

THE NEW VIEW OF ANIMAL PHYLOGENY

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■ **Abstract** Molecular tools have profoundly rearranged our understanding of metazoan phylogeny. Initially based on the nuclear small ribosomal subunit (SSU or 18S) gene, recent hypotheses have been corroborated by several sources of data (including the nuclear large ribosomal subunit, Hox genes, mitochondrial gene order, concatenated mitochondrial genes, and the myosin II heavy chain gene). Herein, the evidence supporting our current understanding is discussed on a clade by clade basis. Bilaterian animals consist of three clades: Deuterostomia, Lophotrochozoa, and Ecdysozoa. Each clade is supported by molecular and morphological data. Deuterostomia is smaller than traditionally recognized, consisting of hemichordates, echinoderms, chordates, and *Xenoturbella* (an enigmatic worm-like animal). Lophotrochozoa groups animals with a lophophore feeding apparatus (Brachiopoda, Bryozoa, and Phoronida) and trochophore larvae (e.g., annelids and mollusk), as well as several other recognized phyla (e.g., platyhelminthes, sipunculans, nemertean). Ecdysozoa comprises molting animals (e.g., arthropods, nematodes, tardigrades, priapulids), grouping together two major model organisms (*Drosophila* and *Caenorhabditis*) in the same lineage. Platyhelminthes do not appear to be monophyletic, with Acoelomorpha holding a basal position in Bilateria. Before the emergence of bilateral animals, sponges, ctenophorans, cnidarians, and placozoans split from the main animal lineage, but order of divergence is less than certain. Many questions persist concerning relationships within Ecdysozoa and Lophotrochozoa, poriferan monophyly, and the placement of many less-studied taxa (e.g., kinorhynch, gastrotrichs, gnathostomulids, and entoprocts).

INTRODUCTION

In the past decade, major new hypotheses of animal evolution have shaken traditional foundations and caused researchers to abandon long-standing hypotheses. This change certainly provoked controversy, and many are critical of these new hypotheses. Skepticism has focused on uncertainty about reliability of molecular data, apparent conflict between morphology and molecular data, lack of robust phylogenetic signal, lack of well-defined morphological synapomorphies, and apparent contradictory conclusions from the same data source. In most cases, the basis for such skepticism is limited. One must keep in mind that systematic

biology is a dynamic field of research, with hypotheses constantly proposed and later falsified. In contrast, many who are interested in animal phylogeny want a well-supported (i.e., static) evolutionary framework that they can use for comparative studies or teaching purposes.

Fortunately, consensus is emerging for many regions of the metazoan tree. This review provides a conceptual framework of the current understanding of animal phylogeny in light of recent advances. In particular, this review supplements invertebrate biology texts (e.g., Brusca & Brusca 2003, Ruppert et al. 2004) that do not adequately convey the recent advances. Owing to space limitations, I do not discuss many of the important traditional hypotheses that are already well reviewed in Willmer (1990) or viewpoints based solely on morphology (Nielsen 2001). I draw mainly on recent analyses to (a) build a basic comparative framework of metazoan phylogeny, (b) discuss support, or lack thereof, of major hypotheses, and (c) catalog significant papers for those less familiar with the field.

Before I discuss animal relationships, a few salient points deserve mention.

1. Traditional understanding of animal phylogeny (Figure 1) was largely based on (a) the concept that evolution proceeds from simple to complex, (b) a suite of purportedly conserved embryological features (e.g., cleavage patterns, blastopore fate, mode of coelom formation), and (c) overall body architecture (e.g., segmentation, type of coelom). These ideas were outlined in Libbie Hyman's (1940, p. 38) figure 5, which presented her understanding of animal body plans. When compared with genetic information (or even cladistic morphological analyses), many traditional morphological and embryological characters are more evolutionarily labile than previously thought (e.g., Halanych 1996a, Valentine 1997, Halanych & Passamanek 2001).
2. "Phyla" are man-made constructs erected because, in part, shared features were lacking between organismal groups. The phylum concept has a long history of being equated to body plans (or Baupläne), which can be misleading as to the age and diversity of a group. For example, despite very different body plans, major lineages of the phylum Arthropoda are put together because of the presence of an exoskeleton. In contrast, the formerly recognized phylum Vestimentifera (tube worms) are highly derived annelids with limited diversity and recent origins (McHugh 1997; Halanych et al. 1998, 2001).
3. Molecular data are more objective and subject to considerably more rigor than morphological data. DNA sequence contains four easily identified and mutually exclusive character states (analyses on protein coding genes usually employ translated nucleotide data). Morphological and embryological character definitions and scoring of character states are far more subjective, and most characters have been repeatedly used without critical evaluation, calling into question the utility of morphological cladistic studies that span Metazoa (Jenner 1999, 2002, 2004). For example, a quick comparison among

morphological cladistic analyses will reveal several characters scored differently by various workers. Furthermore, evolution at nucleotide and amino acid levels of housekeeping and conserved developmental genes (i.e., those used for phylogenetics) is understood to a much better degree than evolutionary forces acting on morphology. As such, more sophisticated and accurate methods of phylogeny reconstruction are available to molecular data, whereas morphological data are generally limited to parsimony methods.

NEW METAZOAN¹ TREE

In 1988, Field and coworkers published on animal phylogeny using the 18S or nuclear small ribosomal subunit (SSU) gene. Although this paper did have some internal inconsistencies, it ushered in the era of molecular systematics for higher-level animal phylogeny. The SSU was chosen at the time because enough RNA could be obtained for sequencing, it was ubiquitous in animals, and regions of the gene were conserved enough to make “universal primers.” Field et al. (1988) were also one of the first to use explicit criteria and algorithms for building a phylogenetic tree of Metazoa (but see Bergstrom 1985). Thus, it provided a means to critically test and evaluate traditional hypotheses of animal relationships.

Figure 2 illustrates our current understanding of animal relationships. The topology presented is a conservative interpretation of available data. Because data come from many different sources, we cannot reconstruct the tree from a single, all-encompassing analysis. Throughout the text, I discuss support for the relationships presented in Figure 2. Similarly, Table 1 summarizes some major changes in our understanding of metazoan phylogeny.

BASAL METAZOAN CLADES

Metazoa constitutes a monophyletic clade closely related to Choanoflagellata. This traditional view and supporting evidence (e.g., morphological synapomorphies, including extracellular matrix, septate junctions, and spermatozoa; and gene trees, including rDNA, heat-shock proteins, and elongation factors) have been recently covered by Cavalier-Smith et al. (1996), Eernisse & Peterson (2004), and Brooke & Holland (2003) and is not elaborated here.

Sponge Paraphyly

The question of poriferan monophyly has recently generated much discussion. Molecular analyses of SSU data suggest that sponges form a basal paraphyletic

¹Note that others recognize a formal distinction between Metazoa and Animalia, using Animalia to represent a more inclusive clade of Choanoflagellata plus Metazoa (e.g., Sørensen et al. 2000, Eernisse & Peterson 2004).

TABLE 1 List of important hypotheses^a supported by our current understanding

Hypothesis	Support ^b
Sponge paraphyly	SSU, LSU, morph.
Cnidaria sister to Bilateria	SSU, SSU 2 ^o structure, Hox, morph.
Platyhelminthes polyphyletic (Acoelomorphs basal)	SSU, LSU, myo II
Deuterostomia	SSU, LSU, Hox, morph.
Ambulacraria (Hemichordata and Echinodermata)	SSU, LSU, tRNA coding, multigene protein coding, morph.
<i>Xenoturbella</i> is a deuterostome	SSU, COI, COII, mtDNA gene order, codon usage
Lophotrochozoa	SSU, LSU, Hox, mtDNA gene trees, mtDNA gene order, myo II, IF gene
Annelida includes siboglinids and echiurids	EF-1 α , SSU, LSU, COI, morph.
Lophophorate polyphyly	SSU, LSU, Hox, morph.
Syndermata (Acanthocephala within Rotifera)	SSU, morph.
Gnathifera (Syndermata, Gnathostomulida, Micrognathozoa)	SSU, morph.
Platyzoa (Platyhelminthes, Gastrotricha, Cycliphora, Entoprocta, Gnathifera)	SSU, LSU, morph.
Ecdysozoa	SSU, LSU, Hox, morph., HRP activity
Scalidophora (Kinorhyncha, Loricifera, Priapula)	SSU, LSU, morph.
Pancrustacea (Hexapoda within Crustacea)	EF-1 α , SSU, LSU

^aHypotheses that are either novel (e.g., based on molecular data) or are different from our traditional understanding.

^bThese are the primary sources of support. See text for full details for any given node.

grade in Metazoa with Hexactinellida (glass sponges), then Demospongia (spongin fibers), and then Calcarea (calcareous sponges) branching off in order from the main metazoan lineage (Borchiellini et al. 2001). Recent combined studies provide support for the claim that Calcarea shares a more recent common ancestor with other animals, but placement of the Demospongia relative to the Hexactinellida is not clear (SSU and LSU, Medina et al. 2001; morphology and SSU, Zrzavy et al. 1998, Peterson & Eernisse 2001). The presence of long cross-striated ciliary

rootlets in calcareous sponge larvae and eumetazoans to the exclusion of other sponges is consistent with the recent SSU hypothesis (Amano & Hori 1992, Nielsen 2001).

In phylogenetic analyses, taxonomic representation of sponges is often limited because of problems with obtaining quality DNA or RNA free of contamination from foreign genomes. In addition to more robust taxon sampling, future studies examining the presence or absence of certain genes in different sponge lineages will also yield valuable insight. As for morphological characters, sponges are often problematic because many phylogenetic characters applied to other animals must be coded as question marks, leading to undesirable effects in tree reconstruction programs.

The paraphyletic nature of sponges has important consequences for understanding early animal evolution. Poriferans are typically considered monophyletic on the basis of their body architecture, with a water canal system, presence of choanocytes, and equatorial polar bodies during development (Nielsen 2001). However, molecular data suggest these features should be considered symplesiomorphies (shared ancestral characters) of Metazoa and not synapomorphies of Porifera. Interestingly, Hox genes, involved in anterior/posterior body patterning, have not been easy to find in sponges despite repeated attempts (Seimiya et al. 1994, Manuel & Le Parco 2000), although Degnan et al. (1995) reports finding three divergent candidates. Thus, transition from an organism with poorly defined or lacking axes to one with an anterior and posterior seems to have been accompanied by expansion of genetic machinery (Finnerty 1998, Finnerty & Martindale 1998).

Cnidarians and Ctenophores

Coelenterata is a dated term that referred to a taxon comprising Cnidaria and Ctenophora (and originally Porifera; Hyman 1940). A Cnidaria/Ctenophora clade is not supported by SSU data (Collins 1998, Kim et al. 1999, Podar et al. 2001), LSU data (Medina et al. 2001), morphology (Eernisse et al. 1992), or combined analyses (Zrzavy et al. 1998, Peterson & Eernisse 2001). However, the hypothesized position of Ctenophora varies depending on which data are analyzed. Morphological analyses place ctenophores closer to bilaterians (Schram 1991, Eernisse et al. 1992, Zrzavy et al. 1998, Peterson & Eernisse 2001) because of sperm morphology and muscle cells, among other characters. In contrast, SSU data support sponges and ctenophores as basal lineages of Metazoa (Collins 1998, Kim et al. 1999, Podar et al. 2001, Medina et al. 2001), leaving cnidarians as sister to bilaterians. Available evidence from Hox genes (reviewed in Martindale et al. 2002), as well as SSU secondary structure (Aleshin & Petrov 2002), also supports the Cnidaria/Bilateria relationship.

The traditional dogma concerning lack of bilateral symmetry and mesoderm in cnidarians and ctenophores is problematic. The issue of symmetry is really an issue of body axis evolution. How many are there? Evolution of developmental mechanisms research has largely focused on how body axes are set up and

maintained during ontogeny. Thus, our understanding and definitions of symmetry are being reshaped by a growing body of evidence that demonstrates that mechanisms used in axial pattern can be found in nonbilaterian animals (Martindale et al. 2002, Brooke & Holland 2003, Wikramanayake et al. 2003, Finnerty et al. 2004). Similarly, we are beginning to appreciate that mesoderm, or mesodermal precursors, may be present in ctenophores and cnidarians (e.g., Martindale & Henry 1999, Spring et al. 2002, Muller et al. 2003, and references therein). Information on relationships within Cnidaria may be found in Bridge et al. (1995) and Collins (2000, 2002). Podar et al. (2001) and Harbison (1985) offer views on molecular and morphological ctenophore phylogeny, respectively.

Placozoa

Placozoans are simple organisms of great interest for understanding very early evolution in animals. As with sponges, the use of placozoans in morphological cladistic analyses can be problematic because they cannot be scored for most characters. Every conceivable placement of placozoans among nonbilaterian metazoans has been proposed. Studies including SSU data variably place placozoans within or sister to the Cnidaria (Bridge et al. 1995, Siddall et al. 1995, Kim et al. 1999, Cavalier-Smith & Chao 2003; but see Zrzavy et al. 1998, Peterson & Eernisse 2001). Reports of a placozoan/cnidarian clade seem less likely based on the circular morphology of the placozoan mtDNA molecule (most cnidarians have a linear mtDNA genome) and secondary structure of the mitochondrial LSU (Ender & Schierwater 2003). Although the exact placement of placozoans is not clear, they are near the base of Metazoa (just before or after the sponge lineages) and are currently receiving considerable attention via genomic tools (e.g., Martinelli & Spring 2003, Jakob et al. 2004).

BILATERIA

Bilateria consists of three main clades that predate the Precambrian/Cambrian boundary, 540 million years ago (Mya) (Balavoine & Adoutte 1998). Unfortunately, the events that led to the last bilaterian ancestor and subsequent diversification into deuterostomes, lophotrochozoans, and ecdysozoans are not well understood. As such, there is considerable interest in determining which extant taxon is the most basal bilaterian lineage. Currently, there are two possible candidates, acoelomorphs and myxozoans (small parasitic group). Very recent data for the chaetognaths, traditionally considered deuterostomes, suggest a fairly basal position. However, arrow worms are discussed in the deuterostome section because of the relevancy of published data.

The placement of acoelomorphs is a controversial topic that highlights some of the potential problems with both morphological and SSU rDNA data. Early SSU analyses suggested Platyhelminthes was polyphyletic, with Acoela being

the most basal bilaterian lineage and separate from other flatworms (Ruiz-Trillo et al. 1999). However, the acoele in question had long-branch lengths owing to high nucleotide substitution rates, a problem that randomizes signal in data and causes long-branched taxa to be artificially placed basal in a tree reconstruction (Felsenstein 1988, Wheeler 1990). Thus, researchers were concerned that the SSU acoele result was an artifact. This long-branch problem was best illustrated in Peterson & Eernisse (2001), who graphically demonstrated that acoele branches for SSU data were so long that they effectively acted as random sequences and were probably rooting the Bilateria incorrectly (see also Giribet et al. 2000). Acoel morphology was not that helpful for higher-level phylogenetic considerations because of their simplified bodies, but within Platyhelminthes morphology supported Acoelomorpha (Acoela and Nemertodermatida) as a distinct clade considerably different from other flatworms (reviewed in Giribet et al. 2000, Ruiz-Trillo et al. 2002). Subsequent work on acoele placement with an independent marker, elongation factor (EF)-1 α , supported platyhelminth monophyly (Berney et al. 2000), but this work soon came under fire because of sequence alignment issues and limited taxon sampling (Littlewood et al. 2001). Recent findings with myosin II heavy chain (Ruiz-Trillo et al. 2002) and combined SSU and LSU data (Telford et al. 2003) have independently confirmed that the Acoelomorpha are a basal lineage of Bilateria. The remaining platyhelminthes appear to be within Lophotrochozoa.

Myxozoans are small enigmatic parasites with a very simple body plan. Thus, even if they are basal bilaterians, their highly derived morphology may limit their utility for understanding the last bilaterian ancestor. Myxozoans were previously considered protozoans, but molecular data demonstrated their metazoan nature (Smothers et al. 1994). Combined SSU and morphology placed them within Cnidaria (Siddall et al. 1995), but other molecular studies placed them at or near the bilaterian root (Schlegel et al. 1996, Kim et al. 1999, Ferrier & Holland 2001). Another mysterious organism, *Buddenbrockia*, has a simple body plan and has vexed scientist since its discovery in 1850. This nondescript worm-like organism is a myxozoan (Monteiro et al. 2002).

DEUTEROSTOMIA

Before the mid-1990s, Deuterostomia was generally considered to consist of three core phyla (Echinodermata, Hemichordata, and Chordata), plus Chaetognatha and lophophorate taxa (Brachiopoda, Phoronida, and Bryozoa²). Lophophorates have typically been regarded as having a mix of traditional protostome and deuterostome characters, but Zimmer (1973), among others, made convincing arguments based on developmental and nervous features (e.g., body regionalization, cleavage

²Technically, the term Bryozoa refers to a clade that includes Ectoprocta and Entoprocta (or Kamptozoa). The terms Ectoprocta and Bryozoa are equated here because one would rather be a bryozoologist than an ectoproctologist!

program, intraepidermal nervous system) for deuterostome affinities. Thus, many considered some or all of them as basal to Deuterostomia *sensu stricto* (Willmer 1990, Nielsen 2001). Building on earlier studies that had included molecular data for a brachiopod (Field et al. 1988, Lake 1990), Halanych et al. (1995) included SSU data from all three major lophophorate lineages and formally proposed that bryozoans, brachiopods, and phoronids were derived protostomes allied to annelids and mollusks. They proposed the node-based clade Lophotrochozoa. The placement of lophophorate taxa and support for Lophotrochozoa are discussed below.

Chaetognatha has also been removed from Deuterostomia. Two independent SSU papers reported that chaetognaths were not deuterostomes, but their exact placement in animal phylogeny remained elusive (Telford & Holland 1993, Wada & Satoh 1994). Further analyses with a broader range of taxa supported a nematode-chaetognath relationship, but the issue of long-branch attraction in SSU data could not be completely ruled out (Halanych 1996b, Peterson & Eernisse 2001). The uncertainty of chaetognath's position is echoed in combined studies that place them within Ecdysozoa (Zrzavy et al. 1998, Peterson & Eernisse 2001, Eernisse & Peterson 2004) or as basal bilaterians (Giribet et al. 2000). Papillon et al. (2003) surveyed Hox genes and reported that chaetognaths are basal in metazoan phylogeny because of the presence of a chimera medial/posterior Hox orthologs. Additional posterior Hox genes have been found, supporting chaetognaths as basal protostomes (D.Q. Matus, K.M. Halanych & M.Q. Martindale, unpublished results), consistent with mtDNA gene order (Helfenbein et al. 2004). Thus, many of the deuterostome-like features of chaetognaths (enterocoelous development, tripartite body, radial cleavage, etc.) are likely bilaterian symplesiomorphies rather than derived features.

Ambulacraria

Hemichordates were once placed within Chordata, but they were removed because some but not all the chordate-like features were present. Perhaps surprisingly these "half-chordates" are much more closely allied to echinoderms than to chordates (e.g., for morphology and SSU data, see Turbeville et al. 1994, Giribet et al. 2000, Peterson & Eernisse 2001; for SSU, see Halanych 1995; for changes in tRNA coding, see Telford et al. 2000; for multigene, see Cameron et al. 2000, Furlong & Holland 2002). Metschnikoff (1881), focusing on similarities between the larvae of echinoderms and enteropneusts (also known as acorn worms), referred to the echinoderm-hemichordate group as Ambulacraria, which Halanych (1995) formalized as a node-based name. Morphology supporting this grouping includes characters pertaining to the tripartite larval coeloms (absent in chordates).

Ambulacraria has profound implications for understanding chordate origins by altering interpretation of the evolution of gill slits, the nervous system, and possibly the notochord. Such chordate features may have been present in the last common ancestor of the Deuterostomia. Now we must address the possibility that echinoderms lack gill slits and a notochord-like structure because these features

were lost during the evolution to a pentaradial body plan. Similarly, Bather's (1913) and later Jefferies's (1986) assertions that primitive echinoderms possessed ciliated gill slits may be true. However, objective cladistic analyses of morphology and/or molecules do not support Jefferies's notion that chordates are direct descendents of echinoderms.

Noteworthy is the large amount of genomic data coming to bear on the issue of deuterostome relationships. For example, an impressive survey of hemichordate genes with orthologs involved in chordate nervous system development demonstrates an amazing amount of conservation in expression domains despite the noncentralized nature of the hemichordate nervous system (Lowe et al. 2003). Additionally, shortly after this publication, completely sequenced genome for each major deuterostome lineage should be available in GenBank.

Tunicata

The tadpole larva is used to unify Tunicata (also known as Urochordata) with Cephalochordata and Craniata. Perhaps the most convincing characters are the notochord and neural development. Except for Larvacea (Appendicularia), commonalities between the adult body form of tunicates, cephalochordates, and craniates are lacking to the point that their inclusion in the same phylum is questioned (Nielsen 2001). Interestingly, as pointed out by Swalla and colleagues (Swalla et al. 2000, Cameron et al. 2000, Winchell et al. 2002), the phylogenetic signal supporting a monophyletic Chordata (including tunicates) clade is weak. In fact, Ambulacraria is repeatedly much more robustly supported than Chordata (e.g., Winchell et al. 2002, Bourlat et al. 2003). The placement of Tunicata is variable: sister to Craniata/Cephalochordata clade (Cameron et al. 2000, SSU of Winchell et al. 2002), sister to Craniata (Giribet et al. 2000), sister to Ambulacraria (Wada & Satoh 1994, Swalla et al. 2000), or the basal deuterostome lineage (LSU of Winchell et al. 2002, Bourlat et al. 2003). Tunicates often display long branches relative to other deuterostomes, making their placement difficult. Despite the consensus that Tunicata, Cephalochordata, and Craniata form a monophyletic clade, this issue deserves more attention. The vast differences between tunicates and other chordates suggest that they possess an evolutionarily distinct body plan. Hence, the term Tunicata is preferred over Urochordata.

Paleontological work of Shu and collaborators (1999, 2001a, 2001b, 2003) is revising our understanding of the early evolution of chordates. They have discovered ascidian (e.g., *Cheungkongella*), cephalochordate-like yunnanozoan (e.g., *Haikouella*), and agnathan (e.g., *Myllokunmingia*) fossils that push the origins of these lineages at least into the Lower Cambrian (approximately 530–540 Mya) and provide evidence of their complexity early in their evolution.

Xenoturbella

Xenoturbella is a small, morphologically nondescript flatworm-like organism that feeds on bivalve eggs and larvae, whose phylogenetic affinities have long been a

mystery. Although the first available molecular data on *Xenoturbella* suggested molluscan affinities (Norén & Jondelius 1997), it appears to be a deuterostome basal to the Ambulacraria. Bourlat et al. (2003) assert that in addition to their SSU, cytochrome oxidase c subunit I (CO I) and CO II data, mitochondrial gene order, and more tentatively, codon usage support this placement. Because of its basal position, questions arise as to whether *Xenoturbella*'s simplified body plan is the result of strong selective constraints on an ancestral body form or the product of secondary simplification. *Xenoturbella* clearly illustrates that many exciting and interesting findings about animal evolution await discovery.

PROTOSTOMIA

Within Bilateria, Lophotrochozoa and Ecdysozoa form a monophyletic clade, typically called Protostomia, supported by SSU and LSU data (Halanych et al. 1995, Mallat & Winchell 2002), combined analyses (Giribet et al. 2000), and Hox genes (de Rosa et al. 1999). In cases where conflicting topologies (e.g., Ecdysozoa with Deuterostomia) have been reported (Ruiz-Trillo et al. 2002), support is weak. Continued use of the term Protostomia is less than ideal but admittedly familiar. From a Hyman-like mindset, the term implies that the ultimate fate of the blastopore is phylogenetically conservative and has traditionally been applied to coelomate animals (to the exclusion of pseudocoelomates and acoelomates). In our current understanding, blastopore fate is irrelevant for many ecdysozoans because the blastopore is usually not retained. Also, both protostome clades contain several former acoelomate or pseudocoelomate taxa. Lastly, lophotrochozoans, ecdysozoans, and deuterostomes were probably all present in Precambrian times and all experienced some degree of a rapid radiation around 580–520 Mya. Thus, it seems more natural to think about bilaterians as having three main clades, given the importance of the Cambrian explosion in metazoan history (Balavoine & Adoutte 1998).

LOPHOTROCHOZOA

Lophotrochozoa is a clade originally identified by SSU data and defined as the last common ancestor of annelids, mollusks, the three lophophorate phyla (Brachiopoda, Phoronida, and Bryozoa), and all the descendants of that ancestor (Halanych et al. 1995). The name of the clade refers to the inclusion of animals that have either a trochophore or a lophophore feeding apparatus. The major implication of this hypothesis is that lophophorate taxa are not allied to the deuterostomes but are highly derived protostomes near annelids, mollusks, and their allies, calling into question many of the classical characters used to split protostomes and deuterostomes.

The Lophotrochozoa hypothesis was contentious at first because analyses of nonmolecular data still grouped some or all lophophorate taxa with deuterostomes

(Schram 1991, Eernisse et al. 1992, Zrzavy et al. 1998, Sørensen et al. 2000, Nielsen 2001). In solely molecular analyses, brachiopods, phoronids, and bryozoans have never been strongly supported with deuterostomes. Moreover, the Lophotrochozoa hypothesis has received strong support from multiple sources of molecular data, including SSU data (Halanych et al. 1995, Mackey et al. 1996, Eernisse 1997), LSU data (Mallat & Winchell 2002; Y.J. Passamanek & K.M. Halanych, unpublished results), Hox gene data (de Rosa et al. 1999, Passamanek & Halanych 2004), mitochondrial sequence and gene arrangement data (Stechmann & Schlegle 1999, Helfenbein et al. 2001, Helfenbein & Boore 2004), myosin II heavy chain sequence data (Ruiz-Trillo et al. 2002), and intermediate filament sequences (Erber et al. 1998). In particular, five Hox genes (*lox2*, *lox4*, *lox5*, *post1*, and *post2*) have been characterized as having lophotrochozoan-specific peptide signatures (de Rosa et al. 1999, Balavoine et al. 2002). Not surprisingly, taxonomic sampling of the above studies varies greatly, and only a few studies include all major (putative) lophotrochozoan taxa. For example, bryozoans seem to be a key taxon for defining Lophotrochozoa but have only recently been examined for data other than the SSU (see below).

Relationships within Lophotrochozoa are not well understood, and short-branch lengths between recognized phyla are a typical result. This lack of phylogenetic signal could be due to a rapid radiation of the taxa (i.e., Cambrian explosion) or indicative of the data in question. As genes other than the SSU and broader taxon sampling have been employed, lophotrochozoan interphyletic relationships have become more apparent, but support via bootstrap analyses or hypothesis testing methods (e.g., likelihood ratio tests) is often limited. For example, the terms Eutrochozoa and Trochozoa have been used to describe subsets of lophotrochozoan taxa (see Peterson & Eernisse 2001), but their weak nodal support precludes detailed discussion here. Nonetheless, within Lophotrochozoa several subclades are emerging (Figure 2).

Annelida

Annelida encompasses a greater diversity of animal body plans than traditionally recognized (McHugh 2000, Halanych et al. 2002). Both McHugh (1997) and Kojima (1998) used EF-1 α data to suggest that "Polychaeta" is not monophyletic. Specifically, Vestimentifera, Pogonophora, and Echiura, which were all considered separate phyla, are within the annelid radiation. SSU (Halanych et al. 2001), CO I (Black et al. 1997), and mitochondrial genome data (Boore & Brown 2000; R.M. Jennings & K.M. Halanych, unpublished data) corroborated placement of vestimentiferans and pogonophorans within annelids. This placement has long been argued by some on the basis of morphology (van der Land & Nørrevang 1977, Southward 1988) and has been supported in a morphological cladistic analysis (Rouse & Fauchald 1997). Furthermore, Vestimentifera is clearly a clade within the recognized Pogonophora (or Frenulata) (Southward 1988; Halanych et al. 1998, 2001; Rouse 2001). McHugh (1997), and Rouse & Fauchald (1997) argue

that the pogonophoran/vestmentiferan clade should revert to its original nomen *Siboglinidae* (Caullery 1914). Additionally, Clitellata (Oligochaeta and Hirudinea) are derived polychaetes (McHugh 2000, Rota et al. 2001, Struck et al. 2002).

McHugh's (1997) EF-1 α data also placed *Echiura* within annelids, which was subsequently supported by morphological work on the larval nervous system (Hessling 2002) and by SSU data (Bleidorn et al. 2003). Another group that deserves brief mention is Myzostomida, commensals/parasites on echinoderms that have often been placed with annelids (Westheide & Rieger 1996, Rouse & Fauchald 1997). Available combined EF-1 α and SSU data suggest an affinity to flatworms (Eeckhaut et al. 2000), but nervous system morphology (Müller & Westheide 2000) as well as larval morphology (Eeckhaut et al. 2003) are annelid-like. A recent combined analysis of SSU and morphology suggests this organism is outside the annelid radiation (Zrzavy et al. 2001). This group will receive future attention because its placement bears on issues of homology for nervous and larval characters within Lophotrochozoa.

Platyzoa

Platyzoa consists of Platyhelminthes (hereafter referred to as excluding Acoelomorpha), Rotifera, Acanthocephala, Gastrotricha, and Gnathostomulida, as recognized by Garey & Schmidt-Rhaesa (1998) and Cavalier-Smith (1998) and further supported by Giribet et al. (2000). Molecular evidence suggests Platyhelminthes are within the lophotrochozoan clade (Balavoine & Telford 1995, Balavoine 1997, de Rosa et al. 1999, Ruiz-Trillo et al. 2002), and Garey and colleagues (Garey et al. 1996b, Garey & Schmidt-Rhaesa 1998) provided strong support for acanthocephalans within rotifers, the Syndermata. The placement of gnathostomulids together with Syndermata has received support (Ahlrichs 1997, Giribet et al. 2000). Members of this group have an internal set of chitinous jaws and are therefore called the Gnathifera. Although molecular data are still wanting, the curious Micrognathozoa is likely to be in the Gnathifera (Kristensen & Funch 2000, Sørensen et al. 2000). However, recent molecular data (Giribet et al. 2004) are ambiguous, with SSU and very small fragments of LSU and Histone 3, placing Micrognathozoa near syndermatans and cyclophorans, whereas CO I placed it near entoprocts. Interestingly, the first report of Gnathostomulid SSU data (Littlewood et al. 1998) placed them next to a chaetognath-nematode clade, whereas in Giribet et al.'s (2000) analyses they clustered with platyhelminthes and gastrotrichs. Peterson & Eernisse (2001) noticed the potential long-branch issues of this group and subsequently removed the acoels, gnathostomulids, and gastrotrichs from their analyses. The LSU data support the grouping of rotifers and acanthocephalans with platyhelminthes (gnathostomulids were not included; Y.J. Passamanek & K.M. Halanych, unpublished observations). The morphological evidence does provide support for the Platyzoa (see Giribet et al. 2000).

One key issue with Platyzoa is its placement relative to Lophotrochozoa. Although some analyses assert that it is the sister clade to Lophotrochozoa (Garey & Schmidt-Rhaesa 1998, Giribet et al. 2000), others support placing Platyzoa within Lophotrochozoa (Peterson & Eernisse 2001; Eernisse & Peterson 2004; Y.J.

Passamaneck & K.M. Halanych, unpublished observations). Given that Lophotrochozoa is a node-based name, the inclusiveness of the group is determined by the position of its basal lineages, in this case Bryozoa. Thus, to determine if Platyzoa is within Lophotrochozoa, we must know its position relative to Bryozoa. The placement of Platyzoa remains an important issue because the position of platyhelminthes, with their simple body plans, shapes perceptions on the evolution of organismal complexity.

Brachipoda, Phoronida, Bryozoa

Another consistent result is that “Lophophorata” is not a monophyletic taxon, as long advocated on the basis of morphology (Nielsen 1985, 1987, 2001). Brachiopods and phoronids form a monophyletic entity, with bryozoans considerably removed. This result has been supported by SSU (Halanych et al. 1995, Mackey et al. 1996, Eernisse 1997), morphology and SSU (Zrzavy et al. 1998, Giribet et al. 2000, Peterson & Eernisse 2001), and morphology (Nielsen et al. 1996, Nielsen 2001). Lophophorate monophyly can be significantly rejected on the basis of combined SSU and LSU data (Y.J. Passamaneck & K.M. Halanych, unpublished results). In these analyses, brachiopods and phoronids tend to be placed near eutrochozoan taxa (e.g., annelids, mollusks; see de Rosa 2001, Eernisse 1997), and bryozoans are the most basal lineage of Lophotrochozoa. Whereas Giribet et al. (2000) suggested that bryozoans were basal protostomes, Hox data from bryozoans confirm their placement in Lophotrochozoa, probably as basal (Passamaneck & Halanych 2004). Cohen and coworkers (Cohen et al. 1998, Cohen 2000) suggested, on the basis of SSU data, that phoronids are derived brachiopods, but subsequent analysis suggests that they are sister taxa (Peterson & Eernisse 2001). Proposals have been put forth to recognize the new clade as either Brachiozoa (Cavalier-Smith et al. 1998), Phoronozoa (Zrzavy et al. 1998), or Lophophorata (Peterson & Eernisse 2001). The first two options represent two sides of the proverbial coin, but the last option is problematic because the term “lophophore” has a long history of being applied to brachiopods, phoronids, bryozoans, and even pterobranch hemichordates (Halanych 1996a). As such, the change in definition of Lophophorata will introduce confusion into the literature.

Other Taxa

Several other taxa deserve attention in the context of the Lophotrochozoa. As with annelids, within mollusks we do not have a good understanding of major relationships (see Haszprunar 2000, Steiner & Dreyer 2003, Medina & Collins 2003, Passamaneck et al. 2004). Although most analyses tend to place annelids and mollusks close to each other (e.g., Zrzavy et al. 1998, Giribet et al. 2000, Peterson & Eernisse 2001), we do not know how several other taxa fit in, for example scipunculans, nemerteans, and the brachiopod/phoronid clade. Examining SSU data alone in this region of the tree is problematic because they do not recover recognized phyla as monophyletic (e.g., mollusks, brachiopods, nemerteans). This situation is highlighted in Eernisse’s (1997) work, which discusses the positive

influence of more robust taxon sampling. A combined analysis of SSU and LSU recovers most “phyla” as monophyletic, but interphylum relationships are based on very short-branch lengths (Y.J. Passamanek & K.M. Halanych, unpublished results). These short-branch lengths would be expected in the case of a rapid radiation such as the Cambrian explosion.

Nemerteans are also lophotrochozoans (Turbeville et al. 1992, Kmita-Cunisse et al. 1998, de Rosa et al. 1999, Balavoine et al. 2002), consistent with embryology and morphology. Sipunculans appear close to mollusks morphologically (Scheltema 1993), but recent mitochondrial gene order data place them near annelids (Boore & Staton 2002). SSU and combined analyses do not provide strong support for a sipunculid placement other than that they are within Lophotrochozoa (Zrzavy et al. 1998, Giribet et al. 2000, Peterson & Eernisse 2001). Dicymids and Orthonectids (also known as Mesozoa) were once thought of as intermediates between protists and metazoans. However, molecular evidence suggests that these two (likely independent) groups are degenerate triploblast animals (Katayama et al. 1995, Hanelt et al. 1996, Pawlowski et al. 1996). Dicymids were shown to contain the lophotrochozoan-specific peptide motif in their *lox5* homolog (Kobayashi et al. 1999). However, the position of orthonectids is less certain and may be basal in Bilateria (Hanelt et al. 1996). Entoprocts and cycliophorans also present a bit of a mystery. The placement of Entoprocta is variable but consistently within Lophotrochozoa (Mackey et al. 1996, Eernisse 1997, Giribet et al. 2000, Peterson & Eernisse 2001). Cycliophorans were first proposed to be close to entoprocts on the basis of morphological similarities (Funch & Kristensen 1995), but preliminary SSU data placed them close to rotifers (Winnepenninckx et al. 1998). This result has been supported by one combined evidence analysis (Peterson & Eernisse 2001) but refuted by two others (Zrzavy et al. 1998, Giribet et al. 2000). LSU data support the Cycliophora/Entoprocta clade (Y.J. Passamanek & K.M. Halanych, unpublished observations), as does morphological cladistic analysis (Sørensen et al. 2000).

Noteworthy lophotrochozoan analyses using solely molecular data have not supported a clade with spiral cleavage *sensu stricto* (i.e., Spiralia).³ This result is partly due to the lack of resolution currently in this region of the tree, yet we must be open to the possibility that spiral cleavage is not strictly evolutionarily conserved. In particular, phoronids and brachiopods seemed to be allied with annelids and mollusks to the exclusion of, at least, platyhelminths (as generally judged by molecules). Given that phoronids and brachiopods have radial or biradial cleavage (Zimmer 1997), we must accept that spiral cleavage, at least, has been lost in some lineages. Thus, there is no monophyletic lineage that includes all spiral cleavers to the exclusion of other cleavage patterns. Recent cell lineage work further demonstrated that phoronids show no vestiges of 4d mesoderm specification expected in spiral cleavage (Freeman & Martindale 2002). For a critical evaluation of spiral cleavage as a phylogenetic character, see Jenner (2004).

³In fact, because of the placement of platyhelminthes, most traditional treatments also do not have a monophyletic Spiralia.

ECDYSOZOA

The clade Ecdysozoa circumscribes those animals that have a cuticle shed through molting (i.e., ecdysis). Aguinaldo et al. (1997) formally proposed this clade, on the basis of SSU rDNA results, to include the last common ancestor of arthropods, tardigrades, onychorans, nematodes, nematomorphs, kinorhynchs, and priapulids, and all the descendants of that last common ancestor. Previous SSU analyses including nematodes typically found round worms clustering at the base of Bilateria (e.g., Winnepenninckx et al. 1995). Aguinaldo et al. (1997) assessed the position of nematodes using a more slowly evolving nematode sequence (in this case *Trichinella*), eliminating the potential problem of long-branch attraction. Interestingly, a previous cladistic morphological study (Eernisse et al. 1992) had also recovered the same clade of molting animals (with the exception that Priapulida was part of a basal polytomy). Initial acceptance of this radical revision was slow because it was based on a single nematode sequence. However, it has been subsequently supported by independent data sets, most notably Hox genes (*Ubx*, *abd-A*, and *Abd-B* have ecdysozoan-specific peptides; de Rosa et al. 1999, Balavoine et al. 2002), LSU rDNA data (Mallat & Winchell 2002, Mallat et al. 2004), and combined morphology and SSU (Zrzavy et al. 1998, Giribet et al. 2000, Peterson & Eernisse 2001). Additionally, Haase et al. (2001) report that Ecdysozoa show neural expression of horseradish peroxidase (HRP) immunoreactivity that is absent in other animals. They suggest that the presence of anti-HRP-reactive glycoprotein(s) is a synapomorphy for Ecdysozoa.

The presence of a multimeric form of β -thymosin that was hypothesized as an ecdysozoan synapomorphy (Manuel et al. 2000) has been shown to be present in other metazoans (Telford 2004a). Also, genome-scale analyses have claimed to refute the Ecdysozoa hypothesis (e.g., Blair et al. 2002, Wolf et al. 2004), but unfortunately these analyses are flawed owing to limited taxon sampling (containing only three or four metazoan taxa total) and the inability to correct adequately for highly derived *Caenorhabditis elegans* sequences (i.e., long-branch issues). Wolf et al. (2004) did try to address the long-branch issue, but their effort was hampered by their limited number of metazoan taxa, three. For additional discussion concerning the problems of these papers, see Telford (2004b).

The Ecdysozoa hypothesis has had perhaps the most far-reaching effects on comparative biology because of the large amount of work on *Drosophila* and *Caenorhabditis*. Before the Ecdysozoa hypothesis, if a common genetic mechanism was found in both flies and round worms, it was presumed to be present throughout Bilateria. With the placement of these two taxa as members of the same lineage, the common machinery may be representative of only the ecdysozoan lineage, thereby limiting the inferences drawn from these model organisms.

Scalidophora and Nematoida

Researchers have limited knowledge of interrelationships within Ecdysozoa because many of the taxa are poorly studied. For example, in early 2004, only 1

GenBank entry existed for Kinorhyncha, 19 for Nematomorpha, and 26 for Priapulida. Nonetheless, there seems to be a general consensus which awaits further conformation. The Priapulida/Kinorhyncha/Loricifera clade should be referred to as Scalidophora (Lemburg 1995, Schmidt-Rhaesa 1996, Ehlers et al. 1996). Nielsen (2001) has used the name Cephalorhyncha for this clade, but previous usage of this term included the Nematomorpha (Malakhov 1980). Within this group, loriciferans are most likely sister taxon to kinorhynchs (Schram 1991, Sørensen et al. 2000, Peterson & Eernisse 2001), but no loriciferan molecular data have been collected because of difficulty in obtaining tissue. When loriciferans are not considered, priapulids and kinorhynchs form a monophyletic clade (Aguinaldo et al. 1997, Aleshin et al. 1998, Giribet et al. 2000, Peterson & Eernisse 2001).

The horsehair worms, Nematomorpha, are allied with Nematoda. This group, termed Nematoida (Schmidt-Rhaesa et al. 1998), has been supported in some analyses (Zrzavy et al. 1998; Giribet et al. 2000, combined data; Garey 2001) but not others (Giribet et al. 2000, SSU only; Peterson & Eernisse 2001). Using combined SSU and LSU data, Mallat et al. (2004) found strong support for Nematoida. The combined group of Scalidophora and Nematoida has been referred to as either the Introverta or Cycloneuralia (Nielsen 2001), but it is refuted by available data (Zrzavy et al. 1998, Peterson & Eernisse 2001, Mallat et al. 2004; but see Giribet et al. 2000). Instead, the Scalidophora is the most basal branch in the Ecdysozoa, with Nematoida and Panarthropoda (Tardigrada, Onychophora, and Arthropoda) as sister clades.

Panarthropoda

Within the Panarthropoda, the placement of tardigrades, onychophorans, and arthropods relative to each other has generated considerable debate. Early mitochondrial SSU data suggested that Onychophora were inside the Arthropoda (Ballard et al. 1992), and papers that reported a Tardigrada/Arthropoda relationship did not include an onychophoran (Garey et al. 1996a, Giribet et al. 1996). The rDNA analyses of Mallat et al. (2004) suggested an onychophoran/tardigrade clade, but the authors were tentative about this result because onychophoran rDNA appears very derived, and nodal support is weak. This result was also recovered by Giribet et al.'s (2000) combined morphology and SSU analyses.

Although of great interest, I do not discuss the relationships within the arthropods in detail. Recent work on the subject has used a variety of molecular markers (rDNA, Hox genes, mtDNA arrangement), and these are discussed elsewhere (Giribet & Ribera 2000, Giribet et al. 2001, Hwang et al. 2001, Cook et al. 2001, Mallett et al. 2004). The most notable change in arthropod phylogeny is the placement of Hexapoda within Crustacea to form Pancrustacea. This hypothesis was convincingly put forth by Regier & Shultz (1997) on the basis of EF-1 α and has received considerable support (e.g., Cook et al. 2001, Mallett et al. 2004). Also the previously recognized phylum Pentastomida, parasites on vertebrates, is a derived crustacean clade (Abele et al. 1989, Lavrov et al. 2004).

Dis-Articulating

One reason many were resistant to the Lophotrochozoa and Ecdysozoa hypotheses is that they contradicted the Articulata (Arthropoda and Annelida) as a real clade. Despite the overwhelming evidence that annelids are much more closely related to mollusks than to arthropods (based on morphology as well as at least five independent molecular markers), there are still attempts to maintain an Articulata-like clade (e.g., Nielsen 2003). The segmented nature of annelids and arthropods has a long history of being used to unite these taxa (Willmer 1990). Although genes involved in the segmentation program in arthropods (e.g., *Drosophila*) are well studied (for a general review, see Carroll et al. 2001), very little is known about segmentation in annelids. What we do know is that segmentation-related genes (e.g., *engrailed* and *hunchback*) in *Drosophila* appear to be doing something different in annelids (Seaver et al. 2001, Werbrock et al. 2001, but see Prud'homme et al. 2003). Seaver (2003) reviews the possibilities of independent origins of segmentation in annelids, arthropods, and chordates. As she points out, all these taxa are nested within several nonsegmented taxa. Thus, any attempt to infer that an ancestor deep in the bilaterian tree was segmented also required multiple losses of segmentation in numerous different lineages. Given that genetic machinery for segmentation does not appear to be the same, a segmented protostome or bilaterian ancestor is not likely.

Proponents of the Articulata hypothesis assert that segmentation is a very strong morphological character and thus a good phylogenetic indicator. Yet from an objective point of view there is no reason segmentation should be a better indicator of phylogenetic history than molting (in ecdysozoans) or a trochophore larva (in a subset of lophotrochozoans). The Articulata hypothesis also suggests that cleavage patterns are not immutable, as arthropods lack spiral cleavage. All these characters are intricately tied to constraints in functional morphology for which we have little understanding of the selective forces or evolutionary plasticity.

CAMBRIAN EXPLOSION

As should be clear from the previous discussion, understanding the early evolutionary events of animal history is difficult. This situation has not been made any easier by the lack of a substantial fossil record before about 570 Mya. At roughly 543 Mya, the fossil record shows a sudden diversity of animal forms that represent most of the major lineages, with some taxa displaying great diversity and derived body plans (reviewed in Grotzinger et al. 1995, Knoll & Carroll 1999, Erwin & Davidson 2002). This sudden appearance of diversity in the fossil record is called the Cambrian explosion.

Recent finds for the Lower Cambrian Chengjiang and Sirius Passet faunas (approximately 520 Mya) have provided exquisitely preserved samples of animals (Conway Morris & Peel 1995, Chen & Zhou 1997, Bengtson & Zhao 1997, Shu et al. 1999). Older fossils from the Ediacaran and Doushantuo formations (up to 570 Mya) predate the Cambrian boundary and show that some animal lineages were present well before the Cambrian (Xiao et al. 1998). For the period before the

Doushantuo formation (which yielded sponges and fossil embryos; Li et al. 1998, Xiao et al. 1998), fossils for animals are generally lacking, despite the presence in the fossil record of several other eukaryotic crown groups dating back to 1200 Mya (Erwin & Davidson 2002). [The report by Seilacher et al. (1998) of ancient worm trace fossils was erroneous (Rai & Gautam 1999, Rasmussen et al. 2002).]

In contrast to the fossil data, several recent works have reported using a molecular clock to date animal diversification (Wray et al. 1996, Nikoh et al. 1997, Ayala et al. 1998, Gu 1998, Bromham et al. 1998, Lynch 1999). These works report that bilaterians diverged between 630 and 1200 Mya. Several workers (e.g., Philippe et al. 1994, Smith & Peterson, 2002; see Graur & Martin 2004 for a particularly colorful discussion) have highlighted some of the problems with using a molecular clock to date deep divergences. In particular, clock studies have been plagued by assumptions of rate homogeneity in nucleotide substitution patterns. Even when nucleotide substitution models can correct for rate variation across different positions in the same gene, available molecular tools are not sophisticated enough (yet) to deal adequately with rate variation across lineages within the same tree. Furthermore, many molecular clock analyses made the mistake of overgeneralizing their results. Specifically, they used only a limited number (3–5) of fossil calibration points, typically within craniates or other deuterostomes, whereas the Cambrian explosion was mainly lophotrochozoan and ecdysozoan in nature and involved numerous lineages (although we must recognize the diversity of echinoderms and presence of early chordates).

On the positive side, studies of molecular clocks have called our attention to a hidden history of early animal evolution. Although the fossil data suggested this hidden history was very short in nature (~30 My), molecular clock analyses in general suggested a much longer hidden history (up to 700 My; see Erwin & Davidson 2002). This last scenario seems unlikely because fossils of other crown eukaryotes are known from this period, necessitating ad hoc hypotheses to account for the dearth of animal fossils in particular.

The fact that there is any hidden history, regardless of duration, suggests that early animals were very small organisms, likely meiofaunal or small epibenthic dwellers. Such organisms would not fossilize easily (but the Chengjiang, Sirius Passet, and Doushantuo formations have proven the exception) and were probably direct developers. Another argument against a segmented last common bilaterian ancestor is that miniaturization can apparently reduce segmentation (Westheide 1997).

CONCLUSIONS

Our understanding of metazoan phylogeny is far from complete. However, in the past 15 years we have made tremendous progress toward understanding the general framework of animal evolution. Relationships among the most basal lineages of animals are not entirely clear, but poriferans are likely a paraphyletic grade that led to a Cnidarian/Bilaterian clade. Within Bilateria, we have three major clades. Lophotrochozoa is the most diverse clade in terms of body plans, and

understanding their internal relationships will take considerable work. How Platyzoa fit into this group also remains to be seen. Ecdysozoa groups nematodes and arthropods and has implications for how we extrapolate information from model systems. Chaetognaths are likely to be basal to Ecdysozoa and Lophotrochozoa. Deuterostomia only contains a limited number of lineages, but the presence of the Ambulacraria (echinoderms and hemichordates) suggests that several chordate features evolved earlier than traditionally believed. At the base of Bilateria, we have the Acoelomorpha and possibly Myxozoa.

Compared with the Hyman-like concept of animal phylogeny, this new view underscores the evolutionary plasticity of embryology and functional morphology. Many of the dogmatic concepts in invertebrate biology must be questioned (e.g., cleavage patterns are immutable, evolution proceeds from simple to complex, segmentation is highly conserved).

Clearly, several relationships still need to be worked out. To promote additional research on metazoan evolution, below are ten provocative hypotheses that are likely to provide considerable insight into animal evolution when tested in a rigorous manner:

1. Placozoans branched off from the main animal lineage before sponges.
2. Mesoderm first arose in ctenophores.
3. Acoelomorphs are secondarily simplified animals.
4. Chaetognatha is the sister to the Lophotrochozoa/Ecdysozoa clade.
5. Spiral cleavers do not form a monophyletic clade exclusive of other cleavage patterns.
6. Genetic mechanisms controlling annelid segmentation are different than in arthropods.
7. The Brachiopoda/Phoronida clade is sister to Mollusca.
8. Panarthropoda evolved from a small infaunal organism.
9. Platyzoa is a derived subclade of Lophotrochozoa.
10. The hidden history of early bilaterian evolution was less than 50 My.

NOTE ADDED IN PROOF

While this work was in press, Anderson et al. (2004) published data from the sodium-potassium ATPase α -subunit gene, which also supports the Lophotrochozoa and Ecdysozoa hypotheses, but did not recover deuterostomes or Arthropods as monophyletic.

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LITERATURE CITED

- Abele LG, Kim W, Felgenhauer BE. 1989. Molecular evidence for inclusion of the phylum Pentastomida in the Crustacea. *Mol. Biol. Evol.* 6:685–91
- Aguinaldo AMA, Turbeville JM, Linford LS, Rivera MC, Garey JR, et al. 1997. Evidence for a clade of nematodes, arthropods and other moulting animals. *Nature* 387:489–93
- Ahlrichs WH. 1997. Epidermal ultrastructure of *Seison nebaliae* and *Seison annulatus*, and a comparison of epidermal structures within the Gnathifera. *Zoomorphology* 117:41–48
- Aleshin VV, Milyutina IA, Kedrova OS, Vladychenskaya NS, Petrov NB. 1998. Phylogeny of *Nematoda* and *Cephalorhyncha* derived from 18S rDNA. *J. Mol. Evol.* 47:597–605
- Aleshin VV, Petrov NB. 2002. Molecular evidence of regression in evolution of Metazoa. *Zh. Obshch. Biol.* 63:195–208
- Amano S, Hori I. 1992. Metamorphosis of calcareous sponges. I. Ultrastructure of free-swimming larvae. *Invertebr. Reprod. Dev.* 21:81–90
- Anderson FE, Cordoba AJ, Thollesson M. 2004. Bilaterian phylogeny based on analyses of a region of the sodium-potassium ATPase α -subunit gene. *J. Mol. Evol.* 58:252–68
- Ayala FJ, Rzhetsky A, Ayala FJ. 1998. Origin of the metazoan phyla: Molecular clocks confirm paleontological estimates. *Proc. Natl. Acad. Sci. USA* 95:606–11
- Balavoine G. 1997. The early emergence of plathelminths is contradicted by the agreement between 18S rRNA and Hox genes data. *C. R. Acad. Sci.* 320:83–94
- Balavoine G, Adoutte A. 1998. One or three Cambrian radiations? *Science* 280:397–98
- Balavoine G, de Rosa R, Adouette A. 2002. Hox clusters and bilaterian phylogeny. *Mol. Phylogenet. Evol.* 24:366–73
- Balavoine G, Telford MJ. 1995. Identification of planarian homeobox sequences indicates the antiquity of most Hox/homeotic gene subclasses. *Proc. Natl. Acad. Sci. USA* 92:7227–31
- Ballard JWO, Olsen GJ, Faith DP, Odgers WA, Rowell DM, Atkinson PW. 1992. Evidence from 12S ribosomal RNA sequences that onychophorans are modified arthropods. *Science* 258:1345–48
- Bather FA. 1913. Caradocian Cystidea from Girvan. *Trans. R. Soc. Edinburgh* 49:359–529
- Bengtson S, Zhao Y. 1997. Fossilized metazoan embryos from the earliest Cambrian. *Science* 277:1645–48
- Bergstrom J. 1985. Metazoan evolution—a new model. *Zool. Scr.* 15:189–200
- Berney C, Pawlowski J, Zaninetti L. 2000. Elongation factor 1- α sequences do not support an early divergence of the Acoela. *Mol. Biol. Evol.* 17:1032–39
- Black MB, Halanych KM, Maas PAY, Hoeh WR, Hashimoto J, et al. 1997. Molecular systematics of vestimentiferan tubeworms from hydrothermal vents and cold-water seeps. *Mar. Biol.* 130:141–49
- Blair JE, Ikeo K, Gojoberi T, Hedges SB. 2002. The evolutionary position of nematodes. *BMC Evol. Biol.* 2:7
- Bleidorn C, Vogt L, Bartolomaeus T. 2003. A contribution to sedentary polychaete phylogeny using 18S rRNA sequence data. *J. Zool. Syst. Evol. Res.* 41:186–95
- Boore JL, Brown WM. 2000. Mitochondrial genomes of *Galathealinum*, *Helobdella*, and *Platynereis*: Sequence and gene arrangement

- comparisons indicate that Pogonophora is not a phylum and annelida and arthropoda are not sister taxa. *Mol. Biol. Evol.* 17:87–106
- Boore JL, Staton JL. 2002. The mitochondrial genome of the Sipunculid *Phascolopsis gouldii* supports its association with Annelida rather than Mollusca. *Mol. Biol. Evol.* 19:127–37
- Borchiellini C, Manuel M, Alivon E, Boury-Esnault N, Vacelet J, Le Parco Y. 2001. Sponge paraphyly and the origin of Metazoa. *J. Evol. Biol.* 14:171–79
- Bourlat S, Nielsen C, Lockyer A, Littlewood DT, Telford M. 2003. *Xenoturbella* is a deuterostome that eats molluscs. *Nature* 424:925–28
- Bridge D, Cunningham CW, DeSalle R, Buss LW. 1995. Class-level relationships in the phylum Cnidaria: molecular and morphological evidence. *Mol. Biol. Evol.* 12:679–89
- Bromham LD, Rambault A, Fortey R, Cooper A, Penny D. 1998. Testing the Cambrian explosion hypothesis by using a molecular dating technique. *Proc. Natl. Acad. Sci. USA* 95:12386–89
- Brooke NM, Holland PW. 2003. The evolution of multicellularity and early animal genomes. *Curr. Opin. Genet. Dev.* 13:599–603
- Brusca RC, Brusca GJ. 2003. *Invertebrates*. Sunderland, MA: Sinauer. 936 pp. 2nd ed.
- Cameron CB, Garey JR, Swalla BJ. 2000. Evolution of the chordate body plan: new insights from phylogenetic analyses of deuterostome phyla. *Proc. Natl. Acad. Sci. USA* 97:4469–74
- Carroll SB, Grenier JK, Weatherbee SD. 2001. *From DNA to Diversity*. London: Blackwell. 214 pp.
- Caulley M. 1914. Sur les Siboglinidae, type nouveau d'invertébrés recueilli par l'expédition du Siboga. *C.R. Acad. Sci.* 158:2014–17
- Cavalier-Smith T. 1998. A revised six-kingdom system of life. *Biol. Rev.* 73:203–66
- Cavalier-Smith T, Allsopp M, Chao E, Boury-Esnault N, Vacelet J. 1996. Sponge phylogeny, animal monophyly, and the origin of the nervous system: 18S rRNA evidence. *Can. J. Zool.* 74:2031–45
- Cavalier-Smith T, Chao EE. 2003. Phylogeny of Choanozoa, Apusozoa, and other Protozoa and early eukaryote megaevolution. *J. Mol. Evol.* 56:540–63
- Chen J-Y, Zhou G-Q. 1997. Biology of the Chengjiang fauna. In *The Cambrian Explosion and the Fossil Record*, ed. J-Y Chen, Y-N Chen, H Van Iten, 10:11–116. Taiwan: Bull. Natl. Mus. Nat. Sci.
- Cohen BL. 2000. Monophyly of brachiopods and phoronids: reconciliation of molecular evidence with Linnaean classification (the subphylum Phoroniformea nov.). *Proc. R. Soc. London* 267:225–31
- Cohen BL, Gawthrop A, Cavaliersmith T. 1998. Molecular phylogeny of brachiopods and phoronids based on nuclear-encoded small subunit ribosomal RNA gene sequences. *Philos. Trans. R. Soc. London Ser. B* 353:2039–61
- Collins AG. 1998. Evaluating multiple alternative hypotheses for the origin of Bilateria: An analysis of 18S rRNA molecular evidence. *Proc. Natl. Acad. Sci. USA* 95:15458–63
- Collins AG. 2000. Towards understanding the phylogenetic history of Hydrozoa: hypothesis testing with 18S gene sequence data. *Sci. Mar.* 4:1–22
- Collins AG. 2002. Phylogeny of Medusozoa and the evolution of cnidarian life cycles. *J. Evol. Biol.* 15:418–32
- Conway Morris S, Peel JS. 1995. Articulated halkieriids from the lower Cambrian of north Greenland and their role in early protostome evolution. *Philos. Trans. R. Soc. London Ser. B* 347:305–58
- Cook CE, Smith ML, Telford MJ, Bastianello A, Akam M. 2001. Hox genes and the phylogeny of the arthropods. *Curr. Biol.* 11:759–63
- Degnan BM, Degnan SM, Giusti A, Morse DE. 1995. A hox/hom homeobox gene in sponges. *Gene* 155:175–77
- de Rosa R. 2001. Molecular data indicate the protostome affinity of brachiopods. *Syst. Biol.* 50:848–59

- de Rosa R, Grenier JK, Andreeva T, Cook CE, Adoutte A, et al. 1999. HOX genes in brachiopods and priapulids and protostome evolution. *Nature* 399:772–76
- Eckhaut I, Fievez L, Muller MC. 2003. Larval development of *Myzostoma cirriferum* (Myzostomida). *J. Morphol.* 258:269–83
- Eckhaut I, McHugh D, Mardulyn P, Tiedemann R, Monteyne D, et al. 2000. Myzostomida: a link between trochozoans and flatworms? *Proc. R. Soc. London* 267:1383–92
- Eernisse DJ. 1997. Arthropod and annelid relationships re-examined. In *Arthropod Relationships*, ed. RA Fortey, RH Thomas, pp. 43–56. London: Chapman & Hall
- Eernisse DJ, Albert JS, Anderson FE. 1992. Annelida and Arthropoda are not sister taxa: a phylogenetic analysis of spiralian metazoan phylogeny. *Syst. Biol.* 41:305–30
- Eernisse DJ, Peterson KJ. 2004. The history of animals. In *Assembling the Tree of Life*, ed. J Cracraft, MJ Donoghue, pp. 197–208. New York: Oxford Univ. Press
- Ehlers U, Ahlrichs W, Lemburg C, Schmidt-Rhaesa A. 1996. Phylogenetic systematization of the Nematelminthes (Aschelminthes). *Verh. Dtsch. Zool. Ges.* 89:8
- Ender A, Schierwater B. 2003. Placozoa are not derived cnidarians: evidence from molecular morphology. *Mol. Biol. Evol.* 20:130–34
- Erber A, Riemer D, Bovenschulte M, Weber K. 1998. Molecular phylogeny of metazoan intermediate filament proteins. *J. Mol. Evol.* 47:751–62
- Erwin DH, Davidson EH. 2002. The last common bilaterian ancestor. *Development* 129:3021–32
- Felsenstein J. 1988. Phylogenies from molecular sequences: inference and reliability. *Annu. Rev. Genet.* 22:521–65
- Ferrier D, Holland PWH. 2001. Sipunculan ParaHox genes. *Evol. Dev.* 3:263–70
- Field KG, Olsen GJ, Lane DJ, Giovannoni SJ, Ghiselin MT, et al. 1988. Molecular phylogeny of the animal kingdom. *Science* 239:748–53
- Finnerty JR. 1998. Homeoboxes in sea anemones and other nonbilaterian animals: implications for the evolution of the Hox cluster and zootype. *Curr. Top. Dev. Biol.* 40:211–54
- Finnerty JR, Martindale MQ. 1998. The evolution of the Hox cluster: insights from outgroups. *Curr. Opin. Genet. Dev.* 8:681–87
- Freeman G, Martindale MQ. 2002. The origin of mesoderm in phoronids. *Dev. Biol.* 252:301–11
- Funch P, Kristensen RM. 1995. Cyclophora is a new phylum with affinities to Entoprocta and Ectoprocta. *Nature* 378:711–14
- Furlong RF, Holland PW. 2002. Bayesian phylogenetic analysis supports monophyly of Ambulacraria and of cyclostomes. *Zool. Sci.* 19:593–99
- Garey JR. 2001. Ecdysozoa: the relationship between Cycloneuralia and Panarthropoda. *Zool. Anz.* 240:321–30
- Garey JR, Krotc M, Nelson DR, Brooks J. 1996a. Molecular analysis supports a tardigrade-arthropod association. *Invert. Biol.* 115:79–88
- Garey JR, Near TJ, Nonnemacher MR, Nadler SA. 1996b. Molecular evidence for Acanthocephala as a subtaxon of Rotifera. *J. Mol. Evol.* 43:287–92
- Garey JR, Schmidt-Rhaesa A. 1998. The essential role of “minor” phyla in molecular studies of animal evolution. *Am. Zool.* 38:907–17
- Giribet G, Carranza S, Bagaña J, Riutort M, Ribera C. 1996. First molecular evidence for the existence of a Tardigrada + Arthropoda clade. *Mol. Biol. Evol.* 13:76–84
- Giribet G, Distel DL, Polz M, Sterrer W, Wheeler WC. 2000. Triploblastic relationships with emphasis on the acoelomates and the position of Gnathostomulida, Cyclophora, Plathelminthes, and Chaetognatha: a combined approach of 18S rDNA sequences and morphology. *Syst. Biol.* 49:539–62
- Giribet G, Edgecombe GD, Wheeler WC. 2001. Arthropod phylogeny based on eight molecular loci and morphology. *Nature* 413:157–61
- Giribet G, Ribera C. 2000. A review of Arthropod phylogeny: new data based on

- ribosomal DNA sequences and direct character optimization. *Cladistics* 16:204–31
- Giribet G, Sørensen MV, Funch P, Kristensen RM, Sterrer W. 2004. Investigations into the phylogenetic position of Micrognathozoa using four molecular loci. *Cladistics* 20:1–13
- Graur D, Martin W. 2004. Reading the entrails of chickens: molecular timescales of evolution and the illusion of precision. *Trends Genet.* 20:80–86
- Grotzinger JP, Bowring SA, Saylor BZ, Kaufman AJ. 1995. Biostratigraphic and geochronologic constraints on early animal evolution. *Science* 270:598–604
- Gu X. 1998. Early metazoan divergence was about 830 million years ago. *J. Mol. Evol.* 47:369–71
- Haase A, Stern M, Wachtler K, Bicker G. 2001. A tissue-specific marker of Ecdysozoa. *Dev. Genes Evol.* 211:428–33
- Halanych KM. 1995. The phylogenetic position of the pterobranch hemichordates based on 18S rDNA sequence data. *Mol. Phylogeny Evol.* 4:72–76
- Halanych KM. 1996a. Convergence in the feeding apparatuses of lophophorates and pterobranch hemichordates revealed by 18S rDNA: an interpretation. *Biol. Bull.* 190:1–5
- Halanych KM. 1996b. Testing hypotheses of chaetognath origins: long branches revealed by 18S ribosomal DNA. *Syst. Biol.* 45:223–46
- Halanych KM, Bacheller JD, Aguinaldo AMA, Liva SM, Hillis DM, Lake JA. 1995. Evidence from 18S ribosomal DNA that the lophophorates are protostome animals. *Science* 267:1641–43
- Halanych KM, Dahlgren TG, McHugh D. 2002. Unsegmented annelids? Possible origins of four lophotrochozoan worm taxa. *Integr. Comp. Biol.* 42:678–84
- Halanych KM, Feldman RA, Vrijenhoek RC. 2001. Molecular evidence that *Scelerolinum brattstromi* is closely related to vestimentiferans, not frenulate pogonophorans (Siboglinidae, Annelida). *Biol. Bull.* 201:65–75
- Halanych KM, Lutz RA, Vrijenhoek RC. 1998. Evolutionary origins and age of vestimentiferan tube-worms. *Cah. Biol. Mar.* 39:355–58
- Halanych KM, Passamanek Y. 2001. A brief review of metazoan phylogeny and future prospects in Hox-research. *Am. Zool.* 41:629–39
- Hanelt B, Van Schyndel D, Adema CM, Lewis LA, Loker ES. 1996. The phylogenetic position of *Rhopalura ophiocomae* (Orthonectida) based on 18S ribosomal DNA sequence analysis. *Mol. Biol. Evol.* 13:1187–91
- Harbison GR. 1985. On the classification and evolution of the Ctenophora. In *The Origins and Relationships of Lower Invertebrates*, ed. S Conway Morris, JD George, R Gibson, HM Platt, pp. 78–100. Oxford: Oxford Univ. Press
- Haszprunar G. 2000. Is the Aplousobranchia monophyletic? A cladistic point of view. *Am. Malacol. Bull.* 15:115–30
- Helfenbein KG, Boore JL. 2004. The mitochondrial genome of *Phoronis architecta*—comparisons demonstrate that phoronids are lophotrochozoan protostomes. *Mol. Biol. Evol.* 21:153–57
- Helfenbein KG, Brown WM, Boore JL. 2001. The complete mitochondrial genome of the articulate brachiopod *Terebratalia transversa*. *Mol. Biol. Evol.* 18:1734–44
- Hessling R. 2002. Metameric organisation of the nervous system in developmental stages of *Urechis caupo* (Echiura) and its phylogenetic implications. *Zoomorphology* 121:221–34
- Hwang UW, Friedrich M, Tautz D, Park CJ, Kim W. 2001. Mitochondrial protein phylogeny joins myriapods with chelicerates. *Nature* 413:154–57
- Hyman LH. 1940. *The Invertebrates: Protozoa through Ctenophora*. New York: McGraw-Hill
- Jakob W, Sagasser S, Dellaporta S, Holland P, Kuhn K, Schierwater B. 2004. The Trox-2 Hox/ParaHox gene of Trichoplax (Placozoa) marks an epithelial boundary. *Dev. Genes Evol.* 214:170–75
- Jefferies RPS. 1986. *The Ancestry of the Vertebrates*. Cambridge, UK: Br. Mus. Nat. Hist.

- Jenner RA. 1999. Metazoan phylogeny as a tool in evolutionary biology: current problems and discrepancies in application. *Belg. J. Zool.* 129:245–62
- Jenner RA. 2002. Boolean logic and character state identity: pitfalls of character coding in metazoan cladistics. *Contrib. Zool.* 71:67–91
- Jenner RA. 2004. Towards a phylogeny of the Metazoa: evaluating alternative phylogenetic positions of Platyhelminthes, Nemertea, and Gnathostomulida, with a critical reappraisal of cladistic characters. *Contrib. Zool.* 73:3–163
- Katayama T, Wada H, Furuya H, Satoh N, Yamamoto M. 1995. Phylogenetic position of the dicyemid Mesozoa inferred from 18S rDNA sequences. *Biol. Bull.* 189:81–90
- Kim J, Kim W, Cunningham CW. 1999. A new perspective on lower metazoan relationships form 18S rDNA sequences. *Mol. Biol. Evol.* 16:423–27
- Kmita-Cunisse M, Loosli F, Bierne J, Gehring WJ. 1998. Homeobox genes in the ribbonworm *Lineus sanguineus*: Evolutionary implications. *Proc. Natl. Acad. Sci. USA* 95:3030–35
- Knoll A, Carroll SB. 1999. Early animal evolution: Emerging views from comparative biology and geology. *Science* 284:2129–37
- Kobayashi M, Furuya H, Holland PWH. 1999. Dicyemids are higher animals. *Nature* 401:762
- Kojima S. 1998. Paraphyletic status of Polychaeta suggested by phylogenetic analysis based on the amino acid sequences of elongation factor-1-alpha. *Mol. Phylogenet. Evol.* 9:255–61
- Kristensen RM, Funch P. 2000. Micrognathozoa: a new class with complicated jaws like those of Rotifera and Gnathostomulida. *J. Morphol.* 246:1–49
- Lake JA. 1990. Origin of Metazoa. *Proc. Natl. Acad. Sci. USA* 87:763–66
- Lavrov DV, Brown WM, Boore JL. 2004. Phylogenetic position of the Pentastomida and (pan)crustacean relationships. *Proc. R. Soc. London Ser. B Biol. Sci.* 271:537–44
- Lemburg C. 1995. Ultrastructure of the introvert and associated structures of the larvae of *Halicryptus spinulosus* (Priapulida). *Zoomorphology* 115:11–29
- Li C-W, Chen J-Y, Hua T-E. 1998. Precambrian sponges with cellular structures. *Science* 279:879–82
- Littlewood DTJ, Olson PD, Telford MJ, Herniou EA, Riutort M. 2001. Elongation factor 1- α sequences alone do not assist in resolving the position of the Acoela within the Metazoa. *Mol. Biol. Evol.* 18:437–42
- Littlewood DTJ, Telford MJ, Clough KA, Rohde K. 1998. Gnathostomulida—an enigmatic metazoan phylum from both morphological and molecular perspectives. *Mol. Phylogenet. Evol.* 9:72–79
- Lowe CJ, Wu M, Salic A, Evans L, Lander E, et al. 2003. Anteroposterior patterning in hemichordates and the origins of the chordate nervous system. *Cell* 113:853–65
- Lynch M. 1999. The age and relationships of the major animal phyla. *Evolution* 53:319–25
- Mackey LY, Winnepenickx B, De Wachter R, Beckeljau T, Emschermann P, Garey JR. 1996. 18S rRNA suggests that Entoprocta are protostomes, unrelated to Ectoprocta. *J. Mol. Evol.* 42:552–59
- Malakhov VV. 1980. Cephalorhyncha, a new type of animal kingdom uniting Priapulida, Kinorhyncha, Gordiacea, and a system of aschelminthes worms. *Zool. Zh.* 59:485–99
- Mallatt J, Winchell CJ. 2002. Testing the new animal phylogeny: first use of combined large-subunit and small-subunit rRNA gene sequences to classify the protostomes. *Mol. Biol. Evol.* 19:289–301
- Mallatt JM, Garey JR, Shultz JW. 2004. Ecdysozoan phylogeny and Bayesian inference: first use of nearly complete 28S and 18S rRNA gene sequences to classify the arthropods and their kin. *Mol. Phylogenet. Evol.* 31:178–91
- Manuel M, Kruse M, Muller WEG, Parco YL. 2000. The comparison of β -thymosin homologues among Metazoa supports an arthropod-nematode clade. *J. Mol. Evol.* 51:378–81

- Manuel M, Le Parco Y. 2000. Homeobox gene diversification in the calcareous sponge, *Sycon raphanus*. *Mol. Phylogenet. Evol.* 17:97–107
- Martindale MQ, Finnerty JR, Henry JQ. 2002. The Radiata and the evolutionary origins of the bilaterian body plan. *Mol. Phylogenet. Evol.* 24:358–65
- Martindale MQ, Henry JQ. 1999. Intracellular fate mapping in a basal metazoan, the ctenophore *Mnemiopsis leidyi*, reveals the origins of mesoderm and the existence of indeterminate cell lineages. *Dev. Biol.* 214:243–57
- Martinelli C, Spring J. 2003. Distinct expression patterns of the two T-box homologues *Brachyury* and *Tbx2/3* in the placozoan *Trichoplax adhaerens*. *Dev. Genes Evol.* 213:492–99
- McHugh D. 1997. Molecular evidence that echiurans and pogonophorans are derived annelids. *Proc. Natl. Acad. Sci. USA* 94:8006–9
- McHugh D. 2000. Molecular phylogeny of the Annelida. *Can. J. Zool.* 78:1873–84
- Medina M, Collins AG. 2003. The role of molecules in understanding molluscan evolution. In *Molecular Systematics and Phylogeography of Mollusks*, ed. C Lydeard, DR Lindberg, pp. 14–44. Washington, DC: Smithsonian Inst.
- Medina M, Collins AG, Silberman JD, Sogin ML. 2001. Evaluating hypotheses of basal animal phylogeny using complete sequences of large and small subunit rRNA. *Proc. Natl. Acad. Sci. USA* 98:9707–12
- Metschnikoff VE. 1881. Über die systematische Stellung von *Balanoglossus*. *Zool. Anz.* 4:139–57
- Monteiro AS, Okamura B, Holland PWH. 2002. Orphan worm finds a home: *Buddenbrockia* is a Myxozoon. *Mol. Biol. Evol.* 19:968–71
- Müller MC, Westheide W. 2000. Structure of the nervous system of *Myzostoma cirriferum* (Annelida) as revealed by immunohistochemistry and cLSM analyses. *J. Morphol.* 245:87–98
- Muller P, Seipel K, Yanze N, Reber-Muller S, Streitwolf-Engel R, et al. 2003. Evolutionary aspects of developmentally regulated helix-loop-helix transcription factors in striated muscle of jellyfish. *Dev. Biol.* 255:216–29
- Nielsen C. 1985. Animal phylogeny in the light of the trochaea theory. *Biol. J. Linn. Soc.* 25:243–99
- Nielsen C. 1987. Structure and function of metazoan ciliary bands and their phylogenetic significance. *Acta Zool.* 68:205–62
- Nielsen C. 2001. *Animal Evolution: Interrelationships of the Living Phyla*. Oxford: Oxford Univ. Press. 561 pp. 2nd ed.
- Nielsen C. 2003. Proposing a solution to the Articulata-Ecdysozoa controversy. *Zool. Scr.* 32:475–82
- Nielsen C, Scharf N, Eibye-Jacobsen D. 1996. Cladistic analyses of the animal kingdom. *Biol. J. Linn. Soc.* 57:385–410
- Nikoh N, Iwabe N, Kuma K, Ohno M, Sugiyama T, et al. 1997. An estimate of divergence time of Parazoa and Eumetazoa and that of Cephalochordata and Vertebrata by aldolase and triose phosphate isomerase clocks. *J. Mol. Evol.* 45:97–106
- Norén M, Jondelius U. 1997. *Xenoturbella*'s molluscan relatives. *Nature* 390:31–32
- Papillon D, Perez Y, Fasano L, Le Parco Y, Caubit X. 2003. Hox gene survey in the chaetognath *Spadella cephaloptera*: evolutionary implications. *Dev. Genes Evol.* 213:142–48
- Passamaneck YJ, Halanych KM. 2004. Evidence from Hox genes that bryozoans are lophotrochozoans. *Evol. Dev.* 6:275–81
- Passamaneck YJ, Schander C, Halanych KM. 2004. Investigation of molluscan phylogeny using large-subunit and small-subunit nuclear rRNA sequences. *Mol. Phylogenet. Evol.* 32:25–38
- Pawlowski J, Montoya-Burgos JI, Fahrni JF, Wuest J, Zaninetti L. 1996. Origin of the Mesozoa inferred from 18S rRNA gene sequences. *Mol. Biol. Evol.* 13:1128–32
- Peterson KJ, Eernisse DJ. 2001. Animal phylogeny and the ancestry of bilaterians: inferences from morphology and 18S rDNA gene sequences. *Evol. Dev.* 3:170–205

- Philippe H, Chenuil A, Adouette A. 1994. Can the Cambrian explosion be inferred through molecular phylogeny? *Development* 1994(Suppl.):15–25
- Podar M, Haddock SH, Sogin ML, Harbison GR. 2001. A molecular phylogenetic framework for the phylum Ctenophora using 18S rRNA genes. *Mol. Phylogenet. Evol.* 21:218–30
- Prud'homme B, de Rosa R, Arendt D, Julien JF, Pajazit R, et al. 2003. Arthropod-like expression patterns of *engrailed* and *wingless* in the annelid *Platynereis dumerilii* suggest a role in segment formation. *Curr. Biol.* 13:1876–81
- Rai V, Gautam R. 1999. Evaluating evidence of ancient animals. *Science* 284:A1235
- Rasmussen B. 2002. Discoidal impressions and trace-like fossils more than 1200 million years old. *Science* 296:1112–15
- Regier JC, Shultz JW. 1997. Molecular phylogeny of the major arthropod groups indicates polyphyly of crustaceans and a new hypothesis for the origin of hexapods. *Mol. Biol. Evol.* 14:902–13
- Rota E, Martin P, Erséus C. 2001. Soil-dwelling polychaetes: enigmatic as ever? Some hints on their phylogenetic relationship as suggested by a maximum parsimony analysis of 18S rRNA gene sequences. *Contrib. Zool.* 70:127–38
- Rouse GW. 2001. A cladistic analysis of Siboglinidae Caullery, 1914 (Polychaeta, Annelida): formerly the phyla Pogonophora and Vestimentifera. *Zool. J. Linn. Soc.* 132:55–80
- Rouse GW, Fauchald K. 1997. Cladistics and polychaetes. *Zool. Scr.* 26:139–204
- Ruiz-Trillo I, Paps J, Loukota M, Ribera C, Jondelius U, et al. 2002. A phylogenetic analysis of myosin heavy chain type II sequences corroborates that Acoela and Nemertodermatida are basal bilaterians. *Proc. Natl. Acad. Sci. USA* 99:11246–51
- Ruiz-Trillo I, Riutort M, Littlewood TJ, Herniou EA, Bagnuà J. 1999. Acoel flatworms: earliest extant bilaterian metazoans, not members of platyhelminthes. *Science* 283:1919–23
- Ruppert EE, Fox RS, Barnes RD. 2004. *Invertebrate Zoology, a Functional Evolutionary Approach*. Belmont, CA: Brooks/Cole-Thomson Learn. 963 pp. 7th ed.
- Scheltema AH. 1993. Aplacophora as progenetic aculiferans and the coelomate origin of mollusks as the sister taxon of Sipuncula. *Biol. Bull.* 184:57–78
- Schlegel M, Lom J, Stechmann A, Bernhard D, Leipe D, et al. 1996. Phylogenetic analysis of complete small subunit ribosomal RNA coding region of *Myxidium lieberkuehni*: evidence that Myxozoa are Metazoa and related to the Bilateria. *Arch. Protistenkd.* 147:1–9
- Schmidt-Rhaesa A. 1996. The nervous system of *Nectonema munidae* and *Gordius aquaticus*, with implications for the ground pattern of Nematomorpha. *Zoomorphology* 116:133–42
- Schmidt-Rhaesa A, Bartolomaeus T, Lemburg C, Ehlers U, Garey JR. 1998. The position of the Arthropoda in the phylogenetic system. *J. Morphol.* 238:263–85
- Schram FR. 1991. Cladistic analysis of metazoan phyla and the placement of fossil problematica. In *The Early Evolution of Metazoa and the Significance of Problematic Taxa*, ed. AM Simonetta, S Conway Morris, pp. 35–46. New York: Cambridge Univ. Press
- Seaver EC. 2003. Segmentation: mono- or polyphyletic? *Int. J. Dev. Biol.* 47:583–95
- Seaver EC, Paulson DA, Irvin SQ, Martindale MQ. 2001. The spatial and temporal expression of Ch-en, the *engrailed* gene in the polychaete *Chaetopterus*, does not support a role in body axis segmentation. *Dev. Biol.* 236:195–209
- Seilacher A, Bose PK, Pflüger F. 1998. Triploblastic animals more than 1 billion years ago: trace fossil evidence from India. *Science* 282:80–83
- Seimiya M, Ishiguro H, Miura K, Watanabe Y, Kurosawa Y. 1994. Homeobox-containing genes in the most primitive metazoa, the sponges. *Eur. J. Biochem.* 221:219–25
- Shu D-G, Chen L, Han J, Zhang X-L. 2001a. An early Cambrian tunicate from China. *Nature* 411:472–73

- Shu D-G, Luo H-L, Morris SC, Zhang X-L, Hu S-x, et al. 1999. Lower Cambrian vertebrates from south China. *Nature* 402:42–46
- Shu D-G, Morris SC, Han J, Chen L, Zhang XL, et al. 2001b. Primitive deuterostomes from the Chengjiang Lagerstätte (Lower Cambrian, China). *Nature* 414:419–24
- Shu D-G, Morris SC, Zhang ZF, Liu JN, Han J, et al. 2003. A new species of yunnanozoan with implications for deuterostome evolution. *Science* 299:1380–84
- Siddall ME, Martin DS, Bridge D, Desser SS, Cone DK. 1995. The demise of a phylum of protists: phylogeny of Myxozoa and other parasitic Cnidaria. *J. Parasitol.* 81:961–67
- Smith AB, Peterson KJ. 2002. Dating the time of origin of major clades: molecular clocks and the fossil record. *Annu. Rev. Earth Planet. Sci.* 30:65–88
- Smothers JF, von Dohlen CD, Smith LHJ, Spall RD. 1994. Molecular evidence that the myxozoan protists are metazoans. *Science* 265:1719–21
- Sørensen MV, Funch P, Willerslev E, Hansen AJ, Olesen J. 2000. On the phylogeny of the metazoa in the light of Cycliophora and Micrognathozoa. *Zool. Anz.* 239:297–318
- Southward EC. 1988. Development of the gut and segmentation of newly settled stages of *Ridgeia* (Vestimentifera): implications for relationship between Vestimentifera and Pogonophora. *J. Mar. Biol. Assoc. UK* 68: 465–87
- Spring J, Yanze N, Josch C, Middel AM, Winninger B, Schmid V. 2002. Conservation of Brachyury, Mef2, and Snail in the myogenic lineage of jellyfish: a connection to the mesoderm of bilateria. *Dev. Biol.* 244:372–84
- Stechmann A, Schlegel M. 1999. Analysis of the complete mitochondrial DNA sequence of the brachiopod *Terebratulina retusa* places Brachiopoda within the protostomes. *Proc. R. Soc. London Ser. B* 266:2043–52
- Steiner G, Dreyer H. 2003. Molecular phylogeny of Scaphopoda (Mollusca) inferred from 18S rDNA sequences: support for a Scaphopoda-Cephalopoda clade. *Zool. Scr.* 32:343–56
- Struck TH, Westheide W, Purschke G. 2002. Progenesis in Eunicida (“Polychaeta,” Annelida)—separate evolutionary events? Evidence from molecular data. *Mol. Phylogenet. Evol.* 25:190–99
- Swalla BJ, Cameron CB, Corley LS, Garey JR. 2000. Urochordates are monophyletic within the deuterostomes. *Syst. Biol.* 49:52–64
- Telford MJ. 2004a. The multimeric β -thymosin found in nematodes and arthropods is not a synapomorphy of the Ecdysozoa. *Evol. Dev.* 6:90–94
- Telford MJ. 2004b. Animal phylogeny: back to the coelomata? *Curr Biol* 14: R274–76
- Telford MJ, Herniou EA, Russell RB, Littlewood DTJ. 2000. Changes in mitochondrial genetic codes as phylogenetic characters: two examples from the flatworms. *Proc. Natl. Acad. Sci. USA* 97:11359–64
- Telford MJ, Holland PWH. 1993. The phylogenetic affinities of the chaetognaths: a molecular analysis. *Mol. Biol. Evol.* 10:660–76
- Telford MJ, Lockyer AE, Cartwright-Finch C, Littlewood DT. 2003. Combined large and small subunit ribosomal RNA phylogenies support a basal position of the acoelomorph flatworms. *Proc. R. Soc. London Ser. B* 270: 1077–83
- Turbeville JM, Field KG, Raff RA. 1992. Phylogenetic position of phylum Nermertini, inferred from 18S rRNA sequences: molecular data as a test of morphological character homology. *Mol. Biol. Evol.* 9:235–49
- Turbeville JM, Schulz JR, Raff RA. 1994. Deuterostome phylogeny and the sister group of the chordates: evidence from molecules and morphology. *Mol. Biol. Evol.* 11:648–55
- Valentine JW. 1997. Cleavage patterns and the topology of the Metazoan tree of life. *Proc. Natl. Acad. Sci. USA* 94:8001–5
- van der Land J, Nørrevang A. 1977. The systematic position of *Lamellibrachia* (Annelida, Vestimentifera). *Z. Zool. Syst. Evol.* 1975:85–101
- Wada H, Satoh N. 1994. Details of the evolutionary history from invertebrates to vertebrates, as deduced from the sequences of 18S rDNA. *Proc. Natl. Acad. Sci. USA* 91:1801–4

- Werbrock AH, Meiklejohn DA, Sainz A, Iwasa JH, Savage RM. 2001. A polychaete hunchback ortholog. *Dev. Biol.* 235:476–88
- Westheide W. 1997. The direction of evolution within the Polychaeta. *J. Nat. Hist.* 31:1–15
- Westheide W, Rieger R. 1996. *Spezielle Zoologie. Teil 1: Einzeller und Wirbellose Tiere.* Stuttgart: Fischer
- Wheeler WC. 1990. Nucleic acid sequence phylogeny and random outgroups. *Cladistics* 6:363–68
- Willmer P. 1990. *Invertebrate Relationships, Patterns in Animal Evolution.* New York: Cambridge Univ. Press. 400 pp.
- Winchell CJ, Sullivan J, Cameron CB, Swalla BJ, Mallatt J. 2002. Evaluating hypotheses of deuterostome phylogeny and chordate evolution with new LSU and SSU ribosomal DNA data. *Mol. Biol. Evol.* 19:762–76
- Winnepenninckx B, Backeljau T, Mackey LY, Brooks JM, De Wachter R, et al. 1995. 18S rRNA data indicate that Aschelminthes are polyphyletic in origin and consist of at least three distinct clades. *Mol. Biol. Evol.* 12:1132–37
- Winnepenninckx BMH, Backeljau T, Kristensen RM. 1998. Relations of the new phylum Cycliophora. *Nature* 393:636–38
- Wolf YI, Rogozin IB, Koonin EV. 2004. Coelomata and not Ecdysozoa: evidence from genome-wide phylogenetic analysis. *Genome Res.* 14:29–36
- Wray GA, Levinton JS, Shapiro LH. 1996. Molecular evidence for deep Precambrian divergences among metazoan phyla. *Science* 274:568–73
- Xiao S, Zhang Y, Knoll AH. 1998. Three-dimensional preservation of algae and animal embryos in a Neoproterozoic phosphorite. *Nature* 391:553–58
- Zimmer RL. 1973. Morphological and developmental affinities of the lophophorates. In *Living and Fossil Bryozoa*, ed. GP Larwood, pp. 593–99. New York: Academic
- Zimmer RL. 1997. Phoronids, brachiopods, and bryozoans, the lophophorates. In *Embryology, Constructing the Organism*, ed. SF Gilbert, AM Raunio, pp. 279–305. Sunderland, MA: Sinauer
- Zrzavy J, Hypsa V, Tietz DF. 2001. Myzostomida are not annelids: molecular and morphological support for a clade of animals with anterior sperm flagella. *Cladistics* 17:170–98
- Zrzavy J, Milhulka S, Kepka P, Bezdek A, Tietz DF. 1998. Phylogeny of the Metazoa based on morphological and 18S ribosomal DNA evidence. *Cladistics* 14:249–85

NOTE ADDED IN PROOF

- Finnerty JR, Pang K, Burton P, Paulson D, Martindale MQ. 2004. Origins of bilateral symmetry; Hox and dpp expression in a sea anemone. *Science* 304:1335–37
- Helfenbein KG, Fourcade HM, Vanjani RG, Boore JL. 2004. The mitochondrial genome of *Paraspadella gotoi* is highly reduced and reveals that chaetognaths are a sister group to protostomes. *Proc. Natl. Acad. Sci. USA.* 101:10639–43
- Wikramanayake AH, Hong M, Lee PN, Pang K, Byrum CA, et al. 2003. An ancient role for nuclear beta-catenin in the evolution of axial polarity and germ layer segregation. *Nature* 426:446–50

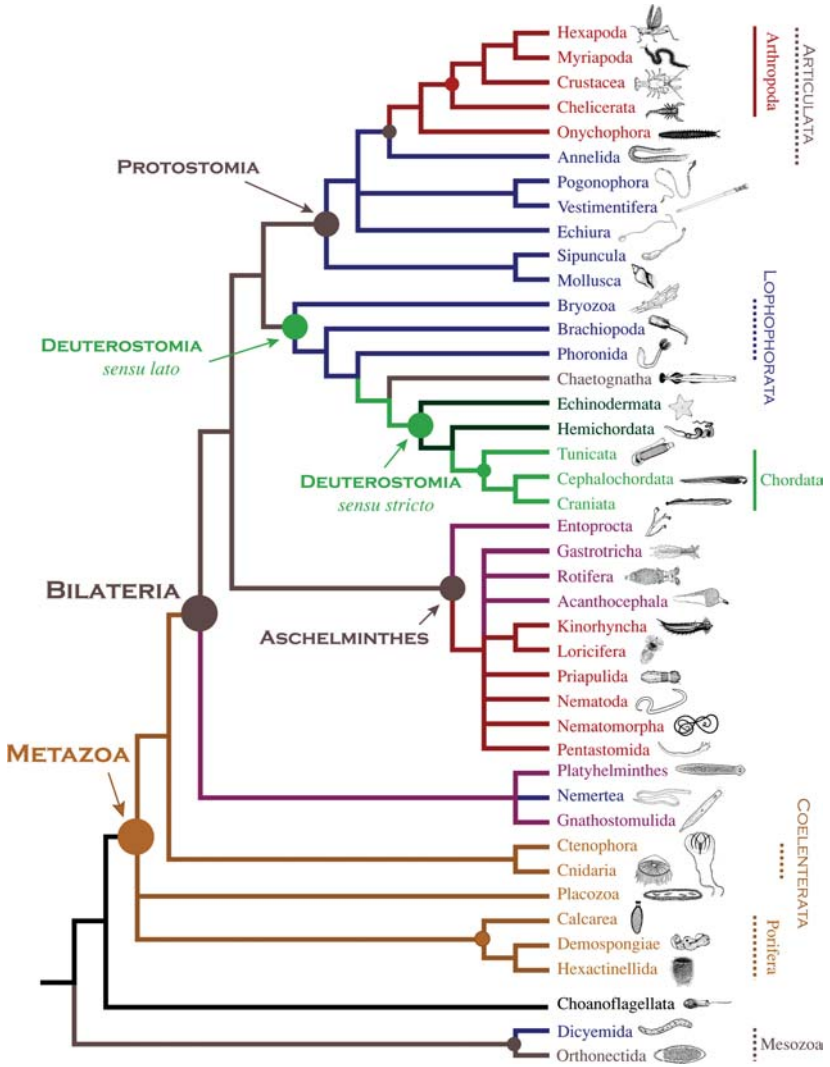


Figure 1 Traditional synthesis. The understanding of metazoan evolution prior to Field et al. (1988). This tree is drawn to illustrate major concepts. The tree is color coded to match the clades consistent with our current understanding as shown in Figure 2: Brown is Metazoa, gray is Bilateria, green is Deuterostomia, dark green is Ambulacraria, red is Ecdysozoa, blue is Lophotrochozoa, magenta is Platyzoa, and black is nonmetazoan. Researchers have proposed many variations of this general hypothesis. For example, many viewed Bryozoa, Brachiopoda, and Phoronida as forming a monophyletic Lophophorata. Also, it was widely recognized that the Aschelminthes was probably not a natural group. Note that, for illustrative purposes, not all taxa shown in Figure 2 are shown here. Filled circles correspond to labeled nodes. Dashed vertical lines indicate groups that are not monophyletic.

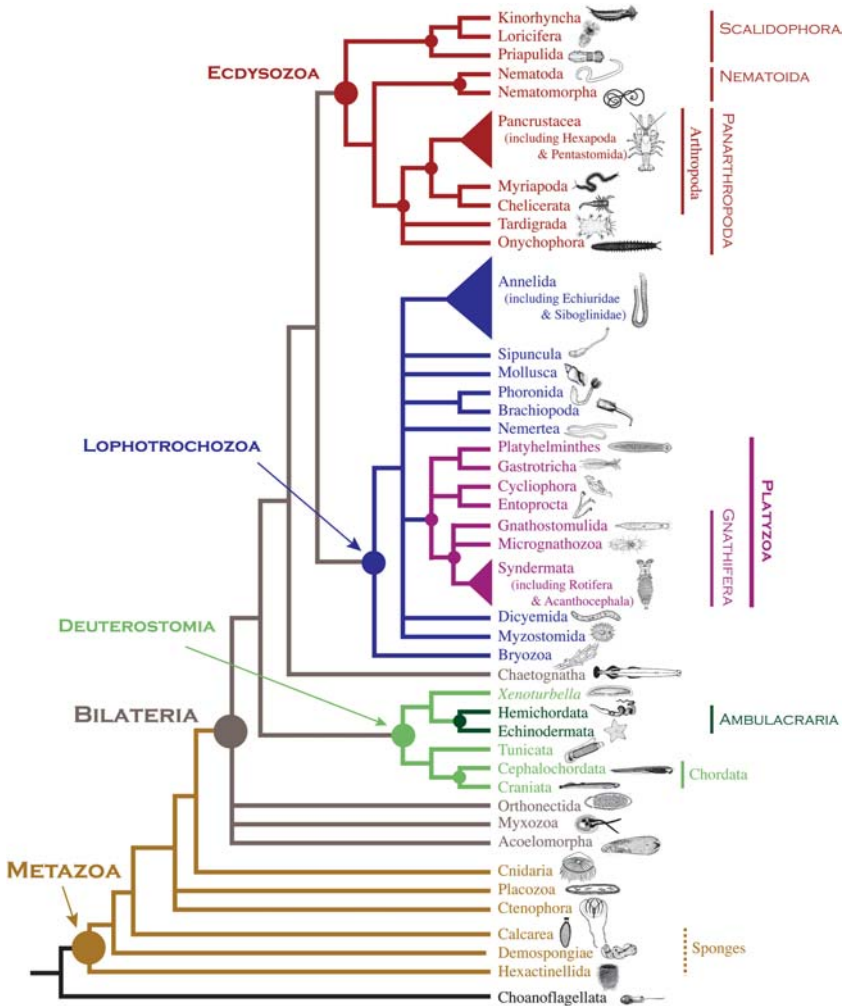


Figure 2 Modern synthesis. The new view of animal phylogeny based largely on molecular data. Details and support for various clades are discussed in the text. This figure is intended to be slightly conservative in nature, as poorly resolved issues are shown as polytomies. The tree is color coded: Brown is Metazoa, gray is Bilateria, green is Deuterostomia, dark green is Ambulacraria, red is Ecdysozoa, blue is Lophotrochozoa, magenta is Platyzoa, and black is nonmetazoan. Nested clades of one color may be inside a more inclusive clade of another color. For example, Echinodermata is dark green because it is within Ambrulacraria, but it is also a deuterostome (*green*), bilaterian (*gray*), and metazoan (*brown*). Note that Siboglinidae is the preferred name for the pogonophoran/vestimentiferan lineage, and the position of Orthonectida as a basal bilaterian needs confirmation. Filled circles correspond to labeled nodes. Dashed vertical lines indicate groups that are not monophyletic.

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