Assembling the Tree of Life

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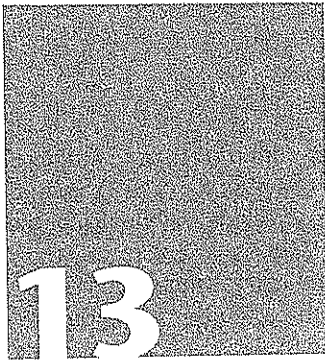
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The History of Animals

This is an exciting time for zoologists. A dramatic upsurge in interest in the interrelationships among animals has occurred across the biological subdisciplines; before the last decade, the topic of high-level animal relationships was one largely confined to zoological texts and older monographs. Revolutionary advances in the fields of phylogenetic analysis, paleontology, developmental biology, and microscopic anatomy, combined with a new wealth of relevant data such as DNA and protein sequences, have led to new insights into animal genealogy. These insights are crucial in this era of "omics": a deeper understanding of any process, including molecular processes, requires an understanding of the underlying pattern, particularly the phylogenetic topology of the systems under consideration.

One of the most significant changes to occur with our understanding of animal evolution is the recognition that animals should be arranged on a phylogenetic tree, and ancestors inferred from character states, rather than the ladder-like progression from protozoans to mammals with ancestors inferred from "archetypes." Despite this new appreciation for the necessity of phylogenetic patterns, it is important to emphasize that even if the topology were somehow precisely known, there would still be uncertainties concerning the appearance or life history attributes of many ancestral metazoan taxa, to say nothing of gene regulatory networks and molecular cascades.

What follows is our attempt to synthesize what is known about high-level (i.e., interphylum) animal relationships, including the controversies that surround some of the crucial cladogenic events. We start from the base of the animal tree and proceed to the individual subclades of bilaterian metazoans, with the latter summarized only briefly because these topics are considered in much greater detail elsewhere in this book. Controversies still remain, but it is also true that agreement among zoologists has never been greater; the basic pattern of animal evolution has largely been resolved into a few major lineages. This congruence is shown in figure 13.1. Figure 13.1A summarizes where the field is with respect to animal interrelationships. This by necessity is a very conservative tree with many polytomies, yet compared with the state of the field just 15 years ago, we have made remarkable progress, and we expect that most of these polytomies will be resolved with the wealth of data being generated. Figure 13.1B is our total-evidence tree, where we combined our morphological data matrix (modified from Peterson and Eernisse 2001) with 335 small subunit (SSU) or 18S ribosomal DNA (rDNA) sequences, and 43 myosin heavy chain type II inferred amino acid sequences (details are provided in the appendix). The common names of many of these taxa are given in table 13.1, as is the number of SSU rDNA and myosin II sequences analyzed for each taxon, and the Bremer support index for selected nodes of interest. Although our data set is able to resolve all of the polytomies, many with high Bremer support (table 13.1), these should be viewed as tentative hypotheses rather than a consensus among workers in the field. We now discuss the interrelationships of the

major animal groups; the reader should refer to figure 13.1 and table 13.1 throughout the remainder of the chapter to see the branching patterns discussed in each section and to compare the consensus nodes with those that are more equivocal.

Are Metazoans Monophyletic?

Until just recently, it seemed possible that sponges arose independently from unicellular ancestors different from those giving rise to all other animals. However, it is now clear from both morphological and molecular analyses that all multicellular animals, including sponges, are monophyletic. The morphological evidence for monophyly consists of many derived attributes that co-occur with the origin of multicellularity at the base of Metazoa ("Met" in fig. 13.1B), including the presence not only of multicellularity but also of the extracellular matrix (Morris 1993) and septate junctions (Nielsen 2001), as well as reproductive features such as eggs with polar bodies and spermatozoa. Furthermore, the molecular support extends beyond SSU rDNA (e.g., Wainright et al. 1993) to include combined SSU rDNA and large subunit (LSU, or 28S) rDNA (Medina et al. 2001), heat-shock protein HSP70 (Borchiellini et al. 1998, Snell et al. 2001), the largest subunit of RNA polymerase II (Stiller et al. 2001, Stiller and Hall 2002), and EF-2 and β-tubulin proteins (King and Carroll 2001). Because the monophyly of Metazoa is robust, multicellularity evolved just once within the animal lineage.
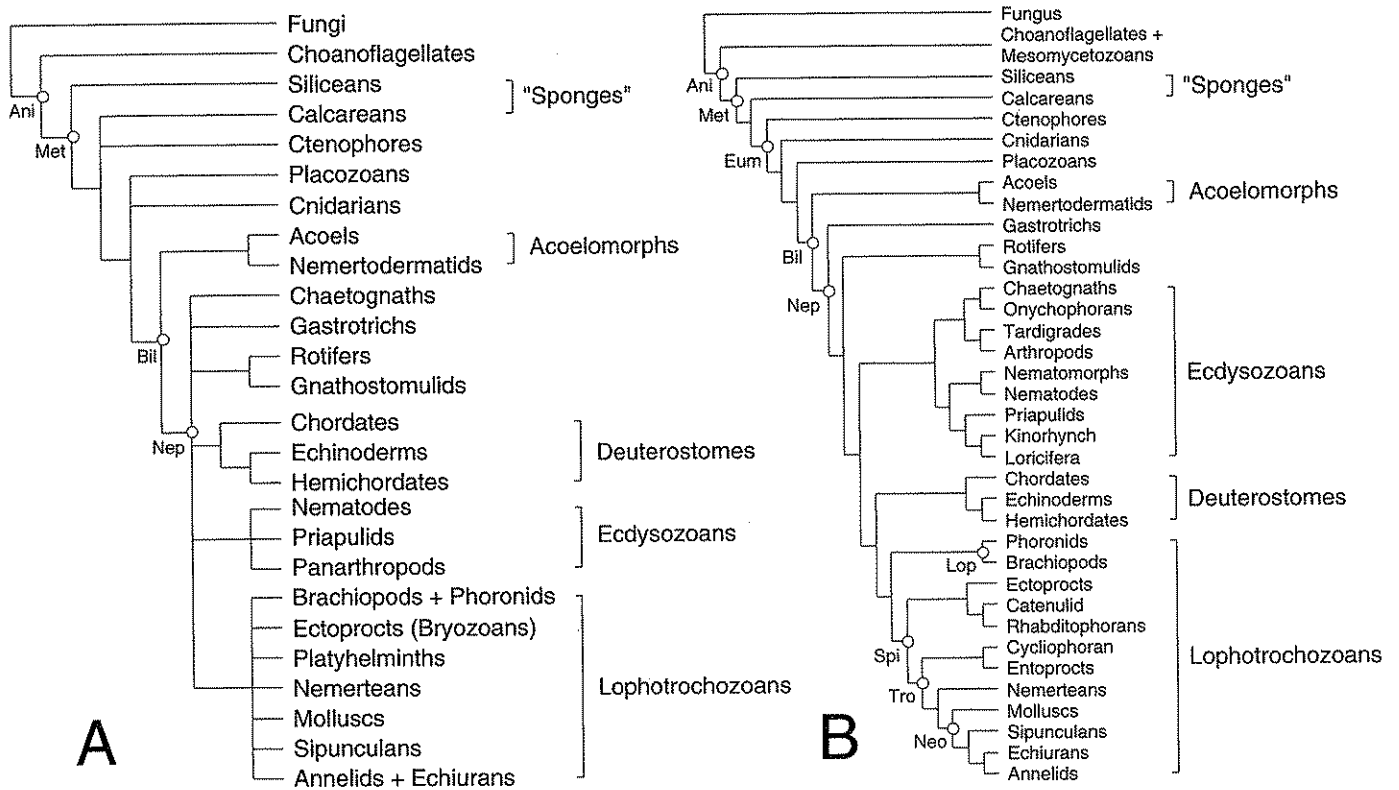
Figure 13.1. The interrelationships among major animal groups. (A) The consensus view from the literature. Although the general structure is apparent, there are several places where much controversy (and work) exists, including the base of Eumetazoa, and especially among the lophotrochozoan taxa. (B) Summary of our combined data set analysis of metazoans. This is the strict consensus summary of first 2000 most parsimonious trees (1115 parsimony-informative characters for 337 taxa, including two with only morphology data; branch length, L = 12,700). To simplify results, the resolution of some terminal taxa scored and analyzed separately are not depicted (see text for details). Bremer support indices and the number of taxa analyzed for SSU rDNA and myosin II are given in table 13.1. Some selected nodes have been labeled with a three-letter taxon abbreviation: Ani, Animalia; Bil, Bilateria; Eum, Eumetazoa; Lop, Lophophorata; Met, Metazoa; Neo = Neotrochozoa; Nep = Nephrrozoa; Spi, Spiralia; Tro, Trochozoa. Nexus format data matrices, search blocks, and full consensus tree descriptions as well as details of sequences analyzed are available from D.J.E.

Table 13.1

Bremer, Support Indices (BSI) for Terminal and Selected Higher Metazoan Taxa for Combined Analysis of Morphology, SSU rDNA, and Myosin II Data Sets.

Taxa	Common name	BSI
Terminal Taxa (No. SSU/myosin II)		
Silicea (10/0)	Siliceous sponges	2
Calcarea (4/0)	Calcareous sponges	4
Ctenophora (3)	Comb jellies	23
Cnidaria (27/3)	Cnidarians	8
Placozoa (2/0)	Trichoplax	22
Acoela (11/3)	Acoel flatworms	28
Nemertodermatida (2/1)	Nemertodermatid flatworms	37
Gastrotricha (2/0)	Gastrotrichs	12
Rotifera (6/1)	Rotifers	19
Gnathostomulida (3/0)	Gnathostomulids	13
Chaetognatha (3/0)	Arrow worms	15
Onychophora (2/0)	Velvet worms	27
Tardigrada (6/0)	Water bears	18
Arthropoda (47/9)	Arthropods	1
Nematomorpha (3/0)	Horsehair worms	14
Nematoda (17/3)	Round worms	20
Priapulida (6/1)	Priapulids	4
Kinorhyncha (1/0)	Kinorhynchs	—
Loricifera (0/0)	Loriciferans	—
Chordata (24/6)	Chordates	5
Echinodermata (6/0)	Echinoderms	12
Hemichordata (6/0)	Hemichordates	3
Phoronida (3/1)	Phoronids	9
Brachiopoda (20/1)	Brachiopods	10
Ectoprocta (2/0)	Bryozoans	4
Catenulida (1/0)	Catenulid flatworms	—
Rhabditophora (38/5)	Rhabditophoran flatworms	11
Cycliophora (1/0)	Cycliophorans	—
Entoprocta (2/0)	Entoprocts	15
Nemertea (4/1)	Ribbon worms	5
Mollusca (12/3)	Mollusks	1
Sipuncula (7/1)	Peanut worms	27
Echiura (3/1)	Spoon worms	18
Annelida (39/3)	Segmented worms	1
Selected higher taxa		
Metazoa	Multicellular animals	6
Eumetazoa	Eumetazoans	6
Bilateria	Bilaterians	36
Acoelomorpha	Acoelomorphs	1
Nephrozoa	Nephrozoans	6
Ecdysozoa	Ecdysozoans	4
Deuterostomia	Deuterostomes	6
Lophotrochozoa	Lophotrochozoans	1
Lophophorata	Brachiopods + phoronids	6
Spiralia	Spiralians	1
Trochozoa	Trochozoans	1
Neotrochozoa	Neotrochozoans	3

Although animal monophyly is firmly established, controversies still remain. One crucial issue relates to whether particular features shared by sponges and all other animals are truly derived for animals or whether they could be more primitive (i.e., found outside of Metazoa). A good example is the presence of receptor tyrosine kinases, a group of mol-ecules involved in cell–cell signaling and thought to be apomorphic for Metazoa (Suga et al. 1999). King and Carroll (2001) recently found a receptor tyrosine kinase in the choanoflagellate *Monosiga*, raising the possibility that many molecules (including those involved in such traditional multicellular activities as cell-to-cell communication and development) currently thought to exist only in animals (and known to be absent in fungi) might be present in choanoflagellates as well. This problem is not restricted to choanoflagellates: the absence of molecules that characterize higher level metazoan groups in "poriferans" is often the result of negative PCR experiments, and until we have a genome sequence from a sponge, all absences fall into the category of "absence of evidence" rather than the preferable "evidence of absence." As a point in fact, nerve cell genes such as *Pax* transcription factors have recently been isolated in sponges (Gröger et al. 2000), suggesting that they might be much more complex than usually presupposed (e.g., Müller 2001).

What Is the Sister Taxon of Metazoans?

Molecular data support the monophyly of a subclade of eukaryotes called Opisthokonta (Baldauf and Palmer 1993, Baldauf et al. 2000, Atkins et al. 2000, Zettler et al. 2001; see Loytynoja and Milinkovitch 2001), which includes metazoans, choanoflagellates, fungi, and several other poorly known unicellular eukaryotic taxa. Within Opisthokonta, metazoans and choanoflagellates appear quite closely related compared with the more distantly related fungi. The morphology of choanoflagellates has long suggested an affinity with animals, specifically sponges. The similarity between the feeding "collar" cells of sponges and those single-celled but frequently colonial choanoflagellates, first noticed more than a century ago (James-Clark 1866, 1868), is striking, and all morphological and molecular analyses conclude that this similarity is not due to convergence but instead was present in the last common ancestor of animals ("Ani" in fig. 13.1B: Animalia = Choanoflagellata + Metazoa; Nielsen 1995).

There is also another recently recognized group, the mesomycetozoans (alternatively known as ichthyosporeans), which are closely related to choanoflagellates and/or metazoans. Mesomycetozoans are parasites of various fish, birds, mammals, and snails (reviewed in Mendozoa et al. 2002; see also Hertel et al. 2002). In some analyses, Mesomycetozoa is resolved as the sister taxon of choanoflagellates, whereas in others it is the sister taxon of metazoans (Medina et al. 2001, Peterson and Eernisse 2001). King and Carroll (2001) argued that, even if mesomycetozoans comprise the sister taxon of metazoans, choanoflagellates are still the most appropriate metazoan outgroups to study because, as parasites, mesomycetozoans are more likely to have experienced general genomic simplification events. Nonetheless, it is prudent to include both choanoflagellates and mesomycetozoans as outgroups when estimating metazoan basal branching patterns. The diversity of

choanoflagellates and mesomycetozoans is still poorly known, and it is possible that additional opisthokont taxa will be discovered (Moon-van der Staay et al. 2001).

Are Sponges Monophyletic?

Porifera is usually assumed to be monophyletic, and this notion is supported by their possession of the water-canal system, a unique arrangement of canals and pores not found in other metazoans. Nonetheless, recent analyses of SSU rDNA that have included an appropriate assortment of sponges, other animals such as cnidarians, and non-metazoan outgroups have instead found sponges to be paraphyletic (e.g., Borchiellini et al. 2001, Peterson and Eernisse 2001, Medina et al. 2001). In particular, those sponges whose skeleton is composed of calcareous spicules (Calcarea) have been supported as comprising the sister taxon of Eumetazoa ("Eum" in fig. 13.1B), the clade composed of all "nonsponge" metazoans, whereas the remaining sponges with a skeleton composed of siliceous spicules (Silicea) comprise the monophyletic sister taxon of the Calcarea + Eumetazoa clade. If the recent SSU rDNA analyses are accurate, then the name "Porifera" should be abandoned and replaced by Calcarea and Silicea. The controversy has important implications. Sponge paraphyly would simplify the optimization of ancestral conditions in ancient metazoans because then the last common ancestor of eumetazoans and calcareans would be more confidently spongelike, complete with a water-canal system. This is because the most proximal outgroup to the Calcarea + Eumetazoa clade, Silicea, also has a water-canal system indistinguishable from the calcarean water-canal system. Furthermore, sponge paraphyly would suggest that the last common ancestor of all animals had a water-canal system as well, and that the acquisition of a spongelike body plan occurred during the early evolution of metazoans and was lost early in the evolution of eumetazoans. Despite the prevailing textbook view of sponge monophyly, as well as our morphology-only analysis (Peterson and Eernisse 2001), sponge paraphyly is consistent with the presence of cross-striated rootlets in calcareous sponges and eumetazoans, but not in siliceous sponges or choanoflagellates (Nielsen 2001). Even if sponges are monophyletic, the near certain monophyly of metazoans and the placement of spongelike choanoflagellates as a near outgroup together imply that our ancient ancestors were "sponges." If living sponges represent a paraphyletic grade, not a clade, of basal metazoans, then the similarities between Silicea and Calcarea reflect only what they lack: the derived traits associated with the eumetazoan body plan.

What Are the Basal Relationships within Eumetazoa?

As for Metazoa, the monophyly of Eumetazoa is strongly supported by morphological evidence. Eumetazoans have clear body symmetry (either radial or bilateral), a mouth and gut, a nervous system, and tissues with characteristic organization, including a basement membrane layer as well as gap junctions and belt desmosomes, all of which are lacking in sponges (Nielsen 2001). Eumetazoa consists of four monophyletic groups whose interrelationships are still unresolved: Cnidaria (anemones and jellies), Ctenophora (comb jellies), Placozoa (a taxon of simple two-layered animals represented by the genus Trichoplax), and Bilateria (i.e., all remaining eumetazoans, which primitively have bilateral symmetry; also referred to as the triploblasts because of their three-layered bodies).

Although cnidarians, like sponges, have been popularly represented as models for our ancient ancestors, there is a fundamental difference: unlike sponges, there is substantial molecular evidence for cnidarian monophyly (Collins 2002). This is consistent with various morphological synapomorphies (Schuchert 1993), including their unique production of nematocysts, extracellular encapsulated structures that cnidarians produce in association with their predatory feeding (Tardent 1995). Also unequivocal is the close relationship between cnidarians and bilaterians to the exclusion of the sponges.

What is equivocal is how ctenophores and placozoans fit into the eumetazoan topology. SSU rDNA studies often find that ctenophores group either with the calcareous sponges (e.g., Wainright et al. 1993, Cavalier-Smith et al. 1996, Collins 1998, Kim et al. 1999, Medina et al. 2001, Podar et al. 2001) or basal to calcareous sponges and the remaining eumetazoan taxa (e.g., Peterson and Eernisse 2001), resulting in a paraphyletic Eumetazoa. In contrast, morphological studies have strongly supported ctenophores as comprising the sister taxon of bilaterians (Nielsen et al. 1996, Zrzavý et al. 1998, Peterson and Eernisse 2001). The almost insurmountable difficulty with clade Ctenophora + Calcarea is that complex systems like the nervous system, in addition to many other characters such as tissues, must have evolved twice, once in ctenophores and once in the remaining eumetazoans (or secondarily lost in calcareous sponges), a conclusion advocated by Cavalier-Smith et al. (1996). When a combined analysis of morphology and SSU rDNA sequence data is attempted, the multiple morphological synapomorphies for Eumetazoa, as well as the few supporting Ctenophora + Bilateria, cancel out the SSU rDNA synapomorphies such that neither cnidarians nor ctenophores are robustly supported as comprising a sister taxon of bilaterians (e.g., Peterson and Eernisse 2001). In fact, our new combined analysis (fig. 13.1B) finds a topology distinct from, but influenced by, both data sets: Eumetazoa is monophyletic, but ctenophores are basal to the remaining eumetazoans. This placement is also consistent with newly emerging data on Hox and Parahox genes, which appear to support a basal eumetazoan position because ctenophores seem to lack most, if not all, of these genes (Martindale et al. 2002). As above, we emphasize that this absence might not be primary because it

is a possible secondary loss or merely absence due to methodological problems.

Placozoans are equally problematic. As discussed above, molecular results tend to suggest an affinity with either bilaterians or (more rarely) cnidarians, whereas morphologists and morphological cladistic analyses have favored a basal position among eumetazoans (Bonik et al. 1976, Grell and Ruthmann 1991, Nielsen et al. 1996, Collins 1998, Zrzavý et al. 1998, Peterson and Eernisse 2001). A position within Cnidaria, specifically within the Medusazoa (*sensu* Collins 2002; e.g., Bridge et al. 1995) is convincingly rejected by Ender and Schierwater (2003), who show that placozoans have a normal circular mitochondrial genome, not the derived linear version known exclusively from medusozoans. Contrary to morphology, analysis of SSU rDNA suggests a more apical position for placozoans, often as comprising the sister taxon of Bilateria, and the addition of morphology does not change this result (fig. 13.1B). Therefore, their simplicity might be better explained by reduction from a more complex body plan than by primitive simplicity relative to the other more complex eumetazoan taxa.

Resolving the interrelationships among eumetazoans is crucial because only by doing so will we elucidate which eumetazoan subgroup is the sister group of bilaterians. It appears that comparisons with cnidarians will remain most productive (Martindale et al. 2002) even should placozoans be found more proximal to bilaterians than are cnidarians. This is because of the similarities between cnidarians and bilaterians in developmental complexity and because the placozoan body plan is likely highly reduced.

Bilaterian Relationships

Of all the nodes found on the metazoan tree, none are more strongly supported than the monophyly of Bilateria ("Bil" in fig. 13.1B). Characters supporting the monophyly of Bilateria include (1) distinct anterior-posterior, dorsoventral, and left right axes [but see Martindale et al. (2002) for possible antecedents in cnidarians and ctenophores]; (2) mesoderm as a distinct germ layer giving rise to, for example, circular and longitudinal muscles; (3) nerves organized into distinct ganglia; (4) an expansion of the *Hox* complex to include at least seven genes; (5) the polar bodies positioned on the animal pole; and (6) the specification of one body axis during oogenesis (Peterson and Eernisse 2001). Two other characters, the presence of nephridia and a through-gut with mouth and anus, depend on the phylogenetic position of acoelomorph flatworms, as discussed below. Hence, all morphological studies find strong support for bilaterian monophyly (e.g., Nielsen et al. 1996, Zrzavý et al. 1998, Peterson and Eernisse 2001). SSU rDNA data are equally unequivocal (reviewed in Adoutte et al. 1999, 2000), as are myosin heavy-chain data (Ruiz-Trillo et al. 2002).

The traditional "textbook" approach to bilaterian phylogeny is to view the evolution of the coelom as a proxy for the evolution of bilaterians themselves. This view is traditionally ascribed to Hyman (1940; see also Hyman 1951), who in turn credits Schimkewitsch (1891). This is the familiar view that acoelomate flatworms are the most basal group; then come the "pseudocoelomates," including nematodes, priapulids, and most other "aschelminth" groups; and then finally the coelomates, including arthropods, mollusks, annelids, and chordates. Although Hyman (1940) clearly viewed this transition as a grade of increasing complexity, not always corresponding to phylogenetic pattern, she argued forcefully against the notion of acoelomate and pseudocoelomate conditions as secondarily derived. Nonetheless, the first morphological cladistic analyses based on explicit data matrices did not support the "Hyman" hypothesis of progressive acquisition of a coelomic condition. Schram (1991) found the "aschelminths" to be basal to both flatworms and coelomates, and Eernisse et al. (1992; see also for a reanalysis of the Schram data set) found nematodes grouping with the arthropods, and flatworms grouping with the spirally cleaving protostomes such as annelids and mollusks.

Nonetheless, it was not until SSU rDNA studies starting with Field et al. (1988) that a different view of bilaterian evolution began to emerge (Adoutte et al. 1999). Rather than viewing bilaterian evolution as a ladder of coelomic complexity, instead bilaterians can be divided into three major groups independent of the presence/absence of the coelom: (1) the deuterostomes, composed of echinoderms, hemichordates, and chordates; (2) the lophotrochozoans (Halanych et al. 1995), composed of lophophorates (brachiopods and phoronids), those taxa possessing a trochophore larva (e.g., annelids, mollusks), the catenulid and rhabidophoran flatworms, and many other minor groups, including rotifers, cycliophorans, and possibly gastrotrichs and gnathostomulids; and (3) the ecdysozoans (Aguinaldo et al. 1997), composed of panarthropods, nematodes, priapulids, and other minor aschelminth groups such as kinorhynchs and nematomorphs. Hence, Lophotrochozoa consists of conventional coelomate, pseudocoelomate, and acoelomate groups, and Ecdysozoa consists of "coelomate" groups such as arthropods and most of the pseudocoelomate taxa. This tripartite division removes "intermediate" taxa such that characters thought to apply only to coelomates now characterize all bilaterians (Adoutte et al. 1999). Thus, the story underlying bilaterian evolution seems to be one of an initial complexity followed by numerous simplifications within Ecdysozoa and Lophotrochozoa, as well as Deuterostomia (Takacs et al. 2002).

Although the monophyly of each of these groups is fairly well supported, the interrelationships among the three are not clear. Usually, a monophyletic Protostomia is assumed, and one character supporting this hypothesis is the presence of the UbdA signature peptide, a stretch of about 11 amino acids C-terminal of the homeodomains of the *Ubx*, *Abd-A*, *Lox-2*, and *Lox-4 Hox* genes (de Rosa et al. 1999, Saló et al. 2001). However, not a single SSU rDNA study has demonstrated any appreciable support for the monophyly of

Protostomia, nor has any other arrangement been strongly supported.

The Deuterostomes

Traditionally, deuterostomes consisted of six taxa: echinoderms, hemichordates, chordates, lophophorates, ectoprocts, and chaetognaths. However, both molecular and morphological analyses agree that lophophorates, ectoprocts, and chaetognaths are not deuterostomes. Deuterostomia *sensu stricto* consists of hemichordates and echinoderms (collectively called ambulacrarians), and the chordates, the monophyletic sister group of the ambulacrarians. For further discussion of deuterostome evolution, see Smith et al. (ch. 22 in this vol.).

The Lophotrochozoa

By far the most phylogenetically challenging group is Lophotrochozoa. Named by Halanych et al. (1995) to reflect its primary taxonomic constituents, the lophophorates (brachiopods and phoronids) and trochozoans (i.e., those protostome phyla having trochophore larva, e.g., annelids and mollusks), as well as groups such as ectoprocts that do not fit under either category, this is by far the largest group of higher level metazoan taxa, containing up to about 14 phyla. Furthermore, it is the least studied group with respect to molecular investigations, because none of its members are currently genetic model systems. In general, we can say very little about how lophotrochozoan phyla are related to one another. There are few morphological characters for resolving deep-level lophotrochozoan relationships, and there is virtually no resolution with SSU rDNA (for discussion and references, see Halanych 1998, Peterson and Eernisse 2001, Giribet 2002). Analyses of LSU (Mallat and Winchell 2002) and the myosin heavy chain (Ruiz-Trillo et al. 2002) have also failed to provide robust and biologically reasonable interrelationships among lophotrochozoans. Even the monophyly of some of the more conspicuous phyla, such as Annelida and Mollusca, is rarely recovered using molecular data.

Our best estimate of lophotrochozoan relationships divides this group into three subgroups: lophophorates [restricted in Peterson and Eernisse (2001) to brachiopods and phoronids], platyzoans (rotifers, gnathostomulids, platyhelminths, and possibly gastrotrichs; Cavalier-Smith 1998; but see Zrzavý et al. 2003 for gastotrichs), and the trochozoans (entoprocts, nemerteans, annelids, mollusks, echiurans, and sipunculans, modified from Ghiselin 1988; compare Beklemishev 1969). There is strong morphological support for the monophyly of lophophorates (e.g., Peterson and Eernisse 2001), but the monophyly of Lophophorata, as well as the monophyly of the remaining groups, is still under debate with respect to molecular data. Giribet and colleagues (Giribet et al. (2000, Giribet 2002) recovered a monophyletic Platyzoa, as did Peterson and Eernisse (2001) in their morpho-

logical analysis. With respect to trochozoans, all analyses agree that these taxa are more closely related to one another than to any platyzoan subgroup, but the interrelationships among these taxa are obscure at the moment, as is the taxonomic constituency of such taxa as Annelida (Halanych et al. 2002).

Morphology alone strongly suggests that lophophorates are basal lophotrochozoans, because they lack several important spiralian (Spiralia = Platyzoa + Trochozoa) and trochozoan characters such as spiral cleavage and a trochophore larval form, respectively (Peterson and Eernisse 2001). The difficulty is that most SSU rDNA analyses place the lophophorates within the trochozoans, often as the sister group to a mollusk or annelid subgroup, but usually with very little support. Nonetheless, this hypothesis is supported by the possession of annelid-like setae in brachiopods (Ghiselin 1989). The reason the position of the lophophorates is critical is that characters supporting the monophyly of Lophotrochozoa depend heavily on the relative position of lophophorates. If Lophophorata is nested within Trochozoa, then all of the traditional developmental characters, such as spiral cleavage and the possession of a prototroch, would constitute basal lophotrochozoan characters (with the interesting by-product of making Lophotrochozoa equivalent to Spiralia). As Giribet (2002) pointed out, Halanych et al. (1995) did not include any platyzoans in their original analysis when first diagnosing Lophotrochozoa, so the potential membership of platyzoans in Lophotrochozoa must depend on their position relative to lophophorates. If lophophorates are basal to Spiralia, then the only nonsequence characters presently supporting the monophyly of Lophotrochozoa are the possession of two *Abd-B Hox* genes, *post-1* and *post-2* (see Callaerts et al. 2002; note that this is known for only brachiopods, annelids, and mollusks), and the *Lox-5* signature peptide, a stretch of eight amino acids C-terminal of the homeodomain of the *Lox5* gene, known in platyhelminths, nemerteans, annelids, brachiopods, and mollusks (de Rosa et al. 1999, Saló et al. 2001, reviewed in Balavoine et al. 2002).

Although there are several other lophotrochozoan taxa, such as the ectoprocts, virtually nothing can be said about how they fit into the lophotrochozoan tree. One of the problems is that sequences for these taxa have been few and taxonomic sampling has been sparse. In some cases (e.g., ectoprocts), this can be easily remedied. In other cases (e.g., cycliophorans), there are relatively few extant species to sample, so multiple gene sequence comparisons are more apt to help.

The Ecdysozoa

Perhaps the most surprising result of SSU rDNA analyses was the formulation of Ecdysozoa by Aguinaldo et al. (1997). Instead of using long-branch nematode taxa like *Caenorhabditis elegans*, Aguinaldo et al. (1997) found shorter branched taxa that, when analyzed phylogenetically, grouped robustly with arthropods. This was unusual given that all previous

analyses found nematodes to be basal bilaterians, supporting the traditional notion of a basal Pseudocoelomata (e.g., Winnepenninckx et al. 1995). Since Aguinaldo et al.'s (1997) analysis, numerous SSU rDNA studies (e.g., Giribet et al. 2000, Peterson and Eernisse 2001) have found strong support for a clade consisting of panarthropods, nematodes, nematomorphs, priapulids, kinorhynchs, and loriciferans (assumed, based on morphology alone, to be closely related to kinorhynchs and priapulids). Moreover, the monophyly of Ecdysozoa is further supported by phylogenetic analyses of LSU (Mallatt and Winchell 2002) and myosin heavy chain (fig. 13.1B; Ruiz-Trillo et al. 2002). In addition, a monophyletic Ecdysozoa is recovered using morphological data (Zrzavý et al. 1998, Peterson and Eernisse 2001); ecdysozoans share similarities in their cuticle and ecdysis pathways (Schmidt-Rhaesa et al. 1998), a terminal mouth, a distinct *Abd-B* gene (Van Auken et al. 2000), an internal triplication within the [-*thymosin* gene (Manuel et al. 2000), neural expression of horseradish peroxidase (HRP) immunoreactivity (Haase et al. 2001), the absence of cannabinoid receptors (McPartland et al. 2001), and the absence of the *Parahox* gene *Xlox* (Ferrier and Holland 2001)]. They might also share similarities in their circumpharyngeal brain (Eriksson and Budd 2000). Thus, the monophyly of Ecdysozoa is recovered using a variety of data sets (fig. 13.1).

Both morphological and molecular analyses agree on the monophyly of the three main Ecdysozoan groups: (1) Scalidophora (Lemburg 1995, Schmidt-Rhaesa et al. 1998, also referred to as Cephalorhyncha by some authors), consisting of priapulids, kinorhynchs and loriciferans; (2) Nematoida (Schmidt-Rhaesa 1996), consisting of nematodes and nematomorphs; and (3) Panarthropoda (Nielsen 1995), consisting of arthropods, onychophorans, and tardigrades. However, the interrelationships among these three groups are unclear.

The Chaetognath Problem

One of the more difficult groups to place phylogenetically is Chaetognatha. Chaetognaths show an odd mix of deuterostome and aschelminth-type characters (Hyman 1959), but because preference was usually given to embryological characters, chaetognaths were traditionally one of the six major deuterostome groups. Initial studies based on cladistic arguments found grouping with either deuterostomes (e.g., Brusca and Brusca 1990) or aschelminths (Schram 1991). Initial SSU rDNA analyses (Telford and Holland 1993, Turbeville et al. 1994, Wada and Satoh 1994; see also Giribet et al. 2000) did not support a placement within Deuterostomia but could not place them with any significant support elsewhere within Bilateria. Halanych (1996) argued that they were the sister group of the nematodes and argued that this was not due to long-branch attraction. More recent analyses seemed to confirm a placement within Ecdysozoa (e.g., Peterson and Eernisse 2001). Morphological analyses alone also suggest

that chaetognaths are basal ecdysozoans (Peterson and Eernisse 2001, Zrzavý et al. 2001), sharing with Ecdysozoa proper a terminal mouth, possibly a chitinous cuticle, absence of a ciliated epidermis, absence of an apical organ, and other larval structures, and they share with nematoidans the absence of circular muscles. A basal position to Ecdysozoa *sensu stricto* is also supported by the absence of HRP immunoreactivity in the chaetognath nervous system (Haase et al. 2001).

It has recently been shown that two characters usually given for a deuterostome affinity were misunderstood in chaetognaths. First, the presence of a trimeric arrangement of the coeloms is at best questionable in chaetognaths because the septum that divides the trunk into anterior and posterior compartments is not a primary septum but a secondary division derived from coelomic cells (Kapp 2000). Second, radial cleavage does not occur in chaetognaths. Instead, they have a tetrahedral four-cell embryo whose cleavage planes are similar to those of crustacean arthropods and nematodes (Shimotori and Goto 2001), and also comparable with the Precambrian embryos described by Xiao et al. (1998). The remaining deuterostome characters, for example, mouth not derived from blastopore, may represent bilaterian plesiomorphies (Peterson and Eernisse 2001). Thus, all available evidence points to an affinity with ecdysozoans, but where they fall within this group remains speculative at best. Because chaetognaths have the most strongly guanine + cytosine–biased sequences among all animal SSU rDNA sequences sampled to date (Peterson and Eernisse 2001), it would be desirable to test this hypothesis with amino acid comparisons instead of (or in addition to) the traditional SSU rDNA or LSU analyses.

The Acoelomorph Problem

One of the more interesting results to emerge from SSU rDNA analyses is the purported basal position of acoelomorph flatworms (Ruiz-Trillo et al. 1999, Jondelius et al. 2002), a placement that could shed much light on the plesiomorphic state of the early bilaterians (e.g., Ruiz-Trillo et al. 1999, 2002, Adoutte et al. 2000, Jondelius et al. 2002). Acoelomorphs (collectively the acoel and nemertodermatid flatworms) were conventionally considered basal platyhelminths because they possess neoblasts, a unique stem cell found only in flatworms (Ax 1996, Gschwentner et al. 2001, Ramachandra et al. 2002), and morphology-alone analyses confirm a flatworm affinity (e.g., Peterson and Eernisse 2001). Because of their possession of neoblasts, a basal position within Bilateria appeared suspicious, a suspicion that seemed justified given that acoels were also very long-branched taxa (Adoutte et al. 2000, Peterson and Eernisse 2001). Peterson and Eernisse (2001) tested this hypothesis and found that acoels strongly attract random DNA sequences and, to the extent that distant outgroups such as cnidarians might be behaving effectively as random sequences, their attraction to a basal position

was considered to be potentially artifactual. In contrast, the internal branch between protostomes and deuterostomes was never attracted to random outgroups, yet that is where the root attached when acoelomorphs and selected other taxa subject to long-branch attraction were removed.

Nevertheless, Ruiz-Trillo et al. (2002) analyzed myosin heavy-chain type II sequences from a variety of bilaterians, including acoelomorphs, and similar to their SSU rDNA result, found acoelomorphs to be basal bilaterians. Consistent with these results, our total-evidence tree also finds a basal Acoelomorpha (fig. 13.1B). A basal position is only moderately less consistent with the morphological data: placing acoelomorphs basally adds only four steps to the analysis. Furthermore, Saló et al. (2001) reported that they were unable to find more than three *Hox/ParaHox* genes in the acoels *Paratomella* and *Convoluta*, and these observations are consistent with the basal bilaterian position supported for acoelomorphs based on available sequence data sets. Therefore, Jondelius et al. (2002) proposed the name Nephrozoa ("Nep" in fig. 13.1B; reflecting the evolution of nephridia) to include the last common ancestor of all bilaterians except acoelomorphs and all descendants of that last common ancestor living or extinct. Nephrozoa would also be characterized by the possession of a through-gut, complete with mouth and anus, which was most likely lost secondarily in platyhelminths (now restricted to exclude acoelomorphs).

The Biology of the Earliest Bilaterians

The implications for a basal position of Acoelomorpha (or "acoelomorph" grade) are striking. Baguñà et al. (2001) proposed that if their mode of development is primitive then it is likely that the earliest bilaterians were small, benthic, directly developing animals without a coelom, segments, a true brain, or nephridia. Of their conclusions, the proposed lack of a true brain in the earliest bilaterians might need reconsideration in light of the recently demonstrated brain primordium in the acoel *Neochildia*, as assessed by the expression of *POU* genes (Ramachandra et al. 2002). Jondelius et al. (2002) further proposed that acoelomorphs arose via progenesis from a planula-like larva. This is a very different scenario for early bilaterian evolution than that espoused, for example, by Davidson and colleagues (e.g., Davidson et al. 1995, Peterson et al. 2000), which postulated indirect development to be primitive and the earliest bilaterians to be small planktonic larval forms. It also differs from the morphology-biased prediction of Peterson and Eernisse (2001), that the last common ancestor of bilaterians (including acoelomorphs) was a large organism with deuterostome-like development (including possibly the possession of a "dipleurula-like" larva) and a tripartite arrangement of coeloms similar to modern hemichordates. However, trimery can no longer be considered primitive for Bilateria because neither phoronids (Bartolomaeus 2001) nor cha-

etognaths (Kapp 2000) are trimeric, which reduces trimery to a novel synapomorphy for Ambulacraria (see Smith et al., ch. 22 in this vol.). Furthermore, this result suggests that there is no reason to postulate that a coelom is primitive for either Bilateria or Nephrozoa (*contra* Budd and Jensen 2000).

We find it intriguing that if acoelomorphs are basal to other bilaterians, this strengthens the inference that the earliest bilaterians were small, interstitial, or meiofaunal animals. Within the remaining bilaterians, small body size is widespread, so it is at least feasible that the last common ancestor of the most familiar animals (e.g., vertebrates, insects, mollusks) was likewise small and benthic. The results (not shown) of SSU rDNA plus morphology alone still support acoelomorphs as basal bilaterians but differ from the total-evidence tree (fig. 13.1B) in that gastrotrichs, gnathostomulids, and rotifers are basal lophotrochozoans. We also found the more conventional split between protostomes (ecdysozoans + lophotrochozoas) and deuterostomes exclusive of Acoelomorpha. If this topology is further supported, then the case for a small, creeping, and direct-developing last common ancestor of not only Nephrozoa but also Protostomia is strongly supported, because the outgroup(s) (acoelomorphs) and basal lineages of at least Lophotrochozoa are small bodied. This could explain why trace fossils are absent during the earliest phase of bilaterian evolution dating from about 600 million years ago (K. J. Peterson, J. B. Lyons, K. S. Nowak, C. M. Takacs, M. J. Wargo, and M. A. McPeek, unpubl. obs.) to 555 million years ago, when traces make their first appearance in the rock record (Martin et al. 2000). The story underlying bilaterian evolution may be one of initial genetic complexity not manifested until the Cambrian explosion.

Conclusions

What continually strikes us is that, aside from a few minor controversies, disparate data sets lead to a remarkably similar topology of the major animal groups. But equally as important (and interesting) is that no single data set is entirely accurate. For example, morphology alone might be "incorrect" (albeit relatively weak) in supporting a monophyletic Porifera, a sister grouping between ctenophores and bilaterians, and placing acoelomorphs within Platyhelminthes. On the other hand, morphology, but not SSU rDNA, can potentially resolve the interrelationships among trochozoans. Along the same vein as our earlier works (e.g., Eernisse 1997, Peterson and Eernisse 2001), we continue to advocate a total-evidence approach with several different types of data derived from numerous taxa. The ever continual advancement in phylogenetic software, molecular tools, and scientific perspective can only lead to a better understanding of the interrelationships among the major animal lineages and, of course, to animal evolution itself.

Appendix: Materials and Methods

The morphology matrix is a revised version of the "morphology" analysis presented in Peterson and Eernisse (2001). Our new matrix consists of 168 characters; it is not exclusively morphological because it also includes coding of developmental or biochemical variation, as well as coding of some molecular aspects such as inferred *Hox* gene duplication events and genetic code differences. The results of this analysis are only slightly different from our previous study and largely agree with those derived from sequence data despite a general perception that molecular results differ fundamentally from what might be inferred from morphology. The modified matrix is available from either author.

We also analyzed two different molecular data sets: 43 myosin heavy-chain type II inferred amino acid sequences, and a data set of 335 selected and manually aligned SSU rDNA sequences (the full matrix is available upon request from D. J. E.). The myosin heavy-chain data set, recently assembled by Ruiz-Trillo et al. (2002), is the newest non-rDNA data set available for a broad range of metazoan taxa and is probably the most promising current alternative to the widely studied SSU rDNA data set [see Giribet (2002) for a review of the others]. In order to combine these data sets, we matched myosin heavy-chain sequences with sequences from the same or related species whose SSU rDNA sequences we analyzed, and then treated each combined sequence as a single taxon. This is similar to the method employed by Ruiz-Trillo et al. (2002) except that, whereas they limited their analysis to only those taxa represented by myosin heavy-chain sequences, we kept the nearly 300 SSU rDNA sequences not matched by particular myosin heavy-chain sequences in the combined analysis, coding the myosin heavy-chain portion for those sequences as missing data. Also unlike those authors, we also combined these molecular data with our morphology matrix. As in Peterson and Eernisse (2001), we did not attempt to code corresponding morphology scores for each of the 335 taxa whose SSU rDNA sequences we analyzed. Instead, for our morphology analysis we gave equivalent morphology scores to each of the sequenced species within each of our terminal taxa. This will create bias in the combined data set favoring the monophyly of these terminal taxa; usually this was not a problem because most of these taxa were already found to be monophyletic in the molecular analyses. The few exceptions, such as annelids and mollusks, that were monophyletic in the combined but not the SSU rDNA analysis could be monophyletic merely because of the groupwide morphology scores they were given.

Methods used for sequence alignment, exclusion of those sites with ambiguous alignment, data set combination, and two-step heuristic search strategy in PAUP* (ver. 4b10; Swofford 2002), are very similar to those employed in Peterson and Eernisse (2001; see also Eernisse and Kluge 1992, Eernisse 1997). We did not include one of the redundant rodent myosin heavy-chain sequences in the combined analysis. Our SSU rDNA data set consisted of 278 of the 302 SSU rDNA sequences analyzed in Peterson and Eernisse (2001), plus 57 additional SSU rDNA sequences beyond those analyzed previously, added to bolster previously underrepresented taxa. We also varied the taxon composition of the SSU rDNA and myosin heavy-chain sequence data sets, and analyzed a number of these different taxon combinations plus our reported 335 taxon SSU rDNA data set with different algorithms, specifically using minimum evolution heuristic searches (HKY85 and LogDet distances as implemented in PAUP*) and Bayesian inference searches using Mr. Bayes software (ver. 2.01; Huelsenbeck and Ronquist 2001). All of these results were consistent with the general pattern resulting from the reported analyses, with the most substantial differences typically involving where particular "long-branch" sequences (e.g., chaetognaths, nemertodermatids, gnathostomulids, onychophorans) happened to be resolved within Bilateria. For example, the nemertodermatid and gnathostomulid sequences were observed to group together or apart anywhere from basally within Bilateria, to within chordates, to within the panarthropods as sister group to onychophorans, and such movement was characteristic of all algorithms employed in the case of the SSU rDNA analyses.

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