RESEARCH ARTICLE PHYLOGENETICS

Integrative phylogenomics positions sponges at the root of the animal tree

JACOB L. STEENWYK AND NICOLE KING Authors Info & Affiliations

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Editor’s summary

The increased availability of high-quality genomes and improved phylogenetic methods have led to researchers revisiting many taxon relationships. Steenwyk and King took on a highly contested debate: whether sponges or comb jellies (ctenophores) were the first lineage to diverge among animals (see the Perspective by Mulhair and Redmond). Using data from 100 genomes and transcriptomes enriched for sponges, ctenophores, and cnidarians, the authors used an integrative phylogenomic approach to determine which of the nearly universal single-copy genes consistently supported either lineage as a sister taxon. Most tests conducted with this set of genes supported sponges as the sister taxon, and none supported ctenophores. This work supports early trees constructed using morphology, although it is likely not the final word in this debate. —Corinne Simonti

Abstract

Determining whether sponges or ctenophores root the animal tree has important implications for understanding early animal evolution. Here, we examined support for these competing hypotheses by constructing large and highly informative data matrices containing sequences from sponges, ctenophores, cnidarians, bilaterians, and diverse animal relatives. The new data matrices and 10 published datasets were analyzed in 785 topology tests conducted using integrative phylogenomics, a method that unifies concatenation and coalescence to identify genes with a consistent phylogenetic signal. All 490 statistically significant tests supported the sponge-sister hypothesis and none supported the ctenophore-sister hypothesis; the remaining 295 tests were inconclusive. These results provide compelling evidence for the sponge-sister hypothesis and suggest that integrative phylogenomics provides a robust and powerful approach for disentangling branches in the tree of life.

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DEC. 22, 2025

Re. Integrative phylogenomics positions sponges at the root of the animal tree

CASEY W. DUNN Professor, Yale University
XIAOFAN ZHOU Associate Professor, South China Agricultural University
JINGXUAN CHEN PhD Student, Zhejiang University
JACOB M. MUSSER Assistant Professor, Yale University
YUANNING LI Professor, Shandong University
STEVEN H.D. HADDOCK Senior Scientist, Monterey Bay Aquarium Research Institute
DANIEL S. ROKHSAR Professor, University of California Berkeley
XING-XING SHEN Professor, Zhejiang University

Steenwyk and King (*1*) (SK) addressed a fundamental question in animal evolution: were ctenophores or sponges the first lineage to split from other animals at the root of the metazoan tree? Modifying the approach of (*2*), they presented metrics to quantify the degree to which individual genes sampled across diverse species support the ctenophore-sister or sponge-sister hypotheses using two different phylogenomic frameworks (“concatenation” and “coalescence”). Genes that support the same hypothesis in both frameworks were deemed “consistent”, while genes that support different hypotheses in the two frameworks were “inconsistent” and not considered further. The counts of consistent sponge-sister and ctenophore-sister genes were then evaluated with chi-squared tests. The authors applied this approach to a new dataset of 869 BUSCO (*3*) genes sampled across 100 animal and outgroup species, as well as derivatives of this new dataset and ten previously published datasets. Although they found that the vast majority of genes were inconsistent in all datasets, they observed a statistically significant excess of consistent genes supporting the sponge-sister hypothesis in most analyses and no significant difference in others; none of their analyses supported ctenophore-sister (their Fig. 4). SK concluded that their “integrative phylogenomics” approach provides compelling evidence for the sponge-sister hypothesis.

Here we note several problems that compromise their conclusions. Supporting material, including figures, for our new analyses can be found at https://github.com/caseywdunn/sk25 (archived at https://doi.org/10.5281/zenodo.18022339). We focus our analyses on their “92.5” matrix (the top left matrix in their Fig. 4A), as this is the most inclusive version of their new 869-gene dataset, from which all results in their Fig. 4A–D are derived. In their scoring, they found that this matrix had 82 genes consistent for sponge-sister but only 6 consistent for ctenophore-sister, and that this difference was significant according to the chi-squared test.

As a first step toward understanding their phylogenetic signal, we inferred concatenation-based phylogenies with the same tool (iqtree2) and model (LG+I+G4+C60) they used. We found that phylogenetic trees based on the 88 consistent genes identified by SK in the “92.5” matrix support the ctenophore-sister hypothesis (Fig. 1a), even though most of these genes were scored as consistent for sponge-sister. Strikingly, we found that the phylogenetic tree based solely on the 82 genes SK scored as consistent for sponge-sister also strongly supports the ctenophore-sister hypothesis (Fig. 1b).

To further explore this discrepancy, we examined the taxonomic coverage of genes. As a matter of principle, testing the relative support for ctenophore-sister vs. sponge-sister requires sampling at least one species from each of four groups: sponges, ctenophores, other animals (Placozoa + Cnidaria + Bilateria), and the outgroup (non-animals). Yet 56 of the 869 genes analysed by SK are missing ctenophores, outgroups, or both. Surprisingly, 45 of these genes that have no information about the animal root are scored by SK as consistent for sponge-sister. This indicates problems with their scoring procedure. We were able to trace the causes of these problems to issues in both their quartet-based (coalescent) and likelihood-based (concatenation) analyses.

In the coalescence framework, SK reported problematic quartet scores, where genes without ctenophore sequences nevertheless support sponge-sister. These genes should not support either hypothesis. We found that this error arises from the interaction of three methodological choices: structurally inappropriate reference trees for evaluating quartets, imbalanced taxon sampling, and the inclusion of all induced quartets. Together these choices create a systematic bias in favor of sponge-sister due to the substantially greater number of 29 sponges than 13 ctenophores in the dataset. The critical issue is the scoring of quartets based on their concordance with two incorrectly defined reference trees. For scoring quartets against the ctenophore-sister hypothesis, SK collapsed “sponges + other animals” into a single clade, and alternatively scored quartets for sponge-sister by combining “ctenophores + other animals”. With this scoring system, quartets that contain two sponges and two other animals are systematically incompatible with the collapsed ctenophore-sister tree but will often match the collapsed sponge-sister tree (despite being inherently uninformative about the root). While other uninformative quartets effectively cancel out, this specific subset generates a spurious directional bias in favor of sponge-sister. This error is eliminated when quartets are properly scored relative to reference trees that maintain ctenophores, sponges, and other animals as separate clades.

In the concatenation framework, SK reported log-likelihood differences $|\Delta \ln L|$ between the sponge- and ctenophore-sister hypotheses that are orders of magnitude larger than typically observed for single genes, reaching into the thousands (Fig. 1). This was due to a procedural error in the use of iqtree (*4*). As SK note in their supplementary methods, "phylogenetic trees [used to calculate site log-likelihoods] were specified using the -z argument." The trees specified with -z should be fully resolved phylogenies; in this case these should be the two maximum likelihood phylogenies inferred under the ctenophore- and sponge-sister constraint trees. Inspection of SK's iqtree log files, however, indicates that the tree file specified with -z was `Ctenophore_and_Sponge_first_trees.tre`, which contains the constraint trees themselves (available in the `TRADITIONAL_TOPOLOGY_TESTS` folder in their figshare (*5*)). A critical step was therefore skipped. Instead of using the constraint trees to build maximum-likelihood trees to calculate the site log likelihoods on, they calculated the site log likelihoods on the constraint trees themselves. The unresolved internal branches of the constraint trees do not yield interpretable site log likelihoods. When we reran the analyses by first inferring maximum-likelihood trees under the ctenophore-sister and sponge-first constraints and then calculating site log-likelihoods on those inferred trees, the resulting $|\Delta \ln L|$ values fell back into typical single-gene ranges, most with magnitude less than 10 (Fig. 2). These corrected results are consistent with the original presentation of these methods in (*3*).

After correcting both likelihood and quartet scoring, the sponge-sister signal reported by SK disappears and is replaced by strong support for ctenophore-sister. Using corrected scoring, 544 genes are classified as consistent (out of 813 genes in the "92.5" matrix that sample all four groups required to test the animal root), indicating substantially less conflict within the data than SK reported. Furthermore, in the reanalyzed "92.5" matrix, significantly more genes are consistent with the ctenophore-sister hypothesis (370 genes) than with the sponge-sister hypothesis (174 genes). As expected, and in contrast to the results obtained using SK's reported sponge-sister genes (Fig. 1b), phylogenetic analyses of the consistent sponge-sister gene set recover sponge-sister (Fig. 2b), whereas analyses of the consistent ctenophore-sister gene set recover ctenophore-sister (Fig. 2c). A combined phylogenomic analysis of all 544 consistent genes strongly supports ctenophores as the sister group of all other animals (Fig. 2a). Although we focus here on their "92.5" matrix, these methodological issues apply to SK's other analyses.

A more comprehensive analysis will be presented elsewhere. We are grateful to Steenwyk and King for discussions about their work, their quick responses to our questions, and for their feedback on this letter.

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"Integrative Phylogenomics" Integrates Sources of Error

NATHAN V. WHELAN Associate Professor, The Ohio State University

Steenwyk and King (SK; *1*) used unsound methods to claim strong support for sponges being sister to other Metazoa. SK's methods and conclusions were flawed because 1) "Integrative Phylogenomics" has no theoretical basis for being used to assess hypotheses *(2)*; 2) SK's gene trees are mostly erroneous *(3)*, making them unsuitable for SK's methods *(4)*; 3) Ignoring 1 and 2, using unresolved reference trees biased SK's results.

First, "Integrative Phylogenomics" is only useful for identifying loci causing incongruence when concatenation and summary-coalescent results differ *(2)*. Yet, both approaches on SK's datasets produce trees supporting ctenophores-sister *(5)*.

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Second, gene-tree error is a known problem for SK's coalescent-based methods *(4)*. Yet, most of SK's gene trees that "support" sponges-sister are obviously wrong, including errors like Ctenophora sister to a mite *(1, 3)*. It defies reason, and methodological assumptions, to use such trees for testing SK's hypotheses.

Third, SK used unresolved trees to test support for alternative hypotheses, which SK never justified or explained. Only one gene (127766at33208) tree inferred by SK in each new dataset had all of their benchmark relationships inferred correctly *(3)*. This gene tree unambiguously had the lowest error and had ctenophores-sister. Yet, SK claimed this gene consistently supported sponges-sister. This claim makes no sense. When using SK's methods, but with fully inferred trees and a sponge-ctenophore swap for the alternative hypothesis, no gene supports either hypothesis using SK's quartet score threshold *(5)*. If minimum quartet score is lowered, then significantly more "consistent" genes in SK's newly generated datasets support ctenophores sister, including 127766at33208 as expected *(5)*. Ctenophores sister is also favored by older datasets *(5)*. Using unresolved trees was clearly a poor methodological choice.

SK's conclusions are not supported by robust methodologies. Available data continue to support Ctenophora as the sister group to other Metazoa.

References and Notes

1. J. L. Steenwyk, N. King, Integrative phylogenomics positions sponges at the root of the animal tree. *Science* **390**, 751–756 (2025).

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