HOME > SCIENCE > VOL. 390, NO. 6774 > INTEGRATIVE PHYLOGENOMICS POSITIONS SPONGES AT THE ROOT OF THE ANIMAL TREE

RESEARCH ARTICLE PHYLOGENETICS

Integrative phylogenomics positions sponges at the root of the animal tree

JACOB L. STEENWYK AND NICOLE KING Authors Info & Affiliations

SCIENCE 13 Nov 2025 Vol 390, Issue 6774 pp. 751-756 DOI: 10.1126/science.adw9456

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.

RELATED PERSPECTIVE

Sponging away phylogenomic incongruence

BY PETER O. MULHAIR, ANTHONY K. REDMOND • SCIENCE • 13 NOV 2025

Supplementary Materials

The PDF file includes:

Materials and Methods

Figs. S1 to S27

References (*60*–*97*)

DOWNLOAD | **5.02 MB**

Other Supplementary Material for this manuscript includes the following:

Tables S1 to S10

DOWNLOAD | **43.36 KB**

References and Notes

1 A. Rokas, B. L. Williams, N. King, S. B. Carroll, Genome-scale approaches to resolving incongruence in molecular phylogenies. *Nature* **425**, 798–804 (2003).

CROSSREF • PUBMED • WEB OF SCIENCE • GOOGLE SCHOLAR

2 J. E. Bond, N. L. Garrison, C. A. Hamilton, R. L. Godwin, M. Hedin, I. Agnarsson, Phylogenomics resolves a spider backbone phylogeny and rejects a prevailing paradigm for orb web evolution. *Curr. Biol.* **24**, 1765–1771 (2014).

CROSSREF • PUBMED • WEB OF SCIENCE • GOOGLE SCHOLAR

3 B. Misof, S. Liu, K. Meusemann, R. S. Peters, A. Donath, C. Mayer, P. B. Frandsen, J. Ware, T. Flouri, R. G. Beutel, O. Niehuis, M. Petersen, F. Izquierdo-Carrasco, T. Wappler, J. Rust, A. J. Aberer, U. Aspöck, H. Aspöck, D. Bartel, A. Blanke, S. Berger, A. Böhm, T. R. Buckley, B. Calcott, J. Chen, F. Friedrich, M. Fukui, M. Fujita, C. Greve, P. Grobe, S. Gu, Y. Huang, L. S. Jermiin, A. Y. Kawahara, L. Krogmann, M. Kubiak, R. Lanfear, H. Letsch, Y. Li, Z. Li, J. Li, H. Lu, R. Machida, Y. Mashimo, P. Kapli, D. D. McKenna, G. Meng, Y. Nakagaki, J. L. Navarrete-Heredia, M. Ott, Y. Ou, G. Pass, L. Podsiadlowski, H. Pohl, B. M. von Reumont, K. Schütte, K. Sekiya, S. Shimizu, A. Slipinski, A. Stamatakis, W. Song, X. Su, N. U. Szucsich, M. Tan, X. Tan, M. Tang, J. Tang, G. Timelthaler, S. Tomizuka, M. Trautwein, X. Tong, T. Uchifune, M. G. Walzl, B. M. Wiegmann, J. Wilbrandt, B. Wipfler, T. K. F. Wong, Q. Wu, G. Wu, Y. Xie, S. Yang, Q. Yang, D. K. Yeates, K. Yoshizawa, Q. Zhang, R. Zhang, W. Zhang, Y. Zhang, J. Zhao, C.

SHOW ALL REFERENCES

eLetters (3)

eLetters is a forum for ongoing peer review. eLetters are not edited, proofread, or indexed, but they are screened. eLetters should provide substantive and scholarly commentary on the article. Neither embedded figures nor equations with special characters can be submitted, and we discourage the use of figures and equations within eLetters in general. If a figure or equation is essential, please include within the text of the eLetter a link to the figure, equation, or full text with special characters at a public repository with versioning, such as Zenodo. Please read our Terms of Service before submitting an eLetter.

LOG IN TO SUBMIT A RESPONSE

JAN. 9, 2026

Response to Dunn et al. eLetter

tingly included errors in our analysis pipeline that artefactually influenced the results. We are grateful to Dunn *et al.* for bringing these issues to our attention and for working with us to identify the errors. In the interest of maintaining the integrity of the scientific record, we have requested that the editors of *Science* retract our study.

In the meantime, we are also exploring the issues raised by Dunn *et al*. in more depth. For example, we have re-run the integrative phylogenomics pipeline on control datasets after implementing the corrections recommended by Dunn *et al.* After seeking feedback from experts in the field (including Dunn *et al.*), we will share our new findings via BioRxiv or GitHub.

This has been a humbling experience, but one that speaks to the self-correcting nature of the scientific endeavor. It is far better that these issues were detected than that they remained in the scientific record. We look forward to following, and perhaps contributing to, ongoing efforts to root the animal tree.

J.L.S. is an advisor to ForensisGroup Inc., a scientific consultant to Edison Scientific Inc., and was a Bioinformatics Visiting Scholar at MantleBio Inc. N.K. declares no competing interests.

view less

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).

lysed 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 Steenwyck and King for discussions about their work, their quick responses to our questions, and for their feedback on this letter.

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

2. X. X. Shen, J. L. Steenwyk, A. Rokas, Dissecting incongruence between concatenation- and quartet-based approaches in phylogenomic data. *Systematic Biology* **70**, 997–1014 (2021).

3. M. Manni, M. R. Berkeley, M. Seppey, F. A. Simão, E. M. Zdobnov, BUSCO update: Novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. *Molecular Biology and Evolution* **38**, 4647–4654 (2021).

4. B. Q. Minh, H. A. Schmidt, O. Chernomor, D. Schrempf, M. D. Woodhams, A. von Haeseler, R. Lanfear, Corrigendum to: IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Mol. Biol. Evol.* **37**, 2461 (2020).

5. J. Steenwyk, Additional information for "Integrative phylogenomics positions sponges at the root of the animal tree". doi:10.6084/m9.figshare.28229990.v4 (2025).

view less