

DISPATCH

Zoology: War of the Worms

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The phylogenetic affinities of Xenacoelomorpha — the phylum comprising *Xenoturbella bocki* and acoelomorph worms — are debated. Two recent studies conclude they represent the earliest branching bilaterally symmetrical animals, but additional tests may be needed to confirm this notion.

The unprepossessing, marine mud dwelling worm, *Xenoturbella bocki*, might seem an unlikely candidate for sustained scientific controversy, and yet its very simplicity — a simple net-like nervous system housed in an unsophisticated body — has been a puzzle that has led to great difficulty placing it and its relatives, the acoelomorph worms, within the animal kingdom[1, 2]. In essence, the point of contention has been whether the simplicity is primary or secondary— that is, are they so simple because they diverged from the main branch of animals before more complex features evolved or because they have lost complex features once shared with other animals? At stake is the nature of the last common ancestor of the bilaterian animals and our understanding of trends in the evolutionary process. Two new papers provide complementary data and another significant step towards finally resolving this issue[3, 4]. Greg Rouse and co-workers have discovered four new species of *Xenoturbella* in the depths of the Pacific ocean [3]. Their phylogenetic analysis, together with that of Cannon *et al.* [4], provide the most complete data sets to date aimed at elucidating the evolutionary affinities of *Xenoturbella* and relatives[4].

Xenoturbella bocki is a small (typically 2 cm long), yellowish-brown, flattened worm first found in 1915 by Sixten Bock on the West coast of Sweden and first described in 1949 [5]. Its name, which translates as ‘strange turbellarian’, references its tenuous similarity to the free living ‘turbellarian’ flatworms, such as the familiar planarians, with which it was initially grouped. Molecular evidence has since shown the similarities between *Xenoturbella* and the equally simple flatworms to be a case of convergent evolution.

The first molecular genetic study of *Xenoturbella's* affinities made the unfortunate mistake of analysing DNA from its prey (bivalve molluscs), rather than from the worm itself, with the inevitable results that it was wrongly linked to the Mollusca [6]. Genuine *Xenoturbella* genes surprisingly linked this simple worm to the morphologically complex members of the Ambulacraria (hemichordate worms and echinoderms) which, together with the Chordata (including vertebrates), make up the great animal branch of Deuterostomia [7]. The deuterostomes are one of two major lineages of bilaterally symmetrical animals — those with mirrored left and right sides; the second branch of the Bilateria are the Protostomia, which includes most familiar invertebrates including flatworms and molluscs. Other molecular studies followed showing that *Xenoturbella* is linked to another group that had also once been linked to the flatworms, the acoelomorphs, in a phylum now named Xenacoelomorpha [8]. This latter result makes great sense of their shared morphology. What is contested is where within the animal tree the combined assemblage of Xenacoelomorpha belongs [9].

Xenoturbella bocki has always been the lone member of the genus and the sole scion of an ancient clade distantly related to the acoelomorphs. Rouse *et al.* [3], however, describe four new species of the genus *Xenoturbella* [3]. All were discovered in the East Pacific between 600 and almost 4000 metres deep. Three were found associated with bivalve molluscs which, as with *X. bocki*, are their most plausible prey, and all are immediately recognisable as clearly similar to *Xenoturbella bocki* (Figure 1A.).

The new *Xenoturbella* species are beautifully coloured ranging from brown to orange to pink to purple and three of the new species are giants compared to *X. bocki* with the largest measuring over 20cm long. In agreement with their conserved morphology, comparisons of their mitochondrial genomes show few differences between all five species suggesting a relatively recent divergence and resulting in the inclusion of all five species in the same genus. These new species are fascinating additions to the genus and their discovery in such inaccessible locations — hydrocarbon seeps, hydrothermal vents and a whale carcass — suggests that more xenoturbellids remain to be found.

The wider interest in the discovery of four new species of simple marine worm, however, lies in the evolutionary relationships of *Xenoturbella* and the acoelomorph worms to the rest of the animal kingdom. There have been two widely supported scenarios (Figure 1): according to the first, Xenacoelomorphs, as their simple body plan suggests, branched from the main stem of the Bilateria early in evolution [10]. This would mean that

they diverged before the invention of some of the more sophisticated aspects of other bilaterians, such as a through gut with separate mouth and anus or nephrocytes, cells that function as 'kidneys'. If such a relationship is true then the xenacoelomorphs hold great interest as a staging post linking the earliest animal branches, such as cnidarians (sea anemones, jellyfish and corals) and sponges, to the more sophisticated bilaterians.

The second scenario is that Xenacoelomorphs are members of the deuterostomes, specifically related to the Ambulacraria [7, 8]. If this contention is true then xenacoelomorphs are descended from the common ancestor of Ambulacraria and Chordata, an animal we infer to be more complex than living xenacoelomorphs, possessing a through gut, nephrocytes, coelomic body cavities, gill slits and perhaps even a centralised nervous system. This would mean that all the deuterostome characters that are missing in xenacoelomorphs must have been lost rather than being primitively absent. So, this otherwise obscure group of worms either gives us a unique insight into the steps leading from cnidarians to bilaterians and the characteristics of our earliest ancestors, or represents a fascinating example of loss of complexity, a common but understudied phenomenon.

The surprise, perhaps, is that this conundrum exists in our days of abundant sequence data [11]. To resolve animal relationships we can readily assemble molecular data sets consisting of hundreds or thousands of genes sampled from across the animal kingdom. Both of the new papers have achieved such scales [3,4]. The problems in answering this particular question must thus stem not from a lack of data, but from difficulties in analysing them.

The position of the xenacoelomorphs bears all the signatures of a difficult phylogenetic problem. The difficulties are caused first by the antiquity and apparent brevity of the events we are trying to reconstruct — short periods of evolution during which groups separated from each other with little opportunity for phylogenetic signal to accumulate — and second by the idiosyncrasies of some of the *dramatis personae* — in particular the rapid evolution that has taken place in the genomes of the acoelomorphs.

Unequal (fast) rates of evolution have been proposed previously to explain the placement of acoelomorphs as the earliest branch within the Bilateria [8] as an artefact caused by the long appreciated problem of long branch attraction. Long branch attraction arises when one lineage has evolved more quickly than others, meaning that more characters have changed, making the branch in the tree of life appear longer. Chance

convergences can then 'attract' this branch to other long branches, in this case to the long branch leading to the evolutionarily distant outgroup taxa at the root of the animal tree of life. The effect of an hypothetical attraction between the long branch leading to xenacoelomorphs and the long branch leading to the outgroup would be to drag the xenacoelomorphs towards the root of the tree. While there is no dispute that the acoelomorphs are a very long branch, it is of course entirely possible for a species to be genuinely early branching and, coincidentally, long-branched.

The two new studies have notably gone to great efforts to rule out the effects of long branch attraction [OK to leave out the details?] and conclude that, whatever experiments they conduct, the xenacoelomorphs branch early, before the ancestor of the protostomes and deuterostomes [3, 4]. They undoubtedly also improve on previously published work in terms of size and completeness of their matrix of genes. But can they be taken as a definitive answer? Some additional analyses do suggest themselves.

Recent work on a very similar problem concerning the position of the Ctenophora (comb jellies) has shown, for example that it is not necessarily sufficient to change one or another factor of the analysis in order to overcome the instances of extreme long branch attraction [12]. The ctenophore analysis needed simultaneously to use the best fitting model of evolution (errors stem ultimately from model mis-specification) and to conduct careful analysis of the effects of removing longer branch outgroups. One example of what more might be done would be to carry out even more extreme taxon sampling — for example removing all long branched acoelomorphs to leave just the non-long-branch *Xenoturbella* (supplemented with data from the new species) in conjunction with the use of the optimal 'CAT' evolutionary model [13]. The use of the shortest branched exemplars from otherwise problematic long branched taxa has a long and successful history and provided the initial evidence for the clade of Ecdysozoa, for example [14].

Ultimately, additional confidence in this result could come from sources of evidence independent of gene sequences. The ctenophore problem provides an example of one such avenue in the form of an analysis of the gene content of genomes to build a matrix recording the presence/absence of a large number of homologous genes [12]. The phylogenetic tree built using these data shows extremely good correspondence with the canonical animal phylogeny, suggesting that this might be an informative source of data.

Another approach would be a search for rare genomic changes to support different groups on the tree [11, 15]. One encouraging example, is the finding of a suite of changes to the mitochondrial NAD5 gene that has occurred in the lineage leading to the protostomes and which can be used to determine membership of this clade [16]. Such a character, with equally complex derived and primitive character states, is essentially immune both to convergent evolution and to secondary reversion to the primitive character state. Genome sequencing efforts across the animals currently underway will no doubt provide raw material for searches for new characters.

The newly discovered xenoturbellid species and the new molecular genetic data are truly important contributions to our understanding of this obscure yet important group of animals, but we may not have found a peaceful resting place for them on the tree of life quite yet

References

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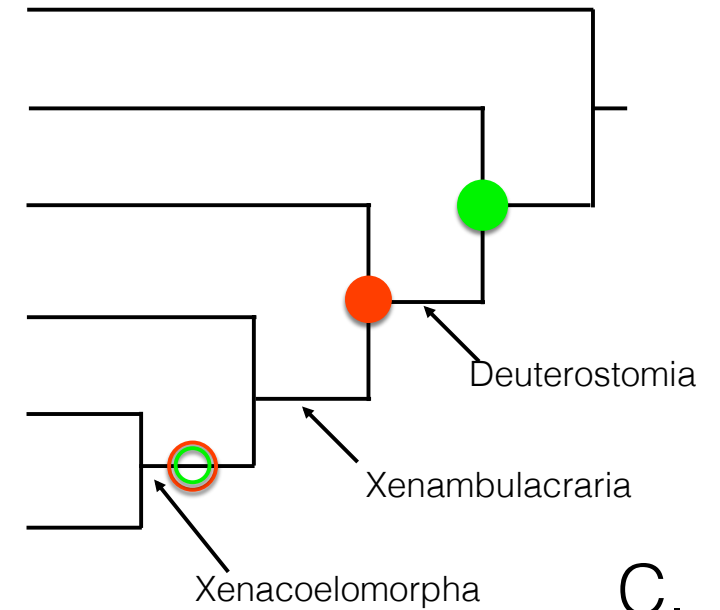
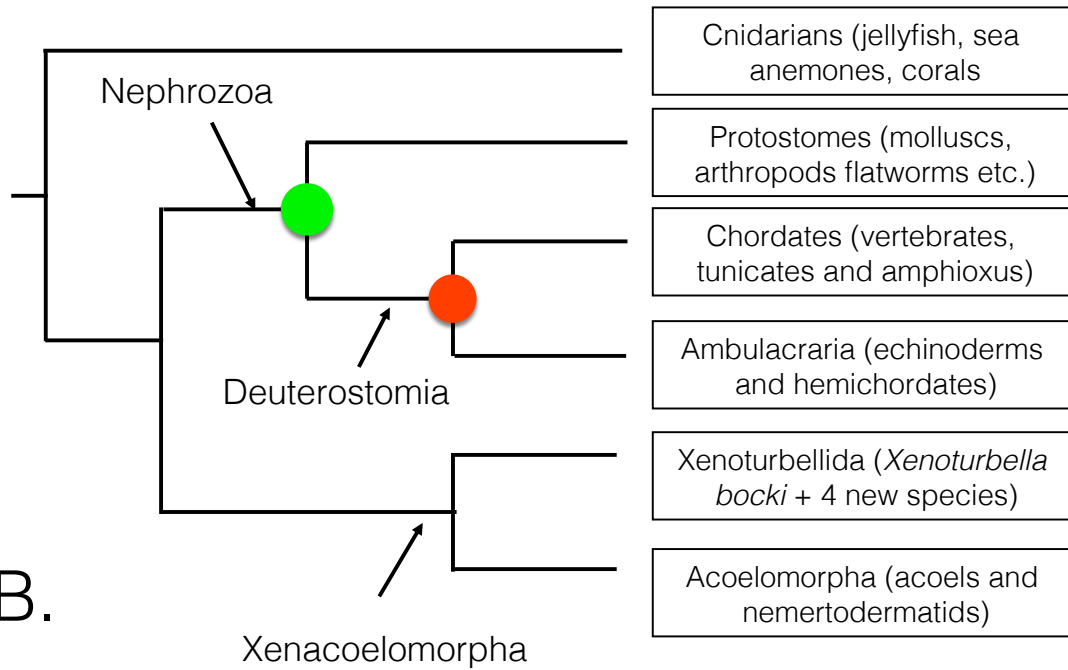
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Figure 1. One of the new *Xenoturbella* species and trees showing possible relationships between Xenacoelomorpha and other major groups of animals. (A) The new species *Xenoturbella hollandorum*. Approx 2.5 cm long. Photo courtesy of Greg Rouse. On the left, the phylogenetic tree supported by Rouse *et al.* [3] and Cannon *et al.* [4] in which Xenacoelomorphs branch before the common ancestor of protostomes and deuterostomes (Nephrozoa). Absences of characters (red and green filled circles) result from diverging before origins of these characters. On the right, the tree supported by previous analyses of large data matrices [8]. Xenacoelomorphs are deuterostomes most closely related to Ambulacraria. Some characters in common to protostomes and deuterostomes (filled green circle) or present in deuterostome common ancestor (filled red circle) are absent from xenacoelomorphs through loss (empty red and green circles).

A.



B.



C.