REVIEW

The ctenophore lineage is older than sponges? That cannot be right! Or can it?

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ABSTRACT

Recent phylogenetic analyses resulting from collection of whole genome data suggest that ctenophores, or comb jellies, are sister to all other animals. Even before publication, this result prompted discussion among researchers. Here, I counter common criticisms raised about this result and show that assumptions placing sponges as the basal-most extant animal lineage are based on limited evidence and questionable premises. For example, the idea that sponges are simple and the reported similarity of sponge choanocytes to Choanflagellata do not provide useful characters for determining the positions of sponges within the animal tree. Intertwined with discussion of basal metazoan phylogeny is consideration of the evolution of neuronal systems. Recent data show that neural systems of ctenophores are vastly different from those of other animals and use different sets of cellular and genetic mechanisms. Thus, neural systems appear to have at least two independent origins regardless of whether ctenophores or sponges are the earliest branching extant animal lineage.

KEY WORDS: Ctenophora, Porifera, Comb jelly, Phylogeny, Choanocytes, Choanoflagellata, Neural evolution, Metazoan origins

Introduction

Ctenophores, or comb jellies, are among the most enigmatic of animal phyla, whereas poriferans, or sponges, have long been considered among one of the simplest. Gelatinous comb jellies are covered by eight rows of ciliary plates composed of compound cilia, called ctene rows. Despite their gelatinous nature, ctenophores are surprisingly complex, with distinct nerve and muscle cells, although Hyman (Hyman, 1940), and thus most subsequent invertebrate textbooks in North America, considered comb jellies to be of the 'tissue grade' of body construction, which implies, incorrectly, that they lack organs. In contrast, sponges are often considered to lack a distinct body plan and even organized tissues, leading to the idea that they are of the cellular grade of construction. Recent research on cellular biology, development and physiology of sponges is reshaping our ideas about these animals. In particular, studies using transcriptomic and genomic data (Ryan et al., 2013; Moroz et al., 2014) suggest that the ctenophore lineage, not sponges, is sister to all other extant metazoan lineages and that early evolution of structures such as nerves, muscles and sensory structures may be very different from what is traditionally understood.

Phylogenetic hypotheses

Understanding relationships between extant lineages near the base of the animal tree focuses mainly on five taxa: Ctenophora, Porifera,

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Placozoa, Cnidaria and Bilateria. There are no well-supported data that place the cnidarian lineage (anemones, corals and their relatives) or the bilaterian lineage (e.g. flatworms, mollusks, vertebrates, arthropods, annelids) at the base of the animal tree, but debate about lineages that lead to placozoans (small very simple amorphous animals), poriferans and ctenophores has been lively during the last 10 years (Dellaporta et al., 2006; Signorovitch et al., 2007; Dunn et al., 2008; Hejnol et al., 2009; Philippe et al., 2009; Schierwater et al., 2009; Srivastava et al., 2010; Kohn et al., 2012; Nosenko et al., 2013). More complete discussion of hypotheses concerning the evolution of early animals and data is presented in Halanych (Halanych, 2004), Kocot et al. (Kocot et al., 2010) and Ryan et al. (Ryan et al., 2013). Much of this debate has been due to alternative taxonomic placements suggested by molecular data (for which most studies have very limited taxon sampling), whereas the longstanding view based on morphology is that the sponge lineage is the most basal among extant animals (e.g. Nielsen, 2012). The first molecular data to bear on the issue were nuclear ribosomal gene data that usually placed poriferan and ctenophore lineages closest to the base of the animal tree (Field et al., 1988; Collins, 1998; Kim et al., 1999; Podar et al., 2001; Medina et al., 2001). Importantly, placozoans were not included in many of these analyses and the concept of Coelenterata (cnidarians plus ctenophores) was typically not supported (Halanych, 2004). Also, nuclear ribosomal data created a debate as to whether sponges were monophyletic or not (Zrzavy et al., 1998; Medina et al., 2001; Peterson and Eernisse, 2001). The currently favored view is that sponges are monophyletic (Philippe et al., 2009; Ryan et al., 2013; Moroz et al., 2014).

Questions of relationships near the base of the animal tree have been addressed with more recent data including mitochondrial DNA (mtDNA) genomes, whole nuclear genomes and transcriptomic data. Phylogenetic inference based on whole mtDNA genomes does not provide clear support for any one hypothesis. Bilaterians have a circular mtDNA genome that is about 15–17 kb in length with 37 genes (13 protein coding genes, two rDNA genes and 22 tRNA genes) (Boore, 1999; Vallès and Boore, 2006). In contrast, other eukaryotes typically have many more mitochondrial genes and are much larger in size. Animal lineages that branched off near the base of the tree have intermediate sized mitochondrial genomes. Placozoans have the largest mtDNA genome (43,079 bp) in animals and have additional genes not found in other animal mtDNA genomes (Dellaporta et al., 2006; Signorovitch et al., 2007). This observation led Dellaporta et al. (Dellaporta et al., 2006) to suggest that placozoans were the basal-most lineage of extant animals. By way of comparison to bilaterians, some sponges and cnidarians have additional mitochondrial genes (e.g. ATPase 9 and extra tRNAs), some sponges have a slightly larger mtDNA genome (approaching 20 kb) (Lavrov et al., 2005) and some cnidarians have linear mtDNA genomes (Bridge et al., 1992). Ctenophores, however, also have a highly divergent mtDNA genome ~11 kb in length and missing several genes – most tRNAs are absent (Pett et al., 2011;

Kohn et al., 2012). Trees based on mtDNA amino acid datasets seem to be sensitive to taxonomic inclusion or phylogenetic methods. For example, recent analyses by Bernt et al. (Bernt et al., 2013) and Osigus et al. (Osigus et al., 2013) of mitochondrial data show variation and even a lack of monophyly for sponges and for cnidarians in some analyses. To date, mitochondrial information has not provided resolution on the issue of early animal relationships.

Analyses based on nuclear transcriptomic and whole genomic data (e.g. Philippe et al., 2009; Schierwater et al., 2009; Srivastava et al., 2010; Nosenko et al., 2013) show variable results, but most of these analyses have been very taxon limited or lack strong support for the deepest nodes in animal phylogeny. Two recent analyses (Ryan et al., 2013; Moroz et al., 2014), however, show considerable support for ctenophores as the basal-most extant lineage within animals and have somewhat better taxon sampling than previous efforts. Ryan et al.'s paper focused on reporting the genome of the ctenophore *Mnemeopsis leidyi* whereas Moroz et al. focused on the comb jelly *Pleurobrachia backei*. Analyses within these papers (based on large amino acid datasets, analyses of gene content, and explicit likelihood approaches for hypothesis testing) show strong support for the ctenophore lineage as basal. Compared with previous work on this topic, these studies employed many more genes (242 for Ryan et al. and 586 for Moroz et al.) and richer taxon sampling. Both studies explored various phylogenetic approaches to look for biases in data and potential sources of error. Ctenophores remained sister to all other animals for the majority of analyses.

A phylogenetic hypothesis that places ctenophores as sister to all other extant animals is contrary to accepted dogma that places sponges in this position. In considering hypotheses that challenge dogma, stepping back and assessing both novel and traditional hypotheses with equal rigor is often prudent. Based on previous experience, some researchers had difficulty critically evaluating a hypothesis they had assumed to be true for the course of their career (e.g. Articulata hypothesis placing arthropods and annelids as sister taxa). Even before the Moroz et al. (Moroz et al., 2014) paper was published, multiple scientists questioned what was 'wrong' with the analyses − before they saw any data or results. The intention of the present paper is not to argue specifically for or against the poriferans-sister-to-all-other-animals (Porifera-sister) hypothesis or ctenophores-sister-to-all-other-animals (Ctenophora-sister) hypothesis but to question some of assumptions that have been, and will be, made concerning the whether sponges or comb jellies are sister to all other animals.

Sponges are simple

Perhaps the primary reason most people accept the Porifera-sister hypothesis is because sponges are considered to be morphologically simple. For eons, scientists have been thinking about how to arrange organisms based on complexity of body plans. This approach has pervaded how we think about animals since Aristotle's *Scala Naturae* through Linnaeus, Haeckle and Hyman to modern times. Libbie Hyman is particularly relevant here as her compilations of previous works and interpretations of animal biology still dominate many invertebrate texts [at least in North America (e.g. Ruppert et al., 2003; Brusca and Brusca, 2003; Pechenik, 2009)]. Thus, her influence has reached across several generations of biologists. In particular, the way she codified the complexity of animals still has a lingering impact on our understanding. She considered animals as belonging to three grades of body plan construction (cellular-level, tissue-level and organ-level), talked about numbers of tissue layers (diploblast and triploblast) and grouped organism by coeloms (acoel, psuedocoel and eucoel). The cellular level, the simplest and most

basic, was reserved for sponges and placozoans, whereas other animals are at the tissue and organ level of construction.

Hyman's treatment of animal complexity is not at issue here and we now have a much deeper understanding of how animal bodies are put together than was possible in Hyman's day. Sponges are less complex than other animals and are clearly far less complex than ctenophores in terms of cell types and arrangement of cells into larger tissues. What is at issue is the assumption that evolution proceeds from simple to complex (see Halanych, 1998). At this point many readers will be thinking, 'Of course evolution does not always go from simple to complex!' Nonetheless, this assumption is often made without realization, especially when discussed on the grand scale across the diversity of animal life. If we want to understand placement of the sponge lineage relative to the ctenophore lineage, consideration of complexity is not relevant to the discussion. In a modern phylogenetic context, we want to focus on synapomorphies (i.e. shared derived characters) or explicit model-driven phylogenetic reconstruction methodologies (e.g. maximum likelihood, Bayesian inference) of appropriate characters. Importantly, there are several other metazoans that arguably have much more simple forms than sponges and we readily accept them as derived animals. For example, multicellular orthonectids, dicyemids and myxozoans were all previously considered lineages potentially near the base of the animal radiation (Halanych, 2004). Once molecular evidence became available (Katayama et al., 1995; Hanelt et al., 1996; Pawlowski et al., 1996), they were shown to be derived.

Obviously, complexity can be discussed on several different levels. Historically, biologists have considered complexity in terms of morphology, but a recent driver of research among lineages that arose early in metazoan evolution has been interest in understanding genome complexity and gene complement of the common ancestor of early animals. As Moroz et al. (Moroz et al., 2014) and Ryan et al. (Ryan et al., 2013) point out, sponges share a more similar complement of genes and gene systems with other animals than do ctenophores. Discussion of genome complexity, in and of itself, between major animal lineages that diverged 600 million years ago is likely not useful for phylogenetic reconstruction of deep nodes. We know genome size and variation can show impressive changes on short time scales (for example, browse the Animal Genome Size Database, http://www.genomesize.com). Thus, consideration of genomic or morphological complexity tells us more about how the evolution of a given lineage has proceeded rather than deep phylogenetic relationships. However, comparison of orthologous elements within those genomes, or across morphological diversity, is proving valuable for understanding evolutionary history.

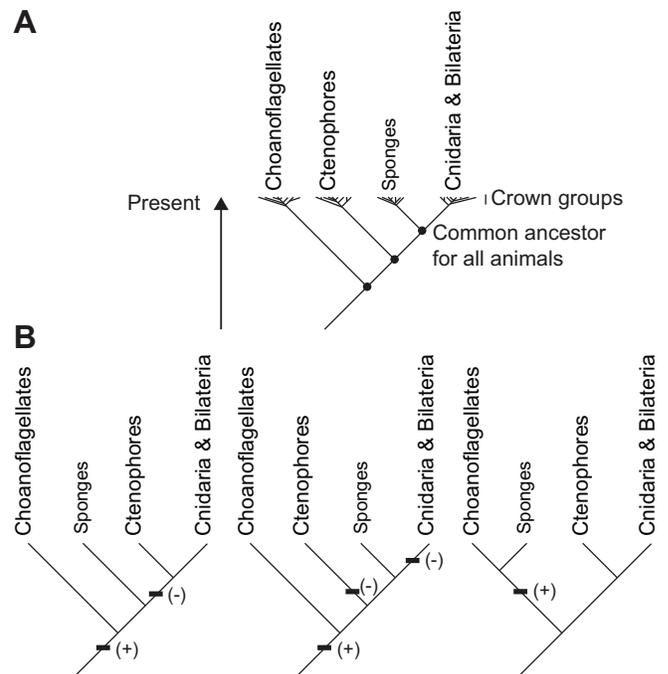### Choanocytes from choanoflagellates

One of the most identifiable characteristics of sponges is the presence of choanocytes. These are cells that possess a flagellum surrounded by microvilli and are used in feeding. Interestingly, they also differentiate into sperm during sexual reproduction (Gaino et al., 1984). Whilst the similarity between sponge choanocytes and choanoflagellates has been long known (James-Clark, 1866), the discovery of multicellular choanoflagellate colonies (Tuzet, 1963) allowed the suggestion that sponges evolved from choanoflagellates to become more widely accepted. The more or less direct relationship between choanocytes and cells of choanoflagellates has long been assumed even though detailed comparisons between these cells are, for the most part, lacking. Maldonado (Maldonado, 2004) covers some of the history of the choanocyte/choanoflagellate homology discussion and points out some interesting, but unlikely,

ideas about the relationship between sponge choanocytes and choanoflagellates. Mah et al. (Mah et al., 2014) compared choanocytes from the sponge *Spongila lacustris* and the choanoflagellate *Monosiga brevicollis* and clarified what other workers missed. To summarize, Mah et al. looked at active feeding, as well as ultrastructural components, in these cells and detailed morphological and functional similarities and differences. They note important functional differences in how the flagellum interacts with the collar in both cells and question whether the observed similarities at the ultrastructure level may be due to selective pressures to optimize feeding efficiency. Selection on ultrastructure and ciliary feeding mechanisms is known in other animals (e.g. Emlet, 1991; Halanych, 1996). After considering their results, Mah et al. concluded, 'that homology [between choanocytes and choanoflagellates] cannot be assumed without question'. Cells that possess a flagellum and are surround by microvilli, collar cells, occur in other organisms as well (e.g. *Phalansterium*). Analyses that compare similar cells outside of poriferans and choanoflagellates may help elucidate how the functional morphology and ultrastructure of these feeding apparatuses were shaped by selective forces acting on efficiency (e.g. Reynolds number, size of particle retention, etc.).

Even if one assumes homology between choanocytes and choanoflagellate cells, the interpretation that sponges evolved as the first metazoans from choanoflagellates is incorrect. Nonetheless, this interpretation seems pervasive. This is well illustrated by everything from lay references (http://en.wikipedia.org/wiki/Choanocyte, 17 July 2014) to the following statement in Brusca and Brusca [see their p. 110 (Brusca and Brusca, 2003)], a leading invertebrate textbook:

> *Most evidence today points to the protist phylum Choanoflagellata as the likely ancestral group from which the Metazoa arose. Choanoflagellates possess collar cells essentially identical to those found in sponges. Genera such as* Proterospongia, Sphaeroeca, *and others are animal-like colonial choanoflagellates and are commonly cited as typifying a potential metazoan precursor.*

This quote, incorrectly, reinforces the idea that modern sponges evolved directly from modern choanoflagellates. Brusca and Brusca do say in the next paragraph that a direct precursor is likely extinct, but the assumption that animals are derived from choanoflagellates is clear. In contrast, these taxa diverged in the Precambrian (prior to 541 million years ago). This viewpoint exemplifies two concepts of evolutionary biology that are often misunderstood (Fig. 1A). (1) Recent taxa share 'common ancestors', and rarely is one taxon directly derived from another. Modern taxa diverged from a common ancestor in the past that possessed characters found in both daughter lineages as well as characters that may not be present in modern lineages. Further, both daughter lineages may not have the same sets of characters or features they inherited from their ancestor, and the ancestor may have been very different from current forms in daughter lineages. (2) Organisms currently on the planet represent 'crown groups'. In other words, most species are the result of relatively recent diversification events as evidenced by the fossil record. The crown-group concept is relevant because ~600 million years have passed between the last common ancestor of Choanoflagellata, Porifera and Ctenophora, but extant species of these taxa are relative young and are likely very derived relative to the common ancestor. Ideally, fossils predating the Choanoflagellata/ Metazoa would help in the interpretation of ancestral features, but such fossils are not likely to be found. These two concepts (shared

Fig. 1. Early animal evolution. (A) Crown groups are typically the result of recent diversifications and lineages diverge from common ancestors. (B) Alternative evolutionary scenarios assuming that choanocytes are homologous to choanoflagellates. The sponge-sister hypothesis requires at least one loss of this character and the Ctenophore-sister hypothesis requires two losses. Only the sponge-sister-to-choanoflagellate topology suggests that the collar cell character is a synapomorphy without homoplasy. This last tree is not supported by current data. Minus signs indicate losses and plus signs indicate gains.

ancestors and crown groups) help clarify that sponges could not have evolved from choanoflagellates, a term we use to delineate an extant crown group. This point is supported by molecular evidence, as choanoflagellates are monophyletic (Carr et al., 2008), not paraphyletic. A paraphyletic grade is the expectation if sponges descended from choanoflagellates.

Moreover, because sponges and ctenophores shared a common ancestor more recently than either did with choanoflagellates (Halanych, 2004; Ryan et al., 2013; Moroz et al., 2014), the collarcells cannot be an unequivocal synapomorphy to show sponges were the first lineage to branch off the animal tree (Fig. 1B). In other words, if choanocytes are homologous (i.e. the same by evolutionary descent) to choanoflagellates, they were lost at least once assuming the sponge-sister hypothesis, or twice assuming the ctenophore-sister hypothesis. Regardless of the number of times choanoflagellate-like cells have been lost in animals, ctenophores and sponges share a more recent common ancestor with each other than with choanoflagellates. Fig. 1B shows that only a tree assuming sponges and choanoflagellates are sister taxa would allow collar cells to be an unequivocal synapomorphy useful for phylogenetic inference. Thus, the similarity assumed between choanocytes and choanoflagellates, based either on current knowledge of their structure or on a parsimony argument based on a length difference of a single step, does not provide a strong argument for the Porifera-sister argument.

Regulation of internal ions

The idea of ctenophores as the basal-most extant lineage in animal phylogeny is controversial in part because of perceived implications

for neural system evolution. Because nerves are polarized, an organism with neurons must be able to maintain an action potential across the cell membrane and thus ions both inside the neuron and in the extracellular matrix must be controlled. In most animals, below epithelial cells is an extracellular matrix or membrane, the basal lamina, that is typically thought of as controlling the movement of substances into and out of an animal's body and as such helps control ionic concentrations of extracellular matrix. [The term 'basal lamina' has a long and convoluted history and is often mixed together with 'basal epithelia', 'basement membrane' or 'basal membrane'. Here, I am not concerned about idiosyncrasies of semantic definitions, but instead ask the question of whether sponges have the propensity to control their intercellular environment.] Sponges are often assumed to be lacking a basal lamina (Mackie, 1984; Cereijido et al., 2004; Srivastava et al., 2010). As a result, I have been subject to arguments that the lack of a basal lamina in sponges attests to the fact that they are basal and that they could not have evolved from an ancestor with nerves. Such arguments assume that functional morphology for osmotic control would have to be gained prior to the evolution of neuronal features, and once osmotic control was obtained it would be too valuable to be lost.

With regards to this argument, the common perception of sponges is incorrect. Despite channeling large amounts of water through their bodies, some sponges do appear to be able to exert ionic control relative to the outside environment and possess an epithelium capable of regulating ionic flow to some degree. Adams et al. (Adams et al., 2010) demonstrate that freshwater haplosclerid sponges possess an epithelium that is able to generate an asymmetrical electrochemical potential. Moreover, resistance to ion passage in these animals was similar to observations from vertebrate tissue. Transmission electron microscopy work in that report appears to confirm the presence of tight junctions [see their fig. 2 (Adams et al., 2010)]. Additionally, evidence is mounting that sponges may have more advanced physiology than previously recognized in that they are capable of some coordinated movement, albeit on a slow time scale (Nickel, 2006; Bond, 2013). Such results imply that physiological state within a sponge body may not be as different from that of other animals as once assumed.

Thus, the argument relating the presence of a basal lamina to neural evolution seems to be based on a flawed assumption about sponge osmotic control. Moreover, the argument essentially boils down to the idea that nerves are so complex (with other specific physiological or morphological requirements like basal lamina) that they could not have evolved twice. This premise of complexity is not defendable in the context of modern evolutionary science.

Long branch attraction

Perhaps the most commonly used excuse to dismiss the tree topologies of Ryan et al. and Moroz et al. (Ryan et al., 2013; Moroz et al., 2014) is to declare that ctenophores, which have elevated rates of nucleotide substitution, are 'obviously' subject to long branch attraction (LBA) (Felsenstein, 1978). The field of molecular systematics has still not devised a good test for detection of LBA, and this is potentially a valid argument. However, a few caveats are needed here. First, in most cases, LBA is often discovered by the addition of more data and more taxa that 'corrects' the tree. In contrast, many prior analyses based on one or a few genes favored the Porifera-sister hypothesis (e.g. Medina et al., 2001; Peterson and Eernisse, 2001; Philippe et al., 2009), and the inclusion of more data, more taxa and more appropriate outgroup testing (Ryan et al., 2013; Moroz et al., 2014) has resulted in support for the Ctenophora-sister hypothesis. There has been some improvement in

testing for LBA. For example, Struck (Struck, 2014) proposed a method for assessing long branches. Whereas the method can identify long branches, determining whether branches are biasing tree reconstruction is still somewhat subjective. Struck showed that ctenophores used in Ryan et al. (Ryan et al. 2013) have longer branches than those of other taxa in their analyses, but additional sampling (e.g. Moroz et al., 2014) helps alleviate this issue. Second, as with any scientific endeavor, there is an expectation that statements can be backed up with results and/or observations. Any valid argument that LBA has influenced topological results should be able to demonstrate that there are problems with substitutional saturation that have caused spurious relationships. One cannot just look at a tree and declare the branches are long because, in LBA situations, attracted branches often appear much shorter. As of this writing, there is no evidence of LBA in the case of ctenophores.

Origins or neural systems

Above, I have detailed some of the discussion about why individuals are reluctant to consider ctenophores as basal. However, one of the main discussions surrounding the Ctenophore-sister hypothesis is whether neural systems have evolved more than once in animals. The question of homology among neural systems is an important one that has the potential to alter our understanding concerning the early evolution of animal life on the planet. To assess homology, we need to be able to show that the same structure(s) descended from a common ancestor. Such an assessment typically combines knowledge of phylogenetic relationships with knowledge of the similarity of the features being examined, which can include development. Even if homologous features are very disparate in two descendants, we can typically identify some similarities or intermediate stages that convince us we are examining the 'same' feature.

Neurons and neural systems are complicated structures and have been defined based on functional grounds (Bullock and Horridge, 1965). As such, neural homology cannot be assumed. Considering the neural system as a single character is grossly over-simplified, and making assessments of gains or losses of 'nerves' or 'neural systems', as a single character, is of little value in helping to understand metazoan evolution. If neurons are homologous across all animals with a neural system, then we expect congruence in mechanisms and structures especially given that bilaterian neural systems seem to be highly evolutionary conserved in function and cellular mechanisms. However, this congruence is not observed. Ctenophores lack many of genes controlling neuronal fate and patterning (e.g. genes encoding neurogenins, *NeuroD*, *Achaete-scute*, *REST*, *HOX*, *Otx*), lack canonical neurotransmitters found in all other animals (or, if present, they are not acting as neurotransmitters) and lack typical neuroreceptors. Moreover, they have a diverse set iGlutamate receptors not found in other animals that presumably act as receptors for L-glutamate, which acts as a neurotransmitter. That said, there are some elements that are similar between ctenophores and other neuron-bearing animals. However, evolutionary studies of development have repeatedly shown that we can expect some cellular and genetic components to predate the evolution of the feature in question. For example, King et al. (King et al., 2003) showed that many genetic mechanisms (cadherins, C-type lectins, etc.) required for multicellularity also arose prior to multicellular animals. Many of the similarities and differences in neural machinery were detailed in Moroz et al. (Moroz et al., 2014) and therefore will not be discussed further here.

The numerous and profound differences in cellular machinery for functional neurons in ctenophores, compared with other animals, question the assumption that these structures are homologous. Some

might argue that the neuron is homologous and that its underlying machinery has changed through the course of evolution, but this is an unlikely scenario. Neuronal machinery is conserved across cnidarians and bilaterians, and the most basic elements of neuronal function and communication are different. (Note that cellular polarization or a voltage potential across the cell is not specific to neurons or even animals.) Interestingly, accepting the premise that the neural systems of ctenophores and other animals are not homologous renders relationships among basal taxa of little consequence for understanding animal neural evolution.

The goals of this paper were (1) to illustrate some of the problems with criticisms that have been espoused against the Ctenophora-sister hypothesis and (2) to challenge researchers to be equally critical of the long-held idea that sponges are basal in animal phylogeny. Given the recent papers of Ryan et al. (Ryan et al., 2013) and Moroz et al. (Moroz et al., 2014), researchers should consider at least two viable hypotheses for relationships of the deepest nodes in the animal tree. Importantly, this controversy is not settled and there will no doubt be future reports on early animal phylogeny. Obviously, more genetic data and data from more taxa are needed but, importantly, development of improved analytical methods (LBA and reconstructing large datasets) will help address this issue. We are entering the age of assessing evolutionary history with complete genomes, but there will still be considerable debates over which genes to use and how to analyze them. In some ways, these issues are more tractable than those of taxon sampling. Ctenophores are a major constituent throughout the world's oceans. However, dwindling oceanographic resources (e.g. ship and submersible time) make accessing offshore deep water, where ctenophores likely have their greatest diversity, extremely challenging.

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