Phylo-evo-devo, tardigrades and insights into the evolution of segmentation

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Abstract

The concept of phylo-evo-devo highlights the benefits of reciprocal illumination between studies of phylogeny and of developmental biology when studying character evolution. Here we consider the case of the evolution of a segmented body plan within the major animal clade of Ecdysozoa. Specifically, we consider developmental studies supporting the homology of segmentation between Arthropoda, Onychophora and Tardigrada. In parallel, we examine inconclusive results regarding the possible phylogenetic affiliations of the tardigrades. If tardigrade arthropod and onychophoran segmentation is indeed homologous, molecular phylogenies supporting a sister group relationship between tardigrades and nematodes imply a loss of segmentation in the latter. To progress, we need both fully resolved phylogenies, and more developmental studies of 'lesser' groups including tardigrades and even more obscure, segmented ecdysozoan clades such as the Kinorhyncha (mud dragons).

Introduction

The term *phylo-evo-devo* was coined to highlight the potential for reciprocal illumination between the fields of developmental biology and phylogenetics (Minelli, 2009). Minelli's original example of the working of phylo-evo-devo concerned the evolutionary developmental origin of the reduced forewings of the Strepsiptera (twisted wing flies). Early phylogenetic analyses suggested that these unusual parasitic insects were closely related to the Diptera (whose hind wings are themselves reduced to halters) (Whiting *et al.*, 1997). This phylogenetic relationship suggested the intriguing possibility that the common ancestor of Diptera and Strepsiptera, both of which have a single pair of wings, was itself

two winged and that the reduced wings were an homologous character between the two groups. The really intriguing inference from this scenario stems from the observation that the pair of wings that are reduced in the two orders (fore wing in Strepsiptera versus hind wing in Diptera) are not on homologous segments, implying that the reduced wings, if homologous, must have swapped segmental position between the two groups by some amazing homeotic change (Whiting and Wheeler, 1994). Subsequent phylogenetic studies solved the problem by showing that the apparent link between Strepsiptera and Diptera was a tree reconstruction artefact stemming from the unequal rates of evolution amongst the insect lineages (Wiegman *et al.*, 2009). New data and improved analyses showed that the fast evolving Strepsiptera were in fact related to the Coleoptera (as morphologists had originally suggested) and that the reduced forewings of Strepsiptera might therefore be related to the tough elytra (modified forewings) seen in the beetles.

Here we focus on a similar phylo-evo-devo problem involving questions of both phylogeny and of homology. We consider the likely homology of segmentation between Tardigrada (the water bears), Onychophora (velvet worms) and Arthropoda (Chelicerata (arachnids, horseshoe crabs and pycnogonids), Myriapoda (centipedes, millipedes and their allies), and Pancrustacea (crustaceans including insects)). Alongside this, we discuss the, still contentious, question of the phylogenetic position of the tardigrades (Fig. 1). While the solution to the first part of this two-sided problem seems fairly straightforward – phylogeny, morphology and developmental genetics all suggest the homology of tardigrade and arthropod segmentation (and therefore its presence in their common ancestor), a solution to the second part of the problem, a precise phylogenetic placement for the tardigrades, is still lacking. This results in a lack of understanding of the deep evolutionary history of segmentation.

Homology of segmentation in Arthropoda and Tardigrada

As is typical for a phylo-evo-devo question, when considering whether the segmentation in Tardigrada, Onychophora and Arthropoda is homologous, we first need to know whether these lineages are closely related. Phylogenetic proximity implies homology through the simple argument that the distribution of a putatively homologous character can be parsimoniously reconciled with the known phylogeny (Telford and Budd, 2003). As we will see in the following section, whatever the unknowns regarding the exact position of the tardigrades, they are nevertheless generally agreed to be relatively closely related to the Arthropoda and Onychophora, even if not necessarily their sister group (e.g. Telford *et al.*, 2008).
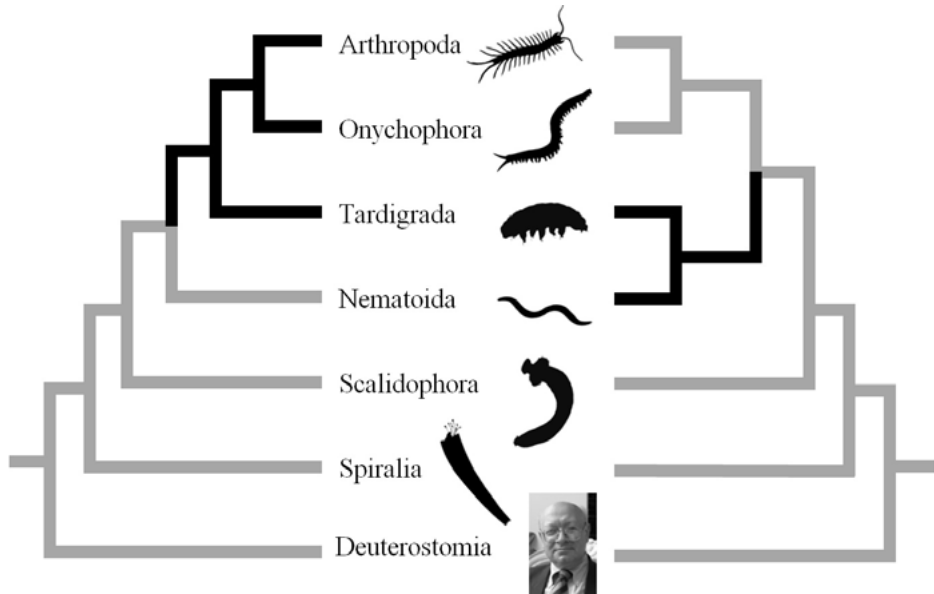
Figure 1. Two hypotheses of Tardigrada affinity. On the left they are shown as sister to the other Panarthropoda, on the right as sister to Nematoida. Animal silhouettes are from PhyloPic.org (Creative Commons, CC0 1.0 Universal).

The second part of the determination of homology depends on a consideration of the degree and detail of similarity of segmentation between the groups at a morphological, embryological and developmental genetic level. Again, detailed similarities would be most parsimoniously explained by homology rather than convergence (Telford and Budd, 2003). On this score, although there are still limited data from the Tardigrada, there is every reason to believe that arthropod and tardigrade segmentation is homologous. While the details of plausibly common features of segmentation vary (and there is reason to believe that tardigrades have changed a lot from the ground state due to miniaturisation), Arthropoda and Tardigrada have similar arrangements of similar components within their segments suggesting homology. Tardigrada, Onychophora and Arthropoda all possess paired ventro-lateral appendages with terminal claws associated with a subset of their segments, all have segments in which the segment polarity gene *engrailed* is expressed in the dorsal portion of each segment (Gabriel and Goldstein, 2007) and there are clear detailed similarities between the segmental ganglia of Tardigrada and Arthropoda at least (Mayer *et al.*, 2013). Finally, albeit a less direct correspondence, the expression domains of orthologous Hox genes coincide with segmental boundaries in a similar manner across all putative panarthropod groups (Smith *et al.*, 2016). Ultimately, the linking of

Arthropoda, Onychophora and Tardigrada within Ecdysozoa and the detailed similarities of their segmental make up mean there is little doubt that their common ancestor was segmented.

The phylogenetic relationships of the segmented animal phyla

Tardigrada, Onychophora and Arthropoda were traditionally grouped in the Articulata (Cuvier, 1817), alongside the other coelomate, segmented protostome phylum, the Annelida (earthworms, leeches, etc.). Articulata was a surprisingly long-lived concept and perhaps the most significant early victim of the application of numerical cladistic methods and molecular phylogenetics (see Minelli 1993 for an overview). Eernisse *et al.* (1992) were the first to find strong morphological evidence for the separation of Annelida from the other segmented phyla. Soon after the study of Eernisse *et al.* (1992), Aguinaldo *et al.* (1997) provided the first molecular evidence (using 18S rRNA) for a clade of ecdysing (i.e., moulting) animals, that they named the Ecdysozoa. The study of Aguinaldo *et al.* (1997) was seminal because of its careful taxon selection and the sophisticated phylogenetic methodologies (including maximum likelihood) it implemented. The same clade was soon after also recovered using broad taxon sampling, i.e., adding many nematodes including "long branched" ones, more ecdysozoan phyla, and using other tree reconstruction methods (Giribet and Ribera, 1998). Ecdysozoa includes a diversity of segmented phyla (Arthropoda, Tardigrada, Onychophora and also the Kinorhyncha or mud dragons, in which segments are referred to as zonites) and four non-segmented phyla (Priapulida – penis worms, Nematoda – roundworms, Nematomorpha – Gordian worms, and Loricifera – the loricated animals).

While the general rejection of Articulata was almost immediate, the monophyletic status of the Ecdysozoa was initially debated, as many early studies failed to find support for the inclusion of the nematodes in Ecdysozoa (e.g., Blair *et al.*, 2002; Wolf *et al.*, 2004; Philip *et al.*, 2005; Zheng *et al.*, 2007). Improved taxon sampling, the development and application of more sophisticated evolutionary models and methods, analyses of rare genomic changes and the presence of specific genes have now broadly confirmed the monophyly of Ecdysozoa (e.g., Telford, 2004; Telford *et al.* 2015; Philippe *et al.*, 2005; Irimia *et al.*, 2007; Holton *et al.*, 2010). While Ecdysozoa is now universally considered a valid lineage, the relationships between the phyla constituting the Ecdysozoa have proven harder to resolve, with the relationships of the Tardigrada being particularly contentious.

Within Ecdysozoa, Priapulida, Kinorhyncha and Loricifera may constitute a monophyletic lineage – the Scalidophora (Schmidt-Rhaesa *et al.*, 1998), al-

though there remains uncertainty over the inclusion of the long branched and poorly sampled Loriciferans within this group (e.g., Yamasaki *et al.*, 2015; Giribet *et al.* 2017). Similarly, Nematoda and Nematomorpha are generally grouped together as the Nematoida (e.g., Dunn *et al.*, 2008; Campbell *et al.*, 2011; Borner *et al.*, 2014; Yoshida *et al.*, 2017). Finally, it is generally agreed that Onychophora and Arthropoda share a common ancestor to the exclusion of Nematoida and Scalidophora (e.g., Dunn *et al.*, 2008; Campbell *et al.*, 2011; Borner et al., 2014; Yoshida *et al.*, 2017). The relationships between Tardigrada, Onychophora + Arthropoda and Nematoida, however, are still debated (Fig. 1). While morphology clearly links the segmented, jointed-legged tardigrades to the Onychophora + Arthropoda in a monophyletic Panarthropoda, molecular phylogenetic analyses have been ambiguous, with some studies recovering Panarthropoda (Campbell *et al.*, 2011; Rota-Stabelli *et al.*, 2011) but most others resolving Tardigrada as the sister group of Nematoida (Dunn *et al.*, 2008; Borner *et al.*, 2014; Yoshida *et al.*, 2017). Considering the long branches leading to both the Nematoida and Tardigrada there is a suspicion that the Nematoida plus Tardigrada grouping is a long branch attraction artefact. Borner *et al.* (2014), for example, found that the signal for Nematoida plus Tardigrada was preferentially found in fast evolving sites (see Philippe *et al.*, 2001; Campbell *et al.*, 2011; Rota-Stabelli *et al.*, 2011). The situation is further complicated by the fact that, while Campbell *et al.* (2011), Borner *et al.* (2014) and one of the analyses of Yoshida *et al.* (2017) found Nematoida plus Tardigrada to be the sister group of Arthropoda, Dunn *et al.* (2008) found Tardigrada to be the sister group of Nematoida within the context of a monophyletic Cycloneuralia (i.e., Scalidophora plus Nematoida).

Tardigrade genomes and implication for phylogeny

A recent important new contribution to the question of Tardigrade affinities came from the analysis of their genomes. Compared with most other animals, tardigrades possess rather compact genomes (55-104 Mb). Interpreting tardigrade genomics in an evolutionary context, however, proved to be challenging, and the results of these interpretations seem to be nothing short of enigmatic. The first genome revealed an unprecedented high level (17%) of genes acquired from other organisms through Horizontal Gene Transfer (Boothby *et al.*, 2015), but subsequent reanalyses and new genomes showed that this estimate was heavily biased by a poor filtering of contaminants (Koutsovolous *et al.*, 2016; Bemm *et al.*, 2017).

Tardigrade genomes seem to contain contradictory phylogenetic signals. While phylogenies based on concatenated genes tend to support tardigrade as sister to Nematoida (even though this result is model dependent), analyses using rare changes, such as presence of specific orthologs, support tardigrades as

sister group to arthropods (Hashimoto *et al.*, 2016; Bemm *et al.*, 2017; see also Borner *et al.*, 2014). Considering that tardigrades and nematodes are obvious candidates for being affected by long branch attraction (Campbell *et al.*, 2011), it is clear that the use of well-fitting models and testing for specific artifacts (as in Campbell *et al.*, 2011 and Feuda *et al.*, 2017) is key to using tardigrade genomic data for phylogenetic analyses.

Discussion

We have seen that, despite limited data from tardigrades, segmentation seems highly likely to have been present in the common ancestor of Tardigrada and Arthropoda. However, and fittingly in this examination of phylo-evo-devo, we need new knowledge of both phylogeny and development if we are to understand the evolution of segmentation in the arthropods better (and its potential loss in Nematoida and perhaps elsewhere).

First is the pressing need to establish the true relationships between major ecdysozoan groups, most obviously to establish whether the Tardigrada are the sister group of Arthropoda + Onychophora or Nematoida. Assuming homology of tardigrade and arthropod segmentation, this latter possibility would force us to conclude that segmentation has been lost in Nematoida.

Second is the wish to know more about the relationship between segmentation in arthropods and other protostomes. Are zonites in kinorhynchs homologs of arthropod segments, making the ecdysozoan ancestor segmented? Could the old concept of homology of annelid and arthropod segmentation be correct, making the protostome ancestor segmented (Balavoine, 2014)?

Minelli observed (Minelli, 2009, p. 2) that "We now have more and more robust phylogenies and deeper insights into evolutionary variations of developmental mechanisms, but the challenge is to understand the data in an integrated phylo-evo-devo framework." What we have seen in this examination of segmentation and phylogeny in Ecdysozoa is that we have yet more work to do to achieve truly robust phylogenies (Telford *et al.*, 2015) and we must gain even deeper insights into developmental mechanisms from more fascinating, if obscure, animal groups such as water bears and mud dragons.

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