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Twenty years into the "new animal phylogeny": Changes and challenges

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Despite an inflated number of current publications that appear in an ever increasing number of scientific journals in the grand field of evolutionary biology, it appears that the half-life of most recent studies (and thus their long-term impact) does not live up to the sheer quantity of papers produced. Thereby, phylogenetic systematics, i.e., the study of the interrelationships and the evolutionary history of organisms, constitutes a particularly drastic example, with a vast number of articles constantly appearing that aim to reveal organismal relationships on almost all phyletic levels. This is undoubtedly the consequence of a shift of the raw data that are used to obtain these phylogenies from morphological characters to (gene) sequences, with the amount of the latter still growing exponentially. Accordingly, what once required taxonomical and morphological expertise as well as a sound and thorough application of basal (albeit, of course, hypothesis-laden) skills such as Remane's criteria of homology (Remane 1952, 1955) in order to produce a data matrix for a phylogenetic analysis in the Hennigian sense (Hennig 1966), the processing of the sequence data that are used today is largely left to computer programs and specifically chosen algorithms. Thus, with an increasing complexity of the respective in silico applications, it becomes more and more difficult for many users to critically review the calculation processes that produce a given phylogenetic tree. In other words: How certain can we be that, e.g., orthology assessments of the gene sequences used in a

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phylogenetic analysis are correct? How certain can we be that the—obviously true—phylogenetic signal that is hidden in the data is correctly interpreted by all the in silico steps downstream of the actual data (i.e., sequence) acquisition? Not to mention the problems with sequencing errors that may occur prior to any computerized analysis.

That such concerns are not merely hypothetical has been shown by several examples. As such, different phylogenetic trees based on (near) identical molecular datasets were produced that largely depended on the in silico tools used (e.g., substitution models applied to the analyses) and/or the outgroups that were chosen (see, e.g., discussion and re-analysis of studies by Ryan et al. 2013 and Moroz et al. 2014 in Pisani et al. 2015). While an a posteriori test as to whether or not the obtained molecular phylogenies make "biological sense" has been suggested as a potential solution to that problem (see Wägele 2005), the subjectivity of such a test is obvious and somewhat contradicts the claim for objectiveness that is often considered one of the major advantages of molecular-based phylogenies.

Apart from such problems and inconsistencies that are intrinsic to in silico-based analyses, another, and so far often ignored, issue that is likely to become more evident the larger the molecular datasets that produce given phylogenetic trees are, is that of the decreasing possibility of verification and falsification, a basic requirement of any scientific discipline. We have already entered an era where thousands of genes from dozens of species are used in phylogenetic analyses (e.g., Misof et al. 2014), and only a limited number of people have currently access to computational power with the required capacity to perform such often weeks- or months-long calculations. Accordingly, it seems as if we are indeed reaching the borders of basic scientific principles where colleagues can freely redo analyses published by their peers. There is a true danger that for many, seeing a published tree

will frankly mean to believe it (or not), with very limited argumentational power to question or even discuss it.

Irrespective of such concerns, there is no doubt that the past 20 years have revolutionized the way as to how we picture animal interrelationships today, with all the associated consequences concerning character evolution, ground pattern reconstruction, and the like. Many of us interested in animal phylogeny will probably agree that two studies from the mid-1990s that proposed two protostomian sister clades, the Lophotrochozoa and the Ecdysozoa, marked the dawning of a new age in our quest to reconstruct the animal tree of life (Halanych et al. 1995, Aguinaldo et al. 1997). The resulting rejection of the hitherto-assumed monophyletic Articulata that had comprised the annelids and arthropods, together with the implied major consequences for the evolution of several key morphological traits, were initially hard to believe for many. For example, all of a sudden we had to assume that segmented bodies can evolve independently—or, alternatively, that a segmented body plan had already been established at the base of the protostomes, with numerous independent losses in the vast majority of animal phyla. Despite these drastic consequences for the evolutionary reconstruction of animal ancestry, this novel "Lophotrochozoa-Ecdysozoa concept" gained almost undisputed support by subsequent molecular-based phylogenetic analyses. For a detailed account as to how Lophotrochozoa came into being, see the paper by Kenneth Halanych in this edition.

With the erection of Lophotrochozoa and Ecdysozoa, considerable interest arose as to how the proposed subclades of these "superphyla" may be related to each other. Questions concerning the sister group of the segmented annelids or the fantastically diverse mollusks remain unsettled until today, and even the intrarelationships of the subordinate taxa within these phyla remain highly controversial (e.g., Kocot et al. 2011, Smith et al. 2011; see also discussion in Haszprunar & Wanninger 2012). The same is true for numerous smaller, little investigated groups such as the gnathiferans or the more recently proposed monophyletic assemblages such as Platyzoa, Polyzoa, or Rouphozoa. Kevin Kocot reviews here the current state-of-the-art of lophotrochozoan intrarelationships and weighs evidence in favor and against some key phylogenetic issues of Lophotrochozoa, thereby also taking into account traditional views such as the monophyly of the lophophorates.

Although their monophyletic status has generally been accepted for a long time, the relationships within Annelida have remained an enigma for many decades. Here, with the aid of phylogenomics, an important breakthrough has recently been achieved, which resulted in the erection of a dichotomous split that divides most annelid groups into two sister clades, Errantia (largely free-living animals) and Sedentaria (mostly tube-forming organisms or sediment dwellers). The basal branches of the annelid tree outside the Sedentaria + Errantia houses some morphologically highly distinct forms, such as the chaetopterids and the amphinomids, that appear very different from the traditional view of simple built, homonomously segmented "archiannelids" as basal annelid offshoots. However, while the issue concerning the morphological ground pattern of the annelids may keep continuing for a while, the inclusion of representatives that are unsegmented as adults within Annelida, such as the sipunculans, echiurans, or myzostomids, is now widely accepted and has in parts also been corroborated by developmental studies (Hessling 2002, Hessling & Westheide 2002, Kristof et al. 2008). Anne Weigert and Christoph Bleidorn provide herein a concise summary of the state-of-the-art of our understanding of annelid phylogeny with a specific focus on the evolutionary implications of these novel findings.

As sequence data became available for an ever increasing number of taxa, the focus was also directed toward the phylogenetic relationships of traditionally difficult taxa. This led to a novel assessment of a group of once-thought platyhelminth flatworms, the acoels (or acoelomorphs). As already previously suggested by morphological analyses (e.g., Haszprunar 1996), this group of worms shows considerable differences to the "classical" platyhelminths, including a unique type of "duet spiral cleavage." When included in a larger phylogenetic analysis using 18S rRNA sequence data at the turn of the millennium, it was found that not only did the acoelomorphs fall outside the Platyhelminthes, but instead formed a monophylum residing at the base of the Bilateria (Ruiz-Trillo et al. 1999). Later, after some years of heated debate, it appeared more and more likely that another cryptic taxon, the xenoturbellids, probably cluster with the acoelomorphs to form the Xenoturbellida (Hejnol et al. 2009). To date, these elusive creatures appear not to have fully found their definite resting place in the tree of life yet, because an alternative view suggests placement of the phylum at the base of the deuterostomes (Philippe et al. 2011). However, a most recent description of four novel xenoturbellid species, and their inclusion in a molecular phylogenetic analysis, supports the "Xenacoelomorpha as basal bilaterians" view (Rouse et al. 2016). Gerhard Haszprunar as well as Iñaki Ruiz-Trillo and Jordi Paps present their views on the topic, which remains of prime importance for any attempts to reconstruct the scenario at the dawn of bilaterian evolution.

Outside the protostomes and the basal-branching metazoans, research into the phylogenetic affinities and evolutionary history of the various deuterostome clades has been equally vibrant. Probably because we, as chordates, are part of this large and diverse taxon, data on the deuterostomes in general, and the chordates in particular, have received considerable attention. Thereby, combined analyses of fossil finds with phylogenomics and dating of phylogenetic splits using molecular clock models have resulted in detailed hypotheses as to how the various subclades are related to each other, but also when the respective

splits in the deuterostome tree occurred. Kevin Peterson and Douglas Eernisse summarize the past 25 years of research into deuterostome phylogenetics and also incorporate some of the recent comparative developmental data. Thus, the resulting picture that emerged strongly suggests monophyly of the Chordata (vertebrates + tunicates together as Olfactores which in turn is the sister group to the cephalochordates) as well as the Ambulacraria (hemichordates and echinoderms), with the three lophophorate phyla (Ectoprocta, Phoronida, and Brachiopoda) neither being a member of nor a transitory branch toward the Deuterostomia. As one of several significant consequences of such a scenario, this renders the evolution of tentacle-based filter feeding between the lophophorates and the respective ambulacrarians a prime example of convergent evolution.

Obviously, we are far from an overall agreement as to how recent and extinct animals are related to each other and which evolutionary pathways they followed. No doubt, however, that the amount of sequence data that will be used in future phylogenetic analyses will keep increasing. Accordingly, phylogeneticists are confronted with key issues such as correct (unbiased, broad) taxon sampling that in particular includes species that are little known, hard to determine, and difficult to collect. This will result in a definite need for taxonomic and morphological expertise on a broad scale (for incorporating fossils into phylogenetic analyses, morphology will even remain the only way to generate relevant data until means to extract nucleotide sequences from animals as old as 600 million years become available). These and other foreseeable, in silico-related challenges, such as the development of novel (faster and more accurate) algorithms, are addressed in the closing chapter of this volume by Gonzalo Giribet.

I thank all contributing authors for sharing their thoughts and insights into specific aspects of past and contemporary research into animal phylogeny. It is still an area of ongoing vibrant discussions with novel hypotheses continuously emerging and traditional ones being either rejected or supported. What could be a better example for this than the current, heated discussion as to whether the sponges or the ctenophores are the earliest branching metazoans (see Ryan et al. 2013 and Moroz et al. 2014 versus Pisani et al. 2015)? Accordingly, while it cannot be expected that most phylogenetic key questions will be settled anytime soon, the obvious importance of a phylogenetic framework for insights into character (state) evolution and transformation, the emergence of evolutionary novelties and character loss, as well as the plasticity of gene expression patterns and the evolution of gene function (including independent events of co-option), will hopefully keep motivating researchers to contribute to our understanding as to how living and extinct organisms are related to each other. The collection of papers in this special issue *The New Animal Phylogeny: The First 20 Years* may be a source of inspiration to do just that.

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