Robust phylogenetic position of the enigmatic hydrozoan, *Margelopsis haeckelii* revealed within the family Corymorphidae

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Keywords: Cnidaria, Hydrozoa, Corymorphidae, Margelopsidae, *Margelopsis haeckelii,* Molecular phylogeny

Abstract

The life-cycle and polyp morphology of Margelopsidae representatives are very different from all other Aplanulata cnidarians. Until recently, their evolutionary origin and phylogenetic position has been a subject of significant speculation. A recent molecular study based only on COI data unexpectedly placed Margelopsidae as a sister group to all Aplanulata, despite the Margelopsid morphology suggests affiliation with Tubulariidae or Corymorphidae. Here we used multigene analyses, including nuclear (18S rRNA and 28S rRNA) and mitochondrial (16S rRNA and COI) markers of the Margelopsidae hydroid *Margelopsis haeckelii* Hartlaub, 1897, to resolve its phylogenetic position with respect to other hydrozoans. Our data provides strong evidence that *M. haeckelii* is a member of the family Corymorphydae, making the family Margelopsidae invalid. Furthermore, we show that medusa previously known as *M. hartlaubii* Browne, 1903 is sister to *Plotocnide borealis,* Wagner, 1885 and might be a member of Boreohydridae. The phylogenetic signal of polyp and medusa stages is discussed in light of concept of inconsistent evolution and molecular phylogenetic analysis.

Introduction

The species in the family Margelopsidae Mayer, 1910 (Aplanulata, Hydrozoa, Cnidaria) have an intriguing lifestyle unusual for the other Aplanulata. The family is exclusively represented by hydrozoans with holopelagic life-cycles, where medusae and solitary vasiform polyps float freely throughout the water column. Interestingly, siphonophore specialists used Margelopsidae species as a model to explain the origin of siphonophoran colonies (Totton and Bargmann, 1965). Margelopsidae is comprised of three genera; *Margelopsis* Hartlaub, 1897; *Pelagohydra*

Dendy, 1902; and *Climacocodon* Uchida, 1924, from which none of them have been sampled for comprehensive molecular analyses. Phylogenetic analysis using only COI sequences (Ortman et al, 2010) of *Margelopsis hartlaubii* Browne, 1903 suggested Margelopsidae is a sister group to the rest of the Aplanulata. However, authors did not recover strong support for this placement (Nawrocki et al., 2013). The systematics and phylogenetic position of Margelopsidae is solely based on insufficient morphological data. Given their polyp morphology, species of Margelopsidae show affinities towards Tubulariidae or Corymorphydae, but their unique medusa morphology justifies their original erection as a separate family. Thus, sampling with more DNA markers and specimens, including the type species, *Margelopsis haeckelii* is needed to determine the scope and phylogenetic position of the family Margelopsidae.

Recognizing the difficulties of sampling Margelopsidae hydroids, we were finally able to collect representatives of *Margelopsis haeckelii* Hartlaub, 1897 for molecular studies. *Margelopsis haeckelii* is the most studied species of its family, yet, documented collection records and morphological examinations have been very few (Hartlaub, 1897; Hartlaub, 1899; Lelloup, 1929; Werner; 1955, Schuchert, 2006). Polyps of *M. haeckelii* closely resemble tubulariid hydranths, having two whorls of tentacles but lacking both a hydrocaulus and stolonal system (Fig. 1, A, B). Free-swimming medusae develop from medusa buds located between whorls of polyp tentacles (Fig. 1, B, C). Eggs of *M. haeckelii* develop on the manubrium of the medusa (Fig. 1, D) and transform directly or through an encysted stage into a hydranth that never fixes to a substrate, exhibiting a continuous planktonic lifestyle (Werner; 1955). It is thought that eggs of this species are parthenogenetic, as no male gonads have ever been reliably documented.

In our study we obtained full-length sequences of 18S rRNA and 28S rRNA and partial sequences of the mitochondrial ribosomal 16S rRNA and cytochrome oxidase subunit I (COI) in order to phylogenetically place *M. haeckelii* within a comprehensive sampling of hydrozoan taxa. Using this approach, we provide the first molecular evidence that *M. haeckelii* should be placed within the family Corymorphydae. Our findings further showed that the previously sequenced *M. hartlaubii* is a relative of the family Boreohydridae, and is not related to *Margelopsis*.

Methods and materials

Maintaining of *Margelopsis haeckelii* in the laboratory

Cultures of *M. haeckelii* are reared and maintained throughout the year in aquaria using artificial sea water (salt Red Sea Coral Pro, salinity 30–32‰) in the Department of Embryology, Lomonosov Moscow State University, Russia, Moscow. For both polyp and medusa stages, *Artemia salina* nauplii, at least 3 days after hatching, were used for feeding. Animals were fed once a day. Cultures originated from individuals collected in the North Sea (51.2154° N, 2.9287° E). Polyps were collected with a planktonic net in the coastal area.

Identification of COI, 16S rRNA, 18S rRNA and 28S rRNA sequences.

Full-length 18S rRNA and 28S rRNA sequences were obtained from the reference transcriptome of *M. haeckelii* available in our laboratory. For transcriptome sequencing, total RNA was extracted from a mixture of various *Margelopsis* life and developmental stages: early cleavage, morula, gastrula, young polyps, mature polyps and mature medusa with oocytes. Total RNA extraction was conducted using the Zymo Research Quick-RNA MiniPrep Plus Kit according to the manufacturer's instructions. The RNA quality and concentration were determined using a NanoDrop2000 (ThermoScientific, USA). Poly-A RNA enrichment, cDNA library construction and sequencing were carried out at Evrogen (Russia). The cDNA library was sequenced using the Illumina NovaSeq 6000 SP flow cell to produce with 150-bp paired-end reads. Quality evaluation of the raw reads with sequencing adaptors and unknown nucleotides was conducted using FastQC v.0.11.5 (Andrews, 2010). Fastp (v.0.20.0) (Shifu et al., 2018) was applied to remove Illumina standard sequencing adapters, low quality bases and polyX

tails. The high-quality bases were employed for the *M. haeckelii* transcriptome assembly with the SPAdes assembler (v.3.13.1) (Bankevich et al., 2012). Assembly was conducted using default parameters.

Mitochondrial COI and 16S rRNA sequence fragments were amplified from genomic DNA of *M. haeckelii* using PCR methods. Genomic DNA was extracted using standard phenol/chloroform protocols. This method involved tissue digestion with proteinase K (20 mg/mL) in a lysis buffer (20 mM Tris-CL pH 8.0, 5 mM EDTA pH 8.0, 400 mM NaCl, 2%SDS), extraction with phenol/chloroform (1:1), precipitation with 0.1 vol 3M Sodium acetate and 1 vol. 100% Isopropanol and elution in mQ water. For amplification, we used the following primers pairs: 16SAR (TCGACTGTTTACCAAAAACATAGC) and 16SBR (ACGGAATGAACTCAAATCATGTAAG) for 16S rRNA (Cunningham and Buss, 1993); and jGLCO1490 (TGTAAAACGACGGCCAGTTNTCNACNAAYCAYAARGAYATTGG) and jGHCO2198 (CAGGAAACAGCTATGACTANACYTCNGGRTGNCCRAARAAYCA) for COI (Geller et al., 2013). Amplification programs used are as previously described (Prudkovsky et al., 2019).

Phylogenetic analyses

Nucleotide sequences were aligned using the MUSCLE algorithm in MUSCLE software (v3.8.31) (Edgar et al., 2004) and trimmed with the TrimAL tool (v.1.2rev59) (Capella-Gutiérrez et al., 2009). A heuristic approach “automated1” was used to select the best automatic method for trimming our alignments. The final alignments yielded fragments of 574, 617, 1778 and 3097 bp for the COI, 16S rRNA, 18S rRNA and 28S rRNA, respectively.

Phylogenetic analyses were performed using Maximum Likelihood methods in IQTree v.2.0-rc2 software (Minh, et al.,2020) according to the optimal models for each gene. Individual marker analyses and a concatenated gene analysis were performed. The best models of nucleotide substitution were chosen using ModelFinder (Kalyaanamoorthy et al., 2017). The GTR+F+I+G4 was found to be optimal for the COI dataset; GTR+F+I+G4 for 16S rRNA; TIM3+F+R3 for 18S rRNA; and TIM3+F+R5 for 28S rRNA. One thousand bootstrap replicates were generated for each individual analysis, as well as for the combined analysis.

The concatenated COI+16S+18S+28S alignment was constructed using Sequence Matrix (https://github.com/gaurav/taxondna). The concatenated dataset was analyzed using IQTree (v.2.0-rc2) with partitioned analysis for multi-gene alignments (Chernomor, et al., 2016). The set of selected species for concatenated analysis was chosen mainly according to Nawrocki et al. (2013) and considering the availability of individual gene sequences in GenBank for COI, 16S rRNA, 18S rRNA and 28S rRNA.

Trees were visualized in FigTree v1.4.4 and processed with Adobe Illustrator CC. No corrections were made to the tree topology or the branch lengths.

An approximately unbiased (AU) test (Hidetoshi, 2002) was performed using IQTree software for testing the phylogenetic hypotheses.

Data availability

Sequences obtained in this study have been deposited in GenBank under the following accession numbers: XXX, XXX, XXX, XXX

Results

Our phylogenetic investigation of Margelopsidae phylogeny was conducted employing Maximum likelihood analysis for all single gene datasets as well as our final concatenated four gene dataset (COI, 16S rRNA, 18S rRNA, 28S rRNA). All taxa used in our analysis are arranged taxonomically in Table 1. All *M. haeckelii* sequences (COI, 16S rRNA, 18S rRNA, 28S rRNA) were newly generated for this study. *M. hartlaubii* had previously only had COI available on GenBank (GQ120059.1) (Ortman et al., 2010). Maximum Likelihood bootstrapping (MLB) analysis of the concatenated dataset recovered a relatively well resolved tree and recovered Margelopsidae paraphyly. *M. hartlaubii* was recovered sister to *Plotocnide borealis* Wagner, 1885 (MLB=100), forming the clade that representative of the family Boreohydridae, a sister taxon to all other Aplanulata genera (MLB = 100) (Fig. 1, E). Individual COI analysis also recovered a strong supported affiliation of *M. hartlaubii* within Boreohydridae (MLB = 100) (Fig. 1S). At the same time, *M. haeckelii* nested within the clade of the Corymorphidae (MLB=75). This clade comprised to two subclades, well supported each, and one for genus *Euphysa*, including the type species *Euphysa aurata* Forbes, 1848*,* and the other for *Corymorpha* + *M. haeckelii,* including the type species, *Corymorpha nutans* M. Sars (Fig 1, E). *M. haeckelii* is nested inside the clade *Corymorpha bigelowi* Maas, 1905, *Corymorpha nutans* M. Sars, 1835, *Corymorpha sarsii* Steenstrup, 1855 and *Corymorpha pendula* L. Agassiz (MLB=75). Clade *Euphysa*+*Corymorpha*+*M.haeckelii* was recovered to be the sister to Tubulariidae, which together with *Branchiocerianthus imperator* Allman, 1885 constitute the superfamily Tubularioidea. Tubularioidea is recovered as sister to Hydridae (MLB=75). General topology of our phylogenetic tree obtained in combined analysis coincides with the Aplanulata tree published by Nawrocki et al., 2013.

We suspected that the grouping of *M. hartlaubii* and *P. borealis* in one clade could be result of a long-branch attraction artefact (LBA). LBA is a form of systematic error whereby distantly related lineages are incorrectly inferred to be closely related. To investigate the possible role of LBA on *M. hartlaubii* placement we removed *P. borealis* from the combined Maximum Likelihood analyses. We have found it did not affect the placement of *M. hartlaubii* at the base of Aplanaulata (not shown). The placement of *P. borealis* was also not affected by the removal of *M. hartlaubii* from the analysis (not shown). These results indicates that the association of *M. hartlaubii* and *P. borealis* in one clade in the root of Aplanulata tree is not an artifact of long-branch attraction.

Phylogenetic hypothesis testing (AU test) were performed to test the statistical significance of tree topologies in our Maximum Likelihood analysis. The AU test rejected the phylogenetic hypothesis of the monophyly of *M. haeckelii* and *M. harlaubii,* what means a polyphyly of *Margelopsis*. Also, as our two individual marker analyses (16S and 28S) (Supp. 2, 3) placed *M. haeckelii* as a sister to *Corymorpha,* two hypotheses of alternative placements of *M. haeckelii* were evaluated: *M. haeckelii* is inside or outside *Corymorpha*. Results of the testing significantly support ($p < 0.05$) the hypothesis of *M. hackelii* placement inside the Corymorpha, between *Corymorpha bigelowi Maas, 1905*, and *Corymorpha pendula* L. Agassiz. (Fig. 5S).

Discussion

Our concatenated dataset (COI+16S+18S+28S), which included a comprehensive taxonomic sampling of hydrozoans, recovered *Margelopsis haeckelii* within Corymorphidae, nested within a clade consisting of several *Corymopha* species. This result is consistent with previous findings based solely on polyp morphology, where Margelopsidae was grouped with Tubulariidae and Corymorphidae in the superfamily Tubularoidea (Rees, 1957). Being quite small (1-2 mm), hydrocaulus-lacking pelagic polyps of the Margelopsidae are similar to those sessile polyps of Corymophids and Tubulariids despite the latter having a well-developed hydrocaulus and reaching up to ten centimeters in height. Among these three families, hydranth tentacles arranged into two, oral and aboral whorls and blastostyles are situated in the inter-tentacular

region (Fig. 2, A, C). Our phylogenetic data suggests that polyp tentacle patterns may be an important morphological character for identifying lineages in Aplanulata (Rees, 1957, Nawrocki et al. 2013).

Interestingly, *M. haeckelii* jellyfish are atypical in having radial symmetry, which more usually is bilateral in Aplanulata. The *M. haeckelii* jellyfish has 3-4 tentacles per bulb instead of one long tentacle per medusa, something typically seen among *Corymorpha* medusae. Even in the *Euphysa*, the sister group to *Corymorpha*, radially symmetric adult medusa develop asymmetrically in contrast to medusae of *M. haeckelii*. The medusae of *Euphysa flammea* Hartlaub, 1902 only have a single tentacle in their youngest stage, with a second, third and fourth being added successively over time (Schuchert, 2010). Radially symmetric medusa is inherent in the species *P. borealis,* which is deeply nested in our phylogenetic analyses of Aplanulata (Pyataeva et al., 2016; this study). Appearance of radial symmetry in *M. haeckelii* may indicate the manifestation of the original body plan of medusa for Aplanulata. The presence of an apical canal in the umbrella may be a phylogenetically significant character warranting further investigation, as this character is shared both by *M. haeckelii* and all *Corymorpha* medusae (Fig. 2, A, C, marked orange). Reproductive characters appear to also be important in determining phylogenetic relationships in Aplanulata. Among all of Tubularoidea, only *Corymorpha* embryos undergo encystment similar to that of *M. haeckelii* (Petersen, 1990).

Surprisingly, our concatenated gene dataset, as well as our single gene COI dataset, recovered the medusa previously known as *M. hartlaubii* to be a close relative of *P. borealis,* and not closely related to *M. haeckelii* nor group within the Corymorphidae. This result is further supported by independent morphological data showing several similarities between medusae of *M. hartlaubii* and *P. borealis,* including thick apical mesoglea of the bell (Fig. 2, marked blue), lack of an umbrella apical canal and nematocyst batteries being located at the distal parts of tentacles (Fig. 2, marked violet) (Schuchert, 2006). Based on our findings, medusa described by Browne (1903) have been wrongly attributed to the genus *Margelopsis*. The affiliation of *M. hartlaubii* with *P. borealis* within the family Boreohydridae solves the former problematic placement of *M. hartlaubii* as a sister species to the rest of Aplanulata, albeit suggested to be a relative of Tubulariidae or Corymorphydae (Nawrocki et al., 2013).

In addition to *M. haeckelii* and *M. hartlaubii,* there are several other suspected species in the genus *Margelopsis,* including *Margelopsis gibbesii* (McCrady, 1859) and *Margelopsis australis* Browne, 1910. However, it is not clear if they are valid species. Unrelated margelopsid polyp and bougainvilliid jellyfish were described under the name *Nemopsis gibbesii*, generating subsequent taxonomic confusion. Polyp of *Nemopsis gibbesii* is supposed to be a *Margelopsis gibbesii* (Calder and Johnson, 2015), but according to World Register of Marine Species this is invalid name. This margelopsid polyp is distributed along the Atlantic Coast of North America and still has an unclear taxonomic status. There are no distinct morphological differences between this polyp, from the western North Atlantic, and *M. haeckelii* from the eastern North Atlantic to state with confidence that they are different species. More biological details and molecular data between the two species are needed in order to resolve this taxonomic question (Schuchert, 2006; Calder and Johnson, 2015). *Margelopsis australis* is only known from its original collection and is based on a single medusa specimen, lacking reliable characters for distinguishing it from *M. hartlaubii* (Browne 1910). Moreover, the single specimen was described as being “somewhat contracted and in a crumbled condition” (Browne 1910). Based on the available morphological data, we cannot state with any degree of certainty that *M. australis* is a valid species, or that it is a member of the *Margelopsis*.

Medusae are a useful means of identifying species, genera and even family ranks (Rees, 1957; Bouillon, et al., 2006). A change in morphology of the typical jellyfish form within a family is usually due to the reduction of the medusa stage, something that is widespread throughout the Anthoathecata and Leptothecata hydrozoans (Cornelius, 1992; Leclere et al., 2009; Cartwright, Nawrocki, 2010). However, *M. haeckelii* is a normally developed medsua, very different from

those typical of *Corymorpha*, despite their close relationship recovered by our phylogenetic analysis. Recent studies using molecular phylogenetic methods have revealed several such cases in which related taxa have very different jellyfishes or species with similar jellyfishes are unrelated. The morphologically aberrant jellyfish *Obelia* is so different from other Companulariidae that a hypothesis was proposed for the re-expression of this jellyfish after its evolutionary reduction (Boero, Sara, 1987). However, this hypothesis was not supported by molecular phylogenetic analysis and *Obelia* may have originated from a *Clytia*-like ancestor (Govindarajan et al., 2006; Leclere et al., 2019). *Larsonia pterophylla* (Haeckel, 1879) was previously assigned to the genus *Stomotoca* due to similarity of their jellyfishes (Larson, 1982). Interestingly, the structure of the polyps in genera *Larsonia* and *Stomotoca* are so dissimilar that they could be attributed to different families (Boero, Bouillon, 1989). According to molecular data, *L. pterophylla* and *Stomotoca atra* L. Agassiz, 1862 are indeed unrelated. Hydroid *L. pterophylla* is closely related to hydroid *Hydrichthys boycei* from the Pandeidae family, and *S. atra* is ungrouped with most species (Schuchert, 2018; Woodstock et al., 2019). Inclusion of the genus *Cytaeis* in Bougainvilliidae or the genera *Polyorchis* and *Scrippsia* in Corynidae is surprising due to the discrepancy between the jellyfishes of these genera and those typical of families (Nawrocki, Cartwright, 2010; Prudkovsky et al., 2017). Finally, we conclude that appearance of atypical jellyfishes in the hydrozoan families can indicate a great evolutionary plasticity of the medusa stage morphology. In contrast, the morphology of the hydroids turns out to be more phylogenetically constant. For example, the morphology of *Cytaeis* hydroid is similar to the structure of Bougainvillidae hydroids with stolonal colonies, and Obelia-like polyps are typical for the family Campanulariidae (Prudkovsky et al., 2017; Leclere et al., 2019).

Concepts of 'mosaic' or 'inconsistent evolution' was proposed for these cases in which closely related hydroids can produce very different medusae or vice versa (Naumov 1956, 1960; Rees, 1957). Inconsistent evolution was explained by differences in rate and direction of evolution in the two stage of life cycle. Some incongruences between hydroid and medusa systems seems were the result of weaknesses in the classification systems (Petersen, 1990). But we found a new reason to return to the discussion of this concept.

Taxonomic recommendations

Based on our results, as well as a number of previous studies, we formally recommend the following changes to the taxonomy of Margelopsidae and its component species:

a) As multigene phylogenetic analyses nested *Margelopsis haeckelii,* the type species of *Margelopsis,* within Corymorphidae, we suggest moving *Margelopsis haeckelii* into Corymorphidae.
b) We suggest moving *Margelopsis hartlaubii* into Boreohydridae family but leave it in *Margelopsis* for the moment until additional molecular markers will be available. The phylogenetic distance to *Plotocnide borealis* is too long to redesignate *Margelopsis hartlaubii* into *Plotocnide hartlaubii.*
c) We suggest that Margelopsidae should no longer be used, and both *Pelagohydra* and *Climacocodon* should be moved to within the Aplanulata incertae sedis until additional molecular phylogenetic analyses can clarify their phylogenetic placement.

Conclusion

Our results present a more inclusive phylogenetic picture of Aplanulata, by further revealing the phylogenetic position of *M. haeckelii.* Although previous molecular phylogenetic results conflicted with the century old hypothesis that *Margelopsis* belongs to Tubulariidae or Corymorphidae lineages (Nawrocki et al., 2013), our investigation presents strong evidence in support of this traditional hypothesis. Our results suggest that *M. haeckelii* is a hydrozoan belonging to Corymorphidae, having lost their hydrocaulus and stolonal systems over the course of evolution, adapting to a holopelagic life-cycle. It was previously suggested that the

foundation for this type of changes in body plan, and accompanying life-style, might lead to speciation and could be reflected by changes in the expression of Wnt signaling components (Duffy, 2011). Based on our results, *M. haeckelii* might be a prime candidate for testing this hypothesis.

Unfortunately, due to the few and extremely irregular documented collection records of the hydroids from the supposedly sister genera *Margelopsis*, *Pelagohydra* and *Climacocodon*, the phylogenetic relationships within this group are still obscured. It remains unclear if this group is monophyletic or if the origin of a secondarily specialized pelagic polyp stage has occurred several times independently. Thus, the possible relationships between these three genera, as well as their phylogenetic placement, still need to be verified by additional studies when molecular data become available.

Acknowledgements

This study was supported by federal project 0108-2019-0003 of the Koltzov Institute of Developmental Biology of the Russian Academy of Sciences.

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Figure legends

Fig. 1. Morphology of *Margelopsis haeckelii* Hartlaub, 1897 and analyses of its phylogenetic position in Aplanulata. (A) Newly hatched *Margelopsis haeckelii* polyp, (B) Mature polyp with medusa buds, (C) Developing medusae buds and young medusae, (D) Mature medusa. (E) Phylogenetic hypothesis of *Margelopsis haeckelii* relationships based on the combined mitochondrial and nuclear dataset (CO1+16S +18S+28S). Node values indicate bootstrap support from 1000 replicates. *Margelopsis haeckelii* and *Margelopsis hartlaubii* are in red and underlined. Abbreviations, ac – apical canal, at – aboral tentacles, e – embryos, h – hypostome, mb – medusa bud, md – medusoid ot – oral tentacles, tb – tentacle bulb.

Fig. 2. Comparison of morphological characters of (A) *Margelopsis hartlaubii*, (B) *Margelopsis haeckelii*, (C) *Corymorpha nutans* and (D) *Plotocnide borealis*. Scalebar – 0.4 mm. Color coding: yellow – oral and aboral whorls of polyp tentacles, pink– region of medusa budding, green – the region of gametes formation, orange – apical canal, blue – medusa umbrella with clusters of exumbrellar nematoblasts, violet – clusters of nematocysts located at the distal parts of tentacles. *Margelopsis hartlaubii, Margelopsis haeckelii, Corymorpha nutans* and *Plotocnide borealis* modified from Schuchert (2006; 2010)

Fig. 1S. Phylogenetic hypothesis of *Margelopsis haeckelii* and *Margelopsis hartlaubii* relationships based on nuclear cytochrome oxidase subunit I (COI). Node values indicate bootstrap support from 1000 replicates. Red arrows indicate *Margelopsis haeckelii* and *Margelopsis hartlaubii* placement. *Margelopsis haeckelii* and *Margelopsis hartlaubii* are in red and underlined.

Fig. 2S. Phylogenetic hypothesis of *Margelopsis haeckelii* and *Margelopsis hartlaubii* relationships based on the mitochondrial 16S rRNA. Node values indicate bootstrap support from 1000 replicates. Red arrow indicates *Margelopsis haeckelii* placement. *Margelopsis haeckelii* is in red and underlined.

Fig. 3S. Phylogenetic hypothesis of *Margelopsis haeckelii* and *Margelopsis hartlaubii* relationships based on the 28S rRNA large ribosomal subunit. Node values indicate bootstrap support from 1000 replicates. Red arrow indicates *Margelopsis haeckelii* placement. *Margelopsis haeckelii* is in red and underlined.

Fig. 4S. Phylogenetic hypothesis of *Margelopsis haeckelii* and *Margelopsis hartlaubii* relationships based on the 18S rRNA small ribosomal subunit. Node values indicate bootstrap support from 1000 replicates. Red arrow indicates *Margelopsis haeckelii* placement. *Margelopsis haeckelii* is in red and underlined.

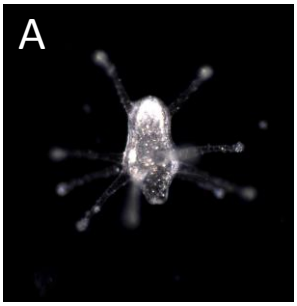
Fig. 5S. Testing of the phylogenetic hypotheses with AU test.

Table 1. List of the species included in the study and corresponding GenBank accession numbers of all analyzed sequences.

suborder	**family**	**species**	**16S rRNA**	**18S rRNA**	**28S rRNA**	**COI**	**vouchers**
Aplanulata	Boreohydridae	*Plotocnide borealis*	KU721822.1	KU721833.1		KU721812.1	RU087.2
	Candelabridae	*Candelabrum cocksii*	EU876535.1	AY920758.1	AY920796.1	GU812438.1	MHNGINVE29591
	Corymorphyda e	*Branchioceriant hus imperator*		JN594046.2	JN594035.2	JX121580.1	MHNG:INVE 74105
		Corymorpha bigelowi	EU448099	EU876564.1	EU272563.1	JX121581.1	KUNHM 2829
		Corymorpha nutans	EU876532.1	EU876558.1	EU879931.1	JX121586.1	MHNG:INVE 48745
		Corymorpha pendula	EU876538.1	EU876565.1	EU305510.1	JX121583.1	KUNHM DIZ2962
		Corymorpha sarsii	KP776787.1	JN594049.2	JN594038.2	JX121585.1	MHNG:INVE 68950
		Euphysa aurata	EU876536.1	EU876562.1	EU879934.1	JX121587.1	MHNG:INVE 48753
		Euphysa intermedia	EU876531.1	AY920759.1	EU879930.1	JX121582.1	
		Euphysa japonica	KP776802.1	EU301605.1	JX122505.1	MF000498.1	
		Euphysa tentaculata	EU876537.1	EU876563.1	EU879935.1	JX121588.1	
		Hataia parva	JN594033.1	JN594045.2	JN594034.2	JX121608.1	UF:5407
	Hydridae	*Hydra hymanae*	GU722762.1	JN594051.2	JN594040.2	GU722849.1	
		Hydra oligactis		JN594052.2	JN594041.2	GU722871.1	
		Hydra utahensis		JN594053.2	JN594042.2	GU722861.1	
		Hydra vulgaris	EU876543.1	JN594054.2	JN594043.2	GU722914.1	
		Hydra viridissima		EU876569.1	EU879940.1	GU722845.1	
	Margelopsidae	*Margelopsis haeckelii*	XXXXXX	XXXXXX	XXXXXX	XXXXXXX	
		Margelopsis hartlaubi				GQ120059.1	

	Protohydridae	*Protohydra leuckarti*	KU721828.1	KU721835.1		KU721813.1	Protohydra20100727.6
	Tubuldariidae	*Ectopleura crocea*	EU876533.1	KF699111.1	EU879932.1	JX121589.1	MHNG:INVE 34010
		Ectopleura dumortierii	FN687542.1	EU876561.1	EU879933.1	JX121590.1	
		Ectopleura larynx		EU876572.1	EU879943.1	JX121591.1	MHNG-INVE-54563
		Ectopleura marina	EU883542.1	EU883547.1	EU883553.1	JX121592.1	
		Ectopleura wrighti	FN687541.1	JN594055.2	JN594044.2	JX121593.1	MHNG:INVE 27331
		Hybocodon chilensis	EU876539.1	EU876566.1	EU879937.1	JX121594.1	MHNG:INVE 36023
		Hybocodon prolifer	FN687544.1	EU876567.1	EU879938.1	JX121595.1	
		Hydractinia sp	EU305477.1	EU305495.1	EU305518.1		KUNHM2876
		Ralpharia gorgoniae	EU305482.1	EU272633.1	EU272590.1	GU812437.1	KUNHM2778
		Tubularia indivisa	FN687530.1	EU876571.1	EU879942.1	JX121596.1	
		Zyzzyzus warreni	EU305489.1	EU272640.1	EU272599.1	JX121597.1	KUNHM 2777
Capitata	Asyncorynidae	*Asyncoryne ryniensis*	EU876552.1	EU876578.1	GQ424289.1		KUNHM 2639
	Cladocorynidae	*Cladocoryne floccosa*	AY512535.1	EU272608.1	EU272551.1		personal:A. Lindner:AL1407
	Cladonematidae	*Staurocladia vallentini*	GQ395332.1	GQ424322.1	GQ424293.1	MF000500.1	Sch522
		Staurocladia wellingtoni	AY787882.1	GQ424323.1	EU879948.1	MF000486.1	
	Corynidae	*Coryne uchidai*	GQ395319.1	GQ424332.1	GQ424305.1	KT981912.1	
		Sarsia tubulosa	EU876548.1	EU876574.1	EU879946.1		MHNGINV35763
		Stauridiosarsia ophiogaster	EU305473.1	EU272615.1	EU272560.1		KUNHM 2803
	Moerisiidae	*Odessia maeotica*	GQ395324.1	GQ424341.1	GQ424314.1		MHNG INVE53642
	Pennariidae	*Pennaria disticha*	AM088481.1	GQ424342.1	GQ424316.1		MHNG INVE29809
	Porpitidae	*Porpita porpita*	AY935322.	GQ424319	EU883551	LT795124.1	RM3_747

			1	.1	.1		
	Solanderiidae	*Solanderia secunda*	EU305484.1	AJ133506.1	EU305533.1	JX121599.1	KUNHM 2611
	Zancleidae	*Zanclea costata*	EU876553.1	EU876579.1	EU879951.1		MHNGINV26507
		Zanclea prolifera	EU305488.1	EU272639.1	EU272598.1		KUNHM 2793
Fillifera	Eudendriidae	*Eudendrium capillare*	AY787884.1		EU305514.1	JX121602.1	KUNHM2625
	Proboscidactylidae	*Proboscidactyla flavicirrata*	EU305480.1	EU305500.1	EU305527.1	JX121600.1	USNM:1074994
	Ptilocodiidae	*Hydrichthella epigorgia*	EU305478.1	EU272622.1	EU272569.1	JX121601.1	KUNHM 2665
	Stylasteridae	*Lepidopora microstylus*	EU645329.1	EU272644.1	EU272572.1	JX121603.1	USNM:1027724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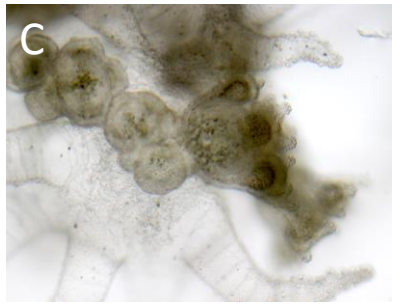
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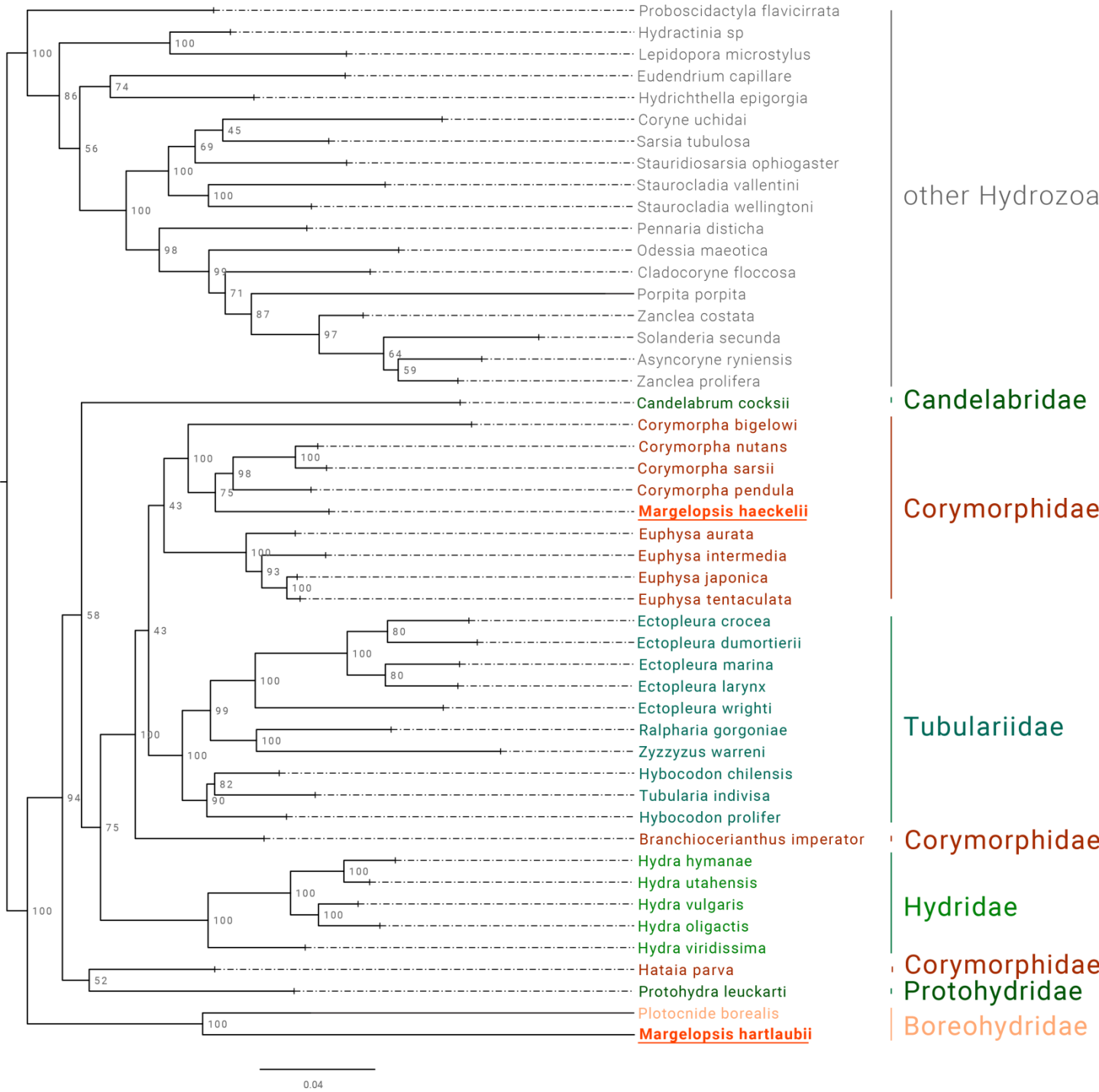
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Coryne uchidai
Sarsia tubulosa
Stauridiosarsia ophiogaster
Staurocladia vallentini
Staurocladia wellingtoni
Pennaria disticha
Odessia maeotica
Cladocoryne floccosa
Porpita porpita
Zanclea costata
Solanderia secunda
Asyncoryne ryniensis
Zanclea prolifera
Candelabrum cocksii
Corymorpha bigelowi
Corymorpha nutans
Corymorpha sarsii
Corymorpha pendula
Margelopsis haeckelii
Euphysa aurata
Euphysa intermedia
Euphysa japonica
Euphysa tentaculata
Ectopleura crocea
Ectopleura dumortierii
Ectopleura marina
Ectopleura larynx
Ectopleura wrighti
Ralpharia gorgoniae
Zyzzyzus warreni
Hybocodon chilensis
Tubularia indivisa
Hybocodon prolifer
Branchiocerianthus imperator
Hydra hymanae
Hydra utahensis
Hydra vulgaris
Hydra oligactis
Hydra viridissima
Hataia parva
Protohydra leuckarti
Plotocnide borealis
Margelopsis hartlaubii
0.04
other Hydrozoa
Candelabridae
Corymorphidae
Tubulariidae
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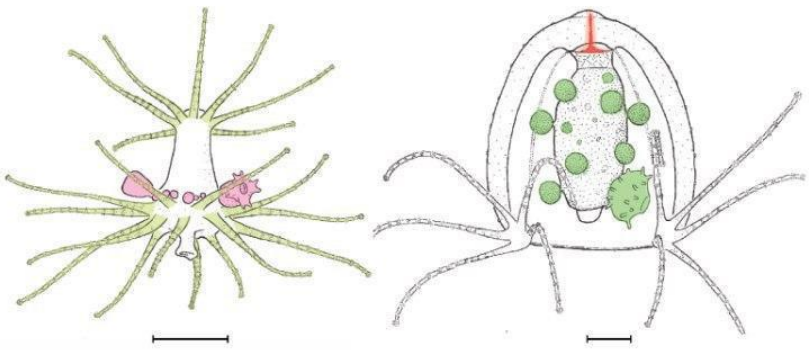
Protohydridae
Boreohydridae

previously fam. Margelopsidae

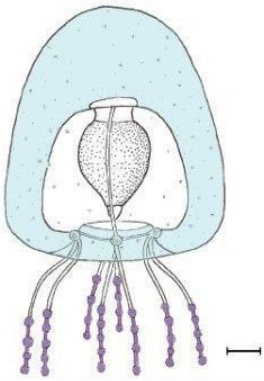
fam. Corymorphidae

Margelopsis haeckelii



fam. Boreohydridae

Margelopsis hartlaubii



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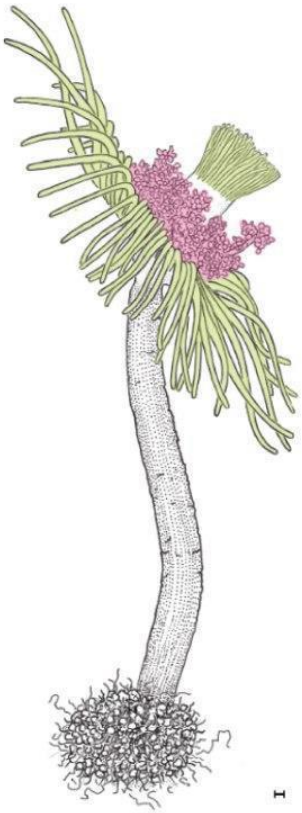
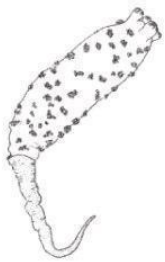


fam.Corymorphidae

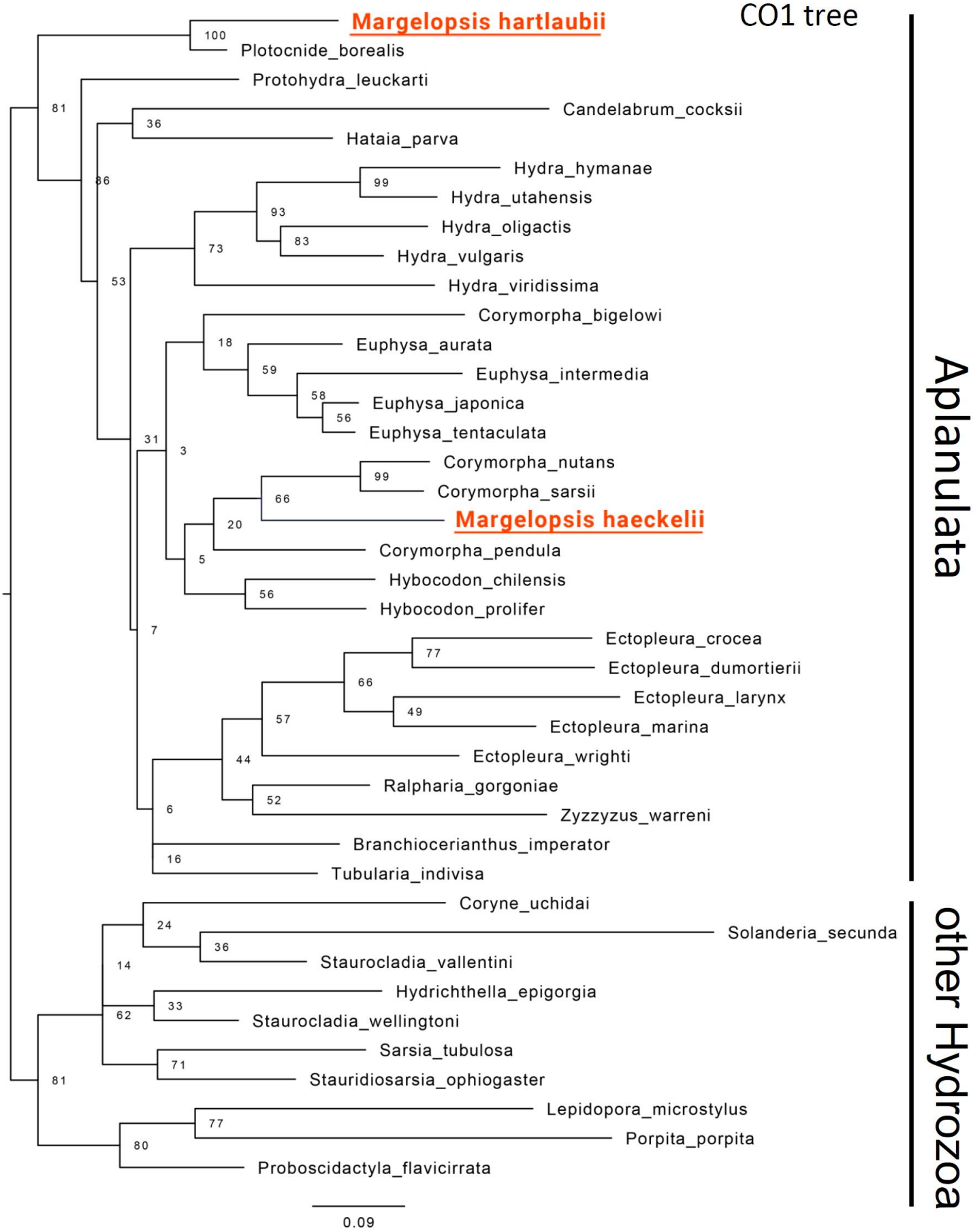
Corymorpha nutans



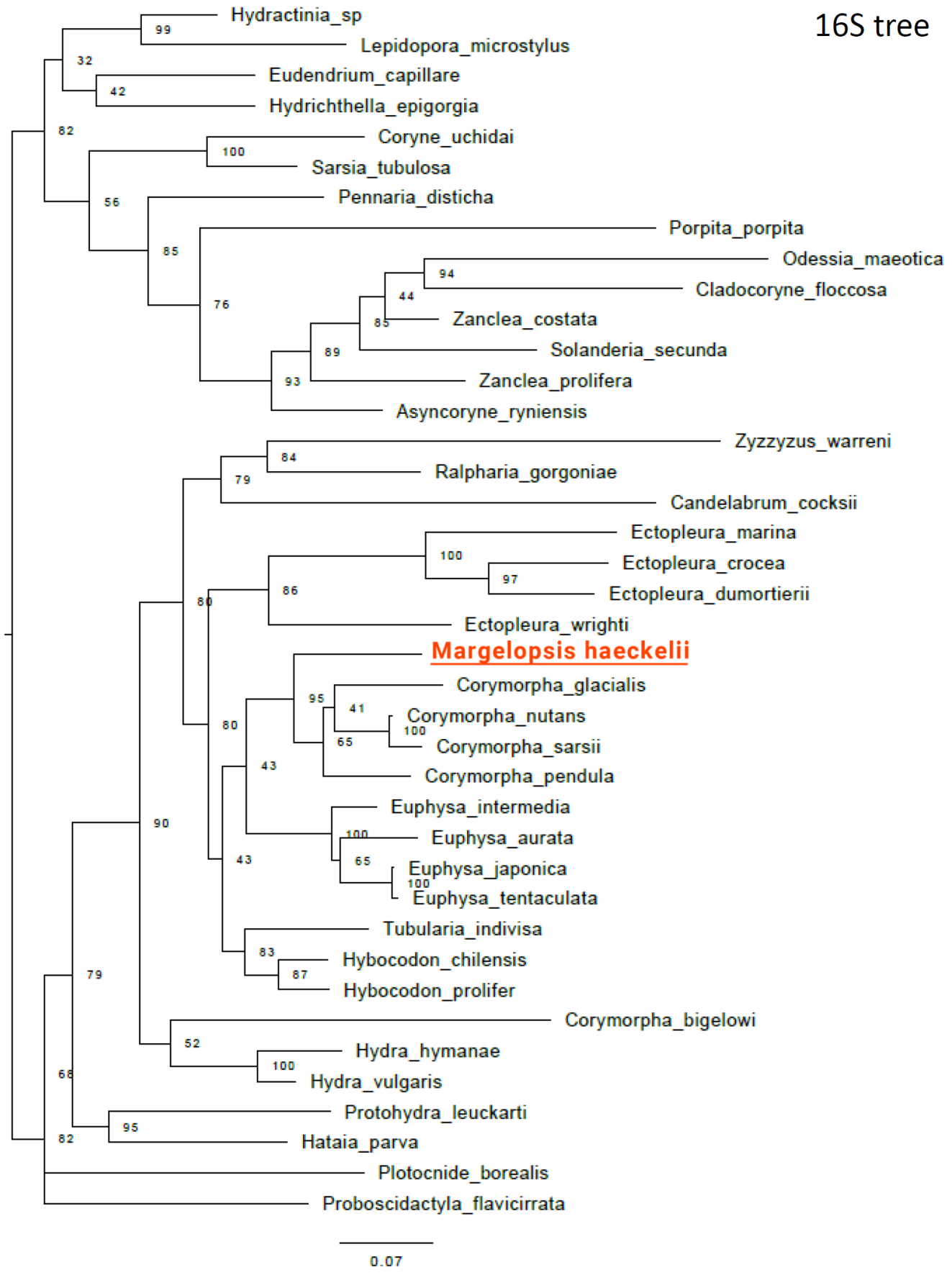
fam. Boreohydridae

Plotocnide borealis

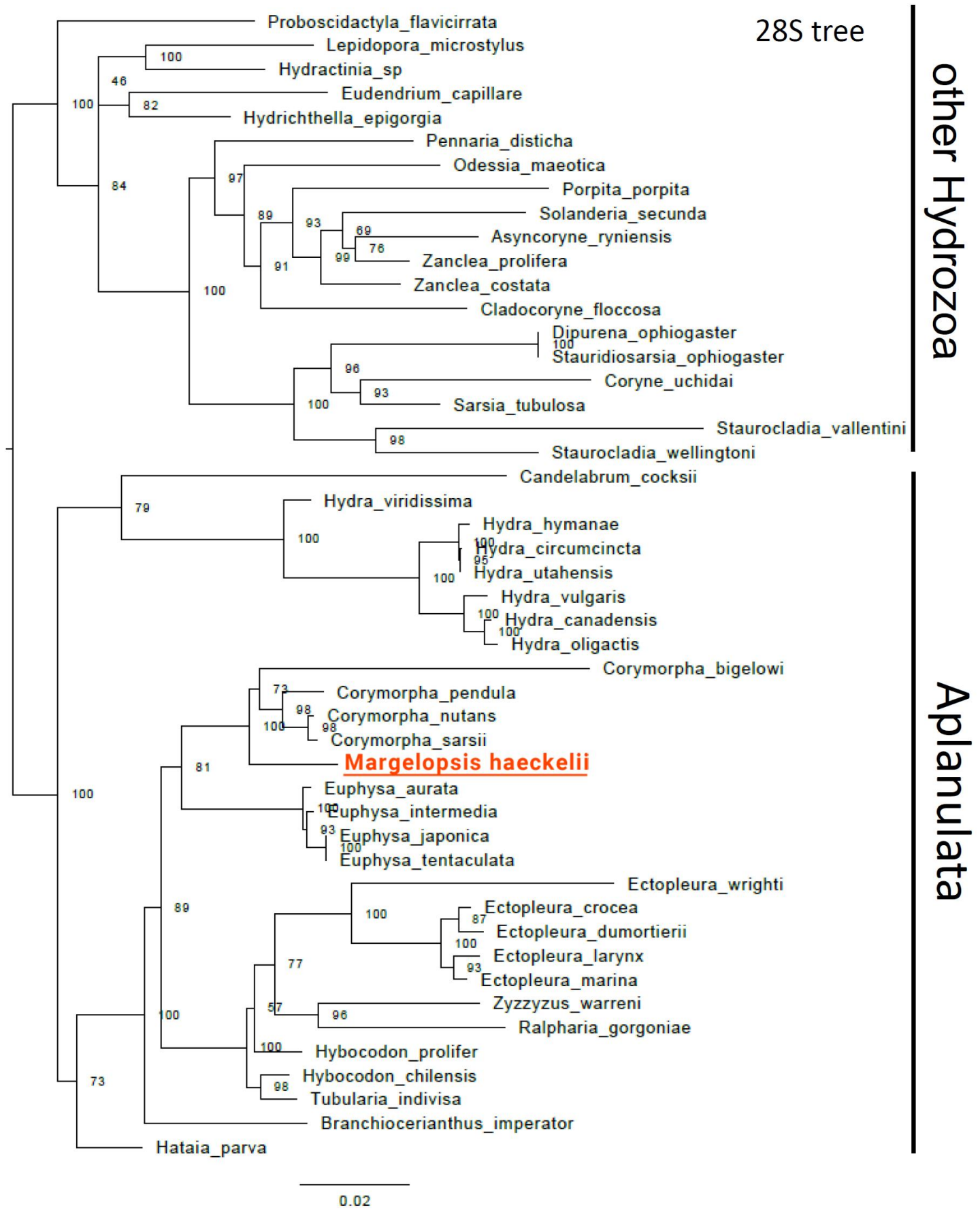
Supplementary figure 1



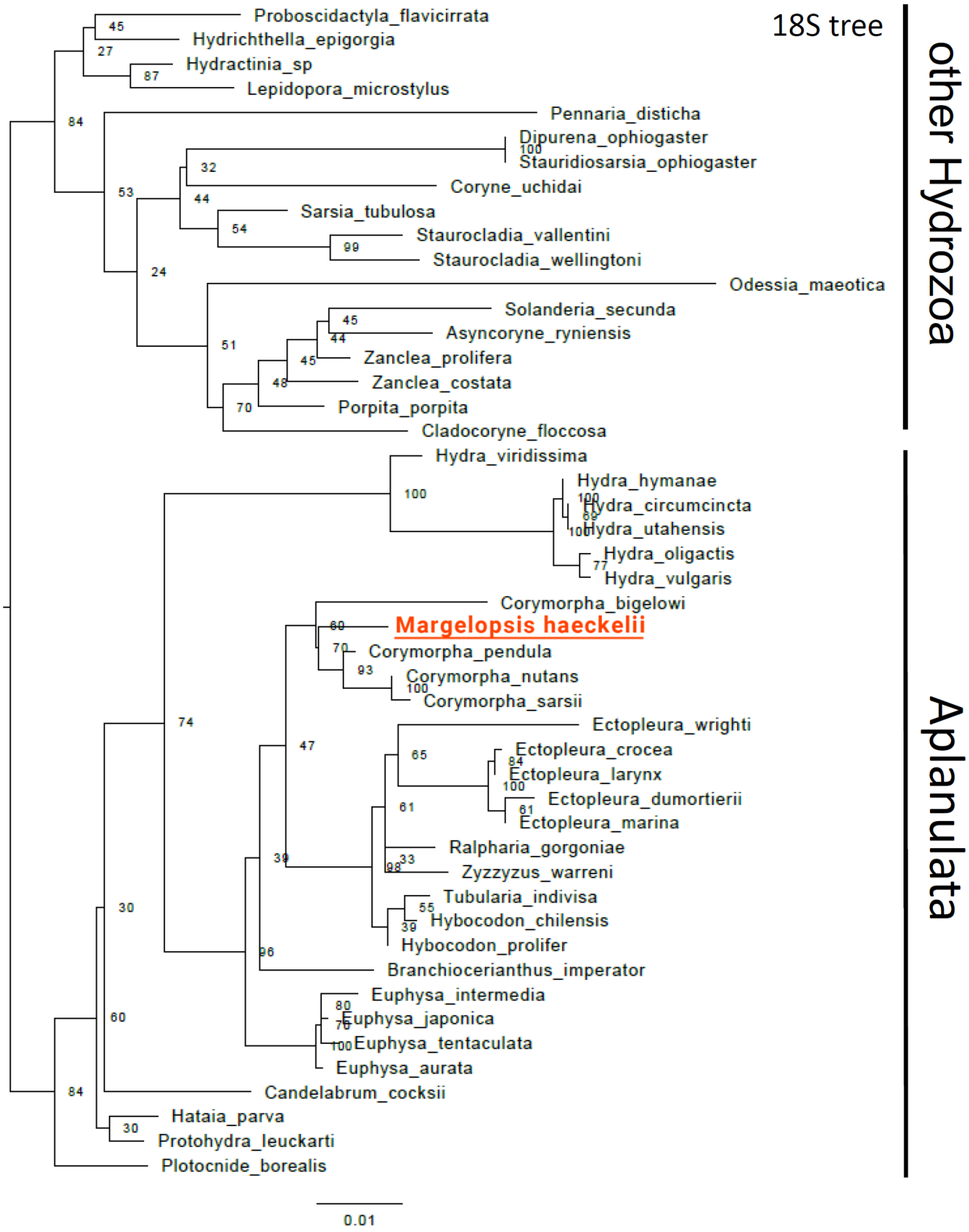
Supplementary figure 2



Supplementary figure 3



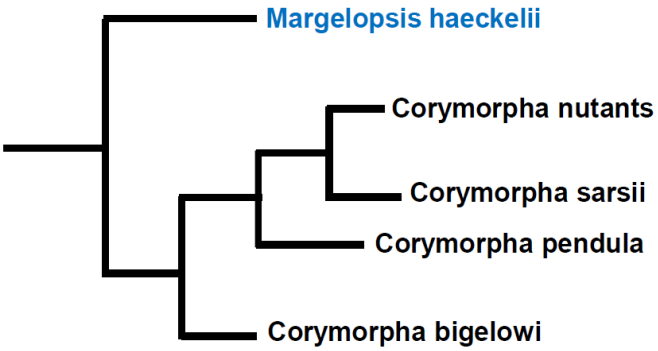
Supplementary figure 4



Supplementary figure 5

Phylogenetic hypothesis #1

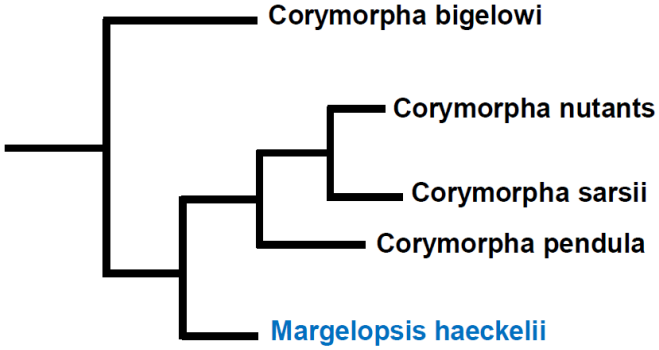
Margelopsis haeckelii is a sister to the genus Corymorpha, AU test



Rejected, p > 0.05

Phylogenetic hypothesis #2

Margelopsis haeckelii nested within the genus Corymorpha, AU test



Supported, p < 0.05