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REVIEW

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How our view of animal phylogeny was reshaped by molecular approaches: lessons learned

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Abstract In the late 1980s, researchers began applying molecular sequencing tools to questions of deep animal phylogeny. These advances in sequencing were accompanied with improvements in computation and phylogenetic methods, and served to significantly reshape our understanding of metazoan evolution. Prior to this time, researchers asserted phylogenetic hypotheses based on their experience with taxa and to some degree, their authority. Molecular phylogenetic tools provided discrete methods and objective characters for reconstructing phylogeny. Nonetheless, major changes to widely accepted views, such as animal phylogeny, take time to be accepted. Development and acceptance of our current understanding of animal evolution occurred in three main phases: initial hypotheses based on 18S data, confirmation with additional molecular markers, and continued refinement with phylogenomics. With the advent of ideas such as Lophotrochozoa and Ecdysozoa, flaws in the traditional view became apparent. We now understand that complex morphological and embryological features (e.g., segmentation, coelom formation, development of body cavities) are much more evolutionarily plastic than previously recognized. Here, I explore how the transition from the traditional to the modern phylogenetic understanding of animal phylogeny occurred and examine some implications of this change in understanding. As the field moves forward, the utility of morphological and embryological characters for reconstruction of deep animal phylogeny should be discouraged. Instead, these characters should be interpreted in the light of independent phylogeny.

Keywords Phylogenetics · Sequence · 18S · Lophotrochozoa · Ecdysozoa · History

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Introduction

There is perhaps a basic human urge to categorize organisms and understand how they relate to each other. This is evident even in young children who, without training, typically do a good job of sorting organisms into groups—hairy things go together, animals with six legs are a group, and plants with flowers belong together. In the absence of a formal methodology, most individuals group organisms by similarity or based on shared morphological characters. Over the course of human history, this general approach has been used from Aristotle to Linneaus to Haeckel and by recent researchers. Organismal expertise, usually gained from spending years working with an organismal group, gave the perceived ability to provide an authoritative understanding of evolutionary history. In the later half of the twentieth century, that changed and researchers started employing explicit methods including morphology based approaches with Hennigian principles (1950; 1966) and distance-based approaches with molecules (e.g., Fitch and Margoliash 1967). By the mid 1980s, systematics was a growing field offering new insights to our understanding of animal evolution. Developments in phylogenetic theory, DNA sequencing, and computation, all played major roles in reshaping our understanding of animal evolution. In this manuscript, I will try to offer some insight as to how and why understanding of higher-level animals has changed since the late 1980s.

This piece will provide a bit of historical perspective and thus is perhaps a bit informal. This was done to provide an anecdotal perspective of how views have changed.

Traditional view

Prior to the mid 1980s, views on deep animal relationships were largely based on decrees by authority figures. Whereas well-reasoned arguments often won the day, these arguments depended on having a comprehensive view of functional morphology, development, paleontology, and physiology. In most cases, formal algorithms for evolutionary analysis of data were not employed, and methods for arriving at a given hypothesis were not repeatable. That said, many individuals informally used an approach where they looked for similarities or shared (hopefully derived) features. As such, many hypotheses from earlier workers are still strongly supported today. However, many ideas came down to conflicting views between authority figures. For example, siboglinid worms (formerly known as the phyla Pogonophora and Vestimentifera), were argued to be annelids by Land and Nørrevang (1977) and Southward (1988) but as deuterostomes by Ivanov (1955; 1988).

One important consequence of this tradition was that different groups of scientists had very different perspectives on animal evolution. Prior to the 1970s and especially earlier in the twentieth century, many scientific programs were more nationalistic than present day. During this time, many scientists were expected to publish in their mother tongue, and, for many, social norms of their culture limited the degree to which younger researchers could challenge and question authority figures. These factors help cement different viewpoints. Thus, those who spoke in English, German, and Russian, for example, viewed animal phylogeny differently and placed different "value" on distinct sets of characters used in evolutionary interpretations. By the early 1980s, a more "global" cohort of biologists was emerging.

In the USA, understanding of animal phylogeny was dominated by the work of Libbie Henrietta Hyman. Hyman worked at the American Museum of Natural History and was very proficient at consolidating and summarizing information from publications in multiple languages, often with colorful and pointed opinions interjected about the quality of science from previous workers. In her 1940 volume (Hyman 1940, p. 38), she presented a "Hypothetical diagram of the relationships of the phyla of the animal kingdom." Although this diagram was only suppose to be "suggestive," authors of invertebrate texts (e.g., Barnes 1967; 1980) picked up on this figure and presented it in a slightly modified form as the phylogeny of animals thus influencing thousands of scientists for several of decades. Several key elements went into Hyman's diagram. Among the most important were the following: (1) evolution proceeds from simple to complex (i.e., grades of construction), (2) embryology and development is very conserved and thus of paramount importance for phylogeny, and (3) overall body architecture (e.g., segmentation, coelomic state) is phylogenetically important. These ideas were synthesized by Hyman from earlier workers going back to the likes of Karl Ernst von Bear and Ernst Haeckel (Halanych 2013). Notably, in the 1980s and 1990s, many senior invertebrate biologists were skeptical of efforts to resolve higher-level phylogeny. Phyla were erected due to the lack of affinities or shared features between the groups of organisms. In other words, phyla are used to emphasize morphological disparity. How much disparity is sufficient to be considered a distinct phylum is arbitrary and decided by people. This is why Arthropoda is ridiculously huge (e.g., the shared feature of an exoskeleton allowed them to be grouped), but Cycliophora was recently given its own phylum (Funch and Kristensen 1995) despite its close affinity (revealed through molecular data) to entoprocts. Furthermore, rank-based classification fails to represent any sense of age or diversity of a clade (see Avise and Johns 1999) and is largely arbitrary. Despite different viewpoints and lack of explicit methodology, by the mid 1980s, a more-or-less common viewpoints had emerged (Wilmer 1990; Halanych 2004).

New approaches

By the late 1980s, several factors converged that laid the ground work for a new view of animal phylogeny. For starters, systematics was rapidly becoming more rigorous and objective. Willie Hennig's work on "Phylogenetic Systematics" (1950; 1966) was becoming more widely known outside Germany, distance-based methods with explicit models of nucleotide and amino acid substitution matured (Jukes and Cantor 1969; Kimura 1980) and foundations for maximum-likelihood approaches were being laid out (Felsenstein 1981). As a result, the ability to simply pontificate one's view of animal relationships was no longer sufficient, characters needed to be presented, and methods explained. Another major advance was the commercial availability of DNA polymerase cloned from *Thermus aquaticus*, or *Taq* polymerase, in 1987. This advance ushered in the "molecular revolution." With *Taq* polymerase, one could rapidly amplify very specific gene fragments. Although the polymerase chain reaction (PCR) opened up a whole new world, getting molecular data in the late 1980s was still often a battle. For example, in the late 1980s and early 1990s, there was only limited information for designing oligonucleotide primers for any given gene; researchers were still learning how to extract quality DNA from, for example, mucous-filled mollusks (Winnepenninckx et al. 1993), and light bulbs and cold water were used to regulate temperature in leading thermocyclers (before peltier control).

Lastly, the importance of computer technology cannot be overstated. Prior to the mid 1980s, some cladistic analyses could be done by hand, but as molecular methods improved and computational power grew, the number of taxa and characters that could be employed in analyses greatly expanded. Despite, the vast development in the systematics, we are still limited by computation (e.g., Whelan et al. 2015).

At about the same time as these advances, Field et al.'s paper "Molecular phylogeny of the animal kingdom" was published in *Science* (1988). This paper employed approaches being used on microbes and sequenced RNA, not DNA, of the nuclear small ribosomal subunit gene. At the time of publication, the paper was criticized because results were not consistent with what was "known" and there were some differences in relationships among results within the paper. Although previous work had examined protein data across a variety of phyla, the Field et al. paper ushered in a new era for metazoan phylogenetics with sequencing of nucleotides. Methodological advancements since this paper have allowed progressive confirmation or rejection of hypotheses resulting in, more or less, three phases of elucidating the animal tree of life.

1988–1997—the early years

As mentioned earlier, in the late 1980s, molecular systematics was just taking off and the initial Field et al. paper was met with both skepticism and excitement. Of course, there were reanalyses of that paper (e.g., Lake 1990) which seemed to clarify results of the 18S data, but these studies also showed phylogenetic methods for analyses of molecular data was in its infancy. Subsequently, the first wave of molecular papers on deep animal relationships focused on phyla that previously had been problematic to place based on morphology (e.g., pentastomids— Abele et al. 1989; onychophorans—Ballard et al. 1992; chaetognaths—Telford and Holland 1993; Wada and Satoh 1994). At the same time, there was an effort to more thoroughly understand the ribosomal genes and their utility for animal phylogeny (Hillis and Dixon 1991; Halanych 1991). The rate of molecular systematic publications addressing deep animal phylogeny was, however, relatively slow for the first 10 years after the Field et al. contribution.

As molecular work was taking off, morphologists also renewed their efforts to examine animal relationships on a number of fronts. Two highly anticipated contributions were Pat Willmer's book *Invertebrate relationships*: *patterns in animal evolution* (1990) and the first edition of Brusca and Brusca's *Invertebrates* (1990). The Willmer book provided a concise review of major evolutionary hypotheses of animal phylogeny by examining different animal groups in turn. However, the last chapter, which sought to synthesize animal relationships, was an unmitigated failure as no clear methodological approach was used to test alternative ideas. The chapter speculated that animals were a polyphyletic assemblage with multiple independent origins from protists, which, even using evidence available at the time, was highly unlikely. The Brusca and Brusca text challenged other invertebrate texts, by including a cladogram depicting synapomorphies for the largely traditional tree presented, moving students to a tree-thinking mindset. Willmer's and Brusca and Brusca's works received more attention than previous efforts on animal phylogeny in large part because they were published after the Field et al. paper. Importantly, Peter Ax, a German meiofauna researcher, had been promoting a cladistic approach to animal phylogeny several years prior to these works (e.g., Ax's 1987). Similarly, Claus Nielsen (1985; 1987) had been coding characters related to ciliary structure to look at animal phylogeny, but he did not use an explicit method to analyze the coded characters. Meglitsch and Schram (1991) also produced a morphological cladistic tree of animal relationships. Perhaps the most important of these morphological cladistics efforts was Eernisse et al.'s (1992) reanalyses of the Meglitsch and Schram dataset (also see Backeljau et al. 1993). For once, the tree was not just a rehash of the traditional viewpoint and more care was placed on character choice and evaluation. Eernisse et al. found that the Eutrochozoan hypothesis, annelids, and mollusks close together, was supported over the more popular Articulata hypothesis, an annelid-arthropod sister relationship.

As an aside, this is also how my career in examining animal phylogeny started. During my first year of graduate school at the University of Texas, I enrolled in a course to collect data and reconstruct animal phylogeny offered by Drs. Tim Rowe and Craig Pease in the Spring of 1989, an outcome of the Field et al. paper. In 1990, I submitted a manuscript on a cladistic analysis of animals which was rejected in part due to using weighted characters in my analyses. Not knowing better and making a rookie mistake, I did not revise and resubmit the paper. That same year, I gave my first scientific talk on this topic to a packed room at The American Society of Zoologists and The Society for Systematic Zoologists meetings in San Antonio, Texas. Unbeknownst to me, Rick Brusca and Fred Schram (authors of leading Invertebrate textbooks at the time) were in the room. One of them asked me how my analyses were different than that of Brusca and Brusca. Not knowing who they were, I proceeded to explain how the Brusca and Brusca cladogram was clearly contrived for teaching and not a proper analyses as there was no homoplasy on the tree. Perhaps an (in)auspicious start to my graduate career.

In the mid-1990s, papers began to come out that more radically reshaped views on animal evolution. I had been working in David Hillis's lab using the 18S and 28S nuclear rDNA genes to place lophophorate taxa (brachiopods, bryozoans, and phoronids) and the pterobranch hemichordates, when I invited Jim Lake to come and give a seminar,

leading the collaboration that resulted in the Halanych et al. (1995) Lophotrochozoa paper. This work place lophophorate taxa well within protostomes and thus challenging long-held views about the conservative nature of embryological patterns (e.g., cleavage pattern, cell fate, coelom formation). Additionally, the placement questioned the value of ciliary characters (see Halanych 1996a) and supported the recent finding of Eernisse et al. for Eutrochozoa. Later the same year, Winnepenninckx et al. (1995b) used 18S data to show that "Aschelminthes," or pseudocoelomate taxa, were polyphyletic, and thus began debunking the notion that overall body architecture as a meaningful phylogenetic character. In 1 year, two of the major premises perpetuated from Hyman, importance of embryology and overall body structure for inferring relationships, were challenged.

For the most part, lophophorates and aschelminthes (except nematodes) did not draw lots of attention from most biologists. When Aguinaldo et al. (1997) showed that nematodes (with *Caenorhabditis elegans*) and arthropods (with *Drosophila melanogaster*) were within the Ecdysozoa clade and much more closely related than previously suspected, the importance of a sound phylogenetic understanding of animal phylogeny became most apparent to a broad scientific community. Prior to this paper, researchers assumed that commonalities observed in round worms and fruits flies were representative of triploblastic, or bilaterian, animals. Given the large sums of funding spent on these model systems, the ability of researchers to extrapolate information across animals was questionable because of closer than suspected relationship between *Drosophila* and *Caenorhabditis*. Noteworthy, almost 20 years after this major revelation of the limited ability to extrapolate from of these model systems, we still lack a comparable model system in Lophotrochozoa.

In addition to the Lophotrochozoa and Ecdysozoa papers, the mid to late 1990s produced several studies that continued to refine the developing molecular phylogenetic view: relationships of vermiform phyla (Winnepenninckx et al. 1995a), a tardigrade-arthropod association (Garey et al. 1996a; Giribet et al. 1996), acathocephalan parasites within rotifers (Garey et al. 1996b), and deuterostomes (Halanych 1995; Swalla et al. 2000). Whereas all of these papers were based on 18S rDNA gene data, work on polychaete annelids was beginning to use other markers. For example, data from elongation factor 1-alpha (McHugh 1997; Kojima 1998) and complete mitochondrial genomes (Boore and Brown 1994; 2000) were beginning to show that polychaetes were a paraphyletic group that included vestimentiferans worms and clitellates. In the 10 years that had passed since the Field et al. paper, a number of papers based on molecular data came out that conflicted with traditional views of animal phylogeny. With few exceptions (e.g., Eernisse et al. 1992), the cladistic morphological analyses were largely confirming traditional views. Although molecular systematics had matured considerably, issues with analyses of nucleotide data were a concern. Many of analyses from the early years had very limited taxon sampling, long branch attraction was known to cause issues (objective test for long branch attraction were lacking), 18S topologies failed to recover monophyly of well support groups (e.g., mollusks, Passamaneck et al. 2004).

Additionally, debates were raging of what type of phylogenetic methods (neighbor joining, parsimony, likelihood) should be used (Felsenstein 2001). In the mid to late 1980s, distance-based approaches such as neighbor-joining were common place, but by 1990, parsimony was in wide use for both molecular and morphological data only to be supplanted by maximum likelihood in the mid to late 1990s. The magnitude of the hypothesized changes to our understanding of animal evolution, combined with rapid developments in phylogenetic and molecular methods, left many skeptical and unsure of how to interpret results. Whereas morphological analyses often just confirmed traditional view points, several molecular studies were implying new relationships or supporting old hypotheses that were less popular. By the late 1990s, many wanted either confirmation of molecular-based hypotheses (e.g., Lophotrochozoa and Edysozoa) or studies explaining why the molecular analyses were wrong.

Era of confirmation 1998–2005

In addition to molecular systematics, growing research on the evolution of developmental mechanisms, or evo-devo, and availability of new fossils from Greenland (Sirius Passet) and China (Doushantuo and Chengjiang) started to impact our understanding of animal evolution. For example, Conway Morris and Peel (1995) had hypothesized that fossils halkieriids were a stem group Eutrochozoan animals close to mollusks and brachiopods, fossil embryos provided a glimpse into early embryology (Bengston and Zhao 1997), and early fossils refined understanding of chordate origins (Shu et al. 1999). Importantly China had become more open to Western scientists starting a steady stream of discoveries of Ediacarian and early Cambrian fossils that were informative in terms of morphology, but did little to resolve relationships among animal phyla. The study of the evolution of developmental mechanisms (evo-devo) on the other hand, made considerable contributions to phylogenetic understanding. As this field was taking off, several researchers thought that different genes accounted for variation in body form across phyla. Instead common sets of genes and machinery were found to control metazoan early development, and more specifically, development of bilaterians (Raff 1996; Wilkins 2002). The homeobox containing transcription factors of the HOX family of genes were one of the first sets of developmental genes to be studied across a broad array of animals. The conserved nature of the

homeobox allowed use of degenerate oligonucleotide primers spanning organismal and gene diversity. This led to several papers out of André Adoutte's lab, among others, using Hox genes for phylogenetic inference (e.g., Balavoine 1997; de Rosa et al. 1999; Balavoine et al. 2002). The de Rosa et al. (1999) paper was particularly important as it showed that Hox genes supported the Lophotrochozoa and Ecdysozoa hypotheses. Whereas 18S rDNA studies compared variation within a given gene, the Hox work revealed that the three major bilaterian clades (Lophotrochozoa, Ecdysozoa, and Deuterostomia) had slightly different compliments of Hox genes; that is, some orthologs are clade-specific. Some still considered embryological characters to faithfully preserve phylogenetic signal, and thus confirmation of the 18S-based hypotheses by genes involved in embryology may have lead to wider acceptance of the new view of animal phylogeny.

During this period, several trends occurred in metazoan phylogenetics. First, while important 18S-based studies continued (e.g., Collins 1998, Kim et al. 1999; Borchiellini et al. 2001), and new markers were being developed and employed (e.g., 28S—Telford et al. 2003; Mallatt et al. 2004; EF-1alpha McHugh 1997; Kojima et al. 1998; mtDNA genomes—Boore and Brown 2000; Myosin II—Ruiz-Trillo et al. 2002; Na/K - pump gene Anderson et al. 2004). The move to new markers was accompanied with increasing amounts of data being collected. In particular, taxon sampling greatly increased which provide more accurate phylogenetic reconstructions (see Graybeal 1998; Rannala et al. 1998). Increased taxon sampling was facilitated by better sequencing technology ("automated" sequencers came on line, e.g., ABI 373, 377), and molecular approaches were starting to be picked up by traditional systematists that had better access to a broad diversity of species for their taxon of interest. Molecular phylogenetic methods were also undergoing rapid development at this time and becoming much more reliable (eg. Huelsenbeck and Rannala 1997; Swofford et al. 2001; Huelsenbeck et al. 2001).

Multiple loci and phylogenomics 2005–present

By 2005, the Lophotrochozoa and Ecdysozoa hypotheses were largely confirmed, but several areas of animal phylogeny remained unresolved. Relationships among lophotrochozoan taxa and among major arthropod lineages remained problematic. Interestingly, multigene analyses became the norm, but the diversity of genes used tended to be limited. The most commonly used genes were still the nuclear ribosomal subunits (18S and 28S) with some work using EF-1alpha, myosin, and other single-copy nuclear genes. The H3 histone protein gene was also used in studies, but it lacks phylogenetic information as a result of limited nucleotide variation across the short fragment that has been typically employed (Dinapoli et al. 2006; Yoshizawa and Johnson 2010; Townsend et al. 2012). Mitochondrial genes (CO1, Cyt b, and 16S) and genomes became widely used, but they were typically not employed for deep animal phylogeny because they were too variable for such ancient events. Although many of these gene have proved powerful phylogenetic tools, the main reason that they were repeatedly and continually used was the availability of conserved primers that worked on a variety of taxa.

The rise of automated sequencing allowed collection of large amounts of genetic material without prior knowledge of the nucleotide sequence (i.e., one could obtain nucleotide data without PCR isolation of targets). This ushered in expressed sequence tag (EST) sequencing of messenger RNA fragments as well as shotgun genome sequencing. Philippe et al. (2005) took advantage of these advances to publish the first higher-level metazoan tree based on genomic data. This study also confirmed the monophyly of Ecdysozoa and Lophotrochozoa as well as the sister relationship of these taxa. Later, Dunn et al. (2008) expanded this sampling and again confirmed these hypotheses with deeper taxon sampling. Both of these analyses were based on data collected via Sanger sequencing. Shortly there after, "next-generation sequencing" machines and subsequent high-throughput technology made collection of transcriptome data routine, and de novo genome sequencing more widely available.

Perhaps the most significant finding to result from phylogenomic analyses is the placement of ctenophores as sister to all other animals. Although shown in Dunn et al. (2008), the result was more thoroughly considered in Hejnol et al. (2009) and then confirmed by Ryan et al. (2013) and Moroz et al. (2014, also see Halanych 2015, Whelan et al. 2015, Dunn et al. 2015). As for relationships within poorly resolved regions of the tree, phylogenomic approaches have proved useful for arthropods (Meusemann et al. 2010; von Reumont et al. 2012; but see Regier et al. 2010 for doing it the hard way), deuterostomes (Telford et al. 2014, Cannon et al. 2014) and has provided utility within lophortochozoans (e.g., Kocot et al. 2011; Smith et al. 2011; Weigert et al. 2015).

Despite advances over the last three decades, there are still regions of the animal tree that have been difficult to resolve. The base of the animal tree and placement of acoels are coming more into focus with recent work, but relationships among Lophotrochozoa are still problematic. In particular, these regions of the tree are characterized by presumed rapid radiations among taxa (meaning little information was likely captured during cladogenic events) and/or long branched taxa. Also taxonomic sampling in these regions of the tree tends to be very limited. Moreover, finding informative morphological synapomorphies that span phyla is problematic. Although improved phylogenetic methods and additional taxon sampling may clarify relationships, we have to realize that there are regions of the tree that may be impossible to resolve with complete confidence.

Altering our understanding

There is little doubt that molecular approaches have radically changed our understanding of animal phylogeny in the last 20 years. Nonetheless, some implications of the new understanding of phylogeny are still working their way through the biological community, and will be for some time. As mentioned earlier, prior to the Field et al. paper views on animal phylogeny were based on assumptions about complexity, morphology, and embryology, many of which have been revealed as incompatible with our current phylogenetic understanding. Whereas some might suggest that such assumptions are no longer an issue, reactions (e.g., Jékely et al. 2015, Pisani et al. 2015) to the recent ctenophore genome papers (Ryan et al. 2013; Moroz et al. 2014) suggested otherwise. One implication of the recent ctenophore data is that neurons in ctenophores and other animals likely have independent origins. At meetings and workshops, several colleagues have voiced opinions basically stating that neurons could not have evolved twice because they are too complex. Similarly, after publication of the lophotrochozoan and ecdysozoan papers, prominent morphologists and developmental biologist insisted segmentation was too complex or too important of a feature to have arisen independently in annelids and arthropods. The irony in such statements is that they are often non-scientific and lack objective methods for distinguishing support for alternative hypothesis (as in Jékely et al. 2015, Pisani et al. 2015), and they fail to acknowledge that highly complex features can be repeatedly produced by evolution (e.g., flagella or camera-style eyes). As has been shown for such features, evolution can produce remarkable complexity.

Moreover, the assumption that overall body structure is representative of phylogeny is clearly incorrect. Body structure (including degree of coelom development, segmentation, appendages, and sensory organs, etc.) is a product of evolutionary selection on functional morphology. Over the course of tens to hundreds of millions of years, this selection can lead to profoundly different architectures or incredible convergences. For example, in contrast to the traditional view of animal evolution, we know that pseudocoelomate animals, are spread across the animal tree (Winnepenninckx et al. 1995b; Halanych 2004; Dunn et al. 2008; Kocot et al. 2010; Struck et al. 2014). We also know that segmentation is evolutionarily plastic as it has been lost or modified repeatedly in annelids (Halanych et al. 2002; Struck et al. 2007; Weigert et al. 2014; Struck et al. 2015).

Embryological and developmental patterns have a long history in interpreting animal phylogeny dating back to von Baer who influenced Darwin and Haeckel (see Halanych 2013). Unfortunately, much of this interpretation is fraught with overgeneralization where a few exemplar taxa are used to represent broad taxonomic groups and the assumption that planktotrophic larvae are representative of diverse taxa and ancestral in form. This ancestral nature of planktotrophic larva maybe likely correct for several taxa, but the assumption has only rigorously been tested with appropriate methods in a few cases, (e.g., Wray 1996). The classic embryological characters of blastopore fate, cleavage pattern, and cell fate (regulative vs mosaic) are evolutionarily labile (for example, due to the amount of yolk in the egg). Perhaps the biggest contradictions to traditional notions of embryology's role in phylogenetic inference were molecular works placing chaetognaths, with purported deuterostome-like embryology, outside of deuterostome and near the base of lophotrochozoans (Telford and Holland 1993; Wada and Satoh 1994; Halanych 1996b; Matus et al. 2006; Marlétaz et al. 2006), and embryologically unique brachiopods, phoronids, and bryozoans in the middle of protostomes (Halanych et al. 1995; Mackey et al. 1996).

Particularly problematic is treatment of spiral cleavage and use of the term "Spiralia." Although the term spiral cleavage has a specific meeting relating to the axis of cell division in the embryo, the application of that term has been wildly inconsistent. Traditionally, protostomes were broadly considered to be spiral cleavers and the term "Spiralia" was essentially equated to Protostomia (Siewing 1976; 1980; Wilmer 1990). Interestingly, arthropods were considered to have spiral cleavage (Anderson 1973; Costello and Henley 1976), a conclusion that was the result of the assumed homology of segmentation placing arthropods next to annelids as members of the Articulata. Of course, we now recognize that arthropods, and the broader ecdysozoans, do not have spiral cleavage. In another iteration the term "Spiralia" was applied to a subgroup of the Lophotrochozoa defined by Peterson and Eernisse (2001) to include ectoprocts, platyzoans, and trochozoans, but excluding brachiopods and phoronids. Then in 2002, Giribet proposed yet another definition of the term that stated "Spiralia" was comprised of Lophotrochozoa and Platyzoa. A footnote was provided to clarify how the term was distinct from Lophotrochozoa, but Giribet did not acknowledge the Peterson and Eernisse (2001) definition or, more importantly, the revision to the term Lophotrochozoa that Aguinaldo et al. proposed in 1997. This oversight was apparently noticed in 2008 when Giribet equated "Spiralia" to Lophotrochozoa, thus again changing the definition of the term. Since then, the term "Spiralia" has been used in a number of papers to represent different membership (e.g., Struck et al. 2014; Laumer et al. 2015). Given that (1) spiral cleavage patterns and fate maps are not as conservative as typically assumed (van den Biggelaar et al. 1997; Seaver 2014), (2) spiral cleavage was previously applied to all protostome animals (albeit incorrectly), and (3) use of the name "Spiralia" lacks consistent use and has multiple definitions (some identical to Lophotrochozoa), use of the term "Spiralia" only breeds

confusion and inaccuracy in the literature and should be avoided as a clade name.

Considerations for animal phylogenetic reconstruction

Ideal characters for phylogenetic reconstruction (1) can be easily scored by an investigator, (2) have a known range of possibilities, (3) contain the appropriate phylogenetic signal, and (4) can be easily applied to all taxa under examination. Jenner (2002) has previously critiqued the non-critical recycling of morphological and developmental characters, but is it important to further consider some of the reasons deep metazoan phylogeny is difficult, if not impossible, to resolve with morphological and developmental data. As mentioned earlier, organisms were previously placed in separate phyla, precisely because there was not enough evidence to unambiguously show affiliations to other phyla. Whereas with nucleotide data (or inferred amino acid data) we know the range of possible scores for a given character (4 for nucleotide, and 20 for amino acids), the range of possible states is completely unknown, and arguably not knowable, with current approaches for most non-molecular characters. Having a known set of possible character states allow calculation of possible random convergences, reversions, or other forms of homoplasy. Thus such noise can be accounted for during phylogeny reconstruction. Perhaps more importantly, characters for nucleotide data can be objectively identified and scored once a gene or set of genes has been chosen based on objective criteria (e.g., signal to noise ratio). In contrast, researchers may disagree a priori as to which non-molecular characters should be employed in analyses, and may disagree with how those characters are scored making the process subjective. Also morphological characters can be hard to score across phyla, and assumptions about primary homology of characters and polarity of characters can be problematic. In retrospect, the major reason some of the first molecular phylogenetic analyses of higher-level animal phylogeny were able to arrive at the Lophotrochozoa and Ecdysozoa hypotheses is because they were free of traditional assumptions and constraints mandated by the traditional assumptions of animal phylogeny based on morphology and embryology. However, if we want to understand the evolutionary patterns across animal lineages, then obtaining detailed comparative morphological and embryological data across several taxa is required. As we move forward with the deep-animal tree, we should not employ morphology and developmental data to reconstruct the tree. There is too much historical baggage and subjectivity with these data. We should reconstruct the tree with molecular data and then use that tree to independently interpret the morphology and development in light of that tree.

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