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Phylogenetic Relationships of Annelids, Molluscs, and Arthropods Evidenced from Molecules and Morphology

Chang Bae Kim,[1] Seung Yeo Moon,[1] Stuart R. Gelder,[2] Won Kim[1]

[1] Department of Molecular Biology, Seoul National University, Seoul 151-742, Korea
[2] Department of Science, University of Maine at Presque Isle, Presque Isle, ME 04769, USA

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Abstract. Annelids and arthropods have long been considered each other's closest relatives, as evidenced by similarities in their segmented body plans. An alternative view, more recently advocated by investigators who have examined partial 18S ribosomal RNA data, proposes that annelids, molluscs, and certain other minor phyla with trochophore larva stages share a more recent common ancestor with one another than any do with arthropods. The two hypotheses are mutually exclusive in explaining spiralian relationships. Cladistic analysis of morphological data does not reveal phylogentic relationships among major spiralian taxa but does suggest monophyly for both the annelids and molluscs. Distance and maximum-likelihood analyses of 18S rRNA gene sequences from major spiralian taxa suggest a sister relationship between annelids and molluscs and provide a clear resolution within the major groups of the spiralians. The parsimonious tree based on molecular data, however, indicates a sister relationship of the Annelida and Bivalvia, and an earlier divergence of the Gastropoda than the Annelida–Bivalvia clade. To test further hypotheses on the phylogenetic relationships among annelids, molluscs, and arthropods, and the ingroup relationships within the major spiralian taxa, we combine the molecular and morphological data sets and subject the combined data matrix to parsimony analysis. The resulting tree suggests that the molluscs and annelids form a monophyletic lineage and unites the molluscan taxa to a monophyletic group. Therefore, the result supports the Eutrochozoa hypothesis and the monophyly of molluscs, and indicates early acquisition of segmented body plans in arthropods.

Key words: Molecular phylogeny — 18S rRNA gene — Annelida — Mollusca — Arthropoda — Combined approach — Morphology

Introduction

The phylogenetic relationships among major spiralian metazoans, the annelids, molluscs, and arthropods, have been the subject of continuous debate in systematic biology. These major spiralians comprise >90% of all living metazoan species (Barnes 1987; Brusca and Brusca 1990), yet the phylogenetic relationships among many of them remain undocumented. The annelids, molluscs, and arthropods are noted for both the trochophore larva and mesodermal segmentation. The segmented body plan occurs in the Arthropoda and Annelida. The Annelida and Mollusca both have trochophore larval stages in at least some of their marine representatives.

Three hypotheses of relationships among the Annelida, Mollusca, and Arthropoda have been suggested. The traditional view is that annelids and arthropods are considered to be each other's closest relatives, as evidenced by similarities in their segmented body plans (Barnes 1987; Brusca and Brusca 1990; Kozloff 1990; Meglitsch and Schram 1991). Although the ''Articulata'' hypothesis has been discussed predominately, it has yet to be supported by an analysis of data using the character congruence approach (Eernisse et al. 1992). An alterna-

Correspondence to: W. Kim

tive view proposes that annelids, molluscs, and certain other less speciose phyla share a more recent common ancestor with one another than any of them do with the arthropods (Ghiselin 1988; Patterson 1989). This assemblage was referred to as ''Eutrochozoa'' and approximately coincides with long-standing grouping of those taxa with a trochophore larval stage in at least some marine representatives of each group. The third logical alternative is that molluscs and arthropods are more closely related to each other than either is to the annelids. The ''Arthropoda–Mollusca'' grouping is somewhat consistent with other character evidence, such as the distribution of hemocyanin respiratory pigments (Mangum 1985; Ghiselin 1989). Because of the incongruent character distribution of trochophore larvae and segmented body plans among spiralian taxa, the Articulata and Eutrochozoa and Arthropoda–Mollusca grouping are mutually exclusive hypotheses of each other.

Hypotheses of spiralian phylogeny have only recently been evaluated with analyses of gene sequence data. The results of analyses of partial 18S rRNA sequences (~1,000 nucleotides) and complete 18S rDNA sequences from some spiralian taxa did somewhat support the Eutrochozoa hypothesis, although relationships among spiralian taxa were not actually resolved (Field et al. 1988; Raff et al. 1989; Lake 1990; Halanych et al. 1995). A molecular study using partial 18S rRNA sequence data suggested that the Mollusca and Arthropoda are sister taxa (Holland et al. 1991). A recent analysis (Wheeler et al. 1993) combining ~660 bp of the 18S rRNA genes and partial ubiquitin sequences with morphological data of some spiralian taxa was accomplished to elucidate the arthropod phylogeny. This analysis weakly supported the Articulata hypothesis, but very few informative sites were present in the region sequenced. More recently, parsimony and distance matrix analyses of 18S rDNA from some protostomes produced contradictory results according to the methods of analysis used, and, moreover, suggested that the Protostomia and Annelida are not monophyletic (Winnepenninckx et al. 1995). On the basis of earlier studies (Eernisse and Kluge 1993; Turbeville et al. 1994), it was argued that the 18S rRNA molecule alone did not resolve high-level phylogeny inference, and the importance of considering both molecular and morphological data in phylogeny reconstruction should be evaluated. However, an integrated approach, combining molecular and morphological characters, has not been utilized to infer the spiralian phylogeny.

To elucidate the phylogeny of major spiralians, we determined 18S rRNA gene sequences for five representative annelid species and added these to published sequences for other spiralian taxa to form a molecular data set. In addition, a traditional data set was assembled. In this paper, we report the first test of phylogenetic relationships among arthropods, molluscs, and annelids with the complete sequences of 18S rRNA genes from major spiralian taxa and include an assessment of the phylogeny among arthropods, molluscs, and annelids with both molecular and morphological characters.

Materials and Methods

Specimens Analyzed. The 18S rRNA gene sequences were compared for the representative annelids, the leeches *Hirudo medicinalis* and *Glossiphonia* sp.; branchiobdellidans *Xironogiton victoriensis* and *Sathodrilus attenuatus*; oligochaetes *Lumbricus rubellus* and *Enchytraeus* sp.; polychaetes *Aphrodita aculeata* and *Neanthes virens*; the representative arthropods; the insects *Drosophila melanogaster* (Genbank accession number: M21017) and *Tenebrio molitor* (X07801); the crustaceans *Berndtia purpurea* (L26511) and *Panulirus argus* (U19182); chelicerates *Eurypelma californica* (X13457) and *Androctonus australis* (X77908); and the representative molluscs, the gastropods *Limicolaria kambeul* (X66374) and *Onchidella celtica* (X70211); and the bivalves *Argopecten irradians* (L11265) and *Chlamys islandica* (L11232). The two flatworms (Platyhelminthes), *Echinostoma caproni* (L06567) and *Opisthorchis viverrini* (X55357), were used for the outgroup. The complete 18S rRNA gene sequences of five annelid (*Glossiphonia* sp., *Sathodrilus attenuatus, Enchytraeus* sp., *Aphrodita aculeata, Neanthes virens*) were determined in the present study, and the sequences of arthropods, molluscs, and flatworms were selected from GenBank. The sequences of the other three annelid taxa were reported in our previous study (Moon et al. 1996). The branchiobdellidan specimens were collected by Ms. Maria M. Ellis and the oligochaete specimens were provided by the Laboratory of Biochemistry, Department of Molecular Biology, Seoul National University. Cultures of live leeches were purchased from Ward's Natural Science International Marketing Group.

Total genomic DNA was isolated from live and ethanol-preserved individuals by using modifications of standard procedure (Sambrook et al. 1989).

DNA Amplification and DNA Sequencing. The 18S rRNA coding region was amplified in polymerase chain reaction (PCR) with oligonucleotide primers that recognize conserved sequences proximal to 5′ and 3′ termini of eukaryotic 18S rRNAs (Medlin et al. 1988). PCR amplifications were performed with Taq polymerase for 30 cycles (94°C for 1 min, 52°C for 2 min, and 72°C for 3 min). The ends of the amplified DNA fragments were modified for blunt-ended ligation using T4 kinase and T4 polymerase. The blunt-ended 18S rRNA genes were inserted into pGEM-3zf(−) plasmid vector and transformed to DH5-α cell lines. Sequencing primers used in this paper were recorded in our previous paper (Moon et al. 1996). 18S rRNA coding regions were sequenced both strands. The DNA sequencing was performed by the dideoxynucleotide chain-termination method (Sanger et al. 1977) using Taq-Track kit (Promega). Sequencing reaction mixtures were electrophoresed on buffer-gradient 6% polyacrylamide gels and visualized by autoradiography.

Phylogenetic Analysis of 18S rDNA Sequences. The nucleotide sequences were aligned with the CLUSTAL V multiple alignment program (Higgins et al. 1992) and refined manually. The alignment-stable regions were identified in a repeatable way by aligning sequences using a range of gap penalties, with toggle transitions unweighted. A data set of alignment-stable positions was produced by excluding those positions that differed between alignments (Gatesy et al. 1993). Analyses were limited to reliably aligned regions from the data set. The sequence alignment used in phylogenetic analyses is available from the authors. Parsimony and evolutionary parsimony (Lake 1987) analyses were conducted with the computer program PAUP, version 3.0 (Swofford 1990). Parsimony analyses were conducted by heuristic search and branch length was optimized according to the Acctrans option.

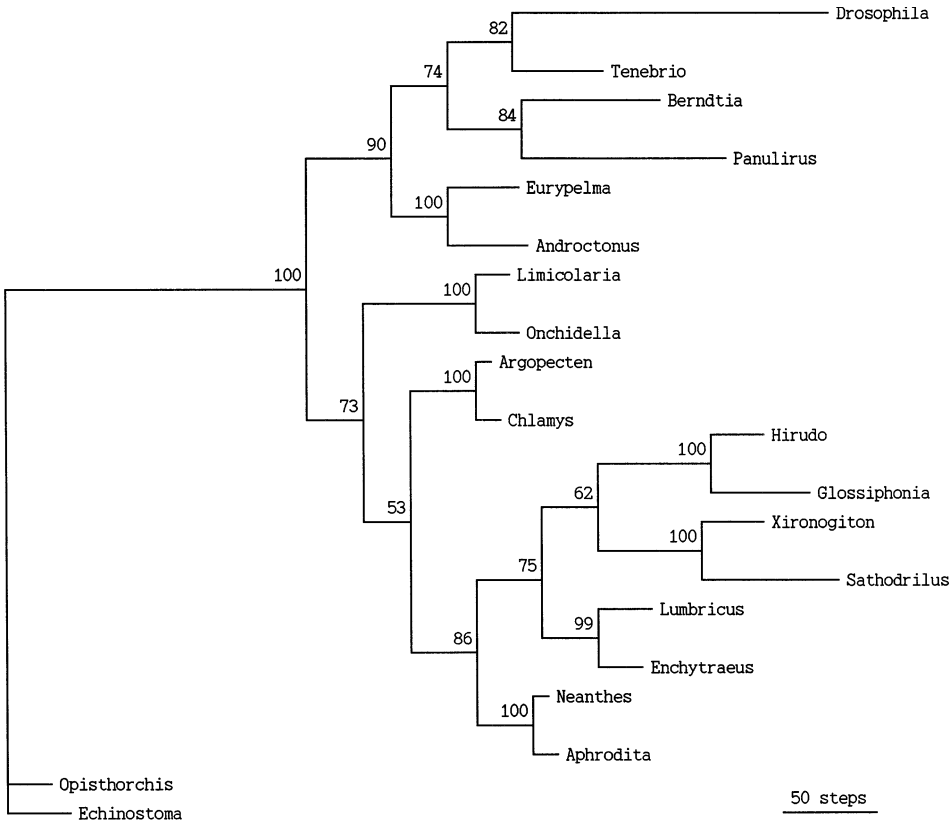

Fig. 1. Phylogenetic relationships among major spiralian taxa based on the 18S rRNA gene sequence data. *Numbers* above each branch indicate the percentage of the most parsimonious trees in which it was found in 100 bootstrap replications performed with PAUP.

PHYLIP version 3.5c (Felsenstein 1993) was used for neighbor-joining (Saitou and Nei 1987) and maximum likelihood (Felsenstein 1981) analyses. The distance analysis was done using a Kimura matrix (Kimura 1980) as input for the neighbor-joining analysis. The parsimony, distance, and maximum likelihood analyses incorporated a transition:transversion ratio of 1.6:1, which was determined in all pairwise comparisons of sequences using the computer program MEGA (Kumar et al. 1993) and calculation of the average ratio. Bootstrap analyses (Felsenstein 1985) were performed to examine the confidence of nodes within the resultant topology with the parsimony and distance analyses. Gaps were treated as missing data in all analyses. One hundred bootstrap replicates were performed for parsimony and distance analyses.

Morphological Data Analysis. Thirty-nine morphological characters were selected from the literature and coded as cladistic characters. Characters were collected whose states are fixed within terminal taxa, and missing values were assigned when information was not available or when a character was not applicable. The single multistate character was treated as unordered and all characters were equally weighted. The resulting data matrix was analyzed using PAUP (Swofford 1990).

Analysis of Combined Data Set. The molecular and morphological data sets were combined directly and analyzed with PAUP. According to this, the overall best-supported hypothesis was determined. In order to identify the phylogenetic signal in the data sets (Hillis 1991; Hillis and Huelsenbeck 1992), the skewness of tree-length distributions was determined in all individual data sets and in a combined data set.

Results

Phylogenetic Analyses of Molecular Data

The parsimony analysis of molecular data resulted in a minimal length tree (length = 1,554, consistency index = 0.616, retention index = 0.615) (Fig. 1) with a highly left-skewed tree-length distribution ($g_1 = -1.0948$). In this tree, the Bivalvia (= *Argopecten* sp. and *Chlamys* sp.), one of several representative classes in the Mollusca, was a sister taxon to the clade representing the Annelida, and the Gastropoda diverged earlier than the Bivalvia–Annelida clade. Moreover, the sister relationship of annelids and bivalves was weakly supported, having a low bootstrap value (53%). A monophyletic Mollusca was found as one of two equally parsimonious trees that require five additional steps. The monophyletic arthropods (bootstrap value: 90%) diverged earlier than the clade comprising the annelids and molluscs. In the annelid group, the polychaetes diverged first, and the remaining clitellate group was monophyletic. In the clitellates, the oligochaetes diverged earlier within the tree, and the leeches and branchiobdellids were left as sister group to each other.

Neighbor joining (NJ) and maximum-likelihood (ML) trees were similar in major respects to the most parsimonious tree. A sister relationship of annelids and molluscs was strongly supported, as suggested by bootstrap result (100% with NJ) (Fig. 2). In these analyses, however, the two molluscan taxa were united in a monophyletic group with a moderate bootstrap value (63%). Evolutionary parsimony found significant support for a sister relationship of annelids and molluscs. But the support

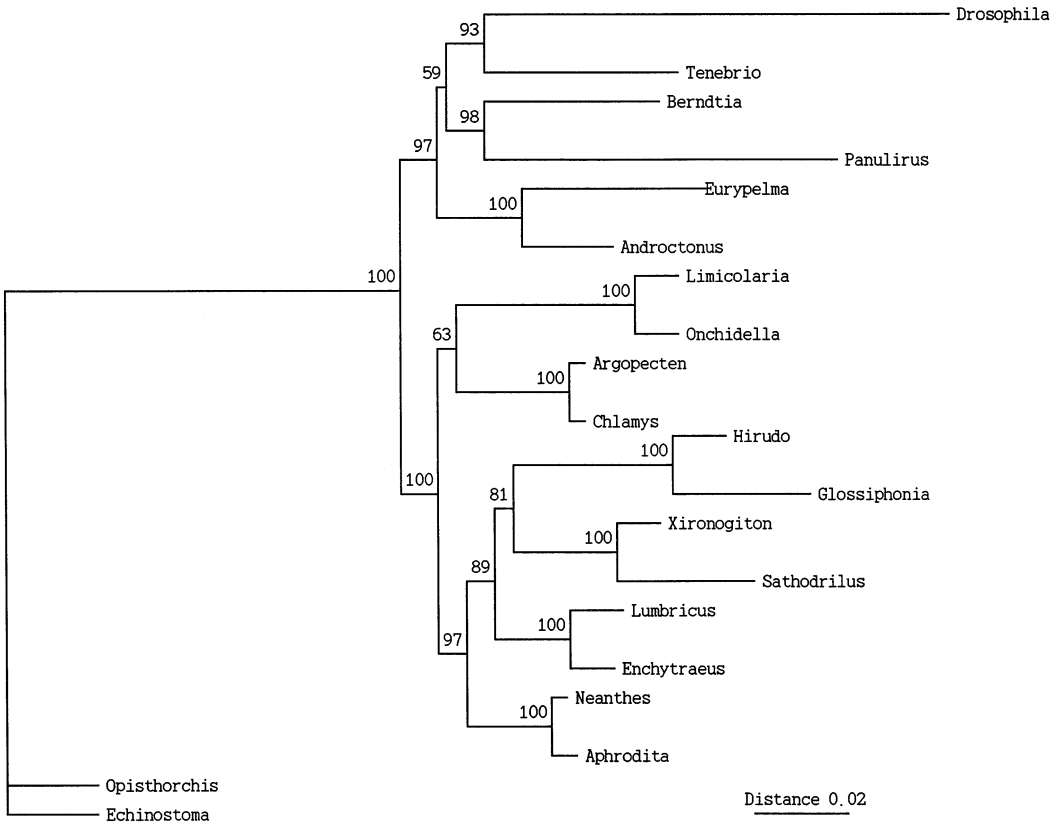

Fig. 2. Phylogenetic relationships among major spiralian taxa based on the 18S rRNA gene sequence data. *Numbers* above branches refer to the results of a bootstrap analysis with neighbor-joining carried with the SEQBOOT and NEIGHBOR programs from PHYLIP.

for the monophyly of molluscs was not significant (Fig. 3).

Morphological Data

The morphological characters and the data matrix for the taxa are shown in the Appendix. The characters uniting the arthropod taxa are excluded from the present analyses because the monophyly or polyphyly of the arthropods is still controversial (Brusca and Brusca 1990). Cladistic analysis of these data produced seven most parsimonious trees (length = 41, consistency index = 0.976, retention index = 0.991), and the strict consensus tree is shown in Fig. 4. The annelids (leeches, branchiobdellidans, and oligochaetes) and molluscs (gastropods and bivalves) each formed a monophyletic group. The relationships among annelids, molluscs, and arthropods were not completely resolved in the tree. In addition, the phylogenetic relationships among arthropod taxa (insects, crustaceans, and chelicerates) were not resolved in the consensus tree. One hundred bootstrap replications gave support at or above 95% level for the six internal branches (Fig. 4). The weakest support (67%) was obtained for the branch uniting two branchiobdellidan annelids. Because almost every internal branch with a bootstrap value greater than 70% represents a true clade according to Hillis and Bull (1993), all branches appear well supported. Tree-length distribution was strongly skewed to the left, with a g_1 index of −0.6475.

Combined Data

Parsimony analysis of the combined data set resulted in a most parsimonious tree (length = 1,600, consistency index = 0.623, retention index = 0.637) (Fig. 5). The tree-length distribution was highly skewed to the left, with a g_1 index of −1.0080. In this tree the annelids and molluscs were each shown as a sister group to the other. Two molluscan groups were clustered in a monophyletic group, although the two groups were separated in parsimony analysis using 18S rDNA data only.

Discussion

Phylogenetic analysis of traditional data does not reveal phylogenetic relationships of the major spiralian taxa but does suggest that the annelids and molluscs form a monophyletic group. The present analyses of molecular data strongly support the monophyly of each Arthropoda and Annelida. NJ and ML analyses of the 18S rDNA sequence data support the Eutrochozoa hypothesis. However, in the molecular tree that resulted from parsimony analysis, two molluscan groups are paraphyletic. The weak support of the paraphyly of the molluscan groups becomes more evident when two data sets are combined. The combined tree unites the molluscan taxa with the

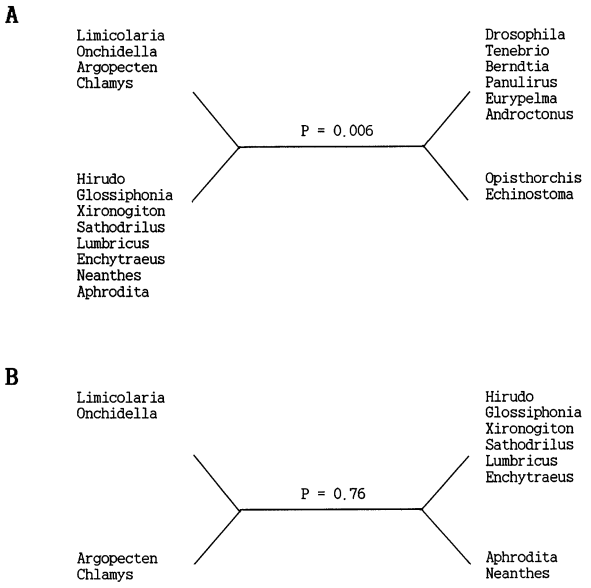

Fig. 3. Results of analyses with evolutionary parsimony. **A** Analysis with evolutionary parsimony to test the spiralian relationships. The favored tree from a combination of 4 molluscs × 8 annelids × 6 arthropods × 2 flatworms (384 quartets) is shown. The sister relationship of annelids and molluscs is significantly supported ($P = 0.006$). The two alternative hypotheses are not significantly supported. The P values for trees linking the annelids and arthropods and the arthropods and molluscs are $P = 0.014$ and $P = 0.918$, respectively. **B** Test of monophyly of molluscs. The favored tree from 4 molluscs × 8 annelids (48 quartets) is shown. The favored tree links two molluscan classes, but it is not significantly supported ($P = 0.76$).

annelid sister group to form a monophyly. This supports the widely held belief of many systematist zoologists (Barnes 1987; Brusca and Brusca 1990; Kozloff 1990; Meglitsch and Schram 1991). Consequently, the tree that resulted from the combined data supports the Eutrochozoa hypothesis and the monophyly of the Mollusca.

The phylogeny inferred from total evidence may be considered the current best combined hypothesis of spiralian relationships. Researchers (Kluge 1989; Jones et al. 1993) advocating a combining approach insist that this best explains all of the available evidence simultaneously. On the other hand, it has been argued that molecular and traditional data should not be combined if the two data sets strongly support conflicting hypotheses of phylogenetic relationship (Bull et al. 1993; De Queiroz 1993). We performed Templeton's (1983) tree comparison test in our data, which showed that molecular and morphological data produced topologies that were not significantly different at the $P < 0.05$ level. This result suggests that there is a general concordance between the two data sets. In addition, in spite of considerable size disparity between molecular (445 characters) and morphological (39 characters) data sets, direct combination of data does not generate the same trees as molecular data alone. The resulting tree (Fig. 5) from analysis of combining data sets strongly supported elements from each of the individual data-set analyses. With respect to the position of molluscs, it is in fact the traditional characters that dominate in the combined data analysis. Thus, the combination of data demonstrates the weak support in the molecular data for the sister relationship between the two molluscan groups in particular.

If the phylogeny inferred from combined data is confirmed, it will suggest a single evolution of the trochophore larva in the selected spiralian taxa and that secondary loss of this larva in the clitellate annelid lineage occurred in the course of their evolution. In addition to these suggestions, the combined data will indicate that segmented body plans in arthropods have been acquired earlier than the annelids.

Several significant findings emerge from this study regarding the phylogeny and classification of the annelids and arthropods. One of the most important questions on annelid phylogeny is, Which of the annelids came first? For a long time it was assumed that archiannelids, a heterogeneous assemblage of minute, highly modified polychaetes, were the most primitive annelids due to their simple body plan (Mettam 1985). However, excluding this assemblage, most hypotheses of annelid origin still assumed that the polychaetes were the earliest segmented worms and that metamerism (= serial segmentation) arose in connection with the development of parapodia (= lateral appendages) (Brusca and Brusca 1990; Pettibone 1982). Although the polychaetes are considered to have the more primitive reproductive system and larval development than the oligochaetes, it has been argued that ancestral annelids were more similar to the oligochaetes in overall body plan and that peristaltic movement evolved because of the metameric coelom (Brinkhurst 1982). In both of our molecular data and combined data-set analyses, the polychaetes diverged earlier than any other annelid taxa. Whether the clitellum, a reproductive structure, is a synapomorphic character for classifying annelid taxa is another subject of debate. The clitellates consist of the oligochaetes, leeches, and branchiobdellidans and are thought to constitute a monophyletic subphylum, superclass, or class (Brusca and Brusca 1990; Brinkhurst and Gelder 1991; Dales 1967). Our combined data-set analysis provides strong support (bootstrap value = 91%) for the monophyly of the Clitellata.

Controversy concerning arthropod evolution raises questions concerning whether the arthropods constitute a monophyletic or polyphyletic group, and, if they are monophyletic, how the major groups are related to one another. The traditional view has been to treat them as a monophyletic taxon at the phylum rank. However, some workers (Manton 1977; Anderson 1973) began to question the arthropod monophyly. Our molecular data and combined data-set analyses provide strong support for the monophyly of the arthropods and the sister relationship of the Chelicerate and the Mandibulata (Insecta plus Crustacea) (Boore et al. 1995).

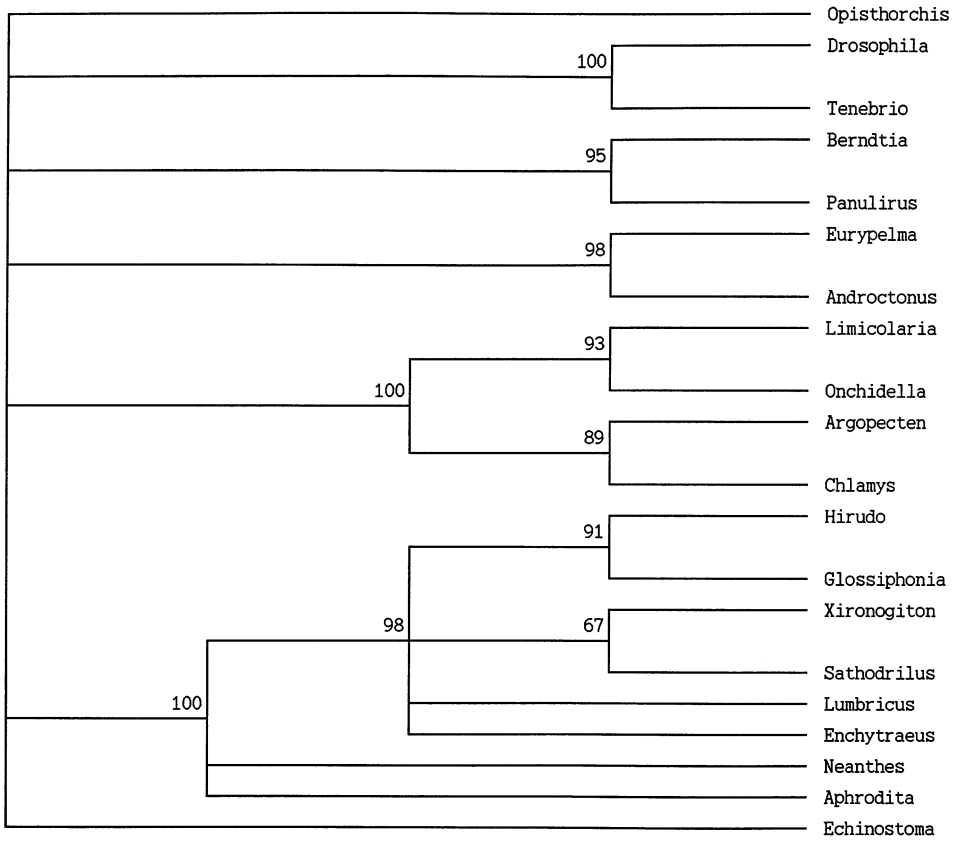

Fig. 4. Strict consensus tree for major spiralian taxa derived from parsimony analysis of morphological data, as listed in the Appendix. Percentage support for branch lengths derived from 100 bootstrap replications are given for each branch.

The support for the Eutrochozoa recognized here does not settle the controversy surrounding the extent to which molluscs are primitively metameric. It is uncertain which taxon is the immediate sister group of the Mollusca and whether the metamerism of this group is a derived loss or retained plesiomorphy. The lack of apparent metamerism in primitive molluscs such as Solenogastres and Caudofoveata and minor spiralian taxa including Sipuncula and Echiura presents difficulties for the argument of a primitively metameric eutrochozoan. In addition, if annelids and arthropods are not sister taxa, then similarities of their segmentation must have been much more ancient, or in many respects independently derived. A thorough evaluation of character evolution will require a strongly supported hypothesis of spiralian relationships. This will require additional 18S rRNA data, which are combinable, from all spiralian taxa in combination with a reassessment of spiralian relationships with morphological characters.

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Appendix. Data matrix for morphological characters[a]

	Characters and Character states
	11111111112222222223333333333
Taxa	12345678901234567890123456789012345678 9
Drosophila	000000000?0000000000000000011111111101
Tenebrio	000000000?0000000000000000011111111101
Berndtia	000000000?0000000000000011100000000001
Panulirus	000000000?0000000000000011100000000001
Eurypelma	000000000?0000000000011100000000000001
Androctonus	000000000?0000000000011100000000000001
Limicolaria	111111110000000000000000000000000000010
Onchidella	111111110000000000000000000000000000010
Argopecten	111111001110000000000000000000000000010
Chlamys	111111001110000000000000000000000000010
Hirudo	000000000?0101111110110000000000000021
Glossiphonia	000000000?0101111110110000000000000021
Xironogiton	000000000?0101111111000000000000000021
Sathodrilus	000000000?0101111111000000000000000021
Lumbricus	000000000?0111111110000000000000000021
Enchytraeus	000000000?0111111110000000000000000021
Neanthes	000000000?0111110000000000000000000011
Aphrodita	000000000?0111110000000000000000000011
Opisthorchis	000000000?0000000000000000000000000000
Echinostoma	000000000?0000000000000000000000000000

[a]Characters and character states of the morphological data-set used in the present study. Each alternative character state was scored as 0, 1, 2.

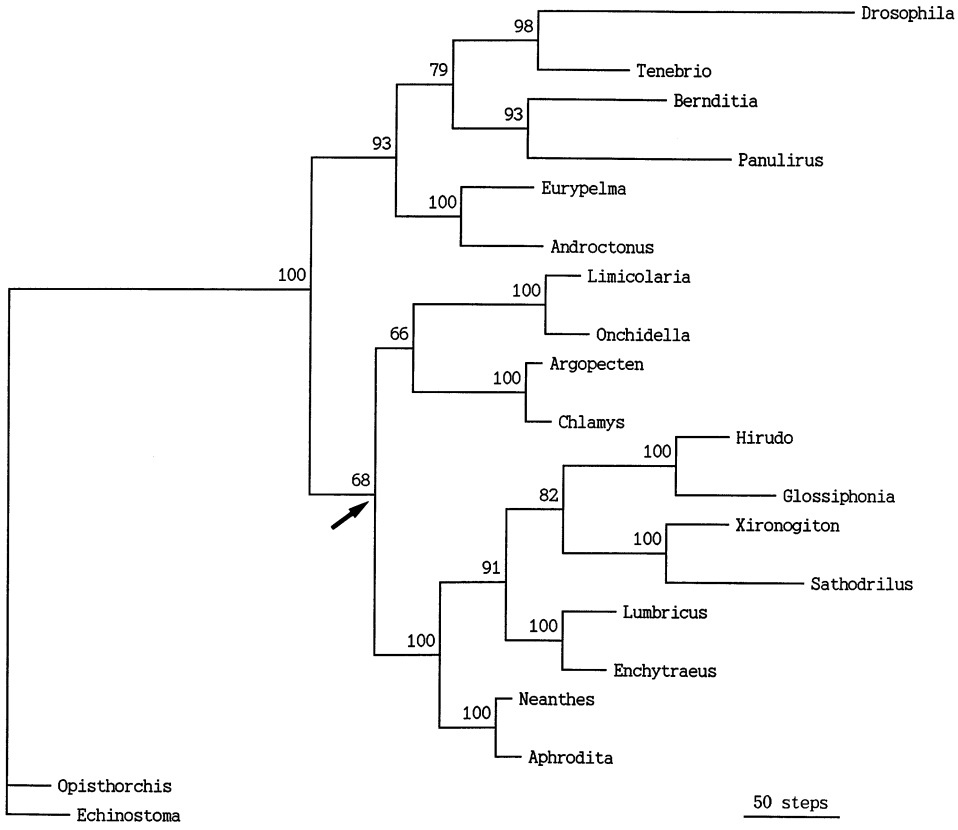

Fig. 5. The most parsimonious tree based on combined molecular and morphological data. *Numbers* above branches represent bootstrap values. The clade of the selected eutrochozoan taxa is indicated by an *arrow*.

Inapplicable states were treated as unknown (?) in analysis. All characters were treated as unordered in the parsimony analysis. The 39 columns correspond to the character numbers in the list below: 1, The coelom and an open hemocoelic circulatory system: 0 = coelom does not reduce and an open hemocoelic circulatory system does not develop, 1 = coelom reduces and an open hemocoelic circulatory system develops (Brusca and Brusca 1990); 2, Dorsal body wall: 0 = does not form a mantle, 1 = forms a mantle (Brusca and Brusca 1990); 3, Calcareous spicules (and shell): 0 = absent, 1 = present (Brusca and Brusca 1990); 4, Ventral body wall muscles: 0 = develops as muscular foot, 1 = develops as muscular foot (Brusca and Brusca 1990); 5, Radula: 0 = absent, 1 = present (Brusca and Brusca 1990); 6, Chambered heart with separate atria and ventricle: 0 = absent, 1 = present (Brusca and Brusca 1990; Hyman 1967); 7, Torsion and its associated anatomical conditions: 0 = absent, 1= present (Brusca and Brusca 1990); 8, Internal organs: 0 = normal, 1 = concentrated as visceral hump (Brusca and Brusca 1990); 9, Bivalve shell and its associated mantle and ctenidial modifications: 0 = absent, 1 = present (Brusca and Brusca 1990); 10, Loss of ladula: 0 = no, 1 = yes (Brusca and Brusca 1990); 11, Byssus; 0 = absent, 1 = present (Brusca and Brusca 1990; 12, Annelid head: 0 = absent, 1 = present (Brusca and Brusca 1990); 13, Epidermal paired setae (or bundles); 0 = absent, 1 = present (Brusca and Brusca 1990); 14, Longitudinal muscles: 0 = form sheets, 1 = broken into bands (Brusca and Brusca 1990; Wheeler et al. 1993); 15, Annelid nephridial system: 0 = absent, 1 = present (Brusca and Brusca 1990); 16, Cuticle with collagen but no chitin except in setae and stomodaeum: 0 = no, 1 = yes (Boudreaux 1979); 17, Clitellum: 0 = absent, 1 = present (Brusca andBrusca 1990); 18, Direct development without intervening larval stages: 0 = no, 1 = yes (Brusca and Brusca 1990); 19, Cerebral ganglion: 0 = does not move into anteriormost trunk segment, 1 = moves into anteriormost trunk segment (Brusca and Brusca 1990; Jamieson 1988); 20, Body segment number fixed at 15 segments: 0 = no, 1 = yes (Brusca and Brusca 1990); 21, Reduction of the coelom to a series of channels or lacunae: 0 = no, 1 = yes (Brusca and Brusca 1990); 22, Body segment number fixed at 34 segments: 0 = no, 1 = yes (Brusca and Brusca 1990); 23, Tagmosis into prosoma and opisthosoma without distinct head: 0 = no, 1 = yes (Wheeler et al. 1993); 24, First appendages chelicerae (or cheliphores) of three articles: 0 = no, 1 = yes (Wheeler et al. 1993); 25, Typically four pairs of walking legs: 0 = no, 1 = yes (Weygolt 1986; Weygoldt and Paulus 1979); 26, Two paris of antennae: 0 = absent, 1 = present (Wheeler et al. 1993); 27, Antennae biramous: 0 = no, 1 = yes (Wheeler et al. 1993); 28, Nauplius or egg-nauplius stage in ontogeny: 0 = no, 1 = yes (Schram 1986); 29, Thorax divided into three segments each with a pair of limbs: 0 = no, 1 = yes (Wheeler et al. 1993); 30, Locomotory limbs six-segmented: 0 = no, 1 = yes (Wheeler et al. 1993); 31, Abdomen with 12 segments: 0 = no, 1 = yes (Wheeler et al. 1993); 32, Distinct thorax and abdomen: 0 = absent, 1 = present (Wheeler et al. 1993); 33, Knee as joint vs segment: 0 = absent, 1 = present (Wheeler et al. 1993); 34, Labium: 0 = absent, 1 = present (Wheeler et al. 1993); 35, Hexapod-type cephalization: 0 = no, 1 = yes (Wheeler et al. 1993); 36, Abdominal cerci: 0 = absent, 1 = present (Wheeler et al. 1993); 37, Two primary pigment cells in ommatidia: 0 = absent, 1 = present (Boudreaux 1979; Kristensen 1975; Paulus 1979); 38; Trochophore larva: 0 = absent, 1 = present; 2 = secondarily lost (Eernisse et al. 1992); 39, Segmented body plan: 0 = absent, 1 = present (Eernisse et al. 1992)

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