"Perspectives in Animal Phylogeny and Evolution": A decade later

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Abstract

Refinements in phylogenomic methods and novel data have clarified several controversies in animal phylogeny that were intractable with traditional PCR-based approaches or early Next Gen analyses. An alliance between Placozoa and Cnidaria has recently found support. Data from newly discovered species of *Xenoturbella* contribute to Xenacoelomorpha being placed as sister group of Nephrozoa rather than within the deuterostomes. Molecular data reinforce the monophyly of Gnathifera and ally the long-enigmatic chaetognaths with them. Platyzoa was an artefactual grouping, and deep relationships within Spiralia now depict Rouphozoa (= Gastrotricha + Platyhelminthes) as sister group to Lophotrochozoa, and Gnathifera (plus Chaetognatha) their immediate sister group. A "divide and conquer" strategy of subsampling clades to optimize gene selection may be needed to simultaneously resolve the many disparate clades of the animal tree of life.

Introduction

In the preface to his textbook *Perspectives in Animal Phylogeny and Evolution*, Minelli (2009) formulated a simple, clear question based on a summary of some "unexpected and arguably controversial hypotheses" in a paper then just co-authored by us (Dunn *et al.*, 2008). He asked, "Will these three phylogenetic hypotheses eventually replace those presented in this book, which have been distilled from the evidence available until last week?", and concluded that "at the moment there is, arguably, nothing like a single best tree for the metazoans." This chapter addresses the major changes over the decade, in relation to our understanding of animal phylogeny and evolution. These changes did not happen in a vacuum, but rather at the interface between amplicon-based (us-

ing PCR) and non-targeted gene sequencing paradigms. In the former, a few markers were selected, and primers designed to amplify them. In the second approach, genes were sequenced from cDNA libraries randomly. The Dunn *et al.* (2008) analysis combined – as other papers did at the time – whole genomes of a selected number of model organisms with a few ESTs (expressed sequence tags), on the order of hundreds to a few thousand, for a growing number of metazoans. This approach was later succeeded by denser gene sampling using next generation sequencing platforms (first Roche's 454 and then Illumina). Today, Illumina and other techniques are routinely producing large numbers of genomes and rather complete transcriptomes. Some of the discussions below focus on recent developments in the field of phylogenomics.
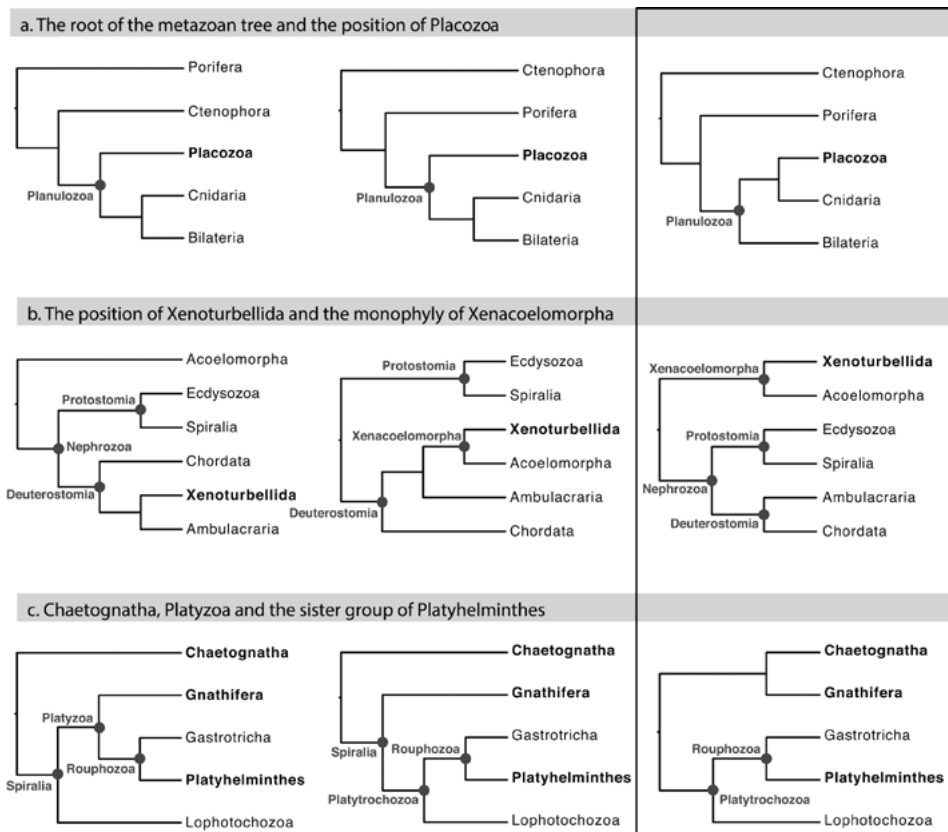

Figure 1. Alternative molecular hypotheses of selected metazoan clades in relation to the base of the animal tree (a), the position of Xenoturbellida (b) and selected spiralian relationships (c). Hypotheses on the left were generally supported by PCR-based phylogenies or early phylogenomic analyses. Rightmost hypotheses are preferred, as they are based on phylogenomic analyses with increased taxon and gene sampling and more sophisticated analytical methods for orthology selection and phylogenetic analyses.

Novel results

Many novel results have been proposed in the past decade, but not all have withstood scrutiny in the same way. Because the question about the position of ctenophores and sponges (which of the two is sister group to all other metazoans) has been debated to exhaustion without firm conclusion – each new paper claiming that the debate has been finally settled – we refrain here from revising such controversy, but refer the reader to recent reviews and the latest analyses (e.g., Dunn *et al.*, 2014; Dunn *et al.*, 2015; Feuda *et al.*, 2017; Shen *et al.*, 2017). Some other controversies have also been discussed, but unlike that of sponges–ctenophores, the addition of new data has provided new insights. Such is the case of the position of Xenacoelomorpha, the clade that includes Xenoturbellida and Acoelomorpha (= Acoela + Nemertodermatida). In addition, new hypotheses are emerging with the addition of genomes, as discussed below for Placozoa. Finally, results related to some clades of Spiralia are also discussed below in reference to Chaetognatha and Gnathifera, the paraphyly of Platyzoa, and the sister group of Platyhelminthes. Some of these hypotheses and alternative views are summarized in Figure 1.

Placozoa and the new animal phylogeny

Placozoans (currently represented by two genera, *Trichoplax* and *Hoilungia*) (Eitel *et al.*, 2018), have traditionally been placed as one of the earliest animal lineages due to their body plan simplicity, yet molecular phylogenetics has, since its early days, placed placozoans as more derived than their morphology suggested (Fig. 1a). In fact, most molecular analyses, and nearly all phylogenomic analyses suggested Placozoa to be the sister group to Cnidaria + Bilateria (= sometimes called Planulozoa[1]) (e.g., Srivastava *et al.*, 2008; Hejnol *et al.*, 2009; Pisani *et al.*, 2015; Simion *et al.*, 2017; Whelan *et al.*, 2017). However, a recent analysis including genomes of four new placozoans found strong support for a sister group relationship between Placozoa and Cnidaria (Laumer *et al.*, 2018), contradicting most previous phylogenomic analyses that supported the monophyly of Cnidaria + Bilateria when only the genome of *T. adhaerens* was sampled (e.g., Hejnol *et al.*, 2009; Feuda *et al.*, 2017). The new analyses suggest that such a grouping may be an analytical artifact, as a majority of genes supporting Cnidaria + Bilateria show evidence of compositional heterogeneity. Further research will be necessary to continue to test this hypothesis, and this should be

[1] There is confusion in the literature about the names Planulozoa and Parahoxozoa, which sometimes are used interchangeably and sometimes Planulozoa is a subclade of Parahoxozoa. Here we follow Wallberg et al. (2004) in identifying Planulozoa as composed of Placozoa, Cnidaria and Bilateria (see their Fig. 2).

facilitated by the recent availability of multiple placozoan genomes (Eitel *et al.*, 2018; Kamm *et al.*, 2018; Laumer *et al.*, 2018).

The position of Xenacoelomorpha in the animal tree

While the monophyly of Xenacoelomorpha is now well accepted, this has not always been the case. Two of its subclades, Acoela and Nemertodermatida, had traditionally been classified within Platyhelminthes (e.g., Karling, 1974), while the position of Xenoturbellida had long been debated, and included an affinity to Platyhelminthes, among many other groups (e.g., Reisinger, 1960; Haszprunar *et al.*, 1991). Early amplicon-based approaches showed that Acoela, first, and Nemertodermatida, later, were not part of Platyhelminthes, but closer to the bilaterian root, as sister group to Nephrozoa (e.g., Ruiz-Trillo *et al.*, 1999; Jondelius *et al.*, 2002; Ruiz-Trillo *et al.*, 2002; Telford *et al.*, 2003). This position seemed settled until *Xenoturbella* came back into play. Molecular accounts of the only accepted species of Xenoturbellida at the time (after a troubled earlier history of contaminations) seemed to suggest that Xenoturbellida was a deuterostome, most probably related to Ambulacraria (Bourlat *et al.*, 2003; Bourlat *et al.*, 2006; Bourlat *et al.*, 2009). This idea resonated with the epidermal ultrastructure of *Xenoturbella*, which bears resemblance to that of hemichordates (Pedersen and Pedersen, 1986, 1988). Subsequent work adding phylogenomic-scale data, mitogenomes and microRNAs of *Xenoturbella* and Acoelomorpha were used to switch the position of acoels from being the sister group of Nephrozoa, to becoming another deuterostome, as the sister group of Xenoturbellida (Philippe *et al.*, 2011). This position was in fact not supported by the microRNA data, which favour Xenacoelomorpha as the sister group to Nephrozoa, and the mitogenomic data only provided marginal support to the deuterostome affinity of Xenacoelomorpha. Much larger mitogenomic sampling has more recently suggested that Xenacoelomorpha are not nested within Deuterostomia, but rather that they are their sister group (Robertson *et al.*, 2017), with marginal nodal support. Deuterostome affinities for Xenacoelomorpha were not supported by other phylogenomic work (Hejnol *et al.*, 2009; Cannon *et al.*, 2016; Laumer *et al.*, submitted), notably when additional species of *Xenoturbella* are added to the analyses (Rouse *et al.*, 2016), and now Xenacoelomorpha is widely regarded as the sister group to Nephrozoa (Brauchle *et al.*, 2018). Since then, Xenoturbellida has now become a clade of six species (Nakano *et al.*, 2017), and many more probably await to be discovered in the deep ocean. They may well turn into a novel model to understand early bilaterian evolution, complementing acoels. Additional genomic data will contribute to definitively place this important animal lineage, and while the weight of phylogenetic evidence has shifted to a

sister group relationship with Nephrozoa (Fig. 1b), mitochondrial gene order and the presence of some genes in xenoturbellids and ambulacrarians have been suggested to support the position of xenoturbellids among deuterostomes (M.J. Telford, pers. comm.).

Chaetognatha and Gnathifera

The monophyly of Gnathifera – a clade uniting Rotifera, Gnathostomulida and Micrognathozoa – has been supported with morphology (Ahlrichs, 1993; Sørensen, 2003) and suspected using molecular approaches that lacked data from micrognathozoans (Witek *et al.*, 2009; Struck *et al.*, 2014). It has only recently been well established that gnathiferans form a clade that is well supported molecularly as well as morphologically (Laumer *et al.*, 2015a), and they constitute the sister group to all other spiralians (Struck *et al.*, 2014; Laumer *et al.*, 2015a) (Fig. 1c). Chaetognaths, on the other hand have been much more difficult to place reliably on the animal tree using molecular approaches, especially due to the long branch separating them from other protostomes (Marlétaz *et al.*, 2006; Matus *et al.*, 2006). A recent analysis of *Hox* genes across metazoans proposed novel synapomorphies between chaetognaths and rotifers, including loss of the *lox5*-parapeptide and the presence of the *MedPost* gene, found in no other animal groups examined to date, suggesting a possible relationship between Chaetognatha and Gnathifera (Fröbius and Funch, 2017). Such *Hox* signatures, however, remain unstudied in Gnathostomulida and Micrognathozoa. Novel phylogenomic analyses using the CAT+GTR model, including substantive data on all gnathiferan phyla (including the first gnathostomulid genome) and new chaetognath sequences, provide support for a sister group relationship between Gnathifera and Chaetognatha (Laumer *et al.*, submitted). This relationship should encourage future research on putative morphological synapomorphies, perhaps those related to the feeding apparatus of gnathiferans and chaetognaths, and additional genome signatures that may help further test this relationship (Fröbius and Funch, 2017; Laumer *et al.*, submitted).

Rouphozoa: Discovering the sister group of flatworms

Not totally unrelated to the resolution and position of Gnathifera and even Acoelomorpha, is the phylogenetic placement of Platyhelminthes – and the dismissal of a clade named Platyzoa (see Struck *et al.*, 2014; Laumer *et al.*, 2015a) proposed by Cavalier-Smith (1998) and endorsed in early amplicon-based (Giribet *et al.*, 2000) and EST-based (Hejnol *et al.*, 2009) analyses. Platyhelminthes have changed membership a few times (see for example the case of Acoelo-

morpha above), but are now understood to be composed of two main clades, Catenulida and Rhabditophora (e.g., Egger *et al.*, 2015; Laumer *et al.*, 2015b). However, their closest relative has been elusive for some time, and they have often been allied to a diversity of acoelomate animal groups, or to nemerteans (which have a coelom but have been considered as functionally acoelomate). Gastrotrichs, on the other hand, have been often grouped with other "aschelminths" due to their cuticle and nervous system of the cycloneuralian type. Using the newest phylogenomic data (well sampled and mostly Illumina-based datasets), results have settled on Gastrotricha being the closest living relative of Platyhelminthes (Struck *et al.*, 2014; Laumer *et al.*, 2015a; Kocot *et al.*, 2017; Laumer *et al.*, submitted) (Fig. 1c), a clade named Rouphozoa by Struck *et al.* (2014) as a derivation of the Greek word *rouphao*, for "ingesting by sucking", referring to the preferred feeding mode of platyhelminths and gastrotrichs. As in many other higher clades, synapomorphies are difficult to identify for these sister taxa, as many shared characters seem to be symplesiomorphic traits for Spiralia, such as lack of coeloms, complete or nearly complete body ciliation, and protonephridia. The presence of a duo-gland organ system (Tyler and Rieger, 1980) may constitute a true synapomorphy of Rouphozoa, even though this was once considered a striking case of convergence between platyhelminths and gastrotrichs (Tyler, 1988).

Discussion of "new old" results

The debate about whether Ctenophora or Porifera constitutes the sister group to all other animals was probably what made Minelli choose the Dunn *et al.* (2008) paper to open his book and to question how long novel results such as the ones presented in that paper might last. A decade later, the number of phylogenetic papers addressing this particular issue, and no doubt more importantly, the amount of research on both Ctenophora and Porifera has grown considerably, at least in non-taxonomic journals. While debate about the phylogenetic position of particular taxa may seem frustrating to many non-systematists who just desire a stable tree, at least in this case it has served to raise interest in nearly all aspects of the biology of sponges and ctenophores.

Many other key aspects have been consistently resolved since, sponge monophyly being one of them. While no PCR-based approach was able to recover monophyly of sponges, nearly all phylogenomic data sets now support the monophyly of Porifera (e.g., Pick *et al.*, 2010; Whelan *et al.*, 2015; Simion *et al.*, 2017; Laumer *et al.*, submitted). The implications of sponge paraphyly were especially relevant for understanding the last common ancestor of Metazoa, especially in light of the "choanoblastaea" theory (Nielsen, 2008), and therefore,

the dismissal of sponge paraphyly has been an important contribution of phylogenomics. The segregation of Homoscleromorpha from Demospongiae is also broadly accepted (Gazave *et al.*, 2012).

The revival of the old taxon Lophophorata (Bryozoa, Brachiopoda and Phoronida) is another contribution of the newest generation of phylogenomic data (Nesnidal *et al.*, 2013; Laumer *et al.*, 2015a; Laumer *et al.*, submitted), although the position of Entoprocta (sometimes allied to Bryozoa, as supported by Nielsen), has introduced some instability to this clade, especially when Cycliophora are introduced in the analyses (Laumer *et al.*, 2015a; Kocot *et al.*, 2017; Laumer *et al.*, submitted). Resolving whether Entoprocta and Cycliophora belong with Lophophorata (possibly as their sister group?), constituting the clade Polyzoa (Hejnol *et al.*, 2009; Laumer *et al.*, submitted), or whether Polyzoa may be artefactual (Nesnidal *et al.*, 2013), remains to be resolved.

Future directions

As eloquently stated recently by Laumer (2018), "Contemporary phylogeneticists enjoy an embarrassment of riches, not only in the volumes of data now available, but also in the diversity of bioinformatic tools for handling these data." These riches thus require more than just brute force, as we now see in most contemporary phylogenomic analyses, where sets of genes are carefully selected according to their properties, taxa need be judiciously selected according to the particular hypothesis to be tested, and methods are thoroughly tested and thoughtfully selected. Yet some questions remain recalcitrant to such treatments, especially when trying to infer relationships of such disparate sets of taxa as Metazoa. Some of our work has thus re-focused towards subsampling clades in order to optimize gene selection and to maximize gene and taxon representation for particular subsets of taxa, whether these are metazoan phyla (Laumer *et al.*, submitted), or subclades of crustaceans (Schwentner *et al.*, 2018). This strategy of divide-and-conquer may seem at odds with much phylogenetic thinking that aimed to build phylogenies as large as possible and may be more allied with some of the supertree aims. The future may decide which strategy is better suited for simultaneously resolving the phylogenetic position of groups such as ctenophores and chaetognaths.

A final reflection has to do with the integration of genome-level data and morphology, a topic that has been debated in several contexts, especially for the integration of fossil and phylogenomic data (e.g., Giribet, 2015; Pyron, 2015), which is essential for "total evidence" dating methods (Ronquist *et al.*, 2016). However, this integration has to date almost entirely been conducted in a Bayesian framework employing standard Markov models of evolution (the Mk

model; Lewis, 2001) that behave well for molecular characters, but not for morphological ones, a debate that has yet to be resolved (e.g., Goloboff *et al.*, 2018; O'Reilly *et al.*, 2018b, a). Some advances have recently been made in modeling transitions between plesiomorphies and apomorphies for morphological characters in a more appropriate manner than assuming equal frequencies through time. The MkA model (for "asymmetrical"), for example, limits reversal in morphological characters (Pyron, 2017). Continuing these efforts to devise better models for morphological characters could be a promising step forward in "total evidence" phylogenetics.

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