PHYLOGENETICS

Integrative phylogenomics positions sponges at the root of the animal tree

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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.

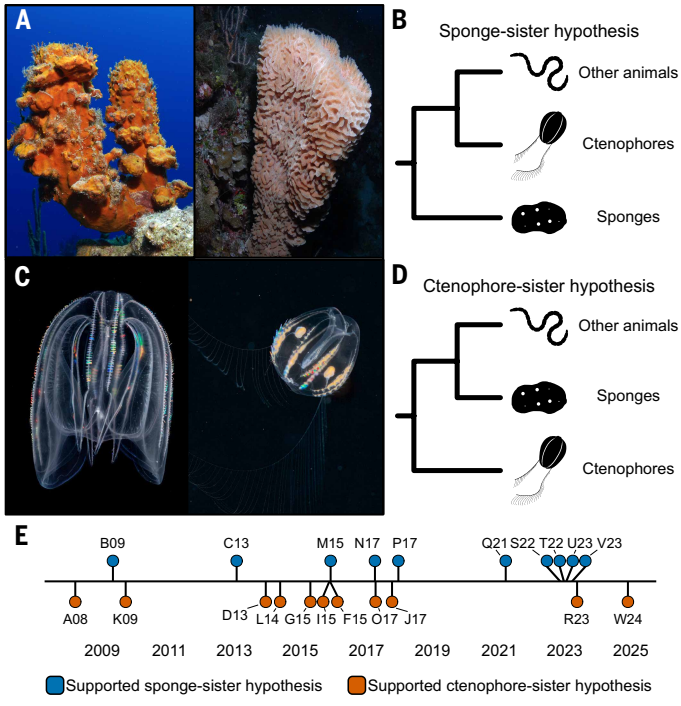

Fig. 1. Major competing hypotheses regarding the root of the animal tree of life. (**A**) Representative images of two sponges (credit: S. Nichols). (**B**) The sponge-sister hypothesis postulates that sponges root the animal tree of life. (**C**) Representative images of two ctenophores (credit: A. Jan). (**D**) The ctenophore-sister hypothesis postulates that ctenophores root the animal tree of life. (**E**) Starting with study A08 in 2008, different phylogenomic studies have provided conflicting support for either the sponge-sister hypothesis or the ctenophore-sister hypothesis (*7*–*13*, *19*, *20*, *22*, *60*–*65*). See table S5 for a key to relevant studies.

Reconstructing the tree of life is a major goal in biology. Although phylogenomics (phylogenetics using genome-scale data) has resolved many branches (*1*–*6*), some remain unresolved and concern key evolutionary episodes. Among the most intensely debated and contentious branches is the root of the animal tree (*7*–*13*).

The debate involves conflicting support for two different hypotheses: the sponge-sister hypothesis, which suggests that sponges were the first lineage to diverge from all other animals (Fig. 1, A and B), and the ctenophore-sister hypothesis, which suggests that ctenophores are sister to all other animals (Fig. 1, C and D). Although early studies based on morphology and single-locus phylogenetics favored the sponge-sister hypothesis (*14*–*18*), the first phylogenomic study of animals supported the ctenophore-sister hypothesis (*7*) (Fig. 1E). The following year, a different phylogenomic study supported the sponge-sister hypothesis (*8*). Since then, numerous investigations have used concatenation, in which multiple gene or protein sequences are combined end to end into a supermatrix and analyzed in a total evidence approach (Table 1). Despite being powered by increasingly large datasets, including genome-scale data from dozens of ctenophores and sponges and the latest advances in analytical approaches, compelling and contradictory evidence for the two competing hypotheses continued to emerge (Fig. 1E) (*9*–*12*, *19*–*24*).

The difficulties in resolving the root of the animal tree likely stem from both the analytical strategies used and the types of data analyzed. For example, whereas the analysis of concatenated datasets has resolved numerous branches in the tree of life, it may fail to recover the correct topology of other branches because of long-branch (LB) attraction, an issue thought to complicate rooting of the animal tree (*19*, *25*, *26*). An alternative framework, coalescence, has also been applied to resolving deep phylogenies (*25*, *26*), but analyses of the root of the animal tree using coalescence alone have also resulted in conflicting results (Table 1) (*27*, *28*). Moreover, coalescence is sensitive to its own errors, such as gene tree estimation errors and LB attraction among single-gene phylogenies (*29*). Finally, incongruent results regarding the root of the animal tree and other poorly understood nodes on the tree of life have been attributed to unreliable markers in datasets, such as those with a weak or erroneous phylogenetic signal caused by phenomena such as saturation by multiple substitutions (*30*, *31*).

In the phylogenomics era, it has become clear that alternative strategies are needed to help clarify the root of the animal tree. Two recent studies analyzed patterns of gene presence and absence, with each finding support for the sponge-sister hypothesis (*28*, *32*), whereas a previous analysis of gene content supported the ctenophore-sister hypothesis (*9*). Another recent study analyzed gene linkages to root the animal phylogeny and identified four linkage groups that supported the ctenophore-sister hypothesis (Table 1) (*13*). Although compelling, analyses of gene linkage can favor hypotheses that have not otherwise been supported (*33*). Moreover, methods to rigorously detect gene linkages and their utility as phylogenomic markers have recently been questioned (*34*, *35*), calling for caution in the use of genome structure as a marker for rooting the animal tree.

Here, we unified diverse phylogenomic methods (*36*) and leveraged newly available, high-quality sequence data across diverse lineages (ctenophores, sponges, cnidarians, and holozoan microeukaryotes) to root the animal tree. Our goals were to build large, highly informative phylogenomic data matrices and analyze the resulting data using integrative phylogenomics (the combination of concatenation and coalescence; Fig. 2) rather than using only concatenation or only coalescence, as was typically done in previous studies (*9*–*12*, *19*–*24*, *28*, *32*) (Table 1). Integrative phylogenomics aims to identify reliable markers by

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determining which genes yield a consistent signal, whether analyzed in the concatenation or the coalescence framework. Consistent genes are further analyzed and inconsistent genes are discarded because, based on simulations, inconsistent genes are prone to gene tree estimation error and incomplete lineage sorting (*36*). Moreover, integrative phylogenomics can overcome errors stemming from LB attraction (figs. S1 to S5) (*12*, *37*, *38*).

The integrative phylogenomics approach was coupled with both traditional and recently developed phylogenomic subsampling strategies and amino acid recoding schemes to uncover bias and to test the stability of any inferences (*39*, *40*). We also reanalyzed 10 previously published datasets, eight of which had been found to support the ctenophore-sister hypothesis and two of which had supported the sponge-sister hypothesis, using the same set of approaches. Among 785 different tests, all 490 statistically significant tests supported the sponge-sister hypothesis. By contrast, no tests supported the ctenophore-sister hypothesis, and the remaining 295 tests were inconclusive. Thus, these analyses converged on support for the sponge-sister hypothesis. These results highlight the potential of integrative phylogenomics to provide new insights into long-standing phylogenetic controversies. We anticipate that these findings will help to refine reconstructions of the origin and early evolution of animals.

Results

Construction of six large and highly informative phylogenomic data matrices

We prioritized taxonomic breadth and data depth in the form of genome-scale phylogenetic markers for the construction of a large data matrix. One hundred genomes and transcriptomes with ≥60% Benchmarking Universal Single-Copy Orthologs (BUSCO) completeness (81.4 ± 10.46% average BUSCO completeness) were selected to span animal diversity, particularly enriching for sponges, ctenophores, and cnidarians ($N = 62$) and phylogenetically relevant outgroups such as choanoflagellates and filastereans ($N = 26$) (table S1 and fig. S7). Representative bilaterians were selected based on the completeness of their genomes. Predicted genes from these taxa were then screened to identify 869 near-universal single-copy orthologs (or BUSCO genes) that were represented in at least 50% of the 100 taxa (*41*, *42*). The median and average taxon occupancy among the 869 orthologs was 84.16 and 81.08%, respectively (fig. S6).

To avoid bias in the trimming strategy, we trimmed gaps from the aligned protein sequences using six different strategies (table S2 and fig. S7E). Four of the six strategies used decreasing stringency in site gappyness thresholds (from 85 to 92.5% gappyness with a step of 2.5%, coded as 85.0, 87.5, 90.0, or 92.5). The remaining two trimming strategies, KPIC ("keep parsimony informative and constant sites") and KPI ("keep parsimony informative sites"), retained phylogenetically informative sites and, in the case of KPIC, constant sites, and removed all others (*43*) (Fig. 3). Individual protein alignments trimmed using each of the six methods were concatenated into separate supermatrices.

The 85.0, 87.5, 90.0, 92.5, KPIC, and KPI data matrices had the longest total alignment length, the highest number of taxa sampled, and the longest average single-protein lengths compared with data matrices from previously published studies of the animal root (Fig. 3A). Key features, such as the number of nonbilaterian animals and parsimony informative sites, were also improved (Fig. 3B).

Generation of 168 data matrices to root the animal tree

The six main data matrices (85.0, 87.5, 90.0, 92.5, KPIC, and KPI) were each subjected to 28 different treatments, using different combinations of phylogenomic subsampling and amino acid recoding strategies, resulting in 168 different data matrices that could be used to test the stability of phylogenomic inferences (fig. S7). Specifically, the 20–amino acid alphabet was recoded to 18-, 12-, and 6-states, a practice that can help to combat the effects of saturation (fig. S7F) (*44*, *45*).

Table 1. Summary of the studies used to root the animal phylogeny. Studies that have aimed to root the animal phylogeny may broadly be assigned to the following methodological categories: concatenation of gene content and multiple sequence alignment data analyzed using a maximum likelihood and Bayesian-based inference, gene linkages in a concatenation framework, coalescence of single-gene trees, or integrative phylogenomics, which integrates concatenation and coalescence. See table S5 for detailed information about example studies.

			Topology supported	
Framework	**Data type**	**Concatenation or coalescence**	**Ctenophore-sister***	**Sponge-sister***
Maximum likelihood	Gene content	Concatenation	D13, E13, X24	
Bayesian statistics	Gene content	Concatenation		T24
Maximum likelihood	Multiple sequence alignments	Concatenation	A08, K09, D13, L14, J17, E13, F15, G15, H15, O17	Q21
Bayesian statistics	Multiple sequence alignments	Concatenation	I15	B09, C13, M15, N17, P17
Maximum likelihood	Single-gene trees	Coalescence	X24	S22
Bayesian statistics	Gene linkages	Concatenation	R23	
Maximum likelihood	Multiple sequence alignments and single-gene trees	Integrated concatenation and coalescence		This study

*See table S5 for codes to studies supporting either the ctenophore-sister or the sponge-sister topology.

The datasets were also subsampled using six distinct strategies (table S3 and fig. S7G) in which the best-scoring 50% of genes based on diverse metrics were used for topology testing. Five of the strategies have been widely used in prior studies: (i) occupancy, in which higher taxon occupancy is favored (*46*); (ii) relative composition variability (RCV), a measure of compositional bias in which lower values are favored (*47*); (iii) saturation, a measure that quantifies the extent of multiple substitutions per site, in which lower saturation values are considered more favorable (*31*); (iv) LB score, which quantifies susceptibility to LB attraction artifacts, favoring genes with low susceptibility (*48*, *49*); and (v) average branch support, a measure of statistical confidence in tree inference in which greater average branch support is considered more favorable (*30*). The last approach, called RemarKIT, is a new strategy that combines machine learning and information theory to rank the informativeness of genes for species tree reconstruction (figs. S8 to S11). Phylogenomic subsampling using RemarKIT outperformed random gene selection (fig. S12).

Robust support for the sponge-sister hypothesis

We then analyzed the 168 data matrices using an integrative phylogenomic framework (*36*) (Fig. 2). Briefly, we examined the data matrices under both a concatenation and a coalescence framework and measured the distributions of single-gene support (fig. S13). In both frameworks, profile mixture models were used to account for site heterogeneity (*50*). Only those genes that provided a consistent and robust phylogenetic signal under both frameworks ["robust" defined as having a stringency threshold (Th1) of absolute difference in log-likelihood score ($|\Delta\ln L| > 2$ for concatenation and an absolute difference in quartet score ($|\Delta QS| > 0.1$ for coalescence] in support of one of the two hypotheses under examination, ctenophore-sister or

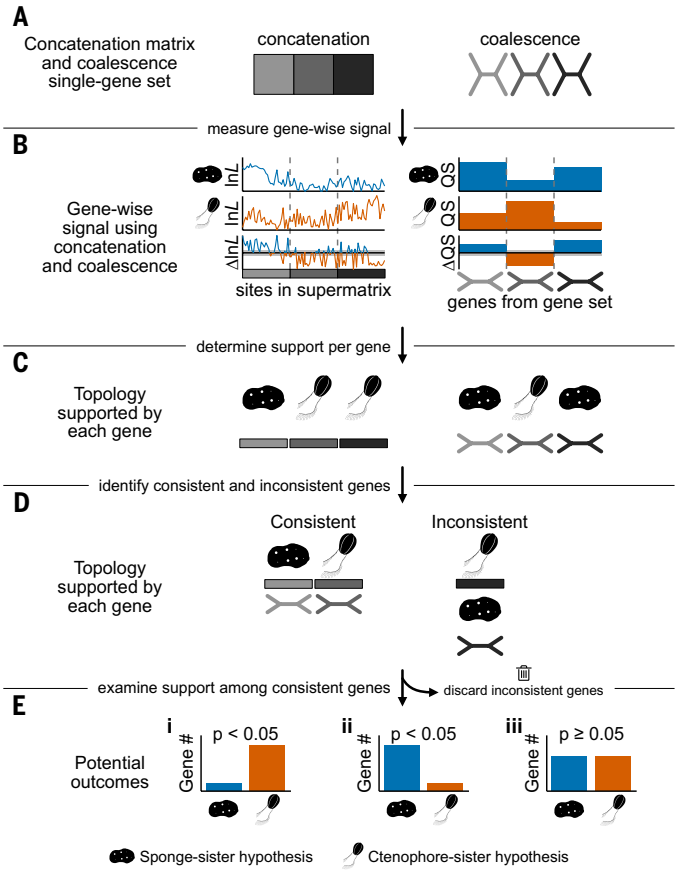

Fig. 2. Hypothesis testing using an integrative phylogenomic workflow. (**A**) A dataset for concatenation or coalescence analysis, i.e., either a supermatrix alignment or a set of gene trees, respectively, was generated. (**B** and **C**) Gene-wise signal was measured in each framework (B) and the topology supported by each gene was determined (C). (**D**) Genes supporting the same topology using both frameworks (consistent genes) were retained for further analysis; genes that conflicted between the two frameworks (inconsistent genes) were discarded. (**E**) Support for the sponge-sister hypothesis or the ctenophore-sister hypothesis was then examined using the counts of genes that supported each hypothesis and chi-square statistics. The possible outcomes were (i) that the ctenophore-sister or (ii) the sponge-sister hypothesis would receive significantly more support than the other or (iii) that the hypotheses may be statistically indistinguishable. Blue indicates support for the sponge-sister hypothesis; orange indicates support for the ctenophore-sister hypothesis.

sponge-sister, were retained for topology tests (Fig. 3, C and D). These genes will hereafter be referred to as the "consistent" genes. Genes that had conflicting support or that lacked robust phylogenetic signal between the two frameworks are referred to as "inconsistent" genes. This gene filtration process unified typically independent phylogenomic frameworks, distinguishing our approach from previous studies.

The average percentage of consistent genes (fig. S14) across the 168 datasets was 4.63 ± 3.84%, suggesting most of the 869 near-universally single-copy orthologs used in the starting datasets were unreliable. Only the consistent genes were used to conduct topology tests. Subsequently, the number of genes supporting each hypothesis was determined and used in a chi-square test with Benjamini-Hochberg multiple-test correction to determine whether there was greater support for one topology over the other (Fig. 2E).

Consistent genes robustly supported the sponge-sister hypothesis (Fig. 4A). For each dataset, the average percentage of consistent genes that supported the sponge-sister hypothesis was 86.32 ± 25.61% (fig. S15). Among statistically significant tests, 135 datasets supported the sponge-sister hypothesis ($P < 0.001$ for all tests, chi-square test with Benjamini-Hochberg multiple-test correction; Fig. 4, A and B). No tests supported the ctenophore-sister hypothesis, and 33 were inconclusive.

To further evaluate the robustness of the outcomes to the methods used in the analysis pipeline, we measured the impact of the thresholds used to identify the genes that had robust phylogenetic signal. For this, we used the 92.5 supermatrix because the multiple sequence alignments were trimmed using the consensus practice of "light" site removal (*43*, *51*). The associated recoded and subsampled datasets were also analyzed. The four different thresholds tested were |ΔlnL| and |ΔQS| values > 1.00 and 0.010 (Th5), 1.25 and 0.025 (Th4), 1.50 and 0.050 (Th3), and 1.75 and 0.075 (Th2), respectively, representing an increase in stringency. This revealed that support for the sponge-sister hypothesis remained robust (Fig. 4, C and D; $P < 0.001$; chi-square test), with 59 tests supporting the sponge-sister hypothesis, none supporting the ctenophore-sister hypothesis, and 53 proving inconclusive. Increased support for the sponge-sister hypothesis was commensurate with stringency. For example, 21 and six tests supported the sponge-sister hypothesis for the Th2 and Th5 thresholds, respectively, whereas the ctenophore-sister hypothesis had no support in both cases. This observation supports the consensus in phylogenomics, which suggests that genes with robust phylogenetic signal are best for inferring ancient divergences (*30*), as well as analyses suggesting that "marginal" statistical significance can mislead inferences (*52*).

To further scrutinize our methodological choices and the relative support for the two topologies, we tested the impacts of additional subsampling approaches, gene function (table S4 and supplementary text) (*53*), removing multiple sequence alignment trimming, and substitution model complexity on the outcomes of integrative and traditional phylogenomics (figs. S16 to S19 and supplementary text). We also investigated whether a subset of the consistent genes analyzed might be driving the results. However, this possibility was ruled out by assessing the overlap in gene selection across various subsetting and amino acid recoding strategies, which revealed that diverse combinations of gene sets were used in this study to root the animal tree (fig. S20). Examination of the genomic distribution of consistent genes revealed that they were dispersed throughout the genomes of representative taxa (fig. S21). Finally, we conducted traditional topology tests of consistent genes in either the concatenation or the coalescence frameworks (fig. S22). Together, these controls added 265 hypothesis tests to our study, of which 166 significantly supported the sponge-sister hypothesis, none supported the ctenophore-sister hypothesis, and 99 were insignificant.

Reanalysis of published datasets supports the sponge-sister hypothesis

Although the analyses of our datasets tested a large fraction of parameter space and robustly supported the sponge-sister hypothesis (Fig. 4, A to D, and figs. S16, S17, and S20), it was possible that the strong signal was the product of undetected biases in dataset construction. To test this, we analyzed 10 previously published datasets using integrative phylogenomics (table S5). Eight of these datasets (A08, D13, E13, F15, G15, H15, I15, and J17) were reported to support the ctenophore-sister hypothesis, and two (B09 and C13) were reported to support the sponge-sister hypothesis (Fig. 4E and Table 1). The methods used in the construction and analysis of these datasets differed from our datasets and from each other in taxon sampling (figs. S23 and S24) and in their approaches to orthology inference, outgroup selection, alignment, and trimming strategy (table S5). Therefore, by reanalyzing these datasets, a wide range of parameter space for dataset construction was tested. The diverse recoding and subsampling strategies described above were used, resulting in 280 data matrices (figs. S7 and S25).

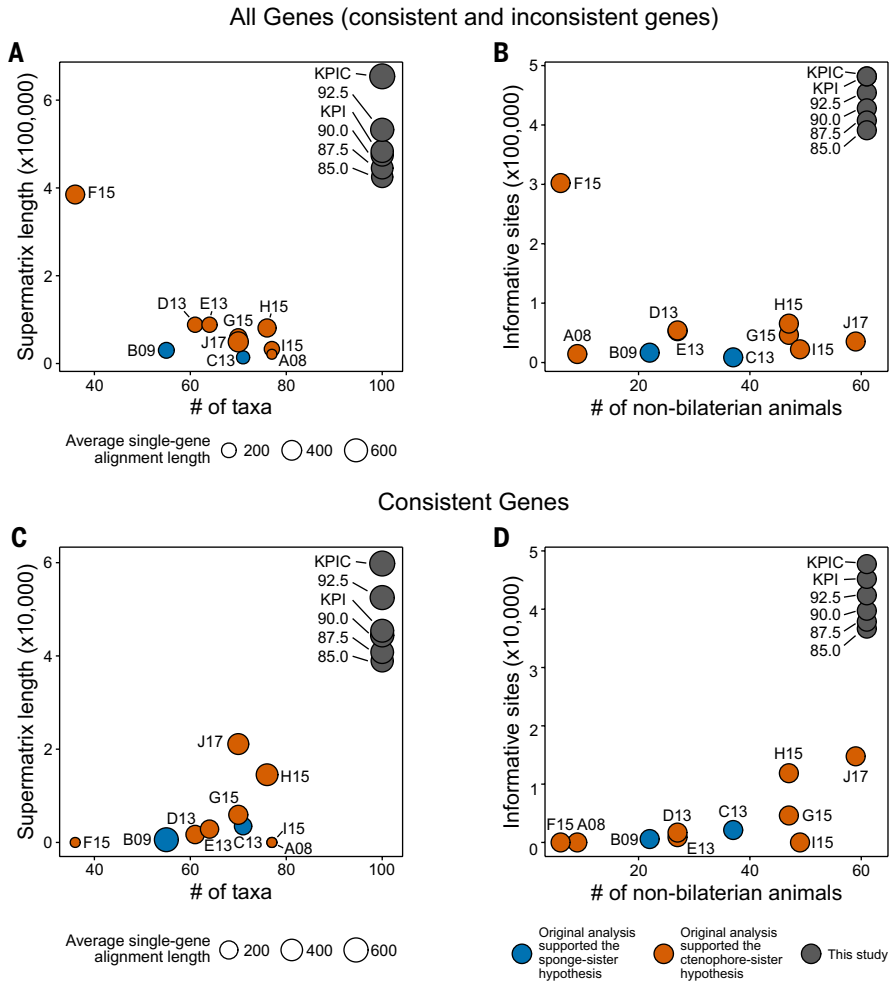

Fig. 3. New large and informative data matrices used to root the animal tree. (**A**) The newly constructed data matrices (this study) have more taxa (*x* axis) and greater supermatrix length (*y* axis) than those previously published (table S5). Moreover, the average protein length was longer (circle size). (**B**) The six data matrices constructed for this study also have the highest number of parsimony informative sites (*y* axis) and the highest number of nonbilaterian animal taxa (*x* axis) compared with previous studies. (**C** and **D**) Consistent genes between concatenation and coalescence were combined into a supermatrix and compared for descriptive metrics. The newly constructed data matrices had a greater total alignment length [(C), *y* axis] and more informative sites [(D), *y* axis] than previously published datasets (table S5). Blue indicates prior support for the sponge-sister hypothesis; orange indicates prior support for the ctenophore-sister hypothesis; and dark gray indicates datasets constructed for this study. Descriptive metrics were calculated using the 20–amino acid alphabet without gene subsampling.

Reanalysis of these published datasets using the Th1 threshold for integrative phylogenomics provided additional support for the sponge-sister hypothesis (Fig. 4E). An average of 14.90 ± 20.03% of genes analyzed in previously published datasets provided consistent results when analyzed by concatenation and coalescence (fig. S26). For the 170 topology tests that provided significant results, all supported the sponge-sister hypothesis; the remaining 110 tests were inconclusive (Fig. 4F). The increased number of inconclusive tests compared with the newly constructed data matrices may be due, in part, to the total alignment length and number of informative sites among consistent genes being substantially lower among previously published datasets (Fig. 3, C and D). Among significant tests, the average percentage of consistent genes that supported the sponge-sister hypothesis was 92.86 ± 7.14% (fig. S27). The observation that many genes are inconsistent suggests that previous analyses supporting the ctenophore-first hypotheses may have suffered from a dearth of phylogenetic signal and an abundance of unreliable genes. Taken together, our results converge on support for the sponge-sister hypothesis.

Conclusions

The use of integrative phylogenomics to analyze diverse datasets robustly supports the sponge-sister hypothesis. The value of integrative phylogenomics arises from its focus on consistent genes, which are less prone to errors from incomplete lineage sorting, gene tree estimation (*36*), and LB attraction artifacts (figs. S1 to S5).

The results of this study support the traditional morphologist's perspective on early animal evolution. The sponge-sister hypothesis was initially proposed because of the resemblance of sponge choanocytes to the closest living relatives of animals, the choanoflagellates: Both have cells with apical microvillar collars surrounding a single flagellum (*54*–*56*). Sponges also lack certain cell types, including myocytes and neurons, that are present in most other animals (*54*, *55*, *57*), including ctenophores. The hypothesized placement of sponges at the root of the animal tree suggests that these cell types evolved after the divergence of sponges from all other animals.

Although a recent analysis of gene linkages provided strong support for the ctenophore-sister hypothesis (*13*), phylogenetic analysis of gene linkages can favor hypotheses that have otherwise not been

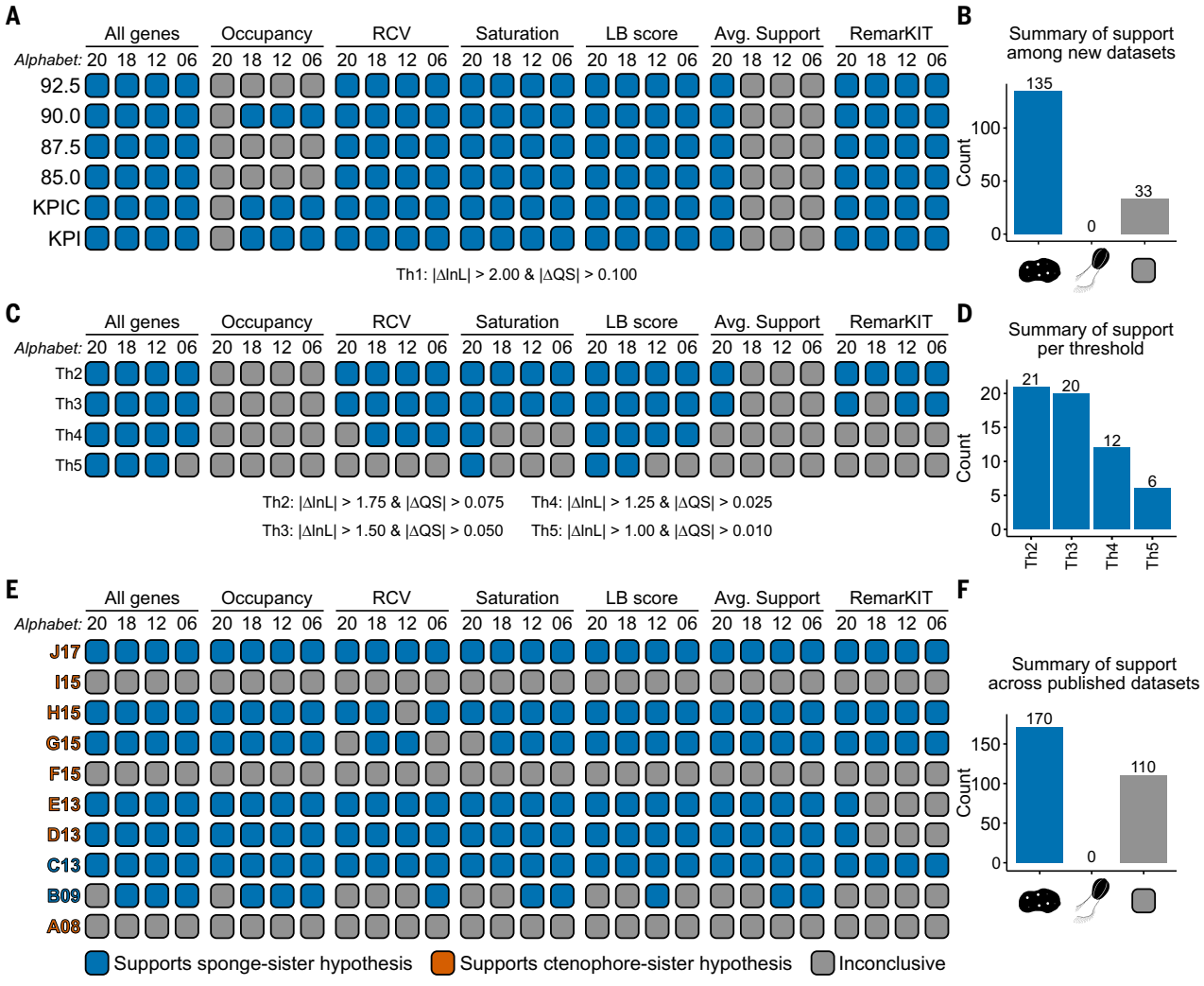

Fig. 4. Integrative phylogenomics supports the sponge-sister hypothesis. (**A**) Each row represents a data matrix; columns depict analyses of the full matrix ("all genes") or the best scoring 50% of genes ranked by one of six metrics: occupancy (taxon representation), RCV (a measure of compositional bias), saturation, LB score (a measure of LB attraction risk), average support (bipartition support), or RemarKIT (machine-learning gene desirability; figs. S8 to S12). Integrative phylogenomic analysis was conducted on genes with robust phylogenetic signal based on a stringent threshold (Th1: $|\Delta\text{lnL}| > 2$ for concatenation and $|\Delta\text{QS}| > 0.1$ for coalescence). The full 20–amino acid alphabet or 18-, 12-, and 6-state recodings were used. Blue indicates support for the sponge-sister hypothesis; orange indicates support for the ctenophore-sister; and gray indicates inconclusive. (**B**) Across all matrices, 135 tests supported the sponge-sister hypothesis, none supported the ctenophore-sister hypothesis, and 33 were inconclusive. (**C** and **D**) In the 92.5 dataset, four other thresholds with lower stringency were applied to identify genes with consistent phylogenetic signal (Th2: $|\Delta\text{lnL}|/|\Delta\text{QS}| \geq 1.75/0.075$, Th3: 1.50/0.050, Th4: 1.25/0.025, and Th5: 1.00/0.010). Support for the sponge-sister hypothesis remained significant ($P < 0.001$; Fisher's exact test): 59 tests favored the sponge-sister hypothesis and none favored the ctenophore-sister hypothesis. As the threshold decreased in stringency, so did support for the sponge-sister hypothesis, whereas the proportion of inconclusive tests increased. (**E**) Ten previously published data matrices (see table S5 for the coding scheme) were reanalyzed. Each row represents one data matrix, with the code colored according to the topology inferred from its original analysis (blue indicates sponge-sister and orange indicates ctenophore-sister). (**F**) Of 280 tests on published datasets, 170 supported the sponge-sister hypothesis and none supported the ctenophore-sister hypothesis.

supported and currently lacks robust models of evolution. Moreover, the homology of chromosomal fusions may not be statistically supported (*34*). Therefore, the utility of gene linkages for phylogenomics and their applicability to root the animal tree should be treated cautiously. Some challenges for using gene linkage may eventually be overcome by using larger samples of chromosome-scale assemblies, advances in modeling chromosomal evolution, and methods for ameliorating sources of phylogenomic error and/or noise (*58*). By contrast, integrative phylogenomics relies on well-established methods and offers a robust framework for analyzing genome-scale data to conduct rigorous topology testing.

In summary, we conducted 785 tests, analyzed >350 million amino acids, and leveraged traditional phylogenomic methods in a new, integrative approach to root the animal phylogeny. All 490 significant tests supported the sponge-sister hypothesis, whereas none supported the ctenophore-sister hypothesis, providing robust support for sponges being the root of the animal tree. We acknowledge that the controversy regarding the root of the animal tree is likely not settled and more research will be needed to reach a consensus view. Nonetheless, integrative phylogenomics provides compelling evidence to root the animal tree and may be useful for addressing other phylogenetic controversies.

REFERENCES AND NOTES

1. A. Rokas, B. L. Williams, N. King, S. B. Carroll, *Nature* **425**, 798–804 (2003).
2. J. E. Bond *et al.*, *Curr. Biol.* **24**, 1765–1771 (2014).
3. B. Misof *et al.*, *Science* **346**, 763–767 (2014).
4. S. M. Crotty *et al.*, *Syst. Biol.* **69**, 249–264 (2020).
5. A. Y. Kawahara *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **116**, 22657–22663 (2019).
6. Y. Li *et al.*, *Curr. Biol.* **31**, 1653–1665.e5 (2021).
7. C. W. Dunn *et al.*, *Nature* **452**, 745–749 (2008).
8. H. Philippe *et al.*, *Curr. Biol.* **19**, 706–712 (2009).
9. J. F. Ryan *et al.*, *Science* **342**, 1242592 (2013).
10. P. Simion *et al.*, *Curr. Biol.* **27**, 958–967 (2017).
11. N. V. Whelan *et al.*, *Nat. Ecol. Evol.* **1**, 1737–1746 (2017).
12. A. K. Redmond, A. McLysaght, *Nat. Commun.* **12**, 1783 (2021).
13. D. T. Schultz *et al.*, *Nature* **618**, 110–117 (2023).
14. P. O. Wainright, G. Hinkle, M. L. Sogin, S. K. Stickel, *Science* **260**, 340–342 (1993).
15. A. G. Collins, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 15458–15463 (1998).
16. M. Medina, A. G. Collins, J. D. Silberman, M. L. Sogin, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 9707–9712 (2001).
17. M. Podar, S. H. D. Haddock, M. L. Sogin, G. R. Harbison, *Mol. Phylogenet. Evol.* **21**, 218–230 (2001).
18. R. C. Brusca, G. J. Brusca, *Invertebrates* (Sinauer, 2002).
19. D. Pisani *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **112**, 15402–15407 (2015).
20. X.-X. Shen, C. T. Hittinger, A. Rokas, *Nat. Ecol. Evol.* **1**, 126 (2017).
21. N. King, A. Rokas, *Curr. Biol.* **27**, R1081–R1088 (2017).
22. R. Feuda *et al.*, *Curr. Biol.* **27**, 3864–3870.e4 (2017).
23. Y. Li, X.-X. Shen, B. Evans, C. W. Dunn, A. Rokas, *Mol. Biol. Evol.* **38**, 4322–4333 (2021).
24. N. V. Whelan, K. M. Halanych, *Nat. Commun.* **14**, 711 (2023).
25. E. D. Jarvis *et al.*, *Science* **346**, 1320–1331 (2014).
26. One Thousand Plant Transcriptomes Initiative, *Nature* **574**, 679–685 (2019).
27. B. Rosenzweig, A. Kern, M. Hahn, Accurate detection of incomplete lineage sorting via supervised machine learning. BioRxiv 515828 [Preprint] (2022); https://doi.org/10.1101/2022.11.09.515828.
28. M. Álvarez-Presas, I. Ruiz-Trillo, J. Paps, Novel genomic approaches support Xenacoelomorpha as sister to all Bilateria. Research Square [Preprint] (2024); https://doi.org/10.21203/rs.3.rs-5529390/v1.
29. J. L. Steenwyk, Y. Li, X. Zhou, X.-X. Shen, A. Rokas, *Nat. Rev. Genet.* **24**, 834–850 (2023).
30. L. Salichos, A. Rokas, *Nature* **497**, 327–331 (2013).
31. H. Philippe *et al.*, *PLOS Biol.* **9**, e1000602 (2011).
32. K. Juravel, L. Porras, S. Höhna, D. Pisani, G. Wörheide, *PLOS ONE* **18**, e0282444 (2023).
33. S. L. Dellaporta *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 8751–8756 (2006).
34. R. R. Copley, Sponges, ctenophores and the statistical significance of syntenies. BioRxiv 652388 [Preprint] (2025); https://doi.org/10.1101/2025.05.07.652388.
35. W. R. Francis, S. Vargas, G. Wörheide, *Peer Community J.* **5**, e56 (2025).
36. X.-X. Shen, J. L. Steenwyk, A. Rokas, *Syst. Biol.* **70**, 997–1014 (2021).
37. N. Lartillot, H. Brinkmann, H. Philippe, *BMC Evol. Biol.* **7** (Suppl 1), S4 (2007).
38. H. Brinkmann, M. van der Giezen, Y. Zhou, G. Poncelin de Raucourt, H. Philippe, *Syst. Biol.* **54**, 743–757 (2005).
39. S. V. Edwards, *Zool. Scr.* **45** (S1), 63–74 (2016).
40. N. Mongiardino Koch, *Mol. Biol. Evol.* **38**, 4025–4038 (2021).
41. R. M. Waterhouse *et al.*, *Mol. Biol. Evol.* **35**, 543–548 (2018).
42. E. V. Kriventseva *et al.*, *Nucleic Acids Res.* **47** (D1), D807–D811 (2019).
43. J. L. Steenwyk, T. J. Buida III, Y. Li, X.-X. Shen, A. Rokas, *PLOS Biol.* **18**, e3001007 (2020).
44. E. Susko, A. J. Roger, *Mol. Biol. Evol.* **24**, 2139–2150 (2007).
45. A. M. Hernandez, J. F. Ryan, *Syst. Biol.* **70**, 1200–1212 (2021).
46. D. D. Pollock, D. J. Zwickl, J. A. McGuire, D. M. Hillis, *Syst. Biol.* **51**, 664–671 (2002).
47. M. J. Phillips, D. Penny, *Mol. Phylogenet. Evol.* **28**, 171–185 (2003).
48. T. H. Struck, *Evol. Bioinform. Online* **10**, 51–67 (2014).
49. J. L. Steenwyk *et al.*, *Bioinformatics* **37**, 2325–2331 (2021).
50. S. Quang, O. Gascuel, N. Lartillot, *Bioinformatics* **24**, 2317–2323 (2008).
51. G. Tan *et al.*, *Syst. Biol.* **64**, 778–791 (2015).
52. V. E. Johnson, *Proc. Natl. Acad. Sci. U.S.A.* **110**, 19313–19317 (2013).
53. A. Hernández-Plaza *et al.*, *Nucleic Acids Res.* **51** (D1), D389–D394 (2023).
54. C. W. Dunn, S. P. Leys, S. H. D. Haddock, *Trends Ecol. Evol.* **30**, 282–291 (2015).
55. C. Nielsen, *R. Soc. Open Sci.* **6**, 190638 (2019).
56. W. Saville-Kent, *A Manual of the Infusoria : Including a Description of All Known Flagellate, Ciliate, and Tentaculiferous Protozoa, British and Foreign, and an Account of the Organization and the Affinities of the Sponges* (D. Bogue, 1880); https://www.biodiversitylibrary.org/bibliography/10143.
57. P. Burkhardt *et al.*, *Science* **380**, 293–297 (2023).
58. J. L. Steenwyk, N. King, *PLOS Biol.* **22**, e3002632 (2024).
59. J. Steenwyk, Data for: Integrative phylogenomics positions sponges at the root of the animal tree, Figshare (2025); https://doi.org/10.6084/M9.FIGSHARE.28229990.

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SUPPLEMENTARY MATERIALS

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Materials and Methods; Figs. S1 to S27; Tables S1 to S10; References (*60*–*97*)

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