 3 = present and equatorial
18. Spermatozoa: 0 = absent; 1 = present; 2 = present without flagellum.
19. Spermatozoa without accessory centriole (Garey et al. 1998). The coding reflects the fact that the primitive condition for monociliated cells is the presence of an accessory centriole (Nielsen 1995). See Lundin and Hendelberg (1998) for nemertodermatids.
20. Acrosome: 0 = absent; 1 = present; 2 = present as a distinct organelle (Ax 1996). The character coding for rotifers reflects only *Seison* (Ahlrichs 1998). Nielsen et al. (1996) coded for the presence in platyhelminthes, entoprocts and kinorhynchs, but it appears that a distinct acrosome is absent in these taxa (see Rieger et al. 1991 for flatworms; Franzén 1983, 2000 for entoprocts; and Nyholm and Nyholm 1983, and Kristensen and Higgins 1991 for kinorhynchs). Wallace et al. (1996) coded gnathostomulids as unknown with respect to the presence or absence of an acrosome, but although the sperm is covered to varying degrees by "protrusions" probably derived from Golgi, the fact that the sperm is orientated with their protrusionless proximal end toward the egg suggests that these protrusions do not perform an acrosome-like function, and hence a distal acrosome is absent (Alvestad-Graebner and Adam 1983).
21. Perforatorium (subacrosomal material) (Baccetti 1979; Baccetti et al. 1986; Ehlers 1993).
22. Germ line and gonads. See Franzén (1996) for sponges, and Littlefield (1985, 1991) and Littlefield and Bode (1986) for data pertaining to cnidarians.
23. Gonads present with gametes passing through coelom and metanephridium. Note that only neotrochozoans are coded for these characters because the metanephridia of these taxa show no similarity with the metanephridia of brachiopods and phoronids, or the sacculus of onychophorans and arthropods. Each of these characters are coded for separately (see Characters 72 and 73).
24. One axis prespecified during oogenesis (Davidson 1991; Goldstein and Freeman 1997; Martindale and Henry 1998).
25. Apical/blastoporal axis. The apical/blastoporal axis of the embryo/larva may or may not coincide with the anterior/posterior axis of the adult (see refs. in Character 24 and Nielsen 1995). We are coding for the presence of an anterior/posterior axis in direct developers. **[Acoels, nemertodermatids, and catenulids should be coded as "1" not as "?".]**
26. Dorsal/ventral axis.
27. Blastula stage. Note that the embryology of kinorhynchs, loriciferans, priapulids, tardigrades, catenulid and nemertodermatid flatworms, and pterobranchs is virtually unknown.
28. Stereotypical cleavage pattern (Davidson 1991; Martindale and Henry 1998). Arthropods lack stereotypical cleavage (Scholtz 1997).
29. Spiral cleavage (see van den Biggelaar et al. 1997 for review). Henry and Martindale's (1994) earlier conclusions regarding nemertean cleavage have been overturned (Martindale and Henry 1998) so there is no fundamental dissimilarity between nemerteans and other quartet spiral cleavers with respect to quadrant specification. See Boyer et al. (1998) for the similarity of polyclad cleavage with the canonical spiral cleavage pattern. Whether the cleavage pattern in rotifers is spiral or not is debatable (cf. Costello and Henley 1976 and Malakhov 1994 vs. Nielsen 1995) and hence we coded them as unknown. Although cleavage is reported to be a monet spiral cleavage in cirripedes (Costello and Henley 1976), this is rejected by Scholtz (1997) and hence we coded arthropods as absent. We agree with Nielsen (1995) and Malakhov (1994) that gastrotrich cleavage, contra Costello and Henley (1976), cannot be described as spiral. Eibye-Jacobsen (1996/1997) suggests that cleavage in tardigrades is consistent with a modified spiral pattern. However, we coded tardigrades as absent because this suggestion appears unfounded. Finally, the "duet" cleavage of acoels appears distinct from quartet spiral cleavage and hence are coded as absent (Henry et al. 2000).
30. Annelid cross (Rouse 1999).
31. Molluscan cross (Rouse 1999). **[Because Characters 30 and 31 are mutally exclusive these characters should be coded as multistate characters in future analyses.]**
32. Gastrulation. See Mainitz (1989) for gnathostomulids. We treat the "inversion" found in Silicea as autapomorphic for that taxon and has no similarity to the gastrulation found in eumetazoans. However, the cellular delamination found in hexactinellids (Boury-Esnault et al. 1999) is a bona fide method of gastrulation.
33. Blastopore associated with larval/adult mouth. The blastopore is associated with the mouth in all brachiopod taxa examined (Long and Stricker 1991; Freeman 1995, 1999) except *Crania* (Nielsen 1991; Freeman 2000).
34. Mesoderm. We coded ctenophores as absent because the endomesodermal muscle cells (Character 35) do not form a germ layer.
35. Endomesodermal muscle cells (Martindale and Henry 1998, 1999).
36. Endomesoderm derived from gut. This character inlcudes both "schizocoelous" and/or "enterocoelous" coelom formation (Ruppert 1991). We are coding for the origin of the coeloms, not the method of coelomgenesis, which can be highly variable even within a single taxon (e.g., enteropneusts, Hyman 1959). See Lüter (2000a) for a recent description of brachiopod mesoderm and a review of lophophorate, ectoproct, and deuterostome mesoderm formation.
37. Ectomesenchyme (Boyer et al. 1996). The evidence for ectomesenchyme in deuterostomes (Ruppert 1991) is very weak and hence we coded these taxa as absent. We also coded cnidarians as absent because the ectodermally derived "muscle" cells in cnidarians are epithelial not mesenchymal. Acoels lack ectomesenchyme (Henry et al. 2000)
38. 4d endomesoderm. See Henry and Martindale (1998) and Boyer et al. (1998) for nemerteans and platyhelminths, respectively.
39. Mesodermal germ bands derived from 4d. See Henry and Martindale (1998) for nemerteans. We coded platyhelminths as absent because the lineage staining of 4d in polyclads, contra nemerteans, is not consistent with distinct mesodermal bands (Boyer et al. 1998).
40. Lateral coelom derived from mesodermal bands (Turbeville 1986).
41. Circular and longitudinal muscles: 0 = absent; 1 = present; 2 = only longitudinal muscle present.
42. Teloblastic segmentation. See Newby (1940) for echiurans. Although it appears that the last three segments or zonites of kinorhynchs develop for a subcaudal growth zone (Neuhaus 1995), we coded them as unknown because the earlier embryonic stages are not known.

43. Somatoblast. The blastomere origins of the adult nervous system in nemerteans and polyclads is unknown (Boyer et al. 1998; Henry and Martindale 1998).
44. Neoblasts (Littlewood et al. 1998). **[Loriciferans should be coded as "?" not as "0".]**
45. Apical organ/tuft. See Lacalli (1994) and Lacalli et al. (1994) for the presence of a modified apical organ in cephalochordates, and Hay-Schmidt (2000) for lamprey. Nielsen et al. (1996) code for the correlation between the apical organ of spiralians and its incorporation into the adult brain. However, more recent investigations in the ontogeny of the larval vs. adult nervous system in the gastropod mollusc *Aplysia* have shown the complete separation between these two systems (Marois and Carew 1997a,b), and thus their character is rejected here. **[See Hay-Schmidt (2000) for a more recent invesigation of the serotonergic neurons of the apical organs of many metazoan taxa. Future analyses should minimally incoporate the fact that the apical ganglion of spiralian protostomes (except nermerteans, but including ectoprocts) generally has three serotonergic neurons with the lateral pair innervating the ciliary band of the prototroch.]**
46. Apical organ with muscles extending to the hyposphere.
47. Pretrochal anlagen. The pretrochal region of the trochophore larva in annelids, echiurans, and sipunculans gives rise to a separate adult body region, which is deliniated from the trunk (derived from posttrochal anlage) sometimes by a septum.
48. Prototroch (Rouse 1999). See Nielsen (1995) for ectoprocts. Unlike Rouse (1999), we consider rotifers and nemerteans to possess a prototroch (Nielsen 1995). For cycliophorans, see Funch (1996) for this and all other larval characters. See Damen et al. (1997) for data regarding the similarity of the trochoblasts of annelids and molluscs.
49. Metatroch (Rouse 1999). Rotifers are coded as present following Nielsen (1995).
50. Adoral ciliary band (Rouse 1999). Rotifers are coded as present following Nielsen (1995).
51. Telotroch (Nielsen 1995; Rouse 1999). Because a telotroch is defined as a perianal ring of compound cilia on multiciliated cells, the perianal ring of phoronids (compound cilia on monociliated cells) does not satisfy a test of similarity and hence are coded as absent. **[Future analyses should code phoronids as "2" instead of "0" for consistency with the rest of the matrix.]**
52. Neurotroch (Rouse 1999).
53. Neotroch (Nielsen 1995). Only larval bands are considered here, adult bands are considered below (Character 57).
54. Nonmuscular peritoneal cells in lateral regions of coelom (Bartolomaeus 1994).
55. Trimery. Because chordates do not express *Brachyury* in any anterior population of mesoderm, unlike both echinoids and enteropenusts (Peterson et al. 1999a,b), chordates are coded as absent, contra Peterson (1995) and Nielsen et al. (1996). Because the septum between the trunk and tail of the chaetognath might be a primary septum, chaetognaths are coded as present, contra Hyman (1959) and Bone et al. (1991). Because the existence of a protocoel is dubious in brachiopods and there is only one coelomic cavity in ectoprocts (Nielsen 1995) the former are coded as questionable (see also Holmer et al. 1995), the latter are coded as absent.
56. Mesocoelomic ducts and pores (Ruppert 1997). Although there is some histological similarity between the echinoderm stone canal and the hemichordate mesocoelomic ducts (Rehkämper and Welsch 1988), only the latter open directly the exterior.
57. Ciliated extensions of the mesocoel. Although the similarity between the tentacles of phoronids, brachiopods and pterobranchs is usually not questioned (e.g., Jefferies 1986; Halanych 1993; Nielsen et al. 1996), there are significant differences between this structure and the tentacles of ectoprocts, and hence we coded ectoprocts as absent (see discussion in Nielsen 1995; Nielsen et al. 1996; Nielsen and Riisgård 1998; Carlson 1995 also notes that the lophophore consists of two arms in phoronids, brachiopods, and *Rhabdopleura*, but the "lophophore" of ectoprocts does not possess arms *per se*, just a ring of tentacles).
58. Lophophore (ciliated extensions of the mesocoel which surrounds the mouth but not the anus). See references in preceding character description.
59. Bipartite lophophore. A mesentery divides the mesocoel of pterobranchs and brachiopods, but not phoronids.
60. Small branchial canals (Carlson 1995).
61. Medial tentacle of lophophore (Carlson 1995).
62. Double row of tentacles (Carlson 1995; Holmer et al. 1995).
63. Lophophore tentacles on only one side of arm axis with a branchial lip bounding a food groove (Holmer et al. 1995; Carlson 1995).
64. Cartilage-like connective tissue in lophophore (Holmer et al. 1995).
65. Striated muscle fibers in lophophore (Holmer et al. 1995).
66. Posterior stalk that develops as a ventral outgrowth (Burdon-Jones 1952). Among enteropneusts only harrimaniids have a stalk.
67. Podocytes/terminal cells/nephrocytes: 0 = absent; 1 = present; 2 = excretory organ of apomorphic design with cells without any obvious similarity to podocytes (Ruppert and Smith 1988; Ruppert 1994). The organization of metanephridia is in many cases autapomorphic for each taxon (Bartolomaeus and Ax 1992), except for Characters 69, 72, and 73. We use a multistate character here because we feel that coding nematodes as equivalent to cnidarians, ctenophores et al. is not appropriate.
68. Protonephridia with channel cell completely surrounding lumen (Ahlrichs 1995).
69. Axial complex (Goodrich 1917; Dilly et al. 1986; Ruppert and Balser 1986; Balser and Ruppert 1990).
70. Hydropore. The presence of a hydropore in chordates (but not urochordates, Ruppert, 1990) is debatable and hence are coded as unknown.
71. Paired hydropores.
72. Metanephridia open through metacoel. Although the metanephrida of brachiopods and phoronids is similar in design to the axial complex of ambulacrarians (a mesodermal nephridial funnel and an ectodermal canal, Lüter and Bartolomaeus 1997), the dissimilarity in position ("protosomal" versus "metasomal") plus the fact that unlike the metanephridia of ambulacrarians they also function as gonoducts warrants separate character status for each.
73. Metanephridia with coelomic compartment restricted to sacculus (Bartolomaeus and Ruhberg 1999).
74. Dorsal and ventral mantles, with canals, that secrete mineralized valves (Carlson 1995).
75. Mantle sinuses with gonads (Williams et al. 1996).
76. Inner epithelium secreting periostracum (Williams et al. 1996).
77. Calcareous valves, which rotate about a hinge axis (Carlson 1995; Holmer et al. 1995).
78. Cuticle with chitin. See Wagner et al. (1993) for chordates; Neuhaus et al. (1997a) for loriciferans; Neuhaus et al. (1997b) for nematodes; Eernisse (1997) for reference to chaetognaths; Haas (1981) and Leise and Cloney (1982) for reference to molluscs (only present in aplacophorans and chitons); and Schmidt-Rhaesa et al. (1998) and Lemburg (1995, 1998) for this and Characters 79–81. **[Entoprocts should be coded as "0" not as "1".]**
79. Trilaminate epicuticle.
80. Trilayered cuticle.
81. Collagenous basal layer.
82. Lorica (Nielsen et al. 1996).
83. Ecdysis (Aguinaldo et al. 1997; Schmidt-Rhaesa et al. 1998).
84. Setae (Lüter and Bartolomaeus 1997; Lüter 2000b).
85. Protrusible and retractible setae (Eernisse et al. 1992).
86. Head divided into three segments (Dewel and Dewel 1996).
87. Terminal mouth (Schmidt-Rhaesa et al. 1998). **[Both onychophorans and arthropods are miscoded—the primitive condition for both is a terminal mouth; the subventral position was derived independently in each clade (Budd 1999; Eriksson and Budd 2001).]**
88. Introvert (Nebelsick 1993; Malakhov 1994). Note that these authors argue, contra Lorenzen (1985) and Nielsen (1995), that the eversible proboscis found in the nematode *Kinonchulus* is not an introvert but an eversible pharynx (see also Schmidt-Rhaesa 1997/1998). Unlike Schmidt-Rhaesa (1997/1998), we find the introverts of nematomorphs and scalidophorans similar and coded accordingly.
89. Oral cone.
90. Jaws elements with cuticular rods with osmophilic cores (Rieger and Tyler 1995; Sørensen 2000; Kristensen and Funch 2000).
91. Food modified with limbs.
92. Digestive gut: 0 = absent; 1 = present; 2 = present without epithelium.
93. Digestive gut without cilia.
94. Anus. See Knauss (1979) for gnathostomulids. Whether the anal pores in ctenophores are similar to the single anus of bilaterians is debatable and hence they are coded as unknown (but the unique development of this region of the ctenophore embryo makes the suggestion of homology unlikely, see Martindale and Henry 1995).
95. Gastroparietal bands (Williams et al. 1996).
96. Pharyngotremy. Because of the possibility that probable stem-group echi-

noderms possessed pharyngeal slits (Jefferies 1986, 1990) we coded echinoderms as unknown. Moreover, because these slits were multiple and U-shaped we do not code this character as multistate as Peterson (1995) did. See Ogasawara et al. (1999) for molecular similarity between the branchial slits of enteropneusts and chordates.

97. Synapticules (Benito and Pardos 1997).
98. Stomochord (Ruppert 1997). See Peterson et al. (1999a) for data suggesting that the stomochord is genetically distinct from the notochord.
99. Nerve cells.
100. Acetylcholine used as a neurotransmitter.
101. Nerve cells organized into distinct ganglia.
102. Circumpharyngeal brain with anterior and posterior rings of perikarya separated by a ring of neuropil (see also Nebelsick 1993; Neuhaus 1994; Schmidt-Rhaesa 1996).
103. Ventral nervous system.
104. Circumoesophageal nerve ring (Rouse 1999).
105. Dorsal and ventral epidermal cords with nerve cords (Schmidt-Rhaesa 1997/1998).
106. Dorsal nervous cord/ganglion associated with the mesosome. Because the adult nervous system of brachiopods and ectoprocts is not obviously associated with a "mesosome" these taxa are coded as unknown, contra Fernández et al. (1996; see also Nielsen 1995).
107. Subenteric ganglion (Carlson 1995).
108. Frontal complex (Ehlers 1992).
109. Tanycytes (elongated epidermal/glial cells with tonofilaments as attachment for the musculature of the introvert with some penetrating the brain, Nebelsick 1993; Neuhaus 1994).
110. Glialinterstitial cell system (Turbeville and Ruppert 1985).*
111. Scalids. See Schmidt-Rhaesa 1997/98 for nematomorphs.
112. Spinoscalids and clavoscalids (see also Nebelsick 1993; Neuhaus 1994; Storch et al. 1994).
113. Flosculi (sensory organs with cuticular micropapillae arranged in a ring around a central pore, Adrianov et al. 1989; Neuhaus 1994). Because the presence of a central pore cannot be confirmed in nematomorphs (Schmidt-Rhaesa, 1997/1998) we coded nematomorphs as unknown.
114. Closed circulatory system with dorsal and ventral blood vessels.
115. Hemerythrin. Of the three primary respiratory proteins found in metazoans (reviewed in van Holde 1997/98), only hemerythrin is considered here because hemoglobin is plesiomorphic (e.g., Hardison 1998; Goodman et al. 1988), and molluscan and arthropod hemocyanins are convergent (Burmester and Scheller 1996; Durstewitz and Terwilliger 1997).
116. Endogenous sialic acids (Warren 1963; Segler et al. 1978).
117. Epithelia binding iodine and secreting iodothyrosine. **[This character is flawed (Eales 1997) and should be deleted from the analysis.]**
118. Nuclear lamins (Erber et al. 1999).
119. Intermediate filament proteins: 0 = absent; 1 = present as S-type (deletion of 42 residues and laminin similarity region absent); 2 = present as L-type (presence of 42 residues in coil 1b subdomain and laminin similarity tail) (Erber et al. 1998).
120. tRNA Lys. For this character and Characters 121 and 122 see Beagley et al. (1998), Castresana et al. (1998a,b), and Watkins and Beckenbach (1999). [For Character 120 all three brachiopod groups should be coded as "0" and for Characters 121–122 all three should be coded as "1" (Saito et al. 2000).]
121. AUA methionine: 0 = absent (i.e., codes for isoleucine); 1 = present. **[Telford et al. (2000) have shown that acoels, nemertodermatids, and catenulids should all be coded as "1", as well as onychophorans, sipunculans, echiurans, and ectoprocts.]**
122. AGA and AGG serine: 0 = absent (i.e., codes for arginine); 1 = present.
123. Nuclear hormone receptors (Escriva et al. 1997; Chervitz et al. 1998; Ruvkun and Hobert 1998).
124. *ETS* gene family (Degnan et al. 1993; Laudet et al. 1999). In addition to the confirmed absence *ETS* genes in the yeast genome, Degnan et al. (1993) report that they could not detect members of this family in the fungus *Aspergillus*, plants or in several protozoans (choanoflagellates were not examined and hence are coded as unknown).
125. *Paired-box* genes (Chervitz et al. 1998; Ruvkun and Hobert 1998; Galliot et al. 1999; Miller et al. 2000; see Hoshiyama et al. 1998 for sponges). **[Placozoans are now known to have a *Pax* gene (Gröger et al. 2000) and should be coded for accordingly.]**
126. *Pax-6* (Callaerts et al. 1999; Galliot et al. 1999; Miller et al. 2000).
127. Hox genes. For this and the following Hox characters see Balavoine (1997), Kourakis et al. (1997), Grenier et al. (1997), Bayascas et al. (1998), Kmita-Cunisse et al. (1998), Martinez et al. (1999), de Rosa et al. (1999), Gauchat et al. (2000), and Finnerty & Martindale (1998) for review. We coded each gene duplication as a separate character because it appears that each was the result of an independent tandem gene duplication. **[Gauchat et al. (2000) argue that thus far no Hox or ParaHox genes are positively identified in sponges. The only homeobox genes confidently identified in sponges are *Msx*, *Tlx* and *Nk2*. Thus, future analyses should consider these genes separately and code sponges as "absent" for Hox genes.]**
128. Hox complex consiting of seven genes.
129. Central Hox class member(s) (see Finnerty et al. 1996 for ctenophores).
130. *Antp.*
131. *Ubx/abd*-A.
132. *Lox2/4.*
133. *Hox 6–8.*
134. *Abd-B* duplication (Lophotrochozoa).
135. *Abd-B* duplication (Deuterostomia).
136. Hexapeptide (Kuhn et al. 1996). The hexapeptide is a six amino acid motiff necessary for proper interaction of Hox proteins with extradenticle (Passner et al. 1999).
137. *T-box* genes (Ruvkun and Hobert 1998). See Technau and Bode (1999) for cnidiarians, and Peterson et al. (1999a,b; 2000b) for hemichordates, echinoderms, and annelids, respectively.
138. *Brachyury* expressed in oral and anal regions of gut (Peterson et al. 1999a; Shoguchi et al. 1999). The sea urchin *Stronglyocentrotus purpuratus* has, in addition to expression in secondary mesenchyme (Peterson et al. 1999b), expression in the oral and anal regions of the gut (Peterson et al. 2000a).

Appendix 2: 18S rDNA sequences analyzed

Higher Taxon (Abbreviated)	Species	Acc. Num.
Fungi; Ascomycota	*Saccharomyces cerevisiae*	J01353
Choanoflagellata; Acanthoecidae	*Acanthocoepsis unguiculata*	L10823
"	*Diaphanoeca grandis*	L10824
Choanoflagellata; Codonosigidae	*Monosiga brevicolis*	AF100940
"	*Sphaeroeca volvox*	Z34900
Choanoflagellata; Salpingoecidae	*Salpingoeca infusionum*	AF100941
Mesomycetozoa ("DRIP" clade)	*rosette agent of chinook salmon*	L29455
"	*Ichthyophonus hoferi*	U25637
"	*Dermocystidium salmonis*	U21337
"	*Dermocystidium* sp.	U21336
"	*Psorospermium haeckelii*	U33180
Porifera; Hexactinellida	*Rhabdocalyptus dawsoni*	AF100949
"	*Farrea occa*	AF159623
Porifera; Demospongia	*Spongilla lacustris*	AF121112
"	*Tetilla japonica*	D15067
"	*Eunapius fragilis*	AF121111
"	*Ephydatia muelleri*	AF121110
"	*Mycale fibrexilis*	AF100946
"	*Suberites ficus*	AF100947
"	*Axinella polypoides*	U43190
"	*Plakortis* sp.	AF100948
Porifera; Calcarea	*Scypha ciliata*	L10827
"	*Sycon calcaravis*	D15066
"	*Leucosolenia* sp.	AF100945
"	*Clathrina cerebrum*	U42452
Placozoa	*Trichoplax adhaerens*	L10828
"	*Trichoplax* sp.	Z22783
Cnidaria; Hydrozoa	*Hydra* sp.	(M20077-79)
"	*Hydra littoralis*	U32392
"	*Coryne pusilla*	Z86107
Cnidaria; Cubozoa	*Tripedalia cystophora*	L10829
Cnidaria; Scyphozoa	*Atolla vanhoeffeni*	AF100942
"	*Craterolophus convolvulus*	AF099104
"	*Haliclystus* sp.	AF099103
Cnidaria; Anthozoa; Alcyonaria	*Bellonella rigida*	Z49195
"	*Lepidisis* sp.	AF052906
"	*Narella bowersi*	AF052905
"	*Protoptilum* sp.	AF052911
"	*Renilla reniformis*	AF052581
Cnidaria; Anthozoa; Ceriantipatharia	*Antipathes fiordensis*	AF052900
"	*Antipathes galapagensis*	AF100943
"	*Ceriantheopsis americana*	AF052898
"	*Cerianthus borealis*	AF052897
Cnidaria; Anthozoa; Zoantharia	*Anemonia sulcata*	X53498
"	*Stomphia* sp.	AF052888
"	*Corynactis californica*	AF052895
"	*Discosoma* sp.	AF052894
"	*Ceratotrochus magnaghii*	AF052886
"	*Phyllangia mouchezii*	AF052887
"	*Fungia scutaria*	AF052884
"	*Parazoanthus axinella*	U42453
"	*Parazoanthus* sp.	AF052893
"	*Palythoa variabilis*	AF052892
"	*Anthopleura kurogane*	Z21671
Ctenophora; Atentaculata	*Beroe cucumis*	D15068
Ctenophora; Tentaculata	*Mnemiopsis leidyi*	L10826
Ctenophora; Cydippida	*Hormiphora* sp.	AF100944
Chaetognatha; Sagittiidea	*Sagitta crassa naikaiensis*	D14363
"	*Sagitta elegans*	Z19551
"	*Paraspadella gotoi*	D14362
Gastrotricha; Chaetonotida	*Lepidodermella squammata*	U29198
Nematomorpha; Gordioidea	*Gordius aquaticus*	X80233
"	*Gordius aquaticus*	X87985
"	*Gordius* sp.	U51005

(*Continued*)

Appendix 2: Continued

Higher Taxon (Abbreviated)	Species	Acc. Num.
Nematoda; Adenophorea; Chromadoria	*Metachromadora* sp.	AF036595
"	*Praeacanthonchus* sp.	AF036612
"	*Plectus acuminatus*	AF037628
"	*Plectus aquatilis*	AF036602
"	*Plectus* sp.	U61761
"	*Prismatolaimus intermedius*	AF036603
"	*Teratocephalus lirellus*	AF036607
"	*Diplolaimelloides meyli*	AF036644
Nematoda; Adenophorea; Enoplia	*Tobrilus gracilis*	none
"	*Bathylaimus* sp.	none
"	*Paratrichodorus anemones*	AF036600
"	*Paratrichodorus pachydermus*	AF036601
"	*Trichodorus primitivus*	AF036609
"	*Longidorus elongatus*	AF036594
Nematoda; Secernentea; Aphelenchida	*Aphelenchus avenae B*	AF036586
Nematoda; Secernentea; Oxyurida	*Dentostomella* sp.	AF036590
Nematoda; Secernentea; Rhabditia	*Anisakis* sp.	U81575
"	*Toxocara canis*	AF036608
"	*Zeldia punctata*	U61760
Priapulida; Priapulidae	*Priapulus caudatus*	X80234
"	*Priapulus caudatus*	Z38009
"	*Priapulus caudatus*	AF025927
Kinorhyncha; Homalorhagida	*Pycnophyes kielensis*	U67997
Loricifera	N/A	N/A
Tardigrada; Heterotardigrada	*Pseudechiniscus suillus*	none
Tardigrada; Eutardigrada	*Thulinia stephaniae* (as *Hysibius* sp.)	none
"	*Macrobiotus* sp. (as *M. hufelandi*)	X81442
"	*Macrobiotus* sp.	U32393
"	*Macrobiotus* sp.	U49912
"	*Milnesium tardigradum*	U49909
Onychophora; Peripatopsidae	*Peripatoides novaezea landiae*	none
Arthropoda; Chelicerata; Merostomata	*Limulus polyphemus*	U91490
"	*Carcinoscorpius rotundicaudatus*	U91491
Arthropoda; Chelicerata; Pycnogonida	*Colossendeis* sp.	AF005440
"	*Callipallene gen.* sp.	AF005439
Arthropoda; Chelicerata; Arachnida; Scorpiones	*Androctonus australis*	X77908
Arthropoda; Chelicerata; Arachnida; Opiliones	*Odiellus troguloides*	X81441
"	*Stylocellus* sp. *'Giribet'*	U91485
"	*Nelima sylvatica*	U91486
"	*Caddo agilis*	U91487
"	*Maiorerus randoi*	U37004
"	*Gnidia holnbergii*	U37006
"	*Parasiro coiffaiti*	U36999
"	*Pachyloides thorellii*	U37007
Arthropoda; Chelicerata; Arachnida; Araneae	*Eurypelma californica*	X13457
"	*Liphistius bicoloripes*	AF007104
Arthropoda; Crustacea; Maxillopoda; Branchiura	*Argulus nobilis*	M27187
Arthropoda; Crustacea; Maxillopoda; Pentastomida	*Porocephalus crotali*	M29931
Arthropoda; Crustacea; Maxillopoda; Ostracoda	*Rutiderma* sp.	L81942
"	*Euphilomedes cacharodonta*	L81941
Arthropoda; Crustacea; Maxillopoda; Cirripedia	*Trypetesa lampas*	L26520
"	*Berndtia purpurea*	L26511
"	*Ulophysema oeresundense*	L26521
"	*Loxothylacus texanus*	L26517
"	*Balanus eburneus*	L26510
"	*Paralepas palinuri*	AF057561
"	*Lepas anatifera*	L26516
Arthropoda; Crustacea; Maxillopoda; Copepoda	*Eucyclops serrulatus*	L81940
"	*Calanus pacificus*	L81939
"	*Cancrincola plumipes*	L81938
Arthropoda; Crustacea; Branchiopoda	*Limnadia lenticularis*	L81934
"	*Branchinecta packardi*	L26512
"	*Daphnia pulex*	AF014011

(*Continued*)

Appendix 2: Continued

Higher Taxon (Abbreviated)	Species	Acc. Num.
Arthropoda; Crustacea; Malacostraca	*Nebalia* sp.	L81945
"	*Anaspides tasmaniae*	L81948
"	*Gonodactylus* sp.	L81947
"	*Squilla empusa*	L81946
"	*Callinectes sapidus*	M34360
"	*Palaemonetes kadiakensis*	M34359
"	*Penaeus aztecus*	M34362
"	*Procambarus leonensis*	M34363
"	*Stenopus hispidus*	M34361
"	*Philyra pisum*	Z25817
"	*Nephrops norvegicus*	Y14812
Arthropoda; Myriapoda; Chilopoda	*Theatops erythrocephala*	AF000776
"	*Cryptops trisulcatus*	AF000775
"	*Scolopendra cingulata*	U29493
"	*Craterostigmus tasmanianus*	AF000774
"	*Lithobius variegatus*	AF000773
"	*Lithobius forficatus*	X90654
"	*Bothropolys asperatus*	none
"	*Scutigera coleoptrata*	AF000772
"	*Nodocephalus doii*	none
Arthropoda; Myriapoda; Diplopoda	*Cylindroiulus punctatus*	AF005448
"	*Polydesmus coriaceus*	AF005449
Arthropoda; Insecta; Thysanura	*Lepisma* sp.	AF005458
Arthropoda; Insecta; Pterygota; Paleoptera	*Aeschna cyanea*	X89481
Arthropoda; Insecta; Pterygota; Orthopterodea	*Carausius morosus*	X89488
Arthropoda; Insecta; Pterygota; Ephemeroptera	*Ephemera* sp.	X89489
Phoronida	*Phoronis psammophila*	AF025946
"	*Phoronis hippocrepia*	U08325
Brachiopoda; Linguliformea; Linguloidea	*Lingula anatina*	U08331
"	*Lingula adamsi*	U08329
Brachiopoda; Linguliformea; Discinoidea	*Discinisca tenuis*	U08327
"	*Discina striata*	U08333
Brachiopoda; Craniiformea; Cranioidea	*Neocrania huttoni*	U08334
"	*Neocrania anomala*	U08328
Brachiopoda; Rhynchonelliformea; Rhynchonellida	*Neorhynchia* sp.	AF025937
"	*Eohemithyris grayii*	AF025936
"	*Hemithyris psittaceae*	U08322
Brachiopoda; Rhynchonelliformea; Terebratulida	*Gryphus vitreus*	AF025932
"	*Gwynia capsula*	AF025940
"	*Megerlina* sp. *D1218*	AF025943
"	*Terebratalia transversa*	AF025945
"	*Platidia anomioides*	AF025933
"	*Calloria inconspicua*	AF025938
"	*Gyrothyris mawsoni*	AF025941
"	*Neothyris parva*	AF025944
"	*Terebratella sanguinea*	U08326
"	*Stenosarina crosnieri*	AF025934
"	*Macandrevia cranium*	AF025942
Ectoprocta; Phylactolaemata; Cristatellidae	*Cristatella mucedo*	AF025947
Rotifera; Monogononta; Ploimida; Brachionidae	*Brachionus plicatilis*	U29235
"	*Brachionus plicatilis*	U49911
"	*Brachionus platus*	AF154568
Rotifera; Monogononta; Ploimida; Lecanidae	*Lecane bulba*	AF154566
Rotifera; Bdelloidea; Bdelloida; Philodinidae	*Philodina roseola*	AF154567
Gnathostomulida; Bursovaginoidea	*Gnathostomula paradoxa*	Z81325
Platyhelminthes; Catenulida	*Stenostomum leucops aquariorum*	AJ012519
Platyhelminthes; Acoela	*Symsagittifera psammophila*	AF102893
"	*Simplicomorpha gigantorhabditis*	AF102894
"	*Atriofonta polyvacuola*	AF102895
"	*Paedomecynostomum bruneum*	AF102896
"	*Philomecynostomum lapillum*	AF102897
"	*Anaperus tvaerminnensis*	AF102898
"	*Postmecynostomum pictum*	AF102899
"	*Haplogonaria syltensis*	AF102900

(*Continued*)

Appendix 2: Continued

Higher Taxon (Abbreviated)	Species	Acc. Num.
Platyhelminthes; Acoela	*Anaperus biaculeatus*	AJ012527
"	*Childia groenlandica*	AJ012529
"	*Convoluta roscoffensis*	AJ012530
"	*Amphiscolops* sp.	AJ012523
"	*Actinoposthia beklemischevi*	AJ012522
"	*Paratomella rubra*	AF102892
Platyhelminthes; Nemertodermatida	N/A	N/A
Platyhelminthes; Rhabditophora; Haplopharyngida	*Haplopharynx rostratus*	AJ012511
Platyhelminthes; Rhabditophora; Lecithoepitheliata	*Geocentrophora wagini*	AJ012509
"	*Geocentrophora baltica*	AF065417
Platyhelminthes; Rhabditophora; Macrostomida	*Macrostomum tuba*	U70080
"	*Paromalostomum fusculum*	AJ012531
Platyhelminthes; Rhabditophora; Polycladida	*Notoplana australis*	AJ228786
"	*Pseudoceros tritriatus*	AJ228794
Platyhelminthes; Rhabditophora; Prolecithophora	*Pseudostomum quadrioculatum*	AF065425
"	*Pseudostomum klostermanni*	AF065424
"	*Cylindrostoma fingalianum*	AF065415
"	*Urastoma* sp.	U70085
"	*Ichthyophaga* sp.	AJ012512
"	*Pseudostomum gracilis*	AF065423
Platyhelminthes; Rhabditophora; Proseriata	*Monocelis lineata*	U45961
"	*Nematoplana coelogynoporvide*	AJ012516
Platyhelminthes; Rhabditophora; Rhabdocoela	*Kronborgia isopodicola*	AJ012513
Platyhelminthes; Rhabditophora; Rhabdocoela	*Diascorhynchus rubrus*	AJ012508
"	*Gyratrix hennaphroditus*	AJ012510
"	*Cheliplana cf, orthocirra*	AJ012507
"	*Temnocephala* sp.	AJ012520
"	*Mariplanella frisia*	AJ012514
Platyhelminthes; Rhabditophora; Seriata	*Paratoplana renatae*	AJ012517
Platyhelminthes; Rhabditophora; Tricladida	*Polycelis tenuis*	Z99949
"	*Phagocata sibirica*	Z99948
"	*Bdelloura candida*	Z99947
"	*Bipalium kewense*	AF033039
"	*Artioposthia triangulata*	AF033044
"	*Caenoplana caerulea*	AF033040
"	*Artioposthia triangulata*	AF033038
Platyhelminthes; Rhabditophora; Udonellida	*Udonella caligorum*	AJ228796
Platyhelminthes; Rhabditophora; Cestodaria	*Gyrocotyle urna*	AJ228782
Platyhelminthes; Rhabditophora; Cestoda	*Abothrium gadi*	AJ228773
"	*Bothriocephalus scorpii*	AJ228776
"	*Grillotia erinaceus*	AJ228781
"	*Echinococcuc granulosus*	U27015
Platyhelminthes; Rhabditophora; Trematoda	*Opisthorchis viverrini*	X55357
"	*Schistosoma haematobium*	Z11976
"	*Schistosoma mansoni*	M62652
Cycliophora	*Symbion pandora*	Y14811
Entoprocta; Barentsiidae	*Barentsia benedeni*	U36272
Entoprocta; Pedicellinidae	*Pedicellina cernua*	U36273
Nemertea; Anopla; Heteronemertea	*Cerebratulus lacteus*	M90051-53
"	*Lineus* sp.	X79878
Nemertea; Enopla; Hoplonemertea	*Prostoma eilhardi*	U29494
Annelida; Polychaeta; Phyllodocida; Aphroditidae	*Aphrodita aculeata*	Z83749
Annelida; Polychaeta; Phyllodocida; Nereididae	*Nereis limbata*	U36270
"	*Neanthes virens*	Z83754
Annelida; Polychaeta; Sabellida; Sabellidae	*Sabella pavonina*	U67144
Annelida; Polychaeta; Sabellida; Serpulidae	*Protula* sp.	U67142
Annelida; Polychaeta; Terebellida; Terebellidae	*Lanice conchilega*	X79873
Annelida; Clitellata; Aeolosomatidae	*Aeolosoma* sp.	Z83748
Annelida; Clitellata; Tubificida; Enchytraeidae	*Enchytraeus* sp.	ESU95948
"	*Enchytraeus* sp.	Z83750
"	*Enchytraeus* sp.	U67325
Annelida; Clitellata; Tubificida; Tubificina	*Stylaria* sp.	U95946
"	*Tubifex* sp.	U67145
"	*Dero digitata*	AF021879

(*Continued*)

Appendix 2: Continued

Higher Taxon (Abbreviated)	Species	Acc. Num.
Annelida; Clitellata; Haplotaxida; Lumbricina	*Eisenia fetida*	X79872
"	*Lumbricus rubellus*	Z83753
Annelida; Clitellata; Hirudinida; Branchiobdellida	*Xironogiton victoriensis*	AF115977
"	*Cronodrilus ogygius*	AF115976
"	*Cambarincola holti*	AF115975
Annelida; Clitellata; Hirudinida; Arynchobdellida	*Hirudo medicinalis*	AF116011
"	*Haemopis lateromaculata*	AF116009
"	*Haemopis marmorata*	AF116008
"	*Chtonobdella bilineata*	AF116006
"	*Haemadipsa sylvestris*	AF116005
"	*Erpobdella punctata*	AF116002
"	*Erpobdella octoculata*	AF116001
"	*Dina dubia*	AF115997
Annelida; Clitellata; Hirudinida; Rhynchobdellida	*Stibarobdella macrothela*	AF115996
"	*Myzobdella lugubris*	AF115994
"	*Ozobranchus margoi*	AF115991
"	*Desmobdella paranensis*	AF115987
"	*Helobdella stagnalis*	AF115986
"	*Marsupiobdella africana*	AF115979
Pogonophora; Obturata; Basibranchia; Ridgeiidae	*Ridgeia piscesae*	X79877
Pogonophora; Perviata; Athecanephria; Siboglinidae	*Siboglinum fiordicum*	X79876
Echiura; Echiuroinea; Echiuridae	*Ochetostoma erythrogrammon*	X79875
Mollusca; Polyplacophora; Chitonida; Chitonidae	*Acanthopleura japonica*	X70210
Mollusca; Polyplacophora; Chitonida; Tonicellidae	*Lepidochitona corrugata*	X91975
Mollusca; Polyplacophora; Lepidopleurida	*Lepidopleurus cajetanus*	AF120502
Mollusca; Gastropoda; Neritospina; Neritidae	*Nerita albicilla*	X91971
Mollusca; Gastropoda; Apogastropoda; Littorinidae	*Littorina littorea*	X91970
Mollusca; Gastropoda; Apogastropoda; Neogastropoda	*Thais clavigera*	X91979
Mollusca; Gastropoda; Apogastropoda; Pulmonata	*Limicolaria kambeul*	X66374
"	*Siphonaria algesirae*	X91973
"	*Fossaria truncatula*	Z73985
Sipuncula; Phascolosomatidae	*Phascolosoma granulatum*	X79874
Chordata; Vertebrata; Actinopterygii; Chondrostei	*Polyodon spathula*	X98838
Chordata; Vertebrata; Actinopterygii; Neopterygii	*Lepisosteus osseus*	X98837
"	*Amia calva*	X98836
"	*Ophichthus rex*	X98843
"	*Hiodon alosoides*	X98840
"	*Elops hawaiiensis*	X98841
Chordata; Vertebrata; Lissamphibia; Anura	*Xenopus laevis*	X04025
Chordata; Vertebrata; Amniota; Reptilia; Lepidosauria	*Sphenodon punctatus*	AF115860
Chordata; Vertebrata; Amniota; Mammalia; Rodentia	*Mus musculus*	X00686
Chordata; Vertebrata; Amniota; Mammalia; Primates	*Homo sapiens*	X03205
Echinodermata; Pelmatozoa; Crinoidea	*Antedom serrata*	D14357
Echinodermata; Eleutherozoa; Ophiuroidea	*Ophioplocus japonicus*	D14361
"	*Ophiopholis aculeata*	L28055
"	*Amphipholis squamata*	X97156
Echinodermata; Eleutherozoa; Asteroidea	*Asterias amurensis*	D14358
Echinodermata; Eleutherozoa; Holothuroidea	*Stichopus japonicus*	D14364
Echinodermata; Eleutherozoa; Echinoidea	*Strongylocentrotus purpuratus*	L28056
Hemichordata; Enteropneusta; Ptychoderidae	*Balanoglossus carnosus*	D14359
Hemichordata; Enteropneusta; Spengelidae	N/A	N/A
Hemichordata; Enteropneusta; Harrimaniidae	*Saccoglossus kowalevskii*	L28054
Hemichordata; Pterobranchia; Rhabdopleurida	N/A	N/A