

The hidden biology of sponges and ctenophores

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Animal evolution is often presented as a march toward complexity, with different living animal groups each representing grades of organization that arose through the progressive acquisition of complex traits. There are now many reasons to reject this classical hypothesis. Not only is it incompatible with recent phylogenetic analyses, but it is also an artifact of ‘hidden biology’, that is, blind spots to complex traits in non-model species. A new hypothesis of animal evolution, where many complex traits have been repeatedly gained and lost, is emerging. As we discuss here, key details of this new model hinge on a better understanding of the Porifera and Ctenophora, which have each been hypothesized to be sister to all other animals, but are poorly studied and often misrepresented.

Viewing all animals through a bilaterian lens distorts the view of animal evolution

We have two windows on early animal evolution: fossils and living animal diversity. Bringing living animal diversity to bear on our understanding of early animal evolution, events that happened hundreds of millions of years ago, requires analyses of phylogenetic relations between animals and the description of morphological, developmental, genomic, and physiological traits across a broad diversity of living animals. Extensive progress has been made in recent years on animal phylogeny [1–3], with particular interest in the deepest relations in the animal tree. All known living animals belong to one of five clades: Porifera (sponges), Ctenophora (comb jellies), Placozoa, Cnidaria, and Bilateria. The monophyly (see [Glossary](#)) of each of these clades is broadly supported, but there has been considerable debate about how they are related to each other and, therefore, what the first splits in the animal tree were. Well-sampled recent phylogenetic analyses place either Ctenophora or Porifera as the sister group to the remaining animals ([Figure 1](#)).

To examine the evolutionary implications of these relations we need more than phylogenies: we also need to describe the biology of these animals so that we can map characters onto the phylogeny and reconstruct their evolutionary history. Unfortunately, ctenophores and sponges are among the least-studied animals and much remains

unknown about their morphology, physiology, and molecular biology ([Box 1](#)). We know less about their unique complex traits than we do about the unique complex traits of many bilaterians, and our ignorance likely extends to complex traits that have yet to be discovered ([Box 1](#)). Making matters worse, what is known about ctenophores and sponges is filtered through the lens of bilaterian biology ([Box 1](#)) and often misrepresented ([Boxes 2 and 3](#)). This leaves considerable gaps in our understanding of traits that are key to reconstructing early animal evolution, and the historical focus on studying complex traits found in Bilateria is often misinterpreted as evidence that there are few unique complex traits found in other animals ([Box 1](#)). These gaps must be closed to answer basic questions, such as: what features did the most recent common ancestor of all animals have? In what order and by what mechanisms were complex traits acquired within each lineage? How many times have these traits been gained or lost?

Why do we know so little about sponges and ctenophores?

There are a few reasons why we know so little about sponges and ctenophores, and why what we do know is often confused. First, most research on animals has focused

Glossary

Aboral end: the end of an animal that is opposite its mouth.

Choanoflagellates: eukaryotic organisms that are the closest known relatives of animals. They are collar cells that live singly or in small colonies.

Choanocyte: collar cells of sponges.

Clade: a monophyletic group of organisms.

Collar cell: a cell with a flagellum surrounded by a collar of microvilli. The motion of the flagellum draws water past the microvilli, serving either a feeding or sensory function. Collar cells are found in multiple animals, including sponges, echinoderms, and cnidarians. They are also found outside animals in the choanoflagellates. Although collar cells are superficially similar, their cytoskeletal structure and functional biology are different in different groups of organisms.

Mesenchymal cells: unpolarized cells dispersed in an extracellular matrix.

Mesoglea: a thick extracellular matrix found in many animals, including ctenophores and cnidarian medusae. It is the jelly in jellyfish. Mesoglea has evolved multiple times in distantly related lineages through the expansion of extracellular matrix.

Monophyletic: for a group of organisms to comprise all the descendants of their most recent common ancestor, and no other organisms. Vertebrates are monophyletic. However, invertebrates are not because they exclude vertebrates, which are descended from the most recent common ancestor of invertebrates.

Picoplankton: plankton on the order of 0.2–2 μm (not on the scale of picometers, as the name implies, which are smaller than the diameter of atoms). Picoplankton includes many organisms, such as bacteria and small eukaryotes.

Synapomorphy: evolutionarily derived traits that are shared by multiple organisms and were present in their most recent common ancestor. These are traits that arose on the stem that gave rise to a clade. Hair, for example, is a synapomorphy of mammals.

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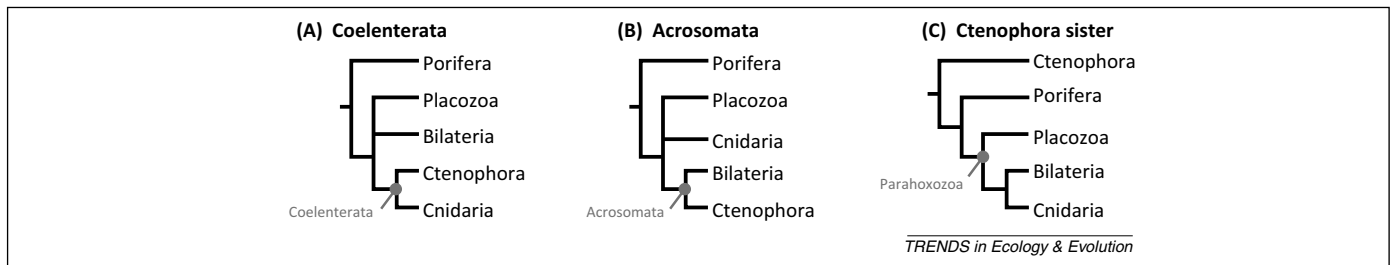


Figure 1. Some of the hypotheses that have been proposed for the phylogenetic relations between sponges, ctenophores, and other animals. **(A)** Porifera is the sister group to all other animals. Ctenophora and Cnidaria are sister groups, forming Coelenterata [14]. **(B)** Porifera is the sister group to all other animals. Ctenophora and Bilateria are sister groups, forming Acrosomata, a relation recovered in some morphological analyses but no molecular analyses [4]. **(C)** Ctenophora is the sister group to all other animals [15–18]. Some analyses that recover this result [17,18] also place Placozoa, Bilateria, and Cnidaria in a clade that has been called ‘Parahoxozoa’ [22].

on Bilateria, while other animals have been relatively neglected. Second, this focus on Bilateria makes it easier to study traits in nonbilaterians that are shared with bilaterians than it is to study nonbilaterian traits that are absent in Bilateria. As a result, there is a large amount of hidden biology in nonbilaterians that we know little about (Box 1). This can give the false impression that nonbilaterians have only a subset of features that are also present in bilaterians, and creates a tendency to shoehorn nonbilaterian biology into bilaterian biology. Third, the diversity within Porifera and Ctenophora is chronically underappreciated. There is no typical species: we must study multiple species strategically sampled across each clade. Fourth, most ctenophore and sponge species are fragile and live in marine environments that are difficult and expensive to study. Bilateria is a diverse group and many of its members are also poorly known, but their closer relation to the best-known model animals means that these shortcomings are, in many cases, easier to overcome and do not have as big an impact on our understanding of the earliest events in animal evolution.

Here, we summarize key aspects of ctenophore and sponge biology, attempt to dispel some common misconceptions about these animals, and explore several aspects of how a biased perspective on complexity in different animal clades affects our understanding of animal evolution.

Phylogenetic placement of sponges and ctenophores

Until recently, there was consensus that Porifera is the sister group to all other animals and the placement of Ctenophora was treated as an independent question. This placement of sponges was based in large part on the hypothesized homology of choanoflagellates and choanocytes, and on the lack in sponges of complex characters that were hypothesized to be synapomorphies of all other animals. Both these morphological arguments have serious problems on closer examination (see sponge section below).

Two hypotheses for the placement of Ctenophora historically have received the most attention [4]: Coelenterata (= Radiata, Figure 1A) [5], with Ctenophora as the sister group to Cnidaria, and Acrosomata (Figure 1B) [6], with Ctenophora as the sister group to Bilateria. Proposed Coelenterata synapomorphies, which reflect an attempt to homologize the body plans of cnidarian medusae and ctenophores, include radial symmetry and the presence of two germ layers separated by a gelatinous extracellular matrix [5]. These proposed Coelenterata synapomorphies

are superficial similarities (see ctenophore section below). For example, ctenophores have rotational symmetry, not radial symmetry, and cnidarians have diverse symmetries [7,8]. Proposed synapomorphies for Acrosomata include sperm and muscle structure [6], although later work brings these interpretations into question [9–11].

Since the first molecular studies to include both sponges and ctenophores [12,13], none have provided strong support for sponges as the sister group to all other animals under all analysis conditions. No molecular studies support Acrosomata, but some have recovered Coelenterata [14]. Recent studies have instead suggested that ctenophores, not sponges, are the sister group to all other animals (Figure 1C) [15–19]. This result is sensitive to taxon sampling (using fewer outgroups generally gives greater support for placing sponges as the sister group to animals), gene sampling, and the analysis method [10,14,17,20,21]. Phylogenetic analyses of gene gain and loss are consistent with this placement of ctenophores [17]. Similar to some earlier studies [13], these also support a clade comprising Placozoa, Cnidaria, and Bilateria that has been named Parahoxozoa [22], although parahox genes may not be a synapomorphy of this group [23].

It is now clear that the phylogenetic placement of Porifera and Ctenophora are not independent questions, and must be addressed together. Improved sampling of genome sequences, coupled with advances in phylogenetic analysis methods, will continue to improve our understanding of these deep relations.

Straightening out ctenophore biology

Ctenophores are marine animals characterized by eight longitudinal rows of ciliary paddles called combs. These are used for locomotion (Figure 2) [24], and give the group its common name: ‘comb jellies’. There are only approximately 200 described species, but many undescribed species are known to exist. Ctenophores are abundant throughout the ocean from pole to pole and down to a depth of at least 7000 m [25]. Nonetheless, they are fragile and gelatinous, which makes them difficult to collect and study. All ctenophores are carnivores. Most are pelagic, while some are benthic. Ctenophore genomes are unique in many respects [17,18]. They lack miRNA and miRNA-processing machinery [26], for example, and their mitochondrial genomes are reduced and derived [27,28].

The gross anatomy of ctenophores (Figure 2) is unlike that of any other animals [29], and it has been interpreted

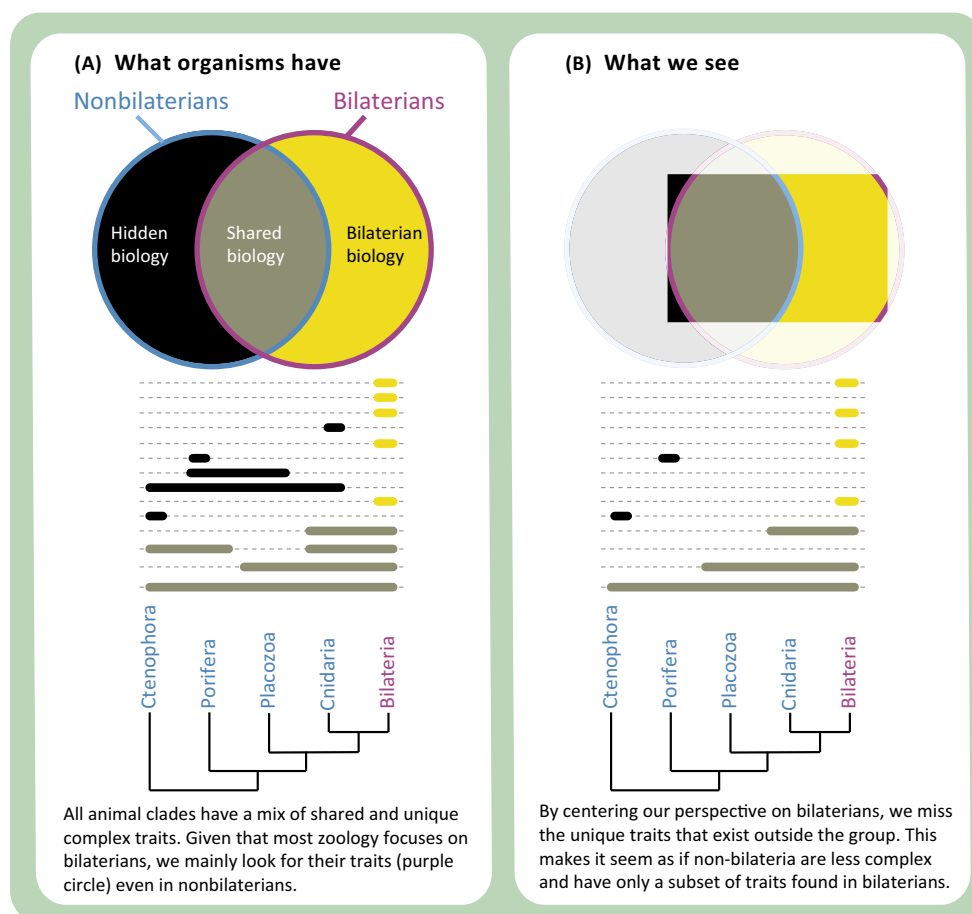
Box 1. The hidden biology of nonbilaterians

All living animals belong to one of five clades: Porifera, Ctenophora, Placozoa, Cnidaria, and Bilateria. To a first approximation, the study of zoology is the study of Bilateria. Humans and all the best-studied model animal species (mouse, *Drosophila melanogaster*, *Caenorhabditis elegans*, and others) are within Bilateria. All of the terrestrial animals, and most freshwater animals that humans regularly encounter are within Bilateria. Most known animal species are within Bilateria (in fact, most known species belong to a single bilaterian clade: Arthropoda). However, if we want to understand the full breadth of animal diversity and the earliest events in animal evolution, we need to study all animals, not just Bilateria.

To a large extent, the focus on the study of bilaterians is a resource allocation decisions: zoologists spend more time and money studying bilaterians than they do nonbilaterians because they comprise most living animal species, including ourselves and the animals we are most familiar with. However, this creates a problem: currently, we see most nonbilaterian biology through the filter of bilaterian biology (Figure 1). All animal clades have a mix of unique traits and traits that are shared with other animals (Figure 1A). It is easier to confirm previously known traits and functions than it is to describe new traits and functions, and most previous studies have been on bilaterians. In addition, many widely used tools and reagents have been optimized for Bilateria. This makes it easier to study the aspects of nonbilaterian biology that are similar to bilaterian biology (Figure 1B, gray), than it is to study traits that are only found outside Bilateria (Figure 1B, black). The candidate gene approach is a widespread example of this. However, just because it is easiest to study the subset of nonbilaterian biology that is shared with bilaterians does not mean that nonbilaterians

only have a subset of bilaterian biology, or that bilaterians are more advanced than other animals. It just means that many of their unique features are currently unknown to us: a 'hidden biology' (Figure 1) that we have only the first glimpses of. This hidden biology includes novel structures and functions, facilitated by novel mechanisms, that are not found in bilaterian model species. It also includes novel mechanisms that underlie shared structures and functions. The problems of hidden biology also extend to nonmodel bilaterians, although it is more severe in nonbilaterians.

What do we miss by letting so much nonbilaterian biology stay hidden? At best, we miss out on some interesting biology, including unique morphology, developmental mechanisms, and physiology. At worst, we are systematically misled. Unfortunately, this is the case when it comes to understanding early animal evolution. It is tempting to mistake our biased perspective (Figure 1B) for the actual distribution of traits (Figure 1A), which gives the false impressions that nonbilaterians have only a subset of the traits found in Bilateria and, therefore, that they are 'lower' or 'simpler'. This in turn plays into the misconception that living animal diversity conforms to a linear aristotelian *scala naturae*, from lower to higher animals, and that animal evolution has proceeded by a step-wise accumulation of complex traits, such that the more distantly an animal is related to Bilateria, the more closely it resembles the most recent common ancestor of all animals. In reality, all living animal lineages have had the same amount of time to evolve since the most recent common ancestor of all animals, and all have gain and lost multiple traits. We need to understand the traits present in all animal groups, not just those that are present in Bilateria, if we are to understand early animal evolution.



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Figure 1. Strong ascertainment bias means that there are many aspects of nonbilaterian biology that we are not equipped to see: we call this 'hidden biology'. This unseen hidden biology leads to a discrepancy between the traits organisms have (A) and the traits we see (B). One consequence is the underestimation of the complexity and diversity of nonbilaterian animals.

Box 2. Ctenophores

Clearing up common misconceptions

- Ctenophores are usually figured upside down. Cydippid ctenophores do not swim with their mouth downward; they swim with their mouth forward and typically rest with their mouth up. Unlike medusae, which swim with the mouth trailing, ctenophores typically forage and transit mouth first.
- Ctenophores share few unique traits with cnidarian medusae, and many of their similarities are superficial and not shared features. For example, most ctenophores and medusae are transparent and gelatinous, but so are many other animals that live in the midwater of the ocean, including salps and pelagic snails.
- Ctenophores do not have radial or bilateral symmetry, they have rotational symmetry. There is no plane that divides them into mirror images, as in animals with bilateral or radial symmetry. Instead, any plane that is drawn through the central oral–aboral axis divides a ctenophore into two halves that are the same, just rotated 180 degrees.
- Ctenophores are not all pelagic (living in the water column), some are benthic (attached to substrates, with tentacles dangling in the water).
- Ctenophores are not ‘primitive’, ‘living fossils’, or the ancestors of other living animals. Neither did humans descend from ctenophores.

Unique traits and hidden biology

- Complex structures made of cilia, including sensory pegs, combs for locomotion, and ‘teeth’ [24];
- Colloblasts: glue cells found only in ctenophores that are used to capture prey;
- Statocysts with unique morphology, function, and development [30];
- Rotational symmetry; and
- Nervous system components.

in many different ways through the centuries. The pharynx is the primary opening to the extensive branched gastrovascular system. Four pairs of endodermal gastrovascular canals extend from the pharynx to underlie the eight comb rows and connect to the central digestive regions via taxon-specific plumbing arrangements (Figure 2E). Many important physiological activities occur in these canals, including gametogenesis, bioluminescence, and the distribution of nutrients. Most species have two tentacles that are anchored with a substantial tentacle bulb on either side of the flattened pharynx. The contractile tentacles usually have side branches with colloblasts, which are specialized prey-capturing adhesive cells only found in ctenophores.

There are several distinctive structures at the aboral end of a ctenophore. These include two small gastrovascular openings, the anal pores (Figure 2D,E). The comb rows radiate from the aboral end near the ‘apical organ’, a domed structure with bundles of cilia supporting suspended carbonate secretions [30] (Figure 2A,B,D). This structure serves primarily as a gravity-sensing organ. Opsins [31] and hypothesized pressure sensors are also found in the apical organ [24]. The name ‘apical organ’ is unfortunate because it perpetuates the misconception that the aboral end is the top of a ctenophore, a false impression that is perpetuated by the fact that most textbooks and other references usually figure ctenophores upside down (Box 2), or that it is homologous to the structure also called an apical organ in larvae of some other animals [32], even though this has not been supported. Although the apical

Box 3. Sponges

Clearing up common misconceptions

- Despite frequent assertions to the contrary, sponges do have epithelia. These seal and control ion flow into the mesohyl.
- Similarities between choanocytes and choanoflagellates, often cited as evidence of homology of these cell types, are largely superficial. There are many key differences, including the tubulin-dense cytoskeleton of choanoflagellates, the labile microvilli of the collar, and interaction of the collar with the flagellum.
- Sponges are often said to lack sensory cells, but cilia that sense and control flow are common across Metazoa, including sponges. Ciliated sensory cells are located on the inside of the osculum. This makes the osculum a sensory organ that provides feedback to the rest of the sponge on water flow through the sponge body. Sponge larvae also have sensory cilia that detect changes in light intensity.
- Sponges have behavior, including contractions of myoid cells. It is too slow to be controlled by rapid neurotransmitter-based signaling mechanisms.
- Sponges use glutamatergic signaling to coordinate slow contractions, and one group, glass sponges, uses calcium/potassium action potentials for rapid arrest of feeding.
- Sponges are not ‘primitive’, ‘living fossils’, or the ancestors of other living animals. Neither did humans descend from sponges.

Unique traits and hidden biology

- Sponges use different minerals (silica or calcium) to build articulated inorganic skeletons comprising spicules. These inorganic mineral skeletons likely arose several times within sponges. Sponges also have organic skeletons, which they use for attachment, support, and even motility.
- Sponges have genes representing many of the families of secreted molecules that are specific to the nervous system of other animals, even though sponges do not have nervous systems. This suggests that these molecules have a different function in sponges, and that sponges have a rich but still unknown secretory biology.
- Sponges develop two- and three-layered larvae largely via methods of sorting at the individual cell level. Control of animal polarity (a polarized aquiferous system and a polarized larva) may involve *Wnt* signaling as in other animals, but the role of other metazoan signaling pathways in development is unclear.
- Sponges have extensive symbioses with microbes, algae, and other animals (including hydrozoans, zoanthids, and isopods). Both sponges and their symbionts produce an array of metabolites, which have a largely unknown function.

organ has been described as a simple ‘brain’ by some [18], there is no indication that it serves to integrate information from other nerve networks or that it is homologous to bilaterian brains. High concentrations of neurons are also found near the mouth and tentacle bulbs [33].

Ctenophores have subtle asymmetries (Figure 2D,E), which require that half of the animal be rotated 180 degrees about the oral–aboral axis for one half to match the other (Figure 2E). This is called rotational symmetry, and is different from the bilateral symmetry found in many other animals (including bilaterians and cnidarians), which requires that the halves to be reflected to match. The anal pores, pole plates (sensory fields on either side of the apical organ), and auricles (ciliated paddles at the oral end of lobate ctenophores) can all show rotational handedness. This distinct pattern may have its roots as early as the third division during development [34].

Ctenophores have two primary tissue layers, the outer ectoderm and inner endoderm, which sandwich

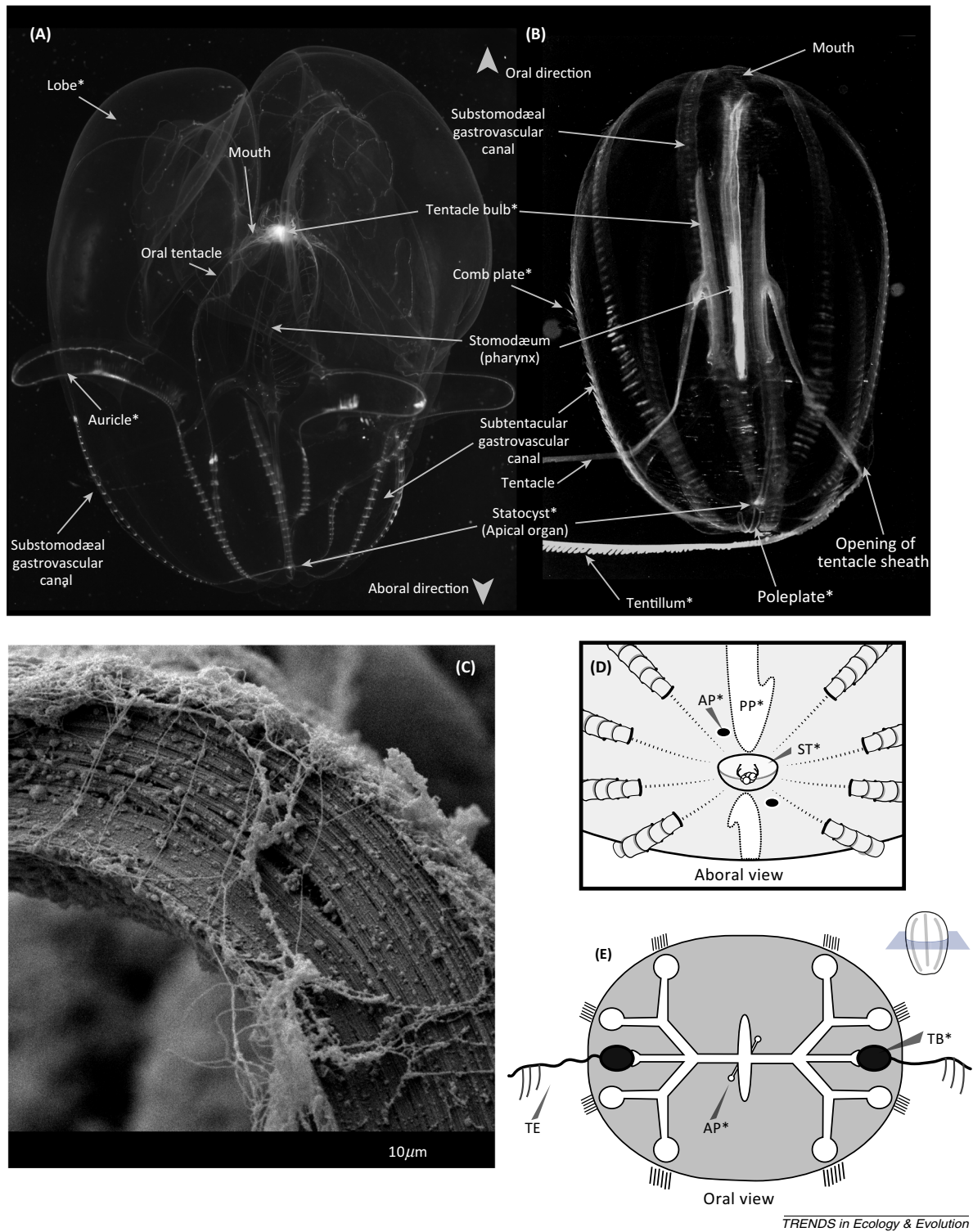


Figure 2. Ctenophore gross anatomy and distinguishing features. Traits unique to ctenophores, or with unique components, are marked with an asterisk. (A) Lobate (*Lampocteis cruentiventer*) and (B) 'cydippid' (*Hormiphora californensis*) ctenophores. (Cydippida is a heavily polyphyletic group.) (C) Scanning electron microscopy of a split comb plate showing the many layers of cilia that constitute each 'paddle'. (D) Close-up schematic view of the aboral region of a *Pleurobrachia* type. (E) Schematic cross section of the internal canal system showing the complexity of the canals and the biradial symmetry of the anal pores. The view is from the oral end [top as shown in (B)] with the section shown as the blue plane of the inset. Note that the anal pores are shown despite not being in the plane of the section. Abbreviations: AP, anal pore; PP, pole plate; ST, statocyst (contained within the aboral apical organ); TB, tentacle bulb.

the gelatinous mesoglea. Nerves, muscles, and mesenchymal cells penetrate the mesoglea. There is no evidence that this layer is homologous to mesoderm [34], and ctenophores lack many genes that are required for mesoderm formation in Bilateria [17,18]. Ctenophores

have giant smooth muscle cells (multinucleate and 4-cm long) [35], and some have striated muscle [36]. The ctenophore nervous system is unique [37], with interconnected nerve nets (not just excitable epithelia) with synapses, but it lacks many of the neurotransmitters

found in Bilateria [17,18]. Even fertilization is different compared with other animals: at least one species has polyspermy, in which multiple sperm fuse with the egg and the female pronucleus moves among multiple male pronuclei before fusing with one [38]. Ctenophore embryogenesis is also unique in many respects [29,34]. Examinations of expression of various gene families are providing a critical bridge for interpreting genomic data in combination with morphology and development [11,18,39–46].

The well-known quote by Krumbach [47] still rings true: ‘Although it is easy in a given case to determine whether or not a particular animal is a ctenophore, it is equally difficult to establish how closely or distantly ctenophores are related to other forms’. Ctenophores have many unique and distinguishing morphological features, but because these features are not shared with other animals, they give little insight into how ctenophores are related to other animals. While ctenophores clearly diverged from other animals early during metazoan evolution, living species radiated relatively recently [48,49]. This means that ctenophores are connected to other animals by a long branch, not due to accelerated rates of molecular evolution but because so much time elapsed between the divergence of ctenophores from other animals and the most recent common ancestor of living ctenophores. This long branch has made it difficult to resolve their relations to other animals. It is unknown whether living ctenophores are the sole remaining subclade of a large diverse group, or if ctenophores have always had a relatively few number of species. Either way, there is no more reason to think that ctenophores are more similar to the most recent common ancestor of animals than are any other living animals [50].

Learning about ctenophores from the fossil record is challenging, because ctenophores preserve so poorly. Most of the fossils ascribed to ctenophores hinge on the presence of ribbed frond-like structure (construed as comb plates) or a stalk at the base, which is interpreted as the apical organ. However, these superficial similarities require complete reinvention of the ctenophore body plan, in some cases to fanciful extremes [51,52], and they are unconvincing to us and others [53]. A few fossils from the Lower Devonian have a clearer resemblance to ctenophores [54,55]. These have structures that have been interpreted as tentacle bulbs, which are tissue rich and would be more likely to fossilize than the tiny apical organ.

Ctenophores should be appreciated as the unique animals that they are, rather than as quasi-cnidarians or stunted bilaterians.

Sponges are not so simple

Sponges (Figure 3) are sessile benthic animals that filter bacteria and other picoplankton. Water is taken into the sponge body through tiny holes in pore cells called porocytes in the surface epithelium, which give the group its name Porifera (‘pore-bearer’). Of the 8500 currently recognized species [56], most are marine, occupying habitats from the deep ocean to intertidal. Many have extensive symbioses. One group has invaded freshwater and inhabits caves, deserts, and lakes world wide [57]. Understanding sponge biology requires not only a comparative and

multidisciplinary approach, but also a solid understanding of a long and multilingual literature.

As with ctenophores, the gross anatomy of sponges is unlike that of any other animals, which makes comparisons to other animals challenging. A primary feature is the extensively branched water canal system, which leads from the porocytes to the pumping and feeding structures, called choanocyte chambers (Figure 3C). The cells that form these chambers, choanocytes, in many cases also give rise to sperm. Given that the chambers and canals fill the entire sponge body, there is little regionalization to a sponge. An exception to this filter-feeding strategy is found in a derived group of deep-sea carnivorous sponges that use hook-shaped spicules to ensnare crustacean prey [58].

The absence of some complex traits found in most other animals, including striated muscle, nerves, and specialized gonads, gives a misleading impression of simplicity. In fact, sponges have typical animal features, including epithelia, coordination, and sensory mechanisms. They accomplish many of the same complex tasks as other animals, but in different ways (Box 1). A fascinating pattern is emerging from the limited genome and transcriptome data now available [59–61]: although sponges have distinct gross morphology, their gene inventory is similar in many respects to that of other animals.

The similarity of sponge choanocytes to unicellular choanoflagellates was first pointed out by James-Clark [62] and, since then, homology of the two cell types has gone largely unquestioned. Each has a collar of microvilli surrounding a flagellum. It is generally considered that these collar cells are a synapomorphy of choanoflagellates and animals, but were then lost in animals other than sponges. This scenario of a single gain (before the divergence of choanoflagellates and animals) followed by a single loss (in a lineage that gave rise to all animals except sponges) is taken as support for the placement of sponges as the sister group to all other animals. However, collar cells do exist in other animals, where they are thought to serve a sensory function [63,64]. Rather than a single gain and a single loss, collar cells might have originated independently multiple times. Whereas the feeding role of choanocytes is identical to that of choanoflagellates, specifics of the cell morphology, function, and development differ significantly (Figure 3D) [65], consistent with multiple independent origins. In all, the morphological and functional similarities between choanocytes and choanoflagellates suggest convergence to an efficient filter-feeding mode [65]. Choanoflagellates are of great interest as the outgroup to animals [66], regardless of the homology of collar cells.

Another entrenched misconception is that sponges lack tissue-level organization. In fact, sponge tissues are well equipped to carry out sealing and sensory functions [67,68] and a basement membrane is even present in some sponge epithelia [69]. Sponges comprise inner and outer epithelia and a middle collagenous layer with motile cells in a thin or thick mesohyl. Some sponge tissues are specialized for transport of nutrients [70] and others are heavily endowed with skeletal parts, presumably for support or protection. Sponge embryos are mostly brooded, so dynamic cell lineage tracing is difficult. In general, early cell movements

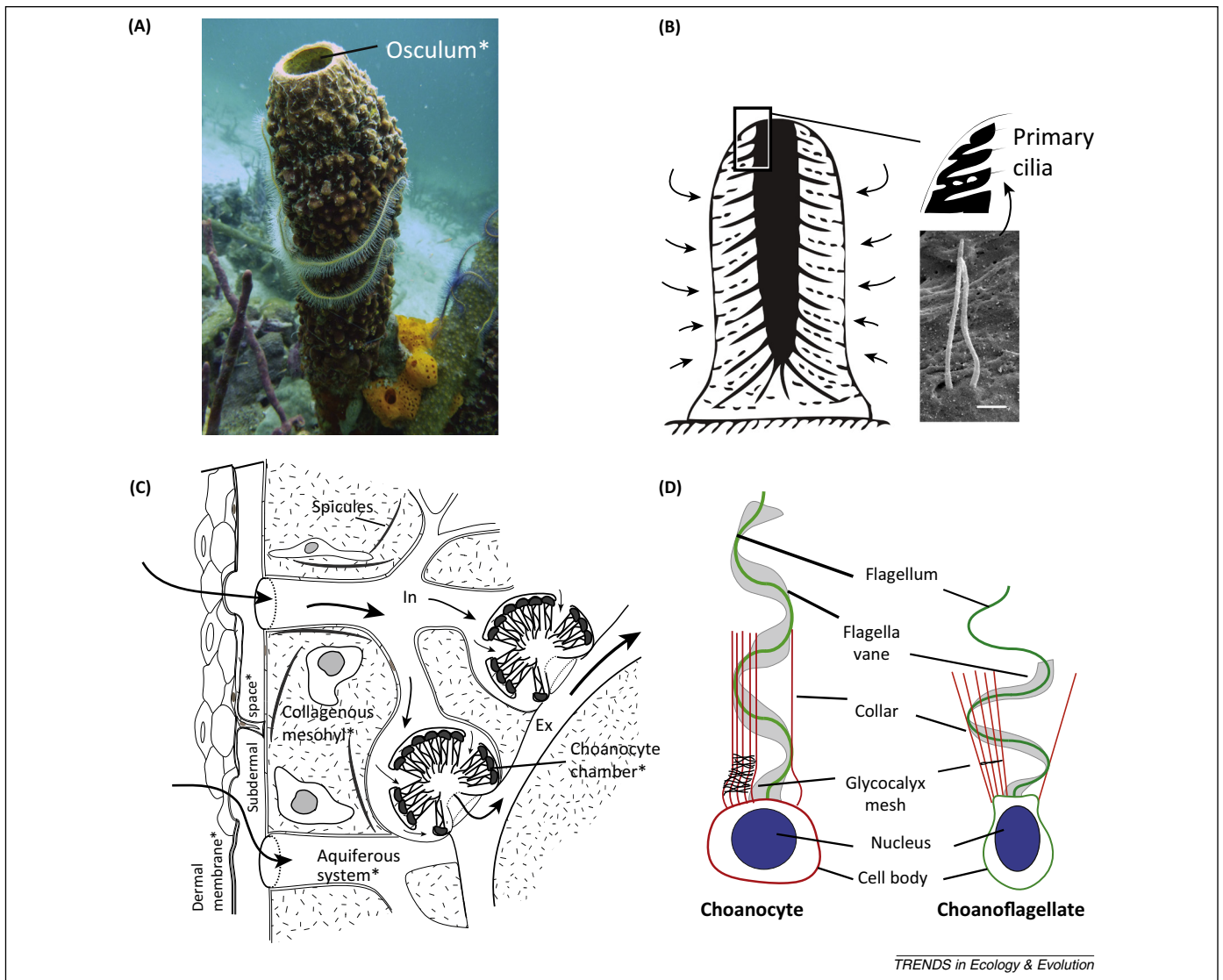


Figure 3. Sponge gross anatomy and distinguishing features. Traits unique to sponges, or with unique components, are marked with an asterisk. **(A)** Photograph of a demosponge (leuconoid) (possibly *Aplysina lacunosa*), which is approximately 50 cm tall. **(B)** Gross anatomy of a tube-form demosponge (after [105]), with oscula region expanded to show primary cilia on the epithelium (right) and a scanning electron micrograph of cilia from another demosponge (*Ephydatia muelleri*). Scale bar = 1 μ m. **(C)** Schematic of the filtration system of a demosponge illustrating the major tissue types. **(D)** Schematic comparing a sponge choanocyte and a unicellular choanoflagellate. The primary differences are: the main component of cytoskeleton of the cell body (actin, shown in red, in sponges and metazoans, tubulin, shown in green, in choanoflagellates), the dynamic nature of the collar in choanoflagellates but not sponges, and different interaction of the flagellum with the collars in each.

that are similar to conventional gastrulation [71] give rise to the larval ciliated epithelial layer, which can generate both the gastrodermis and, in some groups, also the epidermis, of the adult sponge. The molecular mechanisms that differentiate the inner cell layer of sponges and the endoderm of other animals share some features [72], consistent with the suggestion that the endodermal control program is older than that of other cell layers and more widespread than the program of other tissue layers [73]. Differential gene expression has been characterized in one parenchymella [74,75] and one amphiblastula larva [76,77], although interpretation is challenging without a solid understanding of larval structure and behavior.

Sponge epithelia are a major conduit for coordination of behavior. Typical activity of sponges involves periodic contractions of parts or the whole body, sometimes in response to specific stimuli [78,79]. Hexactinellid sponges differ in that their entire body is syncytial [80], which

allows electrical signals to travel unimpeded by membrane boundaries. These cause the feeding current to stop, presumably by the instant arrest of the flagella beat [81]. The 5 s-long action potential is driven by calcium and potassium [82]. Signaling in cellular sponges (i.e., all groups except hexactinellids) is three orders of magnitude slower than neuronal signaling in other animals: between 2×10^{-6} and $3.7 \times 10^{-4} \text{ m s}^{-1}$; by contrast, action potentials in hexactinellid syncytia travel at $2.9 \times 10^{-3} \text{ m s}^{-1}$ [83,84]. Given these slow speeds, it is not surprising that neurons have not been found in sponges [85–88]. Why then do sponge genomes and transcriptomes contain putative homologs of genes involved in synaptic signaling in other animals? The explanation might differ for postsynaptic and presynaptic genes [89]. Sponge genes that are homologous to genes specific to the presynapse in other animals might serve other secretory processes in sponges. In other cases, the sponge homologs lie in families related to but not

involved in synapse structure in other animals. These open questions expose a gross lack of knowledge of synaptic structure and gene homology in invertebrates in general. Could a cryptic synapse exist in sponges and, if it did, would it be possible to recognize it as such? Sponge behavior is slow, so it makes sense that fast neurotransmitters are not needed. Although little is known of neuropeptide homologs in sponges, they might be involved in larval settlement and metamorphosis [90], but not, due to speed, in conventional signaling. Slower signaling toolkits, including conventional glutamatergic and GABA-ergic signalling [78], modulated by nitric oxide synthase [91], are effective at coordinating sponge behavior. Coupled with an elegant system of sensory cilia, which are strategically located in the excurrent oscula to sample excurrent flow rates [68], these traits, although cryptic, provide effective epithelial-based conduction systems.

Many features of sponge physiology are similar to the physiology of other animals, and this is reflected in their genome complement as we know it so far. Unlike the situation for ctenophores, the radically different gross morphology of sponges and their most obvious lack of rapid behavior suggest simplicity, but this picture belies an underlying complexity that remains largely hidden.

Reframing questions about early animal evolution

Accounting for the ascertainment biases of hidden biology (Box 1) helps reframe old questions in critical new ways. Take, for example, the evolution of middle tissue layers. Animals are often described as being triploblastic, with three tissue layers, or diploblastic, with two. Bilaterians are triploblastic, with a mesoderm between the endoderm and ectoderm. There is considerable interest in whether mesoderm is unique to Bilateria, or had an earlier origin and is also present in some other animals. However, we know less about the tissue organization of other animals. To date, the field has largely asked what mesodermal traits nonbilaterians do or do not have, rather than describing the unique traits they do have. We know there is more to the picture: Ctenophora has a unique cellular mesoglea, Porifera has mesohyl, and Placozoa has fiber cells, but no collagenous extracellular matrix [92]. However, the term 'diploblasty' is widely used as a catch-all term, even though it is not a single well-defined character state. These challenges are analogous to the study of gastrulation in Bilateria, where many different modes of gastrulation are lumped under the umbrella of protostomy [93].

Another topic that is undergoing rapid reframing is the evolution of nervous systems. Bilateria, Cnidaria, and Ctenophora have nervous systems. Porifera [84] and Placozoa [92,94] do not. In combination with recent phylogenetic and genomic data, this leads to two conclusions. First, the evolution of the nervous system exhibits homoplasy, that is, it has arisen more than once or has been lost in one or more lineages. (Due to Placozoa, this question does not depend solely on the phylogenetic placement of Porifera and Ctenophora [3].) The field has quickly gone from a classical consensus that the nervous system was gained once and never lost, to a robust debate about how many times the nervous system has been gained or lost [17,18,89,95–97]. Second, earlier concepts of the relations

between nervous system traits and genes were oversimplified. Ctenophores have nervous systems, but lack many genes that are involved in nervous system function in Cnidaria and Bilateria [17,18]. Porifera and Placozoa have many gene families that are characteristic of nervous system development and function, even though they have no nervous system. It is a critical priority to understand what these genes do in Porifera and Placozoa, and to better characterize the structure and function of the ctenophore nervous system. It is also important to recognize that a 'nervous system' is not a single trait, but a complex suite of many traits that can each have independent evolutionary histories. Even if some features that are common to all nervous systems are homologous, others might have arisen by convergent evolution in different clades. A fascinating example of convergence across nervous systems was recently revealed in ion channel gene families [98]. Ctenophores, cnidarians, and bilaterians each have multiple large families of ion channel genes that are critical for nervous system function, but these gene families convergently expanded in each clade from a small number of ancestral sequences.

Concluding remarks

For more than a century, early animal evolution has been presented as a ladder, where 'primitive' living species are thought of as the ancestors of 'complex' living species. This perspective has persisted even though living zoologists have long recognized that complex traits are often reduced or lost [99,100]. As others have noted [50,101–104], this ladder-like perspective has led to considerable confusion, such as the frequent description of some living animals as 'basal', 'living fossils', or ancestors of other living animals, even though they are just as far from the base of the tree as other animals are. These distinctions cannot be dismissed as semantics: they represent fundamental misrepresentations of evolutionary history. We cannot array animals from simple to complex, because there is no single axis of complexity. Organisms have a mix of simple and complex traits, but many are currently hidden to us (Box 1). By identifying our blind spots to hidden biology, a new picture of early animal evolution comes into focus: different animal groups have different complex traits, and complex traits are gained and lost all across the animal tree. The primary scientific benefit of resolving deep animal relations, including the placements of ctenophores and sponges (Figure 1), is to provide a phylogenetic context for reconstructing the evolutionary history of these many characters. However, these character reconstructions will provide limited perspective until we describe unique traits across all clades.

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