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The Essential Role of "Minor" Phyla in Molecular Studies of Animal Evolution[1]

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SYNOPSIS. **Molecular studies have revealed many new hypotheses of metazoan evolution in recent years. Previously, using morphological methods, it was difficult to relate "minor" animal groups representing microscopic metazoans to larger, more well known groups such as arthropods, molluscs, and annelids. Molecular studies suggest that acanthocephalans evolved from rotifers, that priapulids share common ancestry with all other molting animals (Ecdysozoa), and that flatworms, gnathostomulids and rotifers form a sister group to the remaining non-molting protostomes (Lophotrochozoa), together forming Spiralia. The lophophorate phyla (phoronids, brachiopods and bryozoans) appear as protostomes, allied with annelids and molluscs rather than with deuterostomes. These findings present a very different view of metazoan evolution, and clearly show that small and simple animals do not necessarily represent ancestral or primitive taxa.**

INTRODUCTION

What are "minor" phyla? Minor phyla are often referred to as enigmatic or problematic, are usually of uncertain affinity, and generally are treated superficially in invertebrate texts. Minor phyla are considered to be of little consequence to mainstream animal evolution, usually because they are not well represented in present day macrofauna (see *e.g.*, Simonetta and Conway Morris, 1991). This is a major error, since the modern day or paleontological prominence of a taxon does not necessarily reflect its role, or the role of its ancestors in the metazoan radiation. If we use the questionable definition of a phylum as a taxon with a distinctly unique body plan and leave aside the requirement of monophyly, then minor phyla represent the majority of nature's experimentation with animal body plans. In contrast, the "major" phyla are a small number of groups that are prominent among modern macrofauna and are composed of annelids, arthropods, chordates, cnidarians, echinoderms, molluscs, and perhaps platyhelminths.

Several kinds of minor taxa are important to this discussion. Some groups such as mesozoans and placozoans have been considered to be representatives of the stem lineage leading to triploblastic animals (see Ax, 1996). Other groups have uncertain affinities, appear to have simple body plans, and are generally small in size. Many of these historically have been lumped together into the "Aschelminthes" (*e.g.*, Rotifera, Acanthocephala, Nematoda, Nematomorpha, Priapulida, Kinorhyncha, Gastrotricha), based on the dubious assessment that each possesses a pseudocoelom. Other minor phyla appear to be sister taxa to larger and more well defined groups. For example, echiurans, sipunculids, pogonophorans and vestimentiferans have long been considered to be protostomes, possibly allied with annelids or molluscs. Then there are the lophophorates, a group of three phyla (Phoronida, Brachiopoda, Bryozoa) that have been placed either intermediate between protostomes and deuterostomes (see Willmer, 1990), as deuterostomes (Brusca and Brusca, 1990) or have been proposed to be polyphyletic with some being protostomes and others deuterostomes (Nielsen, 1995). Entoprocts have been associated with molluscs (Bartolomaeus, 1993), with aschel-

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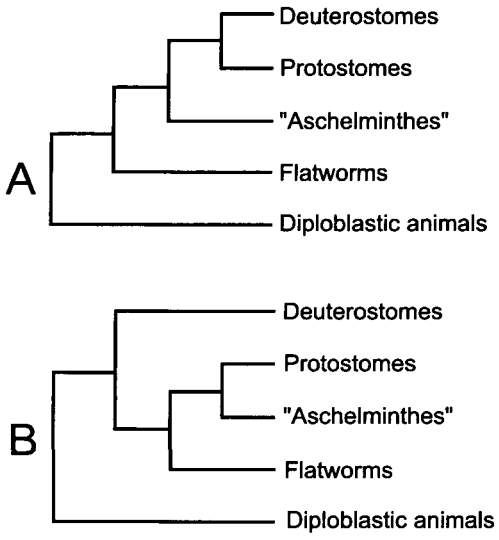

FIG. 1. Common hypotheses of metazoan phylogeny. A. Acoelomate/coelomate tree in which flatworms are basal metazoans (see *e.g.*, Ruppert and Barnes, 1994; Valentine *et al.*, 1996; Gilbert, 1997). B. Protostome/Deuterostome tree in which flatworms are a sister group to protostomes (see *e.g.*, Brusca and Brusca, 1990; Campbell, 1993).

minths (Brusca and Brusca, 1990), or allied with ectoprocts (Nielsen, 1995). Onychophorans are usually allied with annelids or arthropods, while tardigrades have been linked to both "aschelminths" (Ruppert and Barnes, 1994) and arthropods (Brusca and Brusca, 1990) at various times. Some groups such as chaetognaths or the newly discovered Cycliophora (Funch and Kristensen, 1995; Funch, 1996) have only vaguely defined relationships to other taxa.

In general, the perception of basic evolutionary relationships of the major phyla have remained similar to that shown in Figure 1A (acoelomate/coelomate tree) with tribloblastic animals branching from diploblastic animals, leading to an acoelomate ancestor similar to modern day flatworms which then split into the pseudocoelomate ("Aschelminthes") and eucoelomate lineages. The eucoelomates branched from a common eucoelomate ancestor into protostomes and deuterostomes. This succession of acoelomates—pseudocoelomates—coelomates goes back to Hyman (1951, see p. 23), although it was originally not intended to precisely reflect phylogeny. A more modern variation on that theme is shown in Figure 1B (protostome/deuterostome tree), with a protostome/deuterostome split early on, and flatworms as basal protostomes. In this scheme, "aschelminth" taxa are usually ignored.

The phylogeny within the protostomes has been locked into place by the "obvious" relationship between arthropods and annelids as segmented Articulata, sometimes extended to include the molluscs as a third group. A few other phyla (tardigrades and onychophorans) have been invoked as modern day representatives of ancestral forms that were transitional between annelids and arthropods. All the other protostome phyla except perhaps molluscs have essentially played a secondary role to these "mainstream" protostomes in phylogeny for over a century because it has been difficult to integrate most minor groups into overall phylogenetic hypotheses. Part of the reason for this is the difficulty in finding appropriate characters in many of the minor phyla whose members are often tiny, with simple bauplans, of little economic importance and therefore under-studied.

The advent of ultrastructural studies, coupled with cladistic analysis and molecular phylogenetic methods, have dramatically improved our ability to incorporate more minor taxa into phylogenetic hypotheses. Ultrastructural studies suggested that body cavities are more plastic than previously thought and perhaps not a good character for phylogenetic studies (Ruppert, 1991; Kristensen, 1995). They also suggested that aschelminths are polyphyletic, but could not relate them to other phyla because they lacked appropriate character sets (Ruppert, 1991; Kristensen, 1995; Wallace *et al.*, 1996). In a sense, molecular phylogenetic methods democratized phylogeny, providing the theoretical and practical framework to integrate nearly any taxon into phylogenies, regardless of how understudied or unknown it was previously.

The 18S rRNA gene and unequal rate effects

Although there are likely to be other genes better suited for metazoan phylogenetic studies at the level of the phylum, the

18S rRNA gene has been the gene of choice because of the large number of sequences available and because its properties are well known (Hillis and Dixon, 1991; Dixon and Hillis, 1993). The major problems with molecular phylogeny have been the development of adequate methods, the ability to acquire sufficient data and the recognition of the limitations of molecular analysis (*e.g.*, see Maley and Marshall, 1998). Unequal rate effects are one important source of error in the phylogenetic analysis of molecular data (Felsenstein, 1978; Hillis *et al.*, 1994; Aguinaldo *et al.*, 1997) that is often ignored. In unequal rate effects, genes of some taxa (fast evolving) have sequences that have much higher substitution rates than in other taxa (slow evolving). A combination of alignment errors and problems with tree making algorithms cause taxa with long branches to be attracted to one another, not because they are closely related, but because they both have long branches. The 18S rRNA gene is difficult to align among diverse metazoan taxa, particularly when sequences from rapidly evolving taxa are included. Computer simulations have been published that show various tree making algorithms to be immune to unequal rate effects (*e.g.*, Saitou and Nei, 1987; Hillis *et al.*, 1994). However, in these studies the different substitution rates are simulated within previously-aligned sequences which avoids the alignment problem and is unrealistically optimistic. Likewise, the rate calibration method (Van de Peer *et al.*, 1992) does not address the problem of alignment. One way to avoid branch length attraction is to omit fast-evolving gene sequences from the analysis altogether, or at least to test the effect of omitting fast-evolving sequences from an analysis (*e.g.*, see Aguinaldo *et al.*, 1997; Blaxter *et al.*, 1998). In many cases, individual species can be found within a taxon that have more slowly evolving sequences than others. In some taxa such as chaetognaths (Telford and Holland, 1993; Halanych, 1996), all taxa to date examined have rapidly evolving 18S rRNA genes that are difficult if not impossible to place accurately within metazoan phylogeny. A study of evolutionary rates of different protein coding genes within the nematode *Caenorhabditis elegans*, a species with a rapidly evolving 18S rRNA gene, demonstrates that two-thirds of 37 examined genes appear to evolve rapidly, while one-third appear to evolve at a rate comparable to other taxa in the study (human, yeast, *Drosophila*) (Mushegian *et al.*, 1998), so one solution for taxa such as chaetognaths may be to identify and sequence genes that evolve at a rate comparable to that in other metazoans.

MOLECULAR STUDIES

The earliest attempts to describe metazoan phylogeny with 18S rRNA gene sequences lacked the large data sets currently available, and focused on the origin of metazoans rather than the more detailed topology within the metazoan clade (*e.g.*, Field *et al.*, 1988; Raff *et al.*, 1989; Lake, 1989, 1990; Christen *et al.*, 1991; Wainright *et al.*, 1993). A number of hypotheses have been proposed and rejected, but the current consensus appears to be that metazoans are monophyletic (Lake, 1989, 1990) and that fungi are more closely related to metazoans than plants or protists (Wainright *et al.*, 1993; Kumar and Rzhetsky, 1996).

Are diploblastic animals monophyletic?

From morphological evidence, diploblastic animals are generally considered to be a paraphyletic group composed of poriferans, *Trichoplax*, cnidarians and ctenophores, each group branching in turn from the stem leading to triploblastic animals (Fig. 2A) (see *e.g.*, Brusca and Brusca, 1990; Ax, 1996). The ctenophores have been proposed as a sister group to Bilateria based on the ultrastructure of the spermatozoa (Ehlers, 1993), or even as a sister group to deuterostomes but not protostomes (Nielsen, 1995). In contrast, with molecular studies of 18S rRNA (*e.g.*, Winnepenninckx *et al.*, 1995*a*; Garey *et al.*, 1996*a*), partial 28S rRNA (Christen *et al.*, 1991) and elongation factor-1α (EF-1α) genes (Kobayashi *et al.*, 1996), diploblastic animals most often appear to be monophyletic (Fig. 2B). There is a long branch from fungi to diploblastic animals, and another long branch from diploblastic to triploblastic animals, as well as long branches from the stem to poriferans,

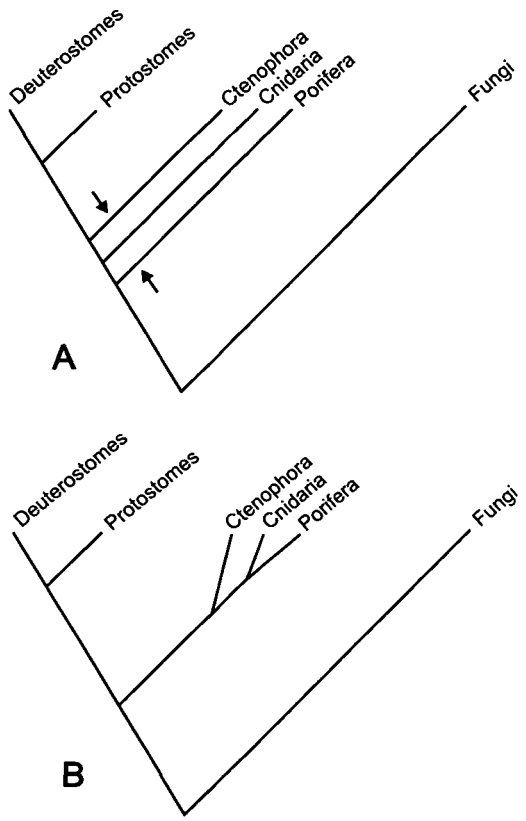

FIG. 2. A. Relationship of diploblastic animals to triploblastic animals from morphological studies. B. Same relationship from molecular studies. The arrows indicate branch length attraction of the long, isolated branches leading to three diploblastic taxa that could explain the discrepancies between the two trees.

cnidarians, and ctenophores (Fig. 2B). It seems likely that the long branches from the stem to poriferans, cnidarians and ctenophores attract each other (arrows in Fig 2A), collapsing into a single branch in molecular studies (Fig. 2B) and the appearance of diploblast monophyly in molecular studies could be due to long branch attraction.

Mesozoa, Myxozoa, and Xenoturbella: *Representatives of ancestral triploblastic animals?*

A number of interesting groups have been considered to represent advanced ciliates, the stem lineage of triploblastic animals, or degenerate triploblastic animals.

Myxozoans are known principally as teleost fish parasites, resembling ciliates in some ways but exhibiting multicellularity and cell differentiation more like metazoans. They are generally thought to be protozoans but not metazoans (Brusca and Brusca, 1990). An 18S rRNA gene study provided sequences from five different myxozoan taxa (Smothers *et al.*, 1994), all displaying extremely long branch lengths in the published trees (and see Pawlowski *et al.*, 1996). The myxozoan sequences formed a sister group relationship with the fast evolving 18S rRNA gene sequence of the rhabditid nematode *Caenorhabditis elegans*, forming a basal triploblastic clade, indicative of long branch attraction. In neighbor-joining trees (unpublished analysis, J.R.G.), the myxozoan sequences produced branches that were an order of magnitude longer than any others in the data set, making it difficult to agree with the authors concerning their conclusions that myxozoans are triploblastic metazoans.

Mesozoans are small ciliated animals parasitic in a number of invertebrates and consist of two groups, the Orthonectida and Rhombozoa. They are bilaterally symmetrical but appear to lack endoderm. Their phylogenetic relationships are uncertain, but mesozoans are often allied with nontriploblastic animals (see Brusca and Brusca, 1990; Ax, 1996). Neighbor Joining analyses of 18S rRNA genes in two published studies (Katayama *et al.*, 1995; Pawlowski *et al.*, 1996) placed dicyemid mesozoans (Rhombozoa) as a sister group to nematodes. The nematodes used in the studies were rhabditid nematodes with fast-evolving 18S rRNA genes. The dicyemid sequences had even longer branches and both groups appeared in the trees as early triploblastic animals, indicative of long branch length attraction. Pawlowski *et al.* (1996) included a sequence from an orthonectid mesozoan which appeared as a basal triploblast in a neighbor-joining analysis but not as a sister group to the dycemids.

Xenoturbella bocki is a small ciliated animal, lacking an anus and having few organs. It was original proposed to be an acoel flatworm (Westblad, 1949), but more recently as a sister taxon to Bilateria based on its subepidermal musculature (Ehlers and Ehlers, 1997). A molecular study using

both 18S rRNA and mitochondrial cytochrome oxidase subunit I genes suggests that *X. bocki* is a degenerate mollusc (Norén and Jondelius, 1997). Branch length attraction does not appear to be a factor in this study, which was also supported by a new embryological study (Israelsson, 1997).

Acoelomates: Not representative of basal triploblastic animals

One of the first major successes in metazoan phylogeny using 18S rRNA genes was to confirm new morphological evidence that nemerteans, the acoelomate ribbon worms, were more closely allied to protostomes such as molluscs and annelids than to the acoelomate flatworms (Turbeville *et al.*, 1992).

Flatworms have long been considered to represent basal triploblasts primarily because they lack a body cavity and have no anus (see *e.g.*, Hadzi, 1963; Ax, 1989; Carranza *et al.*, 1997). Most 18S rRNA genes sequenced from platyhelminths evolve at an accelerated rate, causing the long branches to be attracted to the long branch between diploblastic and triploblastic animals (*e.g.*, see Field *et al.*, 1988; Mackey *et al.*, 1996) so that flatworms appear as basal triploblasts. The 18S rRNA genes of planarians (*e.g.*, *Dugesia*) as well as the terrestrial flatworm *Bipalium*, and the horseshoe crab "fluke" *Bdelloura* appear to evolve rapidly, while the 18S rRNA gene of some flukes (*e.g.*, *Opistorchis*), and the catenulid *Stenostomum* evolve at more moderate rates. When one uses slow evolving 18S rRNA gene sequences of *Opistorchis* (Winnepenninckx *et al.*, 1995*a*) or *Stenostomum* (Aguinaldo *et al.*, 1997) in metazoan trees, the platyhelminths appear within the protostome clade, but not as basal triploblastic animals (see also Balavoine, 1997). The concept of a basal position of platyhelminths in metazoan evolution (see *e.g.*, Willmer, 1990) is so widespread that a platyhelminth species is often used to root molecular trees of other triploblastic taxa. The result may be that deuterostomes and arthropods appear as sister taxa (*e.g.*, see Winnepenninckx *et al.*, 1995*b*), an artifact that can be corrected by rooting the tree to diploblastic animals or between protostomes and deuterostomes.

"Aschelminthes" is not a valid taxon

The first study to specifically address the "Aschelminthes" (Winnepenninckx *et al.*, 1995*a*) included new 18S rRNA gene sequences from a priapulid, rotifer, acanthocephalan, gastrotrich, and nematomorph and also included several previously published rhabditid nematode sequences. Morphological evidence had suggested that "Aschelminthes" were polyphyletic (Ruppert, 1991; Kristensen, 1995; Ehlers *et al.*, 1996), and the molecular study revealed that they formed at least three clades. Rotifers and acanthocephalans appeared as sister taxa, as did gastrotrichs and flatworms, while priapulids, surprisingly, were a sister group of arthropods. Nematodes appeared as basal triploblastic animals, which was attributed to unequal rate effects. This was the first time that several aschelminth groups were described phylogenetically within the context of major metazoan taxa such as annelids, molluscs and arthropods. See below for additional discussion of the "aschelminth subtaxa."

Lophophorates and Lophotrochozoa

The lophophorates have an important role in classical metazoan phylogeny because they have some characters (protostomy) that are protostome-like, others that are deuterostome-like (lophophore as feeding structure, trimeric coelom), and are most often placed as deuterostomes or as basal to deuterostomes. Several 18S rRNA gene studies (Halanych *et al.*, 1995; Mackey *et al.*, 1996) revealed that lophophorates are not monophyletic and appear within the protostomes. Phoronids are closely allied with brachiopods (see also Cohen *et al.*, 1998), while ectoprocts are elsewhere in the protostome branch of the tree, allied with annelids and molluscs. Two ectoproct 18S rRNA gene sequences, one from a phylactolaematan and another from a gymnolaematan are currently available (Halanych *et al.*, 1995; Mackey *et al.*, 1996). The gymnolaematan 18S rRNA sequence from *Alcyonidium gelatinosum* does not group with the phylactolaematan sequence, prob-

ably because it has a long branch that most likely attracted it toward the long branch of a sipunculan also included in the analysis (Mackey *et al.*, 1996). Halanych *et al.* (1995) introduced the name Lophotrochozoa for a taxon within the protostomes including all lophophorate taxa plus annelids and molluscs. The name is derived from Eutrochozoa (Ghiselin, 1988; Eernisse *et al.*, 1992) as a name for protostomes with a trochophore larva (Mollusca, Annelids, Echiurida, Sipuncula, Pogonophora, Vestimentifera). Recently, Lophotrochozoa has been used to include platyhelminths, gastrotrichs and rotifers (Aguinaldo *et al.*, 1997; Valentine, 1997), in which case the name would be synonymous with the older name Spiralia which should be preferred. However, we propose that Lophotrochozoa is a subtaxon of Spiralia (see below).

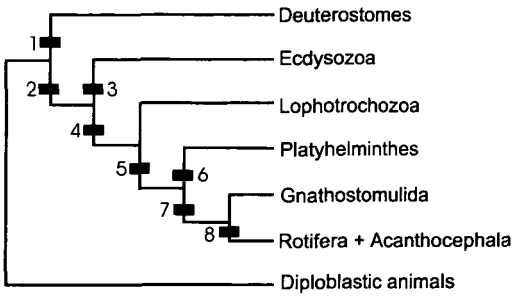

FIG. 3. Proposed phylogeny of protostomes based on morphological and molecular analyses. Platyhelminthes + Gnathostomulida + Rotifera + Acanthocephala may be the sister group to Lophotrochozoa. Only a few key characters are given. 1: Blastopore becomes the anus. 2: Ventral lateral nerve chord. 3: Molting by ecdysis. 4: Spiral cleavage. 5: Filiform sperm without accessory centriole. 6. Biciliary terminal cell in the protonephridia. 7: Jaws composed of rods imbedded in a cuticular matrix. 8: Internal layer in the syncytial epidermis. See Garey *et al.* (1998) for details.

Other taxa associated with Lophotrochozoa

Sipunculids, echiurans, vestimentiferans, and pogonophorans are four minor phyla of protostome worms that are usually associated with annelids or molluscs. Recent 18S rRNA gene studies place echiurans, vestimentiferans, and pogonophorans as a monophyletic group weakly associated with molluscs (Winnepenninckx *et al.*, 1996*b*) or with annelids (Winnepenninckx *et al.*, 1996). The 18S rRNA gene results are contradicted by analyses of a 346 bp fragment of the EF-1α gene (Kojima *et al.*, 1993; McHugh, 1997) in which echiurans and pogonophorans appear to be derived from different polychaete ancestors. Recent morphological analyses support the hypothesis that Vestimentifera and Pogonophora are derived polychaetes (Bartolomaeus, 1995; Rouse and Fauchald, 1997) in accordance with Nielsen (1995).

The position of nemerteans is not completely resolved by morphological or molecular investigations. They could be the sister group to Lophotrochozoa within Spiralia (corresponding to their position as sister group of Trochozoa, if lophophorates are not regarded as protostomes, see Ax, 1996) or a taxon within Lophotrochozoa, as some 18S rRNA and EF-1α gene studies suggest (Winnepenninckx *et al.*, 1995*b*, 1996; McHugh, 1997). For entoprocts (Kamptozoa) various hypotheses about their position within the protostomes exist: related to "aschelminths" (Brusca and Brusca, 1990), to ectoprocts (Nielsen, 1995), to molluscs (Bartolomaeus, 1993; Haszprunar, 1996) and to annelids (Emschermann, 1985). An 18S rRNA gene study clearly places entoprocts within Lophotrochozoa, but not as a sister group of ectoprocts (Mackey *et al.*, 1996).

What is the sister group of the Lophotrochozoa?

Molecular (Winnepenninckx *et al.*, 1995*a*; Garey *et al.*, 1998) and morphological (Wallace *et al.*, 1996; Ahlrichs, 1997) studies show that rotifers form a tightly knit association with acanthocephalans, and it appears that acanthocephalans share a common ancestry with bdelloid rotifers (Garey *et al.*, 1996*b*), making acanthocephalans a sub-taxon of Rotifera rather than an independent phylum. When morphological characters are considered in addition to the molecular data, it appears that there is a clade of protostomes that includes platyhelminths, gnathostomulids, rotifers and acanthocephalans (Rieger and Tyler, 1995; Ahlrichs, 1997; Garey *et al.*, 1998) (Fig. 3). This clade could be a sister taxon to lophotrochozoans because unlike lophotrocho-

zoans, its taxa lack a trochophore larva and a lophophore, but like lophotrochozoans, they have spiral cleavage (at least in platyhelminths and gnathostomulids). Therefore, the entire taxon (Lophotrochozoa + Platyhelminthes + Gnathostomulida + Rotifera + Acanthocephala) would be named Spiralia (see Fig. 3). According to molecular studies, gastrotrichs are closely related to platyhelminths (Winnepenninckx *et al.*, 1996*a*) and are therefore members of Spiralia. Interpretations of morphological evidence suggest that gastrotrichs are closely related to Cycloneuralia (see below) (Nielsen, 1995; Ehlers *et al.*, 1996; Wallace *et al.*, 1996) and could be a sister group to Ecdysozoa (Schmidt-Rhaesa, 1997).

Molting animals: The Ecdysozoa

The discovery that all molting animals form a single clade named Ecdysozoa illustrates the importance of including minor phyla in phylogenetic analyses. In the past, molting was considered a character that could easily evolve convergently, although in retrospect, growth by ecdysis requires adaptations (*e.g.*, loss of locomotory cilia, complex hormonal regulation) that make convergent evolution seem less likely. Ecdysozoa consists of Panarthropoda (arthropods, tardigrades, onychophorans; the last two taxa have been closely associated with arthropods) and five phyla that represent taxa previously associated with "aschelminths", the nematodes, nematomorphs, kinorhynchs, priapulids, and probably loriciferans (see also Schmidt-Rhaesa, 1997). Cladistic analyses of morphological characters have grouped nematodes, nematomorphs, kinrohynchs, priapulids and loriciferans together as Cycloneuralia (Ehlers *et al.*, 1996, see also Nielsen, 1995 and Wallace *et al.*, 1996), but never associated Cycloneuralia with arthropods. Spiral cleavage does not appear among ecdysozoans, so they should be considered nonspiralian protostomes. There is insufficient resolution within 18S rRNA gene trees of Ecdysozoa to determine if Panarthropoda and Cycloneuralia exist as sister taxa, or if they are interspersed within Ecdysozoa.

An association of priapulids and arthropods was observed in an 18S rRNA gene analysis (Winnepenninckx *et al.*, 1995*a*), and a nematomorph appeared closely allied to arthropods in a similar study (Mackey *et al.*, 1996), as was a clade including priapulids, tardigrades and arthropods (Garey *et al.*, 1996a). These were the first molecular indications of the association of taxa with "aschelminth-like" characters to arthropods. It happened that the nematomorph, priapulid and tardigrade 18S rRNA gene sequences used in those studies produced relatively short branches, avoiding problems with branch length attraction. The landmark study by Aguinaldo *et al.* (1997) utilized a set of slow evolving 18S rRNA gene sequences to clearly demonstrate that Ecdysozoa is monophyletic, although the exact relationships within this taxon are incompletely resolved. The association of arthropods and nematodes is supported by a study using partial EF-1α sequences (McHugh, 1997), complete EF-1α sequences (unpublished data, J.R.G.), and a number of additional slow evolving nematode 18S rRNA gene sequences (unpublished data, J.R.G.).

An early 12S rRNA gene sequence study placed onychophorans within the arthropods (Ballard *et al.*, 1992), not inconsistent with the 18S rRNA gene based study (Aguinaldo *et al.*, 1997) although the position of Onychophora within Ecdysozoa remains uncertain. The pentastomids are a group of vertebrate parasites, once considered to be an independent phylum. Analyses of 18S rRNA gene sequences and morphological evidence (Wingstrand, 1972; Abele *et al.*, 1989; Storch and Jamieson, 1992) clearly demonstrated that pentastomids are highly modified branchiuran crustaceans (see also Garey *et al.*, 1996*a*).

CONCLUSIONS

Molecular studies of phylogeny have allowed the integration of "minor" phyla into a theoretical and practical phylogenetic framework that incorporates a more realistic sampling of metazoan taxa. Molecular studies have provided new insight into how animals evolved, and have fundamentally changed our view of the metazoan adaptive radiation (Fig. 4). What are the major implications of this new view? The evolution of growth by molting was a major event in

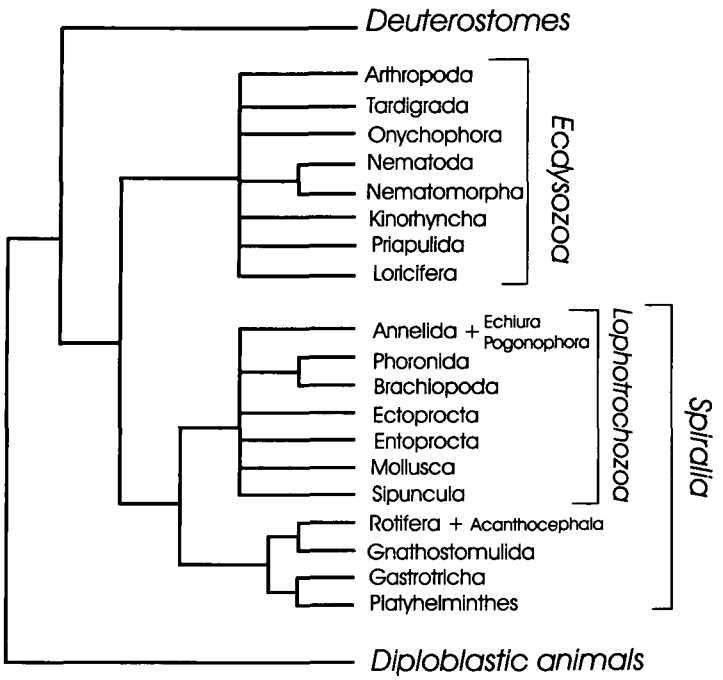

FIG. 4. A current hypothesis of Metazoan evolutionary relationships based on molecular and morphological studies. Polytomies in the tree represent regions where molecular and morphological data disagree or present no clear branching pattern. The bifurcations shown in the tree represent nodes supported by various molecular and/or morphological studies. See text for details and references.

metazoan evolution, and the "Articulata" concept is not valid. The finding that lophophorates are probably not related to deuterostomes has major consequences in how we should view the evolution of protostomes and deuterostomes. Lophophorates have long been considered basal to deuterostomes, and lophophorate characters such as protonephridia have been ascribed to deuterostomes, presenting the illusion that deuterostomes are more similar to protostomes than they really are.

In 18S rRNA gene analyses, there is a long branch between diploblastic and triploblastic animals, and yet no taxon has yet been identified that represents a common triplobastic ancestor to the protostomes and deuterostomes. Recent hypotheses-based on morphological evidence have described the ancestor to be a small benthic organism (Ax, 1996), a small pelagic organism (Nielsen, 1995), or an organism with a biphasic life cycle (Jägersten, 1972; Rieger, 1994). In contrast, the finding of similar patterns of expression of some homeobox genes in insects and vertebrates have led to the suggestion that the common ancestor was complex, with photoreceptors, appendages, segmentation and a circulatory system (De Robertis and Sasai, 1996; De Robertis, 1997). Those studies compared only chordates and arthropods, ignoring more basal deuterostomes and protostome taxa with much less complicated morphology. Perhaps it is time to think of the common ancestor of deuterostomes and protostomes as a much simpler organism, possibly only rudimentarily triploblastic and bilateral. Many of the genes involved in patterning simple features could have been recruited for more complex features later in protostomes and deuterostomes convergently.

A number of additional aspects of metazoan phylogeny should be scrutinized in the next few years. The ecdysozoan and spiralian clades need to be confirmed in detail by analyzing more appropriate genes such as EF-1α and others. The topology within both Ecdysozoa and Lophotrochozoa is currently very uncertain, and should be the fo-

cus of future molecular and morphological studies. It will be especially important to re-evaluate morphological data in light of molecular studies, and to find new morphological characters that will reveal more about metazoan phylogeny. The origin of deuterostomes and protostomes will require intense investigation, as will the relationship among diploblastic taxa, and the relationship between diploblastic and triploblastic animals. Both the 18S rRNA and EF-1α appear to be unable to resolve these ancient metazoan branches, so more informational genes with more clock-like substitution rates will need to be used.

Until recently, molecular phylogenetic studies have used gene sequences simply as markers in which the genes used are unrelated to the evolutionary processes of metazoan radiation. Developmental biologists are now beginning to examine the actual genes involved in patterning that defines morphological characters to understand evolutionary relationships. Many of the homebox genes involved in patterning complex features in chordates and arthropods are members of complex gene families with homologs in diploblastic animals and yeast. It may be premature to homologize the function of these genes among diverse taxa until complete genomes are available so that orthologous forms can be discerned from paralogous forms. However, these functional molecular evolution studies show great promise and may eventually merge molecular and morphological methods of evolutionary analysis.

Finally, there are still a number of taxa that to date cannot be placed accurately by either morphological or molecular studies. These include myxozoans, mesozoans and chaetognaths. Appropriate gene sequence studies will eventually clarify their position and perhaps lead to new insights into the radiation of the Metazoa.

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