Rooting the animal tree of life

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Abstract

Introduction

Over the past decade there has been considerable debate about the position of the root of the animal phylogeny, with Ctenophora-sister and Porifera-sister (Fig XXOverview) emerging as the two primary hypotheses. Historically, there was little debate about the root of the animal tree of life and Porifera-sister was widely accepted though rarely tested. In contrast to the lack of debate about the position of Porifera, there has long been uncertainty about the relationship of Ctenophora to other animals [1].

The first phylogenomic study to include ctenophores [2] suggested a new hypothesis, now referred to as Ctenophora-sister, that ctenophores are our most distant animal relative. Since then many more studies have been published, some supporting Ctenophora-sister, some Porifera-sister, and some neither. As it has become clear that this is a very difficult phylogenetic challenge, and the problem has become better characterized, it has become an interesting test-case to phylogenetic biologists beyond those concerned with this particular biological problem. Work has been hindered, though, because it has been difficult to directly compare results across studies and synthesize findings to understand the broader patterns of support. Here we synthesize data and results from all previous phylogenomic analyses that tested Ctenophora-sister and Porifera-sister, and reanalyze these data using standardized methods, and perform new analyses to characterize differences between studies. We hope that this provides an integrative overview of the challenge and provides direction for future studies. We also hope that the work we have done here, including consolidating all the datasets in one place with consistent formats and species names, will enhance the technical value of this interesting question to methods-focused investigators that look to develop methods to address difficult phylogenetic problems.

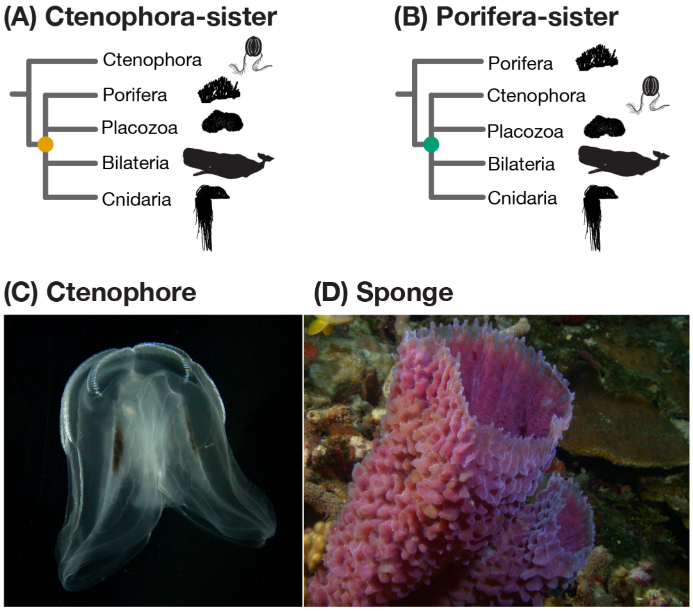


Fig XXOverview. (A) The Ctenophora-sister hypothesis posits that there is a clade (designated by the orange node) that includes all animals except Ctenophora, and that Ctenophora is sister to this clade. (B) The Porifera-sister hypothesis posits that there is a clade (designated by the green node) that includes all animals except Porifera, and that Porifera is sister to this clade. Testing these hypotheses requires evaluating the support for each of these alternative nodes. (C) A ctenophore. (D) A sponge.

Variation across studies

Models of molecular evolution

Models of molecular evolution have several components that each consider different aspects of the evolutionary process. The models that have been used to model protein evolution in studies of the animal root have largely differed according to three components: the exchangeability matrix E, the rate of evolution, and the state equilibrium frequencies Π.

The exchangeability matrix E describes the rate at which one amino acid changes to another. Exchangeability matrices have been used in the studies under consideration here include:

- F81 [3] corresponds to equal rates between all states. The F81 matrix is also sometimes referred to as the Poisson matrix. It has no free parameters to estimate since all off-diagonal elements are set to 1.
- WAG [4] is an empirically derived exchangeability matrix based on a dataset of 182 globular protein families. It has no free parameters to estimate since all off-diagonal elements are set according to values estimated from this particular sample dataset.
- LG [5], like WAG, is an empirically derived exchangeability matrix. It is based on a much larger set of genes, and variation in rates across sites was taken into consideration when it was calculated. It has no free parameters to estimate since all off-diagonal elements are set according to values estimated from this particular sample dataset.
- GTR, the General Time Reversible exchangeability matrix, has free parameters for all off-diagonal elements that describe the exchangeability of different amino acids. It is constrained so that changes are reversible, *i.e.* the rates above the diagonal are the same as those below the diagonal. This leaves 190 parameters that must be estimated from the data long with the other model parameters and the phylogenetic tree topology. This estimation requires a considerable amount of data and computational power, but if successful has the advantage of being based on the dataset at hand rather than a different dataset (as for LG and WAG).

While the exchangeability matrix describes the relative rate of different changes between amino acids, the actual rate can be further scaled. There are couple approaches that have been used in the studies considered here:

- Site homogeneous rates. The rates of evolution are assumed to be the same at all sites in the amino acid alignment.
- Gamma rate heterogeneity. Each site is assigned to a different rate class with its own rate value. This accommodates different rates of evolution across different sites. Gamma is used so commonly that sometimes it isn't even specified, making it difficult at times to know if a study uses Gamma or not.

The vector of equilibrium frequencies Π describes the stationary frequency of amino acids. There are a few approaches that have been used across the studies considered here:

- Empirical site homogeneous. The frequency of each amino acid is observed from the matrix under consideration and applied to homogeneously to all sites in the matrix.
- Estimated site homogeneous. The frequency of each amino acid is inferred along with other model parameters, under the assumption that it is the same at all sites.
- CAT site heterogeneous [6]. Each site is assigned to a class with its own equilibrium frequencies. The number of classes, assignment of sites to classes, and equilibrium frequencies within the data are all estimated in a Bayesian framework.
- C10 to C60 [**??**]. 10 to 60-profile mixture models as variants of the CAT model under the maximum-likelihood framework.

- Six-state amino acid recoding. Amino acids are recoded into six groups based on function to account for both compositional heterogeneity and substitution saturation. Several recoding strategies have been proposed, including Dayhoff 6-state recoding, S&R 6-state recoding, KGB 6-state recoding.

Models can be assembled by selecting different options for all these different components. The models that are applied in practice area heavily influenced by engineering and computational costs, as well as convention. For example, on the questions considered here F81 and GTR exchangeability matrices have only been used in combination with CAT site heterogeneous models of equilibrium frequency. LG and WAG exchangeability matrices have only been used with site homogeneous estimates of equilibrium frequency. This is further confused by the abbreviations that are used for models. Papers often discuss CAT and WAG models as if they are exclusive, but these particular terms apply to non-exclusive model components– CAT refers to variation across sites and WAG a particular exchangeability matrix. CAT is generally shorthand for F81+CAT and WAG is shorthand for WAG+homogeneous equilibrium frequency estimation. One could, though, run a WAG+CAT model.

To avoid confusion on this point, we always specify the exchangeability matrix first, followed by modifiers that describe accommodation of heterogeneity in equilibrium frequencies (*e.g.*, CAT) or rate (*e.g.*, Gamma). If there are no modifiers, then it is implied that site homogeneous models are used.

Gene sampling

Outgroup taxon sampling

XXX

Fig XXOutgroup. The animals and their outgroups, showing the three progressively more inclusive clades Choanimalia, Holozoa, and Opisthokonta.

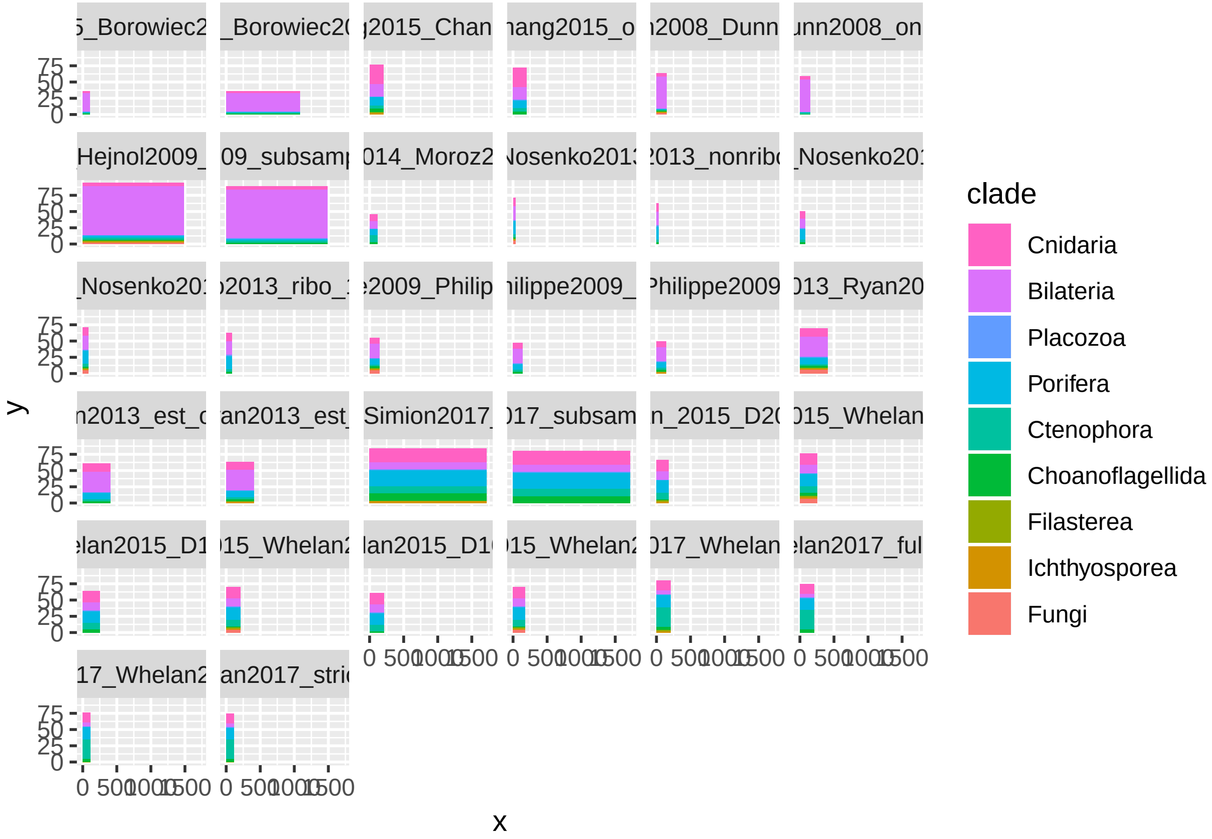
Choanimalia, Holozoa, Opisthokonta

Ingroup taxon sampling

Sensitivity to ingroup sampling has received less attention than sensitivity to outgroup sampling. This may be because results have tended to be more sensitive to outgroup sampling.

Overview of published analyses

Matrix taxon composition



XXX

Fig XXTaxon_composition. Each of the primary matrices considered here, color coded by taxon sampling. Horizontal size is proportional to the number of genes (XXOr should it be sites?) sampled, vertical size to the number of taxa sampled.

Matrix gene composition

```
## # A tibble: 11 x 3
## # Groups:   matrix [6]
##    matrix                  partition                            n
##    <chr>                   <chr>                            <int>
##  1 Borowiec2015_Total1080 OG621.fasta.aln_gene687               2
##  2 Chang2015               l12e_01                              2
##  3 Philippe2009            rpl2                                 2
##  4 Simion2017              V2META12905-42-Calc_Hmm10-BMGE05     2
##  5 Simion2017              V2META14417-42-Calc_Hmm10-BMGE05     2
##  6 Simion2017              V2META14523-42-Calc_Hmm10-BMGE05     2
##  7 Whelan2017_full         Subset18_01                          2
##  8 Whelan2017_strict       Subset19_01                          2
##  9 Whelan2017_strict       Subset4_01                           3
## 10 Whelan2017_strict       Subset5_01                           2
## 11 Whelan2017_strict       Subset9_01                           2
```

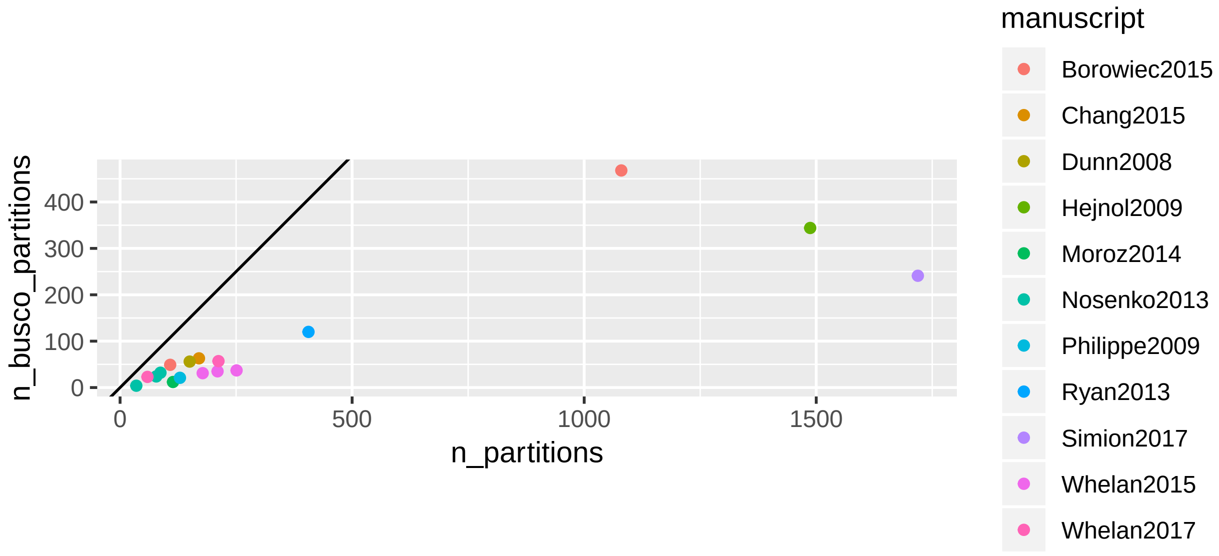


Fig XXBUSCO_annotations. The number of partitions with BUSCO annotations in each matrix, relative to the number of partitions.

Fig XXGene_composition. Each of the primary matrices considered here, color coded by the types of genes sampled (XX Ribosomal proteins, etc). Horizontal size is proportional to the number of genes sampled, vertical size to the number of taxa sampled.

Matrix overlap

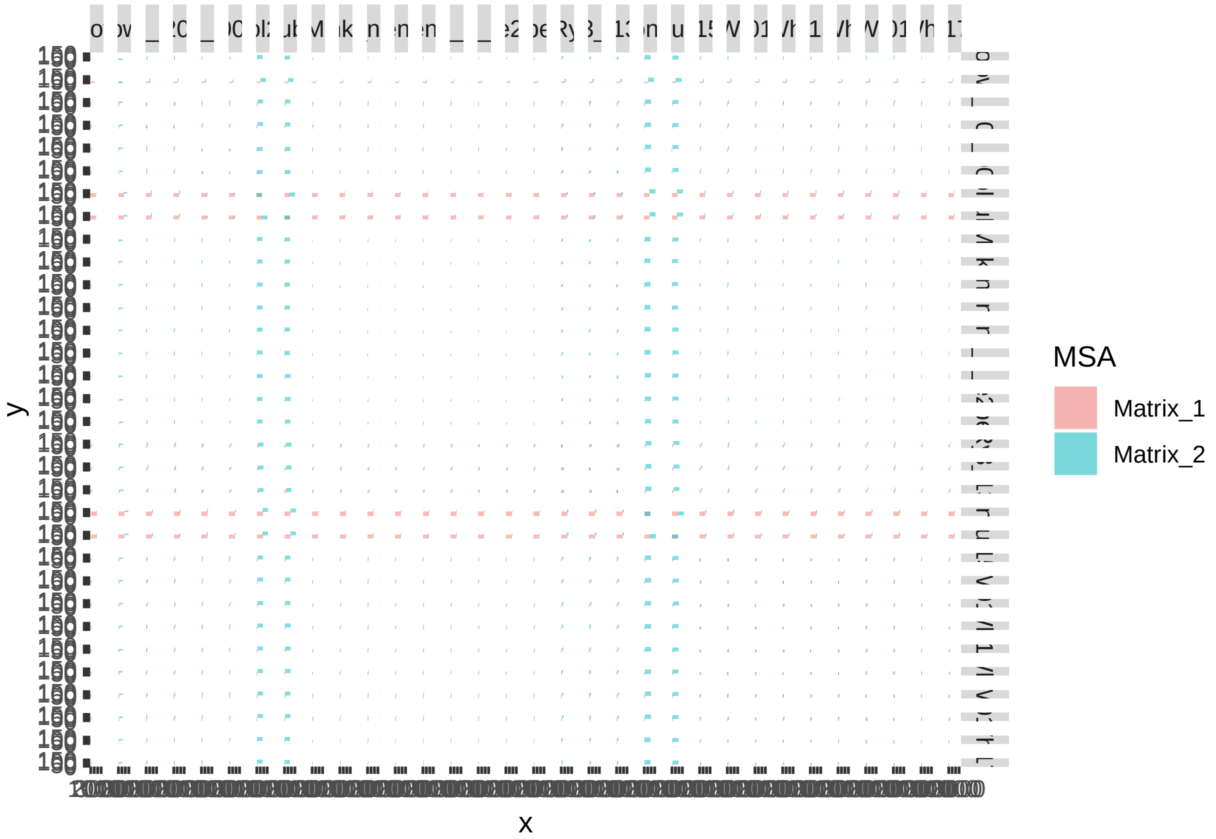
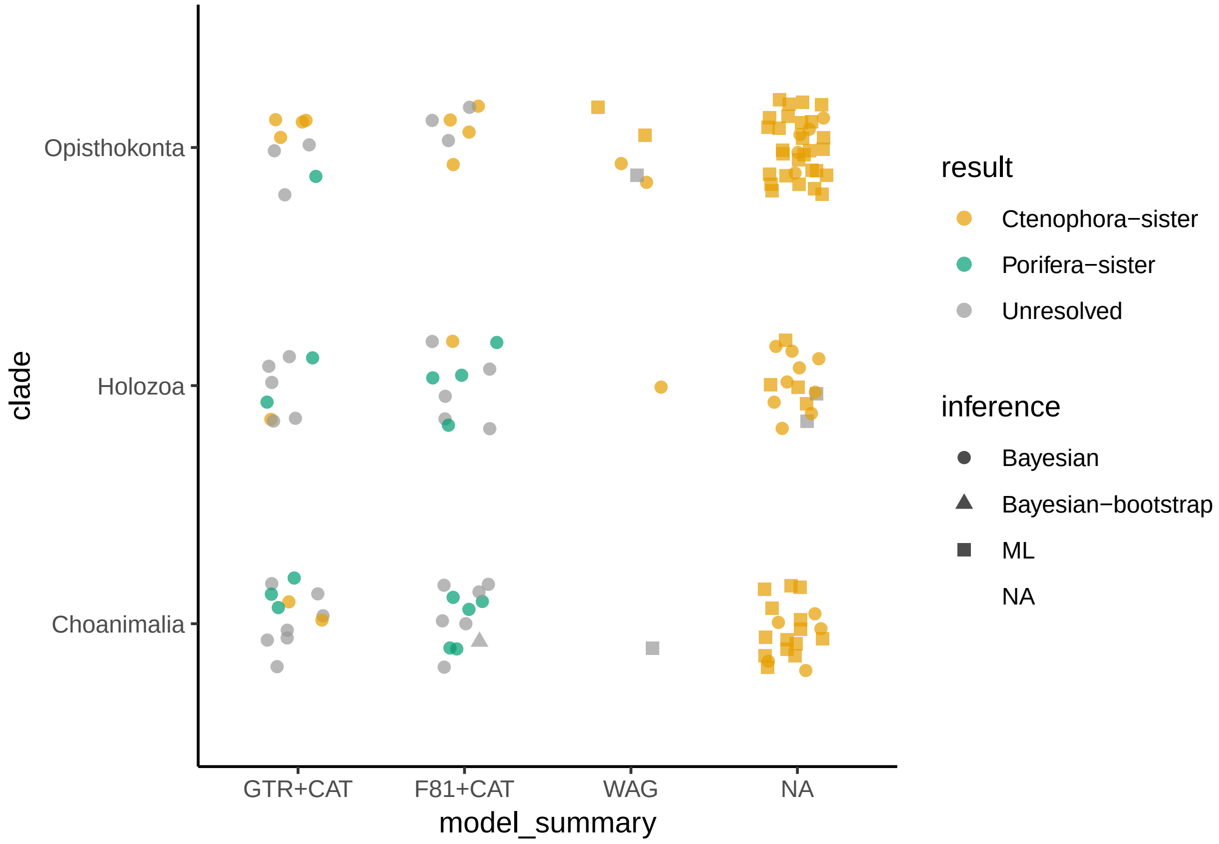


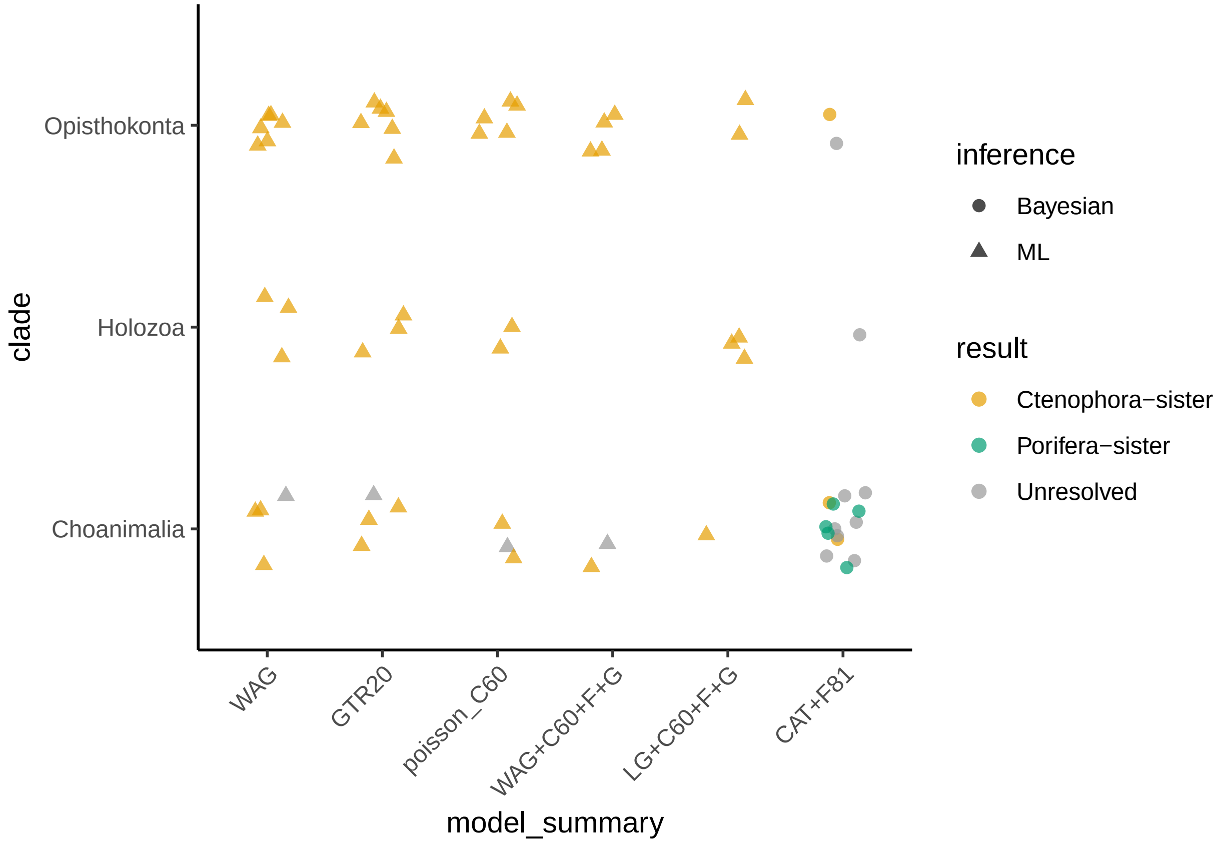
Fig XXAlignment overlap. Pairwise overlap between each of the primary matrices considered here. Horizontal size is proportional to the number of genes sampled, vertical size to the number of taxa sampled. The horizontal intersection shows the proportions of shared genes, the vertical intersection shows the proportions of shared taxa.

Support for Porifera-sister and Ctenophora-sister



A total of 139 analyses were transcribed from the literature.

New analyses of published matrices



One of the challenges of interpreting support for the placement of the animal root across studies is that different programs, software versions, and settings have been used across studies, and phylogenetic analysis decisions have been approached in very different ways. Here we reanalyze the primary matrices from each study under consistent conditions with iqtree. We selected this tool because it has greater model flexibility than other tools and is very fast.

We first tested a variety of models for each matrix, and inferred support under the selected model. We then analyzed every matrix under a panel of standard models, including XXX.

matrix	clade	result	model_summary
Borowiec2015_Best108	Choanimalia	Ctenophora-sister	WAG+C60+F+G
Chang2015	Holozoa	Ctenophora-sister	LG+C60+F+G
Dunn2008	Opisthokonta	Ctenophora-sister	WAG+C60+F+G
Moroz2014_3d	Choanimalia	Unresolved	WAG+C60+F+G
Ryan2013_est	Opisthokonta	Ctenophora-sister	WAG+C60+F+G
Whelan2015_D1	Opisthokonta	Ctenophora-sister	LG+C60+F+G
Whelan2015_D10	Opisthokonta	Ctenophora-sister	WAG+C60+F+G
Whelan2015_D20	Opisthokonta	Ctenophora-sister	WAG+C60+F+G
Whelan2017_full	Holozoa	Ctenophora-sister	LG+C60+F+G
Whelan2017_strict	Choanimalia	Ctenophora-sister	LG+C60+F+G

Table XXModelfinder. The models selected by modelfinder for each matrix.

Comparison of iqtree and phylobayes results

Site heterogeneity in equilibrium frequency has been a major concern in tests of Ctenophora-sister and Porifera-sister. This has been addressed with CAT models. iqtree provides a new family of C models that also address site heterogeneity. Given the extensive computational cost and concerns about overparameterization of CAT models, we compared iqtree C results to CAT results for a subset of matrices to see if they give consistent results. This would be of technical interest because it would reduce the cost of accommodating compositional heterogeneity in future analyses.

New analyses of new matrices

Based on the variation across analyses, we constructed new matrices with altered taxon and gene sampling to test specific hypotheses about differences in support.

The current state of understanding

Interpretting variation support

External criteria, eg posterior predictive scores, model fit etc

Next steps

Conclusion

Methods

All files associated with this analysis are available at https://github.com/caseywdunn/animal_root

Data selection and wrangling

We retreived matrices from each publication (Table XX), storing the raw data in this manuscript's version control repository. We manually edited some minor formatting to make the batch processing of the matrices *en masse*, e.g. standardizing the formatting of charset blocks. All changes made are tracked with git.

Matrix comparison and annotation

Taxon name reconciliation

We programatically queried the NCBI Taxonomy database to standardize names of samples in each matrix. We also use a table where manual entries were needed (Supp Table XX, reconciliation/taxonomy_info/manual_taxonomy_map.tsv), e.g. authors of original matrix indicate species name in original manuscript. For a table summarizing all samples and their new or lengthened names, see Table XX(reconciliation/taxonomy_info/taxon_table.tsv).

Sequence Comparisons

Using the partition files for each matrix, we isolated each sequence for each taxon from each partition. Because many of the matrices had been processed by the original authors to remove columns that are poorly sampled or highly variable, these matrix-derived sequences can have deletions relative to the actual gene sequences.

We used DIAMOND [7] to compare each sequence to all others using default diamond blastp parameters. We further filtered DIAMOND results such that we retained hits for 90% of partitions (pident > 78.0, eValue < 1e-15, no self->self). We ran BUSCO with default parameters for all sequences against the provided metazoa gene set. We also ran a BLAST+ blastp search against the SwissProt [cite] database, filtering such that we retain at least one hit for ~97% of partitions (pident > 50.0, eValue < 1e-15).

Partition Network

We used the sequence similarity comparisons described above to compare partitions.

We constructed a network with Python and NetworkX [8] v2.2 where each node is a partition and each edge represents a DIAMOND sequence-to-sequence match between sequences in the partitions. We extracted each connected component from this network. We further split these components if the the most connected node (i.e. most edges) had two times more the standard deviation from the mean number of edges in the component it is a member of and if removing that node splits the component into two or more components. We then decorated every node in the partition network with the most often found SwissProt BLAST+ result and BUSCO results to see which components contain which classes and families of genes. See Table XX [partition_network_summary table] for a summary tally of each part of the comparison.

Phylogenetic analyses

Full data matrices

To investigate the phylogenetic hypotheses and distribution of phylogenetic signal in studies aiming to elucidate the root of animal position, we considered 16 data matrices from recent studies that were constructed from transcriptomic or genomic data (Table). Because different choices of substitution models could largely influence phylogenetic inference of the placement of the root of animal (e.g. site heterogeneous CAT model and site homogeneous model), we first investigated four different sets of substitution models in IQtree [9], including C10 to C60 profile mixture models as variants of the CAT model in ML framework. Phylogenomic analyses of all the datasets were first conducted under WAG+G, GTR+G, associated best-fit substitution model (C10-C60 model were included for model comparison via -madd option) with ModelFinder [10] and Poisson + C60 models using IQtree v [9]. Nodal support was assessed with 1000 ultrafast bootstrap replicates for each analysis.

Outgroup taxa sampling with C10-C60 and CAT models

Because different choices of outgroups could also affect phylogenetic inference as suggested in previous analyses, we parsed the full data matrices into three different types of outgroups: Choanimalia , Holozoa and Opisthokonta. These datasets include the same set of genes but differ in the composition of outgroup species. Choanozoa only includes choanofagellates as outgroup; Holozoa also includes more distantly related holozoans; Opistokonta also includes Fungi. For each Choanozoa data matrice, both C10-C60 models in ML and CAT models in PhyloBayes were conducted. The maximum likelihood analysis was performed using the best-fit substitution model identified as above and nodal support was assessed with 1000 ultrafast bootstrap replicates using IQtree. Moreover, bayesian inference with the site-heterogeneous CAT-Poisson substitution model was done with PhyloBayes MPI. To minimize computational burden, CAT-GTR models were not in this study.

For results of choanozoa matrices indicated a strong support that sponges are the sister group to the remaining Metazoa using CAT model, bayesian inference with CAT-Poisson model was also conducted to Holozoa and Opisthokonta data matrices with the same settings as above. For CAT-poisson analyses on all the data matrices, two independent chains were sampled every generation. Tracer plots of MCMC runs were visually inspected in Tracer v1.6 to assess stationarity and appropriate burn-in. Chains were considered to have reached convergence when the maxdiff statistic among chains was below 0.3 (as measured by bpcomp) and effective sample size > 50 for each parameter (as measured by tracecomp). A 50% majority-rule consensus tree was computed with bpcomp, and nodal support was estimated by posterior probability. All analyses in PhyloBayes were run for at least one month computational time.

phylogenetic signal in conflicted dataset

To investigate the distribution of phylogenetic signal in data matrices, we considered four data matrices from four studies that had different topology between ML and BI using CAT model in our reanalysis, including Philippe2008, Ryan2013, and Whelan_2017 data matrices.We examined two hypotheses: Ctenophora-sister and Porifera-sister to rest of metazoans, under both ML and BI frameworks with different outgroup schemes

(Choanozoa and Opisthokonta). For ML analysis in each dataset, site-wise likelihood scores ere inferred for both hypotheses using IQtree (option -g) with the same best-fit model identified above. The two different phylogenetic hypotheses passed to IQtree (via -z) were the corresponding tree that the ctenophore as the sister lineage tree and the corresponding tree that was modified to have sponges as the sister to all other metazoans. The constraint trees were conducted by R package. The numbers of genes and sites supporting each hypothesis were calculated with RAxML output and Perl scripts from Shen et al. For BI analysis, we only considered the Whelan_2017 dataset.

Ackowledgements

We thank the Yale Center for Research Computing for use of the research computing infrastructure, specificaly the Farnam cluster.

Author contributions

Supplemental Information

Details of published analyses

Dunn *et al.* 2008

Dunn *et al.* [2] added Expressed Sequence Tag (EST) data for 29 animals. It was the first phylogenomic analysis that included ctenophores, and therefore that could test the relationships of both Ctenophora and Porifera to the rest of animals. It was also the first phylogenetic analysis to recover Ctenophora as the sister group to all other animals.

The data matrix was constructed using a semi-automated approach. Genes were translated into proteins, promiscuous domains were masked, all gene sequences from all species were compared to each other with blastp, genes were clustered based on this similarity with TribeMCL [11], and these clusters were filtered to remove those with poor taxon sampling and high rates of lineage-specific duplications. Gene trees were then constructed, and in clades of sequences all from the same species all but one sequence were removed (these groups are often due to assembly errors). The remaining gene trees with more than one sequence for any taxon were then manually inspected. If strongly supported deep nodes indicative of paralogy were found, the entire gene was discarded. If the duplications for a a small number of taxa were unresolved, all genes from those taxa were excluded. Genes were then realigned and sites were filtered with Gblocks [12], resulting in a 77 taxon matrix. Some taxa in this matrix were quite unstable, which obscured other strongly-supported relationships. Unstable taxa were identified with leaf stability indices [13], as implemented in phyutility [14], and removed from the matrix. This resulted in the 64-taxon matrix that is the focus of most of their analyses. Phylogenetic analyses were conducted under the F81+CAT model in phylobayes [6], and under the WAG model in MrBayes [15] and RAxML [16].

Regarding the recovery of Ctenophora-sister, the authors concluded:

> The placement of ctenophores (comb jellies) as the sister group to all other sampled metazoans is strongly supported in all our analyses. This result, which has not been postulated before, should be viewed as provisional until more data are considered from placozoans and additional sponges.

Note that there was, in fact, an exception to strong support. An analysis of the 40 ribosomal proteins in the matrix recovered Ctenophora-sister with only 69% support. This study did not include *Trichoplax*.

Philippe *et al.* 2009

Philippe *et al.* 2009 [Philippe:2009hh] assembled a 128 EST dataset for 55 species to explore phylogenetic relationship of early diverging animals.

Hejnol et al. 2009

Pick *et al.* 2010

Pick *et al.* [17] sought to test whether Ctenophora-sister was an artefact of insufficient taxon sampling. They added new and additional published sequence data to the 64-taxon matrix of Dunn *et al.* [2]. The new taxa included 12 sponges, 1 ctenophore, 5 cnidarians, and *Trichoplax*. They further modified the matrix by removing 2,150 sites that were poorly sampled or aligned. They considered two different sets of outgroups: Choanoflagellata (resulting in Choanimalia) and the same sampling as Dunn *et al.* (resulting in Opisthokonta).

All their analyses were conducted under the F81+CAT+Gamma model in phylobayes [6], in both a Bayesian framework and with bootstrapping. All analyses have the same ingroup sampling and site removal so it isn't possible to independently assess the impact of these factors. Analyses with Choanimalia sampling recovered Porifera-sister with 72% posterior probability (PP) and 91% bootstrap support (BS). With broader Opisthokonta sampling, support for Porifera-sister is 84% PP. This is an interesting case where increased outgroup sampling leads to increased support for Porifera-sister.

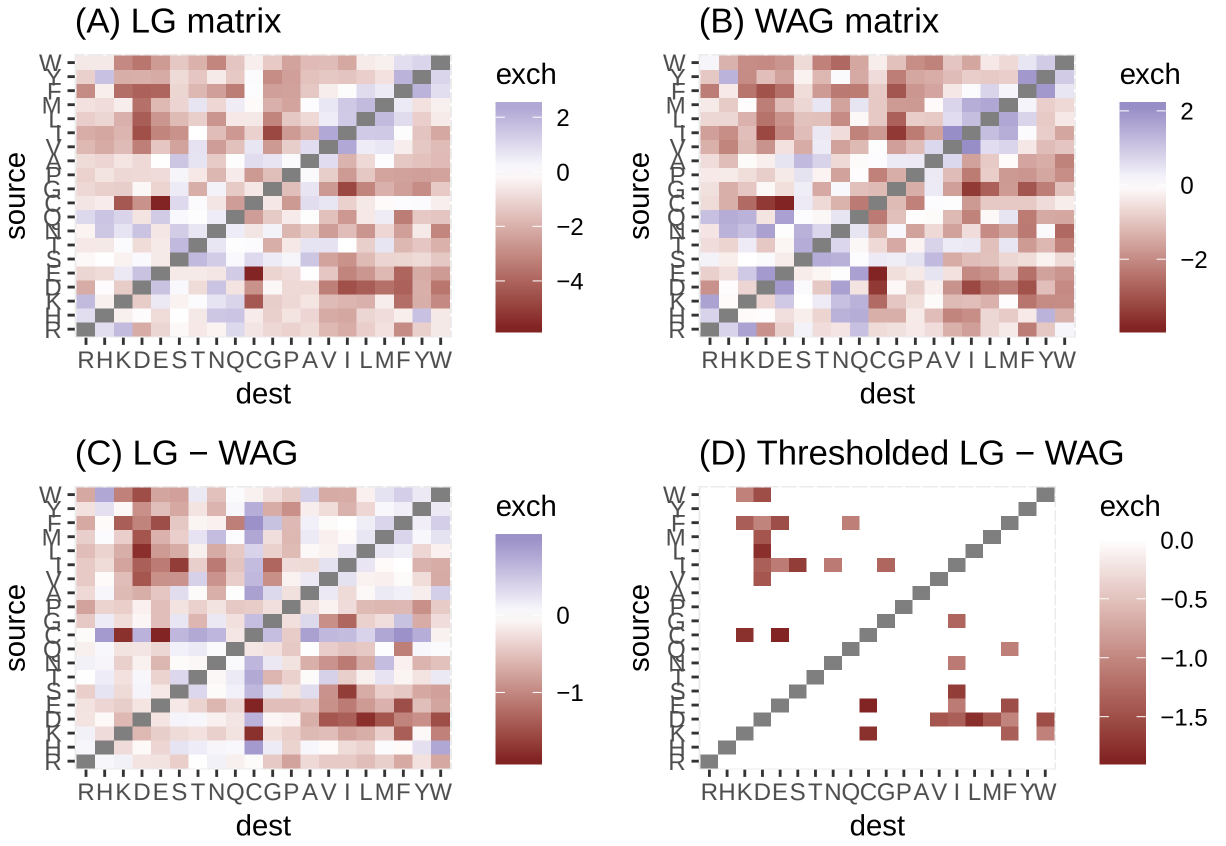
The authors argue that previous results supporting Ctenophora-sister “are artifacts stemming from insufficient taxon sampling and long-branch attraction (LBA)” and that “this hypothesis should be rejected”. Although the posterior probabilities supporting Porifera-sister are not strong, they conclude:

> Results of our analyses indicate that sponges are the sister group to the remaining Metazoa, and Placozoa are sister group to the Bilateria

They also investigated saturation, and conclude that Dunn *et al.* [2] is more saturated than Philippe *et al.* 2009 [Philippe:2009hh]. Note that the Pick *et al.* [17] dataset is not reanalyzed here because partition data are not available, and due to site filtering the partition file from Dunn *et al.* [2] cannot be applied to this matrix.

Model matrix comparison

WAG and LG are both fixed exchange matrices. Their differences are largely limitted to a few amino acid changes.



Matrix mapping

Taxa and partition correspondence across manuscripts was assessed by comparing all sequences for each taxon in each partition across all matrices with diamond blast. Based on inspection of sequence similarity, we excluded all comparisons with less than 99% identity and greater than 10^{-25} e-value.

Taxa comparison across matrices

The primary intent of comparing taxa across matrices was to validate our taxon name reconciliation across studies.

We first considered pairwise similarity between the same species from different matrices in different studies.

Partition comparison across matrices

```
## # A tibble: 3,639 x 3
## # Groups:   matrix [17]
##      matrix                 component_number     n
##      <chr>                  <chr>            <int>
##    1 Philippe2009           21                  14
##    2 Philippe2009           2                    9
##    3 Simion2017             21                   9
##    4 Chang2015              21                   7
##    5 Philippe2009           4                    7
##    6 Philippe2009           1                    6
##    7 Simion2017             2                    6
##    8 Whelan2017_full        21                   6
##    9 Chang2015              2                    5
##   10 Dunn2008               2                    5
##   11 Ryan2013_est           2                    5
##   12 Ryan2013_est           21                   5
##   13 Simion2017             11                   5
##   14 Simion2017             4                    5
##   15 Borowiec2015_Best108   48                   4
##   16 Borowiec2015_Total1080 11                   4
##   17 Borowiec2015_Total1080 2                    4
##   18 Borowiec2015_Total1080 48                   4
##   19 Borowiec2015_Total1080 94                   4
##   20 Chang2015              30                   4
##   21 Nosenko2013_ribo_11057 30                   4
##   22 Nosenko2013_ribo_14615 30                   4
##   23 Philippe2009           12                   4
##   24 Borowiec2015_Best108   2                    3
##   25 Borowiec2015_Best108   4                    3
##   26 Borowiec2015_Total1080 21                   3
##   27 Borowiec2015_Total1080 4                    3
##   28 Borowiec2015_Total1080 47                   3
##   29 Chang2015              0                    3
##   30 Chang2015              12                   3
##   31 Chang2015              49                   3
##   32 Dunn2008               0                    3
##   33 Hejnol2009             2                    3
##   34 Nosenko2013_ribo_11057 0                    3
##   35 Nosenko2013_ribo_11057 2                    3
##   36 Nosenko2013_ribo_11057 47                   3
##   37 Nosenko2013_ribo_11057 49                   3
##   38 Nosenko2013_ribo_14615 0                    3
##   39 Nosenko2013_ribo_14615 2                    3
##   40 Nosenko2013_ribo_14615 47                   3
##   41 Nosenko2013_ribo_14615 49                   3
##   42 Philippe2009           11                   3
##   43 Philippe2009           136                  3
##   44 Philippe2009           137                  3
##   45 Philippe2009           176                  3
##   46 Philippe2009           30                   3
##  47 Philippe2009           47                    3
##  48 Philippe2009           73                    3
##  49 Philippe2009           76                    3
##  50 Simion2017             30                    3
##  51 Simion2017             94                    3
##  52 Simion2017             96                    3
##  53 Whelan2015_D1          21                    3
##  54 Whelan2017_full        11                    3
##  55 Whelan2017_full        83                    3
##  56 Borowiec2015_Best108   11                    2
##  57 Borowiec2015_Best108   21                    2
##  58 Borowiec2015_Best108   30                    2
##  59 Borowiec2015_Total1080 0                     2
##  60 Borowiec2015_Total1080 153                   2
##  61 Borowiec2015_Total1080 275                   2
##  62 Borowiec2015_Total1080 30                    2
##  63 Borowiec2015_Total1080 74                    2
##  64 Borowiec2015_Total1080 91                    2
##  65 Chang2015              1                     2
##  66 Chang2015              101                   2
##  67 Chang2015              11                    2
##  68 Chang2015              176                   2
##  69 Chang2015              177                   2
##  70 Chang2015              23                    2
##  71 Chang2015              3                     2
##  72 Chang2015              38                    2
##  73 Chang2015              53                    2
##  74 Chang2015              70                    2
##  75 Chang2015              73                    2
##  76 Chang2015              75                    2
##  77 Chang2015              76                    2
##  78 Chang2015              88                    2
##  79 Chang2015              89                    2
##  80 Chang2015              91                    2
##  81 Dunn2008               23                    2
##  82 Dunn2008               3                     2
##  83 Dunn2008               30                    2
##  84 Dunn2008               47                    2
##  85 Dunn2008               49                    2
##  86 Dunn2008               53                    2
##  87 Dunn2008               70                    2
##  88 Hejnol2009             11                    2
##  89 Hejnol2009             12                    2
##  90 Hejnol2009             153                   2
##  91 Nosenko2013_ribo_11057 11                    2
##  92 Nosenko2013_ribo_11057 23                    2
##  93 Nosenko2013_ribo_11057 3                     2
##  94 Nosenko2013_ribo_11057 38                    2
##  95 Nosenko2013_ribo_11057 53                    2
##  96 Nosenko2013_ribo_11057 70                    2
##  97 Nosenko2013_ribo_11057 73                    2
##  98 Nosenko2013_ribo_11057 75                    2
##  99 Nosenko2013_ribo_11057 76                    2
## 100 Nosenko2013_ribo_11057 88                    2
## # ... with 3,539 more rows

## # A tibble: 1,150 x 2
##     component_number     n
##     <chr>            <int>
##   1 21                  55
##   2 2                   47
##   3 11                  29
##   4 30                  26
##   5 4                   21
##   6 0                   20
##   7 47                  20
##   8 48                  18
##   9 49                  17
##  10 12                  15
##  11 3                   14
##  12 53                  14
##  13 1                   12
##  14 38                  12
##  15 70                  12
##  16 23                  11
##  17 71                  11
##  18 33                  10
##  19 72                  10
##  20 73                  10
##  21 74                  10
##  22 75                  10
##  23 76                  10
##  24 80                   9
##  25 81                   9
##  26 82                   9
##  27 83                   9
##  28 84                   9
##  29 85                   9
##  30 88                   9
##  31 89                   9
##  32 90                   9
##  33 100                  8
##  34 101                  8
##  35 102                  8
##  36 103                  8
##  37 106                  8
##  38 107                  8
##  39 108                  8
##  40 42                   8
##  41 9                    8
##  42 91                   8
##  43 92                   8
##  44 93                   8
##  45 94                   8
##  46 95                   8
##  47 96                   8
##  48 97                   8
##  49 98                   8
##  50 99                   8
##  51 111                  7
##  52 112                  7
##  53 113                  7
##  54 114                  7
##  55 115                  7
##  56 121                  7
##  57 122                  7
##  58 123                  7
##  59 124                  7
##  60 125                  7
##  61 126                  7
##  62 127                  7
##  63 128                  7
##  64 129                  7
##  65 130                  7
##  66 131                  7
##  67 132                  7
##  68 133                  7
##  69 134                  7
##  70 135                  7
##  71 136                  7
##  72 137                  7
##  73 138                  7
##  74 139                  7
##  75 140                  7
##  76 141                  7
##  77 142                  7
##  78 143                  7
##  79 144                  7
##  80 145                  7
##  81 60                   7
##  82 68                   7
##  83 146                  6
##  84 147                  6
##  85 148                  6
##  86 149                  6
##  87 150                  6
##  88 151                  6
##  89 152                  6
##  90 153                  6
##  91 154                  6
##  92 155                  6
##  93 156                  6
##  94 157                  6
##  95 158                  6
##  96 159                  6
##  97 160                  6
##  98 161                  6
##  99 162                  6
## 100 163                  6
## # ... with 1,050 more rows
```

```
## # A tibble: 17 x 5
##    matrix      n_total_partitio~ n_components_with~     n n_partitions_dis~
##    <chr>                   <int>              <int> <int>             <dbl>
##  1 Borowiec20~               108                 48     6                 0
##  2 Borowiec20~              1080                334    39                 0
##  3 Chang2015                 170                 55    68                 0
##  4 Dunn2008                  150                 52    37                 0
##  5 Hejnol2009               1487                244    27                 0
##  6 Moroz2014_~               114                  9     1                 0
##  7 Nosenko201~                35                  3     0                 0
##  8 Nosenko201~                78                 19    69                 0
##  9 Nosenko201~                87                 25    71                 0
## 10 Philippe20~               129                 19    65                 2
## 11 Ryan2013_e~               406                111    26                 0
## 12 Simion2017               1719                133    24                 0
## 13 Whelan2015~               251                 29     5                 0
## 14 Whelan2015~               210                 28     5                 0
## 15 Whelan2015~               178                 27     5                 0
## 16 Whelan2017~               212                 52     9                 3
## 17 Whelan2017~                59                 13     4                12
```

The count for a partition pair can be much arger than the number of genes in the matrix, which suggests that the count is the number of hsps rather than the number of sequences with hits.

There are 17 matrices. A gene that is perfectly sampled would form a cluster with this size. Very few clusters, though, are this size. This suggests that intersection of genes between matrices is low

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