

DISPATCH

ZOOLOGY: **Worming into the Origin of Bilaterians**

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Xenacoelomorphs, a group of worms with simple body organization, have been proposed to represent the first offshoot of bilaterians. A new study shows that they might instead belong to the deuterostomes, just as echinoderms and vertebrates.

The nature of the last common ancestor of all bilaterally symmetrical animals — the urbilaterian — is one of the key questions in zoology, partly because it reflects on the origin of the key organ systems that make us who we are. There are two opposing views of what this ancestor may have been like — either it was a rather simple organism or a fairly complex one. And, a lot of the debate surrounding this dichotomy revolves around the phylogenetic position of a handful of worms — the xenacoelomorphs. Two alternative positions were successively proposed over the years: one suggests that this group of morphologically simple worms is the sister-group to all other bilaterians. This branching would mean that the bilaterian ancestor was a rather unsophisticated organism. Alternatively, other researchers have proposed that these xenacoelomorphs instead are a derived offshoot of deuterostomes, one of the two main clades of bilaterians, which mean that their simplicity derives from secondary character loss. A new study by Hervé Philippe, Max Telford and colleagues [1] in *Current Biology* brings new elements to support this latter tree, in particular a thorough evaluation of methodological bias that can affect phylogenetics reconstruction — such as the famous long-branch attraction. They conclude that xenacoelomorphs are the sister-group of a clade called ‘Ambulacraria’, which includes sea stars, sea urchins and acorn worms.

The idea that the urbilaterian was a simple organism without a body cavity (coelom) reminiscent of the planula larva of some cnidarians was mentioned a long time ago for instance in the famous zoology textbook of Libbie Hyman [2]. At this time, morphologically simple animals, such as nematode roundworms and flatworms, were considered as early branches of the animal tree of life. Morphological cladistics and molecular phylogeny then helped redefine our understanding of animal evolution by splitting all bilaterian animals into

two main clades: the protostomes (including insects, molluscs, annelids, flatworms) and the deuterostomes (including vertebrates, tunicates, sea urchins and sea stars). This reclassification implied that seemingly simple lineages may have originated through simplification and secondary character losses from a more complex coelom-bearing urbilaterian ancestor [3].

Then, in the late nineties, a study [4] pointed out that a neglected lineage of flatworms — the acoels — might have represented a sister-group to both protostomes and deuterostomes. This reignited the debate concerning the nature of the bilaterian ancestor and propelled these overlooked animals to the front stage of animal evolution. Acoel flatworms indeed show a very simple planula-like organization and do not possess structures that are found in other bilaterians, such as a coelomic cavity, excretory organs, or nerve chords (Figure 1A). All bilaterians that are not acoels were dubbed ‘Nephrozoa’ (highlighting the presence of an excretory system) (Figure 1B). Acoels thus became attractive models for comparative developmental biology [4] or regeneration studies [5].

Over the years, acoels were joined as the possible earliest bilaterian offshoot by another group of worms, the nemertodermatids [6], and later studies supported another group to join the club, the xenoturbellids, which constituted their own phylogenetic enigma [7,8]. Xenoturbellids were originally represented by a single species (*Xenoturbella bocki*; Figure 1B) which was — just like acoels — originally associated with other flatworms (platyhelminthes) these three animal groups make up the Xenoacoelomorpha clade [9].

Philippe and colleagues [1] are not the first to address the position of xenacoelomorphs. The first studies dealing with acoels only used a handful of genes, notably the small subunits of ribosomal RNA (rRNA) [10]. The first phylogenomic studies placed them as the sister-group of bilaterians (the Nephrozoa hypothesis; Figure 1B) [11]. More recently, two new ambitious studies based on next-generation transcriptome sequencing (RNA-seq) introduced an exhaustive dataset of multiple xenacoelomorph species including four new species of *Xenoturbella* [12,13]. Yet, in their paper, Philippe and colleagues [1] show that precautions should be taken to handle such a massive amount of data. A first problem is to decide which genes to use and how best construct a dataset amenable to phylogenetic reconstruction. Instead of using a pre-existing set of genes, Philippe and colleagues [1] committed to detecting all the available single-copy orthologous genes by reciprocally aligning all genes for an array of species including an acoelomorph

and *Xenoturbella*. If fast evolution can affect tree reconstruction, it can also affect the way genes are gained and lost, and existing collection of orthologues can be plagued by hidden duplications or losses in the case of xenacoelomorphs. More directly, the authors meticulously eliminate spurious sequences from their datasets, such as contaminants of technical or biological origin.

While more data generally improve phylogenetic signal, they did not alleviate reconstruction artefacts such as long-branch attraction. This is the second issue addressed by Philippe and colleagues [1]. Phylogenetic reconstruction relies on a probabilistic description of the substitution process between amino acid or nucleotide residues in the dataset, called the evolutionary model. Such a model is used to compute the probability of different trees given the sequence alignment data and to ultimately pick the best tree. Long branch attraction occurs when the model cannot cope anymore with the amount of changes observed in one lineage. This artefact usually results in grouping such fast-evolving lineages together or placing them at the base of the tree. Acoel flatworms are among the fastest evolving lineages of bilaterian animals, closely followed by nemertodermatids, while xenoturbellids, however, evolve at a much slower pace. A possible solution to this problem is to use a more realistic description of the patterns of mutation at play in the genome. For instance, instead of considering the all amino acids in the proteins evolve in the same way, a model named 'CAT' allows each site to use a distinct profile characterized by its own set of amino acid frequencies (Figure 1C) [14]. This new 'site-heterogenous' model appears to be much better at coping with fast evolving phyla and is therefore far less sensitive to long-branch attraction. The only caveat of this model called CAT-GTR is that needs to estimate many parameters from the dataset, which makes it computationally more intensive than simpler models.

The first application of the CAT-GTR model to investigate the position of the xenacoelomorphs rejected their early divergence and suggested instead that they belonged to deuterostomes [15]. In their new study, Philippe and colleagues [1] attempt to apply the CAT-GTR model to the broadest collection of genes so far. To do that, they ran many independent trees using smaller set of genes randomly sampled from their main collection (the 'jack-knife' approach). As they obtained markedly different results with many alternative sets, they showed that genes yielding strong support for unambiguous animal groups also provided a stronger support for Xenambulacraria rather than for Nephrozoa. Another important bias examined in this paper derives from the differences in the amino

acid repertoire observed in each species. Even if all species use the same 20 amino acids, there are subtle but clear differences in how often they use each of them. To limit this problem, Philippe and colleagues [1] tried to reconstruct a tree by only considering six broad categories of amino acids based on their biochemical properties instead of all 20 individual amino acids. They show that this ‘recoding’ actually improves the support for Xenambulacraria. Altogether, these detailed analyses provide evidence that carefully mitigating known phylogenetic biases enhances the support for Xenambulacraria over Nephrozoa (Figure 1B).

While the next-generation sequencing era made data collection easier and raised hopes for definitive answers to many phylogenetic questions, the new analysis by Philippe and colleagues [1] shows that animal phylogeny remains a difficult matter. A striking observation in this study for instance is how limited the support is for the deuterostome clade, even though it is a classical and well-established animal clade. Deuterostomes normally include xenambulacrarians and chordates, but in several analyses they appear paraphyletic: xenambulacrarians are sister-group of all other bilaterians and chordates are sister-group to protostomes (Figure 1B). This controversial topology has been previously reported [16] and could convincingly be attributed to the limited phylogenetic signal and very short branch leading to deuterostomes. While there is no definitive synapomorphy for deuterostomes, except for maybe gill slits, this is food for thought for morphologists and evolutionary developmental biologists. Indeed, this could mean that many of the characters specific to Nephrozoa, such as the excretory system, could have independently evolved in Xenambulacraria and other bilaterians instead of being inherited from a more complex urbilaterian [17].

Can we hope to see these phylogenetic questions solved in the near future? With full genomes now sequenced and compared, a study such as the one by Philippe and colleagues [1] probably comes close to capturing the entire collection of genes available to build the tree of animals. Of course, more species and better data might alleviate some biases and provide marginal gains in phylogenetic resolution but will be unlikely to provide a major step forward. Inference methods, by contrast, remain the major bottleneck of current phylogenetic analysis: computationally tractable but simplistic site-homogeneous models are easy to apply but misrepresent the underlying complexity of the data, while site-heterogeneous models provide a richer framework but remain difficult to deploy on the broadest datasets due to computational limits. Recent attempts have been made at

developing computationally tractable site-heterogeneous models; for instance, the use of approaches derived from machine learning to speed up calculation using the CAT-GTR model [18]. Another attractive path is to account for possible incomplete lineage sorting, for instance by applying multispecies coalescent models [19]. Whole genome sequences conceal many other source of phylogenetic signal (chromosomal rearrangements, intron positions, gene gain, losses and duplications) but their interpretation remains limited by character binary models where more refined explicit models would be desirable [20]. These prospects show that molecular phylogeny is still a lively field.

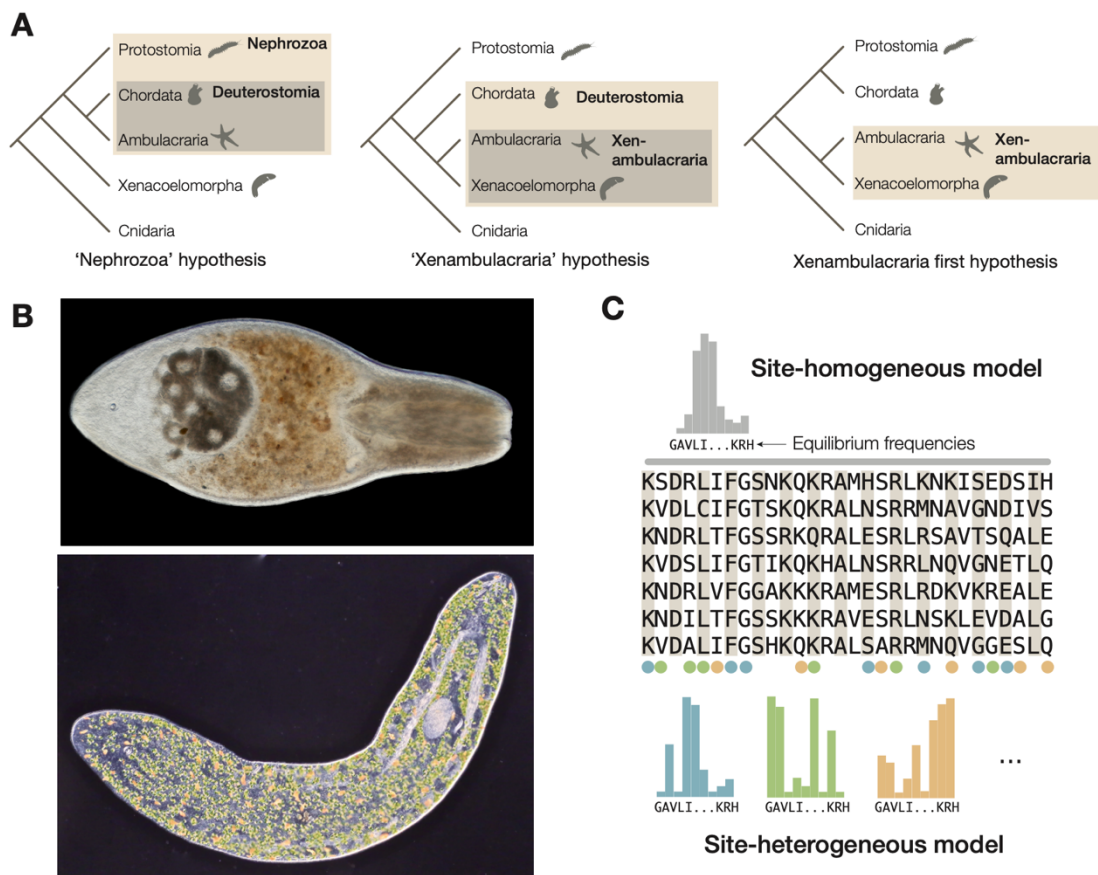


Figure 1. The placement of xenacoelomorphs and the impact of phylogenetic inference models.

(A) Two examples of acoelomorph flatworms: *Diopisthoporus longitubus* (top) shows a planula-like organisation with a posteroterminal mouth deemed to represent a more ancestral organisation in this lineage; *Symsagitiferra schultzei* (bottom) is a symbiotic acoel hosting photosynthetic microalgae (photos: Ulf Jondelius). (B) Alternative hypothesis regarding the phylogenetic position of xenacoelomorphs discussed in [1,12,15]. (C) While site-homogeneous estimate respective abundance of each residue once in the dataset, the equilibrium frequency, the site-heterogeneous approach considers each position could be

assigned to different categories or profiles, each defined by a specific equilibrium frequency.

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