

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

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22

23 **Abstract**

24 The life-cycle and polyp morphology of representatives of Margelopsidae are very different from
25 all other species in the hydrozoan clade Aplanulata. Their evolutionary origin and phylogenetic
26 position has been the subject of significant speculation. A recent molecular study based only on
27 COI data placed Margelopsidae as a sister group to all Aplanulata, an unexpected result because
28 margelopsid morphology suggests affiliation with Tubulariidae or Corymorphidae. Here we used
29 multigene analyses, including nuclear (18S rRNA and 28S rRNA) and mitochondrial (16S rRNA
30 and COI) markers of the hydroid stage of the margelopsid species *Margelopsis haeckelii* Hartlaub,
31 1897 and the medusa stage of *Margelopsis hartlaubii* Browne, 1903 to resolve their phylogenetic
32 position with respect to other hydrozoans. Our data provide strong evidence that *M. haeckelii*, the
33 type species of *Margelopsis*, is a member of the family Corymorphidae. In contrast, *M. hartlaubii*
34 Browne, 1903 is sister to *Plotocnide borealis* Wagner, 1885, a member of Boreohydridae. These
35 results invalidate the family Margelopsidae. The phylogenetic signal of polyp and medusa stages
36 is discussed in light of concept of inconsistent evolution and molecular phylogenetic analysis.

37

38 **Introduction**

39 Species in the family Margelopsidae Mayer, 1910 (Aplanulata, Hydrozoa, Cnidaria) have
40 intriguing life histories. The family is exclusively represented by hydrozoans with holopelagic life-

41 cycles, where medusae and solitary vasisform polyps float freely throughout the water column.
42 Interestingly, siphonophore specialists used margelopsid species as a model to explain the origin
43 of siphonophoran colonies (Totton and Bargmann, 1965). Margelopsidae is comprised of three
44 genera; *Margelopsis* Hartlaub, 1897; *Pelagohydra* Dendy, 1902; and *Climacocodon* Uchida,
45 1924, none of which have been sampled for comprehensive molecular analyses. Phylogenetic
46 analysis using only COI sequences (Ortman et al, 2010) of *Margelopsis hartlaubii* Browne, 1903
47 suggested that Margelopsidae might be the sister group to the rest of Aplanulata. However,
48 authors have not recovered strong support for this placement (Nawrocki et al., 2013). The
49 systematics and phylogenetic position of Margelopsidae is solely based on insufficient
50 morphological data. Given their polyp morphology, species of Margelopsidae show affinities with
51 Tubulariidae or Corymorphyidae, but their unique medusa morphology was used to justify their
52 original erection as a separate family. Thus, sampling with more DNA markers and specimens –
53 especially including the type species *Margelopsis haeckelii* – has been needed to determine the
54 scope and phylogenetic position of the family Margelopsidae.

55 Despite difficulties of sampling margelopsid hydroids, we were finally able to collect
56 representatives of *Margelopsis haeckelii* Hartlaub, 1897 and *Margelopsis hartlaubii* Browne, 1903
57 for molecular studies. *Margelopsis haeckelii* is the most studied species of its family, yet,
58 documented collection records and morphological examinations have been very few (Hartlaub,
59 1897; Hartlaub, 1899; Lelloup, 1929; Werner; 1955, Schuchert, 2006). Polyps of *M. haeckelii*
60 closely resemble tubulariid hydranths, having two whorls of tentacles but lacking both a
61 hydrocaulus and stolonial system (Fig. 1, A, B). Free-swimming medusae develop from medusa
62 buds located between whorls of polyp tentacles (Fig. 1, B, C, D). Eggs of *M. haeckelii* develop on
63 the manubrium of the medusa (Fig. 1, C, D) and transform directly or through an encysted stage
64 into a hydranth that never fixes to a substrate, exhibiting a continuous planktonic lifestyle (Werner;
65 1955). It is thought that eggs of this species are parthenogenetic, as no male gonads have ever
66 been reliably documented. There is less information about *M. hartlaubii*, which is only known from
67 the medusa stage. The medusa of *M. hartlaubii* can readily be distinguished from the medusa of
68 *M. haeckelii* by its thick apical mesoglea of the bell without apical canal and two tentacles per bulb
69 (Fig. 1, C, D, E) (Schuchert, 2006).

70 In our study we obtained full-length sequences of 18S rRNA and 28S rRNA and partial sequences
71 of the mitochondrial ribosomal 16S rRNA and cytochrome oxidase subunit I (COI) in order to
72 phylogenetically place *M. haeckelii* and *M. hartlaubii* within as comprehensive sampling of
73 hydrozoan taxa as possible. Using this approach, we provide the first molecular evidence that *M.*
74 *haeckelii* should be placed within the family Corymorphyidae. Our findings further showed that the
75 previously sequenced *M. hartlaubii* is a relative of the family Boreohydridae, and is only distantly
76 related to *Margelopsis haeckelii*, the type species of the genus.

77 **Methods and materials**

78 **Animal sampling.**

79 Some *M. haeckelii* polyps were collected in the North Sea (loc. Belgium, Ostend, 51.218028°,
80 2.879417°) (Fig. 1, F, J). Polyps were collected with a plankton net in the coastal area. Collected
81 animals were used to set up a lab culture. The obtained culture was maintained throughout the
82 year in aquaria using artificial sea water (salt Red Sea Coral Pro, salinity 30–32‰) at the
83 Department of Embryology, Lomonosov Moscow State University, Russia, Moscow. For both
84 polyp and medusa stages, *Artemia salina* nauplii, at least 3 days after hatching, were used for
85 feeding. Animals were fed once a day.

86 Also, *M. haeckelii* medusae were collected in the Atlantic Ocean, Atlantic Coast of North America
87 (loc. USA, New York, 40.560556°, -73.882333°). Medusae were collected with a plankton net in
88 the coastal area, about 10 meters out from the shore. Collected animals were fixed and stored in
89 96% ethanol (Fig. 1, F, J).

90 *M. hartlaubii* DNA was a gift from Dr. Peter Schuchert (Schuchert, 2022). The medusa was
91 collected in Norway, Raunefjord (60.2575°, 05.1393°) with a plankton net from 200 to 0 m depth
92 on 14-JUN-2016.

93 Meiobenthic polyps of *Plotocnide borealis* (formerly known as *Boreohydra simplex*; Pyataeva et
94 al., 2016) were collected in the White Sea near the N.A. Pertsov White Sea Biological Station of
95 the Moscow State University, Kandalaksha Bay, Russia (66.528056°, 33.185556°). Fine mud with
96 polyps was collected with a light hyperbenthic dredge from depth 20-40 m. Collected individuals
97 were fixed and stored in 96% ethanol.

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

99 COI, 16S rRNA, 18S rRNA and 28S rRNA sequence fragments were amplified from genomic
100 DNA using PCR methods. Genomic DNA was extracted using standard phenol/chloroform
101 protocols. This method involved tissue digestion with proteinase K (20 mg/mL) in a lysis buffer
102 (20 mM Tris-CL pH 8.0, 5 mM EDTA pH 8.0, 400 mM NaCl, 2%SDS), extraction with
103 phenol/chloroform (1:1), precipitation with 0.1 vol 3M Sodium acetate and 1 vol. 100% Isopropanol
104 and elution in mQ water.

105 For amplification, we used the following primers pairs:

106 16SAR (TCGACTGTTTACCAAAAACATAGC) and 16SBR
107 (ACGGAATGAACTCAAATCATGTAAG) for 16S rRNA (Cunningham and Buss, 1993); and
108 jGLCO1490 (TITCIACIAAYCAYAARGAYATTGG) and jGHCO2198
109 (TAIACYTCIGGRTGICCRAARAAYCA) for COI (Geller et al., 2013). Amplification programs used
110 for 16S rRNA and COI are as previously described in Prudkovsky et al., 2019.

111 18S-EukF (WAYCTGGTTGATCCTGCCAGT) and 18S-EukR
112 (TGATCCTTCYGCAGGTTACCTAC) for 18S rRNA (Medlin et al., 1988). F97
113 (CCYYAGTAACGGCGAGT), R2084 (AGAGCCAATCCTTTTCC), F1383
114 (GGACGGTGGCCATGGAAGT) and R3238 (SWACAGATGGTAGCTTCG) for 28S rRNA (Evans
115 et al., 2008). Amplification programs used for 18S rRNA and 18S rRNA are as previously
116 described in Evans et al., 2008.

117 Full-length 18S rRNA and 28S rRNA sequences of *M. haeckelii* from the North Sea were obtained
118 from the reference transcriptome available in our laboratory. For transcriptome sequencing, total
119 RNA was extracted from a mixture of various *Margelopsis* life and developmental stages. Total
120 RNA extraction was conducted using the Zymo Research Quick-RNA MiniPrep Plus Kit according
121 to the manufacturer's instructions. Poly-A RNA enrichment, cDNA library construction and
122 sequencing were carried out at Evrogen (Russia). The cDNA library was sequenced using the
123 Illumina NovaSeq 6000 SP flow cell to produce with 150-bp paired-end reads. The high-quality
124 reads were employed for the *M. haeckelii* transcriptome assembly with the SPAdes assembler
125 (v.3.13.1) (Bankevich et al., 2012).

126

127 **Phylogenetic analyses**

128 Nucleotide sequences were aligned using the MUSCLE algorithm in MUSCLE software (v3.8.31)
129 (Edgar et al., 2004) and trimmed with the TrimAL tool (v.1.2rev59) (Capella-Gutiérrez et al., 2009).
130 A heuristic approach “automated1” was used to select the best automatic method for trimming
131 our alignments.

132 Phylogenetic analyses were performed using Maximum Likelihood methods in IQTree v.2.0-rc2
133 software (Minh, et al., 2020) according to the optimal models for each gene. Individual marker
134 analyses and a concatenated gene analysis were performed. The best models of nucleotide
135 substitution were chosen using ModelFinder (Kalyaanamoorthy et al., 2017). The GTR+F+I+G4

136 was found to be optimal for the COI dataset; GTR+F+I+G4 for 16S rRNA; TIM3+F+R3 for 18S
137 rRNA; and TIM3+F+R5 for 28S rRNA. One thousand bootstrap replicates were generated for
138 each individual analysis, as well as for the combined analysis.

139 The concatenated COI+16S+18S+28S alignment was constructed using Sequence Matrix
140 (<https://github.com/gaurav/taxondna>). The concatenated dataset was analyzed using IQTree
141 (v.2.0-rc2) with partitioned analysis for multi-gene alignments (Chernomor, et al., 2016). The set
142 of selected species for concatenated analysis was chosen mainly according to Nawrocki et al.
143 (2013) and considering the availability of individual gene sequences in GenBank for COI, 16S
144 rRNA, 18S rRNA and 28S rRNA.

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

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

149

150 **Data availability**

151 Sequences obtained in this study have been deposited in GenBank under the following accession
152 numbers: *Margelopsis haeckelii* (OK129327, OK139084, OK142735, OK127861, ON391039,
153 ON391070), *Margelopsis hartlaubii* (ON237369, ON237671, ON237710), *Plotocnide borealis*
154 (OK110252).

155

156 **Results**

157 Our phylogenetic investigation of phylogenetic affinities of species of Margelopsidae was
158 conducted employing Maximum likelihood analysis for all single gene datasets as well as our final
159 concatenated four-gene dataset (COI, 16S rRNA, 18S rRNA, 28S rRNA). All taxa used in our
160 analysis are arranged taxonomically in Table 1. All *M. haeckelii* and *M. hartlaubii* sequences (COI,
161 16S rRNA, 18S rRNA, 28S rRNA) were newly generated for this study. *M. hartlaubii* had
162 previously only had COI available on GenBank (Ortman et al., 2010). Maximum Likelihood
163 bootstrapping (MLB) analysis of the concatenated dataset recovered a relatively well resolved
164 tree and recovered Margelopsidae paraphyly. *M. hartlaubii* was recovered sister to *Plotocnide*
165 *borealis* Wagner, 1885 (MLB=100), forming a clade that affiliate with the family Boreohydridae, a
166 sister taxon to all other Aplanulata genera (MLB = 100) (Fig. 2). Each individual COI, 16S rRNA,
167 18S rRNA or 28S rRNA analysis also recovered a strong supported affiliation of *M. hartlaubii*
168 within Boreohydridae (MLB = 100) (Fig. 2). At the same time, both *M. haeckelii* from different
169 locations nested within the clade of the Corymorphidae (MLB=89). This clade comprised two
170 subclades, each well supported, one for genus *Euphysa*, including the type species *Euphysa*
171 *aurata* Forbes, 1848, and the other for *Corymorpha* + *M. haeckelii*, including the type species,
172 *Corymorpha nutans* M. Sars (Fig 2). *M. haeckelii* is nested inside the clade *Corymorpha bigelowi*
173 Maas, 1905, *Corymorpha nutans* M. Sars, 1835, *Corymorpha sarsii* Steenstrup, 1855 and
174 *Corymorpha pendula* L. Agassiz (MLB=89). Clade *Euphysa+Corymorpha+M. haeckelii* was
175 recovered to be the sister to Tubulariidae (MLB=85), which together with *Branchiocerianthus*
176 *imperator* Allman, 1885 constitute the superfamily Tubularioidea. Tubularioidea is recovered as
177 sister to Hydridae (MLB=91). General topology of our phylogenetic tree obtained in combined
178 analysis coincides with the Aplanulata tree published by Nawrocki et al., 2013.

179 Separate COI and 16S rRNA analysis recovered, that individuals of *Margelopsis haeckelii* from
180 the opposite sides of the Atlantic Ocean are representatives of the same species (Fig. 1S, 2S).
181 No nucleotide substitutions were identified in analyzed sequences of *Margelopsis haeckelii* from
182 the waters of Belgium (51.218028°, 2.879417°) and the USA (40.560556°, -73.882333°).

183 At the same time, *M. hartlaubii* COI sequences analysis revealed five mismatches between
184 sequences obtained in this study (ON237369) and sequence published in Ortman et al., 2010
185 (GQ120058.1) (Fig. 1S). However, COI sequences of *M. hartlaubii* published in Ortman et al.,
186 2010 (GQ120058.1 and GQ120059.1) also are not identical and have three mismatches.

187 Phylogenetic hypothesis testing (AU test) was performed to test the statistical significance of tree
188 topologies in our Maximum Likelihood analysis. The AU test rejected the phylogenetic hypothesis
189 of the monophyly of *M. haeckelii* and *M. harlaubii*, providing strong evidence for the polyphyly of
190 *Margelopsis*. Also, as our two individual marker analyses (16S and 28S) (Supp. 2, 3) placed *M.*
191 *haeckelii* as a sister to *Corymorpha*, two hypotheses of alternative placements of *M. haeckelii*
192 were evaluated: *M. haeckelii* is inside or outside *Corymorpha*. Results of the testing significantly
193 support ($p < 0.05$) the hypothesis that *M. haeckelii* is within *Corymorpha*. (Fig. 5S).

194

195 Discussion

196 Our concatenated dataset (COI+16S+18S+28S), which included a comprehensive taxonomic
197 sampling of hydrozoans, recovered *Margelopsis haeckelii* within Corymorphidae, nested within a
198 clade consisting of several *Corymorpha* species. This result is consistent with previous findings
199 based solely on polyp morphology, where Margelopsidae was grouped with Tubulariidae and
200 Corymorphidae in the superfamily Tubularoidea (Rees, 1957). Being quite small (1-2 mm),
201 hydrocaulus-lacking pelagic polyps of the Margelopsidae are similar to those sessile polyps of
202 corymorphids and tubulariids despite the latter having a well-developed hydrocaulus and reaching
203 up to ten centimeters in height. For all three families, hydranth tentacles are arranged into two,
204 oral and aboral whorls and blastostyles are situated in the inter-tentacular region (Fig. 3, A, C).
205 Our phylogenetic data support assertions that polyp tentacle patterns may be an important
206 morphological character for identifying lineages in Aplanulata (Rees, 1957, Nawrocki et al. 2013).

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

222 Surprisingly, our concatenated gene dataset, as well as our single gene COI dataset, recovered
223 the medusa known as *M. hartlaubii* to be a close relative of *Plotocnide borealis*, and not closely
224 related to *M. haeckelii* nor group within Corymorphidae. This result is further supported by
225 independent morphological data showing several similarities between medusae of *M. hartlaubii*
226 and *P. borealis*, including thick apical mesoglea of the bell (Fig. 3, marked blue), lack of an
227 umbrella apical canal and nematocyst batteries being located at the distal parts of tentacles (Fig.
228 3, marked violet) (Schuchert, 2006). Based on our findings, medusae described by Browne (1903)
229 have been wrongly attributed to the genus *Margelopsis*. Nawrocki et al. (2013) suggested that the
230 hypothesis of *M. hartlaubii* as the sister to the rest of Aplanulata was uncertain due to low
231 bootstrap support and that more genetic markers were needed to understand the phylogenetic

232 placement of the species. Based on our multi-marker phylogenetic analysis and morphological
233 data (Browne, 1903; Schuchert, 2006) we hypothesize that *M. hartlaubii* has a mud-dwelling,
234 meiobenthic polyp like *P. borealis* (Fig. 3), and that the two species combined represent the sister
235 group to the rest of Aplanulata.

236 In addition to *M. haeckelii* and *M. hartlaubii*, there are several other suspected species in the
237 genus *Margelopsis*, including *Margelopsis gibbesii* (McCrary, 1859) and *Margelopsis australis*
238 (Browne, 1910). Following Schuchert (2007), the World Register of Marine Species
239 (<https://www.marinespecies.org/>) lists *Margelopsis gibbesii* as invalid. This stems from the fact
240 that the original material used to describe this species, as *Nemopsis gibbesii*, consisted of a
241 margelopsid polyp and a bougainvilliid medusa, the latter subsequently recognized as a medusa
242 of *Nemopsis bachei* (L. Agassiz, 1862). This situation has generated subsequent nomenclatural
243 confusion. More recently, Calder and Johnson (2015) stabilized the situation by designating the
244 hydroid specimen illustrated by McCrary (1859) in Plate 10, Figure 7 as a lectotype for the
245 margelopsid species. Calder and Johnson (2015) went on to provide evidence casting doubt on
246 the distinction between *M. gibbesii* and *M. haeckelii* but maintained the two species given the
247 geographic locations on either side of the north Atlantic and pending further study. In this study,
248 however, using molecular phylogenetics, we have shown that *Margelopsis* from the western North
249 Atlantic, and *M. haeckelii* from the eastern North Atlantic is the same species as *M. haeckelii*,
250 *Margelopsis gibbesii* invalid. The lack of any nucleotide substitution in COI and 16S sequences
251 of *Margelopsis* representatives from both sides of Atlantic Ocean makes it possible to suggest
252 that these two populations are not isolated.

253 *Margelopsis australis* is only known from its original collection and is based on a single medusa
254 specimen, lacking reliable characters for distinguishing it from *M. hartlaubii* (Browne 1910).
255 Moreover, the single specimen was described as being “somewhat contracted and in a crumbled
256 condition” (Browne 1910). Based on the available morphological data, we cannot state with any
257 degree of certainty that *M. australis* is a valid species, or that it is a member of *Margelopsis*.

258 Medusae are a useful means of identifying species, genera and even family ranks (Rees, 1957;
259 Bouillon, et al., 2006). A change in morphology of the typical jellyfish form within a family is usually
260 due to the reduction of the medusa stage, something that is widespread throughout
261 Anthoathecata and Leptothecata (Cornelius, 1992; Leclere et al., 2009; Cartwright, Nawrocki,
262 2010). However, *M. haeckelii* is a normally developed medusa, distinctly different from those
263 typical of *Corymorpha*, despite their close relationship recovered by our phylogenetic analysis.
264 Recent studies using molecular phylogenetic methods have revealed several such cases in which
265 related taxa have very different jellyfishes or species with similar jellyfishes are only distantly
266 related. The morphologically aberrant jellyfish *Obelia* is so different from other Campanulariidae
267 that a hypothesis was proposed for the re-expression of this jellyfish after its evolutionary
268 reduction (Boero, Sara, 1987). However, this hypothesis was not supported by molecular
269 phylogenetic analysis and *Obelia* may have originated from a *Clytia*-like ancestor (Cunha et al.
270 2017; Govindarajan et al., 2006; Leclere et al., 2019). *Larsonia pterophylla* (Haeckel, 1879) was
271 previously assigned to the genus *Stomotoca* due to similarity of their jellyfishes (Larson, 1982).
272 Interestingly, the structure of the polyps in the genera *Larsonia* and *Stomotoca* are so dissimilar
273 that they could be attributed to different families (Boero, Bouillon, 1989). And indeed, according
274 to molecular data, *L. pterophylla* and *Stomotoca atra* L. Agassiz, 1862 are not closely related.
275 Rather, *L. pterophylla* is closely related to *Hydrichthys boycei* from the Pandeidae family, and *S.*
276 *atra* is ungrouped with most species (Schuchert, 2018; Woodstock et al., 2019). Inclusion of the
277 genus *Cytaeis* in Bougainvilliidae or the genera *Polyorchis* and *Scrippisia* in Corynidae is
278 surprising due to the discrepancy between the jellyfishes of these genera and those typical of the
279 respective families (Nawrocki, Cartwright, 2010; Prudkovsky et al., 2017). Finally, we conclude
280 that appearance of atypical jellyfishes in hydrozoan families can indicate a great evolutionary
281 plasticity of the medusa stage morphology. In contrast, the morphology of the hydroids appear to
282 be more phylogenetically constant. For example, the morphology of *Cytaeis* hydroids is similar to

283 the structure of Bougainvillidae hydroids with stolonial colonies, and Obelia-like polyps are typical
284 for the family Campanulariidae (Prudkovsky et al., 2017; Leclere et al., 2019).

285 Concepts of 'mosaic' or 'inconsistent evolution' were proposed for these cases in which closely
286 related hydroids can produce very different medusae or vice versa (Naumov 1956, 1960; Rees,
287 1957). Inconsistent evolution was explained by differences in the rate and direction of evolution
288 in the two life cycle stages. Some incongruences between hydroid and medusa systems seem to
289 result from weaknesses in a classification system (Petersen, 1990), but our work provides new
290 reason to return to the discussion of this concept.

291 Taxonomic recommendations

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

294 a) As multigene phylogenetic analyses nested *Margelopsis haeckelii*, the type species of
295 *Margelopsis*, within genus *Corymorpha*, we recommend to redesignate it into *Corymorpha*
296 *haeckelii*.

297

298 *Corymorpha* M. Sars, 1835

299 Type species: *Corymorpha nutans* M. Sars, 1835 by monotypy.

300 *Diagnosis*: Solitary hydroids with more or less vasiform hydranth, with long caulus or with
301 short, squat polyp with broad head. **Rarely a hydrant without a caulus**. Hydranth with
302 one or several closely set whorls of 16 or more **moniliform** or filiform tentacles and one
303 or more aboral whorls of 16 or more long, non-contractile moniliform or filiform tentacles.
304 Gastrodermal diaphragm parenchymatic or **without parenchymatic specializations of**
305 **the gastrodermis**. Hydrocaulus, **if present**, stout, covered by thin perisarc, filled with
306 parenchymatic gastrodermis, with long peripheral canals; aboral end of caulus with
307 papillae turning more aborally into rooting filaments, rooting filaments scattered or
308 gathered in a whorl, rooting filaments composed of epidermis and solid gastrodermis,
309 sometimes tips with non-ciliated statocysts. **Otherwise, hydroid planktonic and**
310 **hydrocaulus reduced, with a central depression**. With or without asexual reproduction
311 through constriction of tissue from aboral end of hydrocaulus. Gonophores develop on
312 blastostyles arranged in a whorl over aboral tentacles. Gonophores remain either fixed as
313 sporosacs, medusoids, or are released as free medusae. Medusa bell apex dome-shaped
314 or pointed, **with apical canal**. Four marginal bulbs present, lacking long exumbrellar
315 spurs. With a single tentacle or three short tentacles and one long tentacle that differs not
316 merely in size, but also in structure. **Rarely with 1-6 tentacle per bulb**. Manubrium thin-
317 walled, sausage-shaped with flared mouth rim, reaching to umbrella margin. Cnidome
318 comprises stenoteles, desmonemes, and haplonemes, **with or without euryteles**.

319 *Remarks*: This diagnosis for the most part corresponds to Schuchert, 2010 (Schuchert,
320 2010), Petersen, 1990 (Petersen, 1990) and Nawrocki et al., 2013 (Nawrocki et al., 2013),
321 but with modifications (indicated in bold) to polyp and medusa body shape, and cnidome
322 description to include *Margelopsis (Corymorpha) haeckelii*.

323

324 b) We suggest moving *Margelopsis hartlaubii* into family Boreohydridae and recommend to
325 redesignate it into *Plotocnide hartlaubii*.

326

327 *Plotocnide* Wagner, 1885

328 Type species: *Plotocnide borealis* Wagner, 1885 by monotypy.

329 *Diagnosis*: Medusa umbrella evenly rounded with thick apical jelly and scattered groups
330 of exumbrellar nematocysts; manubrium half as long as bell cavity, with or without broad,
331 dome-shaped apical chamber; **without apical canal**; mouth simple, with ring of
332 nematocysts; gonad forming thick ring around manubrium; four narrow radial canals and

333 narrow ring canal; four marginal bulbs each **with 1-3 solid tentacles per bulb**; tentacles
334 terminate in ovoid knob studded with nematocysts. No ocelli. Cnidome comprises
335 desmonemes and stenoteles, **with or without mastigophores**. Hydroids, **if known**,
336 solitary, small, with one whorl of reduced tentacles, capitate or not, located in the oral or
337 median part of body; perisarc covering of base filmy or absent; gametes in body wall.
338 Remarks: This diagnosis for the most part corresponds to Schuchert, 2006, 2010
339 (Schuchert, 2006; Schuchert, 2010), but with modifications (indicated in bold) to medusa
340 body shape, and cnidome description to include *Margelopsis (Plotocnide) hartlaubii*.
341
342 c) We suggest that Margelopsidae should no longer be used, and both *Pelagohydra* and
343 *Climacocodon* should be moved to Aplanulata *incertae sedis* until additional molecular
344 phylogenetic analyses can clarify their phylogenetic placement.

345 Conclusion

346 Our results clarify the phylogenetic picture of Aplanulata, by revealing the phylogenetic position
347 of *M. haeckelii*, type species of the genus *Margelopsis* as falling within *Corymorpha* and *M.*
348 *hartlaubii* as being a close relative of *Plotocnide* in the family Boreohydridae. On the case of the
349 latter species, this phylogenetic result conflicts with the century old hypothesis that *Margelopsis*
350 belongs to Tubulariidae or Corymorphidae (Nawrocki et al., 2013). However, by showing that *M.*
351 *haeckelii* falls within the genus *Corymorpha*, our investigation presents strong evidence in support
352 of this traditional hypothesis. Because *M. haeckelii* is a hydrozoan belonging to Corymorphidae,
353 we can infer that this lineage evolutionarily lost their hydrocaulus and stolon, likely as an
354 adaptation to a holopelagic life-cycle. It was previously suggested that the foundation for this type
355 of changes in body plan, and accompanying life-style, might lead to speciation and could be
356 reflected by changes in the expression of Wnt signaling components (Duffy, 2011). Based on our
357 results, *M. haeckelii* might be a prime candidate for testing this hypothesis.

358 Unfortunately, due to the few and extremely irregular documented collection records of hydroids
359 from the supposedly sister genera of *Margelopsis*, *Pelagohydra* and *Climacocodon*, the
360 phylogenetic relationships within this group are still obscured. It remains unclear if *Pelagohydra*
361 and *Climacodon* form a clade with either *M. hartlaubii* or *M. haeckelii*, or neither. Thus, the number
362 of origin of a secondarily specialized pelagic polyp stage is still not known. The possible
363 relationships between these three genera, as well as their phylogenetic placement, still need to
364 be verified by additional studies when molecular data become available.

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506 **Figure legends**

507 Fig. 1. Morphology of collected Margelopsidae representatives and the locations of its samplings.
 508 (A-D) *Margelopsis haeckelii* Hartlaub, 1897. (A) Newly hatched polyp, (B) Mature polyp with
 509 medusa buds, (C, D) Mature medusa. (E) Mature medusa of *Margelopsis hartlaubii* Browne, 1903.
 510 Photo Credit: Dr. Peter Schuchert (Schuchert, 2022). (F, J) Geographic locations of sampling
 511 sites. Abbreviations, ac – apical canal, at – aboral tentacles, e – embryos, h – hypostome, md –
 512 medusoid ot – oral tentacles, tb – tentacle bulb, yp – young polyp.

513 Fig.2. Analysis of phylogenetic position of *Margelopsis haeckelii* and *Margelopsis hartlaubii* in
 514 Aplanulata. Phylogenetic hypothesis of *Margelopsis haeckelii* relationships based on the
 515 combined mitochondrial and nuclear dataset (CO1+16S+18S+28S). Node values indicate
 516 bootstrap support from 1000 replicates. *Margelopsis haeckelii* and *Margelopsis hartlaubii* are in
 517 red. *WGS84 51.218028°, 2.879417°, ** WGS84 40.560556°, -73.882333°

518 Fig. 3. Comparison of morphological characters of (A) *Margelopsis hartlaubii*, (B) *Margelopsis*
 519 *haeckelii*, (C) *Corymorpha nutans* and (D) *Plotocnide borealis*. Scalebar – 0.4 mm. Color coding:
 520 yellow – oral and aboral whorls of polyp tentacles, pink– region of medusa budding, green – the
 521 region of gametes formation, orange – apical canal, blue – medusa umbrella with clusters of
 522 exumbrellar nematoblasts, violet – clusters of nematocysts located at the distal parts of tentacles.
 523 *Margelopsis hartlaubii*, *Margelopsis haeckelii*, *Corymorpha nutans* and *Plotocnide borealis*
 524 modified from Schuchert (2006; 2010)

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

529 Fig. 2S. Phylogenetic hypothesis of *Margelopsis haeckelii* and *Margelopsis hartlaubii*
 530 relationships based on the mitochondrial 16S rRNA. Node values indicate bootstrap support from
 531 1000 replicates. *Margelopsis haeckelii* and *Margelopsis hartlaubii* are in red.

532 Fig. 3S. Phylogenetic hypothesis of *Margelopsis haeckelii* and *Margelopsis hartlaubii*
 533 relationships based on the 28S rRNA large ribosomal subunit. Node values indicate bootstrap
 534 support from 1000 replicates. *Margelopsis haeckelii* and *Margelopsis hartlaubii* are in red.

535 Fig. 4S. Phylogenetic hypothesis of *Margelopsis haeckelii* and *Margelopsis hartlaubii*
 536 relationships based on the 18S rRNA small ribosomal subunit. Node values indicate bootstrap
 537 support from 1000 replicates. *Margelopsis haeckelii* and *Margelopsis hartlaubii* are in red.

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

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

541

suborder	family	species	16S rRNA	18S rRNA	28S rRNA	COI	vouchers
Aplanulata	Boreohydridae	<i>Plotocnide borealis</i>	KU721822.1	KU721833.1	OK110252	KU721812.1	RU087.2
	Candelabridae	<i>Candelabrum cocksii</i>	EU876535.1	AY920758.1	AY920796.1	GU812438.1	MHNGINVE29591
	Corymorphydae	<i>Branchioceriant hus imperator</i>		JN594046.2	JN594035.2	JX121580.1	MHNG:INVE74105
		<i>Corymorpha bigelowi</i>	EU448099	EU876564.1	EU272563.1	JX121581.1	KUNHM 2829

		<i>Corymorpha nutans</i>	EU876532.1	EU876558.1	EU879931.1	JX121586.1	MHNG:INVE 48745
		<i>Corymorpha pendula</i>	EU876538.1	EU876565.1	EU305510.1	JX121583.1	KUNHM DIZ2962
		<i>Corymorpha sarsii</i>	KP776787.1	JN594049.2	JN594038.2	JX121585.1	MHNG:INVE 68950
		<i>Euphysa aurata</i>	EU876536.1	EU876562.1	EU879934.1	JX121587.1	MHNG:INVE 48753
		<i>Euphysa intermedia</i>	EU876531.1	AY920759.1	EU879930.1	JX121582.1	
		<i>Euphysa japonica</i>	KP776802.1	EU301605.1	JX122505.1	MF000498.1	
		<i>Euphysa tentaculata</i>	EU876537.1	EU876563.1	EU879935.1	JX121588.1	
		<i>Hataia parva</i>	JN594033.1	JN594045.2	JN594034.2	JX121608.1	UF:5407
Hydridae		<i>Hydra hymanae</i>	GU722762.1	JN594051.2	JN594040.2	GU722849.1	
		<i>Hydra oligactis</i>		JN594052.2	JN594041.2	GU722871.1	
		<i>Hydra utahensis</i>		JN594053.2	JN594042.2	GU722861.1	
		<i>Hydra vulgaris</i>	EU876543.1	JN594054.2	JN594043.2	GU722914.1	
		<i>Hydra viridissima</i>		EU876569.1	EU879940.1	GU722845.1	
Margelopsidae		<i>Margelopsis haeckelii</i>	OK129327 ON391070	OK139084	OK142735	OK127861 ON391039	
		<i>Margelopsis hartlaubii</i>	ON287278	ON237671	ON237710	ON237369 GQ120058.1	
Protohydridae		<i>Protohydra leuckarti</i>	KU721828.1	KU721835.1		KU721813.1	Protohydra2010072 7.6
Tubuldariidae		<i>Ectopleura crocea</i>	EU876533.1	KF699111.1	EU879932.1	JX121589.1	MHNG:INVE 34010
		<i>Ectopleura dumortierii</i>	FN687542.1	EU876561.1	EU879933.1	JX121590.1	
		<i>Ectopleura larynx</i>		EU876572.1	EU879943.1	JX121591.1	MHNG-INVE-54563
		<i>Ectopleura marina</i>	EU883542.1	EU883547.1	EU883553.1	JX121592.1	
		<i>Ectopleura wrighti</i>	FN687541.1	JN594055.2	JN594044.2	JX121593.1	MHNG:INVE 27331

		<i>Hybocodon chilensis</i>	EU876539.1	EU876566.1	EU879937.1	JX121594.1	MHNG:INVE36023
		<i>Hybocodon prolifer</i>	FN687544.1	EU876567.1	EU879938.1	JX121595.1	
		<i>Hydractinia sp</i>	EU305477.1	EU305495.1	EU305518.1		KUNHM2876
		<i>Ralpharia gorgoniae</i>	EU305482.1	EU272633.1	EU272590.1	GU812437.1	KUNHM2778
		<i>Tubularia indivisa</i>	FN687530.1	EU876571.1	EU879942.1	JX121596.1	
		<i>Zyzyzus warreni</i>	EU305489.1	EU272640.1	EU272599.1	JX121597.1	KUNHM 2777
Capitata	Asyncorynidae	<i>Asyncoryne ryniensis</i>	EU876552.1	EU876578.1	GQ424289.1		KUNHM 2639
	Cladocorynidae	<i>Cladocoryne floccosa</i>	AY512535.1	EU272608.1	EU272551.1		personal:A. Lindner:AL1407
	Cladonematidae	<i>Staurocladia vallentini</i>	GQ395332.1	GQ424322.1	GQ424293.1	MF000500.1	Sch522
		<i>Staurocladia wellingtoni</i>	AY787882.1	GQ424323.1	EU879948.1	MF000486.1	
	Corynidae	<i>Coryne uchidai</i>	GQ395319.1	GQ424332.1	GQ424305.1	KT981912.1	
		<i>Sarsia tubulosa</i>	EU876548.1	EU876574.1	EU879946.1		MHNGINV35763
		<i>Stauridiosarsia ophiogaster</i>	EU305473.1	EU272615.1	EU272560.1		KUNHM 2803
	Moerisiidae	<i>Odessia maeotica</i>	GQ395324.1	GQ424341.1	GQ424314.1		MHNG INVE53642
	Pennariidae	<i>Pennaria disticha</i>	AM088481.1	GQ424342.1	GQ424316.1		MHNG INVE29809
	Porpitidae	<i>Porpita porpita</i>	AY935322.1	GQ424319.1	EU883551.1	LT795124.1	RM3_747
	Solanderiidae	<i>Solanderia secunda</i>	EU305484.1	AJ133506.1	EU305533.1	JX121599.1	KUNHM 2611
	Zanclidae	<i>Zanclaea costata</i>	EU876553.1	EU876579.1	EU879951.1		MHNGINV26507
		<i>Zanclaea prolifera</i>	EU305488.1	EU272639.1	EU272598.1		KUNHM 2793
Fillifera	Eudendriidae	<i>Eudendrium capillare</i>	AY787884.1		EU305514.1	JX121602.1	KUNHM2625
	Proboscidactylidae	<i>Proboscidactyla flavicirrata</i>	EU305480.1	EU305500.1	EU305527.1	JX121600.1	USNM:1074994

	Ptilocodiidae	<i>Hydrichthella epigorgia</i>	EU305478.1	EU272622.1	EU272569.1	JX121601.1	KUNHM 2665
	Stylasteridae	<i>Lepidopora microstylus</i>	EU645329.1	EU272644.1	EU272572.1	JX121603.1	USNM:1027724

542

Figure 1

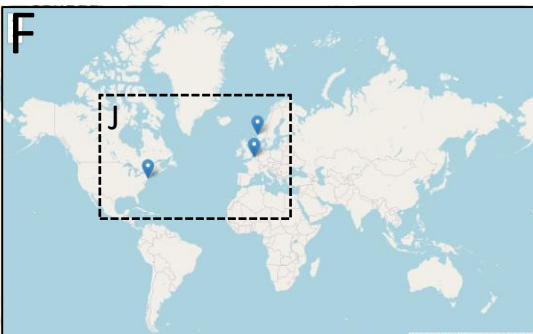
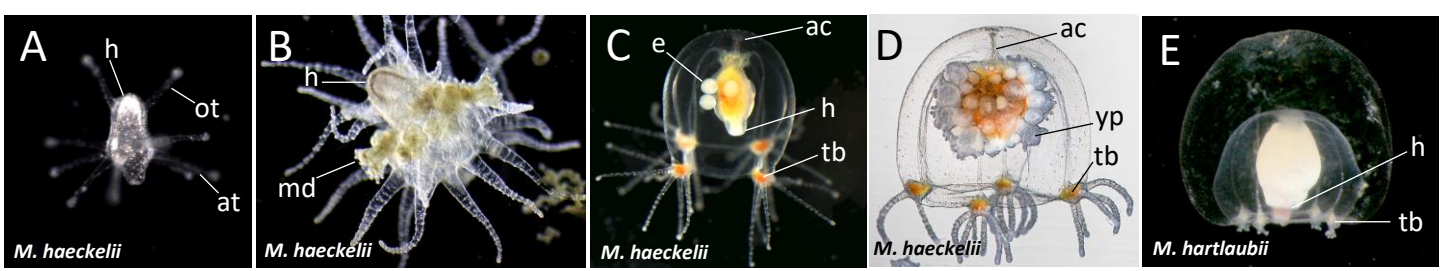
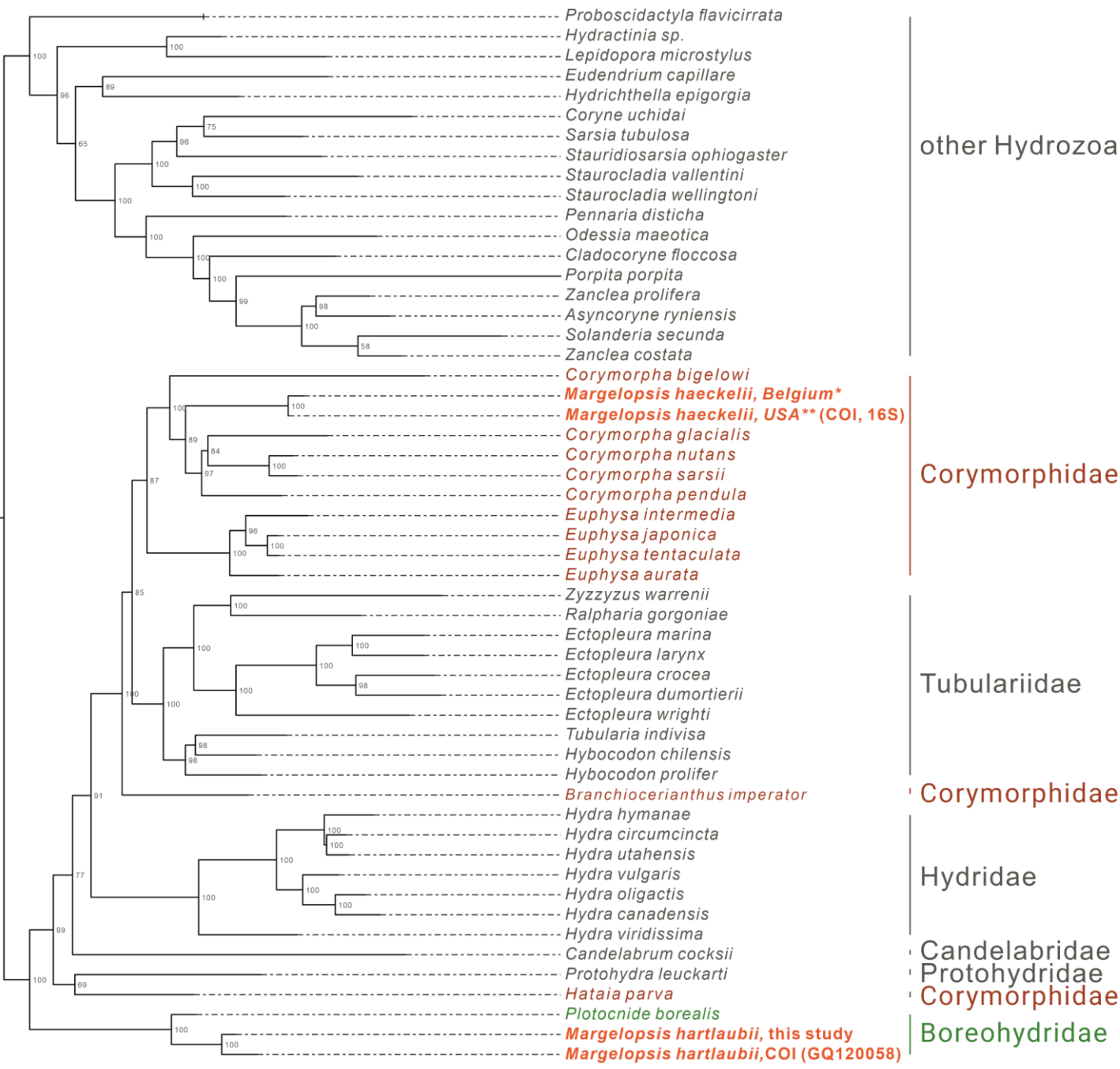


Figure 2



other Hydrozoa

Corymorphidae

Tubulariidae

Corymorphidae

Hydridae

Candelabridae

Protohydridae

Corymorphidae

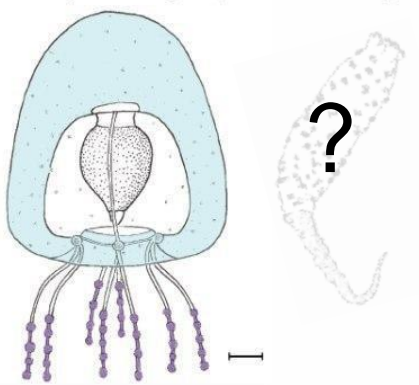
Boreohydridae

Figure 3

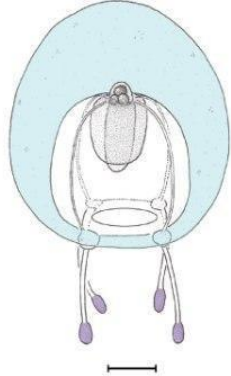
fam. Corymorphidae
Margelopsis (Corymorpha) haeckelii



fam. Boreohydridae
Margelopsis (Plotocnide) hartlaubii



fam. Corymorphidae
Corymorpha nutants



fam. Boreohydridae
Plotocnide borealis

Supplementary figure 1

Solanderia secunda

COI tree

other Hydrozoa

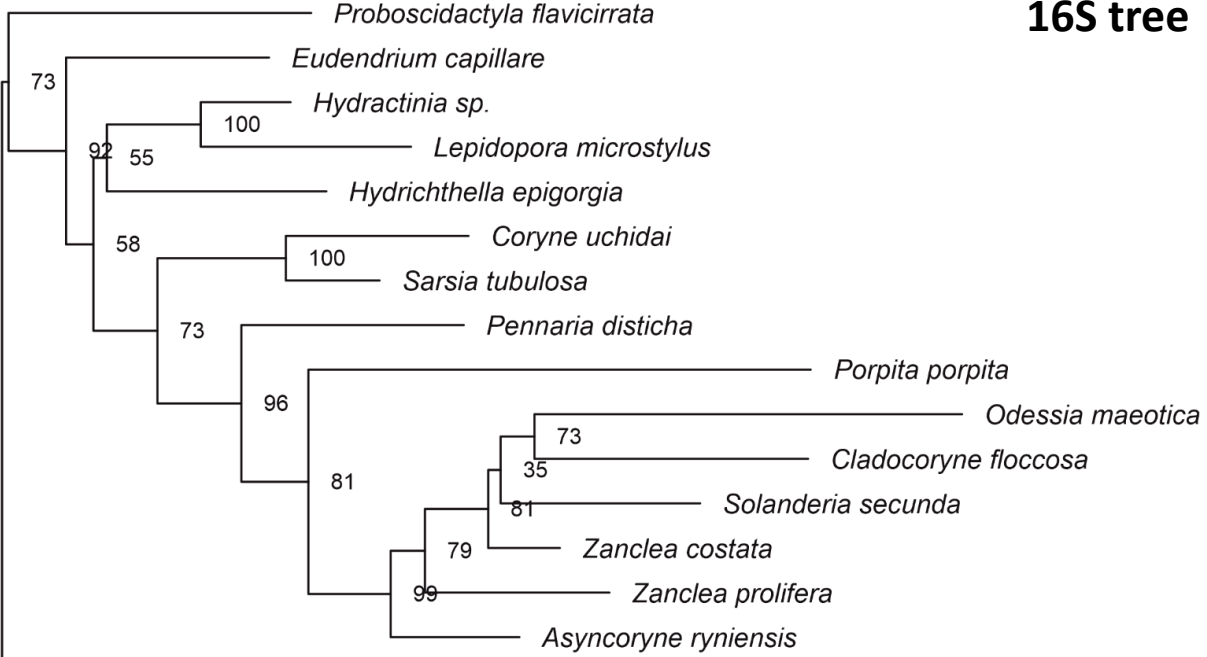
Aplanulata



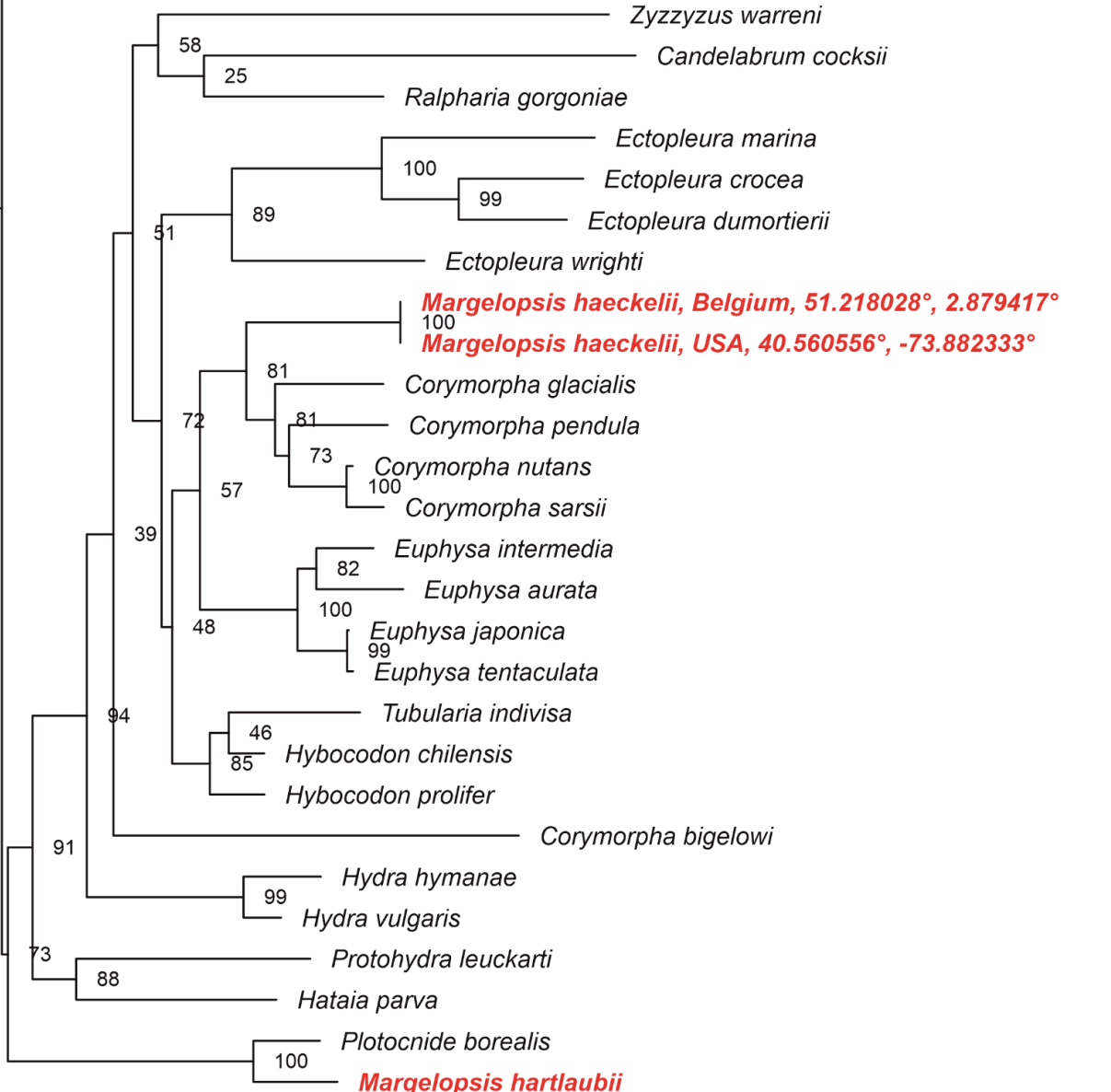
Supplementary figure 2

16S tree

other Hydrozoa



Aplanulata

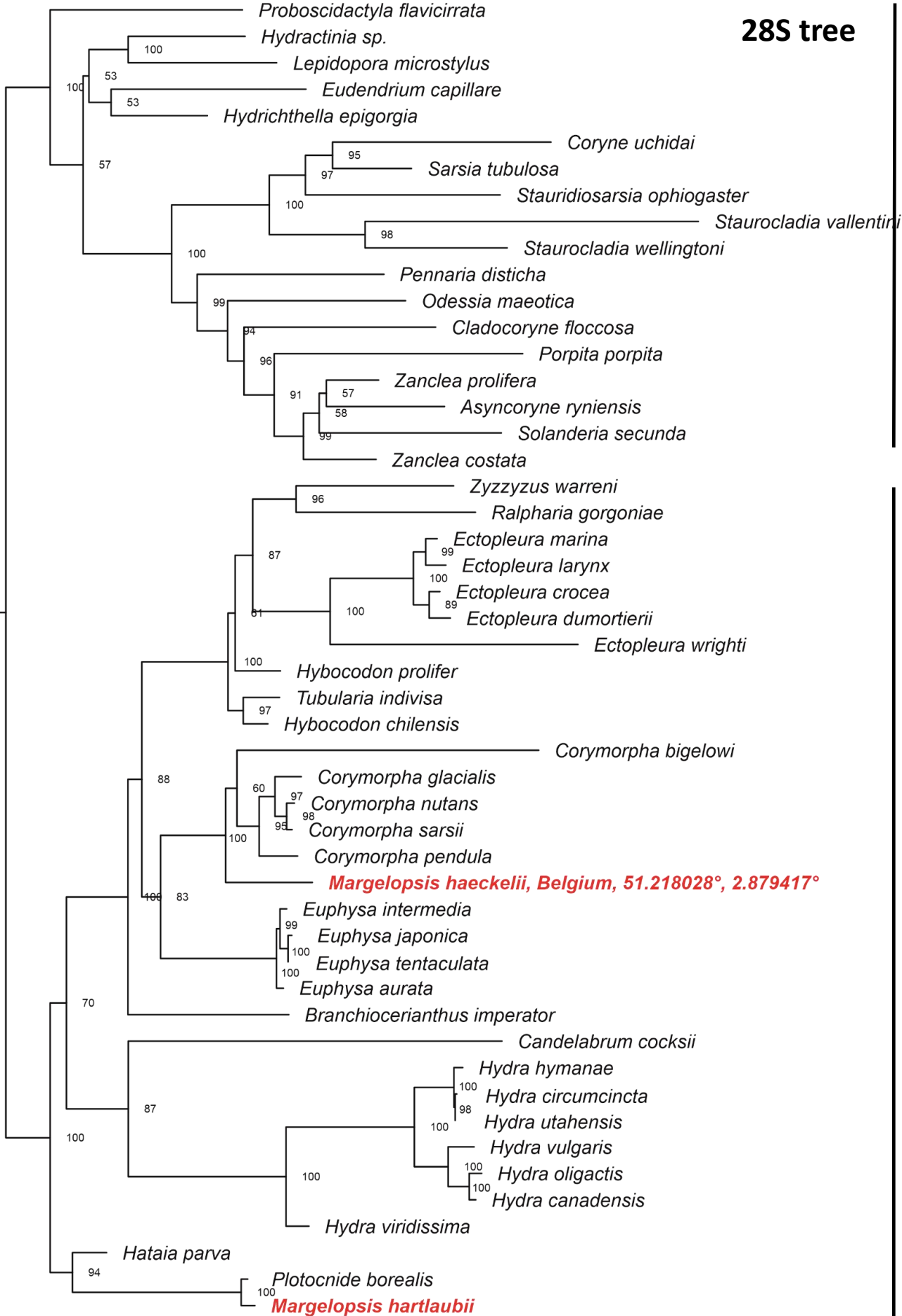


Supplementary figure 3

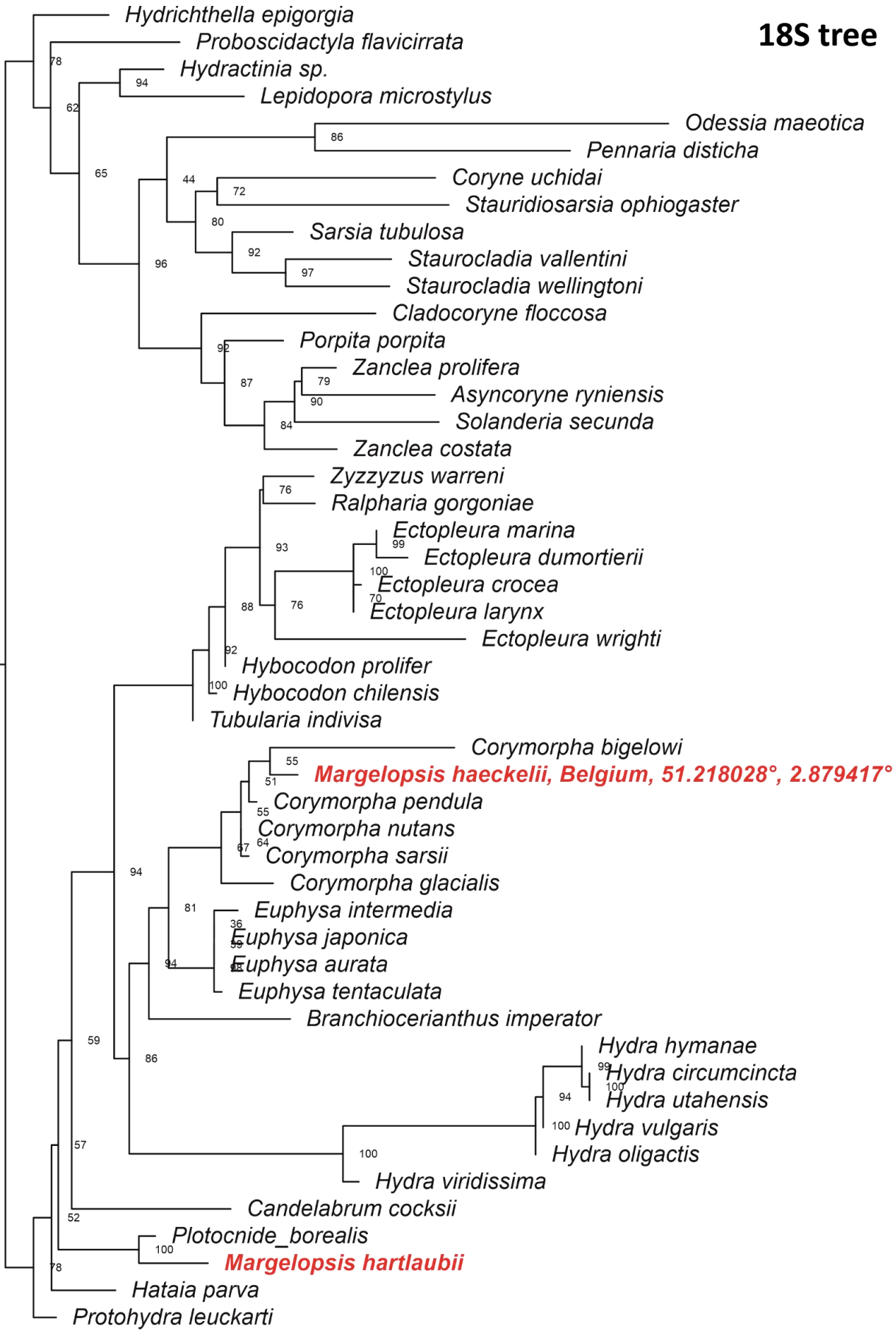
28S tree

other Hydrozoa

Aplanulata



Supplementary figure 4



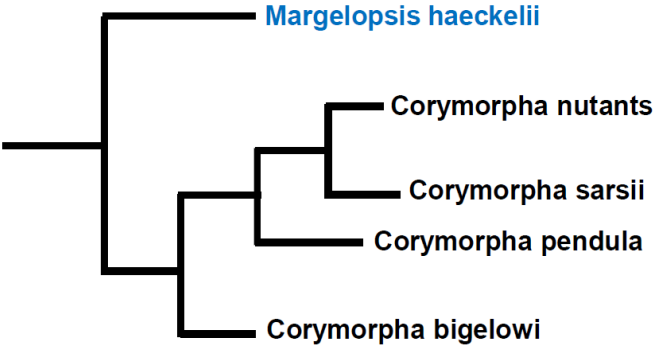
other Hydrozoa

Aplanulata

Supplementary figure 5

Phylogenetic hypothesis #1

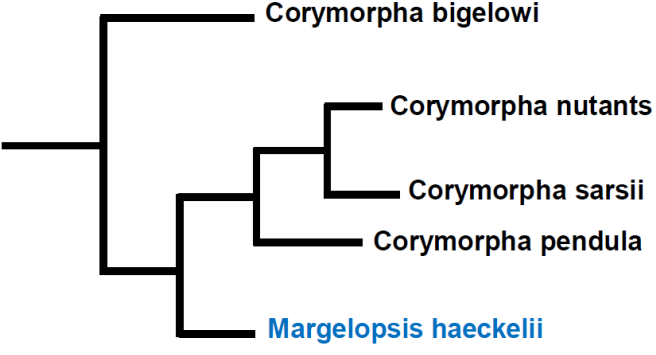
Margelopsis haeckelii is a sister to the genus *Corymorpha*,
AU test



Rejected, $p > 0.05$

Phylogenetic hypothesis #2

Margelopsis haeckelii nested within the genus *Corymorpha*,
AU test



Supported, $p < 0.05$