# Animal evolution

## the end of the intermediate taxa?

**Contrary to general belief, there has not been a reliable, global phylogeny of animals at hand within the past few decades. Recent progress in molecular phylogeny is rapidly changing the situation and has provided trees that constitute a reference frame for discussing the still controversial evolution of body plans. These trees, once purged of their possible artefacts, have already yielded confirmation of traditional, anatomically based, phylogenies as well as several new and quite significant results. Of these, one of the most striking is the disappearance of two superphyla (acoelomates such as flatworms, pseudocoelomates such as nematodes) previously thought to represent grades of intermediate complexity between diploblasts (organisms with two germ layers) and triploblasts (organisms with three germ layers). The overall image now emerging is of a fairly simple global tree of metazoans, comprising only a small number of major branches. The topology nicely accounts for the striking conservation of developmental genes in all bilaterians and suggests a new interpretation of the 'Cambrian explosion' of animal diversity.**

There is an apparent paradox in animal phylogeny: the individual groups making up the animal kingdom, large ensembles named 'phyla', are fairly easy to recognize but the evolutionary relationships between the phyla have been very difficult to establish; the phyla were first described in the 19th century, and have been refined since, on the basis of a shared 'body plan' within each phylum. That is, all species belonging to a given phylum share a set of characters unmistakably allowing their assignment to the phylum. Straightforward examples are arthropods with a segmented body, chitin cuticle and jointed appendages, molluscs with a radula (a rigid cuticular band used for feeding), mantle (usually giving rise to the shell) and foot, echinoderms with fivefold body symmetry and vertebrates with their notochord, gill slits and vertebral column.

By contrast with the clear subdivision of animals into phyla, the kinship relationships between these phyla has been a matter of controversy for decades. The fundamental reason for this is the lack of homologous characters between phyla, preventing their grouping into successive nodes, each corresponding to a set of shared derived characters (synapomorphies, in cladistic terminology; Box 1).

In the absence of such discrete nested characters in adults, zoologists have relied until recently on other criteria inspired by the idea of recapitulation, the popular hypothesis that the successive embryological stages of organisms recapitulated their phylogenetic history. They looked for broad-ranging similarities, especially in early embryological stages. This enabled them, as we shall briefly describe, to erect some very large groups. And they organized these groups into an order reflecting what they

### BOX 1. Glossary

**Bilaterians**
The bilaterally symmetrical metazoans, including the echinoderms, which have bilaterally symmetrical larvae. It is a clade.

**Cambrian explosion**
The sudden appearance in the fossil record of a large diversity of metazoan phyla, about 550 million years ago.

**Clade or natural group**
A monophyletic taxon.

**Diploblasts**
The metazoans whose development goes through a two germ-layer stage. It is probably not a clade.

**Evolutionary radiation**
The explosive historical diversification of a clade.

**Metazoans**
The animals, as multicellular organisms, including sponges but excluding the unicellular 'protozoans'.

**Monophyletic taxon**
A group of organisms including all the descendants of its last common ancestor. Also called natural group or clade.

**Phylum**
A group of species sharing a common organization of the body.

**Superphylum**
A group of phyla sharing some morphological or genetic characters.

**Synapomorphy**
A derived character shared by the organisms of a clade and absent in other organisms, such as the feathers for birds.

**Taxon**
A group of organisms recognized at any level of the classification.

**Triploblasts**
The metazoans whose development goes through a three germ-layer stage. It is now synonymous with bilaterians.

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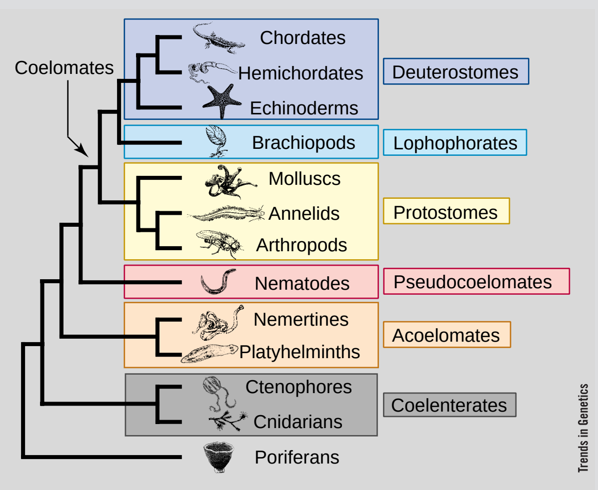
considered to be 'increasing complexity', with extant simple animals emerging at the base of the tree and more complex ones at the top (Fig. 1). In this way, the first demarcation within metazoans was placed between sponges (loose tissue organization, few differentiated cell types, absence of nerve cells), considered to be the simplest animals, and all the others. The rest of metazoans were then subdivided according to the number of germ layers. Cnidarians (jelly fishes, sea anemones, corals, hydras) apparently have only two tissue layers: an external ectoderm, giving rise principally to the epidermis and to nerve cells; and an internal endoderm, forming the digestive cavity. Cnidarians are therefore sometimes called diploblastic animals. All the other animals have a third germ layer, the mesoderm, lying between the other two and allowing the elaboration of muscles and internal organs. They are therefore called triploblastic animals. Perhaps more significant is that cnidarians are for the most part radially symmetric while all triploblastic animals are primitively bilaterally symmetric (Bilateria is a synonym of Triploblastica). The status of ctenophores (comb jellies), placozoan (a single species, *Trichoplax*) and mesozoans (small worm-like acoelomates made of very few cells), has been ambiguous, but they were most often placed together with cnidarians at the base of the metazoan tree.

Within bilaterians, the next big subdivision was between acoelomates and coelomates. Starting from that point, much weight was placed on the presence or absence of the coelom and its mode of formation (especially in the influential work of Libbie Hyman[1]). In acoelomates, basically comprising the flatworms (and often the nemertines), the internal cavity lying between the ectoderm and the endoderm is entirely filled with loosely arranged mesodermal cells. In coelomates, the mesoderm is 'hollow', containing a fluid-filled compartment of variable size, lined with a mesodermal epithelium, known as the coelom. True coelomates were further subdivided into two large clades, the protostomes and deuterostomes, first according to the way the mouth is generated during embryogenesis but also according to a congruent set of other characteristics. Many protostomes have spiral egg cleavage while deuterostomes display radial cleavage. The coelom is often formed by the hollowing of a mesodermal mass in protostomes (schizocoely), whereas it pouches out from the primitive gut in deuterostomes (enterocoely). The nerve cords are typically ventral in protostomes while there is a dorsal neural tube in many deuterostomes. Protostomes contain arthropods, annelids, molluscs and a host of more obscure 'worm-like' phyla (sipunculans, pogonophorans, tardigrades, onychophorans). Deuterostomes contain echinoderms, hemichordates (pterobranchs and enteropneusts such as *Balanoglossus*), and the chordates. The latter are subdivided into urochordates (ascidians), cephalochordates (amphioxus) and vertebrates. Things were slightly more complicated for a group of small bilaterians, apparently devoid of a true coelom but nevertheless displaying some internal cavities often derived from the embryonic blastocoel. These organisms, collectively known as pseudocoelomates (or aschelminths), comprise the nematodes, rotifers, nematomorphs, priapulids and a few other small groups. The prevailing view was to place them somewhere between the acoelomates and the true coelomates, usually as a single super-phylum.

The overall tree that could be found in most influential textbooks[1,2] was organized as in Fig. 1, from sponges at the bottom to two terminal branches at the top, one corresponding to arthropods, the other to vertebrates. In between, there were several lateral branches emerging successively, corresponding to grades of increasing complexity. Initial authors had often been more careful than this simplification might suggest. But animal evolution was too easy to recount as a glorious saga of progressive increase in complexity that generations of zoology students could not understand it differently. More recent cladistic treatment of animal phylogeny has moderated this bias but has not completely eliminated it[3,4]. Molecular phylogeny has recently incited a vigorous reevaluation of this scenario.

**FIGURE 1. A traditional phylogenic tree**



An example of 'traditional' metazoan phylogeny illustrating the principle of a gradual increase in the complexity of the body plan, still represented in modern organisms. The topology is mainly based on the reasoning of Hyman[1], although similar topologies, with some minor disagreements, can be found in more modern publications[30,31]. The grouping of the lophophorates with the deuterostomes is also retained in several recent zoology textbooks[4].

## A short history of metazoan molecular phylogeny

Metazoan molecular phylogeny is a relatively recent exercise whose beginnings can be dated rather precisely with the paper by Field *et al.*[5] in 1988. There, the first broad phylogeny of animals, based on partial 18S rRNA sequences of 20 taxa belonging to 10 phyla, was proposed. This was an era of naive optimism and great expectations.

The first molecular trees displayed expected and surprising results. Diploblastic organisms were deeply split from triploblastic ones, to the point that the monophyly of metazoans was not recovered [as was also the case using 28S rRNA (Ref. 6)]. This was rectified in later work using longer sequences, more taxa and, above all, when the long-branch attraction artefact was better taken into account (see below). Coelomates appeared to be monophyletic and, as expected, had the acoelomate platyhelminths as their sister group. Finally, four clades were recognized among coelomates: arthropods, spiralian coelomate protostomes, echinoderms and chordates. The order of emergence of these clades was nevertheless difficult to resolve, suggesting that the 'bushes' of phyla might be a reflection of the Cambrian explosion. From the start, the deuterostome branch appeared to be fairly solid and some resolution was observed within it, confirming long-established views. For example, echinoderms were consistently found to emerge as the sister group to chordates, a remarkable confirmation of traditional insight when one realizes that this grouping was based on very few developmental characters. It is within the large protostome assemblage that the situation was confused and controversial.

With the expansion of the rRNA database, trees containing an increasing number of taxa and phyla could be built and, while a large number of expected groupings emerged, two problems arose: first, different methods of tree construction or different sampling of analyzed species did not yield identical topologies; second, some well-established monophyletic groups, such as annelids or molluscs, were not recovered as monophyletic in the molecular trees, a conclusion that no morphologist would accept. These two worrisome results appear to result from very different causes, some clearly artifactual, and others related to intrinsic difficulties of phylogenetic reconstruction. Among the most devastating artifacts is the long-branch attraction, a phenomenon that had been known about for a long time[7] but whose pervasiveness has only been appreciated recently. This is the phenomenon whereby species that have higher mutation rates in the gene under study than the other species analyzed will tend to be displaced to a wrong position (because they appear as being very distant from all others) and to group together often at the base with the outgroup (because of chance convergence at some nucleotide positions). This artifact can be alleviated to some extent by first checking the rates of evolution of the various taxa under analysis through the relative rate test[8] and eliminating the ones displaying anomalously rapid rates. As for the lack of resolution at some nodes in trees, we have argued in detail previously that it could reflect the occurrence of an evolutionary radiation[9]. However, this particular issue is still under debate: it has also been proposed that mutational saturation and unequal rates of evolution along the 18S rRNA molecule could cause this lack of resolution[10].

In spite of these difficulties, several important new findings were made. First, sponges were firmly united with metazoans[11], a result also supported by numerous protein data[12]. Second, and contrary to a two-century old tradition[13], the 'Articulata' (i.e. the clade of segmented organisms grouping annelids and arthropods) exploded, annelids becoming instead solidly united with molluscs and several other protostome phyla that share spiral cleavage of their egg and a trochophore larva. It is noteworthy that recent cladistic reanalysis of the relationships within protostomes using morphological characters has confirmed the reality of this vast group of spiralians, all sharing a trochophore larva, to which the name Eutrochozoa was attributed[14]. Interestingly, during that same period, nemertines, which were thought to belong to acoelomates, were raised within Eutrochozoa[15], in accord with morphological reanalysis suggesting that their rhynchocoel is indeed a coelom[16]. Third, the lophophorates found a home in an unexpected neighbourhood. Lophophorates comprise three phyla, brachiopods, bryozoans and phoronids, that share a lophophore (i.e. a set of tentacles surrounding the mouth, used for feeding). They had been difficult to classify either within protostomes or deuterostomes but were generally thought to belong to the latter (especially brachiopods) because of the mode of cleavage of their egg, the formation of their mouth and the tripartite organization of their coelom. Now, rRNA clearly affiliated them to the protostomes[17] and, more precisely, to the broad group just mentioned which contains annelids and molluscs. Thus, an even larger monophyletic group emerged to which the name Lophotrochozoa was given because it united organisms displaying a lophophore and those going through a trochophore larva[17].

Thus, the general tree of coelomate metazoans started to take shape and to simplify with a monophyletic group of deuterostomes on the one hand and monophyletic protostomes on the other. The protostomes were further subdivided into lophotrochozoans and arthropods, in a broad sense. Finally, the aschelminths or pseudocoelomates also exploded. This group had always been problematic to zoologists, who could not find clear characters uniting them to one or the other of the metazoan supergroups and whose apparent 'simplicity' was difficult to interpret in terms of primitive characters. Several papers showed that pseudocoelomates were not, in fact, a natural group, and that they split into several independent clades, some joining the newly born Lophotrochozoa and others leaning more towards arthropods[18,19]. Throughout that period, the nematodes, whose well known representative, *Caenorhabditis elegans*, is a key organism for developmental biology as well as genomic studies, were very difficult to place. Because all the representatives available of this phylum displayed 'long branches', it was difficult to be confident in their usually basal position in trees. These studies culminated in the article by Aguinaldo *et al.*[19] who found that nematodes rose much higher when a species with a slow evolving rRNA was finally found. In addition, this nematode emerged within the arthropods. This article confirmed also some earlier trees showing some 'pseudocoelomate' phyla (nematomorphs, priapulids) emerging with the arthropods[18]. Aguinaldo *et al.* noted that this new clade of metazoans comprises taxa that all undergo molting of their cuticle and so they named it 'Ecdysozoa'. Similarly, a slowly evolving platyhelminth also rose in the tree to a position with the lophotrochozoans. These results have been confirmed in two independent papers presenting very broad metazoan rRNA phylogenies[20,21]. Shortly before, Balavoine[20] obtained two other results that fitted remarkably the new position of flatworms: the flatworms contain an almost full set of *Hox* genes. This surprising complexity of the cluster in an unsegmented, 'simple' looking organism provided a first hint that it might have been wrongly positioned as a primitive bilaterian. In addition, and even more significantly, careful analysis of amino acid and peptide signatures within and close to the homeodomain showed several similarities with protostome genes and, more specifically, to lophotrochozoan ones. These combined results appear to signal the demise of acoelomates and pseudocoelomates as early grades of metazoans, pre-dating the protostome/deuterostome split and suggest instead that they might correspond to secondarily simplified organisms[22,23]. It should be stressed that, in spite of these modifications in the trees, the resolution within the two large protostome clades did not improve: in particular, neither annelids nor molluscs appeared as monophyletic groups within lophotrochozoans, while branching orders remained basically unresolved within Ecdysozoa[19].

In summary, the 18S rRNA phylogeny appears to support the existence of three fundamental clades in the bilaterians. Within Bilateria, the protostome/deuterostome and, within protostomes, the Lophotrochozoa–Ecdysozoa splits are consistently recovered, although not with very high statistical confidence. Thus, while the reality of each of the three clades appears to be reliable, their definitive interrelationships must await further studies. On the other hand, the intra-Lophotrochozoa and intra-Ecdysozoa phylogeny remains quite uncertain at the moment and might turn out to be very difficult to solve if the diversification within each of these clades indeed corresponds to a radiation. A topology that can be taken as the consensus of all the presently available information is shown in Fig. 2 (compare with Fig. 1).

As for the position of the Bilateria in the early diversification of metazoans, all markers used so far indicate a large distance between them and the other metazoans (sponges, cnidarians, ctenophores). The depth of the split might be exaggerated, however, in the rRNA tree because of the long branch uniting all bilateria, by contrast with the short branches of all the diploblasts. This long branch, in turn, makes it difficult to establish which of the diploblast groups is closest to the triploblasts.

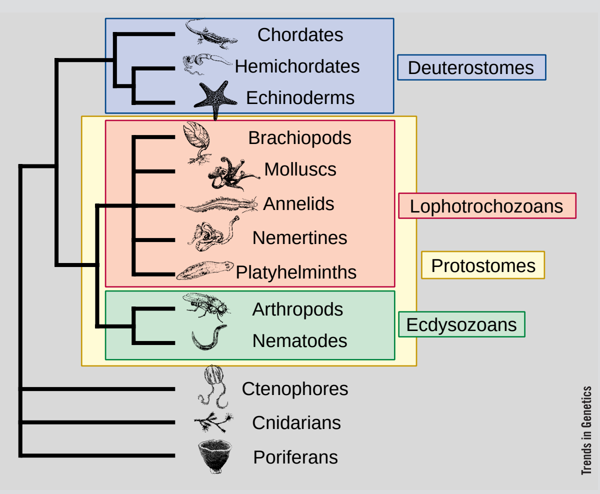
Is the rRNA data confirmed by other types of evidence? Here, we will not discuss in detail congruence between rRNA and morphology, except for pointing out that gross discrepancies have eroded with better molecular trees, as can be seen by comparing Figs 1 and 2. Conversely, the new and sometimes surprising placement of some phyla can be readily accounted for by reasonable assumptions of morphological evolution. Testing the rRNA trees with other genes is only just beginning because it is hard to find genes that can be analyzed over such an evolutionary span and because it requires a substantial effort to build databases as comprehensive as the rRNA database. At the moment, therefore, there are very few, if any, comprehensive phylogenies of metazoans achieved with the analysis of genes other than rRNA genes.

Another approach is to test some broad aspects of the topology using qualitative genetic signatures. In this respect, one type of data that has proven highly valuable, at least within some metazoan clades, is from the qualitative arrangement of mitochondrial genes, as exemplified by the work of Boore *et al.*[24] The analysis of the number and type of *Hox* genes, as mentioned above[20], also appears to provide a very powerful qualitative tool.

## Developmental and paleontological implications

The new phylogeny has several profound implications for understanding animal evolution. First, it suggests that the hope of finding extant 'intermediates' in the bilaterian lineage could well be doomed. We should consider the possibility that the tree might lead from diploblastic organisms to the coelomate bilaterians without any of the long-cherished intermediate phyla. This makes it more difficult to picture the origin of the bilaterian body plan but, at the same time, accounts much better for a number of remarkable genetic similarities uncovered between all bilaterians. Who would have thought that, for example, apparently simple, unsegmented 'worms', such as platyhelminths[23] or nemertines[25], would also turn out to possess what appears to be a large complement of the *Hox* cluster genes? These findings, somewhat difficult to interpret in the old scheme, are now easily understood: because these apparently simple phyla all emerge after the protostome/deuterostome split, they must have shared, at least originally, the same gene complement as is found in more 'complex' representatives of these two lineages, such as arthropods and vertebrates. In that case, the small number of *Hox* genes found in nematodes is also interpreted quite differently from what has often been assumed so far: instead of being the testimony of an intermediate stage in complexity of the *Hox* cluster, it would represent a degenerate state. This might be related to the particular mode of development of nematodes and to their patterning processes. It will be of great interest to test, in this respect, a number of other pseudocoelomate phyla.

**FIGURE 2. A phylogenic tree based on 18S rRNA**



A consensus of 18S rRNA phylogenies produced by several authors[17–20]. Note the absence of resolution at the base of the tree, contrasting with the certainties of the 'traditional' tree, the monophyly of the protostomes and their subdivision in two great branches: the lophotrochozoans and the ecdysozoans. Unresolved phylogeny is represented by a 'comb' topology within the lophotrochozoan ensemble.

Second, the topology of the tree suggests a new interpretation of the famous Cambrian explosion that we have described elsewhere[22]. Briefly, instead of a single radiation, the topology suggests that at least three independent events (corresponding to the diversification of the lophotrochozoans, ecdysozoans and deuterostomes) have occurred in a poorly diversified stem lineage. It follows that animal diversification cannot be due to a single 'genetic revolution' but, instead, must have been triggered primarily by external factors acting on a 'pre-adapted' (meaning possessing many features prone to further specialization or functional co-option), already genetically complex metazoan. How long before the Cambrian the initial protostome/deuterostome split occurred is still a matter of harsh debate[26,27], but even a conservative estimate[27] suggests a long precambrian history of these two branches.

Third, by separating the annelids from the arthropods and grouping them into a broad clade with other, mostly spiralian, organisms, the phylogeny indicates that protostome evolution has not been a linear progression, as was often envisaged, from an unsegmented worm, through annelids, to insects. Instead, one has to deal with a major split between two very large groups. This revives the interest in the taxa contained in the lophotrochozoans, such as annelids and molluscs, because they become the sister group of the well-studied ecdysozoans (containing arthropods and nematodes). In particular, because there are segmented phyla both in lophotrochozoans and ecdysozoans, one can ponder as to whether the common ancestor to both lineages was itself segmented (and segmentation was later lost in many of the phyla belonging to the two groups) or whether it was invented twice, independently (or more) in each of them. In the first case, this would make segmentation much older than commonly assumed, bringing it back to the base of all the protostomes. This, in turn, raises the question of the attributes of the last common ancestor of the bilaterians, that is, the Urbilateria. Because many deuterostomes are also segmented, this suggests that the Urbilateria might have been segmented itself, although such an opinion might be considered heretical.

Indeed, the genetic homologies between protostomes and deuterostomes have now been extended much beyond the *Hox* cluster. There are two extreme interpretations of such similarities: either these genes were already involved in carrying out that same function in the Urbilateria, which implies an already quite elaborate ancestor[28], or they were independently recruited in protostomes and deuterostomes from an ancestor that could have been a much simpler animal[29], using them for other related purposes. The new metazoan phylogeny will provide the necessary framework to help us draw this picture of the Urbilateria.

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