DeBiasse, M. B., Buckenmeyer, A., Macrander, J., Babonis, L. S., Bentlage, B., Cartwright, P., Prada, C., Reitzel, A. M., Stampar, S. N., Collins, A. G., Daly, M., & Ryan, J. F. (2024). A cnidarian phylogenomic tree fitted with hundreds of 18S leaves. *Bulletin of the Society of Systematic Biologists*. https://doi.org/10.18061/bssb.v3i2.9267

Investigations

A Cnidarian Phylogenomic Tree Fitted With Hundreds of 18S Leaves

Melissa B. DeBiasse[1,2], Ariane Buckenmeyer[1,3], Jason Macrander[4], Leslie S. Babonis[5], Bastian Bentlage[6], Paulyn Cartwright[7], Carlos Prada[8], Adam M. Reitzel[9], Sergio N. Stampar[10], Allen G. Collins[11], Marymegan Daly[12], Joseph F. Ryan[1,13]

[1] Whitney Laboratory for Marine Bioscience, Department of Biology, University of Florida, [2] Department of Biology, Radford University, [3] Department of Zoology, Swedish Museum of Natural History, [4] Biology Department, Florida Southern College, [5] Department of Ecology and Evolutionary Biology, Cornell University, [6] Marine Laboratory, University of Guam, [7] Department of Ecology and Evolutionary Biology, University of Kansas, [8] Department of Biological Sciences, University of Rhode Island, [9] Department of Biological Sciences, University of North Carolina Charlotte, [10] Department of Biological Sciences, São Paulo State University, [11] National Systematics Lab of NOAA Fisheries and Department of Invertebrate Zoology, Smithsonian Institution National Museum of Natural History, [12] Department of Evolution, Ecology, and Organismal Biology, The Ohio State University, [13] Department of Biology, University of Florida

Keywords: Ceriantharia, Coralliformes, Anthozoa, Endocnidozoa, Medusozoa, Operculozoa

https://doi.org/10.18061/bssb.v3i2.9267

Bulletin of the Society of Systematic Biologists

Abstract

Cnidarians are critical members of aquatic communities and have been an experimental system for a diversity of research areas ranging from development to biomechanics to global change biology. Yet, we still lack a well-resolved, taxonomically balanced cnidarian tree of life to place this research in appropriate phylogenetic context. To move towards this goal, we combined data from 26 new anthozoan transcriptomes with 86 previously published cnidarian and outgroup datasets to generate two 748-locus alignments containing 123,051 (trimmed) and 449,935 (untrimmed) amino acids. We estimated maximum likelihood phylogenies for both matrices under partitioned and unpartitioned site-homogeneous and site-heterogenous models of substitution. We used the resulting topology to constrain a phylogenetic analysis of 1,814 small subunit ribosomal (18S) gene sequences from GenBank. Our results confirm the position of Ceriantharia (tube-dwelling anemones), a historically recalcitrant group, as sister to the rest of Hexacorallia across all phylogenies regardless of data matrix or model choice. We find unanimous support for the sister relationships of Scleractinia and Corallimorpharia and of Endocnidozoa and Medusozoa. We propose the name Coralliformes for the clade uniting scleractinians and corallimorpharians and the name Operculozoa for the clade uniting endocnidozoans and medusozoans. Of the 229 genera with more than a single representative in our 18S hybrid phylogeny, 47 (21%) were identified as monophyletic, providing a starting point for a number of taxonomic revisions. Together, these data are an invaluable resource for comparative cnidarian research and provide perspective to guide future refinement of cnidarian systematics.

Introduction

Cnidarians have been evolving independently from other animals for at least 600 million years (Dohrmann & Wörheide, 2017; Erwin, 2015; McFadden et al., 2021) and have diversified into an astonishingly wide assemblage of forms, including hard and soft corals, anemones, siphonophores, hydroids, jellyfish, and myxozoan parasites. Cnidaria consists of 12,789 extant, accepted species (WoRMS Editorial Board, 2022) and approximately 8,000 additional predicted species (estimated from Appeltans et al., 2012). These diverse species form a well-supported clade and are united by their ability to produce stinging cells called cnidocytes (Collins et al., 2020). The species richness of Cnidaria, the ecological importance of many of its species, and its phylogenetic position as sister to Bilateria have made Cnidaria the focus of a range of basic biological research questions. As such, the long-standing goal of establishing a complete cnidarian tree of life is becoming more urgent but also more tractable as maturing sequencing technologies allow for the collection of more phylogenetic characters from more species.

There is a rich history of research involving cnidarians, with centuries of studies on topics such as regeneration

(e.g., Trembley et al., 1744; Zeleny, 1907; Zoja, 1895), embryogenesis (e.g., Hargitt, 1904; Murbach, 1896), coral reef formation (e.g., Darwin, 1851), life history (e.g., Sars, 1829), physiology (e.g., Romanes, 1880), systematics (e.g., Müller, 1862), and morphology (e.g., Hargitt, 1901). Scientific interest in these animals has not waned over time. Research on broad biological questions using cnidarians as a focal system continues in all fields of biology, with striking recent examples including studies of allorecognition (Karadge et al., 2015), biogeography (Martínez et al., 2010), biomechanics (Hamlet & Miller, 2014), circadian clock (Peres et al., 2014), development (Helm et al., 2013), early animal evolution (Bebenek et al., 2004; Collins & Valentine, 2001; Gröger & Schmid, 2001; Martin et al., 1997), evolutionary novelty (Babonis et al., 2016), genomics (Chapman et al., 2010; Leclère et al., 2019; Putnam et al., 2007), germ cell evolution (C.-Y. Chen et al., 2020; Extavour et al., 2005), global change (Bellwood et al., 2004; Hoegh-Guldberg, 1999), human health (e.g., Miller et al., 2005; Sullivan & Finnerty, 2007), life history (Sanders & Cartwright, 2015), natural products (Jouiaei et al., 2015; Mariottini & Grice, 2016), neurobiology (Grimmelikhuijzen et al., 2004; Marlow et al., 2009), regeneration (Bradshaw et al., 2015; Chera et al., 2009), stem cell biology (Gahan et al., 2016; Siebert et al., 2019), symbiosis (Davy et al., 2012; Gault et al., 2021; Lehnert et al., 2012; Newkirk et al., 2018), venom (Klompen et al., 2020; Macrander et al., 2015, 2016), and vision (Picciani et al., 2018). This growing community of researchers and an expanding taxonomic breadth applied to a diversity of questions (e.g., He et al., 2019) underscores the importance of an accurate and comprehensive cnidarian tree of life.

Early efforts to reconstruct the phylogeny of Cnidaria emphasized broad-scale patterns, including work by Siddall et al. (1995) who used 18S sequence data to demonstrate that Myxozoa belonged to Cnidaria after Smothers et al. (1994) showed them to be metazoans. Bridge et al. (1995) combined 18S and 16S sequence data with morphological characters to test class-level relationships within Cnidaria. Many multi-locus studies followed, including those that used two or more nuclear ribosomal genes (e.g., 5S, 18S, 28S, ITS), two or more mitochondrial genes (e.g., 12S, 16S, COI, COIII), or a combination of both ribosomal and mitochondrial genes to resolve relationships within individual cnidarian lineages. The earliest molecular phylogenetic studies to employ complete mitochondrial genome sequences focused on relationships within Scleractinia (Medina et al., 2006), Antipatharia (Mercer R. Brugler & France, 2007), Hydrozoa (Kayal et al., 2015), and across all of Cnidaria (Kayal et al., 2013; Kayal & Lavrov, 2008). More recently, cnidarian systematics has entered the phylogenomics age, with studies using data from hundreds (and sometimes thousands) of loci from transcriptome sequences (Chang et al., 2015; Kayal et al., 2018; Zapata et al., 2015) and target-capture sequencing approaches (Bentlage & Collins, 2021; Cowman et al., 2020; Glon et al., 2021; Horowitz et al., 2020; Quattrini et al., 2017, 2020). See Table S1 for an extensive, but non-exhaustive, list of 142 published cnidarian molecular phylogenetic studies.

At higher taxonomic levels, an accumulating body of phylogenetic evidence based on one or a few loci consistently recovers monophyletic Anthozoa, Hexacorallia, Octocorallia, Antipatharia, Ceriantharia, Zoantharia, Medusozoa, Staurozoa, Scyphozoa, Cubozoa, Hydrozoa, Endocnidozoa, and Myxozoa. However, the inferred phylogenetic relationships among and within these lineages differ in various studies. For example, many of the early cladistic and likelihood analyses of sequence data that included representatives of Staurozoa, Scyphozoa, Cubozoa, and Hydrozoa did not resolve the position of Staurozoa (Bridge et al., 1995; Collins, 2002; Kim et al., 1999) (Fig. S1). Through analyses of morphology and 18S ribosomal RNA sequences, Marques and Collins (2004) found support for a clade consisting of Cubozoa and Staurozoa, with Scyphozoa as sister to this clade. Subsequent analyses of 28S ribosomal genes by Collins et al. (2006) supported Cubozoa and Scyphozoa as sister lineages, with Staurozoa as the sister group to this clade plus Hydrozoa. Kayal et al. (2013) found support in analyses of complete mitochondrial genome sequences for a clade that consisted of Staurozoa and Cubozoa, sister to a clade consisting of Hydrozoa and Scyphozoa. More recently, based on analyses of phylogenomic datasets, Zapata et al. (2015), Kayal et al. (2018), and Quattrini et al. (2020) all found support for Acraspeda (i.e., the clade uniting Staurozoa, Cubozoa and Scyphozoa), with Staurozoa sister to Rhopaliophora (i.e., the clade that unites Cubozoa and Scyphozoa) (Fig. S1).

No phylogenetic analysis published to date provides evidence to support recent taxa erected as part of a re-classification of acrasped cnidarians proposed by Straehler-Pohl and Jarms (2022a, 2022b). Two main clades of hydrozoans, Trachylina and Hydroidolina, are consistently recovered as monophyletic, although studies using traditional Sanger sequencing markers have failed to recover relationships between the main lineages within Hydroidolina with sufficient support (e.g., Cartwright et al., 2008; Collins et al., 2008; Picciani et al., 2018). Multiple studies have confirmed that most of the major groups of Hydroidolina—Leptothecata, Siphonophorae, Capitata, and Aplanulata—are monophyletic. However, Filifera has been found to be polyphyletic (Bentlage & Collins, 2021; Cartwright et al., 2008; Collins et al., 2008; A. M. Nawrocki et al., 2010, 2013). Historically, there has been little consistency in inferred relationships between higher-level groups within Hydroidolina. Recent phylogenetic analyses of Trachylina found congruent relationships among the major groups Limnomedusae, Trachymedusae, Narcomedusae, and Actinulida (Bentlage et al., 2018; Collins et al., 2008). Trachymedusae was found to be non-monophyletic, with one lineage derived from within Limnomedusae and the rest of Trachymedusae paraphyletic with respect to Narcomedusae. To address part of this issue, Bentlage et al. (2018) revised Limnomedusae to include members of Geryonidae that were previously classified as Trachymedusae. As presently understood, Trachymedusae is still a paraphyletic assemblage that gave rise to a monophyletic Narcomedusae.

There has also been discordance in reconstructions of relationships within Anthozoa, perhaps with the most in-

triguing phylogenetic question being the placement of Ceriantharia. Analyses of 18S and 28S ribosomal RNA sequences by Stampar et al. (2014) recovered Ceriantharia as sister to all other Hexacorallia. However, mitochondrial datasets placed Ceriantharia as sister to the rest of Anthozoa (Stampar et al., 2014). Nuclear exon data from Zapata et al. (2015) and Kayal et al. (2018), ultraconserved element (UCE) data from Quattrini et al. (2020), and studies of complete mitochondrial sequences from Stampar et al. (2019) recovered the same result found in the ribosomal rRNA studies, with Ceriantharia as sister to the rest of Hexacorallia.

Phylogenomic studies (i.e., those with hundreds or thousands of loci sampled across the genome) have brought higher resolution to the cnidarian tree of life; but all of them lack taxonomic balance, and many omit key lineages (Fig. 1). For example, Zapata et al. (2015) included minimal ceriantharian and staurozoan data and did not include Myxozoa. Chang et al. (2015) added seven representatives of Myxozoa and a *Polypodium* transcriptome but lacked Staurozoa and Ceriantharia. Kayal et al. (2018) combined previous data sets and added five deeply sequenced transcriptomes from Staurozoa but included very few cerianthiarian and octocoral data and had limited sampling within the most diverse clade of Hydrozoa, Hydroidolina. Kayal et al. (2018) and Zapata et al. (2015) resolved Aplanulata as the sister to a limited sampling of other Hydroidoloina. Bentlage and Collins (2021) addressed this deficiency using a bait capture approach focusing on Hydroidolina and also recovered strong support for Aplanulata as the sister group to the remainder of Hydroidolina. Using over 100 loci, Bentlage and Collins (2021) found Filifera to be polyphyletic, but recovered support for a topology uniting Filifera I with Filifera II, as sister to Capitata, with these three taxa united in a clade sister to Leptothecata. This study also found support for Filifera III plus IV, as the closest relatives of Siphonophorae (Bentlage & Collins, 2021). To comprehensively understand the evolutionary relationships among Cnidaria clades, it is essential to generate a phylogenetic tree that includes a comprehensive sampling across all major lineages.

Here, we combine 26 de novo transcriptome datasets and previously published transcriptome and gene model datasets to increase taxon sampling for underrepresented clades and improve the balance of taxon sampling across Cnidaria. In a hybrid approach designed to further increase taxon sampling (see McFadden et al., 2022), we use the topology resulting from phylogenomic analyses of our transcriptome data to constrain a phylogenetic analysis of more than 1,800 small subunit ribosomal DNA (18S) sequences. Our resulting phylogenies and new transcriptomic data provide a solid framework for present understanding of the evolutionary history of Cnidaria and for guiding future research on the phylogenetics of Cnidaria.

Methods

Reproducibility and transparency statement

Custom scripts, command lines, and data used in these analyses, including transcriptomes and alignment and tree files, are available at our GitHub repository (github.com/josephryan/DeBiasse_cnidophylogenomics; doi:10.5281/zenodo.10794451) and Dryad (https://doi.org/10.6071/M3K39S). To maximize transparency and minimize confirmation bias, we planned analyses *a priori* using a phylotocol (DeBiasse and Ryan 2019) and posted this original document and any subsequent changes to our GitHub repository.

Sample collection and data generation

Specimens were collected under permits as required by local jurisdictions. Full metadata for collection locations and dates, RNA extraction, library prep, and sequencing is available in Table S2. Raw reads are available under accession number PRJNA1023279.

Transcriptome assembly and processing

We generated new transcriptome data for 26 anthozoans (Table S3). We trimmed FASTQ sequences and assembled transcriptomes using the Trimmomatic (Bolger et al., 2014) option in Trinity v2.8.5 (Grabherr et al., 2011). We applied the 'include_supertranscripts' parameter to generate superTranscripts as part of each Trinity run. SuperTranscripts provide a single all-inclusive transcript for genes with multiple isoforms (Davidson et al., 2017). We translated the superTranscripts into amino acid sequences in TransDecoder v5.0.2 (github.com/TransDecoder). We set the TransDecoder '-m' flag (minimum length of open reading frame) to 50 and used the results from BLASTP (McGinnis & Madden, 2004) searches to inform the final TransDecoder prediction step. Using Alien Index v2.1 (https://github.com/josephryan/alien_index) we filtered potential contaminants in these translated sequences by removing sequences that were the top BLASTP hit to v0.02 of the curated alien_index database. was to a non-metazoan sequence. We assessed the completeness of each transcriptome by searching against the eukaryote database in BUSCO v2 (Simão et al., 2015) as implemented in gVolante v1.2.0 (Nishimura et al., 2017).

Phylogenomic matrix construction and phylogeny estimation

Our original dataset consisted of 26 new anthozoan transcriptomes (18 actiniarians, 4 ceriantharians, and 4 octocorallians), four cnidarian transcriptomes that we assembled from data publicly available on the NCBI Sequence Read Archive, 75 previously assembled and published transcriptomes, and seven previously published amino acid gene model data sets (112 sequences total, Table S3). The dataset included 104 ingroup cnidarians and the following eight outgroup taxa: the ctenophore *Mnemiopsis leidyi* (GCA_000226015.1), the sponge *Amphimedon queenslandica*

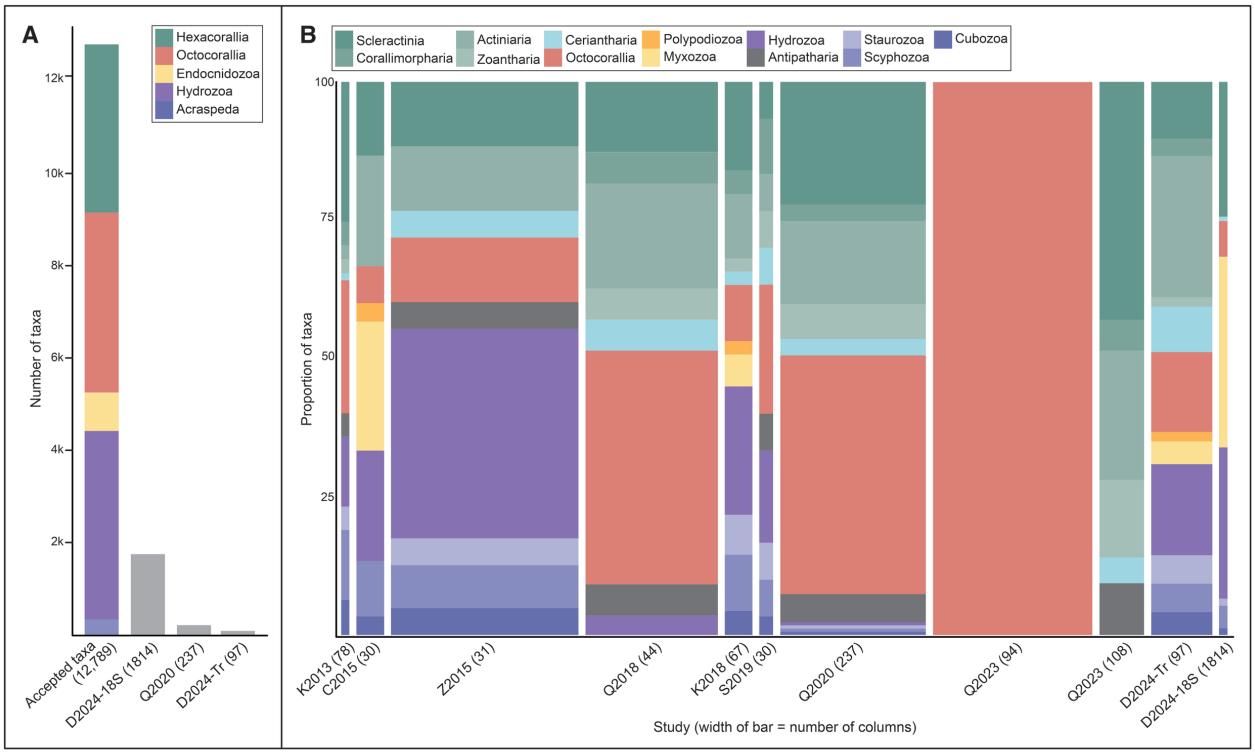

Figure 1. A graphical survey of cnidarian phylogenomic datasets over time as compared to species richness across its major lineages.

A) The colored bar represents the total number of cnidarian species described for four major taxonomic groups. The number of accepted taxa is based on the World Register of Marine Species database as of September 2022. The height of each grey bar represents the number of species from the total described included in the corresponding study. B) The height of each colored section represents the proportion of a particular taxonomic group included in the study. The width of the bar represents the number of nucleotide or amino acid columns in the dataset. For panels A and B, the studies are abbreviated with the first letter of the first author's surname and the publication year (e.g., K2013 represents Kayal et al., 2013). The number in parentheses indicates the number of cnidarian taxa included in the alignment. Columns labeled D2024-18S and D2024-Tr indicate the 18S and transcriptomic datasets generated in this study, respectively. The other studies included are Chang et al., 2015; Kayal et al., 2018; Quattrini et al., 2017, 2020, 2023; Zapata et al., 2015, and Stampar et al., 2019.

(GCF_000090795), the placozoan *Trichoplax adhaerens* (ABGP00000000), the fruit fly *Drosophila melanogaster* (GCF_000001215), the marine annelid *Capitella teleta* (PRJNA175705), the limpet *Lottia gigantea* (PRJNA175706), the purple sea urchin *Strongylocentrotus purpuratus* (https://metazoa.ensembl.org, release 47), and the zebra finch *Taeniopygia guttata* (ABQF00000000) (Table S3). We used diamond v0.9.22.123 (Buchfink et al. 2015) to perform reciprocal best BLAST searches and generated FASTA files of orthologous sequences (i.e., orthogroups) in OrthoFinder v2.2.3 (Emms & Kelly, 2019) using all 112 sequences as input.

We filtered the orthogroups inferred by OrthoFinder as follows: Using an automated script, sequences within each orthogroup were aligned using MAFFT v7.309 (Katoh & Standley, 2013) using the mafft-linsi alias with parameters –localpair and –maxiterate 1000, and, in the multicore version of IQ-TREE v1.5.5 (Nguyen et al., 2014), a maximum likelihood (ML) tree for each alignment that had no more than 50% sequence gaps was estimated. Only the orthogroup trees that had at least 50% of the total taxa and no more than eight paraphyletic duplicates per species were retained (there were no limits on the number of duplicates if they were monophyletic). In PhyloTreePruner v1.0 (Kocot et al., 2013), we used the -u flag to remove all but the longest sequence in taxa with monophyletic duplicates (e.g., paralogs), which produced a set of orthologous loci with one sequence per species in at least 50% of our taxa. The initial run of the orthogroup-filtering pipeline produced a small number of orthogroups that included single-copy loci per species and many orthogroups that contained duplicate (i.e., two or more) transcript copies per locus. These duplicate transcript copies can be artifacts produced by Trinity due to misassemblies or sequencing errors, or they can be paralogs that evolved by gene duplication events. Regardless of their source, including duplicate transcript copies per locus interferes with phylogenetic inference because their evolutionary history is unknown. Using the BUSCO scores as a guide, we removed five cnidarian species that had a high number of transcript duplicates per core gene (Table S3). This increased the number of single-copy loci produced by subsequent runs of the orthogroup-filtering pipeline (see below). We also removed *Heteractis crispa* because it clustered with the outgroup taxa in preliminary trees, and subsequent BLAST analyses suggested the *H. crispa* transcriptome was substantially contaminated with vertebrate sequences. *Muricea muricata* was also removed due to suspected non-target cnidarian contamination. We reran OrthoFinder and the filtering pipeline with the parameters described above for the 105 species remain-

ing, assigning 3,670,777 of 4,892,912 sequences (75%) to orthogroups and retaining 4,117 orthogroups that had at least 53 of 105 species present and no more than eight duplicates per species. After removing within-species duplicates, we were left with 748 single-copy orthogroups.

We concatenated alignments of all the single-copy orthologs for the remaining 105 species using the fasta2phylomatrix utility v0.02 (github.com/josephryan/JFR-PerlModules) and aligned these sequences with MAFFT v7.309 using the mafft-linsi alias with parameters –localpair and –maxiterate 1000. This dataset did not involve any column trimming as it has been shown that current methods for filtering multiple sequence alignments lead to suboptimal alignments (Tan et al., 2015). To test if removing divergent and ambiguously aligned columns affected our results, we generated a trimmed version of this matrix with Gblocks v0.91b (Castresana, 2000) using dynamic parameters generated by Gblockswrapper v0.03 (https://goo.gl/fDjan6). The untrimmed and trimmed matrices consisted of 449,935 and 123,051 amino acid columns, respectively.

We used the untrimmed and trimmed matrices and two models (partitioned and unpartitioned) to estimate four ML phylogenies in IQ-TREE v1.5.5 (Nguyen et al. 2015). In the first and second analyses, we used the IQ-TREE parameter '-m TEST' to determine site-homogeneous models of amino acid substitution for each gene partition applied to the (i) untrimmed and (ii) trimmed data matrices. In the third and fourth analyses, we used the C60 model in IQ-TREE, which accounts for across-site compositional heterogeneity in equilibrium frequencies, applied to the (iii) untrimmed, unpartitioned data matrix and the (iv) untrimmed, partitioned data matrix. Support values for all phylogenies were determined from 1000 ultrafast bootstrap replicates.

Small subunit (18S) ribosomal DNA matrix construction

We ran the following search at GenBank (NT) on July 8, 2020: ((Cnidaria[ORGN] AND (18S OR "small subunit ribosomal")) BUTNOT Nematostella[ORGN]) OR AF254382. We downloaded these results in GenBank format. To these 13,717 sequences, we added accessions AF254382 (*Nematostella vectensis*) and AF052581 (*Renilla reniformis*) and chose a single representative from sets of sequences from the same species name (retaining the longest sequence of a set of duplicates). We used a custom script (get_18S_fasta_from_genbank.pl) to convert GenBank format to FASTA and remove the following sequences: (1) all duplicates of a species except for the longest, (2) AY935208 (*Aurelia* sp.), (3) sequences shorter than 1000 nucleotides, (4) sequences that include the patterns 'environ,' 'parasite,' or 'proliferative' in their definition line, (5) sequences that did not include a class designation, and (6) sequences from taxa that include species *affinis* (abbreviated sp. or cf.) unless those sequences were the only representative of a genus.

The following changes were made based on prior knowledge: (1) *Virgularia gustaviana* was removed as it is erroneously annotated (clearly a ceriantharian) in GenBank, (2) *Carybdea marsupialis* was renamed *Alatinidae* indet., (3) *Alatina philippina* was removed as it was shown to be the same as *Alatina morandinii* (Straehler-Pohl & Toshino, 2015), (4) *Darwin* sp. was renamed to *Gerongia rifkinae*, and (5) accession AF099104 (*Craterolophus convolvulus*) is a contaminant of *Haliclystus* so it was replaced with AY845344. After running an initial tree, we identified one long-branched clade of octocoral sequences that contained *Junceella aquamata* (AY962535), *Junceella fragilis* (AY962533), and *Subergorgia ornata* (AY962537), which fell within Hexacorallia instead of Octocorallia. We determined that these three sequences were likely contaminants and removed them based on the following criteria: (1) all were from the same NCBI PopSet (accession=63148780), (2) the top BLAST hits of each of these were to other sequences from this PopSet including bivalves and crustaceans, (3) *Junceella* and *Subergorgia*, which were sister in our preliminary tree, are distantly related genera in Quattrini et al. (2020), and (4) there is no obvious voucher available for these sequences. We included a reduced set of octocoral sequences given the low phylogenetic signal for 18S in this group demonstrated previously (Berntson et al., 2001; McFadden et al., 2010).

We used ssu-align v0.1.1 (E. P. Nawrocki & Farm, 2010) with default settings to align 1,814 18S gene sequences (15 ceriantharians, 442 non-ceriantharian hexacorallians, 117 octocorallians, 496 hydrozoans, 22 cubozoans, 24 staurozoans, 73 scyphozoans, and 625 endocnidozoans) representing 702 genera. We used ssu-mask v0.01 from the same package with default settings to remove columns that likely include a significant number of misaligned nucleotides as recommended in the ssu-align manual. We used esl-format v0.43 from the Easel sequence library (https://github.com/EddyRivasLab/easel) to convert stockholm formatted alignments to FASTA format. The resulting alignment contained 1,324 columns (959 parsimony-informative).

Small subunit (18S) ribosomal DNA phylogeny estimation

We constructed a constraint tree based on the topology of the transcriptomic phylogeny estimated in the untrimmed, partitioned site homogeneous analysis above (Fig 2). We pruned *Ceriantheopsis americana* from the constraint tree because there has been confusion regarding the distinction between this taxon and *Pachycerianthus borealis* (Klompen et al., 2020) that complicates downstream interpretations. Additional sequences were pruned if a corresponding sequence did not exist in the 18S dataset. In total, there were 23 sequences pruned from the constraint tree (Table S2). We also collapsed the three cubozoan species into a polytomy to reflect discrepancies that we encountered among the phylogenomic trees (discussed in Results). The final constraint tree contained 75 taxa that were present in both our phylogenomic and 18S datasets.

We next generated a phylogeny of 18S sequences using IQ-TREE multicore v1.6.12 (Nguyen et al., 2014) applying the parameter -m TEST to identify the best fitting model of nucleotide substitution. The final dataset, the accessions of all sequences, the constraint tree, the final tree, and the scripts used to create the dataset and constraint tree, are

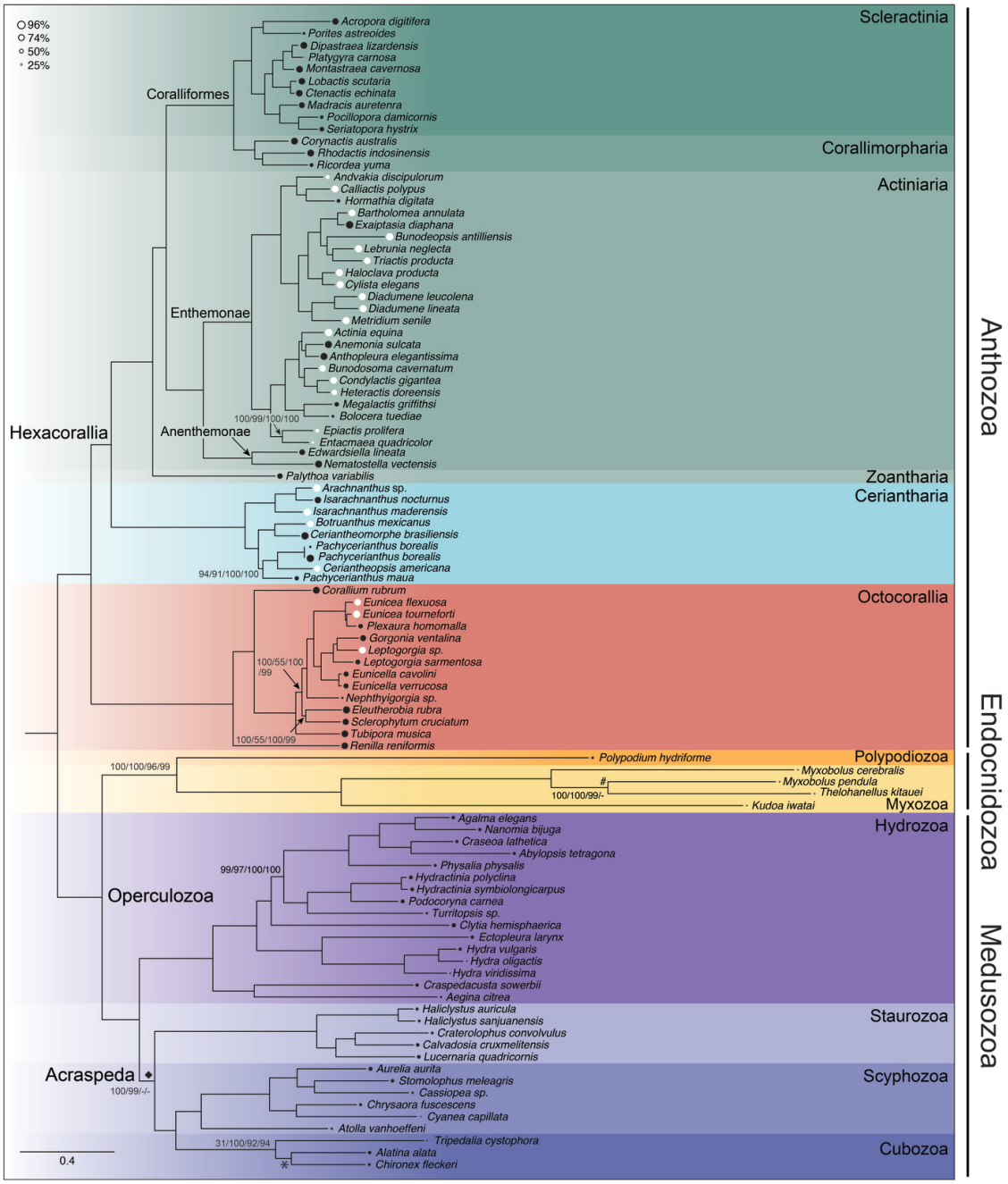

Figure 2. Maximum-likelihood phylogeny of Cnidaria estimated from an untrimmed, concatenated matrix of 748 ortholog alignments analyzed under a site-homogeneous partitioned model (rooted based on the outgroup, which is not shown).

Circles at the branch tips are proportional to the occupancy of that taxon in the data matrix, with black circles indicating previously published data and white circles indicating data generated for this study. Occupancy for previously published Myxozoa, *Cyanea capillata*, *Tripedalia cystophora*, and *Platygyra carnosa* sequences was below 15% (Table S2), making circles for these taxa very small and appearing to be missing. Bootstrap values are indicated at nodes if support is less than 100% for any of the analyses (partition-specific site-homogeneous models run on untrimmed matrix listed first, partition-specific site-homogeneous models run on trimmed matrix listed second, C60 analysis listed third, and C60 partitioned analysis listed fourth). The hash indicates a conflicting relationship where *T. kitauei* is sister to *M. cerebralis* in the C60 analysis (Fig. S3).The diamond indicates a conflicting relationship in the two phylogenies estimated under the C60 model where Staurozoa is sister to Hydrozoa (Fig. S3-S4). The asterisk indicates a conflicting relationship where *A. alata* is sister to *T. cystophora* in the three other phylogenies (Fig. S2-S4).

posted to our GitHub repository (see the URL above), and the transcriptomes are available at Dryad (https://doi.org/10.6071/M3K39S).

Results and Discussion

Cnidaria is an ancient lineage that encompasses a wide range of phenotypic and genomic diversity. Research interests in cnidarian organisms are extensive and taxonomically broad, presenting an excellent opportunity to study a

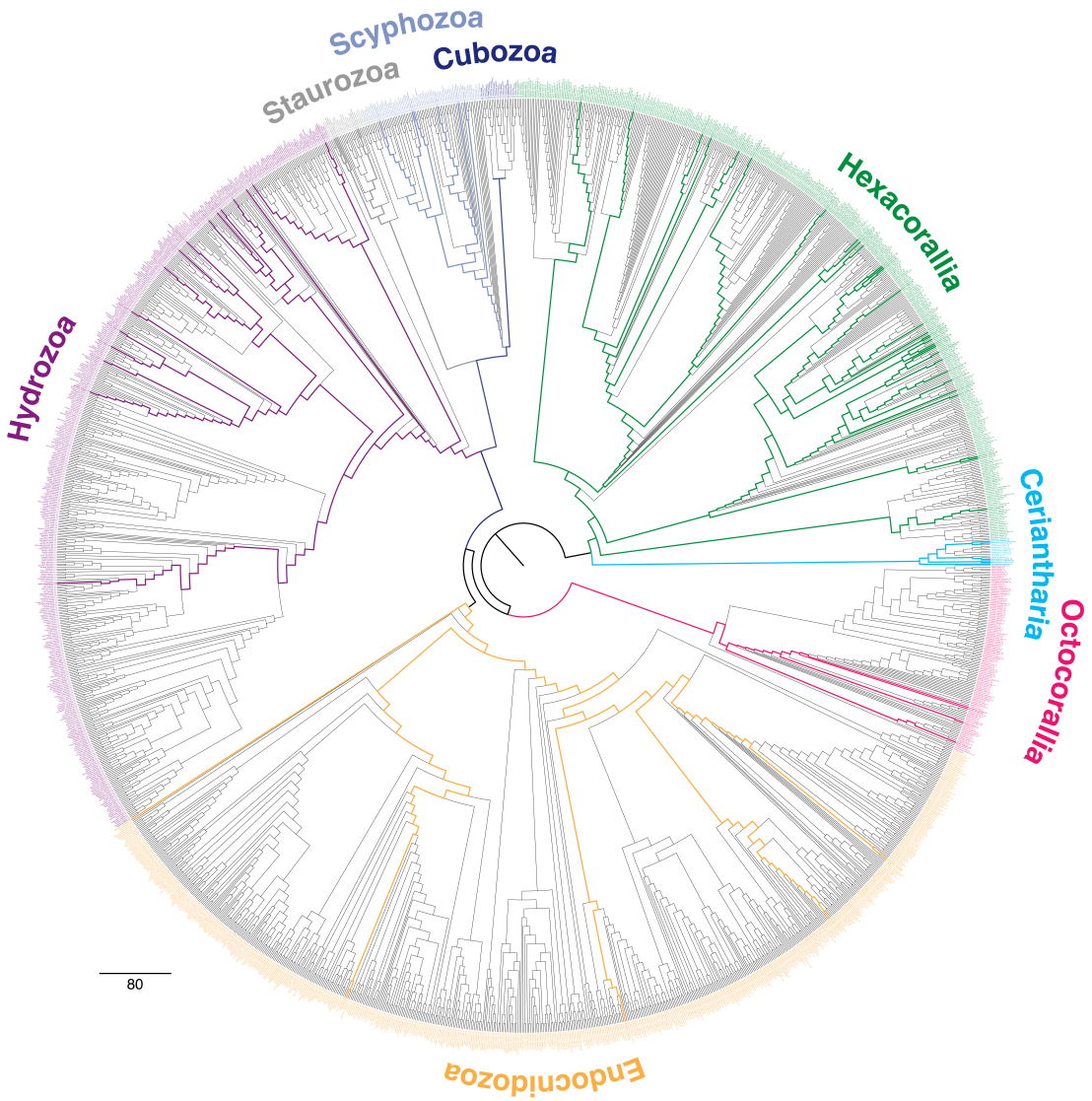

Figure 3. Maximum-likelihood phylogeny estimated using small subunit ribosomal (18S) gene sequences from 1,814 cnidarian species with higher-level relationships constrained according to the relationships in the multi-locus, untrimmed, partitioned, site homogeneous analysis (Fig 2).

Bolded branches indicate the constraint tree applied, with colors representing higher-level taxonomic groups as in Fig. 1 and 2.

wide variety of biological processes. However, an accurate and extensive phylogenetic framework is necessary to promote and contextualize such research. Existing phylogenies encompass less than 2% of species (Fig. 1A), and most, if not all, are taxonomically skewed relative to the actual representation of major cnidarian groups (Fig. 1B). In addition, there are conflicting relationships that emerge from these previous studies, which is not surprising given that they differ in taxon sampling and fail to represent the diversity of the group. Here, we apply a hybrid approach to present phylogenetic relationships for nearly 15% of described cnidarian species. Building a reliable phylogeny for any group, particularly one as diverse and species rich as Cnidaria, requires the cumulative efforts of researchers working to improve inference methods and taxonomic and genomic sampling. In comparing results across studies and incorporating previously applied sequences with newly acquired data, support for phylogenetic relationships becomes stronger. Our most important contributions in this paper, which we discuss below, include strong support in both phylogenies for the sister relationship of the Hexacorallia and Octocorallia, the position of Ceriantharia as sister to the remaining Hexacorallia, the sister relationship of the Medusozoa and Endocnidozoa, and the newly coined clades Coralliformes and Operculozoa.

Anthozoa

Hexacorallia

The four transcriptomic phylogenies generated here (Fig. 2, S2-4) recovered all sampled hexacoral lineages (i.e., Scleractinia, Corallimorpharia, Actiniaria, Zoantharia, Ceriantharia) as monophyletic and found Scleractinia and

Corallimorpharia as sister taxa (discussed further below), concurring with many recent phylogenomic studies (Kayal et al., 2018; Quattrini et al., 2017, 2020, 2023; Zapata et al., 2015). In the 18S tree, Zoantharia was non-monophyletic with a sample labeled as *Zoanthus* falling out among the Antipatharia; we suspect that this is a contaminant or mislabeled sequence rather than evidence for zoantharian polyphyly. Our transcriptomic analyses did not include representatives from Antipatharia as the one transcriptome available when we were assembling our matrix had very low BUSCO scores (*Antipathes griggi*, Zapata et al., 2015; Table S3). A more recent study (Drake and Mass 2022) included a high-quality *A. griggi* transcriptome, however, by the time this study was published, our transcriptomic phylogenetic analyses were complete. In the 18S tree, we recover Antipatharia as the sister to the Actiniaria-Scleractinia-Corallimorpharia clade, rather than as sister to Scleractinia and Corallimorpharia as in previous target-enrichment (Quattrini et al., 2017, 2020, 2023), transcriptome-based (Zapata et al., 2015), and whole mitochondrial genome analyses (Quattrini et al., 2023). If true, our 18S topology suggests either that a number of characters related to skeletonization evolved independently in Scleractinia and Corallimorpharia or that there were multiple independent losses of these characters in the clade that includes Antipatheria, Scleractinia, and Corallimorpharia. A recent comparison of anthozoan phylogenies estimated from UCE and whole mitochondrial genomes also demonstrated instability in the relationships of Zoantharia, Actiniaria, and the enigmatic giant deep-sea anemone *Relicanthus daphneae*, which grouped with Zoantharia in the UCE dataset and Antipatharia in the mtDNA dataset (Quattrini et al., 2023), highlighting the need for more thorough taxon and more phylogenetically-informative loci to solidify the evolutionary history of these clades.

Corallimorpharia and Scleractinia

Reconciling molecular phylogeny with morphology in Scleractinia has been a long term, persistent, and challenging task (Fukami et al., 2004, 2008; Kitahara et al., 2010; McMillan et al., 1991), with Huang et al. (2011) coining the tongue-in-cheek term "Bigmessidae" to describe this group. Some of the earliest molecular phylogenies based on mitochondrial rRNA genes resulted in two major scleractinian groups that conflict with morphologically defined subordinal classifications (C. A. Chen et al., 2002; McMillan et al., 1991; Romano & Cairns, 2000; Romano & Palumbi, 1996, 1997). One group, called the robust corals, contains platelike and massive taxa with thick, heavily calcified skeletons. The second group, called the complex corals, contains corals with more porous, less calcified skeletal walls. We recovered these robust and complex groups (Fig. 2, S2-4) as have recent multilocus studies (e.g., M. F. Lin et al., 2016; Quek & Huang, 2022). An important area of future research attention is resolving the position of deep-sea aposymbiotic taxa, which fall out as the earliest branching scleractinians in studies based on mitogenomes and rRNA (Barbeitos et al., 2010; Kitahara et al., 2010; Seiblitz et al., 2020; Stolarski et al., 2011) but have been absent or underrepresented in recent phylogenomic studies.

Introducing Coralliformes (Corallimorpharia + Scleractinia)

Using whole mitochondrial genomes, Medina et al. (2006) found Corallimorpharia sister to the complex corals, rendering Scleractinia non-monophyletic. Based on these relationships, Medina et al. (2006) resurfaced the "naked coral hypothesis," suggesting the soft-bodied corallimorpharians evolved from a scleractinian ancestor and subsequently lost the stony skeleton trait during historical periods of increased CO_2 concentrations in the marine environment. Following studies using a range of loci (nuclear rRNA: Fukami et al. (2008); whole mitochondrial genomes: Kayal et al. (2013); Lin et al. (2014); Seiblitz et al. (2020); nuclear protein coding: Lin et al. (2016); Kayal et al. (2018); UCEs: Quattrini et al.(2017); Quattrini et al. (2020)) have found Scleractinia to be monophyletic. Like these studies, we recovered a monophyletic Scleractinia across all four phylogenetic analyses (Fig. 2, S2-4), demonstrating this relationship is robust to model choice and refuting the naked coral topology, which was likely a result of saturation in the mitochondrial protein sequences (but see Quattrini et al., 2023), long-branch attraction, and/or model violations (Kitahara et al., 2014). We propose the name Coralliformes to represent the clade that unites Corallimorpharia and Scleractinia.

Actiniaria

Although we found monophyly for Actiniaria as a whole, internal relationships differed between marker types and from previous studies. For example, Enthemonae and Anenthemonae were monophyletic in all phylogenies based on transcriptomic data (Fig.2, S2-4), corroborating previous studies (Gusmão et al., 2020; Rodríguez et al., 2014). In contrast, in the 18S phylogeny Enthemonae and Anenthemonae were non-monophyletic, with the difference stemming from the placement of the actinostolideans *Hormosoma* and *Anthosactis* and the actinernoideans *Actinernus*, *Isactinernus*, and *Synactinernus* at the base of the actiniarian tree rather than within Enthemonae and Anenthemonae, respectively. This novel topology recalls historical groupings of these taxa within "Mesomyaria," (see Carlgren, 1949; Rodríguez et al., 2014) and, if confirmed, would significantly change the inferred history and homology of marginal musculature in Actiniaria. Based on the 18S tree, the marginal sphincter would be inferred to be present and mesogleal at the root of Actiniaria, with subsequent losses and multiple shifts to become concentrated and embedded in the endoderm. In the phylogenomic tree, internal relationships for Enthemonae contradict historical taxonomy, with *Haloclava producta* (Actiniodea) nested within Metridioidea and *Megalactis griffithsi* (Actinodendridae) nested within Actiniidae (Fig. 2, S2-4). These discordant relationships are well known from previous studies (Barragán et al., 2019; Izumi et al., 2020; Rodríguez et al., 2014; Yap et al., 2014), and the taxonomy of Haloclavidae has recently

been updated to better reflect the position of *H. producta* (Hamilton et al., 2022). While multilocus phylogenies are generally preferred to those based on a single marker, taxon sampling in the phylogenomic dataset is almost an order of magnitude lower for Actinioidea and Metridioidea than that of the 18S tree and mesomyarian taxa, which typically branch near the base of the actiniarian tree. Resolving relationships at the suborder and below in Actiniaria will require generating data for species in more sparsely sampled regions of the tree.

Ceriantharia

Among anthozoan lineages, Ceriantharia has been the most challenging to interpret phylogenetically. Instability in the resolution of Ceriantharia confounds attempts to understand two key aspects of ceriantharian biology. Cerianthariansproduce spirocytes and ptychocytes in addition to nematocytes, with these additional kinds of capsules having different structural properties and functions (Mariscal, 1984). All hexacorals produce spirocytes, but ptychocytes are unique to Ceriantharia (Mariscal et al., 1977). The inferred relationship among these cell types depends on the topology of the anthozoan tree (Babonis et al., 2023; Reft & Daly, 2011) and is worth investigating given the functional importance and unparalleled complexity of these microscopic machines. Similarly, the medusiform, long-lived larval stage of some ceriantharians is complicated to interpret if the phylogenetic position of Ceriantharia is not well resolved.

Here, we recovered this historically labile group as the sister lineage to the rest of Hexacorallia in all our analyses with strong bootstrap support (Fig. 2, 3, S2-4). This result corroborates the results of previous phylogenomic studies (Kayal et al., 2018; Quattrini et al., 2017, 2020, 2023; Zapata et al., 2015) and solidifies the placement of this clade. Previously, Stampar et al. (2019) found a different relationship based on complete mitochondrial genomes with ceriantharians sister to a clade containing octocorals and other hexacorals. Possible explanations provided for this relationship included unique mitochondrial features such as multipartite linear genome structure (Stampar et al., 2019), saturation (Pratlong et al., 2016), and remarkably slow rates of mitochondrial genome evolution (Mercer Robert Brugler, 2004; Hellberg, 2006; Shearer et al., 2002), a trend echoed across most anthozoan groups. Interestingly, a more recent phylogeny also inferred using complete mitochondrial genome sequences agreed with phylogenomic studies, recovering ceriantharians as the first hexacorallian lineage (Quattrini et al., 2023). In their study, Quattrini et al., 2023 explicitly tested for saturation in the mitochondrial loci and found none, lending support to the hypothesis that saturation might lead to outlier relationships for Ceriantharia and other cnidarian taxa.

While we recovered the same internal relationships for Ceriantharia across all four phylogenomic trees, those relationships conflicted with taxonomy. For example, Penicillaria and Spirularia are monophyletic, but, within Spirularia, the two Cerianthidae species are not as *Pachycerianthus borealis* (Cerianthidae) is sister to a clade that contains *Botruanthus benedeni* (Botrucnidiferidae) and *Ceriantheomorphe brasiliensis* (Cerianthidae). We also find the genus *Isarachnanthus* non-monophyletic as *I. nocturnus* is sister to *Archnanthus* sp. Our constrained 18S tree presents further conflicts between ceriantharian taxonomy and phylogeny. Here, the genus *Pachycerianthus* is non-monophyletic as a clade of six *Pachycerianthus* species is sister to the two *Isarachnanthus* (Penicillaria) species. In addition, the two species of Botrucnidiferidae (*Botruanthus benedeni* and *Botrucnidifer* sp.) and the genus *Cerianthus* are non-monophyletic. These problems echo results of previous molecular phylogenetic relationships within Ceriantharia, which generally find conflict between taxonomic groups and phylogenetic results (e.g., Forero Mejia et al., 2020; Stampar et al., 2012, 2014). The disconnect between taxonomy and phylogeny in both the phylogenomic and 18S data are paralleled in recent discoveries of significant plasticity in morphology within the life history of a species and persistent taxonomic confusion, even at a narrow geographic scale (reviewed in Stampar et al., 2016).

Octocorallia

In all four of our phylogenomic analyses, we found Octocorallia sister to Hexacorallia (Fig. 2, S2-4), as have recent phylogenomic studies (Quattrini et al., 2017, 2020, 2023), demonstrating the stable position of octocorals in the cnidarian tree. However, ordinal and familial-level taxonomy has been, and continues to be, uncertain (reviewed in McFadden et al., 2021). Recently, Quattrini et al. (2017, 2020) made major strides in octocoral phylogenomics, increasing the number of taxa and loci (933 UCE loci, 278,819bp), and McFadden et al. (2022) published a thorough taxonomic revision of Octocorallia guided by 739 UCE and exon loci and including 185 octocoral taxa representing 55 of 63 currently recognized families. In this monograph, the authors dissolve the three historical orders (Alcyonacea, Pennatulacea, Helioporacea) and reassign taxa to two new orders: Scleralcyonacea and Malacalcyonacea. Within these orders, McFadden et al. (2022) identify 79 families, 18 that are newly described and 3 that the authors elevated or reinstated. Our results for Malacalcyonacea, while limited in taxon sampling, concur with the relationships defined by McFadden et al. (2022). In our transcriptomic phylogeny, Eunicellidae sensu McFadden et al. (2022) and Gorgoniidae form a clade sister to Plexauriidae within the Malacalcyonacea. For genera where we had multiple octocoral species, neither *Eunicea* nor *Leptogorgia* were monophyletic, which matches known evolutionary patterns described previously (Poliseno et al., 2017). We did not recover a monophyletic Scleralcyonacea, which is perhaps expected given we had only two representatives of this order (Coralliidae and Pennatuloidea). In light of the extensive UCE-based phylogeny (McFadden et al., 2022; Quattrini et al., 2017, 2020, 2023) and the long-realized unreliability of 18S for constructing octocoral relationships due to its lack of sufficient variation among octocoral taxa (McFadden et al., 2010), we do not go into extensive details of the octocoral relationships in our 18S tree.

Endocnidozoa

We found support for Endocnidozoa, recovering Myxozoa sister to Polypodiozoa across all four transcriptomics analyses (Fig. 2, S2-4) and the 18S phylogeny, concordant with Chang et al. (2015) and Kayal et al. (2018). In the transcriptomic analyses, we included data for the same five species included in Kayal et al. (2018), which all had low matrix occupancy (5-35%, Table S2). Across our four analyses, we found two topologies, both with paraphyletic *Myxobolus*. In the site-heterogeneous unpartitioned analyses, *Thelohanellus kitauei* and *M. cerebralis* formed a clade (Fig. S3), and, in all other analyses, *T. kitauei* formed a clade with *M. pendula* (Fig. 2, S2, S4). In the 18S phylogeny, which contained 624 myxozoan tips, Malacosporea and Myxosporea were monophyletic, but most genera, including those most frequently represented, were not (*Myxobolus* (n = species 185), *Ceratomyxa* (n = 85), *Kudoa* (n = 69), and *Henneguya* (n = 64)). Only 5 genera were monophyletic (*Sphaeromyxa* (n = 10), *Ellipsomyxa* (n = 10), *Soricimyxum* (n = 2), *Gadimyxa* (n = 2), *Gastromyxum* (n = 2) (Figure 3). Given that early descriptions of myxosoan species were based on spore characteristics (Lom and Noble 1984), which are often plastic (Mitchell 1989), discordance between morphological taxonomy and molecular phylogeny are not surprising. In fact, one of the first phylogenetic attempts to place Myxozoa on the tree of life using 18S sequence data found *Myxobolus* and *Henneguya* to be paraphyletic 29 years before our study (Smothers et al., 1994). Clearly, with plastic and reduced traits that evolved under a parasitic lifestyle (Kent 2005), reconciling myxozoan species boundaries will remain a challenge in cnidarian systematics.

Introducing Operculozoa (Medusozoa + Endocnidozoa)

A relationship consistent in past studies (Chang et al., 2015; Kayal et al., 2018) and in all phylogenies estimated here is the sister relationship of Endocnidozoa and Medusozoa (Fig. 2, S2-4). Based on this result, and noting that nematocytes of Medusozoa and Polypodiozoa (see Raikova and Raikova 1990) and polar capsules of Myxozoa all possess an operculum (Reft and Daly 2012), we propose the name Operculozoa for the clade uniting Endocnidozoa and Medusozoa.

Medusozoa

Our results highlight the need for more taxon sampling within Medusozoa. Relationships of the major medusozoan lineages have been increasingly refined in recent decades (Bridge et al., 1995; Collins, 2002; Collins, Bentlage, et al., 2006; Kayal et al., 2015; Kim et al., 1999; Marques & Collins, 2004), with an emerging consensus that Medusozoa consists of two major lineages, Acraspeda and Hydrozoa (Kayal et al., 2018). In our analyses that applied a site-homogeneous model of amino acid substitution to each gene partition (i & ii), we recovered a monophyletic Acraspeda with Staurozoa as sister to the clade containing Scyphozoa and Cubozoa (Fig. 2, S2), matching the relationships found by four recent studies (Chang et al., 2015; Kayal et al., 2018; Quattrini et al., 2020; Zapata et al., 2015). However, we recovered an intriguing, albeit weakly supported, finding when applying the C60 site-heterogeneous model (iii & iv) in which Hydrozoa and Staurozoa formed a clade sister to a clade containing Scyphozoa and Cubozoa (Fig. S1, S3, S4), a result also recovered by Miranda et al. (2016) analyzing concatenated mitochondrial (COI, 16S) and nuclear (ITS, 18S, 28S) loci under a Bayesian framework. None of our results corroborate the ribosomal RNA-based hypothesis that Staurozoa is sister to the remaining medusozoans (Collins, Schuchert, et al., 2006; Picciani et al., 2018).

Site-heterogeneous models that partition data at the level of sites (as opposed to at the level of genes) have been suggested to alleviate problems of long-branch attraction (Lartillot & Philippe, 2004; Le & Gascuel, 2008). It is possible that our recovery of a non-monophyletic Acraspeda was due to applying a site-heterogeneous approach. However, Kayal et al. (2018) applied the site-heterogeneous CAT model in PhyloBayes (Lartillot et al., 2009) using largely the same medusozoan taxa as we used here and recovered a monophyletic Acraspeda. These conflicting relationships within Medusozoa may be a result of a number of factors including: (1) increased anthozoan sampling in our study, (2) increased gene sampling in our study, or (3) differences between the C60 model implemented in IQ-TREE and the CAT model implemented in PhyloBayes. Nevertheless, taxon sampling within Medusozoa is poor in all phylogenomic analyses to date, and a true understanding of medusozoan relationships will require substantial increase in data from this group (Fig. 1).

Cubozoa

In addition to conflicting relationships between the major medusozoan lineages, we also find unstable relationships within Cubozoa across our analyses. We included three cubozoans in our analyses: *Chironex fleckeri*, *Tripedalia cystophora*, and *Alatina alata*. In our untrimmed site-homogeneous analysis, we recovered *Tripedalia cystophora* as sister to a clade containing *Chironex fleckeri* and *Alatina alata* (Fig. 2). We also recovered this relationship in our 18S phylogeny, which did not constrain relationships within Cubozoa. These results conflict with the rest of our analyses as well as with Bentlage et al. (2010) and Kayal et al. (2018), which recovered *C. fleckeri* (Chirodropidae) as sister to a clade containing *A. alata* (Alatinidae) and *T. cystophora* (Tripedaliidae). This inconsistency is almost certainly due to the poor taxon sampling (3 of 48 described species represented) and low data matrix occupancy (4-46%, Table S3) of Cubozoa in our analyses (Fig. S2–S4) and Kayal et al. (2018).

Staurozoa

The phylogenetic placement of staurozoans has been recalcitrant (Fig. S1). Historically, these so-called stalked jellyfish were considered scyphozoans until Marques and Collins (2004) elevated the group to the class level (Miranda et al., 2010). Despite the variable position of Staurozoa

in our phylogenies (see above), the relationships within the clade were constant and matched those of Kayal et al. (2018), the source of the transcriptomic data analyzed here. The relationships among the genera sampled here were discordant with those inferred by Miranda et al. (2016), which to date has the best taxon sampling for Staurozoa based on a concatenated matrix of mitochondrial (COI, 16S) and nuclear genes (ITS, 18S, 28S) under parsimony, maximum likelihood, and Bayesian analyses.

Within Staurozoa, transcriptome-based analysis suggests Myostaurida is sister to Amyostaurida. This result would suggest that muscles in the stalks have been lost in Amyostaurida (also suggested in Miranda, Collins, et al., 2016; Miranda et al., 2018; Miranda, Hirano, et al., 2016) because scyphozoan polyps possess these muscles. However, the constrained 18S tree paints a more complicated picture, as neither Amyostaurida nor Myostaurida is monophyletic (Fig. 3).

Scyphozoa

Relationships within Scyphozoa are consistent and highly supported across all our transcriptomic phylogenies (Fig. 2, S2-4) and agree with prior studies that found Discomedusae sister to Coronatae (Bayha et al., 2010, 2017). Despite removing *Periphylla periphylla* due to high numbers of transcript copies and/or paralogs (Table S3), we recovered the same relationships among the remaining 6 scyphozoan species as Kayal et al. (2018), the source of the transcriptomic data analyzed here. Interestingly, despite frequent topological discordance between nuclear and mitochondrial phylogenies, we find the same phylogenetic relationships (a clade containing *Cyanea* and *Chrysaora* sister to a clade containing *Aurelia* and *Cassiopea*) as Kayal et al. (2013), who inferred phylogenies using mitogenomes, and Daglio and Dawson (2017), who used mitochondrial and nuclear ribosomal genes (16S, 18S, 28S). Comparisons to other recent phylogenomic studies are limited due to low taxon sampling in this group (Zapata et al., 2015, p. 2 scyphozoans).

Hydrozoa

The transcriptomic phylogeny (Fig. 2) recovered Hydrozoa split into Trachylina and Hydroidolina, in agreement with analyses of other previous studies (Bentlage & Collins, 2021; Collins et al., 2008; Kayal et al., 2018; Picciani et al., 2018; Zapata et al., 2015), although the 18S topology recovered the trachyline *Halammohydra* as falling outside of these two clades, rendering Trachylina paraphyletic. The 18S topology fails to recover the monophyly of and relationships among many well-established taxa. For example, the 18S topology includes part of a grade of Limnomedusae (including Geryonidae) plus Actinulida as sister to the remainder of Hydrozoa, rendering Trachylina paraphyletic while Narcomedusae was monophyletic, derived from within the grade of Trachymedusae, which is congruent with previous studies (Bentlage et al., 2018; Collins et al., 2008) (including Actinulida). Within Hydroidolina, two distinct clades (Siphonophorae and Leptothecata) were monophyletic, but Aplanulata, Capitata, and Filifera were not. The latter was as expected given that earlier studies established Filifera as paraphyletic (e.g., Bentlage & Collins, 2021; Cartwright et al., 2008). The high degree of conservation, and resulting lack of resolution, from 18S has hampered inferences of hydrozoan relationships previously, an issue that has been partially overcome by using additional markers such as 16S and 28S (Cartwright et al., 2008; Collins, Bentlage, et al., 2006; Collins et al., 2008) and most recently target capture data (Bentlage & Collins, 2021).

While the 18S topology failed to recover monophyly of most of the traditionally recognized groups within Hydrozoa (Fig. 3), the transcriptomic phylogeny (Fig. 2) was consistent with many previous studies. Within Hydroidolina, Aplanulata (*Ectopleura* and *Hydra*) is shown to be the sister to the rest of Hydroidolina, consistent with previous phylogenomic studies (Bentlage and Colllins 2021; Kayal et al., 2018; Zapata et al., 2015). In addition, Filifera III and IV (*Hydractinia*, *Podocoryne*, *Turritopsis*) were recovered as monophyletic (Fig. 2), consistent with Bentlage and Collins (2021), Cartwright et al. (2008), Kayal et al. (2018), and Zapata et al. (2015). Sampling of genomic data has been particularly sparse for Leptothecata, the most species rich of all roughly ordinal-level medusozoan clades (just one of 2,140 leptothecate species sampled herein) and Filifera I and II, which are not represented in the genomic data analyzed herein. Bentlage and Collins (2021) filled some of these gaps with target capture data, but more sampling will be necessary to settle some of the long-standing debates in hydrozoan systematics. The sparse taxonomic representation of hydrozoans, and resulting sparse backbone, may at least partially explain the lack of resolution of the 18S phylogeny for Hydrozoa.

Future of phylogenomics for cnidarians and other organisms

One critical need for future studies is to improve taxon sampling across the cnidarian tree. Currently, we have just scratched the surface of capturing the species richness of Cnidaria in our phylogenomic analyses (Fig. 1A). Of the 12,000-plus cnidarian species known, the largest phylogenomic study to date (Quattrini et al., 2020) includes just 2% of cnidarian species richness and our 18S hybrid phylogeny includes less than 15% (Fig. 1A). Recent studies have dramatically improved the representation of certain clades, for example Anthozoa and Octocorallia (Quattrini et al., 2017, 2020) and Actiniaria (this study), while other clades like Medusozoa have lagged (Fig. 1B), particularly the species-rich hydrozoan clade Leptothecata. Cubozoans also have been poorly represented (Fig. 1b, Chang et al., 2015 *Tripedalia cystophora* only, Zapata et al., 2015 *Alatina alata* only, Stampar et al., 2019 *Alatina moseri* only), making relationships within the clade difficult to resolve with confidence. Zoantharia, Endocnidozoa, Antipatharia, and Ceriantharia are other groups that have historically been underrepresented in phylogenomic studies (Fig. 1B, see also Quek & Huang, 2022). Furthermore, sampling bias due to uneven geographic accessibility presents a major challenge in building a complete tree of Cnidaria.

The 18S phylogeny we estimated employed a hybrid approach leveraging the strength of the multi-locus phylogenomic backbone and the vast number of 18S sequences publicly available. McFadden et al., 2022 used a similar approach to increase taxonomic representation in octocorals, using a UCE-based topology to constrain the phylogeny of 284 *mtMutS* sequences. These hybrid methods are useful for identifying areas where taxonomy can be improved, although instances of contamination and misidentification in large databases like GenBank can produce spurious results in phylogenetic trees. In some cases, the topology based on our 18S was unconventional, differing from results in focused analyses of this marker. This discordance might have been due to mis-identified specimens and/or differences in alignment and models in a phylum-scale data set, highlighting areas of the tree where molecular evolution differs from that of the clade.

All approaches that estimate phylogenies based on genomic, transcriptomic, single-gene, morphological, target-capture data as well as super trees have a unique set of limitations. Despite these limitations, there is value in exploring sequence data using the hybrid "transcriptomic trunk with 18S leaves" approach we have outlined here. The biggest contribution of this analysis is that many of the taxa in our 18S analysis have hitherto not been incorporated in a large-scale phylogeny. As such, our analysis provides a position, albeit provisional, for these taxa relative to hundreds of other cnidarians.

Target capture methods like UCEs are likely the way forward in phylogenomic studies in cnidarians (Quek & Huang, 2022) and other taxa given their cost effectiveness, coverage, and flexibility, particularly in that they can be used on older and/or less optimally preserved specimens (Derkarabetian et al., 2019; McCormack et al., 2015; Ruane & Austin, 2017). Target capture approaches also reduce problems with multiple transcript isoforms and paralogous loci, which we encountered mining transcriptomes for phylogenetic markers. Generating genome and transcriptome sequences will remain important since these data aid in the design of target capture probe sets (e.g., Quattrini et al., 2017) and are required for other analyses (e.g., selection, structural variants, gene family evolution, among others). Our transcriptome-based phylogenies confirm backbone relationships resolved in recent studies using target capture, which is a promising approach for reaching the goal of total taxon sampling.

Conclusions

Building a reliable phylogeny for any group, particularly one as diverse and species rich as Cnidaria, requires the cumulative efforts of researchers working to improve inference methods and taxonomic and genomic sampling. In comparing results across studies and incorporating previously applied sequences with newly acquired data, support for phylogenetic relationships becomes stronger. The goal of generating a cnidarian tree that includes every species increasingly seems like a reality that will happen within the next 10 or 20 years. In the meantime, generating trees (i.e., hypotheses of relationships) that encompass as many species as possible is critical for moving the field of cnidarian systematics and supplying cnidarian researchers with a framework from which to interpret evolutionary patterns.

Funding

This work was supported by the National Science Foundation under grant number DBI-1156528 to JFR. AB contributed to this project while an intern in the Whitney Laboratory's National Science Foundation Research Experience for Undergraduates (REU) Program (grant number 1560356 to JFR). CP has been supported by NSF-OIA 2032919 and USDA-NIFA 1017848. SNS was supported by São Paulo Research Foundation (FAPESP), grant number 2019/03552-0, 2022/16193-1 and CNPq (Research Productivity Scholarship) grant number 301293/2019-8, 304267/2022-8.

Acknowledgements

We would like to acknowledge the many researchers who generated and/or analyzed the public genomic, transcriptomic, and 18S data used in these analyses. We thank Gustav Paulay and Jessie Whelpley for sample collection and RNA extraction for *Leptogorgia* sp. and Renato Nagata for sample collection of *Arachnanthus* sp. We thank Antonis Rokas for discussions about phylogenomic analyses and Barbara Battelle for her 30-year leadership of the Whitney Lab REU program. We would like to acknowledge David Plachetzki for input on this manuscript. We thank two anonymous reviewers and editor Frank E. Anderson for their constructive comments, which improved the manuscript. The color palette of figures was inspired by *The Warmth of Other Sons* by Bisa Butler, 2020.

Supporting Information

All analysis scripts, alignments, and trees are available at https://github.com/josephryan/DeBiasse_cnidophylogenomics and transcriptomes are available on Dryad (https://doi.org/10.6071/M3K39S). A snapshot of the GitHub repo is included here as supplemental file 1. All accessions are available in Table S3.

Submitted: September 30, 2022 EDT, Accepted: October 24, 2023 EDT

References

Appeltans, W., Ahyong, S. T., Anderson, G., Angel, M. V., Artois, T., Bailly, N., Bamber, R., Barber, A., Bartsch, I., Berta, A., Błażewicz-Paszkowycz, M., Bock, P., Boxshall, G., Boyko, C. B., Brandão, S. N., Bray, R. A., Bruce, N. L., Cairns, S. D., Chan, T.-Y., … Costello, M. J. (2012). The magnitude of global marine species diversity. *Current Biology*, *22*(23), 2189–2202. https://doi.org/10.1016/j.cub.2012.09.036

Babonis, L. S., Enjolras, C., Reft, A. J., Foster, B. M., Hugosson, F., Ryan, J. F., Daly, M., & Martindale, M. Q. (2023). Single-cell atavism reveals an ancient mechanism of cell type diversification in a sea anemone. *Nature Communications*, *14*(1), 885. https://doi.org/10.1038/s41467-023-36615-9

Babonis, L. S., Martindale, M. Q., & Ryan, J. F. (2016). Do novel genes drive morphological novelty? An investigation of the nematosomes in the sea anemone Nematostella vectensis. *BMC Evolutionary Biology*, *16*(1), 1–22. https://doi.org/10.1186/s12862-016-0683-3

Barbeitos, M. S., Romano, S. L., & Lasker, H. R. (2010). Repeated loss of coloniality and symbiosis in scleractinian corals. *Proceedings of the National Academy of Sciences*, *107*(26), 11877–11882. https://doi.org/10.1073/pnas.0914380107

Barragán, Y., Sánchez, C., & Rodríguez, E. (2019). *Tenactis*gen. nov. (Actiniaria, Haloclavidae) a new genus of sea anemones from the Gulf of California, Mexico. *Journal of the Marine Biological Association of the United Kingdom*, *99*(3), 563–574. https://doi.org/10.1017/s0025315418000437

Bayha, K. M., Collins, A. G., & Gaffney, P. M. (2017). Multigene phylogeny of the scyphozoan jellyfish family Pelagiidae reveals that the common U.S. Atlantic sea nettle comprises two distinct species (*Chrysaora quinquecirrha*and*C. chesapeakei*). *PeerJ*, *5*, e3863. https://doi.org/10.7717/peerj.3863

Bayha, K. M., Dawson, M. N., Collins, A. G., Barbeitos, M. S., & Haddock, S. H. D. (2010). Evolutionary relationships among scyphozoan jellyfish families based on complete taxon sampling and phylogenetic analyses of 18S and 28S ribosomal DNA. *Integrative and Comparative Biology*, *50*(3), 436–455. https://doi.org/10.1093/icb/icq074

Bebenek, I. G., Gates, R. D., Morris, J., Hartenstein, V., & Jacobs, D. K. (2004). sine oculis in basal Metazoa. *Development Genes and Evolution*, *214*(7), 342–351. https://doi.org/10.1007/s00427-004-0407-3

Bellwood, D. R., Hughes, T. P., Folke, C., & Nyström, M. (2004). Confronting the coral reef crisis. *Nature*, *429*(6994), 827–833. https://doi.org/10.1038/nature02691

Bentlage, B., Cartwright, P., Yanagihara, A. A., Lewis, C., Richards, G. S., & Collins, A. G. (2010). Evolution of box jellyfish (Cnidaria: Cubozoa), a group of highly toxic invertebrates. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1680), 493–501. https://doi.org/10.1098/rspb.2009.1707

Bentlage, B., & Collins, A. G. (2021). Tackling the phylogenetic conundrum of Hydroidolina (Cnidaria: Medusozoa: Hydrozoa) by assessing competing tree topologies with targeted high-throughput sequencing. *PeerJ*, *9*, e12104. https://doi.org/10.7717/peerj.12104

Bentlage, B., Osborn, K. J., Lindsay, D. J., Hopcroft, R. R., Raskoff, K. A., & Collins, A. G. (2018). Loss of metagenesis and evolution of a parasitic life style in a group of open-ocean jellyfish. *Molecular Phylogenetics and Evolution*, *124*, 50–59. https://doi.org/10.1016/j.ympev.2018.02.030

Berntson, E. A., Bayer, F. M., McArthur, A. G., & France, S. C. (2001). Phylogenetic relationships within the Octocorallia (Cnidaria: Anthozoa) based on nuclear 18S rRNA sequences. *Marine Biology*, *138*(2), 235–246. https://doi.org/10.1007/s002270000457

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bradshaw, B., Thompson, K., & Frank, U. (2015). Distinct mechanisms underlie oral vs aboral regeneration in the cnidarian Hydractinia echinata. *Elife*, *4*, e05506. https://doi.org/10.7554/elife.05506

Bridge, D., Cunningham, C. W., DeSalle, R., & Buss, L. W. (1995). Class-level relationships in the phylum Cnidaria: molecular and morphological evidence. *Molecular Biology and Evolution*, *12*(4), 679–689.

Brugler, Mercer R., & France, S. C. (2007). The complete mitochondrial genome of the black coral Chrysopathes formosa (Cnidaria:Anthozoa:Antipatharia) supports classification of antipatharians within the subclass Hexacorallia. *Molecular Phylogenetics and Evolution*, *42*(3), 776–788. https://doi.org/10.1016/j.ympev.2006.08.016

Brugler, Mercer Robert. (2004). *The complete mitochondrial DNA sequence of the black coral Chrysopathes formosa (Antipatharia) and six non-contiguous mitochondrial genes of the tube anemone Ceriantheopsis americanus (Ceriantharia): implications for cnidarian phylogeny*. Graduate School of the College of Charleston.

Carlgren, O. (1949). A survey of the Ptychodactiaria, Corallimorpharia and Actiniaria. *Kungl. Svenska Vetenskapsakademiens Handlingar*, *3*, 1–121.

Cartwright, P., Evans, N. M., Dunn, C. W., Marques, A. C., Miglietta, M. P., Schuchert, P., & Collins, A. G. (2008). Phylogenetics of Hydroidolina (Hydrozoa: Cnidaria). *Journal of the Marine Biological Association of the United Kingdom*, *88*(8), 1663–1672. https://doi.org/10.1017/s0025315408002257

Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, *17*(4), 540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Chang, E. S., Neuhof, M., Rubinstein, N. D., Diamant, A., Philippe, H., Huchon, D., & Cartwright, P. (2015). Genomic insights into the evolutionary origin of Myxozoa within Cnidaria. *Proceedings of the National Academy of Sciences*, *112*(48), 14912–14917. https://doi.org/10.1073/pnas.1511468112

Chapman, J. A., Kirkness, E. F., Simakov, O., Hampson, S. E., Mitros, T., Weinmaier, T., Rattei, T., Balasubramanian, P. G., Borman, J., Busam, D., Disbennett, K., Pfannkoch, C., Sumin, N., Sutton, G. G., Viswanathan, L. D., Walenz, B., Goodstein, D. M., Hellsten, U., Kawashima, T., … Steele, R. E. (2010). The dynamic genome of Hydra. *Nature*, *464*(7288), 592–596. https://doi.org/10.1038/nature08830

Chen, C. A., Wallace, C. C., & Wolstenholme, J. (2002). Analysis of the mitochondrial 12S rRNA gene supports a two-clade hypothesis of the evolutionary history of scleractinian corals. *Molecular Phylogenetics and Evolution*, *23*(2), 137–149. https://doi.org/10.1016/s1055-7903(02)00008-8

Chen, C.-Y., McKinney, S. A., Ellington, L. R., & Gibson, M. C. (2020). Hedgehog signaling is required for endomesodermal patterning and germ cell development in the sea anemone Nematostella vectensis. *Elife*, *9*, e54573. https://doi.org/10.7554/elife.54573

Chera, S., Ghila, L., Dobretz, K., Wenger, Y., Bauer, C., Buzgariu, W., Martinou, J.-C., & Galliot, B. (2009). Apoptotic cells provide an unexpected source of Wnt3 signaling to drive hydra head regeneration. *Developmental Cell*, *17*(2), 279–289. https://doi.org/10.1016/j.devcel.2009.07.014

Collins, A. G. (2002). Phylogeny of Medusozoa and the evolution of cnidarian life cycles. *Journal of Evolutionary Biology*, *15*(3), 418–432. https://doi.org/10.1046/j.1420-9101.2002.00403.x

Collins, A. G., Bentlage, B., Lindner, A., Lindsay, D., Haddock, S. H. D., Jarms, G., Norenburg, J. L., Jankowski, T., & Cartwright, P. (2008). Phylogenetics of Trachylina (Cnidaria: Hydrozoa) with new insights on the evolution of some problematical taxa. *Journal of the Marine Biological Association of the United Kingdom*, *88*(8), 1673–1685. https://doi.org/10.1017/s0025315408001732

Collins, A. G., Bentlage, B., Matsumoto, G. I., Haddock, S. H. D., Osborn, K. J., & Schierwater, B. (2006). Solution to the phylogenetic enigma of *Tetraplatia*, a worm-shaped cnidarian. *Biology Letters*, *2*(1), 120–124. https://doi.org/10.1098/rsbl.2005.0372

Collins, A. G., Daly, M., Dunn, C. W., de Queiroz, K., Gauthier, J. A., & Cantino, P. D. (2020). Cnidaria Verrill 1865. In *Phylonyms: a companion to the Phylocode*. University of California Press.

Collins, A. G., Schuchert, P., Marques, A. C., Jankowski, T., Medina, M., & Schierwater, B. (2006). Medusozoan phylogeny and character evolution clarified by new large and small subunit rDNA data and an assessment of the utility of phylogenetic mixture models. *Systematic Biology*, *55*(1), 97–115. https://doi.org/10.1080/10635150500433615

Collins, A. G., & Valentine, J. W. (2001). Defining phyla: evolutionary pathways to metazoan body plans. *Evolution & Development*, *3*(6), 432–442. https://doi.org/10.1046/j.1525-142x.2001.01048.x

Cowman, P. F., Quattrini, A. M., Bridge, T. C. L., Watkins-Colwell, G. J., Fadli, N., Grinblat, M., Roberts, T. E., McFadden, C. S., Miller, D. J., & Baird, A. H. (2020). An enhanced target-enrichment bait set for Hexacorallia provides phylogenomic resolution of the staghorn corals (Acroporidae) and close relatives. *Molecular Phylogenetics and Evolution*, *153*, 106944. https://doi.org/10.1016/j.ympev.2020.106944

Darwin, C. (1851). *Geological Observations on Coral Reefs, Volcanic Islands, and on South America: Being the Geology of the Voyage of the Beagle, Under the Command of Captain Fitzroy RN, During the Years 1832 to 1836*. Smith, Elder & Company.

Davidson, N. M., Hawkins, A. D. K., & Oshlack, A. (2017). SuperTranscripts: a data driven reference for analysis and visualisation of transcriptomes. *Genome Biology*, *18*(1), 1–10. https://doi.org/10.1186/s13059-017-1284-1

Davy, S. K., Allemand, D., & Weis, V. M. (2012). Cell biology of cnidarian-dinoflagellate symbiosis. *Microbiology and Molecular Biology Reviews*, *76*(2), 229–261. https://doi.org/10.1128/mmbr.05014-11

Derkarabetian, S., Benavides, L. R., & Giribet, G. (2019). Sequence capture phylogenomics of historical ethanol-preserved museum specimens: Unlocking the rest of the vault. *Molecular Ecology Resources*, *19*(6), 1531–1544. https://doi.org/10.1111/1755-0998.13072

Dohrmann, M., & Wörheide, G. (2017). Dating early animal evolution using phylogenomic data. *Scientific Reports*, *7*(1), 1–6. https://doi.org/10.1038/s41598-017-03791-w

Emms, D. M., & Kelly, S. (2019). OrthoFinder: phylogenetic orthology inference for comparative genomics. *Genome Biology*, *20*(1), 1–14. https://doi.org/10.1186/s13059-019-1832-y

Erwin, D. H. (2015). Early metazoan life: divergence, environment and ecology. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1684), 20150036. https://doi.org/10.1098/rstb.2015.0036

Extavour, C. G., Pang, K., Matus, D. Q., & Martindale, M. Q. (2005). *vasa* and *nanos* expression patterns in a sea anemone and the evolution of bilaterian germ cell specification mechanisms. *Evolution & Development*, *7*(3), 201–215. https://doi.org/10.1111/j.1525-142x.2005.05023.x

Forero Mejia, A. C., Molodtsova, T., Östman, C., Bavestrello, G., & Rouse, G. W. (2020). Molecular phylogeny of Ceriantharia (Cnidaria: Anthozoa) reveals non-monophyly of traditionally accepted families. *Zoological Journal of the Linnean Society*, *190*(2), 397–416. https://doi.org/10.1093/zoolinnean/zlz158

Fukami, H., Budd, A. F., Paulay, G., Solé-Cava, A., Allen Chen, C., Iwao, K., & Knowlton, N. (2004). Conventional taxonomy obscures deep divergence between Pacific and Atlantic corals. *Nature*, *427*(6977), 832–835. https://doi.org/10.1038/nature02339

Fukami, H., Chen, C. A., Budd, A. F., Collins, A. G., Wallace, C., Chuang, Y.-Y., Chen, C., Dai, C.-F., Iwao, K., Sheppard, C., & Knowlton, N. (2008). Mitochondrial and nuclear genes suggest that stony corals are monophyletic but most families of stony corals are not (Order Scleractinia, Class Anthozoa, Phylum Cnidaria). *PloS One*, *3*(9), e3222. https://doi.org/10.1371/journal.pone.0003222

Gahan, J. M., Bradshaw, B., Flici, H., & Frank, U. (2016). The interstitial stem cells in Hydractinia and their role in regeneration. *Current Opinion in Genetics & Development*, *40*, 65–73. https://doi.org/10.1016/j.gde.2016.06.006

Gault, J. A., Bentlage, B., Huang, D., & Kerr, A. M. (2021). Lineage-specific variation in the evolutionary stability of coral photosymbiosis. *Science Advances*, *7*(39), eabh4243. https://doi.org/10.1126/sciadv.abh4243

Glon, H., Quattrini, A., Rodríguez, E., Titus, B. M., & Daly, M. (2021). Comparison of sequence-capture and ddRAD approaches in resolving species and populations in hexacorallian anthozoans. *Molecular Phylogenetics and Evolution*, *163*, 107233. https://doi.org/10.1016/j.ympev.2021.107233

Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., Chen, Z., Mauceli, E., Hacohen, N., Gnirke, A., Rhind, N., di Palma, F., Birren, B. W., Nusbaum, C., Lindblad-Toh, K., … Regev, A. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology*, *29*(7), 644–652. https://doi.org/10.1038/nbt.1883

Grimmelikhuijzen, C. J. P., Williamson, M., & Hansen, G. N. (2004). Neuropeptides in cnidarians. In *Cell signalling in prokaryotes and lower Metazoa* (pp. 115–139). Springer Netherlands. https://doi.org/10.1007/978-94-017-0998-9_5

Gröger, H., & Schmid, V. (2001). Larval development in Cnidaria: a connection to Bilateria? *Genesis*, *29*(3), 110–114. https://doi.org/10.1002/gene.1013

Gusmão, L. C., Van Deusen, V., Daly, M., & Rodríguez, E. (2020). Origin and evolution of the symbiosis between sea anemones (Cnidaria, Anthozoa, Actiniaria) and hermit crabs, with additional notes on anemone-gastropod associations. *Molecular Phylogenetics and Evolution*, *148*, 106805. https://doi.org/10.1016/j.ympev.2020.106805

Hamilton, N., Gusmão, L. C., Izumi, T., Rodríguez, E., Yap, N. W. L., & Daly, M. (2022). Phylogeny and taxonomy of Haloclavidae (Verrill, 1899) with a redescription of the parasitic, burrowing sea anemone, Peachia chilensis Carlgren, 1931. *Plos One*, *17*(9), e0266283. https://doi.org/10.1371/journal.pone.0266283

Hamlet, C., & Miller, L. (2014). Effects of Grouping Behavior, Pulse Timing, and Organism Size on Fluid Flow Around the Upside-Down Jellyfish Cassiopea xamachana. *Contemporary Mathematics*, *628*, 173–189. https://doi.org/10.1090/conm/628/12560

Hargitt, C. W. (1901). Variation among hydromedusae. *The Biological Bulletin*, *2*(5), 221–255. https://doi.org/10.2307/1535717

Hargitt, C. W. (1904). The early development of Pennaria tiarella McCr. *Archiv Für Entwicklungsmechanik Der Organismen*, *18*(4), 453–488. https://doi.org/10.1007/bf02161728

He, S., Grasis, J. A., Nicotra, M. L., Juliano, C. E., & Schnitzler, C. E. (2019). Cnidofest 2018: the future is bright for cnidarian research. *EvoDevo*, *10*(1). https://doi.org/10.1186/s13227-019-0134-5

Hellberg, M. E. (2006). No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation. *BMC Evolutionary Biology*, *6*(1), 1–8. https://doi.org/10.1186/1471-2148-6-24

Helm, R., Siebert, S., Tulin, S., Smith, J., & Dunn, C. (2013). Characterization of differential transcript abundance through time during Nematostella vectensis development. *BMC Genomics*, *14*(1), 266. https://doi.org/10.1186/1471-2164-14-266

Hoegh-Guldberg, O. (1999). Climate change, coral bleaching and the future of the world's coral reefs. *Marine and Freshwater Research*, *50*(8), 839–866. https://doi.org/10.1071/mf99078

Horowitz, J., Brugler, M. R., Bridge, T. C. L., & Cowman, P. F. (2020). Morphological and molecular description of a new genus and species of black coral from the mesophotic reefs of Papua New Guinea (Cnidaria: Anthozoa: Hexacorallia: Antipatharia: Antipathidae: Blastopathes). *Zootaxa*, *4821*(3), 553–569. https://doi.org/10.11646/zootaxa.4821.3.7

Huang, D., Licuanan, W. Y., Baird, A. H., & Fukami, H. (2011). Cleaning up the "Bigmessidae": Molecular phylogeny of scleractinian corals from Faviidae, Merulinidae, Pectiniidae and Trachyphylliidae. *BMC Evolutionary Biology*, *11*(1), 1–13. https://doi.org/10.1186/1471-2148-11-37

Izumi, T., Yanagi, K., & Fujita, T. (2020). Comprehensive revision of Anemonactis (Cnidaria: Anthozoa: Actiniaria: Haloclavidae) in Japan: reestablishment of Anemonactis minuta (Wassilieff, 1908) comb. nov. and description of Anemonactis tohrui sp. nov. *Marine Biodiversity*, *50*(5), 1–16. https://doi.org/10.1007/s12526-020-01085-5

Jouiaei, M., Yanagihara, A., Madio, B., Nevalainen, T., Alewood, P., & Fry, B. (2015). Ancient venom systems: a review on cnidaria toxins. *Toxins*, *7*(6), 2251–2271. https://doi.org/10.3390/toxins7062251

Karadge, U. B., Gosto, M., & Nicotra, M. L. (2015). Allorecognition proteins in an invertebrate exhibit homophilic interactions. *Current Biology*, *25*(21), 2845–2850. https://doi.org/10.1016/j.cub.2015.09.030

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, *30*(4), 772–780. https://doi.org/10.1093/molbev/mst010

Kayal, E., Bentlage, B., Cartwright, P., Yanagihara, A. A., Lindsay, D. J., Hopcroft, R. R., & Collins, A. G. (2015). Phylogenetic analysis of higher-level relationships within Hydroidolina (Cnidaria: Hydrozoa) using mitochondrial genome data and insight into their mitochondrial transcription. *PeerJ*, *3*, e1403. https://doi.org/10.7717/peerj.1403

Kayal, E., Bentlage, B., Sabrina Pankey, M., Ohdera, A. H., Medina, M., Plachetzki, D. C., Collins, A. G., & Ryan, J. F. (2018). Phylogenomics provides a robust topology of the major cnidarian lineages and insights on the origins of key organismal traits. *BMC Evolutionary Biology*, *18*(1), 1–18. https://doi.org/10.1186/s12862-018-1142-0

Kayal, E., & Lavrov, D. V. (2008). The mitochondrial genome of Hydra oligactis (Cnidaria, Hydrozoa) sheds new light on animal mtDNA evolution and cnidarian phylogeny. *Gene*, *410*(1), 177–186. https://doi.org/10.1016/j.gene.2007.12.002

Kayal, E., Roure, B., Philippe, H., Collins, A. G., & Lavrov, D. V. (2013). Cnidarian phylogenetic relationships as revealed by mitogenomics. *BMC Evolutionary Biology*, *13*(1), 1–18. https://doi.org/10.1186/1471-2148-13-5

Kim, J., Kim, W., & Cunningham, C. W. (1999). A new perspective on lower metazoan relationships from 18S rDNA sequences. *Molecular Biology and Evolution*, *16*(3), 423–427. https://doi.org/10.1093/oxfordjournals.molbev.a026124

Kitahara, M. V., Cairns, S. D., Stolarski, J., Blair, D., & Miller, D. J. (2010). A comprehensive phylogenetic analysis of the Scleractinia (Cnidaria, Anthozoa) based on mitochondrial CO1 sequence data. *PloS One*, *5*(7), e11490. https://doi.org/10.1371/journal.pone.0011490

Kitahara, M. V., Lin, M.-F., Forêt, S., Huttley, G., Miller, D. J., & Chen, C. A. (2014). The "naked coral" hypothesis revisited–evidence for and against scleractinian monophyly. *PLoS One*, *9*(4), e94774. https://doi.org/10.1371/journal.pone.0094774

Klompen, A. M. L., Macrander, J., Reitzel, A. M., & Stampar, S. N. (2020). Transcriptomic analysis of four cerianthid (Cnidaria, Ceriantharia) venoms. *Marine Drugs*, *18*(8), 413. https://doi.org/10.3390/md18080413

Kocot, K. M., Citarella, M. R., Moroz, L. L., & Halanych, K. M. (2013). PhyloTreePruner: a phylogenetic tree-based approach for selection of orthologous sequences for phylogenomics. *Evolutionary Bioinformatics*, *9*, EBO.S12813. https://doi.org/10.4137/ebo.s12813

Lartillot, N., Lepage, T., & Blanquart, S. (2009). PhyloBayes 3: a Bayesian software package for phylogenetic reconstruction and molecular dating. *Bioinformatics*, *25*(17), 2286–2288. https://doi.org/10.1093/bioinformatics/btp368

Lartillot, N., & Philippe, H. (2004). A Bayesian mixture model for across-site heterogeneities in the amino-acid replacement process. *Molecular Biology and Evolution*, *21*(6), 1095–1109. https://doi.org/10.1093/molbev/msh112

Le, S. Q., & Gascuel, O. (2008). An improved general amino acid replacement matrix. *Molecular Biology and Evolution*, *25*(7), 1307–1320. https://doi.org/10.1093/molbev/msn067

Leclère, L., Horin, C., Chevalier, S., Lapébie, P., Dru, P., Peron, S., Jager, M., Condamine, T., Pottin, K., Romano, S., Steger, J., Sinigaglia, C., Barreau, C., Quiroga Artigas, G., Ruggiero, A., Fourrage, C., Kraus, J. E. M., Poulain, J., Aury, J.-M., … Copley, R. R. (2019). The genome of the jellyfish Clytia hemisphaerica and the evolution of the cnidarian life-cycle. *Nature Ecology & Evolution*, *3*(5), 801–810. https://doi.org/10.1038/s41559-019-0833-2

Lehnert, E. M., Burriesci, M. S., & Pringle, J. R. (2012). Developing the anemone Aiptasia as a tractable model for cnidarian-dinoflagellate symbiosis: the transcriptome of aposymbiotic A. pallida. *BMC Genomics*, *13*(1), 1–10. https://doi.org/10.1186/1471-2164-13-271

Lin, M. F., Chou, W. H., Kitahara, M. V., Chen, C. L. A., Miller, D. J., & Forêt, S. (2016). Corallimorpharians are not "naked corals": insights into relationships between Scleractinia and Corallimorpharia from phylogenomic analyses. *PeerJ*, *4*, e2463. https://doi.org/10.7717/peerj.2463

Lin, M.-F., Kitahara, M. V., Luo, H., Tracey, D., Geller, J., Fukami, H., Miller, D. J., & Chen, C. A. (2014). Mitochondrial genome rearrangements in the Scleractinia/Corallimorpharia complex: implications for coral phylogeny. *Genome Biology and Evolution*, *6*(5), 1086–1095. https://doi.org/10.1093/gbe/evu084

Macrander, J., Broe, M., & Daly, M. (2016). Tissue-specific venom composition and differential gene expression in sea anemones. *Genome Biology and Evolution*, *8*(8), 2358–2375. https://doi.org/10.1093/gbe/evw155

Macrander, J., Brugler, M. R., & Daly, M. (2015). A RNA-seq approach to identify putative toxins from acrorhagi in aggressive and non-aggressive Anthopleura elegantissima polyps. *BMC Genomics*, *16*(1), 1–19. https://doi.org/10.1186/s12864-015-1417-4

Mariottini, G., & Grice, I. (2016). Antimicrobials from Cnidarians. A new perspective for anti-infective therapy? *Marine Drugs*, *14*(3), 48. https://doi.org/10.3390/md14030048

Mariscal, R. N. (1984). Cnidaria: cnidae. In *Biology of the Integument* (pp. 57–68). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-51593-4_6

Mariscal, R. N., McLean, R. B., & Hand, C. (1977). The form and function of cnidarian spirocysts. *Cell and Tissue Research*, *178*(4), 427–433. https://doi.org/10.1007/bf00219566

Marlow, H. Q., Srivastava, M., Matus, D. Q., Rokhsar, D., & Martindale, M. Q. (2009). Anatomy and development of the nervous system of *Nematostella vectensis*, an anthozoan cnidarian. *Developmental Neurobiology*, *69*(4), 235–254. https://doi.org/10.1002/dneu.20698

Marques, A. C., & Collins, A. G. (2004). Cladistic analysis of Medusozoa and cnidarian evolution. *Invertebrate Biology*, *123*(1), 23–42. https://doi.org/10.1111/j.1744-7410.2004.tb00139.x

Martin, V. J., Littlefield, C. L., Archer, W. E., & Bode, H. R. (1997). Embryogenesis in hydra. *The Biological Bulletin*, *192*(3), 345–363. https://doi.org/10.2307/1542745

Martínez, D. E., Iñiguez, A. R., Percell, K. M., Willner, J. B., Signorovitch, J., & Campbell, R. D. (2010). Phylogeny and biogeography of Hydra (Cnidaria: Hydridae) using mitochondrial and nuclear DNA sequences. *Molecular Phylogenetics and Evolution*, *57*(1), 403–410. https://doi.org/10.1016/j.ympev.2010.06.016

McCormack, J. E., Tsai, W. L. E., & Faircloth, B. C. (2015). Sequence capture of ultraconserved elements from bird museum specimens. *Molecular Ecology Resources*, *16*(5), 1189–1203. https://doi.org/10.1111/1755-0998.12466

McFadden, C. S., Quattrini, A. M., Brugler, M. R., Cowman, P. F., Dueñas, L. F., Kitahara, M. V., Paz-García, D. A., Reimer, J. D., & Rodríguez, E. (2021). Phylogenomics, origin, and diversification of Anthozoans (Phylum Cnidaria). *Systematic Biology*, *70*(4), 635–647. https://doi.org/10.1093/sysbio/syaa103

McFadden, C. S., Sanchez, J. A., & France, S. C. (2010). Molecular phylogenetic insights into the evolution of Octocorallia: a review. *Integrative and Comparative Biology*, *50*(3), 389–410. https://doi.org/10.1093/icb/icq056

McFadden, C. S., van Ofwegen, L. P., & Quattrini, A. M. (2022). Revisionary systematics of Octocorallia (Cnidaria: Anthozoa) guided by phylogenomics. *Bulletin of the Society of Systematic Biologists*, *1*(3). https://doi.org/10.18061/bssb.v1i3.8735

McGinnis, S., & Madden, T. L. (2004). BLAST: at the core of a powerful and diverse set of sequence analysis tools. *Nucleic Acids Research*, *32*, W20–W25. https://doi.org/10.1093/nar/gkh435

McMillan, J., Mahony, T., Veron, J. E. N., & Miller, D. J. (1991). Nucleotide sequencing of highly repetitive DNA from seven species in the coral genusAcropora (Cnidaria: Scleractinia) implies a division contrary to morphological criteria. *Marine Biology*, *110*(3), 323–327. https://doi.org/10.1007/bf01344350

Medina, M., Collins, A. G., Takaoka, T. L., Kuehl, J. V., & Boore, J. L. (2006). Naked corals: skeleton loss in Scleractinia. *Proceedings of the National Academy of Sciences*, *103*(24), 9096–9100. https://doi.org/10.1073/pnas.0602444103

Miller, D., Ball, E., & Technau, U. (2005). Cnidarians and ancestral genetic complexity in the animal kingdom. *Trends in Genetics*, *21*(10), 536–539. https://doi.org/10.1016/j.tig.2005.08.002

Miranda, L. S., Collins, A. G., Hirano, Y. M., Mills, C. E., & Marques, A. C. (2016). Comparative internal anatomy of Staurozoa (Cnidaria), with functional and evolutionary inferences. *PeerJ*, *4*, e2594. https://doi.org/10.7717/peerj.2594

Miranda, L. S., Collins, A. G., & Marques, A. C. (2010). Molecules clarify a cnidarian life cycle–the “hydrozoan” Microhydrula limopsicola is an early life stage of the staurozoan Haliclystus antarcticus. *PLoS One*, *5*(4), e10182. https://doi.org/10.1371/journal.pone.0010182

Miranda, L. S., Hirano, Y. M., Mills, C. E., Falconer, A., Fenwick, D., Marques, A. C., & Collins, A. G. (2016). Systematics of stalked jellyfishes (Cnidaria: Staurozoa). *PeerJ*, *4*, e1951. https://doi.org/10.7717/peerj.1951

Miranda, L. S., Mills, C. E., Hirano, Y. M., Collins, A. G., & Marques, A. C. (2018). A review of the global diversity and natural history of stalked jellyfishes (Cnidaria, Staurozoa). *Marine Biodiversity*, *48*(4), 1695–1714. https://doi.org/10.1007/s12526-017-0721-4

Müller, F. (1862). II.—*On the systematic position of the* Charybdeidæ. *Annals and Magazine of Natural History*, *10*(55), 6–12. https://doi.org/10.1080/00222936208681271

Murbach, L. (1896). Observations on the development and migration of the urticating organs of sea nettles, Cnidaria. *Proceedings of the United States National Museum*, *18*(1097), 733–740. https://doi.org/10.5479/si.00963801.1097.733

Nawrocki, A. M., Collins, A. G., Hirano, Y. M., Schuchert, P., & Cartwright, P. (2013). Phylogenetic placement of Hydra and relationships within Aplanulata (Cnidaria: Hydrozoa). *Molecular Phylogenetics and Evolution*, *67*(1), 60–71. https://doi.org/10.1016/j.ympev.2012.12.016

Nawrocki, A. M., Schuchert, P., & Cartwright, P. (2010). Phylogenetics and evolution of Capitata (Cnidaria: Hydrozoa), and the systematics of Corynidae. *Zoologica Scripta*, *39*(3), 290–304. https://doi.org/10.1111/j.1463-6409.2009.00419.x

Nawrocki, E. P., & Farm, H. J. (2010). *SSU-ALIGN User’s Guide*.

Newkirk, C. R., Frazer, T. K., & Martindale, M. Q. (2018). Acquisition and proliferation of algal symbionts in bleached polyps of the upside-down jellyfish, Cassiopea xamachana. *Journal of Experimental Marine Biology and Ecology*, *508*, 44–51. https://doi.org/10.1016/j.jembe.2018.08.010

Nguyen, L.-T., Schmidt, H. A., Von Haeseler, A., & Minh, B. Q. (2014). IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, *32*(1), 268–274. https://doi.org/10.1093/molbev/msu300

Nishimura, O., Hara, Y., & Kuraku, S. (2017). gVolante for standardizing completeness assessment of genome and transcriptome assemblies. *Bioinformatics*, *33*(22), 3635–3637. https://doi.org/10.1093/bioinformatics/btx445

Peres, R., Reitzel, A. M., Passamaneck, Y., Afeche, S. C., Cipolla-Neto, J., Marques, A. C., & Martindale, M. Q. (2014). Developmental and light-entrained expression of melatonin and its relationship to the circadian clock in the sea anemone Nematostella vectensis. *EvoDevo*, *5*(1), 1–18. https://doi.org/10.1186/2041-9139-5-26

Picciani, N., Kerlin, J. R., Sierra, N., Swafford, A. J. M., Ramirez, M. D., Roberts, N. G., Cannon, J. T., Daly, M., & Oakley, T. H. (2018). Prolific origination of eyes in Cnidaria with co-option of non-visual opsins. *Current Biology*, *28*(15), 2413-2419.e4. https://doi.org/10.1016/j.cub.2018.05.055

Poliseno, A., Feregrino, C., Sartoretto, S., Aurelle, D., Wörheide, G., McFadden, C. S., & Vargas, S. (2017). Comparative mitogenomics, phylogeny and evolutionary history of Leptogorgia (Gorgoniidae). *Molecular Phylogenetics and Evolution*, *115*, 181–189. https://doi.org/10.1016/j.ympev.2017.08.001

Pratlong, M., Rancurel, C., Pontarotti, P., & Aurelle, D. (2016). Monophyly of Anthozoa (Cnidaria): why do nuclear and mitochondrial phylogenies disagree? *Zoologica Scripta*, *46*(3), 363–371. https://doi.org/10.1111/zsc.12208

Putnam, N. H., Srivastava, M., Hellsten, U., Dirks, B., Chapman, J., Salamov, A., Terry, A., Shapiro, H., Lindquist, E., Kapitonov, V. V., Jurka, J., Genikhovich, G., Grigoriev, I. V., Lucas, S. M., Steele, R. E., Finnerty, J. R., Technau, U., Martindale, M. Q., & Rokhsar, D. S. (2007). Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. *Science*, *317*(5834), 86–94. https://doi.org/10.1126/science.1139158

Quattrini, A. M., Faircloth, B. C., Dueñas, L. F., Bridge, T. C. L., Brugler, M. R., Calixto-Botía, I. F., DeLeo, D. M., Forêt, S., Herrera, S., Lee, S. M. Y., Miller, D. J., Prada, C., Rádis-Baptista, G., Ramírez-Portilla, C., Sánchez, J. A., Rodríguez, E., & McFadden, C. S. (2017). Universal target-enrichment baits for anthozoan (Cnidaria) phylogenomics: New approaches to long-standing problems. *Molecular Ecology Resources*, *18*(2), 281–295. https://doi.org/10.1111/1755-0998.12736

Quattrini, A. M., Rodríguez, E., Faircloth, B. C., Cowman, P. F., Brugler, M. R., Farfan, G. A., Hellberg, M. E., Kitahara, M. V., Morrison, C. L., Paz-García, D. A., Reimer, J. D., & McFadden, C. S. (2020). Palaeoclimate ocean conditions shaped the evolution of corals and their skeletons through deep time. *Nature Ecology & Evolution*, *4*(11), 1531–1538. https://doi.org/10.1038/s41559-020-01291-1

Quattrini, A. M., Snyder, K. E., Purow-Ruderman, R., Seiblitz, I. G. L., Hoang, J., Floerke, N., Ramos, N. I., Wirshing, H. H., Rodriguez, E., & McFadden, C. S. (2023). Mito-nuclear discordance within Anthozoa, with notes on unique properties of their mitochondrial genomes. *Scientific Reports*, *13*(1), 7443. https://doi.org/10.1038/s41598-023-34059-1

Quek, Z. B. R., & Huang, D. (2022). Application of phylogenomic tools to unravel anthozoan evolution. *Coral Reefs*, *41*(3), 475–495. https://doi.org/10.1007/s00338-021-02072-3

Reft, A. J., & Daly, M. (2011). Morphology, distribution, and evolution of apical structure of nematocysts in hexacorallia. *Journal of Morphology*, *273*(2), 121–136. https://doi.org/10.1002/jmor.11014

Rodríguez, E., Barbeitos, M. S., Brugler, M. R., Crowley, L. M., Grajales, A., Gusmão, L., Häussermann, V., Reft, A., & Daly, M. (2014). Hidden among sea anemones: the first comprehensive phylogenetic reconstruction of the order Actiniaria (Cnidaria, Anthozoa, Hexacorallia) reveals a novel group of hexacorals. *PloS One*, *9*(5), e96998. https://doi.org/10.1371/journal.pone.0096998

Romanes, G. J. (1880). The New Freshwater Jelly Fish: Physiology of the Freshwater Medusa. *Nature*, *22*(556), 179–181. https://doi.org/10.1038/022179a0

Romano, S. L., & Cairns, S. D. (2000). Molecular phylogenetic hypotheses for the evolution of scleractinian corals. *Bulletin of Marine Science*, *67*(3), 1043–1068.

Romano, S. L., & Palumbi, S. R. (1996). Evolution of scleractinian corals inferred from molecular systematics. *Science*, *271*(5249), 640–642. https://doi.org/10.1126/science.271.5249.640

Romano, S. L., & Palumbi, S. R. (1997). Molecular evolution of a portion of the mitochondrial 16S ribosomal gene region in scleractinian corals. *Journal of Molecular Evolution*, *45*(4), 397–411. https://doi.org/10.1007/pl00006245

Ruane, S., & Austin, C. C. (2017). Phylogenomics using formalin-fixed and 100+ year-old intractable natural history specimens. *Molecular Ecology Resources*, *17*(5), 1003–1008. https://doi.org/10.1111/1755-0998.12655

Sanders, S. M., & Cartwright, P. (2015). Patterns of Wnt signaling in the life cycle of *Podocoryna carnea* and its implications for medusae evolution in Hydrozoa (Cnidaria). *Evolution & Development*, *17*(6), 325–336. https://doi.org/10.1111/ede.12165

Sars, M. (1829). Bidrag til Soedyrenes naturhistorie. In *Med Sex Illuminerede Steentryktavler* (pp. 1–63). Chr. Dahl's bogtrykkeri.

Seiblitz, I. G. L., Capel, K. C. C., Stolarski, J., Quek, Z. B. R., Huang, D., & Kitahara, M. V. (2020). The earliest diverging extant scleractinian corals recovered by mitochondrial genomes. *Scientific Reports*, *10*(1), 1–11. https://doi.org/10.1038/s41598-020-77763-y

Shearer, T. L., Van Oppen, M. J. H., Romano, S. L., & Wörheide, G. (2002). Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). *Molecular Ecology*, *11*(12), 2475–2487. https://doi.org/10.1046/j.1365-294x.2002.01652.x

Siddall, M. E., Martin, D. S., Bridge, D., Desser, S. S., & Cone, D. K. (1995). The demise of a phylum of protists: phylogeny of Myxozoa and other parasitic Cnidaria. *The Journal of Parasitology*, *81*(6), 961–967. https://doi.org/10.2307/3284049

Siebert, S., Farrell, J. A., Cazet, J. F., Abeykoon, Y., Primack, A. S., Schnitzler, C. E., & Juliano, C. E. (2019). Stem cell differentiation trajectories in *Hydra* resolved at single-cell resolution. *Science*, *365*(6451), eaav9314. https://doi.org/10.1126/science.aav9314

Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., & Zdobnov, E. M. (2015). BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics*, *31*(19), 3210–3212. https://doi.org/10.1093/bioinformatics/btv351

Smothers, J. F., von Dohlen, C. D., Smith, L. H., Jr., & Spall, R. D. (1994). Molecular evidence that the myxozoan protists are metazoans. *Science*, *265*(5179), 1719–1721. https://doi.org/10.1126/science.8085160

Stampar, S. N., Broe, M. B., Macrander, J., Reitzel, A. M., Brugler, M. R., & Daly, M. (2019). Linear mitochondrial genome in Anthozoa (Cnidaria): a case study in Ceriantharia. *Scientific Reports*, *9*(1), 1–12. https://doi.org/10.1038/s41598-019-42621-z

Stampar, S. N., Maronna, M. M., Kitahara, M. V., Reimer, J. D., Beneti, J. S., & Morandini, A. C. (2016). Ceriantharia in current systematics: life cycles, morphology and genetics. *The Cnidaria, Past, Present and Future*, 61–72. https://doi.org/10.1007/978-3-319-31305-4_5

Stampar, S. N., Maronna, M. M., Kitahara, M. V., Reimer, J. D., & Morandini, A. C. (2014). Fast-evolving mitochondrial DNA in Ceriantharia: a reflection of Hexacorallia paraphyly? *PLoS One*, *9*(1), e86612. https://doi.org/10.1371/journal.pone.0086612

Stampar, S. N., Maronna, M. M., Vermeij, M. J. A., Silveira, F. L. d., & Morandini, A. C. (2012). Evolutionary diversification of banded tube-dwelling anemones (Cnidaria; Ceriantharia; Isarachnanthus) in the Atlantic Ocean. *PLoS One*, *7*(7), e41091. https://doi.org/10.1371/journal.pone.0041091

Stolarski, J., Kitahara, M. V., Miller, D. J., Cairns, S. D., Mazur, M., & Meibom, A. (2011). The ancient evolutionary origins of Scleractinia revealed by azooxanthellate corals. *BMC Evolutionary Biology*, *11*(1), 316. https://doi.org/10.1186/1471-2148-11-316

Straehler-Pohl, I., & Jarms, G. (2022a). Back to the roots, Part 1—early life cycle data of Rhopaliophora (Scyphozoa, Cubozoa and Staurozoa). *Plankton and Benthos Research*, *17*(1), 1–33. https://doi.org/10.3800/pbr.17.1

Straehler-Pohl, I., & Jarms, G. (2022b). Back to the roots, Part 2—Rhopaliophora (Scyphozoa, Cubozoa and Staurozoa) reborn based on early life cycle data. *Plankton and Benthos Research*, *17*(2), 105–126. https://doi.org/10.3800/pbr.17.105

Straehler-Pohl, I., & Toshino, S. (2015). Carybdea morandinii—New investigations on its life cycle reveal its true genus: Carybdea morandinii Straehler-Pohl & Jarms, 2011 becomes Alatina morandinii (Straehler-Pohl & Jarms, 2011). *Plankton and Benthos Research*, *10*(4), 167–177. https://doi.org/10.3800/pbr.10.167

Sullivan, J. C., & Finnerty, J. R. (2007). A surprising abundance of human disease genes in a simple "basal" animal, the starlet sea anemone (*Nematostella vectensis*). *Genome*, *50*(7), 689–692. https://doi.org/10.1139/g07-045

Tan, G., Muffato, M., Ledergerber, C., Herrero, J., Goldman, N., Gil, M., & Dessimoz, C. (2015). Current methods for automated filtering of multiple sequence alignments frequently worsen single-gene phylogenetic inference. *Systematic Biology*, *64*(5), 778–791. https://doi.org/10.1093/sysbio/syv033

Trembley, A., Bayer, F. M., Lyonet, P., Pronk, C., & Schley, J. van der. (1744). *Mémoires pour servir à l'histoire d'un genre de polypes d'eau douce, à bras en forme de cornes* (Vol. 1). Chez Jean & Herman Verbeek. https://doi.org/10.5962/bhl.title.64073

WoRMS Editorial Board. (2022). World Register of Marine Species. Available from https://www.marinespecies.org at VLIZ. *WoRMS Editorial Board*.

Yap, N. W. L., Fautin, D. G., Ramos, D. A., & Tan, R. (2014). Sea anemones of Singapore:*Synpeachia temasek*new genus, new species, and redescription of*Metapeachia tropica*(Cnidaria: Actiniaria: Haloclavidae). *Proceedings of the Biological Society of Washington*, *127*(3), 439–454. https://doi.org/10.2988/0006-324x-127.3.439

Zapata, F., Goetz, F. E., Smith, S. A., Howison, M., Siebert, S., Church, S. H., Sanders, S. M., Ames, C. L., McFadden, C. S., France, S. C., Daly, M., Collins, A. G., Haddock, S. H. D., Dunn, C. W., & Cartwright, P. (2015). Phylogenomic analyses support traditional relationships within Cnidaria. *PloS One*, *10*(10), e0139068. https://doi.org/10.1371/journal.pone.0139068

Zeleny, C. (1907). *The effect of degree of injury, successive injury and functional activity upon regeneration in the scyphomedusan, Cassiopea xamachana*. publisher not identified.

Zoja, R. (1895). Sullo sviluppo dei blastomeri isolati dalle uova di alcune meduse (e di altri organismi). *Archiv für Entwicklungsmechanik der Organismen*, *2*(1), 1–37. https://doi.org/10.1007/bf02084178

Supporting Information

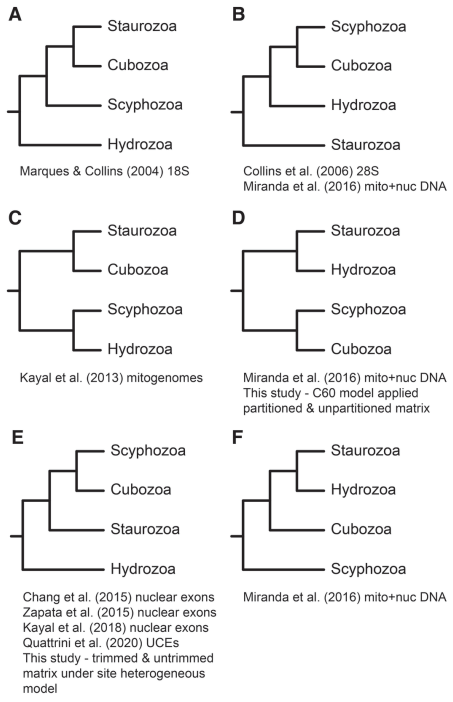

Figure S1. Cladograms representing the historical phylogenetic relationships found within Medusozoa

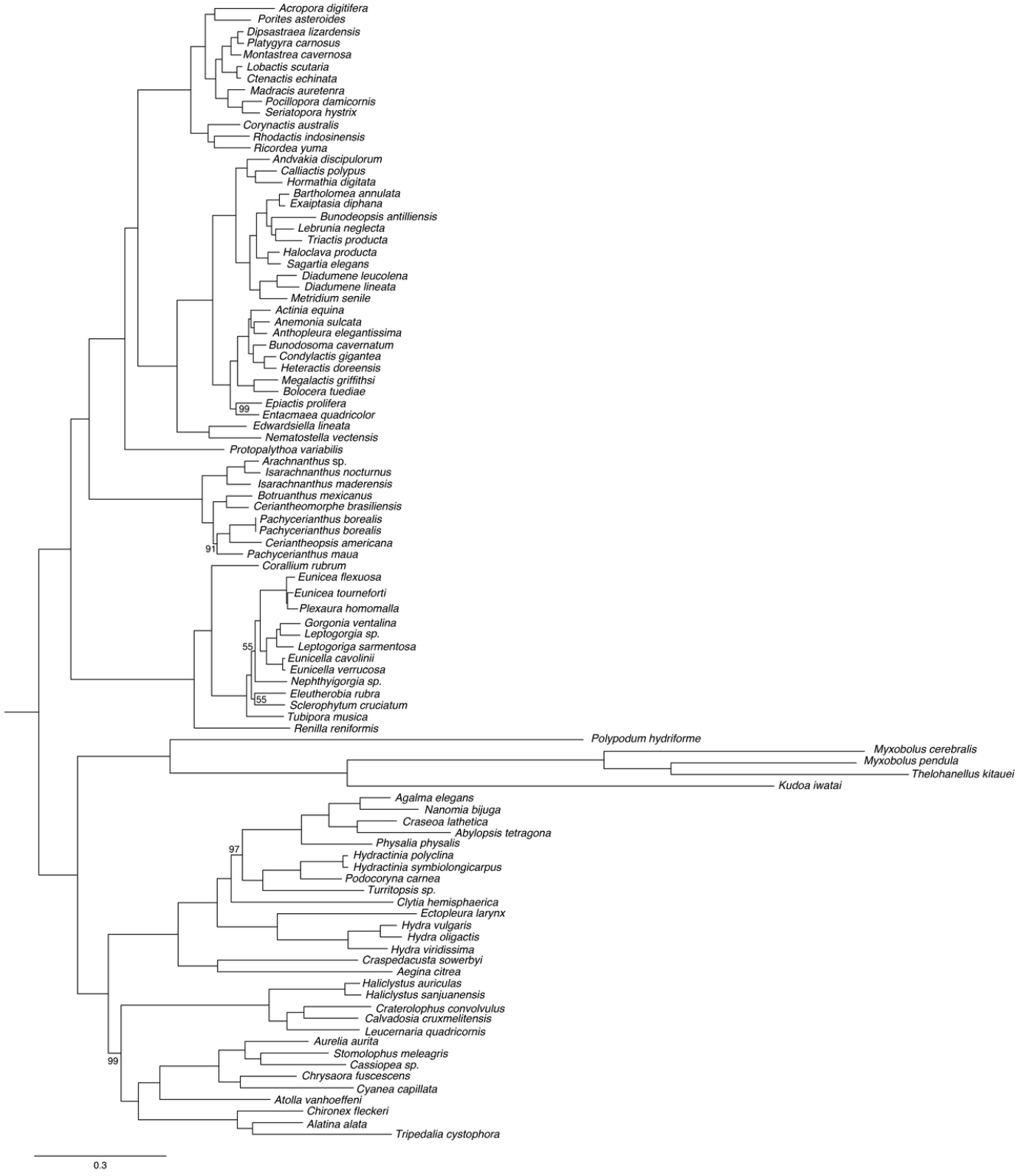

Figure S2. Maximum-likelihood phylogeny of cnidarian estimated from a concatenated matrix of 748 trimmed ortholog alignments generated partition-specific models. Bootstrap values are indicated at nodes with less than 100% support.

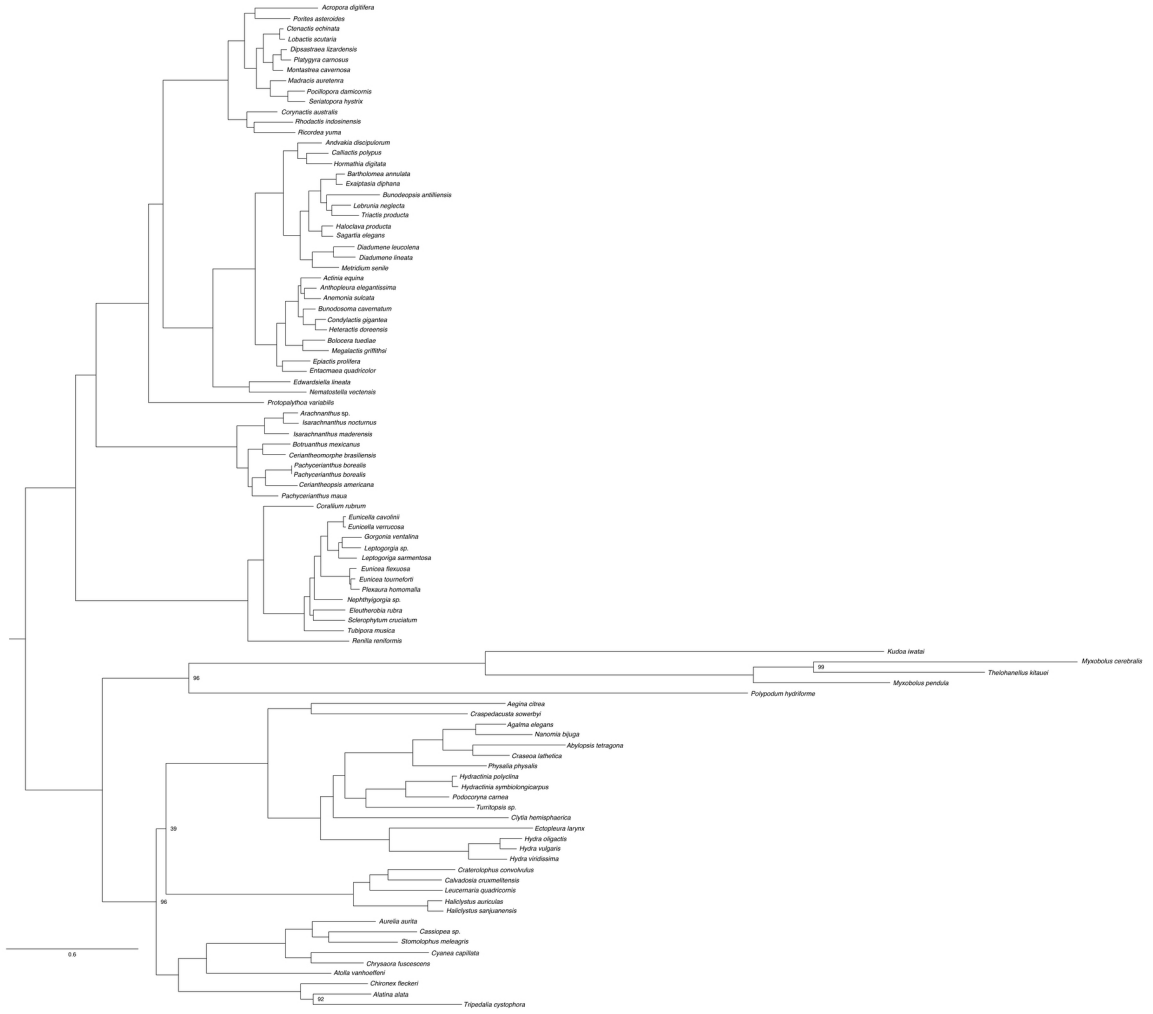

Figure S3. Maximum-likelihood phylogeny of cnidarians estimated from a concatenated matrix of 748 untrimmed ortholog alignments under the C60 model of amino acid substitution. Bootstrap values are indicated at nodes with less than 100% support.

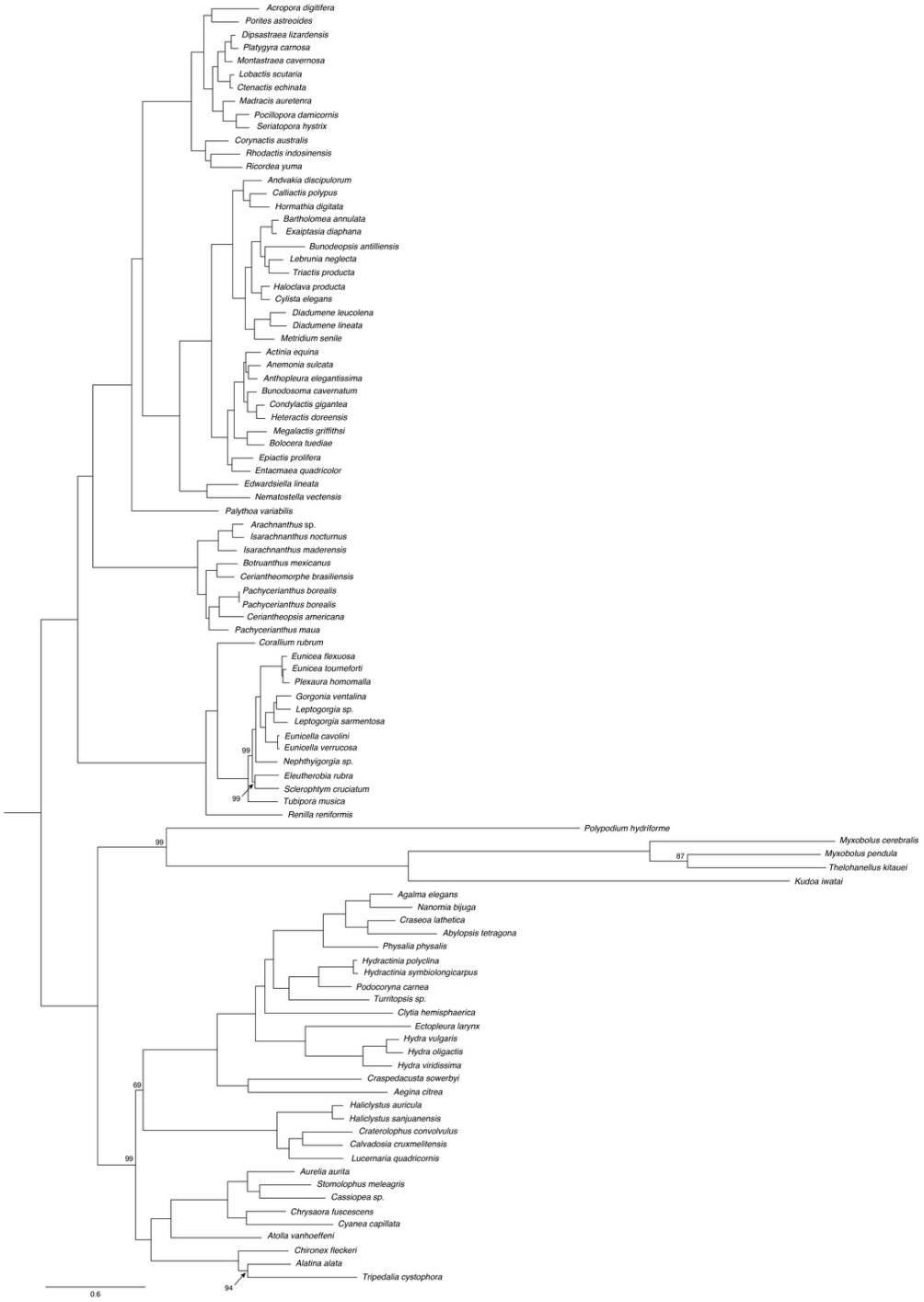

Figure S4. Maximum-likelihood phylogeny of cnidarians estimated from a concatenated matrix of 748 untrimmed ortholog alignments under the C60 model of amino acid substitution applied to a partitioned matrix. Bootstrap values are indicated at nodes with less than 100% support.

Supplementary Materials

Table S1

Download: https://ssbbulletin.scholasticahq.com/article/94008-a-cnidarian-phylogenomic-tree-fitted-with-hundreds-of-18s-leaves/attachment/197660.xlsx?auth_token=fHoHUaDCIGRRSkbto5Wz

Table S2

Download: https://ssbbulletin.scholasticahq.com/article/94008-a-cnidarian-phylogenomic-tree-fitted-with-hundreds-of-18s-leaves/attachment/197662.docx?auth_token=fHoHUaDCIGRRSkbto5Wz

Table S3

Download: https://ssbbulletin.scholasticahq.com/article/94008-a-cnidarian-phylogenomic-tree-fitted-with-hundreds-of-18s-leaves/attachment/197661.xlsx?auth_token=fHoHUaDCIGRRSkbto5Wz