

FORUM Evolution

A sisterly dispute

Which phylum first branched off from the animal phylogenetic tree is a contested issue. A new analysis challenges the proposal that comb jellies are the sister group to all other animals, and emphasizes a 'sponges-first' view. Three evolutionary biologists weigh up the evidence.

THE PAPER IN BRIEF

- There are five major branches of animals: Porifera (sponges), Cnidaria (jellyfishes, corals and related species), Ctenophora (comb jellies), Placozoa (*Trichoplax*) and Bilateria (all other animal phyla).
- Writing in *Proceedings of the National Academy of Sciences*, Pisani *et al.*¹ reanalyse some existing data and support the case

that Porifera are the sister group to all other animals.

- The authors propose that this conclusion fits with the fact that sponges lack features present in the other phyla, such as a nervous system and muscles.
- However, other recent genomic analyses have suggested that the more complex Ctenophora are the sister group.

Fighting over a comb

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The Ctenophora, also known as comb jellies or sea gooseberries, are a phylum of beautiful marine animals. Several molecular phylogenetic studies^{2–4} have made the extraordinary claim that Ctenophora, despite sharing 'advanced' characteristics — such as muscles, nerves and epithelial tissues — with Bilateria and Cnidaria, are more distantly related to these groups than are the simple Porifera, which lack these features.

In contrast to the conventional 'Porifera-sister' hypothesis, the Ctenophora-sister tree, in which the Ctenophora are the sister group of all other animals, implies either that muscles, nerves and epithelia evolved independently in two animal lineages (in Ctenophora and in the ancestor of Bilateria and Cnidaria), or that these characteristics are ancient and have been lost by sponges (Fig. 1). Considering these surprising implications, it was inevitable that the Ctenophora-sister idea would be highly controversial^{5–7}.

Critics of the original studies suggest that they were affected by errors in tree reconstruction caused by the use of inadequate phylogenetic models. Pisani *et al.*¹ investigate this possibility by reanalysing several published data sets comprising concatenated alignments of many genes^{2–5}, and a data matrix recording the presence or absence of more than 23,000 genes in different animal species³. The authors

present three lines of evidence to suggest that the Ctenophora-sister trees are an artefact.

First, recognizing that tree-reconstruction errors can stem from a poor fit between an evolutionary model and real data, they use the statistical technique of cross-validation to show that a 'site-heterogeneous' model⁸ best fits the data. These CAT models (named for modelling multiple categories of site) avoid the assumption of a homogeneous process of amino-acid substitution across sites within genes, and they have repeatedly been shown to outperform site-homogeneous models. The CAT model provided the best fit to the published data and supported the Porifera-sister hypothesis; less-well-fitting models better suit a Ctenophora-sister situation.

The authors' second approach considers the common artefact of long-branch attraction (LBA), which could cause the long ctenophore branch to be attracted downwards towards the long branch leading to the non-animal species that form the root of the tree⁹ — these groups are referred to as outgroups. The authors reason that, the longer the branch leading to the outgroups, the higher the likelihood of LBA. Removing the most distant outgroups shifts support from the Ctenophora-sister to the Porifera-sister picture, suggesting that the Ctenophora-sister result is an effect of LBA.

Third, the authors show that previous analyses of the gene presence-or-absence matrix, cited as an independent source of evidence supporting the Ctenophora-sister tree³, were affected by an unintentional bias: genes present in only one species were not recorded in the original matrix, and the prior existence of genes that have been lost in all sampled

species was ignored. These exclusions lead to a systematic underestimation of the likelihood of a gene being lost in any given species. The effect is that missing genes (such as those absent from ctenophores) are more likely to be interpreted as never having existed than as having been lost during evolution — effectively pushing species that have lost many genes towards the root of the tree. When this known problem with presence-absence data¹⁰ is corrected, the result is a much more credible tree (the previous work had several odd features), and independent support for the Porifera-sister hypothesis.

The position of Ctenophora has proved so difficult to determine because it involves three confounding circumstances: the main animal groups separated more than 540 million years ago during the Precambrian period, meaning that the phylogenetic signal supporting any clade is faint; Ctenophora was a fast-evolving taxon that seems to have lost features along the way; and there is a lack of informative intermediates on a long branch leading to the recent radiation of modern ctenophore species. Careful use of models designed to avoid the worst effects of these problems, and experiments designed to detect these issues, are essential for us to understand the true affinities of this phylum. The analyses that fit these requirements support the Porifera-sister camp, providing a parsimonious interpretation of morphological evolution.

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Methodological misconceptions

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Each new analysis of early animal phylogeny fuels debate about animal origins and the parallel evolution of animal complexity. Pisani *et al.*¹ have used phylogenetic methods that we

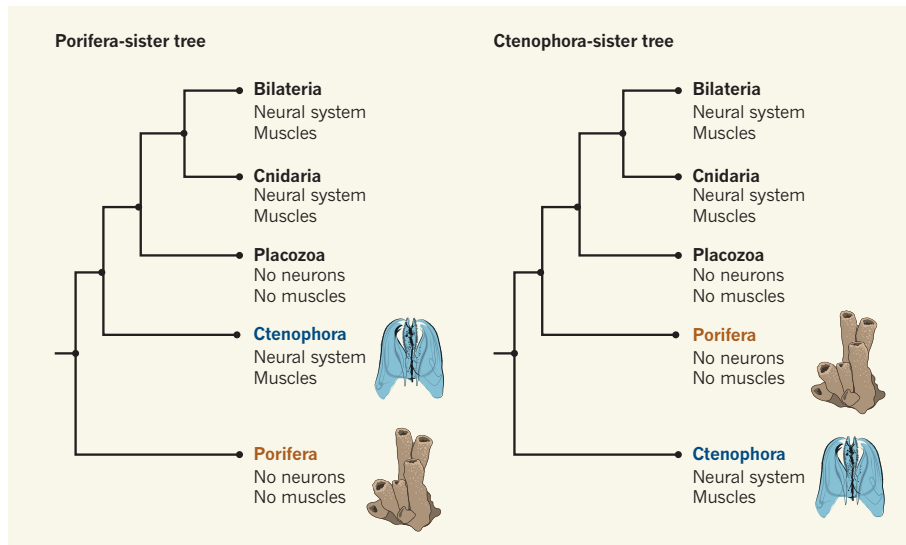


Figure 1 | The animal tree. There is debate over whether Porifera (sponges) or Ctenophora (comb jellies) were the first group to branch off from the animal tree. **a**, Porifera have typically been considered the simplest group, because they lack muscles and neurons, which are present in Ctenophora, Cnidaria (jellyfishes, corals and related species) and Bilateria (other animals). **b**, However, some phylogenetic analyses have suggested that Porifera are more closely related than Ctenophora to Bilateria and Cnidaria. (Placozoa, generally classified as the single species *Trichoplax adhaerens*, are consistently placed as the sister group of Cnidaria and Bilateria, but also lack neurons and muscles.)

consider unproven to reanalyse a small fraction of published data sets^{2–4}, and they reject the hypothesis that ctenophores are the sister group to other animals. But the authors' assertions are based on incomplete analyses and inappropriate assumptions, and their conclusion is at odds with other studies, including two recent analyses^{11,12}.

A key point is that convergent evolution of complex characteristics is compatible with both the Ctenophora-sister and Porifera-sister hypotheses^{4,13}. Morphological, functional or molecular complexity does not correlate with phylogeny. More than 550 million years of animal evolution has produced countless examples of independent gains and losses of complex structures¹⁴, and hypotheses based on the idea that morphologically simpler animals came first should not be blindly accepted, nor should complex features be treated as single characteristics in phylogenetic analyses.

Convergent evolution can be surmised if the system in question differs in structure, molecular composition and mechanisms. This case is met for genes expressed in neurons and in smooth and striated muscles in ctenophores versus those in cnidarians and bilaterians^{15,16}, implying that these features were not shared in the common ancestor. Integrative multiple-trait analysis and direct microanalytical measurements have demonstrated that ctenophores possess distinct neural machinery⁴, and there are no known pan-neuronal or neural genes that are shared by all animals¹⁷.

In fact, neural systems should not be considered as a single character in evolutionary reconstructions. They are composed of highly distinct cell populations with

different histories and origins, and relationships between these different cell lineages across phyla must be reconstructed to decipher neuronal genealogies. Furthermore, the placement of the nerveless Placozoa in Pisani and colleagues' tree challenges the assumption of a single origin of the nervous system (Fig. 1). Placing either sponges or ctenophores as sister to all other animals does not alter the possible scenario of independent origins of neural systems^{13,15} nor of muscles in ctenophores^{4,16}.

Several uncertainties about phylogenomic analyses are also illustrated by Pisani and colleagues' approaches. The authors assert that CAT models, which correct for variation between amino-acid positions⁸, outperform models used in other studies^{2–4}. However, other studies^{2,4} applied a different model to each partition in the data set, whereas Pisani and colleagues' validation approach uses only a single model across the entire data set. The authors' justification for superiority of CAT models is also based on assumptions about the 'true tree', including the placement of sponges, leading to a circular argument⁷. Moreover, CAT models are computationally demanding, and can produce conflicting results^{1,7,11,12} or fail to run to completion^{1–4}, and Pisani *et al.* discuss several incomplete analyses as providing support for the Porifera-sister hypothesis.

The authors chose to use only the taxon most closely related to animals (unicellular and colonial choanoflagellates) as the outgroup on their animal tree. It is not clear whether this assumption was made because it yielded a Porifera-sister tree or in response to objective criteria. The approach used does not allow for

the control of potential artefacts, such as the fact that choanoflagellates and some sponges have markedly different amino-acid composition from most animals, which could lead to incorrect rooting of the animal tree. Prior analyses^{2,4} that have thoroughly explored outgroup choice with objective criteria support the ctenophore-sister hypothesis.

Finally, it is worth considering other lines of evidence alongside phylogenetic reconstructions. Morphological characteristics that seem to unite the choanocyte cells of sponges with choanoflagellates may not actually be homologous¹⁸. And palaeontological evidence for the appearance of sponges during the Cryogenian period (around 850 million to 635 million years ago) has been challenged¹⁹. Confirmed sponge fossils appeared only with most other animal groups, near the transition from the Ediacaran to the Cambrian period, around 540 million years ago¹⁹. The Porifera-sister hypothesis, as promoted by Pisani *et al.*, needs further scrutiny from both phylogenomic and other standpoints. By contrast, analyses based on objective criteria favour the Ctenophore-sister hypothesis and parallel evolution of animal complexities across phyla. ■

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