

1 Title: Phylogenetic relationships of Proboscoida Broch, 1910 (Cnidaria, Hydrozoa): are
2 traditional morphological diagnostic characters relevant for the delimitation of lineages at the
3 species, genus, and family levels?
4

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18 **Abstract**

19 Overlapping variation of morphological characters can lead to misinterpretation in
20 taxonomic diagnoses and the delimitation of different lineages. This is the case for
21 hydrozoans that have traditionally been united in the family Campanulariidae, a group known
22 for its wide morphological variation and complicated taxonomic history. In a recently
23 proposed phylogenetic classification of leptothebate hydrozoans, this family was restricted to
24 a more narrow sense while a larger clade containing most species traditionally classified in
25 Campanulariidae, along with members of Bonneviellidae, was established as the suborder
26 Proboscoida. We used molecular data to infer the phylogenetic relationships among
27 campanulariids and assess the traditional classification of the family, as well as the new
28 classification scheme for the group. The congruity and relevance of diagnostic characters
29 were also evaluated. While mostly consistent with the new phylogenetic classification of
30 Proboscoida, our increased taxon sampling resulted in some conflicts at the family level,
31 specially regarding the monophyly of Clytiidae and Obeliidae. Considering the traditional
32 classification, only Obeliidae is close to its original scope (as subfamily Obeliinae). At the
33 genus level, *Campanularia* and *Clytia* are not monophyletic. Species with *Obelia*-like
34 medusae do not form a monophyletic group, nor do species with fixed gonophores, indicating
35 that these characters do not readily diagnose different genera. Finally, the species *Orthopyxis*
36 *integra*, *Clytia gracilis*, and *Obelia dichotoma* are not monophyletic, suggesting that most of
37 their current diagnostic characters are not informative for their delimitation. Several
38 diagnostic characters in this group need to be reassessed, with emphasis on their variation, in
39 order to have a consistent taxonomic and phylogenetic framework for the classification of
40 campanulariid hydrozoans.

41 **Keywords:** DNA, classification, diagnosis, interspecific variation.

42

43 **1. Introduction**

44 Studies associating molecular and morphological data have contributed to solve many
45 taxonomical difficulties involving species delimitation in Cnidaria (e.g., Miglietta et al. 2007,
46 2009; Benzoni et al. 2010; Moura et al. 2011a, b; Ardila et al. 2012; Miranda et al. 2016),
47 especially in groups with wide morphological variation (Kim et al. 2004; Gutiérrez-
48 Rodríguez et al. 2009; Forsman et al. 2009; Schmidt-Roach et al. 2013). Some studies have
49 shown that the relevance of morphological characters used to delimit species is frequently
50 misinterpreted, and some traditional diagnostic characters are inadequate (e.g., Fukami et al.
51 2004; Bo et al. 2012).

52 Among hydrozoan species, characters such as colony size, branching pattern, length
53 of the hydrotheca and number of pedicel rings have been traditionally used in the diagnoses
54 of many species and genera (cf. Ralph 1957; Naumov 1969; Millard 1966, 1975; Calder
55 1991, 1997; Cornelius 1995a,b), even though they were also shown to be intraspecifically
56 variable, often in relation to flow rate/direction, nutrition, substrate, latitude, and water
57 temperature (Naumov 1969; Ralph 1956; Hughes 1986; Silveira and Migotto 1991; Bumann
58 and Buss 2008). Consequently, morphological variability presented by species may result in
59 overlap of their diagnostic characters, hampering species identification and generating
60 taxonomic confusion.

61 The family Campanulariidae Johnston, 1836 (Cnidaria, Hydrozoa) is known for the
62 wide morphological variability of its species, which, in addition, have simple and similar
63 morphological characters (cf. Cornelius 1982). Not surprisingly, Campanulariidae has a
64 complicated taxonomic history, with recurrent disagreements among taxonomists on the
65 relevance of the morphological characters used to diagnose and delimit genera and species
66 (Nutting 1915; Millard 1975; Cornelius 1982, 1995b; Calder 1991). At the species level, the
67 validity and scope of some taxa are frequently questioned (e.g., *Obelia longissima*, Cornelius
68 1975, 1990; *O. dichotoma*, Calder 2013; Calder et al. 2014; *Orthopyxis integra*, Cunha et al.
69 2015), while others were described as potentially cryptic (e.g., *O. integra*, *Obelia geniculata*,
70 *Clytia gracilis*, Govindarajan et al. 2005, 2006; Lindner et al. 2011). At the genus level,
71 several generic divisions were considered doubtful (e.g., *Orthopyxis* and *Campanularia*,
72 Millard 1975, Schuchert 2001; *Laomedea*, *Hartlaubella*, *Gastroblasta*, *Tulpa*, *Rhizocaulus*,
73 Boero et al. 1996), as well as some nominal genera (*Orthonia*, *Eucalix*, Cornelius 1982,
74 Calder 1991). Finally, at the suprageneric level, molecular studies with representatives of
75 Campanulariidae have shown a disputable monophyly of the family (Govindarajan et al.
76 2006; Peña Cantero et al. 2010), and even its phylogenetic placement among Leptothecata
77 was questioned (Collins 2000; Leclère et al. 2009). This scenario has posed the question of
78 whether the classification of Campanulariidae is based on relevant diagnostic characters that
79 reliably reflect its evolutionary history.

80 Campanulariid hydroids are traditionally known for their stolonial or upright colonies,
81 campanulate hydrothecae and trumpet-shaped hypostomes (Millard 1975; Cornelius 1982;
82 Bouillon 1985; Calder 1991). The family comprises 11 genera (up to 13 if different
83 taxonomic proposals are considered, cf. Cornelius 1982), divided into three subfamilies,
84 Campanulariinae Johnston, 1836, Clytiinae Cockerell, 1911, and Obeliinae Haeckel, 1879
85 (cf. Cornelius 1982, 1995b). A recent and comprehensive phylogenetic inference of

86 Leptothecata, however proposed a new classification for the order Proboscoida Broch, 1910,
87 dividing campanulariids into two infraorders, viz., Campanulariida Bouillon, 1984 and
88 Obeliida Maronna et al. 2016, and three families, viz., Campanulariidae, Clytiidae and
89 Obeliidae (Maronna et al. 2016), with similar scope to the former subfamilies. Although
90 originally included in Proboscoida (Bouillon 1985), Phialuciidae was not covered by their
91 analysis, and its inclusion in this order still needs confirmation (Maronna et al. 2016).

92 The muddled taxonomical history of Campanulariidae prevents an indisputable
93 estimation of the number of valid species, although WoRMS (World Register of Marine
94 Species) accounts approximately 150 species (Schuchert 2015). Despite the lack of precision,
95 campanulariids are frequently among the richest and dominant groups in marine epibenthic
96 communities (e.g., Llobet et al. 1991; Watson 1992; Calder 1995; Gravier-Bonnet 1999;
97 Migotto et al. 2001; Cunha and Jacobucci 2010; Fernandez et al. 2014, 2015), and their
98 medusae are commonly reported in the plankton (e.g., Segura-Puertas and Damas-Romero
99 1997; Palma et al. 2014; Laakmann and Holst 2014; Nagata et al. 2014), occasionally in large
100 populations (Genzano et al. 2008). Despite the richness, abundance, and ubiquitousness of
101 the campanulariids, the basic knowledge on their phylogenetic relationships and taxonomy is
102 still highly deficient.

103 This study aims to propose a phylogenetic hypothesis for campanulariid hydroids
104 based on a large molecular dataset. With this hypothesis we evaluate the congruity and
105 relevance of diagnostic characters from traditional classifications over the last 100 years, at
106 the family, genus, and species levels. We evaluate the classification of Campanulariidae both
107 in its traditional sense (i.e., primarily based on studies without formal phylogenetic analyses,
108 e.g., Cornelius 1982), as well as the recently proposed phylogenetic classification (Maronna
109 et al. 2016).

110

111 **2. Material and methods**

112 2.1 Taxonomic sampling

113 Sequence data of the family Campanulariidae were obtained during this study and
114 from published works, comprising several localities (Tables 1-2). Most of the sequences
115 assigned to Campanulariidae and available in Genbank were considered in the analysis,
116 including those from Collins (2002), Collins et al. (2005, 2006), Govindarajan et al. (2006),
117 Evans et al. (2008), Leclère et al. (2009), Ortman et al. (2010), Peña Cantero et al. (2010),
118 Lindner et al. (2011), Zhou et al. (2013), Laakmann and Holst (2014) and He et al. (2015).

119 GenBank sequences from closely related taxa, such as species of Campanulinidae, Eirenidae,
120 Mitrocomidae, Lovenellidae and Phialellidae, were included as outgroups. Sequences
121 obtained during this study are deposited in GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>;
122 Table 1).

123 Specimens included in this study were generally sampled at low tide, although a few
124 colonies were collected at 20-30m deep with scuba diving (*Clytia* sp.1 and *Clytia noliformis*,
125 see Table 1). The hydroids occurred on a variety of substrates, including rocks, algae (mostly
126 Phaeophyceae, such as kelps and *Sargassum* sp.), mussel shells, cirripeds, sponges, floating
127 docks, *Rhizophora* sp. support roots, as well as other hydroids (e.g. *Ectopleura* sp.,
128 *Plumularia setacea*), and were preserved in 95-100% ethanol. Specimens were primarily
129 identified based on traditional morphological diagnostic characters, in accordance with
130 previous studies (Vervoort 1972; Millard 1971, 1975; Cornelius 1975, 1990, 1982, 1995b;
131 Calder 1991; Schuchert 2001; Vervoort and Watson 2003; Bouillon et al. 2004). Voucher
132 specimens were deposited in the Museu de Zoologia da Universidade de São Paulo, Brazil
133 (MZUSP) and in the National Museum of Natural History, Smithsonian Institution, United
134 States of America (USNM). Vouchers from previously published sequences were studied
135 whenever possible (see Table 2). This study comprises 35 nominal species, including
136 representatives of the three traditionally recognized subfamilies of Campanulariidae (cf.
137 Cornelius 1982; Calder 1991), as well as specimens of *Bonneviella* (family Bonneviellidae),
138 which has fallen within Campanulariidae in previous studies (Govindarajan et al. 2006). In
139 addition, all accepted genera were included, with the exception of *Gastroblasta* Keller 1883.
140 *Orthonia* Stechow, 1923 and *Eucalix* Stechow, 1921 have a doubtful taxonomic status, and
141 are not considered in this analysis. Similarly, *Billardia* Totton, 1930 is assigned to
142 Campanulariidae by many authors (Ralph 1957; Bouillon 1985; Vervoort and Watson 2003),
143 but previous phylogenetic analyses have consistently placed it well outside Campanulariidae
144 (Govindarajan et al. 2006; Leclère et al. 2009; Peña Cantero et al. 2010), and, therefore, it
145 was not included in this analysis. With respect to a recent phylogenetic study (Maronna et al.
146 2016), we have included data comprising the three families proposed (Campanulariidae,
147 Clytiidae, and Obelliidae), with a substantial increase in the number of sampled taxa within
148 each group.

149

150 2.2 Molecular data

151 Samples were processed in the Laboratory of Molecular Evolution (University of São
152 Paulo) and in the Laboratories of Analytical Biology (National Museum of Natural History).

153 DNA was extracted either with Agencourt DNAdvance (Beckman Coulter, Beverly, MA,
154 USA) or DNeasy (QUIAGEN, Valencia, CA, USA) extraction kits following the
155 manufacturer's protocol. Mitochondrial genes 16S and COI and nearly complete sequences
156 of nuclear 18S and 28S genes were obtained using standard PCR and sequencing primers
157 (Table S1).

158 PCRs were performed either in a total volume of 25 μ l (with 75mM Tris-HCL (pH 8.8
159 at 25°C), 20mM (NH₄)SO₂, 2.5mM MgCl₂, 0.26U/ μ l Taq polymerase (Thermo Fisher
160 Scientific, Waltham, MA, USA), 0.2mM dNTP and 0.4 μ M primers), 20 μ l (with 1x Phusion
161 Buffer, 0.02U/ μ l Taq polymerase Phusion (FinnZymes, Thermo Fisher Scientific, Waltham,
162 MA, USA), 1.1mM MgCl₂, 0.2mM dNTP, 0.4 μ M primers) or 10 μ l (with 10x NH₄ Buffer,
163 3mM MgCl₂, 0.05U/ μ l Biolase Taq polymerase (Bioline, London, UK), 0.1mM dNTP, 1x
164 bovine serum albumin (BSA), 3 μ M primers). Dimethyl sulfoxide (DMSO) was included in
165 some PCRs for amplification of nuclear genes (1.25 μ l for 25 μ l reactions, 0.5 μ l for 10 μ l
166 reactions). Subsequent steps were either conducted as described in Cunha et al. (2015), or by
167 the following procedure: PCR products were purified with ExoSapIT (Affymetrix, Santa
168 Clara, CA, USA), and used in cycle sequencing reactions with Big Dye Terminator v3.1 kit
169 (Applied Biosystems, Foster City, CA, USA) and diluted primers from PCR (0.03 μ M). Cycle
170 sequencing products were purified with Sephadex G-50 (Sigma-Aldrich, Buchs, Switzerland)
171 and sequenced on an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA,
172 USA). Both strands were sequenced for all samples.

173 Sequences were assembled and edited using Geneious v. 7.1 (Biomatters, Auckland,
174 New Zealand), and compared with those deposited in GenBank using the Basic Local
175 Alignment Search Tool (BLAST, Altschul et al. 1990) to confirm genes and species of
176 interest. Sequences were aligned using MAFFT (Kato et al. 2002), implemented in
177 Geneious R7, and missing ends were removed from the alignments using GBlocks
178 (Castresana 2000), implemented in SeaView (Gouy et al. 2010), with settings for a less
179 stringent selection. Gaps within the contiguous sequences of 18S and 28S were coded as
180 missing data in the alignments. For specimens with multiple sequences from the same locality,
181 only unique haplotypes were included in the analysis (mainly present in single-gene
182 phylogenies).

183

184 2.3 Phylogenetic analysis

185 Phylogenetic analyses were performed on (a) individual markers and (b) the
186 concatenated dataset (16S+COI+18S+28S). This last dataset was analyzed based on (b1) taxa

187 with sequences available for at least 3 markers (Dataset 1), and (b2) taxa with sequences
188 available for all 4 markers (Dataset 2). In the first case (b1), absent fragments in the
189 alignment were coded as missing data. The datasets were analyzed using parsimony (P),
190 maximum likelihood (ML) and Bayesian (B) criteria. Parsimony analyses were performed in
191 PAUP* v4b10 (Swofford 2002), with heuristic searches with 1,000 replicates of random-
192 addition-sequence, saving up to 100 trees per replicate, and branch-swapping by TBR (Tree
193 Bisection-Reconnection). Gaps were treated as a fifth state. For 16S and COI alignments,
194 10,000 replicates of random-addition-sequence were performed to improve search. Branch
195 support was estimated with bootstrap based on 1,000 replicates (with 10 replicates of
196 random-addition-sequence). ML analyses were performed in GARLI v2.01 (Zwickl 2006)
197 and consisted of 10 replicate searches with taxa randomly added to the starting tree. Branch
198 support was estimated with Bootstrap based on 100 replicates. Bayesian analyses were
199 performed using MrBayes 3.2 (Ronquist et al. 2012). For each dataset, two independent
200 searches were run for 5,000,000 generations and trees were sampled every 1,000 generations.
201 For convergence diagnostics and calculation of posterior probabilities and branch lengths, the
202 first 25% of the trees was discarded as burnin. For both ML and B, concatenated datasets
203 were analyzed as different partitions, corresponding to each gene. Models of molecular
204 evolution for each dataset were chosen using jModeltest v2.1.7 (Guindon and Gascuel 2003;
205 Darriba et al. 2012), with the Akaike Informarion Criterion (AIC, Table 3). For Bayesian
206 analyses, a GTR+G+I model or partitioned GTR+G+I models were used for the single-gene
207 and concatenated datasets, respectively. Uncorrected p-distances for COI and 16S were
208 calculated using PAUP* v4b10.

209

210 **3. Results**

211 All single-gene phylogenies agree in the lineages identified on less inclusive levels,
212 although there is lack of resolution and/or support for relationships among higher lineages,
213 especially at genus and family levels. Resolution and support enhances with the
214 concatenation of all genes. The concatenated dataset including taxa with sequences for at
215 least three genes (Dataset 1) comprises 181 taxa (Figures 1-2, Table 3), while the combined
216 dataset including taxa with sequences for all four genes (Dataset 2) has only 80 taxa (Figures
217 S1-S2, Table 3). The topologies generated from both datasets are highly congruent, and
218 minor contradictions usually occur among weakly supported groups. Therefore, we base our
219 conclusions largely on the results from Dataset 1, because its inclusiveness allows a broader
220 discussion. Similarly, since ML and B topologies are nearly identical for all datasets, we only

221 show the trees that resulted from ML analyses, with posterior probabilities plotted on these
222 trees. The 16S phylogeny derived from ML analysis is presented for further discussions on
223 specific lineages (Figures 3), and the remaining single-gene phylogenies are remarked upon
224 when relevant (Figures S3-9).

225

226 3.1 Family level

227 Topologies derived from the combined dataset show three main well supported
228 groups, which nearly agree with the three traditionally recognized subfamilies, as well as the
229 change of their status to family level (Figures 1-2). However, some discrepancies occur. The
230 P topology derived from Dataset 1 has the species *Clytia hummelincki*, *C. paulensis* and
231 Obeliida indet. ambiguously placed at the base of the Clytiidae+Obeliidae clade (Infraorder
232 Obeliida Maronna et al. 2016) (Figure 1), whereas the ML topology shows *C. hummelincki*
233 placed outside Clytiidae+Obeliidae, with high support (Figure 2) and Obeliida indet. at the
234 base of Obeliidae. Both topologies derived from Dataset 2 (Figures S1-S2) are congruent
235 with the last scenario. Additionally, sequences of *Bonneviella* (Bonneviellidae) are placed
236 within Campanulariidae, and are closely related to *Campanularia volubilis* and *Rhizocaulus*
237 *verticilatus* (Figures 1-2; Govindarajan et al. 2006). Three main clades are also recovered in
238 single-gene phylogenies, with the exception of COI (P topology) and 18S, in which some of
239 the recently proposed families and traditional subfamilies are not monophyletic with
240 negligible support (Figures S4-7). Also, most of the single-gene phylogenies have outgroup
241 representatives placed within the ingroup, but this is never well supported (Figures 3, S3-S9).

242

243 3.2 Genus level

244 Four of 11 genera included in the analysis were recovered as monophyletic in the
245 concatenated phylogenies: *Silicularia*, *Orthopyxis*, *Gonothyraea*, and *Hartlaubella* (Figures
246 1-2, S1-S2). *Tulpa* and *Rhizocaulus* are only represented by one species, so their monophyly
247 still needs testing. Although *Orthopyxis* was not recovered monophyletic in the P topology
248 derived from Dataset 2 (Figure S1), it is monophyletic in accordance with topologies derived
249 from Dataset 1 (Figures 1-2), which are more informative at the genus level. Similarly,
250 *Silicularia* was recovered as monophyletic in the concatenated phylogenies, while
251 *Campanularia* is clearly not monophyletic (Figures 1-2). *Bonneviella* was not recovered as
252 monophyletic in the P topology of Dataset 1, but this scenario is weakly supported (Figure 1).

253 *Clytia* is not monophyletic because two of its species are ambiguously placed: *C.*

254 *hummelincki* (always placed outside *Clytia* in concatenated phylogenies; Figures 1-2, S1-2),

255 and *C. paulensis* (placed at the base of *Clytia* in the ML topology of Dataset 1, but its
256 placement is unresolved in the P topology; Figure 1). There is no congruence and little
257 support for the position of these species in single gene phylogenies, although *C. paulensis* is
258 frequently placed inside *Clytia* (Figures 2-3, S6-S7). Similarly, specimens from Belize
259 identified as Obeliida indet. (CBC40.2 and CBC45_BLZ) were ambiguously placed at the
260 base of Clytiidae plus Obellidae (P topology of Dataset 1; Figure 1) or at the base of
261 Obeliidae (ML topologies, Figures 2 and 3), and this prevented us from reliably assigning the
262 specimens to either *Clytia* or *Obelia*.

263 Regarding Obellidae, only *Gonothyraea* and *Hartlaubella* were recovered as
264 monophyletic, with high posterior probabilities and bootstrap supports in most of the
265 phylogenies, including those of individual genes in which these lineages were sampled
266 (Figures 1-2, S4-9). *Laomedea* is not monophyletic because *L. flexuosa* falls outside the main
267 *Laomedea* clade (*L. angulata* + *L. calceolifera*) (Figures 1-2). *Obelia* is also not
268 monophyletic, since many of its lineages are more closely related to different genera than to
269 other species of *Obelia*. It is important to note that species of *Obelia* are distributed into four
270 different, well supported and rather distant (considering branch lengths in the ML topology,
271 Figure 2) monophyletic clades: *Gonothyraea*+*Obelia* (clade S), *Obelia*+*Laomedea* (clade
272 AA), *Obelia bidentata* (clade Z), *Obelia*+*Laomedea flexuosa*+*Hartlaubella* (clade AB)
273 (Figures 1-3). Relationships among these clades vary between ML and P topologies derived
274 from Dataset 1 (Figures 1-2), and only P topologies recover clade S at the base of Obeliidae.
275 However, both ML and P topologies derived from Dataset 2 (Figures S1-S2) also place clade
276 S at the base of the group, giving further support to this hypothesis.

277

278 3.3 Species level

279 Lineages at the species level are highly congruent among single-gene and
280 concatenated phylogenies (Figures 1-3, S1-S9). In Campanulariidae, *Silicularia rosea* is
281 formed by one clade from New Zealand (B) and another from Argentina (C), which are not
282 monophyletic in 16S topologies (Figures 3, S3). The maximal intra-clade distance,
283 considering the 16S, is 1.58% for clade B and 0.99% for clade C, and they have a minimum
284 inter-clade distance of 6.37% (Figure 4, clade D). The species is monophyletic, though, in
285 concatenated phylogenies (Figures 1-2, clade D). Similarly, *Orthopyxis integra* was
286 recovered in three different and relatively distant clades. One of these clades is closely related
287 to *O. crenata*, suggesting that misidentifications might have occurred (Clade H, Figure 1-3,
288 S4-5). Indeed, specimens of *O. integra* and *O. crenata* from clade H have low (<2%) intra-

289 clade distances considering the 16S, and 3.85% intra-clade distances on average for COI
290 (Figure 4). In addition, they form a monophyletic group with another *O. crenata* clade (G) in
291 concatenated phylogenies (Figures 1-2, clade I). Specimens identified as *O. integra* also
292 cluster with *O. everta* and *Orthopyxis* sp.1 (clade J, Figures 1-3), with maximum intra-clade
293 distances of 1.82% for 16S and 9.41% for COI (Figure 4).

294 Numerous lineages were recovered in Clytiidae, including seven identified as *Clytia*
295 *gracilis* that do not form a clade (Figures 1-2). Although these lineages seem to be
296 geographically structured, one species identified as *Clytia* sp. from China falls into *C.*
297 *gracilis* clade from Slovenia (clade K, Figures 1-3). This clade shows maximum intra-clade
298 distances of 5.41% for 16S and 6.62% for COI (Figure 4). Similarly, the species *Clytia*
299 *gulangensis*, also from China, clusters with specimens of *C. gracilis* from Brazil (clade N,
300 Figures 1-3), with maximum intra-clade distances of 2.67% for 16S and 5.71% for COI
301 (Figure 4). Also, specimens of *C. gracilis* from the Mediterranean split into two closely
302 related clades (K and L, minimum inter-clade distances are 6.60% for 16S and 9.65% for
303 COI, Figure 4, clade M), which are monophyletic in the ML concatenated phylogeny, but not
304 in the P concatenated phylogeny, as well as 16S and COI topologies (Figures 1-3, S3-5).
305 Additionally, specimens of *C. gracilis* also fall within a clade (O) comprising *C.*
306 *hemisphaerica* and the recently described *C. xiamenensis* (Figures 3-4). Intra-clade distances
307 (<2%) indicate close affinities between these specimens (Figure 4). Finally, *C. hemisphaerica*
308 split into two main reciprocally monophyletic clades (O and P, inter-clade distances in Figure
309 4, clade Q).

310 Four lineages corresponding to *Obelia dichotoma* were recovered in the Obeliidae
311 (Figures 1-3). Clade R is formed exclusively by Brazilian specimens, and is closely related to
312 the species *Gonothyraea loveni* in the concatenated phylogenies (Figures 1-2, clade S). Clade
313 T and U are closely related to each other and to the species *O. geniculata*, with which they
314 form a monophyletic group (W, intra and inter-clade distances in Figure 4). These two clades
315 also seem to be geographically structured, although USA specimens of *O. dichotoma* fall
316 within the Mediterranean clade (T). Specimens from the USA and Uruguay are also present in
317 a fourth *O. dichotoma* clade (X), which is more closely related to the species *Laomedea*
318 *flexuosa* and *O. longissima* (clade Y, Figures 1-3). *Obelia geniculata* lineages form three
319 different clades, unambiguously monophyletic in most of the phylogenies (Figures 1-3, S1-
320 S9). They show intra-clade distances ranging from 0 to 3.02% for 16S and 0.16% to 7.29%
321 for COI (clade V, Figure 4). Finally, *O. bidentata* forms a monophyletic clade in nearly all

322 topologies (except for COI, Figures 1-3, S1-S9), but its intra-clade distances are comparable
323 to inter-clade distances of other lineages (clade Z, Figure 4).

324

325 **4. Discussion**

326 The molecular phylogeny of the family Campanulariidae (in its traditional sense)
327 obtained in this study is incompatible with many current morphology-based taxonomic
328 hypotheses at the family, genus and species levels. Some morphological diagnostic characters
329 traditionally used for the delimitation of campanulariid species and genera are not
330 informative. The phylogenetic relationships presented herein are largely congruent with
331 previous molecular studies (Govindarajan et al. 2006; Zhou et al. 2013; He et al. 2015; Cunha
332 et al. 2015), but our increased taxon sampling provides a much more thorough test of prior
333 hypotheses. We found that mitochondrial markers (16S and COI) were informative for
334 delimitation of lineages at the species level, supporting their use as barcoding genes (e.g.,
335 *Obelia* and some *Clytia* medusae, Laakmann and Holst 2014; He et al. 2015).

336 Although the 16S is also considered useful for inferring relationships among
337 hydrozoan lineages at less inclusive levels (e.g., Moura et al. 2008; Peña Cantero et al. 2010;
338 Zhou et al. 2013; Calder et al. 2015; Cunha et al. 2015), our 16S-only phylogenies show
339 important inconsistencies with combined nuclear and mitochondrial genes phylogenies,
340 always at nodes with little support (Figures 3, S3). For instance, specimens of *Silicularia*
341 *rosea* (clade D) and *Clytia gracilis* (clade M) present high intra-clade distances (Figure 4)
342 and are not recovered as monophyletic in 16S topologies, but are well-supported
343 monophyletic lineages in combined nuclear and mitochondrial genes phylogenies (Figures 1-
344 2). Therefore, while 16S results could be interpreted as evidence for numerous cryptic
345 species, the use of more conserved, nuclear markers may indicate the existence of great
346 population subdivision within an otherwise morphologically cohesive species, which would
347 reconcile more readily with current taxonomy (see Schuchert 2014). Nuclear markers (18S
348 and 28S) were more informative at the genus and family levels, even though Obeliidae was
349 not recovered as monophyletic in the 18S analysis (Govindarajan et al. 2006, as subfamily
350 Obeliinae; this study, Figures S6-S7). In this particular case, signal from the 28S proved to be
351 more informative, increasing resolution and support at this level of the tree.

352

353 4.1 Delimiting campanulariids at the family level

354 Phylogenetic relationships at the family level obtained from our analyses are
355 congruent with the traditional taxonomy, which divides the family Campanulariidae in three

356 subfamilies (cf. Cornelius 1982; Calder 1991), as well as with the phylogenetic classification
357 of Proboscoida (cf. Maronna et al. 2016), in which the three main monophyletic groups that
358 comprise campanulariids were elevated to families. This classification improves the
359 taxonomy of the group when compared to the former subfamily division, especially
360 considering that there are few characters that are unique to Campanulariidae (in its traditional
361 sense), and the presence of a campanulate/bell-shaped hydrotheca is probably a
362 symplesiomorphy of the group. In this sense, including the three clades in the family rank
363 contributes to better communication by allowing for more direct reference and assessment of
364 the wide morphological diversity they exhibit. Our results further agree with the classification
365 of Maronna et al. (2016) in their recovery of clades that comprise the infraorders
366 Campanulariida and Obeliida, confirming the close affinities between Bonneviellidae and
367 Campanulariidae, as well as Clytiidae and Obeliidae. As a result, we follow Maronna et al.'s
368 classification, pointing out its incongruencies when compared to the traditional taxonomy and
369 our current results.

370 The family Campanulariidae is monophyletic in both its traditional sense and
371 according to Maronna et al. (2016), so long as *Bonneviella* is included and *Billardia* excluded
372 from its scope (Govindarajan et al. 2006; Penã Cantero et al. 2010; this study). However,
373 there is not a consensus about the taxonomic affinities of *Bonneviella* and *Billardia*, probably
374 because their phylogenetic relationships are not congruent with former morphological studies
375 (see Broch 1909, 1918; Cornelius 1995b: 221; Marques et al. 2006 for *Bonneviella*; Totton
376 1930; Vervoort 1972; Vervoort & Watson 2003 for *Billardia*). Our analysis supports
377 *Bonneviella* in the Campanulariidae, and if further sampling continues to support monophyly
378 of *Bonneviella* lineages, the presence of a pre-oral cavity in the hydranth (viz., veloid, Broch
379 1909; Yamada 1969; Schuchert 2001) would be a likely synapomorphy and diagnostic
380 character for the genus. *Billardia* is not part of the Campanulariidae (cf. Moura et al. 2011c),
381 even though the campanulate hydrotheca with a large but completely retractable hydranth
382 (Vervoort 1972; Vervoort and Watson 2003) may explain its initial inclusion in that family.
383 However, based on its phylogenetic relationships, these characters are probably
384 plesiomorphic in Leptothecata and should not be regarded as diagnostic of Proboscoida, at
385 least if other characters are not present for a reliable identification.

386 Although the three families comprising campanulariids were recovered in our
387 analysis, only Obeliidae is close to its original scope (Obeliida indet. is frequently placed at
388 the base of the group, but with little support, see Figures 1-3). The family Campanulariidae,
389 traditionally characterized by a subhydrothecal spherule and annular perisarc thickening at

390 the hydrothecal base, but lacking a true diaphragm (see Cornelius 1982; Calder 1991;
391 Bouillon 1985, as subfamily Campanulariinae), is monophyletic if *Tulpa* and *Bonneviella* are
392 included, even though these genera do not have subhydrothecal spherules (Stechow 1921;
393 Vervoort 1972; Schuchert 2001; Vervoort and Watson 2003). Annular perisarc thickening
394 occurs in *Tulpa* and, although not universal (i.e., absent in *Bonneviella*), would appear to be
395 the best available morphological character to delimit the family (Boero et al. 1996).

396 Clytiidae becomes monophyletic if *Clytia hummelincki* is excluded from the family.
397 This scenario, however, is ambiguously supported by our results. The subhydrothecal
398 spherule of *C. hummelincki*, a character commonly associated with Campanulariidae, as well
399 as a diaphragm and medusae with tentacle bulbs, characteristic of Clytiidae, led Cornelius
400 (1982) to regard this species as having uncertain taxonomic affinities, although he followed
401 Millard (1966) and kept the species in Clytiidae (as subfamily Clytiinae) based on characters
402 of the medusa stage. Govindarajan et al. (2006, concatenated phylogeny) recovered this
403 species at the base of the Clytiidae (as subfamily Clytiinae), and concluded that the
404 subhydrothecal spherule is plesiomorphic of campanulariids in general. Given the
405 phylogenies that resulted from our analysis (Figures 1-2), the hypothesis of plesiomorphy is a
406 possibility, as well as the hypothesis of convergence (Cornelius 1982: 83), since it is also
407 possible that the character originated at the base of Campanulariidae, and again at the lineage
408 leading to *C. hummelincki*. Additional evidences are necessary to corroborate one of these
409 hypotheses.

410 The ambiguous placement of several *Clytia* (e.g., *C. hummelincki*, *C. paulensis*,
411 Figures 1-2) and Obeliida indet. within Clytiidae plus Obellidae suggests these groups have
412 close taxonomic affinities. In fact, the two families are only differentiated based on medusa
413 characters, since their polyps are mainly characterized by a true hydrothecal diaphragm
414 (Cornelius 1982; Calder 1991, as subfamilies Clytiinae and Obeliinae). Following previous
415 authors, Boero et al. (1996) suggested that Clytiidae and Obellidae, as former subfamilies,
416 should be merged, considering that lineages in Obellidae that lost their medusa stage (e.g.,
417 *Laomedea*, *Hartlaubella*) can not be differentiated from *Clytia* exclusively based on hydroid
418 characters. Indeed, establishing diagnostic characters of Obelliidae based on medusa
419 characters is problematic, because most of its genera do not produce free medusae. Given the
420 phylogenetic patterns presented herein, Clytiidae and Obeliidae are not unequivocally
421 supported as monophyletic groups, and their classification as two distinct families still needs
422 further assessment and refinement.

423 Specimens identified as Obeliida indet. (CBC40.2 and CBC45_BLZ) are similar to
424 *Clytia stolonifera* (Blackburn, 1938) (Figure S10), a species described from Australia
425 (Blackburn 1938; Watson 2005), but recently recorded in Brazil by Fernandez et al. (2014,
426 2015). Although this species was originally considered in the genus *Clytia*, its gonophores
427 have not been described, and our material lacks gonothecae as well. Considering the
428 ambiguous phylogenetic position of these specimens, frequently placed at the base of
429 Obeliidae, we conservatively considered them non-identified, at least until more information
430 and specimens (particularly from the type locality) are available for a reliable identification
431 of the species.

432

433 4.2 Generic limits in Proboscoida

434 As in other groups of Hydrozoa, gonophore morphology has long been used to
435 distinguish genera of Campanulariidae (cf. Cornelius 1982). Nevertheless, the topic is much
436 debated and there are significant doubts that species with different types of gonophores (fixed
437 sporosacs or free medusae) should be assigned to separate genera (Levinsen 1893; Kramp
438 1935; Rees 1957; Petersen 1990). Campanulariidae remarkably has gonophores varying from
439 fixed sporosacs, released or retained medusoids, and meconidia to free medusae, including
440 the singular medusae of *Obelia* (Cornelius 1990; Boero et al. 1996). Even though the
441 occurrence of gonophore reduction, from free medusae to fixed gonophores, was
442 hypothesized to reflect phylogenetic patterns in the family (Boero and Sarà 1987), subsequent
443 studies showed that taxonomical classification based on types of gonophores does not result
444 in monophyletic genera, because medusa reduction can happen multiple times within the
445 same genus (Petersen 1990; Cunningham and Buss 1993). Following these ideas, *Laomedea*,
446 *Clytia*, and *Obelia* were thought not to be monophyletic (Boero et al. 1996), and these
447 hypotheses were indeed corroborated by molecular studies (Govindarajan et al. 2006; this
448 study). Our phylogenies shows that species with *Obelia*-like medusae do not form a
449 monophyletic group, as well as those species with fixed gonophores, indicating that these
450 characters are inappropriate for diagnosing different genera.

451 Even if we consider some of the main classifications proposed during the last 100
452 years, there are no or few classifications in which the scope of *Campanularia*, *Clytia*, *Obelia*,
453 and *Laomedea* could be considered monophyletic based on our phylogenetic analyses
454 (Figures 1-3, Table 4). In contrast, *Orthopyxis*, *Silicularia*, *Gonothyraea*, and *Hartlaubella*
455 are consistent with most of the proposed classifications (Table 4). These inconsistencies and

456 variation occur because most of the classifications separate genera based on the type of
457 gonophore, and conspicuous morphological diagnostic characters are absent in some groups.

458 *Campanularia*, the most problematic genus of Campanulariidae, appears to be
459 polyphyletic, and its current diagnostic characters are symplesiomorphies of the family (e.g.,
460 stolonial colonies, campanulate hydrotheca, annular perisarc thickening, subhydrothecal
461 spherule, fixed sporosacs; Ralph 1957; Cornelius 1982; Bouillon 1985; Calder 1991).
462 Considering the phylogenetic patterns of *Campanularia* presented in this study, the definition
463 of separate genera for the different clades would probably be the best taxonomic decision to
464 establish monophyletic genera in Campanulariidae, other than including all species of the
465 family, with a wide morphological diversity, into a large and single genus. There is little
466 basis for this decision, however, at the moment, given that there are few morphological
467 characters that could differentiate the new genera, and, most importantly, the typical
468 *Campanularia* Lamouroux, 1812. In addition, *Campanularia volubilis*, the type species of the
469 genus, appears to be more closely related to species of *Bonneviella* than to other
470 *Campanularia* (Figures 1-3), forming a clade that was thought to represent a local radiation
471 (Govindarajan et al. 2006). In order to make sound taxonomic decisions, it is important that
472 the phylogenetic relationships and morphological characters of more representatives of the
473 typical *Campanularia* are studied. This will lead to a better characterization of
474 *Campanularia*, contributing to the definition of new genera.

475 Considering the phylogenetic patterns of *Clytia* presented in this study, the only
476 classifications that are congruent with a monophyletic genus are those that disregard the
477 subhydrothecal spherule as part of the diagnostic characters of *Clytia*, which excludes *C.*
478 *hummelincki* (Nutting 1915; Ralph 1957; Hirohito 1995). In spite of that, traditional
479 diagnostic characters of the hydroids (e.g., stolonial or erect colonies and true hydrothecal
480 diaphragm; Millard 1975; Cornelius 1982, 1995b; Bouillon 1985; Calder 1991; Bouillon et
481 al. 2004) are not entirely relevant to delimit the genus, because they are shared with species
482 of Obeliidae. Characters of the medusa stage, on the other hand, are important diagnostic
483 features for *Clytia*, and they support the inclusion of *C. hummelincki* in the genus (Gravili et
484 al. 2008). Nevertheless, additional studies on *C. hummelincki* are crucial to ascertain its
485 precise phylogenetic positions.

486 Obeliidae is the most problematic family within Proboscoida, including nearly all
487 types of gonophores, but this variation is clearly not informative to delimit genera.
488 *Gonothyraea* might be the only exception, considering that meconidia are exclusive in that
489 genus, and therefore it is regarded as distinct in most of the classifications proposed (Nutting

490 1915; Ralph 1957; Millard 1975; Cornelius 1982, 1995b; Bouillon 1985; Table 4).
491 *Hartlaubella* is considered a distinct genus in most classifications because of its polysiphonic
492 colonies, clearly differentiating this genus from *Laomedea*, although both have gonophores as
493 fixed sporosacs (Cornelius 1982, 1995b; Bouillon 1985; Bouillon et al. 2004). Trophosomal
494 characters, however, are irrelevant for the delimitation of *Laomedea* and *Obelia*, both
495 described as presenting erect, sympodial colonies, with a true hydrothecal diaphragm
496 (Nutting 1915; Millard 1975; Cornelius 1975, 1982; Calder 1991; Bouillon 1985; Bouillon et
497 al. 2004). Indeed, *Obelia* would become monophyletic only by the inclusion of *Laomedea*,
498 *Hartlaubella*, and *Gonothyraea*, similar to what was proposed by Naumov (1969). In this
499 sense, there are no conspicuous or unambiguous morphological characters, neither from
500 hydroids nor medusae, that support *Obelia* or *Laomedea* as monophyletic genera. The
501 reassessment of their scope and morphological diagnostic characters is critical to reflect the
502 phylogenetic patterns of the family.

503

504 4.3 Species boundaries in Proboscoida

505 At least three campanulariid species are not monophyletic and include cryptic
506 lineages: *Orthopyxis integra*, *Clytia gracilis*, and *Obelia dichotoma*. Indeed, some of these
507 species were shown to be polyphyletic in previous molecular studies (Govindarajan et al.
508 2005, 2006; Lindner et al. 2011; Cunha et al. 2015). Several other species showed signs of
509 population subdivision (*Silicularia rosea*, *Orthopyxis crenata*, *Clytia hemisphaerica*, *Obelia*
510 *geniculata*), although they resulted as monophyletic in the concatenated analysis (Figures 1-
511 2). Also, most of them have identification problems related to their wide morphological
512 variability and/or lack of conspicuous diagnostic characters (Ralph 1957; Cornelius 1982,
513 1995b). These problems contribute to misinterpretations about intra and interspecific
514 variations, leading to the discovery of cryptic species that are frequently a result of previously
515 overlooked morphological differences (e.g., Lindner et al. 2011; Cunha et al. 2015; also see
516 Cunha et al. 2016). Sequences from type localities help to link the identification of clades as
517 the typical species, and we tried to include them whenever possible, as well as comparisons
518 with the presumably typical diagnostic morphological characters.

519 Cornelius (1982, 1995b) included more than 15 nominal species in the synonymy of
520 *Orthopyxis integra*, a species believed to be cosmopolitan and to comprise several different
521 morphotypes as a result of its wide morphological variability (e.g., thickened to unthickened
522 hydrothecal walls, sinuous to smooth pedicels, smooth to completely spirally grooved
523 gonotheca). Govindarajan et al. (2006) were the first to show that this species comprised

524 several cryptic lineages, and Cunha et al. (2015) remarked that much of the variation within
525 *O. integra* was overestimated, attributing part of its former morphotypes to two different
526 species (*O. caliculata* (Hincks, 1853) and *O. mianzani* Cunha, Genzano & Marques, 2015).
527 The “true” *O. integra* was assigned to the morphotype with a spirally grooved gonotheca
528 (Cunha et al. 2015: 21). Following these ideas, we argue that the clade comprising the
529 specimen of *O. integra* from the Aleutian Islands (*O.integra_1_USA* =*O.integra*(AK),
530 Govindarajan et al. 2006) probably corresponds to the “true” *O. integra*, which comprises
531 specimens from the USA, Iceland, and Argentina (see 16S phylogenies, Figure 3, S3). The
532 specimen of *O. integra* from New Zealand (*O.integra_NZ*, Govindarajan et al. 2006) clusters
533 with a specimen of *O. crenata* also from New Zealand, the type locality of *O. crenata*
534 (Hartlaub 1901; Vervoort & Watson 2003; see 16S phylogenies, Figures 3, S3), and with an
535 unidentified specimen from Argentina (*Orthopyxis* sp._Co1_ARG, Cunha et al. 2015),
536 believed to have close affinities with *O. crenata* (Cunha et al. 2015). This evidence suggests
537 that *O.integra_NZ* is a misidentification, also because this clade clusters with specimens of
538 *O. crenata* from Brazil. The variation of the hydrothecal cusps of *O. crenata* may lead to
539 confusion with *O. integra* for the occasional occurrence of even hydrothecal margins among
540 its specimens (Ralph 1957; Millard 1975). Finally, the specimen of *O. integra* from Italy
541 (*O.integra_IT*, Govindarajan et al. 2006), clusters with specimens of *O. everta* and
542 *Orthopyxis* sp. 1, all from the Mediterranean (Italy and Slovenia, Table 1 and 2), and they are
543 clearly separated from the “true” *O. integra* (clade F, Figures 1-3). This suggests the clade
544 comprising *O.integra_IT* corresponds to a different species occurring in the Mediterranean
545 Sea (e.g., *O. asymmetrica*; cf. Peña Cantero and García Carrascosa 2002; Bouillon et al.
546 2004).

547 Similarly to *O. integra*, *Clytia gracilis* is also considered to be widely distributed
548 (Calder 1991; Cornelius 1995b), and was long regarded as conspecific with *C.*
549 *hemisphaerica*, based on the variation of its hydrothecal cusps and gonothecal shape (Ralph
550 1957, as *C. johnstoni*; Millard 1966; Cornelius 1982). Several subsequent studies, however,
551 found consistent differences among characters of the hydranths and nematocysts of the two
552 species, and demonstrated that many trophosomal characters previously regarded as
553 intraspecific variations were actually more likely to be diagnostic at the species level
554 (Östman 1979; Cornelius 1987a,b, 1995b). Unfortunately, based on our molecular
555 phylogenies, we are unable to assign any of the lineages to the “true” *C. gracilis*, because
556 none of our sequences come from the type locality of the species (i.e. Lofoten, Norway, Sars
557 1850) or any close locality. This would give further support for the identification of the

558 typical species, since the generally accepted concept of *C. gracilis* probably still comprises
559 morphological variations erroneously interpreted as intraspecific. Recently, the location of
560 the insertion of the gonotheca (hydrorhiza or stem) was shown to differentiate *C. gracilis*
561 from *C. elsaeoswaldae* (Lindner et al. 2011), which is corroborated as a distinct,
562 monophyletic lineage in this study (Figures 1-3). The shape of the hydrothecal cusps and
563 gonothecae of the polyps, as well as size, shape of gonads and number of tentacles of the
564 medusae, were considered diagnostic for two new species of *Clytia*, *C. xiamenensis* and *C.*
565 *gulangensis*, differentiating them from *C. hemisphaerica* and *C. gracilis*, their presumed
566 closest congeners (Zhou et al. 2013; He et al. 2015; see Figures 1-3). The fact that *C.*
567 *gulangensis* clusters with specimens identified as *C. gracilis* from Brazil, and *C. xiamenensis*
568 clusters with specimens identified as *C. hemisphaerica* from the USA (as well as *C. cf.*
569 *gracilis*_sp.A, Lindner et al. 2011; Figures 1-3) suggests that morphological characters
570 distinguishing these species as separate and valid are still unclear. Although the shape of
571 hydrothecal cusps and gonothecae were traditionally used to differentiate *C. gracilis* and *C.*
572 *hemisphaerica*, these characters are not informative for the delimitation of these species,
573 considering the phylogenies presented herein.

574 A similar situation occurs among Obeliidae, in which *Obelia dichotoma* is not
575 monophyletic (Figures 1-3). This would be expected, considering that several diagnostic
576 characters of species of *Obelia* are frequently reported as intraspecifically variable,
577 hampering species identification (Cornelius 1975, 1982, 1990, 1995b). Cornelius (1975) was
578 the first to conduct a formal revision of the genus, in which he regarded several diagnostic
579 characters of the polyp and medusa of distinct species as intraspecific variations, lumping
580 more than 80 nominal species of *Obelia* into three (*O. bidentata*, *O. geniculata*, and *O.*
581 *dichotoma*). Among these characters, colony size, branching, shape of the hydrothecal rim
582 and number of annulations in the pedicels of the polyps, as well as number of tentacles and
583 position of the gonads on the medusae were shown to be variable, and correlated with
584 changes in environmental factors (Ralph 1956; Ralph and Thomson 1968; Hughes 1980;
585 Kubota 1981). Later, Östman (1982a,b) showed consistent differences in the nematocyst
586 types and isoenzyme patterns among *O. dichotoma* and *O. longissima*, regarding both species
587 as valid, and was followed by Cornelius (1990, 1995b), who also corroborated the validity of
588 the species based on characters of the hydranths (Cornelius 1987b). Currently, the four
589 species are separated based solely on polypoid characters, since medusa characters do not
590 seem to be reliable for their morphological distinction (Cornelius 1975, 1990, 1995b).
591 Diagnostic characters of *O. bidentata* and *O. geniculata* are usually conspicuous and were

592 corroborated to delimit monophyletic lineages, but there is still much confusion in the
593 separation of the other two species. *Obelia dichotoma* is mainly distinguished from *O.*
594 *longissima* by its branching patterns and shape of the hydrothecal rim (Cornelius 1990,
595 1995b), but our analysis shows these characters are not informative for the delimitation of the
596 species. In fact, further discriminations of these characters have recently corroborated the
597 revalidation of former synonyms of *O. dichotoma* (Calder 2013, Calder et al. 2014), and this
598 might also prove to be the case for the cryptic lineages of *O. dichotoma* presented in this
599 study.

600

601 **5. Conclusions**

602 Taxonomic investigation on Hydrozoa benefits from the inclusion of molecular data,
603 as a matter of achieving a more complete understanding of species boundaries and the
604 relevance of diagnostic characters. We corroborated previous results and presented novel
605 evidence on the phylogenetic relationships within the suborder Proboscoida. Considering the
606 traditional morphological diagnostic characters of the group and the phylogenetic patterns
607 presented in this study, the scope of the family Campanulariidae (cf. Cornelius 1982) should
608 be changed, as well as the scope of its subfamilies and, in this sense, the phylogenetic
609 classification recently proposed by Maronna et al. (2016), which raises the subfamilies to
610 families, improves the taxonomy of the group. However, the relationships among taxa within
611 Clytiidae and Obeliidae still remain open questions. Additionally, generic limits will only
612 reflect phylogenetic patterns if different types of gonophores are disregarded as generic
613 characters, especially within Obeliidae. Finally, diagnostic characters of several species have
614 to be reassessed based on a detailed study of their patterns of morphological variation,
615 including material from type localities whenever possible. Further morphological studies are
616 essential to establish a solid taxonomic and phylogenetic framework for the classification of
617 Proboscoida, contributing to a broader discussion on morphological variation and species
618 delimitation, particularly in extensively variable groups.

619

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640

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Figure 1. Strict consensus of 725 most parsimonious trees based on 16S, COI, 18S and 28S data. Only taxa with sequences for at least 3 genes (Dataset 1) were analyzed. Bootstrap values are shown for each node. Nodes without numbers indicate support below 70%. Node letters in accordance with Figure 4. Taxa in bold indicate specimens obtained during this study. Colors indicate taxa traditionally included in the subfamilies Campanulariinae, Clytiinae and Obeliinae (cf. Cornelius 1982), which were elevated to family level by Maronna et al. (2016). For specimens codes and site abbreviations see Tables 1-2.

Figure 2. Maximum likelihood tree based on 16S, COI, 18S and 28S data. Only taxa with sequences for at least 3 genes (Dataset 1) were analyzed. Bootstrap values/posterior probabilities (Bayesian analysis) are shown for each node, with dots (/.) representing the same value for both measures. Nodes without numbers (-) indicate support below 70/95%. Node letters in accordance with Figure 4. Taxa in bold indicate specimens obtained during this study. Colors indicate taxa traditionally included in the subfamilies Campanulariinae, Clytiinae and Obeliinae (cf. Cornelius 1982), which were elevated to family level by Maronna et al. (2016). For specimens codes and site abbreviations see Tables 1-2.

Figure 3. Maximum likelihood tree based on 16S data. Branch colors, support values, and other notations are described in Figure 2. For specimens codes and site abbreviations see Tables 1-2.

Figure 4. Intra and inter-clade uncorrected p-distances based on 16S and COI data. Mean distances with minimum and maximum values are presented for each clade. Letters are in accordance with clades shown in Figures 1-3, S3-S4).

Table 1. Codes, sampling sites, museum vouchers and GenBank accession numbers ([-]=no sequence) for the sequences obtained during this study. Sequences marked with * were obtained in Cunha et al. (2015). MZUSP=Museu de Zoologia da Universidade de São Paulo, USNM=National Museum of Natural History, Smithsonian Institution.

| Code in tree | Species | Locality | Geographic coordinate | Voucher | GenBank Acession Numbers | | | |
|--------------|-----------------------------------|---------------------------------------|-----------------------|------------|--------------------------|------------|----------|----------|
| | | | | | 16S | COI | 18S | 28S |
| IT10_IT | <i>Campanularia hincksii</i> | Ancona, Italy | not available | MZUSP 2759 | KX665304 | - | KX665403 | KX665500 |
| IT14_IT | <i>Campanularia hincksii</i> | Paraggi, Italy | not available | MZUSP 2760 | KX665308 | - | KX665407 | - |
| PT10_ARG | <i>Campanularia</i> sp. | Punta Cuevas, San Julián, Argentina | not available | MZUSP 2761 | KX665335 | KX665233 | KX665434 | - |
| SJ2_ARG | <i>Campanularia subantarctica</i> | La Mina, Puerto San Julián, Argentina | -49.1569 -67.6331 | MZUSP 2639 | KM405574 * | KM405569 * | KX665450 | - |
| SJ4_ARG | <i>Campanularia</i> sp. | La Mina, Puerto San Julián, Argentina | -49.1569 -67.6331 | MZUSP 2641 | KM405572 * | KM405571 * | KX665154 | KX665523 |
| PM36_BRA | <i>Clytia elsaeoswaldae</i> | Palmas Island, Brazil | not available | MZUSP 2762 | KX665328 | KX665227 | KX665428 | KX665516 |
| CB19_BRA | <i>Clytia elsaeoswaldae</i> | Cabras Island, Ilhabela, Brazil | not available | MZUSP 2763 | KX665260 | KX665163 | KX665365 | KX665466 |
| PM18_BRA | <i>Clytia elsaeoswaldae</i> | Palmas Island, Brazil | not available | MZUSP 2764 | KX665327 | KX665226 | - | - |
| Me26_BRA | <i>Clytia elsaeoswaldae</i> | Mel Island, Brazil | -25.5561 -48.2987 | MZUSP 2765 | KX665315 | KX665215 | - | - |
| EL05_SLV | <i>Clytia gracilis</i> | Mund Bay, Piran, Slovenia | not available | MZUSP 2766 | KX665278 | KX665181 | KX665377 | KX665477 |
| EL14_SLV | <i>Clytia gracilis</i> | Strunjan, Piran, Slovenia | 45.5370 13.6014 | MZUSP 2767 | KX665282 | KX665185 | KX665381 | KX665481 |
| EL15_SLV | <i>Clytia gracilis</i> | Strunjan, Piran, Slovenia | 45.5370 13.6014 | MZUSP 2768 | KX665283 | KX665186 | KX665382 | KX665482 |
| EL31_SLV | <i>Clytia gracilis</i> | Slovenia | 45.5912 13.6998 | MZUSP 2769 | KX665289 | KX665192 | KX665388 | KX665488 |
| EL32_SLV | <i>Clytia gracilis</i> | Slovenia | 45.5912 13.6998 | MZUSP 2770 | KX665290 | KX665193 | KX665389 | KX665489 |
| EL38_SLV | <i>Clytia gracilis</i> | Piran, Slovenia | 45.5303 13.5675 | MZUSP | KX665292 | KX665195 | KX665391 | KX665491 |

| Code in tree | Species | Locality | Geographic coordinate | Voucher | GenBank Accession Numbers | | | |
|--------------|------------------------|--|-----------------------|-----------------|---------------------------|----------|----------|----------|
| | | | | | 16S | COI | 18S | 28S |
| IT12_IT | <i>Clytia gracilis</i> | Ancona, Italy | not available | 2771 MZUSP | KX665306 | KX665208 | KX665405 | KX665502 |
| IT13_IT | <i>Clytia gracilis</i> | Ancona, Italy | not available | 2772 MZUSP | KX665307 | - | KX665406 | KX665503 |
| CBC13_BLZ | <i>Clytia gracilis</i> | Twin Cays, Belize | 16.8282 -88.1073 | 2773 USNM1 | KX665262 | KX665166 | KX665367 | KX665468 |
| CBC20_BLZ | <i>Clytia gracilis</i> | Carrie Bow Cay, Belize | 16.8080 -88.0630 | 420648 USNM1 | KX665263 | KX665167 | - | - |
| CBC26_BLZ | <i>Clytia gracilis</i> | Twin Cays Fisheries Dock, Belize | 16.8235 -88.1060 | 420655 USNM1 | KX665265 | KX665169 | - | - |
| MAP01_BRA | <i>Clytia gracilis</i> | Panaquatira, São Luís do Maranhão, Brazil | -2.4984 -44.0239 | 420660 MZUSP | KX665310 | KX665210 | KX665411 | KX665506 |
| MAP11_BRA | <i>Clytia gracilis</i> | Panaquatira, São Luís do Maranhão, Brazil | -2.4984 -44.0239 | 2774 MZUSP | KX665312 | KX665212 | - | - |
| PAF03_BRA | <i>Clytia gracilis</i> | Farol Velho, Salinópolis, Brazil | -0.591 -47.3248 | 2775 MZUSP | KX665321 | KX665221 | KX665422 | KX665512 |
| T1_BRA | <i>Clytia gracilis</i> | Flexeiras, Trairí, Brazil | -3.217 -39.2671 | 2776 MZUSP | KX665352 | KX665249 | KX665456 | KX665527 |
| T5_BRA | <i>Clytia gracilis</i> | Flexeiras, Trairí, Brazil | -3.2222 -39.2502 | 2777 MZUSP | KX665354 | KX665251 | KX665458 | KX665529 |
| T6_BRA | <i>Clytia gracilis</i> | Flexeiras, Trairí, Brazil | -3.2222 -39.2502 | 2778 MZUSP | KX665355 | KX665252 | - | - |
| CE1_BRA | <i>Clytia gracilis</i> | Náutico, Fortaleza, Brazil | not available | 2779 MZUSP | KX665271 | KX665173 | KX665372 | - |
| CE2_BRA | <i>Clytia gracilis</i> | Caponga, Cascavel, Brazil | -4.0391 -38.1929 | 2780 MZUSP | KX665272 | KX665174 | - | - |
| CE3_BRA | <i>Clytia gracilis</i> | Náutico, Fortaleza, Brazil | not available | 2781 MZUSP | KX665273 | KX665175 | - | - |
| CE5_BRA | <i>Clytia gracilis</i> | Caponga, Cascavel, Brazil | -4.0391 -38.1929 | 2782 MZUSP | KX665275 | KX665177 | - | - |
| Me24_BRA | <i>Clytia gracilis</i> | Mel Island, Brazil | -25.5729 -48.3091 | 2783 MZUSP | KX665314 | KX665214 | KX665414 | - |

| Code in tree | Species | Locality | Geographic coordinate | Voucher | GenBank Accession Numbers | | | |
|--------------|-----------------------------|-------------------------------------|-----------------------|-----------------|---------------------------|----------|----------|----------|
| | | | | | 16S | COI | 18S | 28S |
| PT9_ARG | <i>Clytia gracilis</i> | Punta Cuevas, San Juliàn, Argentina | not available | 2784 MZUSP | KX665334 | KX665232 | KX665433 | - |
| FLT03_USA | <i>Clytia hemisphaerica</i> | Westport, USA | 41.5129 -71.0765 | 2785 MZUSP | KX665296 | KX665199 | KX665395 | KX665495 |
| HCM04_USA | <i>Clytia hemisphaerica</i> | Salem, USA | 42.5219 -70.8822 | 2786 MZUSP | KX665301 | KX665204 | KX665400 | - |
| MMA05_USA | <i>Clytia hemisphaerica</i> | Bourne, USA | 41.7397 -70.6242 | 2787 MZUSP | KX665318 | KX665218 | KX665417 | - |
| PTJ01_USA | <i>Clytia hemisphaerica</i> | Point Judith, Rhode Island, USA | 41.3877 -71.5171 | 2788 MZUSP | - | KX665240 | KX665441 | - |
| EL06_SLV | <i>Clytia hemisphaerica</i> | Mund Bay, Piran, Slovenia | not available | 2789 MZUSP | KX665279 | KX665182 | KX665378 | KX665478 |
| EL08_SLV | <i>Clytia hemisphaerica</i> | Mund Bay, Piran, Slovenia | not available | 2790 MZUSP | KX665280 | KX665183 | KX665379 | KX665479 |
| EL12_SLV | <i>Clytia hemisphaerica</i> | Strunjan, Piran, Slovenia | 45.5370 13.6014 | 2791 MZUSP | KX665281 | KX665184 | KX665380 | KX665480 |
| EL20_SLV | <i>Clytia hemisphaerica</i> | Strunjan, Piran, Slovenia | 45.5370 13.6014 | 2792 MZUSP | KX665285 | KX665188 | KX665384 | KX665484 |
| EL28_CRO | <i>Clytia hemisphaerica</i> | Croatia | not available | 2793 MZUSP | KX665287 | KX665190 | KX665386 | KX665486 |
| EL35_SLV | <i>Clytia hemisphaerica</i> | Slovenia | 45.5912 13.6998 | 2794 MZUSP | KX665291 | KX665194 | KX665390 | KX665490 |
| CBC1_BLZ | <i>Clytia hemisphaerica</i> | Carrie Bow Cay, Belize | not available | 2795 USNM1 | KX665261 | KX665165 | - | - |
| CBC25_BLZ | <i>Clytia hemisphaerica</i> | Twin Cays Fisheries Dock, Belize | 16.8235 -88.1060 | 420636 USNM1 | KX665264 | KX665168 | KX665368 | KX665469 |
| CBC40.1_BLZ | <i>Clytia hemisphaerica</i> | Cuda Cut, Twin Cays, Belize | not available | 420659 USNM1 | KX665267 | KX665171 | - | - |
| CBC42_BLZ | <i>Clytia hummelincki</i> | Cuda Cut, Twin Cays, Belize | not available | 420673 USNM1 | KX665269 | KX665172 | KX665370 | KX665471 |
| PY10_BRA | <i>Clytia linearis</i> | Paraty, Brazil | not available | 420675 MZUSP | KX665343 | - | KX665444 | KX665519 |

| Code in tree | Species | Locality | Geographic coordinate | Voucher | GenBank Accession Numbers | | | |
|--------------|--------------------------------|--|-----------------------|-----------------------|---------------------------|----------|----------|----------|
| | | | | | 16S | COI | 18S | 28S |
| SP3_BRA | <i>Clytia noliformis</i> | Barão Tefé Island, São Pedro and São Paulo Archipelago, Brazil | not available | 2796 MZUSP 2797 | KX665349 | KX665246 | KX665453 | KX665525 |
| SP9_BRA | <i>Clytia noliformis</i> | Barão Tefé Island, São Pedro and São Paulo Archipelago, Brazil | not available | MZUSP 2798 | KX665350 | KX665247 | KX665454 | KX665526 |
| SP1_BRA | <i>Clytia</i> sp.1 | Boca da Enseada, São Pedro and São Paulo Archipelago, Brazil | not available | MZUSP 2799 | KX665348 | KX665245 | KX665452 | KX665524 |
| CE4_BRA | <i>Clytia</i> sp.2 | Caponga, Cascavel, Brazil | -4.0391 -38.1929 | MZUSP 2800 | KX665274 | KX665176 | KX665373 | KX665473 |
| NAT05_BRA | <i>Clytia</i> sp.3 | Jenipabú, Natal, Rio Grande do Norte | -5.70 -35.19 | MZUSP 2801 | KX665320 | KX665220 | KX665419 | KX665509 |
| CBC45_BLZ | Obeliida indet. | Cuda Cut, Twin Cays, Belize | not available | USNM1 420678 | KX665270 | - | KX665371 | KX665472 |
| CBC40.2_BZ | Obeliida indet. | Cuda Cut, Twin Cays, Belize | not available | USNM1 420685 | KX665268 | - | - | - |
| BPM03_USA | <i>Gonothyraea loveni</i> | Plymouth, USA | not available | MZUSP 2802 | KX665257 | KX665161 | KX665362 | KX665464 |
| SWM03_USA | <i>Gonothyraea loveni</i> | Sandwich, USA | 41.7703 -70.5036 | MZUSP 2803 | KX665351 | KX665248 | KX665455 | - |
| PT13_ARG | <i>Hartlaubella gelatinosa</i> | Río Gallegos, Argentina | not available | MZUSP 2804 | KX665337 | - | - | - |
| PT14_ARG | <i>Hartlaubella gelatinosa</i> | Río Gallegos, Argentina | not available | MZUSP 2805 | KX665338 | KX665235 | KX665436 | - |
| PT16_ARG | <i>Hartlaubella gelatinosa</i> | Río Gallegos, Argentina | not available | MZUSP 2806 | KX665339 | KX665236 | KX665437 | - |
| EL40_SLV | <i>Laomedea angulata</i> | Piran, Slovenia | 45.515 13.5794 | MZUSP 2807 | KX665293 | KX665196 | KX665392 | KX665492 |
| EL50_SLV | <i>Laomedea angulata</i> | Mund Bay, Piran, Slovenia | not available | MZUSP 2808 | KX665294 | KX665197 | KX665393 | KX665493 |

| Code in tree | Species | Locality | Geographic coordinate | Voucher | GenBank Accession Numbers | | | |
|--------------|------------------------------|--|-----------------------|-----------------|---------------------------|----------|----------|----------|
| | | | | | 16S | COI | 18S | 28S |
| IT11_IT | <i>Laomedea angulata</i> | Ancona, Italy | not available | MZUSP 2809 | KX665305 | KX665207 | KX665404 | KX665501 |
| FTA01_USA | <i>Laomedea calceolifera</i> | Newport, USA | 41.4780 -71.3355 | MZUSP 2810 | KX665298 | KX665201 | KX665397 | KX665496 |
| GFP01_USA | <i>Laomedea calceolifera</i> | Gloucester, USA | 42.6151 -70.6504 | MZUSP 2812 | KX665299 | KX665202 | KX665398 | KX665497 |
| HRM06_USA | <i>Laomedea calceolifera</i> | Hampton, USA | 42.54 -70.495 | MZUSP 2813 | KX665303 | KX665206 | KX665402 | - |
| MMA06_USA | <i>Laomedea calceolifera</i> | Bourne, USA | 41.7397 -70.6242 | MZUSP 2814 | KX665319 | KX665219 | KX665418 | - |
| ROW03_USA | <i>Laomedea calceolifera</i> | Boston, USA | 42.3569 -71.0408 | MZUSP 2815 | KX665344 | KX665242 | KX665446 | - |
| RYE02_USA | <i>Laomedea flexuosa</i> | Rye, USA | 42.9768 -70.7656 | MZUSP 2816 | KX665346 | - | KX665448 | - |
| CBC35_BLZ | <i>Obelia bidentata</i> | Cuda Cut, Twin Cays, Belize | not available | USNM1 420668 | KX665266 | KX665170 | KX665369 | KX665470 |
| MAR02_BRA | <i>Obelia bidentata</i> | Raposa Channel, São Luís do Maranhão, Brazil | -2.4271 -44.0700 | MZUSP 2817 | KX665313 | KX665213 | KX665412 | KX665507 |
| MAP10_BRA | <i>Obelia bidentata</i> | Panaquatira, São Luís do Maranhão, Brazil | -2.4984 -44.0239 | MZUSP 2818 | KX665311 | KX665211 | - | - |
| PAF09_BRA | <i>Obelia dichotoma</i> | Farol Velho, Salinópolis, Brazil | -0.591 -47.3248 | MZUSP 2819 | KX665324 | KX665223 | KX665424 | KX665514 |
| MA03_BRA | <i>Obelia dichotoma</i> | Calhau, São Luís Maranhão, Brazil | -2.4798-44.2429 | MZUSP 2820 | KX665309 | KX665209 | KX665410 | KX665505 |
| PAF07_BRA | <i>Obelia dichotoma</i> | Farol Velho, Salinópolis, Brazil | -0.591 -47.3248 | MZUSP 3334 | KX665322 | KX665222 | KX665423 | KX665513 |
| MMA03_USA | <i>Obelia dichotoma</i> | Bourne, USA | 41.7397 -70.6242 | MZUSP 3335 | KX665316 | KX665216 | KX665415 | - |
| FLT04_USA | <i>Obelia dichotoma</i> | Westport, USA | 41.5129 -71.0765 | MZUSP 3336 | KX665297 | KX665200 | KX665396 | - |
| PIM01_USA | <i>Obelia dichotoma</i> | New Bedford, USA | 41.6579 -70.9308 | MZUSP 3337 | KX665325 | KX665224 | KX665426 | - |

| Code in tree | Species | Locality | Geographic coordinate | Voucher | GenBank Accession Numbers | | | |
|--------------|--------------------------|-------------------------------------|-----------------------|---------------|---------------------------|----------|----------|----------|
| | | | | | 16S | COI | 18S | 28S |
| PIM02_USA | <i>Obelia dichotoma</i> | New Bedford, USA | 41.6579 -70.9308 | MZUSP 3338 | KX665326 | KX665225 | KX665427 | KX665515 |
| PTJ03_USA | <i>Obelia dichotoma</i> | Point Judith, Rhode Island, USA | 41.3877 -71.5171 | MZUSP 3339 | KX665342 | KX665241 | KX665442 | KX665517 |
| ROW04_USA | <i>Obelia dichotoma</i> | Boston, USA | 42.3569 -71.0408 | MZUSP 3340 | KX665345 | KX665243 | KX665447 | KX665521 |
| S1.1_USA | <i>Obelia dichotoma</i> | Providence, USA | not available | MZUSP 3341 | KX665347 | KX665244 | KX665449 | KX665522 |
| EL30_SLV | <i>Obelia dichotoma</i> | Slovenia | 45.5912 13.6998 | MZUSP 3342 | KX665288 | KX665191 | KX665387 | KX665487 |
| PT2_ARG | <i>Obelia dichotoma</i> | Punta Cuevas, San Julián, Argentina | not available | MZUSP 3343 | KX665330 | KX665229 | - | - |
| PT3_ARG | <i>Obelia dichotoma</i> | Punta Cuevas, San Julián, Argentina | not available | MZUSP 3344 | KX665331 | KX665230 | KX665430 | - |
| UR1_URG | <i>Obelia dichotoma</i> | Rocha, Uruguay | -34.6523 -54.1416 | MZUSP 3345 | KX665359 | KX665255 | KX665462 | KX665532 |
| UR6_URG | <i>Obelia dichotoma</i> | Rocha, Uruguay | -34.6541 -54.1435 | MZUSP 3346 | KX665360 | KX665256 | KX665463 | KX665533 |
| BSF05_USA | <i>Obelia geniculata</i> | South Freeport, USA | 43.8215 -70.1079 | MZUSP 3347 | KX665258 | - | KX665363 | - |
| BZ5_BRA | <i>Obelia geniculata</i> | João Gonçalves, Búzios, Brazil | not available | MZUSP 3348 | KX665259 | KX665162 | KX665364 | KX665465 |
| EL23_SLV | <i>Obelia geniculata</i> | Mund Bay, Piran, Slovenia | not available | MZUSP 3349 | KX665286 | KX665189 | KX665385 | KX665485 |
| PT5_ARG | <i>Obelia geniculata</i> | Punta Cuevas, San Julián, Argentina | not available | MZUSP 3350 | KX665332 | KX665231 | KX665431 | - |
| UNH01_USA | <i>Obelia geniculata</i> | New Castle, USA | 43.0723 -70.7157 | MZUSP 3351 | KX665358 | - | KX665461 | - |
| PT1_ARG | <i>Obelia longissima</i> | San Julián, Argentina | not available | MZUSP 3352 | KX665329 | KX665228 | KX665429 | - |
| GFP04_USA | <i>Obelia longissima</i> | Gloucester, USA | not available | MZUSP 3353 | KX665300 | KX665203 | KX665399 | KX665498 |

| Code in tree | Species | Locality | Geographic coordinate | Voucher | GenBank Accession Numbers | | | |
|--------------|--------------------------------|-------------------------------------|-----------------------|---------------|---------------------------|---------------|----------|----------|
| | | | | | 16S | COI | 18S | 28S |
| HRM05_USA | <i>Obelia longissima</i> | Hampton, USA | not available | MZUSP 3354 | KX665302 | KX665205 | KX665401 | KX665499 |
| MMA04_USA | <i>Obelia longissima</i> | Bourne, USA | 41.7397 -70.6242 | MZUSP 3355 | KX665317 | KX665217 | KX665416 | - |
| T2_BRA | <i>Obelia</i> sp.1 | Flexeiras, Trairí, Brazil | -3.217 -39.2671 | MZUSP 3356 | KX665353 | KX665250 | KX665457 | KX665528 |
| PAF08_BRA | <i>Obelia</i> sp.1 | Farol Velho, Salinópolis, Brazil | -0.591 -47.3248 | MZUS P3357 | KX665323 | - | - | - |
| AB_BRA | <i>Orthopyxis caliculata</i> | Armação, Penha, Brazil | -26.7833 -48.6167 | MZUSP 2565 | KM405578 * | KM405567 * | KX665361 | - |
| JGB3_BRA | <i>Orthopyxis caliculata</i> | João Gonçalves, Búzios, Brazil | not available | MZUSP 2614 | KM405584 * | KM405565 * | KX665408 | KX665504 |
| CB_BRA | <i>Orthopyxis crenata</i> | Caponga, Cascavel, Brazil | -4.0391 -38.1929 | MZUSP 2633 | KM405590 * | KX665164 | KX665366 | KX665467 |
| PAB2_BRA | <i>Orthopyxis crenata</i> | Paciência, Penha, Brazil | -26.7772 -48.6028 | MZUSP 2551 | KM405593 * | KM405559 * | KX665420 | KX665510 |
| PT19_ARG | <i>Orthopyxis crenata</i> | Comodoro Rivadavia, Argentina | not available | MZUSP 3359 | - | KX665238 | KX665439 | - |
| PT20_ARG | <i>Orthopyxis integra</i> | San Julián, Argentina | not available | MZUSP 3358 | KX665341 | KX665139 | KX665440 | - |
| MB1_BRA | <i>Orthopyxis mianzani</i> | Mel Island, Brazil | -25.5561 -48.2987 | MZUSP 2570 | KM405603 * | KM405549 * | KX665413 | KX665508 |
| PAB6_BRA | <i>Orthopyxis mianzani</i> | Paciência, Penha, Brazil | -26.7772 -48.6028 | MZUSP 2559 | KM405607 * | KM405545 * | KX665421 | KX665511 |
| PTY2_BRA | <i>Orthopyxis sargassicola</i> | Paraty, Brazil | not available | MZUSP 2606 | KM405629 * | KM405523 * | KX665443 | KX665518 |
| LB9_BRA | <i>Orthopyxis sargassicola</i> | Lázaro, Ubatuba, Brazil | -23.5091 -45.1385 | MZUSP 2602 | KM405618 * | KM405534 * | KX665409 | - |
| PB1_BRA | <i>Orthopyxis sargassicola</i> | Padres, Aracruz, Brazil | -19.9323 -40.1221 | MZUSP 2617 | KM405622 * | KM405531 * | KX665425 | - |
| Co1_ARG | <i>Orthopyxis</i> sp. | Caleta Olivia, Argentina | -46.4256 -67.5197 | MZUSP 2644 | KM405635 * | KX665178 | KX665374 | KX665474 |

| Code in tree | Species | Locality | Geographic coordinate | Voucher | GenBank Acession Numbers | | | |
|------------------|--------------------------|---|-----------------------|------------|--------------------------|----------|----------|----------|
| | | | | | 16S | COI | 18S | 28S |
| EL02_SLV | <i>Orthopyxis</i> sp.1 | Mund Bay, Piran, Slovenia | not available | MZUSP 3360 | KX665276 | KX665179 | KX665375 | KX665475 |
| EL04_SLV | <i>Orthopyxis</i> sp.1 | Mund Bay, Piran, Slovenia | not available | MZUSP 3361 | KX665277 | KX665180 | KX665376 | KX665476 |
| EL16_SLV | <i>Orthopyxis</i> sp.1 | Strunjan, Piran, Slovenia | 45.5370 13.6014 | MZUSP 3362 | KX665284 | KX665187 | KX665383 | KX665483 |
| EL52_SLV | <i>Orthopyxis</i> sp.1 | Strunjan, Piran, Slovenia | 45.5370 13.6014 | MZUSP 3363 | KX665295 | KX665198 | KX665394 | KX665494 |
| PT11_ARG | <i>Silicularia rosea</i> | Río Deseado, San Julián, Argentina | not available | MZUSP 3364 | KX665336 | KX665234 | KX665435 | - |
| RG4_ARG | <i>Silicularia rosea</i> | Río Grande, Cabo Santo Domingo, Argentina | -53.6888 -67.8445 | MZUSP 2645 | KM405636* | - | KX665445 | KX665520 |
| PT8_ARG | <i>Silicularia rosea</i> | San Julián, Argentina | not available | MZUSP 3365 | KX665333 | - | KX665432 | - |
| PT18_ARG | <i>Tulpa tulipifera</i> | Patagonia, Argentina | not available | MZUSP 3366 | KX665340 | KX665237 | KX665438 | - |
| OUTGROUPS | | | | | | | | |
| U10_URG | <i>Eucheilota</i> sp. | Uruguay | not available | MZUSP 3367 | KX665356 | KX665253 | KX665459 | KX665530 |
| U11_URG | <i>Eucheilota</i> sp. | Uruguay | not available | MZUSP 3368 | KX665357 | KX665254 | KX665460 | KX665531 |

Table 2. Codes ([-]=no code), sampling sites and GenBank accession numbers ([-]=no sequence) for published sequences included in the analysis. For *Clytia* sp. (Code=USA), sequences of different specimens were used in the concatenated analysis. Vouchers deposited in museum collections of these species (*) were studied.

| Code in tree | Species | Locality | GenBank Acession Numbers | | | | Reference |
|--------------|---|---------------------------------------|--------------------------|----------|----------|-----|---|
| | | | 16S | COI | 18S | 28S | |
| USA | <i>Bonneviella regia</i> * | Aleutians, USA | AY789805 | AY789890 | AY789740 | - | Govindarajan et al. (2006) |
| USA | <i>Bonneviella</i> sp.2* | Aleutians, USA | AY789806 | AY789891 | AY789741 | - | Govindarajan et al. (2006) |
| USA | <i>Bonneviella</i> sp.3 | Aleutians, USA | AY789807 | AY789892 | AY789742 | - | Govindarajan et al. (2006) |
| USA | <i>Bonneviella</i> sp.4* | Aleutians, USA | AY789808 | AY789893 | AY789743 | - | Govindarajan et al. (2006) |
| IT | <i>Campanularia hincksii</i> * | Otranto, Italy | AY789794 | AY789882 | AY789729 | - | Govindarajan et al. (2006) |
| SJ5_ARG | <i>Campanularia</i> sp.* | La Mina, Puerto San Julián, Argentina | KM405573 | KM405570 | - | - | Cunha et al. (2015) |
| AN | <i>Campanularia</i> sp. | Low Island, Antarctica | FN424118 | - | - | - | Penã Cantero et al. (2010) |
| SJ6_ARG | <i>Campanularia subantarctica</i> * | La Mina, Puerto San Julián, Argentina | KM405575 | KM405568 | - | - | Cunha et al. (2015) |
| USA | <i>Campanularia volubilis</i> * | Monterey, USA | AY789804 | AY789889 | AY789739 | - | Govindarajan et al. (2006); Lindner et al. (2011) |
| SJ1_ARG | <i>Campanulariidae</i> sp. indet.* | La Mina, Puerto San Julián, Argentina | KM405576 | - | - | - | Cunha et al. (2015) |
| SJ3_ARG | <i>Campanulariidae</i> sp. indet.* | La Mina, Puerto San Julián, Argentina | KM405577 | - | - | - | Cunha et al. (2015) |
| MA_USA | <i>Clytia</i> cf. <i>gracilis</i> sp.A | Woods Hole, USA | AY789812 | AY789900 | AY789751 | - | Govindarajan et al. (2006); Lindner et al. (2011) |
| ME_USA | <i>Clytia</i> cf. <i>gracilis</i> sp.A | Maine, USA | DQ068061 | DQ068054 | DQ068051 | - | Lindner et al. (2011) |
| BRA | <i>Clytia</i> cf. <i>gracilis</i> sp.B | São Sebastião, Brazil | DQ068062 | DQ068055 | DQ068052 | - | Lindner et al. (2011) |
| USA | <i>Clytia</i> cf. <i>gracilis</i> sp.B* | Beaufort, USA | AY789813 | AY789901 | AY789752 | - | Govindarajan et al. (2006); Lindner et al. (2011) |
| BRA | <i>Clytia</i> cf. <i>gracilis</i> sp.C | São Sebastião, Brazil | DQ068063 | DQ068056 | DQ068053 | - | Govindarajan et al. (2006); Lindner et al. (2011) |
| USA | <i>Clytia</i> cf. <i>gracilis</i> sp.D | Georges Bank, USA | AY789811 | AY789899 | AY789750 | - | Govindarajan et al. (2006); Lindner et al. (2011) |
| 1_BRA | <i>Clytia elsaeoswaldae</i> * | São Sebastião, Brazil | DQ064793 | DQ064800 | DQ064796 | - | Govindarajan et al. (2006); Lindner et al. (2011) |

| Code in tree | Species | Locality | GenBank Accession Numbers | | | | Reference |
|--------------|-------------------------------|------------------------------|---------------------------|---------------|-------------|-------------|---|
| | | | 16S | COI | 18S | 28S | |
| 2_BRA | <i>Clytia elsaeoswaldae</i> * | São Sebastião, Brazil | DQ068064 | - | - | - | Lindner et al. (2011) |
| 1_CHI | <i>Clytia folleata</i> | China | - | JQ716211 | - | - | Zhou et al. (2013) |
| 2-6_CHI | <i>Clytia folleata</i> | China | JQ716051-55 | KF962081-85 | KF962213-17 | - | Zhou et al. (2013); He et al. (2015) |
| IT | <i>Clytia gracilis</i> | Italy | AY346364 | AY789898 | AY789749 | - | Govindarajan et al. (2006) |
| XMCG1-15_CHI | <i>Clytia gulangensis</i> | Xiamen Bay, China | KF962425-39 | KF962086-2100 | KF962218-32 | KF962318-32 | He et al. (2015) |
| NS | <i>Clytia hemisphaerica</i> * | North Sea | AY789814 | AY789902 | AY789753 | - | Govindarajan et al. (2006); Lindner et al. (2011) |
| FR | <i>Clytia hemisphaerica</i> | Villefranche-sur-mer, France | - | - | FJ550601 | FJ550457 | Leclère et al. (2009) |
| IT | <i>Clytia hummelincki</i> | S. Caterina, Italy | AY346363 | AY789895 | AY789745 | - | Govindarajan et al. (2006); Lindner et al. (2011) |
| SA | <i>Clytia hummelincki</i> | South Africa | AY789809 | AY789894 | AY789744 | - | Govindarajan et al. (2006) |
| - | <i>Clytia languida</i> | no precise information | - | GQ120064-65 | - | - | Ortman et al. (2010) |
| USA | <i>Clytia linearis</i> * | Beaufort, USA | AY789810 | AY789897 | AY789748 | - | Govindarajan et al. (2006); Lindner et al. (2011) |
| IT | <i>Clytia linearis</i> | Torre Inserraglio, Italy | AY346362 | - | AY789747 | - | Govindarajan et al. (2006); Lindner et al. (2011) |
| BRA | <i>Clytia linearis</i> | São Sebastião, Brazil | DQ064791 | - | DQ064794 | - | Govindarajan et al. (2006); Lindner et al. (2011) |
| 1_BRA | <i>Clytia noliformis</i> * | São Sebastião, Brazil | DQ064792 | - | DQ064795 | - | Govindarajan et al. (2006); Lindner et al. (2011) |
| 2_BRA | <i>Clytia noliformis</i> | São Sebastião, Brazil | - | - | EU272554 | EU272611 | Evans et al. (2008) |
| IT | <i>Clytia paulensis</i> * | Otranto, Italy | AY346361 | AY789896 | AY789746 | - | Govindarajan et al. (2006); Lindner et al. (2011) |
| XMCL1-3_CHI | <i>Clytia</i> sp. | China | KF962440-42 | KF962101-3 | KF962233-35 | - | He et al. (2015) |
| KC1-5_CHI | <i>Clytia</i> sp. | China | JQ716046-50 | JQ716206-10 | KF962238-47 | - | He et al. (2015) |
| AGC_USA | <i>Clytia</i> sp. | California, USA | AY512519 | - | AF358074 | - | Collins (2002); Collins et al. (2005) |

| Code in tree | Species | Locality | GenBank Accession Numbers | | | | Reference |
|--------------|--------------------------------|----------------------------|---------------------------|------------------|----------|----------|--|
| | | | 16S | COI | 18S | 28S | |
| USA | <i>Clytia</i> sp. | California, USA | AY800195 | AY789903 | AF358074 | - | Collins et al. (2005); Govindarajan et al. (2006) |
| 1-15_HR | <i>Clytia</i> sp.1 | Helgoland Roads, North Sea | - | KC439960- 74 | - | - | Laakmann and Holst (2014) |
| 1-4_HR | <i>Clytia</i> sp.2 | Helgoland Roads, North Sea | - | KC439975- 78 | - | - | Laakmann and Holst (2014) |
| 1-8_CHI | <i>Clytia xiamenensis</i> | Xiamen Bay, China | JQ716037- 44 | JQ716198- 205 | - | - | Zhou et al. (2013) |
| IC | <i>Gonothyrea loveni</i> | Sandgerdi, Iceland | FJ550480 | - | FJ550547 | FJ550404 | Leclère et al. (2009) |
| USA | <i>Gonothyrea loveni</i> * | Dennis, USA | AY789826 | - | AY789765 | - | Govindarajan et al. (2006) |
| FR | <i>Gonothyrea loveni</i> | Roscoff, France | AY789827 | - | AY789766 | - | Govindarajan et al. (2006) |
| FR | <i>Laomedea calceolifera</i> * | Herquemoulin, France | FJ550504 | - | FJ550590 | FJ550447 | Leclère et al. (2009) |
| USA | <i>Laomedea calceolifera</i> * | Woods Hole, USA | AY789829 | AY789914 | AY789768 | - | Govindarajan et al. (2006) |
| FR | <i>Laomedea flexuosa</i> | Roscoff, France | AY789823 | AY789910 | AY789762 | - | Govindarajan et al. (2006) |
| IC | <i>Laomedea flexuosa</i> * | Iceland | AY789824 | AY789911 | AY789763 | - | Govindarajan et al. (2006) |
| WS | <i>Laomedea flexuosa</i> * | White Sea | AY789825 | AY789912 | AY789764 | - | Govindarajan et al. (2006) |
| USA | <i>Laomedea inornata</i> * | Friday Harbor, USA | AY789822 | - | AY789761 | - | Govindarajan et al. (2006) |
| USA | <i>Obelia bidentata</i> * | Beaufort, USA | AY789815 | AY789904 | AY789754 | - | Govindarajan et al. (2006) |
| FR | <i>Obelia bidentata</i> | Utah Beach, France | FJ550503 | - | FJ550589 | FJ550446 | Leclère et al. (2009) |
| NS | <i>Obelia bidentata</i> * | North Sea | AY789816 | AY789905 | AY789755 | - | Govindarajan et al. (2006) |
| IT | <i>Obelia dichotoma</i> * | Otranto, Italy | AY789828 | AY789913 | AY789767 | - | Govindarajan et al. (2006) |
| FR | <i>Obelia geniculata</i> | Roscoff, France | AY530359 | AY530410 | AY789769 | - | Govindarajan et al. (2006) |
| NB_CAN | <i>Obelia geniculata</i> * | New Brunswick, Canada | AY530344 | AY530395 | AY789770 | - | Govindarajan et al. (2006) |
| IC | <i>Obelia geniculata</i> | Sandgerdi, Iceland | FJ550481 | - | FJ550548 | FJ550405 | Leclère et al. (2009) |
| JP | <i>Obelia geniculata</i> * | Japan | AY530335 | AY530386 | AY789771 | - | Govindarajan et al. (2006) |
| NZ | <i>Obelia geniculata</i> * | New Zealand | AY530378 | AY530429 | AY789772 | - | Govindarajan et al. (2006) |
| NZ | <i>Obelia longissima</i> | Dunedin, New Zealand | AY789817 | AY789906 | AY789756 | - | Govindarajan et al. (2006) |
| AN | <i>Obelia longissima</i> * | Antarctic Peninsula | AY789821 | AY789909 | AY789760 | - | Govindarajan et al. (2006) |
| IC | <i>Obelia longissima</i> * | Sandgerdi, Iceland | AY789820 | AY789908 | AY789759 | - | Govindarajan et al. (2006) |

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|--------------|--|---------------------------------|---------------------------|-----------------|----------|----------|----------------------------|
| | | | 16S | COI | 18S | 28S | |
| WS | <i>Obelia longissima</i> * | White Sea | AY789819 | AY789907 | AY789758 | - | Govindarajan et al. (2006) |
| USA | <i>Obelia longissima</i> * | Ryders Cove, USA | AY789818 | - | AY789757 | - | Govindarajan et al. (2006) |
| 1-11_HR | <i>Obelia</i> sp.1 | Helgoland Roads, North Sea | - | KC439979-89 | - | - | Laakmann and Holst (2014) |
| 1-5_HR | <i>Obelia</i> sp.2 | Helgoland Roads, North Sea | - | KC439990-94 | - | - | Laakmann and Holst (2014) |
| 1-9_HR | <i>Obelia</i> sp.3 | Helgoland Roads, North Sea | - | KC439995-440003 | - | - | Laakmann and Holst (2014) |
| JGB1_BRA | <i>Orthopyxis caliculata</i> * | João Gonçalves, Búzios, Brazil | KM405582 | - | - | - | Cunha et al. (2015) |
| JGB2_BRA | <i>Orthopyxis caliculata</i> * | João Gonçalves, Búzios, Brazil | KM405583 | - | - | - | Cunha et al. (2015) |
| JGB4_BRA | <i>Orthopyxis caliculata</i> * | João Gonçalves, Búzios, Brazil | KM405585 | - | - | - | Cunha et al. (2015) |
| PAB1_BRA | <i>Orthopyxis caliculata</i> * | Paciência, Penha, Brazil | KM405586 | KM405564 | - | - | Cunha et al. (2015) |
| PAB3_BRA | <i>Orthopyxis caliculata</i> * | Paciência, Penha, Brazil | KM405587 | KM405563 | - | - | Cunha et al. (2015) |
| PAB4_BRA | <i>Orthopyxis caliculata</i> * | Paciência, Penha, Brazil | KM405588 | KM405562 | - | - | Cunha et al. (2015) |
| PAB5_BRA | <i>Orthopyxis caliculata</i> * | Paciência, Penha, Brazil | KM405589 | KM405561 | - | - | Cunha et al. (2015) |
| GB_BRA | <i>Orthopyxis caliculata</i> * | Grande Beach, Penha, Brazil | KM405581 | KM405566 | - | - | Cunha et al. (2015) |
| BB_BRA | <i>Orthopyxis caliculata</i> * | Bombas Beach, Bombinhas, Brazil | KM405579 | - | - | - | Cunha et al. (2015) |
| COB_BRA | <i>Orthopyxis caliculata</i> * | Conceição, Bombinhas, Brazil | KM405580 | - | - | - | Cunha et al. (2015) |
| LB5_BRA | <i>Orthopyxis crenata</i> * | Lázaro, Ubatuba, Brazil | KM405591 | - | - | - | Cunha et al. (2015) |
| LB8_BRA | <i>Orthopyxis crenata</i> * | Lázaro, Ubatuba, Brazil | KM405592 | - | - | - | Cunha et al. (2015) |
| PAB7_BRA | <i>Orthopyxis crenata</i> * | Paciência, Penha, Brazil | KM405594 | KM405558 | - | - | Cunha et al. (2015) |
| LG_BRA | <i>Orthopyxis crenata</i> * | Praia, Laguna, Brazil | - | KM405560 | - | - | Cunha et al. (2015) |
| NZ | <i>Orthopyxis crenata</i> | Wellington, New Zealand | FJ550466 | - | - | FJ550383 | Leclère et al. (2009) |
| IT | <i>Orthopyxis everta</i> * ^A | Torre del Serpe, Italy | AY789793 | AY789881 | AY789728 | - | Govindarajan et al. (2006) |
| IT | <i>Orthopyxis integra</i> * ^B | Italy | AY789799 | AY789884 | AY789734 | - | Govindarajan et al. (2006) |
| 1_USA | <i>Orthopyxis integra</i> | Aleutians, USA | AY789800 | AY789885 | AY789735 | - | Govindarajan et al. (2006) |

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|--------------|--|--------------------------|---------------------------|----------|----------|-----|---|
| | | | 16S | COI | 18S | 28S | |
| 2_USA | <i>Orthopyxis integra</i> | Friday Harbor, USA | AY789798 | - | AY789733 | - | Govindarajan et al. (2006) |
| IC | <i>Orthopyxis integra</i> | Sandgerdi, Iceland | AY789802 | AY789887 | AY789737 | - | Govindarajan et al. (2006) |
| NZ | <i>Orthopyxis integra</i> * ^C | New Zealand | AY789801 | AY789886 | AY789736 | - | Govindarajan et al. (2006) |
| USA | <i>Orthopyxis integra</i> CA sp.1* | Monterey, USA | AY789796 | - | AY789731 | - | Govindarajan et al. (2006) |
| USA | <i>Orthopyxis integra</i> CA sp.2 | Monterey, USA | AY789797 | - | AY789732 | - | Govindarajan et al. (2006) |
| MB2_BRA | <i>Orthopyxis mianzani</i> * | Mel Island, Brazil | KM405603 | KM405549 | - | - | Cunha et al. (2015) |
| MB3_BRA | <i>Orthopyxis mianzani</i> * | Mel Island, Brazil | KM405604 | KM405548 | - | - | Cunha et al. (2015) |
| MB4_BRA | <i>Orthopyxis mianzani</i> * | Mel Island, Brazil | KM405605 | KM405547 | - | - | Cunha et al. (2015) |
| MB5_BRA | <i>Orthopyxis mianzani</i> * | Mel Island, Brazil | KM405606 | KM405546 | - | - | Cunha et al. (2015) |
| FOB1_BRA | <i>Orthopyxis mianzani</i> * | Mel Island, Brazil | KM405595 | KM405557 | - | - | Cunha et al. (2015) |
| FOB2_BRA | <i>Orthopyxis mianzani</i> * | Mel Island, Brazil | KM405596 | KM405556 | - | - | Cunha et al. (2015) |
| FOB3_BRA | <i>Orthopyxis mianzani</i> * | Mel Island, Brazil | KM405597 | KM405555 | - | - | Cunha et al. (2015) |
| FOB4_BRA | <i>Orthopyxis mianzani</i> * | Mel Island, Brazil | KM405598 | KM405554 | - | - | Cunha et al. (2015) |
| FOB5_BRA | <i>Orthopyxis mianzani</i> * | Mel Island, Brazil | KM405599 | KM405553 | - | - | Cunha et al. (2015) |
| FOB6_BRA | <i>Orthopyxis mianzani</i> * | Mel Island, Brazil | KM405600 | KM405552 | - | - | Cunha et al. (2015) |
| FOB7_BRA | <i>Orthopyxis mianzani</i> * | Mel Island, Brazil | KM405601 | KM405551 | - | - | Cunha et al. (2015) |
| BRA | <i>Orthopyxis sargassicola</i> | São Sebastião, Brazil | AY789795 | AY789883 | AY789730 | - | Govindarajan et al. (2006); Lindner et al. (2011) |
| FB1_BRA | <i>Orthopyxis sargassicola</i> * | Formosa, Aracruz, Brazil | KM405610 | KM405542 | - | - | Cunha et al. (2015) |
| FB2_BRA | <i>Orthopyxis sargassicola</i> * | Formosa, Aracruz, Brazil | KM405611 | KM405541 | - | - | Cunha et al. (2015) |
| PB2_BRA | <i>Orthopyxis sargassicola</i> * | Padres, Aracruz, Brazil | KM405623 | KM405530 | - | - | Cunha et al. (2015) |
| PB3_BRA | <i>Orthopyxis sargassicola</i> * | Padres, Aracruz, Brazil | KM405624 | KM405529 | - | - | Cunha et al. (2015) |
| PB4_BRA | <i>Orthopyxis sargassicola</i> * | Padres, Aracruz, Brazil | KM405625 | KM405528 | - | - | Cunha et al. (2015) |
| PB5_BRA | <i>Orthopyxis sargassicola</i> * | Padres, Aracruz, Brazil | KM405626 | KM405527 | - | - | Cunha et al. (2015) |
| PB6_BRA | <i>Orthopyxis sargassicola</i> * | Padres, Aracruz, Brazil | KM405627 | KM405526 | - | - | Cunha et al. (2015) |
| PB7_BRA | <i>Orthopyxis sargassicola</i> * | Padres, Aracruz, Brazil | - | KM405525 | - | - | Cunha et al. (2015) |

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|------------------|------------------------------------|--|---------------------------|----------|----------|----------|---|
| | | | 16S | COI | 18S | 28S | |
| PTY1_BRA | <i>Orthopyxis sargassicola</i> * | Paraty, Brazil | KM405628 | KM405524 | - | - | Cunha et al. (2015) |
| PTY3_BRA | <i>Orthopyxis sargassicola</i> * | Paraty, Brazil | KM405630 | KM405522 | - | - | Cunha et al. (2015) |
| PTY4_BRA | <i>Orthopyxis sargassicola</i> * | Paraty, Brazil | KM405631 | KM405521 | - | - | Cunha et al. (2015) |
| PTY5_BRA | <i>Orthopyxis sargassicola</i> * | Paraty, Brazil | KM405632 | KM405520 | - | - | Cunha et al. (2015) |
| RI_BRA | <i>Orthopyxis sargassicola</i> * | Ratos Island, Paraty, Brazil | KM405633 | KM405519 | - | - | Cunha et al. (2015) |
| MI_BRA | <i>Orthopyxis sargassicola</i> * | Meros Island, Paraty, Brazil | KM405621 | KM405532 | - | - | Cunha et al. (2015) |
| LB1_BRA | <i>Orthopyxis sargassicola</i> * | Lázaro, Ubatuba, Brazil | KM405612 | KM405540 | - | - | Cunha et al. (2015) |
| LB2_BRA | <i>Orthopyxis sargassicola</i> * | Lázaro, Ubatuba, Brazil | KM405613 | KM405539 | - | - | Cunha et al. (2015) |
| LB3_BRA | <i>Orthopyxis sargassicola</i> * | Lázaro, Ubatuba, Brazil | KM405614 | KM405538 | - | - | Cunha et al. (2015) |
| LB4_BRA | <i>Orthopyxis sargassicola</i> * | Lázaro, Ubatuba, Brazil | KM405615 | KM405537 | - | - | Cunha et al. (2015) |
| LB6_BRA | <i>Orthopyxis sargassicola</i> * | Lázaro, Ubatuba, Brazil | KM405616 | KM405536 | - | - | Cunha et al. (2015) |
| LB7_BRA | <i>Orthopyxis sargassicola</i> * | Lázaro, Ubatuba, Brazil | KM405617 | KM405535 | - | - | Cunha et al. (2015) |
| LB10_BRA | <i>Orthopyxis sargassicola</i> * | Lázaro, Ubatuba, Brazil | KM405619 | - | - | - | Cunha et al. (2015) |
| LB11_BRA | <i>Orthopyxis sargassicola</i> * | Lázaro, Ubatuba, Brazil | KM405620 | KM405533 | - | - | Cunha et al. (2015) |
| SS_BRA | <i>Orthopyxis sargassicola</i> * | Preta Beach, São Sebastião, Brazil | KM405634 | KM405518 | - | - | Cunha et al. (2015) |
| CI1_BRA | <i>Orthopyxis sargassicola</i> * | Campeche Island, Florianópolis, Brazil | KM405608 | KM405544 | - | - | Cunha et al. (2015) |
| CI2_BRA | <i>Orthopyxis sargassicola</i> * | Campeche Island, Florianópolis, Brazil | KM405609 | KM405543 | - | - | Cunha et al. (2015) |
| USA | <i>Rhizocaulus verticillatus</i> * | Aleutians, USA | AY789803 | AY789888 | AY789738 | - | Govindarajan et al. (2006); Lindner et al. (2011) |
| 1_NZ | <i>Silicularia rosea</i> * | Bay of Islands, New Zealand | AY789792 | - | AY789727 | - | Govindarajan et al. (2006) |
| 2_NZ | <i>Silicularia rosea</i> | Wellington, New Zealand | FJ550482 | - | FJ550549 | FJ550406 | Leclère et al. (2009) |
| OUTGROUPS | | | | | | | |
| FR | <i>Calycella syringa</i> | Roscoff, France | FJ550460 | - | FJ550519 | FJ550372 | Leclère et al. (2009) |
| USA | <i>Calycella syringa</i> | Woods Hole, USA | AY789833 | AY789916 | AY789776 | - | Govindarajan et al. (2006) |
| - | <i>Mitrocomella niwai</i> | Devonport, New Zealand | FJ550473 | - | FJ550536 | FJ550392 | Leclère et al. (2009) |
| - | <i>Phialella quadrata</i> | Whangaparoa, New Zealand | FJ550474 | - | FJ550537 | FJ550393 | Leclère et al. (2009) |



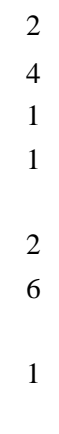

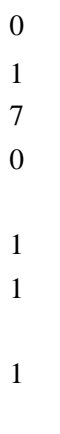
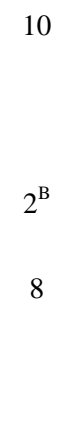

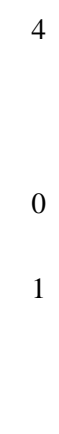







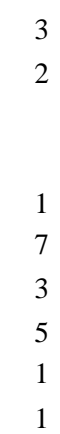







| Code in tree | Species | Locality | GenBank Accession Numbers | | | | Reference |
|--------------|-------------------------------|--------------------------|---------------------------|----------|----------|----------|---|
| | | | 16S | COI | 18S | 28S | |
| - | <i>Eugymnanthea inquilina</i> | Taranto, Italy | AY789832 | AY789915 | AY789775 | - | Govindarajan et al. (2006) |
| - | <i>Eucheilota maculata</i> | Luc-sur-mer, France | FJ550501 | - | FJ550587 | FJ550444 | Leclère et al. (2009) |
| - | <i>Eirene viridula</i> | Luc-sur-mer, France | FJ550502 | - | FJ550588 | FJ550445 | Leclère et al. (2009) |
| - | <i>Blackfordia virginica</i> | Northern California, USA | AY512516 | - | AF358078 | AY920800 | Collins (2002); Collins et al. (2005); Collins et al. (2006) |
| - | <i>Aequorea aequorea</i> | Woods Hole, USA | AY512518 | - | AF358076 | EU305505 | Collins (2002); Collins et al. (2005); Cartwright et al. (2008) |
| - | <i>Aequorea victoria</i> | not available | EU305469 | - | AF358077 | AY920799 | Collins (2002); Collins et al. (2006); Cartwright et al. (2008) |
| - | <i>Opercularella pumila</i> | Woods Hole, USA | AY789834 | - | AY789777 | - | Govindarajan et al. (2006) |
| - | <i>Lovenella gracilis</i> | Wildwood Crest, USA | AY789830 | - | AY789773 | - | Govindarajan et al. (2006) |
| - | <i>Eucheilota bakeri</i> | California, USA | AY789831 | - | AY789774 | - | Govindarajan et al. (2006) |
| - | <i>Tiaropsidium kelsey</i> | not available | - | - | AF358079 | - | Govindarajan et al. (2006) |

^{A, B, C}These specimens were misidentified and most likely correspond to ^{A, B}*Orthopyxis asymmetrica* Stechow, 1919 (accepted as *Campanularia breviscyphia* Sars, 1857 in WoRMS, Schuchert 2015) and ^C*Orthopyxis crenata* (Hartlaub, 1901) (see section 4.3 of the text for more details).

Table 3. Details of datasets used in the phylogenetic analyses. (P) Parsimony, (ML) Maximum Likelihood.

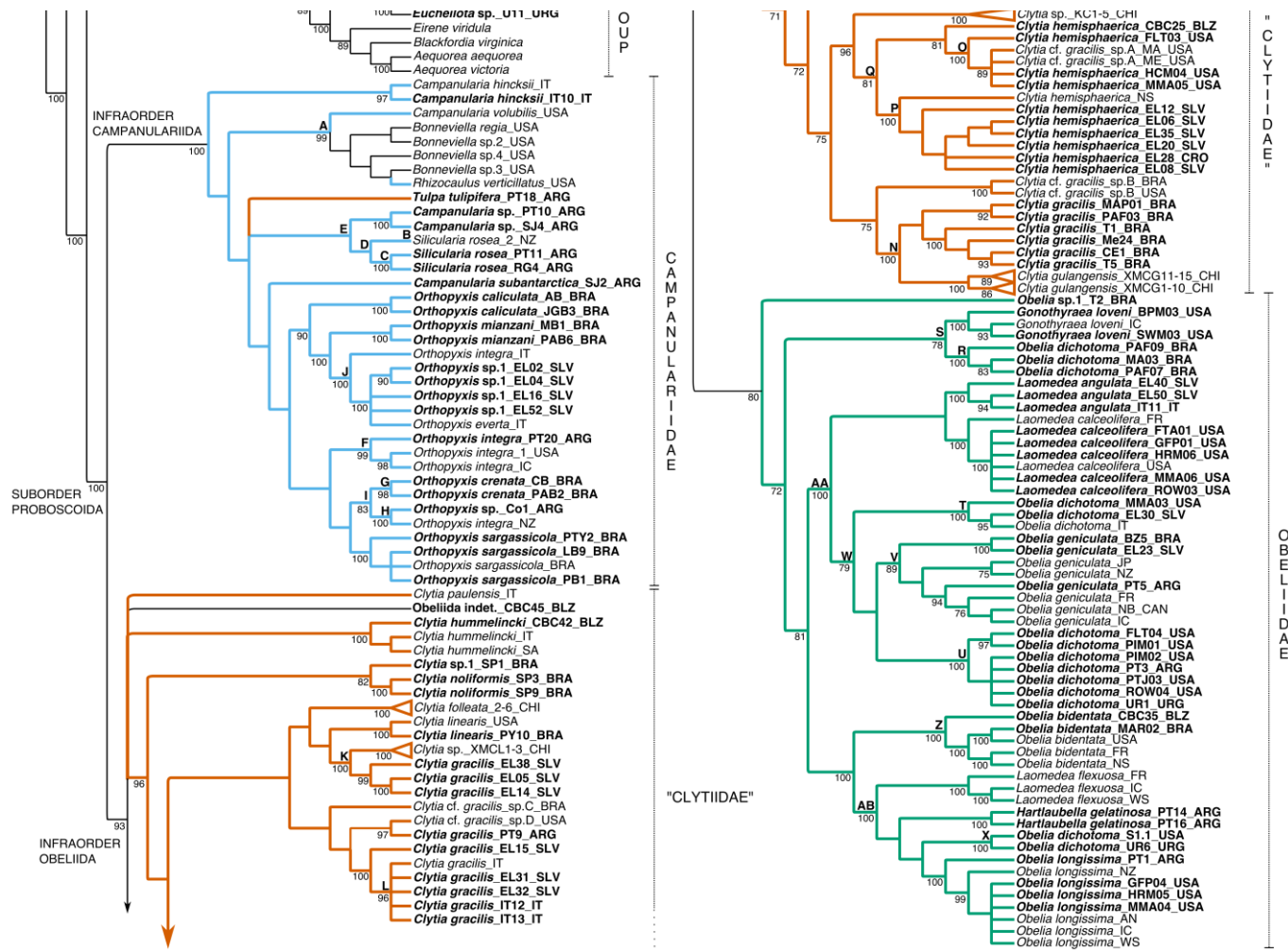
| | 16S+COI+18S+28S | | | | | | | | | | | |
|---------------------------------------|-----------------|-------------|--------------|-------------|----------------|--------------|-------------|--------------|----------------|----------------|----------------|----------------|
| | Dataset 1 | | | | Dataset 2 | | | | 16S | COI | 18S | 28S |
| | 16S | COI | 18S | 28S | 16S | COI | 18S | 28S | | | | |
| Number of taxa | 181 | | | | 80 | | | | 220 | 202 | 182 | 88 |
| Total number of characters | 627 | 669 | 1829 | 3396 | 632 | 680 | 1891 | 3391 | 641 | 665 | 1840 | 3443 |
| Number of informative characters (ML) | 234 | 263 | 412 | 904 | 191 | 248 | 332 | 835 | 247 | 262 | 422 | 918 |
| Model of nucleotide evolution (ML) | TPM3uf+ I+G | GTR+I+ G | TIM1+I +G | GTR+I+ G | TPM2uf+ I+G | TIM2+I +G | TrN+I+ G | TIM2+I +G | GTR+I +G | GTR+I +G | TIM1+I +G | GTR+I+ G |
| Log Likelihood (ML) | -64219.9805 | | | | -42777.3438 | | | | 11165.2 990 | 13700.0 579 | 13659.9 397 | 26737.05 06 |
| Number of informative characters (P) | 1954 | | | | 1736 | | | | 264 | 262 | 467 | 1010 |
| Number of most parsimonious trees (P) | 725 | | | | 8 | | | | 182263 | 4000 | 16424 | 9 |
| Minimum length (P) | 13092 | | | | 7922 | | | | 2409 | 3082 | 2449 | 5501 |

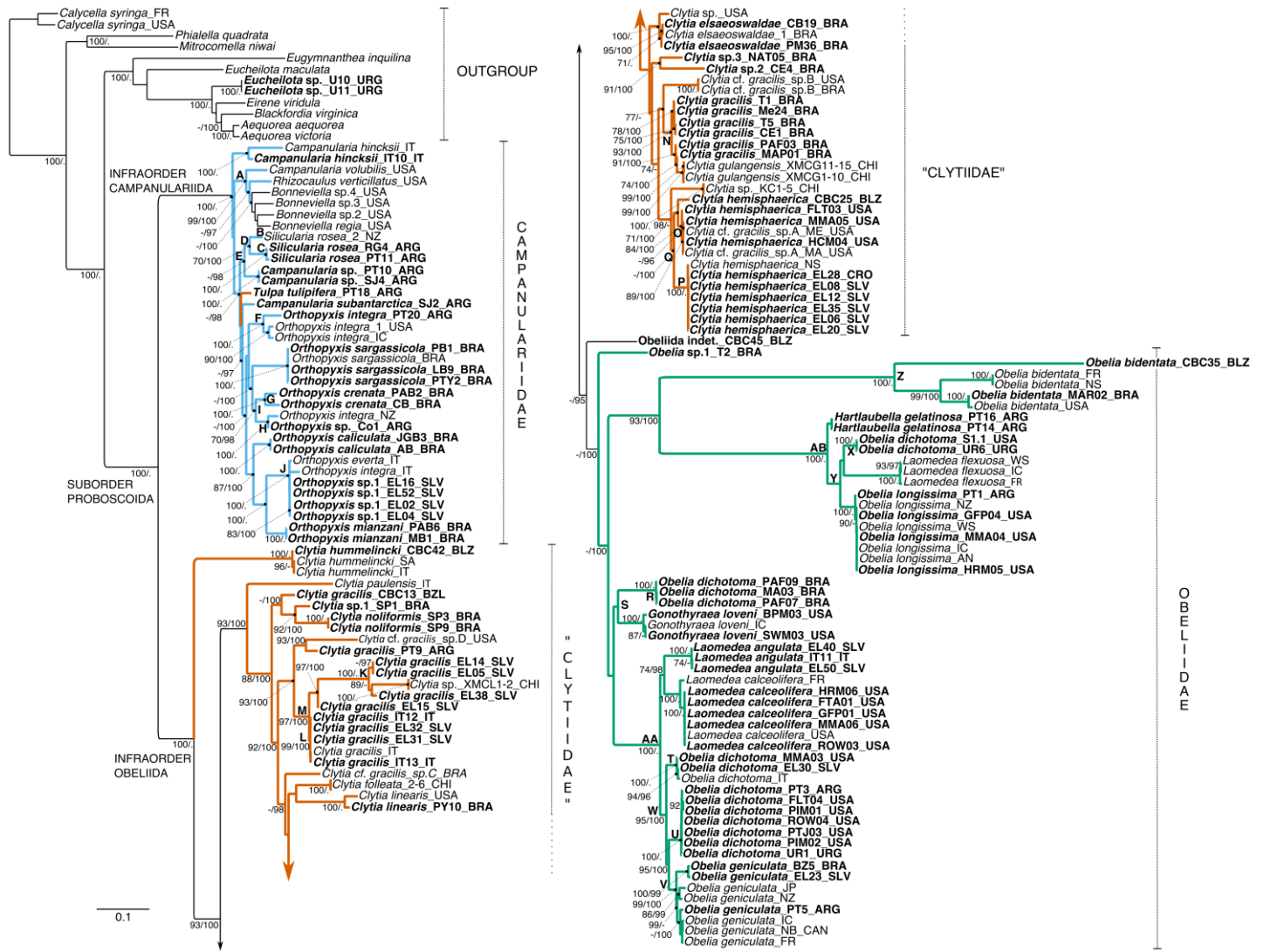
Table 4. Comparison between the resulted phylogenetic topologies (Figures 1-2) and genera proposed in previous classifications of Campanulariidae. Black cells – monophyletic in the topologies; white cells – non monophyletic in the topologies; * – synonymized genera; grey striped cells – genera not included in the corresponding classification. Numbers on the left of each cell indicate total number of species described; numbers on the right indicate total number of synonymized species.

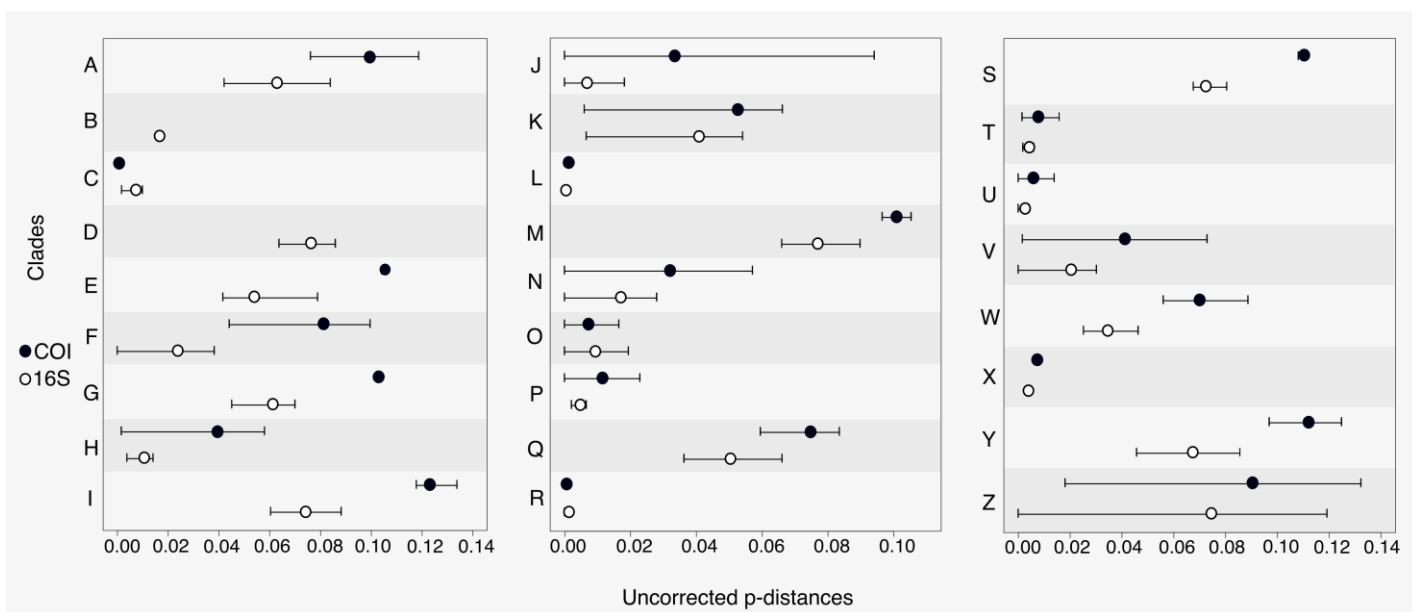
| | Nutting (1915) | Ralph (1957) | Naumov (1969) | Millard (1975) | Cornelius (1975) | Cornelius (1982) |
|-------------------------------------|--|---|--|---|---|---|
| <i>Campanularia</i> Lamarck, 1816 | 35  | 12  | 2  | 10  | 9  | 5  |
| <i>Orthopyxis</i> L. Agassiz, 1862 | 5  | 2  | 4  | 1  | 0  | 10  |
| <i>Silicularia</i> Meyen, 1834 | 6  | 3  | 1  | 7  | 5  | 9  |
| <i>Tulpa</i> Stechow, 1921 | *  | 1  | 0  | 0  | 0  | 0 |
| <i>Rhizocaulus</i> Stechow, 1919 | * | | 2 ^B | 0 | 0 | 0 |
| <i>Clytia</i> Lamouroux, 1812 | 13 | 2 | 2 | 1 | 7 | 1 |
| <i>Obelia</i> Péron & Lesueur, 1810 | 17 | 6 | 1 | 8 | 1 | 1 |
| <i>Laomedea</i> Lamouroux, 1812 | * | | 1 | 1 | 3 | 3 |
| <i>Gonothyraea</i> Allman, 1864 | 4 | 1 | 1 | 1 | 0 | 0 |
| <i>Hartlaubella</i> Poche, 1914 | 1 ^A | 3 | * | 1 | 0 | 0 |
| | | | | | 3 | +80 |
| | | | | | | 7 |
| | | | | | | 3 |
| | | | | | | 5 |
| | | | | | | 1 |
| | | | | | | 1 |

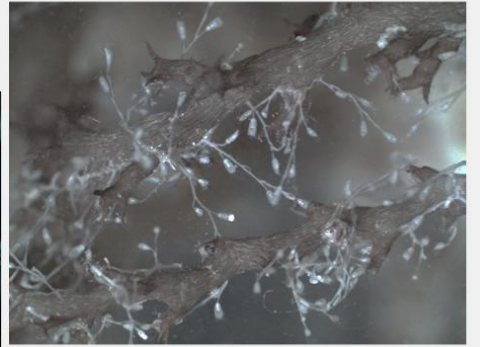
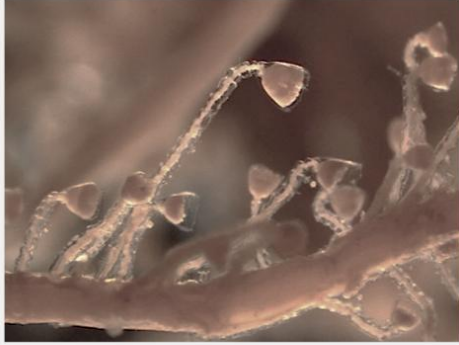
| | Calder 1991 | Bouillon (1985) ^D | Cornelius (1995) | Hirohito (1995) | Vervoort and Watson (2003) ^H | Bouillon et al. (2004) ^I | This study ^J |
|-------------------------------------|-------------|------------------------------|------------------|-----------------|---|-------------------------------------|-------------------------|
| <i>Campanularia</i> Lamarck, 1816 | 1 | 8 | 2 | 9 | 2 | 3 | |
| <i>Orthopyxis</i> L. Agassiz, 1862 | 1 | 1 | 1 | * | 4 | 5 | |
| <i>Silicularia</i> Meyen, 1834 | | | | | 1 | | |
| <i>Tulpa</i> Stechow, 1921 | | | | 1 ^G | 1 | | |
| <i>Rhizocaulus</i> Stechow, 1919 | | | 1 | * | | | |
| <i>Clytia</i> Lamouroux, 1812 | 6 | 40 | 4 | 9 | 7 | 10 | |
| <i>Obelia</i> Péron & Lesueur, 1810 | 2 | 50 | 4 | 4 | 7 | 4 | |
| <i>Laomedea</i> Lamouroux, 1812 | | | 5 | * | | 5 | |
| <i>Gonothyraea</i> Allman, 1864 | | | 1 | * | 1 | 1 | |
| <i>Hartlaubella</i> Poche, 1914 | | | 1 | | 1 | 1 | |

^Aas *Obelaria* Hartlaub, 1987; ^Bas *Verticillina* Naumov, 1960; ^Cas *Eulaomedea* Broch, 1909; ^DBouillon (1985) provided diagnosis for all genera but did not describe any species; ^Emore synonyms in Cornelius (1982); ^Fmore synonyms in Cornelius (1975) and (1982); ^GHirohito (1995) only mentions one species for this genus, *Tulpa* (*Campanularia*) *speciosa*, which was not originally included in the genus; ^Hthe diagnosis of the genera were inferred based on diagnoses of the species, and the grey cell indicate the inference of monophyly is unclear; ^Ino synonyms are provided in this study; ^J*Tulpa* and *Rhizocaulus* are represented by only one specimen, and their monophyly needs to be confirmed.









CAMPANULARIIDAE

CLYTIIDAE

OBELIIDAE

