

Molecular phylogeny of the Annelida

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Abstract: Traditionally, the Annelida has been classified as a group comprising the Polychaeta and the Clitellata. Recent phylogenetic analyses have led to profound changes in the view that the Annelida, as traditionally formulated, is a natural, monophyletic group. Both molecular and morphological analyses support placement of the Siboglinidae (formerly the Pogonophora) as a derived group within the Annelida; there is also evidence, based on molecular analysis of the nuclear gene elongation factor-1 α , that the unsegmented echiurids are derived annelids. While monophyly of the Clitellata is well-supported by both molecular and morphological analyses, there is no molecular evidence to support monophyly of the polychaete annelids; the Clitellata fall within a paraphyletic polychaete grade. Relationships among groups of polychaete annelids have not yet been resolved by molecular analysis. Within the Clitellata, paraphyly of the Oligochaeta was indicated in a phylogenetic analysis of cytochrome *c* oxidase I, which supported a sister relationship between the leeches, including an acanthobdellid and a branchiobdellid, and two of the four oligochaetes in the analysis. There is some evidence from analysis of 18S rRNA sequences for a sister-group relationship between the clitellates and the taxon *Aeolosoma*. There is no agreement regarding the body form of the basal annelid, and while molecular analyses provide strong support for the Eutrochozoa, the identity of sister-group to the Annelida among the Eutrochozoa remains enigmatic. It is recommended that future investigations include additional conserved gene sequences and expanded taxon sampling. It is likely that the most productive approach to resolving annelid phylogeny, and thus increasing our understanding of annelid evolution, will come from combined analyses of several gene sequences.

Resume : Traditionnellement, les Annélides sont classifiés en un groupe qui englobe les Polychaeta et les Clitellata. Les analyses phylogénétiques récentes, cependant, ont abouti à des bouleversements profonds de l'interprétation traditionnelle des Annélides comme groupe monophylétique naturel. Les analyses moléculaires et morphologiques appuient l'inclusion des Siboglinidae (autrefois les Pogonophora), comme un groupe dérivé parmi les Annélides; de plus, l'analyse moléculaire du gène nucléaire facteur d'élongation-1 α démontre que les échiuridés non segmentés sont des annélides dérivés. Alors que le monophylétisme des Clitellata est appuyé par les analyses moléculaires et morphologiques, il n'existe pas de preuve moléculaire que les annélides polychètes soient monophylétiques; les Clitellata forment un groupe paraphylétique parmi les polychètes. Les analyses moléculaires n'ont pas encore permis d'établir les relations entre les groupes d'annélides polychètes. Parmi les Clitellata, le paraphylétisme des Oligochaeta est indiqué par une analyse phylogénétique de la cytochrome *c* oxydase I, qui suggère la formation d'une relation de type groupe soeur entre les sangsues, dont un acanthobdellidé et un branchiobdellidé, et deux des quatre oligochètes étudiés. L'analyse des séquences de 18S ARNr indique aussi l'existence d'une relation de type groupe soeur entre les Clitellata et *Aeolosoma*. Il n'y a pas de consensus sur la structure corporelle d'origine d'un Annélide, et bien que les analyses moléculaires suggèrent que cette structure se retrouve chez les Eutrochozoa, le groupe soeur des Annélides parmi les Eutrochozoa demeure une énigme. Nous recommandons lors d'analyses futures de tenir compte d'autres séquences génétiques conservatrices et d'étendre l'échantillonnage à plus de taxons. Il est probable que l'approche la plus productive pour résoudre la phylogénie des annélides et donc augmenter notre compréhension de leur évolution, sera de faire des analyses combinées de plusieurs séquences de gènes.

Introduction

The most striking thing about the phylogeny of the Annelida is how poorly resolved are the evolutionary relationships of this large, ancient, and ecologically important metazoan group. While some trace fossils attributed to annelids are known as far back as the Ediacaran period, the fossil record provides limited insight into the pattern of radiation of the

major annelid groups. Body fossils are rare, although the increasing diversity of annelids throughout the past 600 million years is clearly reflected in tube and trace fossils over that time period (Robison 1987). Currently, approximately 15 000 species of annelids are known, and it is likely that many hundreds more remain undiscovered and undescribed.

Morphological analyses of deep-level annelid relationships are difficult for a number of reasons. As is the case for many metazoan groups, homology of some morphological characters among extant annelid groups is difficult to assess, and scoring of character states for phylogenetic analyses is open to conflicting interpretations (e.g., Eernisse 1997; Rouse and

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Fauchald 1995). Furthermore, convergent or parallel secondary losses of morphological characters likely occurred in some annelid lineages, and scoring such characters as simply absent in a phylogenetic analysis would be misleading.

Molecular data provide characters for phylogenetic analysis for which homology assessments of numerous characters across a wide range of taxa are very feasible, secondary character losses are not problematic, and character coding is largely uncontroversial (Hillis et al. 1996). For these reasons, phylogenetic analyses of molecular data can be a very productive approach to understanding annelid evolution. Relatively few studies of deep-level annelid phylogeny using molecular data have yet been published, however. So far, these molecular studies of early annelid divergences have drawn on DNA or amino acid sequence data for highly conserved genes, particularly the structural gene 18S ribosomal RNA (18S rRNA) (e.g., Winnepenninckx et al. 1995, 1998; Kim et al. 1996; Moon et al. 1996; Eernisse 1997); the nuclear protein-coding gene elongation factor-1 α (EF-1 α) (Kojima et al. 1993; McHugh 1997; Kojima 1998); and the slowly evolving mitochondrial gene cytochrome *c* oxidase I (COI) (e.g., Siddall and Burreson 1998). Recently, combined analyses of these (Martin et al. 2000) and other nuclear genes (histone H3, U2 snRNA, and 28S rDNA) (Brown et al. 1999) have been published. This review focuses on these and other studies, the insights that have been gained from them regarding the branching patterns among the major annelid groups, and the outstanding questions concerning annelid evolution that future molecular phylogenetic analyses can address.

Annelida

The Annelida has been generally recognized as a group of segmented coelomate worms in which the nerve cord is located ventrally and paired chitinous chaetae occur segmentally (e.g., see Brusca and Brusca 1990). They range in size from millimetres to several metres long, and because many are deposit-feeders, they are ecologically important bioturbators of aquatic and terrestrial sediments. Traditionally, the Annelida has been classified as a group comprising the Polychaeta and Clitellata (including the Oligochaeta and Hirudinea) (for details of the taxonomic history of the Annelida see Fauchald and Rouse 1997).

Most polychaetes have parapodia that bear chitinous chaetae on each segment, and many bear a pair of anterior ciliated sensory structures called nuchal organs. Polychaete annelids exhibit a great diversity of forms, ranging from sedentary tube-dwellers to pelagic worms; in some, the segments along the body are of uniform morphology, while in others, segments are fused to form specialized regions for feeding or respiration or for brooding of embryos. Polychaetes are equally diverse in their habitats, which are mostly marine but range from estuarine mud flats to deep-sea hydrothermal vents. Some polychaetes are suspension-feeders, others are scavengers or predators, and a few, like the histriobdellids, are parasitic on other invertebrates (Fauchald and Jumars 1979).

Clitellates are recognized by means of the clitellum, a glandular girdle involved in cocoon production; they also share a complex reproductive system. The majority of clitel-

lates live in freshwater and terrestrial habitats, and they are detritivores, direct deposit-feeders, predators, or parasites. In many classification schemes, the Clitellata is divided into the Oligochaeta, which includes the familiar earthworms and also many aquatic groups, and the Hirudinea, which includes the true leeches (Euhirudinea) and other parasitic groups (Branchiobdellida and Acanthobdellida) (Brusca and Brusca 1990).

Monophyly of the Annelida

Recent phylogenetic analyses have led to a profound change in the view that the Annelida, as traditionally formulated to include the polychaetes and clitellates, is a natural, monophyletic group. These analyses were prompted by a lack of derived characters shared by the polychaetes and clitellates, and because of the proposed affinities of several other worm-like groups with the annelids, e.g., the deep-sea siboglinid tube worms (formerly the Pogonophora (see McHugh 1997; Rouse and Fauchald 1997)) and the echiurid spoon worms.

In siboglinids, both larval and adult features suggest annelid affinities, but the phylogenetic position of this group of worms has been a contentious issue since they were first discovered. Siboglinids are tentaculate segmented tube-dwelling worms that are found at ocean depths from 200 to 10 000 m; adults lack a functional gut and derive their nutrition from endosymbiotic chemoautotrophic bacteria (Cavanaugh 1994). One of the first siboglinid species described was assigned to a polychaete family, but in 1944 a new phylum was erected for these bizarre worms and this taxonomic rank remained in use until 1997 (McHugh 1997; Rouse and Fauchald 1997). After studying incomplete animals, some authors considered siboglinids to be closely allied with deuterostomes (Ivanov 1955); however, with the description of the chaeta-bearing, segmented posterior end of the worms and studies of their embryology, the protostome condition of siboglinids became clear, and a close relationship with the annelids seemed likely. The vestimentiferan worms of deep-sea hydrothermal vents were also assigned to a separate metazoan phylum based on their unique form of coelomic cavities (Jones 1985), but are now considered siboglinids (Southward 1988; Ivanov 1994).

Echiurids are unsegmented coelomate marine worms that burrow in soft sediments and typically feed on detritus, using a scooplike proboscis. The lack of segmentation in echiurids was considered a primary absence rather than a derived loss of this trait, and their elevation from their initial placement as a class within the Annelida to phylum status was justified by this lack of segmentation, and on the basis of the unique excretory anal vesicles, the proboscis, and the arrangement of body wall muscle layers that they possess (Newby 1940). Until recent molecular analyses were carried out, this taxonomic status of the group has been entrenched in all but a few classification schemes (McHugh 1997).

Early phylogenetic analysis of 18S rRNA established it as a useful gene for resolving deep-branch relationships among metazoans (Field et al. 1988); however, phylogenetic analyses to determine the position of the siboglinids and echiurids in relation to the Annelida based on the 18S rRNA gene have so far been inconclusive or contradictory. In neighbour-joining (NJ) and maximum-parsimony (MP) analyses of 18S

rRNA sequences from 22 taxa, the siboglinids and the echiurid formed a monophyletic group that was more closely related to molluscs than to the polychaete or clitellate in the analysis (Winnepenninckx et al. 1995); bootstrap-proportion (BP) support for the siboglinid–echiurid clade was moderate (BP = 76 and 77 for NJ and MP analyses, respectively). In a later study focusing on molluscan relationships, Winnepenninckx et al. (1996) included three annelids as well as an echiurid and two siboglinids. In the NJ analysis of these data, the siboglinids and the echiurid again formed a monophyletic group (BP = 87), but in this case there was support, albeit weak (BP < 50), for a sister relationship with a clade formed by the three annelids in the analysis. The MP analysis of these data also showed weak support for an annelid–siboglinid–echiurid clade (BP < 50; decay index = 1). In a study of the evolutionary origins of hydrothermal vent siboglinids, Halanych et al. (1998) used MP to analyze 23 18S rRNA gene sequences and found weak support (BP < 50) for placement of a monophyletic siboglinid clade and the echiurid within a paraphyletic polychaete–clitellate clade.

Over the past few years there has been rapid growth in the availability of 18S rRNA gene sequences for most major metazoan groups, and a more consistent effort to align sequences according to the secondary-structure model for 18S rRNA. A recurring pattern in phylogenetic analyses as the number of 18S rRNA sequences for metazoan taxa increased has been poor resolution of relationships among major spiralian groups, including annelid groups. Furthermore, some relationships among major groups differ according to the number and choice of taxa analyzed. Winnepenninckx and her colleagues (1998) recently drew on the increased availability of 18S rRNA data to reassess previous results with respect to worm relationships; they analyzed an expanded sample of 57 taxon sequences aligned according to the secondary-structure model. Their NJ results showed that the siboglinid–echiurid sister relationship was no longer supported, and that both the siboglinids and the echiurid are more closely related to spiralian other than any of the 25 polychaetes and clitellates in the analysis, which themselves do not share a common ancestor (Fig. 1). As Winnepenninckx et al. (1998) point out, however, their results are only weakly supported by BP values, and the results may reflect the limitations of the 18S rRNA molecule for resolving branching patterns dating from more than 500 million years ago; alternatively, radiation among protostomes may have been so rapid as to render resolution of the resultant short internodes difficult with a single gene such as 18S rRNA. At the very least, Winnepenninckx et al.'s (1998) results show the critical role played by taxon sampling in the conclusions drawn from any phylogenetic analysis. This is further demonstrated in parsimony analyses of 103 and 66 18S rRNA metazoan sequences by Eernisse (1997), who investigated annelid–arthropod relationships. Monophyly of the siboglinids was supported, but their position within a protostome clade differed between the two analyses. The single echiurid was sister to a polychaete annelid in the 103-taxon analysis, but its position in the 66-taxon analysis was not resolved. In both analyses the representatives of the traditional Annelida (three polychaetes and one clitellate) formed a polyphyletic group.

Similar results were found by Halanych (1998), who ana-

lyzed 119 metazoan sequences using parsimony methods; nonparametric bootstrap analysis of the parsimony tree showed weak support (BP < 50) for placement of the siboglinids and echiurids on the tree. Like Winnepenninckx et al. (1998), Halanych (1998) suggested that the poor resolution of the tree may reflect rapid radiation of the major protostome groups. If this is the case, the problem of resolving annelid relationships using molecular data would not be limited to 18S rRNA but would also be expected in phylogenetic analyses of other conserved gene sequences.

EF-1 α is highly conserved in its amino acid sequence and has been used in several studies of ancient metazoan divergences (e.g., Kobayashi et al. 1996; Regier and Shultz 1997, 1998), including divergences among annelid groups. Using EF-1 α amino acid sequence data (146 residues), Kojima et al. (1993) inferred that the siboglinids are closely related to the annelids, but any further conclusions were precluded by the very limited taxon sample (8) in that study. In a further examination of phylogenetic relationships among polychaetes, clitellates, echiurids, and siboglinids, McHugh (1997) analyzed the same coding region of EF-1 α from 20 taxa. Using a chordate as the outgroup, the parsimony analysis of first and second codon positions yielded two equally parsimonious trees in which the siboglinids and the echiurids each formed a monophyletic clade within a paraphyletic grade of polychaetes (Fig. 2). Decay indices ranging from 3 to 7 supported these results, and when the parsimony analysis was constrained to a topology that forced a traditional polychaetes + clitellates clade, the resultant trees were statistically significantly longer than the most parsimonious trees (McHugh 1997). Using molluscs and arthropods as outgroups yielded the same results from parsimony analysis, and NJ analysis also supported placement of siboglinids and echiurids within a monophyletic worm clade (McHugh 1997). Based on these results, McHugh (1997) proposed that the pogonophoran tube worms assume the group name erected in the first species description for the group, Siboglinidae (Caullery 1914), and that the group name for echiuran spoon worms be changed to that used in early references to these worms as members of the Annelida, Echiuridae (Hatschek 1880). (Note that Siddall et al. (1998) recently published a critique of McHugh (1997) in which they made several misleading and obfuscatory statements about the EF-1 α analysis and omitted information that supports the EF-1 α results presented; a complete rebuttal of Siddall (1998) is provided by McHugh (1999).)

As with the earlier 18S rRNA studies, the taxon sampling in the EF-1 α analyses of McHugh (1997) is limited. However, analyses with greater taxon sampling and more sequence data from EF-1 α (~1200 base pairs (bp)) also support the results (Kojima 1998; D. McHugh, unpublished data). Kojima (1998) analyzed amino acid sequences for almost the entire EF-1 α gene (378 residues) to test monophyly of the Polychaeta. The results of his NJ analysis of 25 taxa (including 13 polychaetes, 4 clitellates, and 2 siboglinids) and the result of his likelihood analysis of a smaller sample of annelids (7 polychaetes, 4 clitellates, and 2 siboglinids), although weakly supported, are congruent with those of McHugh (1997) regarding the placement of siboglinids within a paraphyletic grade of polychaetes (Kojima 1998); no echiurids were included in the Kojima (1998) analyses.

Fig. 1. Neighbour-joining tree of Jukes–Cantor distances based on the alignment of 57 metazoan sequences of 18S rRNA, including 25 polychaetes and clitellates (from Winnepenninckx et al. 1998). Numbers above the nodes represent bootstrap proportions. Monophyly of the Oligochaeta, Euhirudinea, Branchiobdellida, and Clitellata is supported; *Aeolosoma* is indicated as the sister-group to the Clitellata. Monophyly of the Siboglinidae (vestimentiferan and pogonophoran) is also supported. Polychaetes do not form a monophyletic group and none of the orders of Fauchald (1977) or the clades proposed by Rouse and Fauchald (1997) are supported.

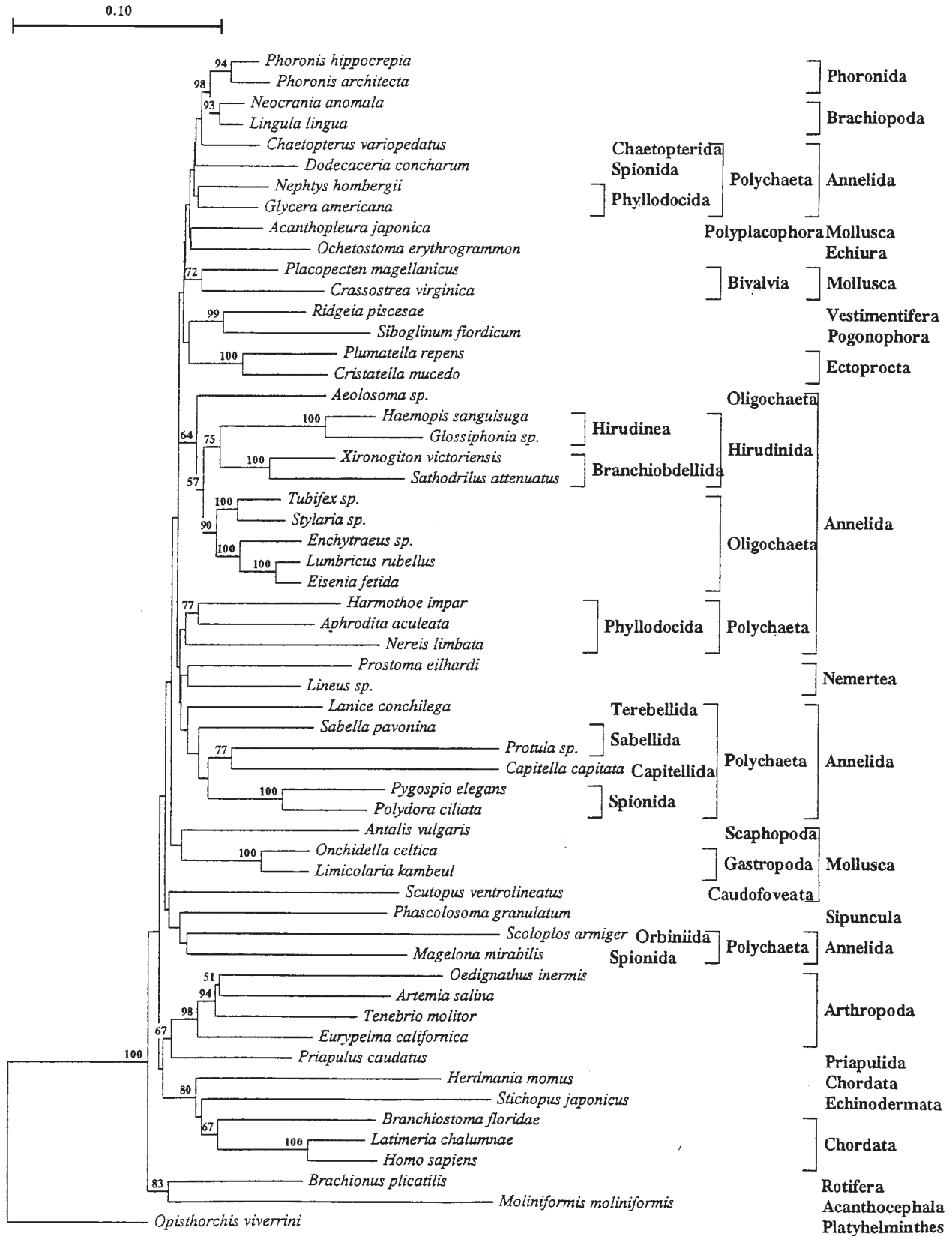
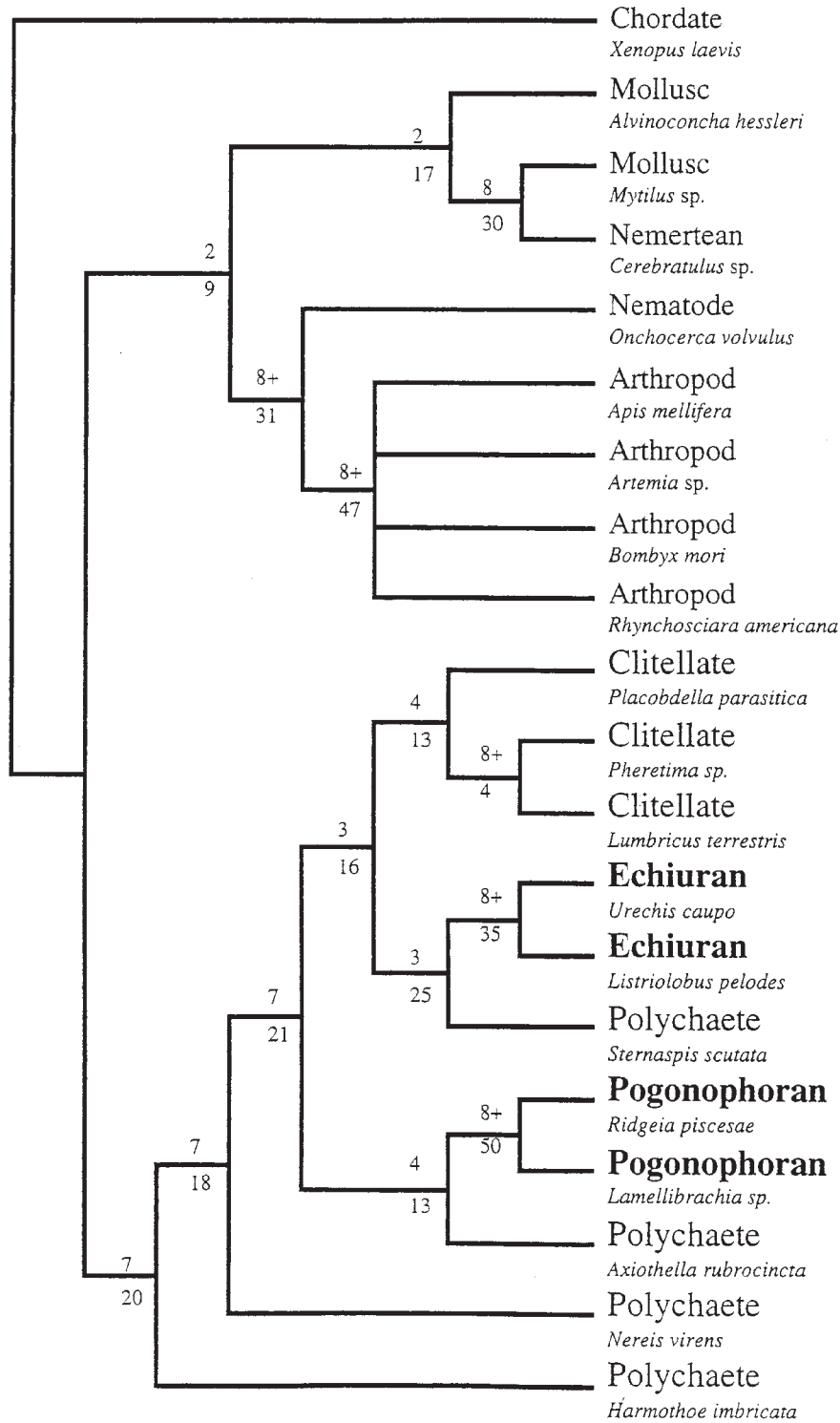


Fig. 2. Results of the parsimony analysis of elongation factor-1 α DNA sequence data showing siboglinids, echiurids, and clitellates as derived groups within the annelid clade (from McHugh 1997). Numbers above and below the internodes are decay indices and branch lengths, respectively.



The results of the EF-1 α sequence analyses of McHugh (1997) and Kojima (1998) support the view, based on early embryology, hooked chaetae, and blood pigments, that siboglinids are closely allied with annelids, and with polychaete annelids in particular (Uschakov 1933; Webb 1969;

van der Land and Nørrevang 1975; Terwilliger et al. 1985; Southward 1988; Suzuki et al. 1989; Gardiner and Jones 1994; Bartolomaeus 1995; Callsen-Cencic and Flügel 1995; Young et al. 1996). This result is also congruent with the phylogenetic analyses of morphological and developmental

characters by Rouse and Fauchald (1997) and Rouse (1999). A close relationship between siboglinids and polychaete annelids is also indicated by the molecular structure of collagen; *Arenicola marina*, the lugworm polychaete, and *Riftia pachyptila*, a hot-vent siboglinid, share a thus-far unique substructural feature in the triple helical domain of interstitial collagen (Sicot et al. 1997). Comparisons of mitochondrial-gene arrangements in a polychaete, two clitellates, a siboglinid, a mollusc, two arthropods, and two chordates, and phylogenetic analyses of these mitochondrial amino acid sequences strongly support inclusion of siboglinids in the Annelida (Boore and Brown 2000). Further support for this relationship is demonstrated in analyses of the DNA sequences from the nuclear genes histone H3, U2 snRNA, and 28S rDNA (Brown et al. 1999; see below).

The results of McHugh (1997) support the inclusion of echiurids in the Annelida as suggested by Nielsen (1995), and imply that the unsegmented echiurid condition is derived from a segmented annelid ancestor. Several morphological and embryological characters provide corroborative evidence that echiurids stem from an annelid ancestor. For example, in their cleavage patterns, chaetal formation, and sperm ultrastructure, echiurids share similarities with some polychaete annelids (Newby 1940; Franzén and Ferraguti 1992; Pilger 1993). A possible ancestral segmented condition in echiurids is indicated by early reports of annelid-like teloblastic development, the arrangement of ganglia along the larval nerve cord coincident with rings of larval ectodermal mucous glands, and repeated pairs of nephridia in adults (Hatschek 1880; Newby 1940). As suggested by Pearse et al. (1987), echiurids may represent neotenous annelids in which the development of segmentation is suppressed during growth. The secondary absence of segmentation may account for the contradictory placement of echiurids basal to a clade of segmented protostomes in the phylogenetic analyses of morphological characters by Rouse and Fauchald (1997).

Based on her results, McHugh (1997) proposed that the Annelida be redefined to include the echiurids, siboglinids, clitellates, and polychaetes, with the presence of paired chitinous chaetae then forming an annelid synapomorphy. The "annelid cross" cleavage pattern of blastomeres $1a^{112}-1d^{112}$ that occurs in echiurids, clitellates, and polychaetes (Newby 1940; Needham 1990) may constitute another synapomorphy for the Annelida; the pattern of cleavage in siboglinids has yet to be fully described. Because paraphyly of the taxon Polychaeta was supported by the EF-1 α analyses, McHugh (1997) also proposed that the term polychaete be used only as an informal name referring to that annelid grade. Further evaluation of this redefined Annelida taxon using additional taxa and different genes is ongoing; preliminary analyses of annelid relationships using a second nuclear coding gene, RNA polymerase II, corroborate the EF-1 α results (M. Diaz and D. McHugh, unpublished data).

A third group for which annelid status has been debated is the myzostomid worms, incompletely segmented, acoelomate worms with chaetae and a trochophore-like larva. Myzostomids are viewed by many authors as derived polychaetes, in which a close association with echinoderms has led to aberrant morphology (Nielsen 1995; Rouse and Fauchald 1997), while others consider them to be a separate taxon altogether

(Haszprunar 1996). A recent investigation of the phylogenetic relationship of the myzostomids and the Annelida based on combined analyses of 18S rDNA and EF-1 α data shows that the myzostomids are not nested within the Annelida; rather, they are closely related to flatworms (Eeckhaut et al. 2000).

Polychaete relationships

The morphologically diverse polychaete annelids have been classified into over 80 families, the most widely accepted grouping of which has been into the orders proposed in a key to polychaetes by Fauchald (1977). However, as stated in Fauchald and Rouse (1997), that key was based on differences among taxa rather than the shared similarities that form the basis for any phylogenetic classification. While Rouse and Fauchald (1997) addressed the need for a phylogenetic analysis of polychaete family relationships based on morphological characters, and presented a reclassification based on their results, very few authors have tackled the same problem using molecular data. In their analyses, Rouse and Fauchald (1997) found that a monophyletic Polychaeta that includes the Siboglinidae was weakly supported. There is also molecular evidence for placing the Siboglinidae within a polychaete clade (see above); however, there is no molecular evidence to support monophyly of the polychaete annelids.

In one of the most comprehensive molecular analyses of annelid relationships to date, Winnepenninckx et al. (1998) included 15 polychaetes in an analysis of 18S rDNA sequences from 57 metazoan taxa. The resultant tree (Fig. 1) did not support monophyly of the Annelida (see above), nor did the seven polychaete orders (sensu Fauchald 1977) represented in the analysis have a common ancestor. Furthermore, none of the polychaete orders for which two or more species were included were monophyletic (Winnepenninckx et al. 1998); groups proposed by Rouse and Fauchald (1997) based on their morphological analysis were poly- or paraphyletic. A similar lack of support for higher level polychaete groups is seen in the results of Kojima (1998), in which EF-1 α sequences from 13 polychaete annelids representing nine orders were analyzed. However, neither study presents strongly supported conflict with the results of Rouse and Fauchald (1997), i.e., bootstrap support for all nodes grouping polychaetes is weak.

Brown et al. (1999) included 24 polychaetes (including one siboglinid) and 1 clitellate in their 29-taxon parsimony analysis of combined data from the nuclear genes histone H3, U2 snRNA, and 28S rDNA (D1 and D9-D10 expansion groups). The resulting tree, rooted using a nematode, supported monophyly of the two families for which more than one member was included: the Terebellidae and the Cirratulidae. It also showed monophyly of the two scale worms in the analysis, and the two eunicomorphs (Brown et al. 1999). Other relationships, none of them strongly supported (BP < 50), were contrary to expected results based on polychaete orders (sensu Fauchald 1977) or on the phylogenetic classification of polychaetes proposed by Rouse and Fauchald (1997). Monophyly of neither the annelids nor the polychaetes (including the siboglinid) was supported; the clitellate fell within a clade containing polychaete annelids,

the sipunculan fell within the polychaete + clitellate clade, and the echiurid fell basal to this grouping. Separate analyses of the three data sets yielded similar results, but in the separate histone H3 and 28S rDNA analyses the echiurid was placed as sister to a terebellid polychaete (Brown et al. 1999).

Clitellate relationships

Monophyly of the Clitellata is well supported by both morphological and molecular analyses, and molecular analyses indicate that clitellates fall within a paraphyletic polychaete grade. As mentioned above, the Clitellata is usually classified as the Oligochaeta, which includes the familiar earthworms but also many aquatic forms, and the Hirudinea, or leeches. Some authors consider the Hirudinea to contain the true leeches (Euhirudinea), the Acanthobdellida, and the crayfish parasites, the Branchiobdellida; others have suggested that the branchiobdellids evolved a parasitic habit independently of the true leeches and acanthobdellids, and that the similarities in morphology between the groups reflect convergent adaptations to their common life-style (see Purschke et al. 1993). In either case, it has been generally accepted that the leech groups stem from within the Oligochaeta, thus rendering the Oligochaeta paraphyletic (e.g., Brinkhurst and Nemeč 1987; Jamieson 1988).

While monophyly of the Clitellata is well-supported by both molecular and morphological analyses (e.g., see Brusca and Brusca 1990; Kim et al. 1996; Winnepeninckx et al. 1998; McHugh 1997; Kojima 1998), the sister-group to the Clitellata remains enigmatic. Nielsen (1995) suggested that the capitellid polychaetes might be considered the closest polychaete group to the clitellates because, like clitellates, some capitellids are hermaphroditic and have reproductive organs restricted to certain segments. However, there is no molecular evidence to support this, and the suggestion has been criticized by Rouse and Fauchald (1997). An alternative candidate for clitellate sistership is the Aeolosomatidae, a group of freshwater oligochaete-like worms that lack a clitellum. In a preliminary analysis of the phylogenetic relationships of the branchiobdellidans and the aeolosomatids, Moon et al. (1996) used 18S rRNA gene sequences for one molluscan outgroup and five ingroup taxa: a polychaete, a true leech, an oligochaete, a branchiobdellidan, and an aeolosomatid. The results of this study supported placement of the aeolosomatid as sister to the monophyletic Clitellata, but such a small taxon sample (six) severely limits the inferences that can be made from this study. In their NJ analysis of 57 18S rRNA sequences, including nine clitellates, Winnepeninckx et al. (1998) found moderate support (BP = 64) for an aeolosomatid–clitellate sister relationship; this analysis also supported monophyly of the Clitellata (BP = 57), the Oligochaeta (BP = 90), and the Hirudinea (BP = 75), with the two true leeches and the two branchiobdellids forming a sister clade to the five oligochaetes (Fig. 1).

The molecular analyses of EF-1 α sequences by McHugh (1997) and Kojima (1998) support placement of the three and four clitellates included, respectively, as a derived clade within the Annelida (Fig. 2), but the sampling of other annelid representatives differs between the two studies and neither

provides strong support for a sister relationship to a particular group of polychaetes. The combined analysis of histone H3, U2 snRNA, and 28S rDNA by Brown et al. (1999) also showed the single clitellate species in the analysis as nested within a clade of polychaetes; bootstrap support for its sister relationship to the siboglinid was weak. The derived annelid position of the Clitellata supports some of the functionally based arguments regarding annelid phylogeny outlined by Westheide (1997) (see also Westheide et al. 1999), and contradicts the hypothesis based on the morphological analyses of Rouse and Fauchald (1997). One of the major implications of a derived position of the clitellates within the Annelida is that features identified as synapomorphies for the polychaetes in the morphological analyses of Rouse and Fauchald (1997), i.e., nuchal organs, parapodia, mixonephridia, are secondarily absent in the clitellates.

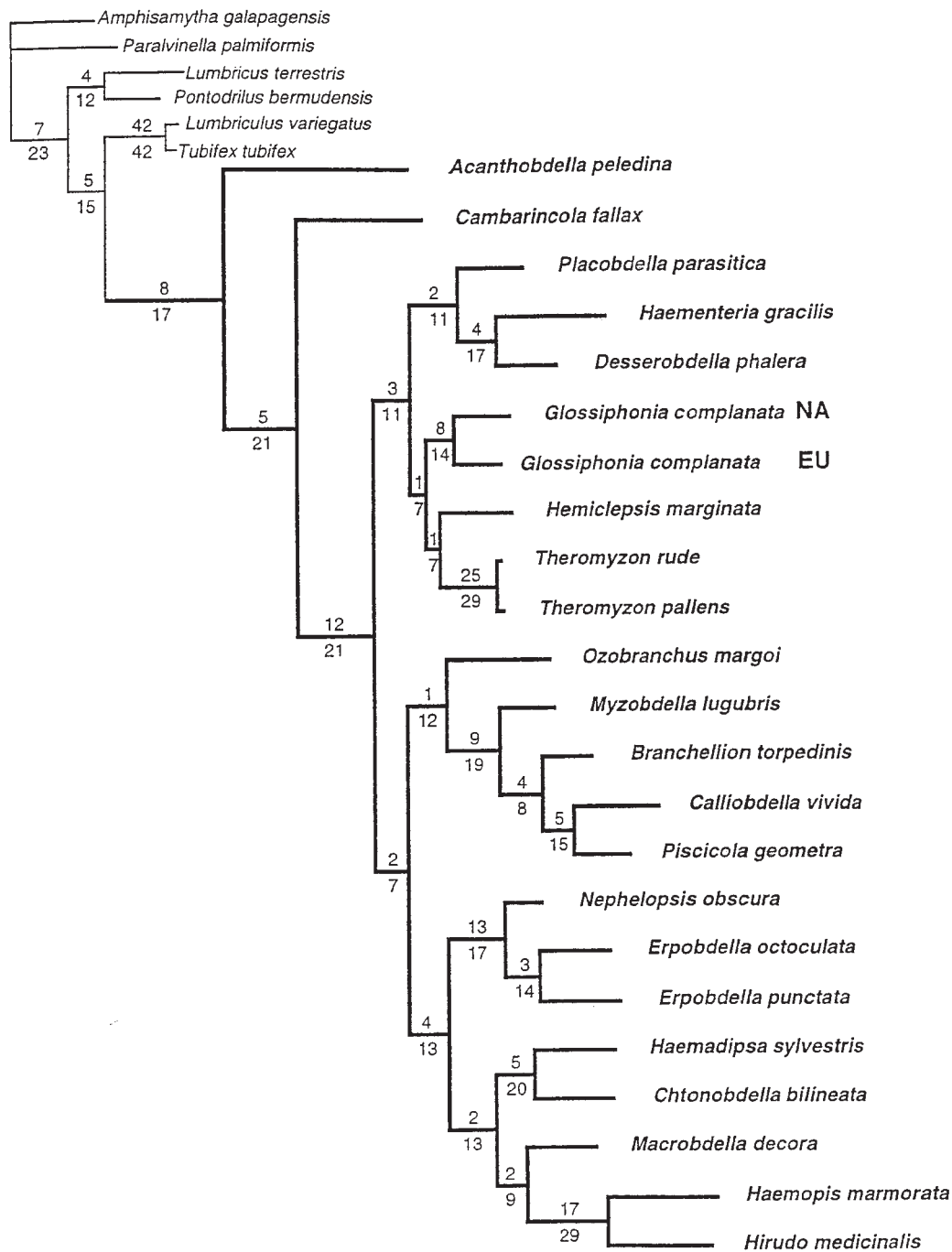
Surveys of HOM/Hox genes in the oligochaete *Stylaria lacustris* and the polychaete annelids *Ctenodrilus serratus* and *Chaetopterus variopedatus* have been undertaken using the polymerase chain reaction (PCR) (Dick and Buss 1994; Snow and Buss 1994; Irvine et al. 1997). From these surveys it appears that polychaetes have representative genes of each of the Hox groups except *Abd-B*, but the oligochaete has a clear *Abd-B* ortholog. The absence of *Abd-B* in the polychaetes may represent a loss of this Hox gene in their common ancestor, and if the oligochaetes arose from within the polychaetes, this result would suggest that the *Abd-B* gene was regained in that group. However, with just three species surveyed, and no guarantee that the PCR surveys were exhaustive, it is premature to draw any conclusions based on these data.

Paraphyly of the Oligochaeta was indicated in a phylogenetic analysis by Siddall and Bureson (1998), who analyzed the relationships of the leeches, using a 651-bp fragment of COI for 29 taxa. The resulting phylogenetic hypothesis supported a sister relationship between the leeches, including an acanthobdellid and a branchiobdellid, and two of the four oligochaetes in the analysis (decay index = 5) (Fig. 3). Monophyly of the true leeches was also supported by the COI analysis (decay index = 12), as was a sister relationship between the branchiobdellid and the Euhirudinea (decay index = 5). Within the Euhirudinea, monophyly of taxonomic groups within the orders Arhynchobdellida and Rhynchobdellida were supported; however, a monophyletic Arhynchobdellida (decay index = 3) fell within the Rhynchobdellida (Fig. 3). Paraphyly of the Rhynchobdellida was also suggested in a recent combined analysis of 18S rRNA, COI, and morphological data by Apakupakul et al. (1999); otherwise, the results of this study supported taxonomic groupings within the Euhirudinea.

Maximum-likelihood analysis of a combined 18S rRNA and COI data set also suggests the inclusion of the Euhirudinea, Acanthobdellida, and Branchiobdellida in the Oligochaeta (Martin et al. 2000). Interestingly, Martin et al. (2000) also report that the leeches, leech-like worms, and gutless worms in their analysis have higher mutation rates than other worms, and this phenomenon may be associated with their commensal or parasitic lifestyle.

In another study of clitellate relationships using COI, Nylander et al. (1999) tested the monophyly of the gutless

Fig. 3. Results of the parsimony analysis of cytochrome *c* oxidase I DNA sequence data from leeches and related taxa showing paraphyly of the Oligochaeta, monophyly of the Euhirudinea, and placement of the branchiobdellid as sister to the Euhirudinea (from Siddall and Bureson 1998). Numbers above and below the internodes are decay indices and branch lengths, respectively. "NA" is North America and "EU" is Europe.



Phalloporinae (Oligochaeta, Tubificidae) using a 573-bp region of the gene. They found that monophyly of this group of Tubificidae is supported, but concluded that otherwise the COI sequences vary too much to be of use for higher levels in the annelid phylogeny. Erséus et al. (2000) analyzed 18S rRNA sequences from 40 protostome worm species to test monophyly of the Tubificidae and taxa within this family of oligochaetes. Their results indicate that the Naididae falls within the Tubificidae, thus rendering the Tubificidae para-

phyletic; an analysis of 23S rRNA and COI sequences by Christensen and Thiesen (1998) also supported placement of the naidids within a paraphyletic tubificid group.

Basal annelid

There is no agreement regarding the body form of the basal annelid. Attempts have been made to reconstruct an

“ancestral annelid” on the basis of functional morphological arguments, but no consensus has been reached. The view that the ancestral annelid was a burrowing form, put forward by Clark (1964, 1969) and Fauchald (1974, 1977), hinges on functional arguments regarding the link between the evolution of the coelom, the origin of segmentation, and the ability to burrow. An implication of this view is that the basal annelids would be homonomous burrowers. Mettam (1985) revived the old view that the first annelids were microscopic interstitial forms, but most authors now agree that interstitial groups represent derived groups within the Annelida (Nielsen 1995; Rouse and Fauchald 1997). More recently, Conway Morris and Peel (1995) and Westheide (1997) proposed that the ancestral annelid condition was morphologically similar to extant epifaunal polychaete groups such as the phyllodocidans. Certainly the first unambiguous annelid fossils to appear were surface-dwelling forms, but earlier traces that could be the products of annelid burrowers are known.

Molecular evidence regarding the basal annelid group is limited, and phylogenetic hypotheses based on molecular analyses conflict on the basalmost taxon in the Annelida. The basal position of the epifaunal polychaete taxa *Nereis* and *Harmothoe* within the worm clade in the study of EF-1 α sequences by McHugh (1997) (Fig. 2) lends support to the view of Conway Morris and Peel (1995) and Westheide (1997). In his analysis of EF-1 α sequences, Kojima (1998) did not sample the same annelid taxa as McHugh (1997), and their results conflict with regard to the basal taxon within the annelid clade. In the NJ analysis of Kojima (1998), a tube-dwelling oweniid falls basally, while a burrowing capitellid is the basal annelid in his restricted likelihood analyses. In the more comprehensive analysis of 25 annelid taxa, Brown et al. (1999) showed a clade of tube-dwelling polychaetes (a sabellid, a serpulid, and a spionid) as the basal taxon in their combined analysis of histone H3, U2 snRNA, and 28S rRNA. Given the lack of resolution of this issue, there is a clear need for more complete sampling of the Annelida in future molecular analyses.

Sister-group to the Annelida

A fundamental question in annelid phylogeny concerns the sister-taxon of this metazoan group. If segmentation in annelids and arthropods is interpreted as being homologous, then the grouping of annelids with arthropods and other segmented animals in a clade termed the Articulata is supported. However, if segmentation arose convergently or in parallel in annelids and arthropods, the sister-taxon of the annelids may be an unsegmented spiralian group, and the Eutrochozoa is supported. According to the Eutrochozoa hypothesis, annelids, molluscs, and other unsegmented, bilateral coelomates that share a trochophore larva are more closely related to each other than to arthropods. Phylogenetic analyses of morphological characters have yielded contradictory results concerning the issue (e.g., Eernisse et al. 1992; Rouse and Fauchald 1995; Eibye-Jacobsen and Nielsen 1996; Rouse 1997). However, molecular data support the Eutrochozoa hypothesis, though the strength of the support depends on the data analyzed.

While 18S rDNA sequence data have not been useful in resolving relationships among all metazoan phyla, all studies

that include arthropods, annelids, and other spiralian present evidence in support of a closer relationship between annelids and other unsegmented spiralian than between annelids and arthropods (e.g., Halanych et al. 1995; Kim et al. 1996; Eernisse 1997; Aguinaldo et al. 1997; Aguinaldo and Lake 1998; Winnepeninckx et al. 1998). For example, Kim et al. (1996) analyzed the 18S rRNA sequences of eight annelids (six clitellates and two polychaetes), four molluscs, and six arthropods, using flatworms as outgroups. Parsimony analysis of the 20 taxa showed the annelids falling within the molluscan clade; a NJ analysis of the 18S rRNA sequences and a parsimony analysis of these molecular data combined with 39 morphological characters supported a sister relationship between the Annelida and the Mollusca (BP = 68) to the exclusion of the Arthropoda.

In more comprehensive analyses of up to 103 metazoan 18S rRNA sequences by Eernisse (1997), annelid monophyly was not supported but annelids were shown to be more closely related to molluscs and other unsegmented spiralian than to arthropods. In their analysis of 18S rRNA sequences, Winnepeninckx et al. (1998) showed 2 annelids and 6 molluscs as monophyletic in a NJ tree of 78 taxa (BP = 84); annelids, molluscs, and various other unsegmented coelomates were also monophyletic in the NJ tree of 57 taxa, to the exclusion of the arthropods, but support for this relationship was weak (BP < 50) (Fig. 1). Regier and Shultz (1998) also found weak support for this relationship in their analysis of arthropod relationships using EF-1 α data. In the EF-1 α analysis of McHugh (1997), a weakly supported grouping of two molluscs and a nemertean with four arthropods and a nematode was unexpected (decay index = 2), but a mollusc–arthropod clade was also supported (BP = 76) in Kojima’s (1998) NJ analysis of EF-1 α data.

Boore and Brown (2000) recently showed that the arrangements of genes in the mitochondrial genome and phylogenetic analysis of the inferred amino acid sequences of those genes strongly support the Eutrochozoa hypothesis, and further support for the Eutrochozoa is emerging from studies of the distribution of Hox genes in bilaterians. Two central Hox genes, *Lox2* and *Lox4*, are shared by only a polychaete, a leech, a gastropod, and a brachiopod; there is also evidence, based on posterior Hox homeodomain sequences, that annelids are more closely related to nemerteans and brachiopods than to arthropods (see de Rosa et al. 1999). These genome-level data support the Lophotrochozoa hypothesis (Halanych et al. 1995), which groups the lophophorates with the eutrochozoans, to the exclusion of the arthropods.

Future directions

A great deal of progress has been made over the past several years in the field of the molecular systematics of annelids: phylogenetic analyses of molecular data have shown support for monophyly of the Eutrochozoa, and derived positions of the siboglinids, echiurids, and Clitellata within a paraphyletic polychaete grade. However, many questions regarding annelid relationships remain unanswered, e.g., what are the relationships among the polychaete annelids, what group is sister to the Clitellata, what extant group is most basal on the annelid tree, and what group is sister to the Annelida? In some cases

these questions have yet to be adequately addressed using molecular data, i.e., more extensive taxon sampling is required. In other cases, molecular phylogenetic analyses of single genes have not resolved the branching patterns among annelid lineages. It has been argued that this lack of resolution reflects rapid radiation of the annelids, which resulted in short internodes with few informative sites at the molecular level (e.g., Halanych 1998; Winnepeninckx et al. 1998). The combination of sequence data from several conserved genes can provide one possible approach to overcoming such a problem and perhaps moving towards improved understanding of early annelid diversification. For example, with the availability of numerous 18S rRNA sequences from annelid species (~25) and a growing data base of EF-1 α sequences, a combined analysis of these two genes will be possible in the near future. The only contraindication for such a combined approach would occur if independent analyses of the two genes gave strongly supported conflicting results (de Queiroz et al. 1995), and this is not the case.

The choice of additional genes for the study of annelid phylogeny is critical. As illustrated by the study of Brown et al. (1999), even a combined analysis of three genes (histone H3, U2 snRNA, and 28S rDNA) did not yield a well-supported hypothesis of annelid relationships, possibly because the genes chosen were too conserved for the level of analysis: for U2 snRNA there are only 47 parsimony-informative sites for an analysis of 36 taxa; for histone H3, when the likely saturated third positions are excluded there are only 23 parsimony-informative sites. Candidate conserved genes that have not yet been exploited in studies of annelid phylogeny include the largest subunit of the RNA polymerase II gene and elongation factor-2 gene (EF-2). RNA polymerase II has proved useful in analyses of arthropod (Regier and Shultz 1997) and annelid (M. Diaz and D. McHugh, unpublished data) relationships. EF-2 has also been indicated as a potential source of phylogenetic information for deep-level analyses of metazoans (Friedlander et al. 1994). Other highly conserved, single or low copy number nuclear genes of >1000 bp, such as enolase and Na⁺,K⁺-ATPase (Friedlander et al. 1994), also await assessment of their usefulness.

Over the coming years, with the accumulation of new molecular data, efforts must also be made to increase taxonomic representation in molecular analyses of the Annelida. Ultimately, we can hope for a robust, stable phylogenetic hypothesis for the Annelida that will not only provide a sound basis for reclassifying the group, but will bring a better understanding of annelid character evolution. For example, the patterns of change through time in morphological characters such as segmentation (e.g., McHugh 1997), behavioural characters such as blood-feeding in leeches (e.g., Arapupakul et al. 1999), and developmental characters such as larval feeding (e.g., McHugh 1998) can all be best inferred in a phylogenetic context.

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