

The first bilaterian organisms: simple or complex? New molecular evidence

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ABSTRACT The quest for the first bilaterian organisms is the biggest riddle in metazoan evolution and in understanding the evolution of developmental mechanisms. Recent molecular work has regrouped the bilaterian phyla into three superphyletic clades: the Deuterostomia, the Lophotrochozoa and the Ecdysozoa. In these trees, Platyhelminthes, for a long time considered basal bilaterians, have a more derived position among the Spiralia. However, a recent 18S rDNA analysis showed Platyhelminthes to be polyphyletic with one of its orders, the Acoela, as the earliest extant bilaterian. To corroborate such position, we have sequenced new 18S and other nuclear genes, two mitochondrial genes, and examined the number and type of Hox cluster genes in acoels, nemertodermatids and other platyhelminthes and metazoans. Results confirm acoels and nemertodermatids as the earliest extant bilaterians. These results imply that the last common bilaterian ancestor was a small, benthic, direct developer without segments, coelomic cavities, nephridia or a true brain. In addition this argues for an extended pre-Cambrian period within which different simple bilaterian stem lineages emerged from which more complex ones diversified during the Cambrian explosion.

Over 150 years, morphologists and embryologists have proposed different hypothesis on the nature of the first bilaterian organism. Such hypotheses hinge into two basic forms. The first, the planuloid-acoeloid theory, posits a small and structurally simple acoel-like ancestor, similar in its organization grade to today's planulae larvae of cnidarians, from which the rest of bilaterian phyla evolved by stages of increased size and complexity. In this view, today's simple unsegmented acoelomate and pseudocoelomate organisms would be basal bilaterians, whereas coelomate segmented bilaterians should be derived. The alternative hypothesis, suggests instead a rather large and complex organism as the ancestral bilaterian (the so-called 'Urbilateria', Kimmel 1996) bearing a mouth and anus, coelom, segments, a primitive heart and, very likely, some sort of appendages (De Robertis, 1997). Under such conception, acoelomate/pseudocoelomate unsegmented organisms would be structurally simplified organisms derived from coelomate bilaterians. A recently proposed third scenario suggest a small ciliated primary larva with a population of set-aside cells as the ancestral bilaterian (reviewed in Petterson *et al.*, 2000). This hypothesis hinges on the assumption that 'maximal indirect development' is ancestral for bilaterians, direct development being derived. However, several difficulties turn untenable this hypothesis.

In the last 10 years and thanks largely to molecular systematic studies based on 18S rDNA sequencing and Hox cluster synapomorphies, the metazoan phylogenetic tree has been reorganized into three main clades of bilaterian organisms: Deuterostomia, Ecdysozoa and Lophotrochozoa (Aguinaldo *et al.*, 1997). The main casualties of this process have been the acoelomate and pseudocoelomate organisms, once considered intermediate forms between diploblasts and higher bilaterians (coelomates), and now displaced to much higher positions inside the tree (Adoutte *et al.*, 1999). This new scenario backs the 'Urbilateria' hypothesis featuring a large and complex bilaterian ancestor. However, the branching order between these three clades and within each of them is still unresolved leaving, at the very least, the position of acoelomates and pseudocoelomates within them still unsettled. This new status quo was recently upset by an 18S rDNA based sequence work showing that platyhelminth Acoela is the most basal extant bilaterian lineage distinct from the other platyhelminths (Ruiz-Trillo *et al.*, 1999). These results rendered the Platyhelminthes polyphyletic. More importantly, they implied that the last common bilaterian ancestor was small, benthic, without segments and coelomic cavities, and having direct development. This invalidated the Urbilateria model and resurrected the idea of an extant evolutionary intermediate of 'simple' design as postulated by the planuloid-acoeloid theory.

To further test the position of acoels as basal bilaterians we have undertaken a multigenic approach together with a search for non-homoplasious molecular synapomorphies. In particular we have obtained sequences of 18S rDNA and the mitochondrial genes Cox1 and Cytb from new acoels and from four nemertodermatids, a group of basal platyhelminths close to acoels. In addition, nuclear genes other than 18S (e.g. TPI, triose phosphate isomerase; and MHC, myosin heavy chain), and two mitochondrial genes (Cox1 and Cytb) have also been sequenced from a large set of metazoans. Finally, the order of genes in the mitochondrial genomes of acoels, nemertodermatids and other platyhelminthes as well as the number and type of Hox cluster genes in acoels are at present under study. Data were analysed with parsimony and maximum likelihood methods.

Main results can be summarized as follows: 1) sequences of 18S rDNA from nemertodermatids mapped onto the rDNA general tree of metazoans branch basal to the rest of bilaterians, second only to acoels (Fig. 1); 2) whereas TPI was found to be uninformative, metazoan trees based on the MHC gene se-

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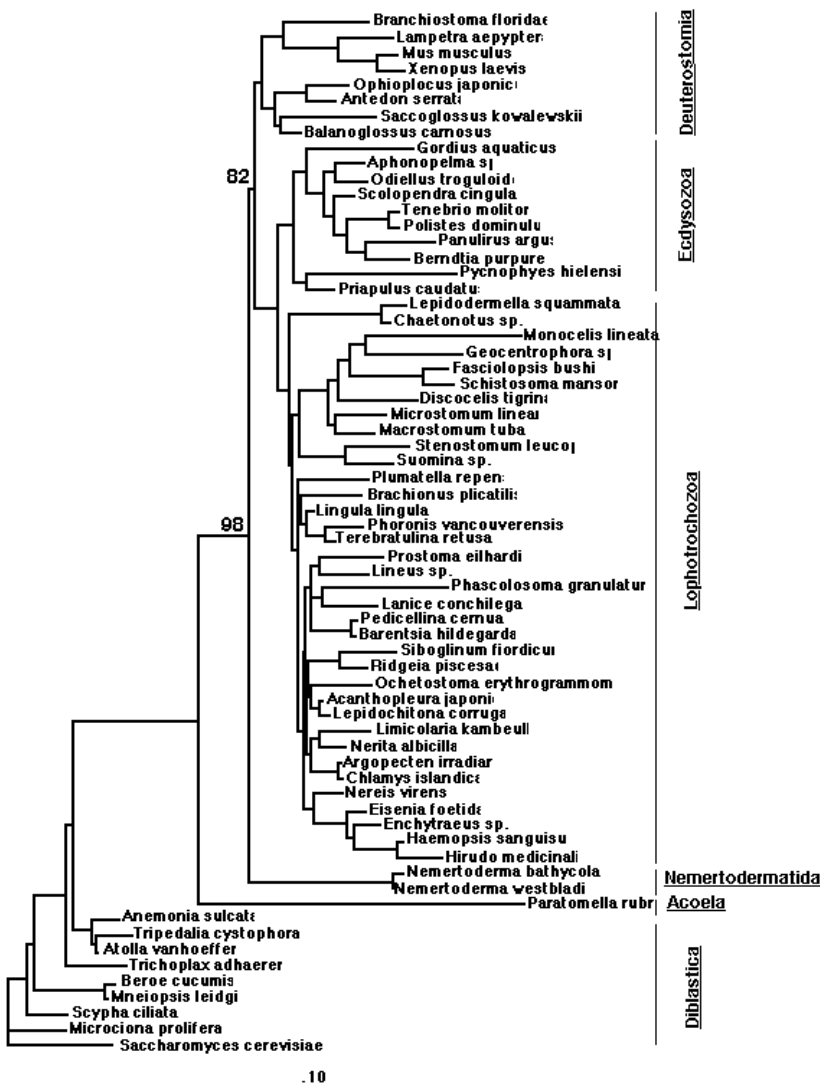


Fig. 1. Maximum Likelihood tree of 18S rDNA sequences from 66 metazoan taxa. Branch support for the Acoela and Nemertodermatida are indicated at the corresponding nodes. The tree illustrates the position of both clades as sister groups to the rest of the Bilateria. The rest of the Platyhelminthes branch within the Lophotrochozoa.

quences resolves into the three bilaterian clades, with acoels branching at the base of the bilaterians; 3) concatenated analyses of 18S rDNA+ two mitochondrial genes show acoels and nemertodermatids to branch separately at the base of the bilaterians; and 4) despite an extensive search carried out in several labs, the number of Hox and ParaHox genes detected in acoels is so far consistently limited to three: a *labial*-like, an *Antennapedia*-like and a *Caudal*-like (Saló et al., 2001). Unfortunately, attempts to detect

lophotrochozoan synapomorphies in the *Antp*-like gene of acoels (e.g. the UbdA peptide and the spiralian peptide; Telford, 2000) have so far been unsuccessful. The presently limited number of Hox genes in acoels, if it holds, may also be indicative of its primitiveness.

If acoels and nemertodermatids are basal bilaterians this argues for an extended pre-Cambrian period within which a few different and simple bilaterian lineages emerged from which more complex ones diversified during the Cambrian explosion (Knoll and Carroll, 1999). Moreover, were acoels and nemertodermatids basal bilaterians, the rest of bilaterians should bear some synapomorphies for which acoels and nemertodermatids must have the plesiomorphic condition in common with an outgroup (e.g. cnidarians). Two of these synapomorphies could be the presence of a true brain and protonephridia. Finally, acoels and nemertodermatids will be instrumental to study how the synapomorphies defining all bilaterians (e.g. bilateral symmetry with two orthogonal body axes compared to radial/biradial symmetry and a single body axis in diploblasts, presence of mesoderm or endomesoderm, and clustering of nerve cells into a sort of primitive brain) came about at the diploblast-triploblast transition.

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