Short running title: Morphometry and species boundaries in Proboscoida

Title: When morphometry meets taxonomy: morphological variation and species boundaries in Proboscoida (Cnidaria, Hydrozoa)

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When morphometry meets taxonomy: morphological variation and species boundaries in Proboscoida (Cnidaria, Hydrozoa)

Abstract

Species delimitation in marine taxa is often problematical given wide intraspecific variation. Based on extensive genetic sampling from specimens of the families Campanulariidae, Clytiidae and Obeliidae recently published, we evaluated morphological variation in this group, correlating morphometric and phylogenetic patterns for species delimitation. Several species within Campanulariidae were confidently delimited based on differences in size (e.g., *Bonneviella* species, *Tulpa tulipifera* and *Rhizocaulus verticillatus*) while others were reidentified and corroborated based on differences in perisarc thickness (e.g., *Silicularia rosea*, *Orthopyxis* and *Campanularia* species). In Clytiidae, the length and diameter of hydrothecae, height of hydrothecal cusps and perisarc thickness delimited the species *Clytia linearis*, *C. elsaeoswaldae* and *C. noliformis*, among others. However, few characters reliably differentiated the lineages associated with the nominal species *C. gracilis* and *C. hemisphaerica*. In Obeliidae, *Obelia geniculata* was distinctive for its higher perisarc thickness, and corroborated as a widely distributed species. *Obelia longissima* and lineages refered to *O. dichotoma* were subtly distinguished, showing a few differences in size and branching of colonies. The taxonomic implications of these results are broadly discussed. With a few exceptions, species could be delimited based on morphometric patterns, once morphological variation was investigated in a comparative manner.

Keywords: morphometrics – Campanulariidae – Clytiidae – Obeliidae – diagnostic characters – morphology – size – perisarc thickness – hydrothecae – hydrothecal cusps – branching

Introduction

Marine taxa frequently have highly variable morphology and/or a paucity of diagnostic characters, often rendering their species delimitation problematic (Yoshioka, 1982; Trussell, 1996; Bruno & Edmunds, 1997; Kaandorp, 1999; Bell & Barnes, 2000; Todd, 2008). Integrative approaches have helped to resolve incongruencies between molecular and morphological data, and many traditional characters considered to be diagnostic are often found to be uninformative (Fukami *et al.*, 2004, 2008; Forsman *et al.*, 2009, 2010; Budd *et al.*, 2010; DeBiasse & Hellberg, 2015; Pérez-Barros *et al.*, 2015). Presumably cosmopolitan species are often found to comprise several cryptic lineages (e.g., Klautau *et al.*, 1999; Barroso *et al.*, 2010; Kawauchi & Giribet, 2014), but excessive splitting of taxa may also occur (e.g., Prada *et al.*, 2014; Willette *et al.*, 2015). Contemporary studies use integrative approaches as taxonomic standards for species delimitation, but delimiting species remains far from simple because population-level variation may commonly be mistaken as interspecific variation or vice-versa, and these patterns are often not easy to differentiate (e.g., Meroz-Fine *et al.*, 2003; Prada *et al.*, 2008; Forsman *et al.*, 2010; Stefani *et al.*, 2011; see also Schuchert, 2014; Cunha *et al.*, 2016).

Species delimitation in Hydrozoa involves similar problems (reviewed by Cunha *et al.*, 2016). Their planktonic medusa stage and hydroid rafting has been for long considered to widen the dispersal capabilities of species (Ralph, 1961; Cornelius 1981a, 1992a; Boero & Bouillon, 1993; Calder, 1993), theoretically enhancing gene flow and supporting the traditional view that most hydrozoan species have nearly cosmopolitan distributions (Cornelius, 1981a, 1992b). However, molecular studies are showing that genetic diversity in Hydrozoa is higher than previously assumed (Schuchert 2005, 2014; Miglietta *et al.*, 2007, 2009, 2015; Postaire *et al.*, 2016; Moura *et al.*, 2018), and that samples from different, usually distant, localities often likely represent their own lineages (Schuchert 2014; Postaire *et al*., 2017a, b; Boissin *et al*.,

67 2018). Molecular studies have also revealed the need for major changes in the classification of
68 the group at several taxonomic levels (Collins *et al.*, 2004, 2006, 2008; Cartwright *et al.*, 2008;
69 Leclère *et al.*, 2009; Maronna *et al.*, 2016; Moura *et al.*, 2018), allowing the description of new
70 species (e.g., Schierwater & Ender, 2000; Cunha *et al.*, 2015) as well as revalidations of former
71 synonyms (e.g., Schuchert, 2005; Miglietta *et al.*, 2007, 2009; Lindner *et al.*, 2011; Moura *et*
72 *al.*, 2012; Cunha *et al.*, 2015).

73 Hydroids that were formerly included in the family Campanulariidae Johnston, 1836
74 have been the subject of important recent taxonomic changes. Because of the supposedly wide
75 intraspecific variation in this group (e.g., Ralph, 1956, 1957; Cornelius, 1982, 1995),
76 taxonomists have frequently disagreed on the importance of diagnostic characters for the
77 species and genera, and many nominal species were either split or lumped excessively (Nutting,
78 1915; Ralph, 1957; Millard, 1975; Östman, 1982a, 1987; Cornelius, 1975, 1990, 1982, 1995;
79 Calder, 1991; Boero *et al.*, 1996). Recent molecular analyses have shown that several species
80 comprise cryptic lineages, and that intraspecific variation has been overestimated
81 (Govindarajan *et al.,* 2005, 2006; Lindner *et al.*, 2011; Cunha *et al.*, 2015). Additionally, their
82 phylogenetic relationships and extensive morphological diversity have led to campanulariids
83 being split into three families within the suborder Proboscoida Broch, 1910: Campanulariidae
84 Johnston, 1836, Clytiidae Cockerell, 1911, and Obeliidae Haeckel, 1879 (Maronna *et al.*,
85 2016).

86 Several morphological characters used in traditional diagnoses have proven to be
87 uninformative to delimit species and genera in these families (Cunha *et al.*, 2017). Besides
88 information from the cnidome (Östman 1982a, 1999; Lindner & Migotto, 2001) and life cycles
89 (Lindner & Migotto, 2002; Lindner *et al.*, 2011; Zhou *et al.*, 2013; He *et al.*, 2015),
90 morphometric data are also promising to delimit species boundaries in the group (e.g., Cunha

91 *et al.*, 2015), especially if the range of variation of morphological characters is investigated
92 (Cunha *et al.*, 2016).
93 This study aimed to evaluate patterns of morphological variation correlated with species
94 delimitation in the suborder Proboscoida (*sensu* Maronna *et al.*, 2016). Morphometric patterns
95 of nearly all specimens included in a previous phylogeny (Cunha *et al.*, 2017) were analyzed
96 based on their phylogenetic relationships, integrating morphological, morphometric and
97 molecular data for the delimitation of species of Campanulariidae, Clytiidae and Obeliidae.
98
99 **Material and Methods**
100 Taxonomic sampling
101 The specimens used in this study are the same vouchers that were included in the
102 molecular phylogenetic analysis by Cunha *et al.* (2017), with a few exceptions (Supporting
103 Information, Table S1). Therefore, materials used for DNA analyses were also used in
104 morphometric analyses whenever possible, and the results of the two studies can be directly
105 compared. Also, vouchers of previously published sequences, deposited in the National
106 Museum of Natural History (USNM), Smithsonian Institution (Govindarajan *et al.*, 2006;
107 Lindner *et al.*, 2011), Muséum d'Histoire Naturelle de Genève (MHNG) (Leclère *et al.*, 2009),
108 and Museu de Zoologia da Universidade de São Paulo (MZUSP) (Cunha *et al.*, 2015) were
109 studied. Additional type and non-type materials from these and other museum collections (see
110 Supporting Information, Table S1) were studied, enhancing taxon sampling and comparisons
111 to delimit specific lineages.
112 In total, we analyzed morphometric data for 291 specimens of the suborder Proboscoida,
113 comprising 16 species of Campanulariidae (and all currently accepted genera, cf. Schuchert,
114 2019), 16 species of Clytiidae (and one out of two accepted genera), and 14 species of
115 Obeliidae (covering all accepted genera). We tried to include in the analysis as many

116 individuals of each species as possible, but this was determined by the number of sequences
117 available for each species, as it was important to have a direct comparison between
118 morphometric data and molecular lineages. In some cases only one voucher representing the
119 species was measured (e.g., *Clytia paulensis*), whereas in other cases up to 26 different
120 individuals were included for comparison (e.g., *Orthopyxis sargassicola*). Additionally, some
121 collection lots had two to three polyps of the same colony measured, allowing for intracolony
122 comparisons (see Supporting Information, Table S1).
123
124 Morphological and morphometric analyses
125 We studied morphological characters of the polyps of species of Proboscoida, in
126 accordance with the previous phylogeny of the group (Cunha *et al.*, 2017). We were not able
127 to study vouchers of published sequences that came from medusae (Zhou *et al.*, 2013;
128 Laakmann & Holst 2014; He *et al.*, 2015). However, their original publications, as well as
129 some additional studies, provided important information on medusa characters that improved
130 the discussion (e.g., Lindner & Migotto, 2002; Lindner *et al.*, 2011; Zhou *et al.*, 2013;
131 Laakmann & Holst, 2014; He *et al.*, 2015).
132 Morphological characters were initially chosen based on measurements of polyps of
133 Proboscoida reported in species descriptions that have been considered informative for species
134 delimitation (e.g., Millard, 1975; Cornelius, 1982, 1990, 1995; Calder, 1991; Migotto, 1996;
135 Lindner & Migotto, 2002; Lindner *et al.*, 2011). Based on our previous experience with the
136 genus *Orthopyxis* (Cunha *et al.*, 2015) and morphological variation in Proboscoida (Cunha *et*
137 *al.*, 2016), further characters were added to the analysis to capture more of the interspecific
138 variation, specially regarding size and shape of hydrothecae and gonothecae, as well as the
139 thickness of the perisarc (by measuring the diameter and thickness in three different positions,
140 see Table 1). Gonosomal characters were included whenever these structures were available,

but the identification of their contents was rarely possible because of their state of maturation and/or preservation. Hydranth characters (e.g., number of tentacles, length and diameter of column) were not considered because all materials studied were preserved in ethanol or formalin, and hydranths were frequently retracted or absent.

Specimens and the corresponding scales were photographed under stereo- and/or compound microscopes for morphometric analysis, and measurements were subsequently taken using Image J (Schneider *et al.*, 2012). Morphometric data were analyzed with a Principal Component Analysis (PCA, see Legendre & Legendre, 1998; Borcard *et al.*, 2011) using the *vegan* package (Oksanen *et al.*, 2015) for the R programming language (R Core Team, 2019). The PCA was conducted on a correlation matrix, and distance biplots were generated for a graphical view of the results. The analysis comprised different levels of comparison within each family, including the complete dataset as well as subsets of data, in order to have a more detailed investigation of patterns of morphological variation in these groups.

Results

Family Campanulariidae

The PCA with all species shows that several measurements of length and diameter (LH, DHMa, DHMe, DHB, LP, TLT) are responsible for the largest amount of variation in the data (PC1), while the presence of cusps (NC, HCMax, HCMin) and perisarc thickness (PPMe, PHMe, PSS) explain another direction of high variation among species (PC2, Fig. 1A, B; Table 1). Differences in size separate *Tulpa tulipifera*, *Bonneviella superba*, *B. ingens* and *B. regia* from other Campanulariidae, based on their larger hydrothecae and pedicels (Figure 1A, C). Similarly, *Rhizocaulus verticillatus* can be distinguished from *Campanularia* and *Orthopyxis* by its larger hydrothecae and trophosome (Fig. 1D, E). Differences in size are not only informative to delimit different genera, but are considerably variable among *Bonneviella*

166 species (Supporting Information, Table S2). The dimensions of the specimens of *B. regia*
167 (USNM 1106181, Govindarajan *et al.*, 2006) are congruent with the type material of this
168 species, while measurements of the unidentified specimens (*Bonneviella* sp.2 and sp.4,
169 Govindarajan *et al.*, 2006) are closer to type materials of the other species examined
170 (Supporting Information, Table S2). *Bonneviella* sp.2 (USNM 1106182), here reidentified as
171 *B. superba*, and *B. grandis* are among the species with larger hydrothecae and trophosome,
172 while *Bonneviella* sp.4 (USNM 1106187), here reidentified as *B. ingens*, have hydrothecae and
173 trophosome almost half the size of the three previous species (Supporting Information, Table
174 S2, Fig. 2A-C).

175 Perisarc thickness, as well as the number and height of hydrothecal cusps, separate
176 several species within Campanulariidae (Fig. 1B). *Silicularia rosea* is clearly distinct from
177 *Campanularia, R. verticillatus, Tulpa* and *Bonneviella* due to its thicker perisarc (Fig. 1C, 2H).
178 Species of *Campanularia*, in contrast, can hardly be differentiated by any of the characters
179 included in the analysis, since they have similar morphological patterns (Fig. 1D). The
180 exception is *C. hincksii*, slightly set apart from the remaining *Campanularia* by its taller
181 hydrothecal cusps (HCMax, HCMin, Fig. 1D), a character that shows little or no overlap among
182 the species when intraspecific variation is considered (Fig. 3B). The remaining characters,
183 however, do not show this pattern (Fig. 3A, C-D).

184 Perisarc thickness is also informative to separate *Orthopyxis* from species of
185 *Campanularia*, although morphological variation may attenuate this difference. Several
186 specimens of *O. sargassicola* and *O. crenata* group together with *Campanularia* because of
187 their thinner perisarc and presence of hydrothecal cusps, compared to the remaining species of
188 *Orthopyxis* (Fig. 1E and Supporting Information, Fig. S1C). Indeed, although *O. sargassicola*
189 and *O. crenata* have a thicker perisarc on average, their range of variation may overlap with
190 *Campanularia* (Fig. 4A). Species of *Campanularia* have, on average, a thinner perisarc in

comparison to most other *Orthopyxis* (except for *O. mianzani*, Fig. 4B), and when there is overlap in the range of variation of perisarc thickness, these taxa can be distinguished by the hydrothecal length and length:diameter ratio (Fig. 4C, D).

When considering only species of *Orthopyxis* without hydrothecal cusps, the variation in size and perisarc thickness distinguish all individual lineages (Figs. 1F): *Orthopyxis mianzani* has larger polyps with larger hydrothecae and a thinner perisarc; *O. asymmetrica* (see reidentified material in Table 2) have shorter polyps and hydrothecae, with thinner perisarcs; *O. caliculata* has shorter polyps and hydrothecae, but a thicker perisarc; and *O. integra* (see reidentified material in Table 2) have larger polyps and hydrothecae, with thicker perisarcs. The specimen from the Aleutian Islands (USNM 1106184, Govindarajan *et al.*, 2006; Cunha *et al.*, 2017, as *Orthopyxis integra*_1_USA) is distinguished by its larger hydrothecae and pedicels (Figs. 1E-F, 4D). However, variation occurs in all species, and some may overlap in their ranges, sometimes contradicting the separation of the lineages (e.g., *O. caliculata* and *O. asymmetrica*, *O. integra* and *O. caliculata*, see Figs. 1F, 4). Additional comparisons with type species and descriptions from the literature (Supporting Information, Table S3) show that the morphological patterns of the specimens identified as *Orthopyxis* sp.1, *O. everta* and *O. integra*_IT by Govindarajan *et al.*, (2006) and Cunha *et al.*, (2017) are congruent with that of *O. asymmetrica* (Stechow, 1919). Differences in hydrothecal length, perisarc thickness and length:diameter ratio of the basal chamber confirm their distinction from *O. angulata* Bale, 1914, *O. compressa* (Stechow, 1919), and *O. caliculata* (Hincks, 1853) (Supporting Information, Table S3).

Additional principal components were evaluated, but they did not show clear patterns of differentiation among species (Supporting Information, Fig. S1). A PCA including only data from specimens with gonothecae separated *S. rosea* for its longer gonothecae, as well as

Orthopyxis and *Bonneviella* for their broader gonothecae (see Supporting Information, Fig. S1F).

Family Clytiidae

When all species of *Clytia* are compared, the PCA shows that most of the variation (PC1) is related to the presence of erect colonies, and the number, length, diameter, and perisarc thickness of the internodes (NIS, LIS, DIS, PIS) separate *Clytia linearis* and some specimens of *C. elsaeoswaldae*, *C.* cf. *gracilis* sp.1, and *C. hemisphaerica* from the remaining Clytiidae (Fig. 6A). However, when data for species of *C.* cf. *gracilis* and measurements related to internodes are excluded from the analysis, further morphological patterns among species with erect colonies appear (Fig. 6C-D). *Clytia linearis* is distinguished by its longer hydrothecae and cusps (LH, HCMax, HCmin, Figs. 6C-D), although the range of variation of the cusps height overlaps with those of other species (Fig. 8A-B). Likewise, *C. elsaeoswaldae* is separated by the larger hydrothecal diameter (DHMa, DHMe, DHB, DBC, Fig. 6A, C-D), but this character is more informative when compared to species of *C.* cf. *gracilis* and *C.* cf. *hemisphaerica*, with which it shows less overlap (Fig. 8C). Further comparisons show that *C. elsaeoswaldae* has a thicker diaphragm on average than *C. linearis*, as well as species of *C.* cf. *gracilis* and *C.* cf. *hemisphaerica* (Fig. 8D). However, morphological variation is high and certainly attenuates these differences, leading to large overlaps among species.

The second direction accounting for most variation (PC2, Fig. 6A-B) is related to perisarc thickness (PHMa, PHMe, PHB, PPMe) and length:diameter ratio of the hydrotheca (HRatio). It sets apart *Clytia* sp.2 and *Clytia noliformis* for their thicker perisarc, and *Clytia* sp.1, *C.* cf. *gracilis* sp.5 and *C. paulensis* for their more cylindrical hydrothecae (Figs. 6A, 8E-F). Although evident when directly compared among these species, differences in HRatio are not

239 evident in all PCAs, probably because of the slight variation shown by the remaining species
240 of *Clytia* (Fig. 8F).

241 Species of *C.* cf. *gracilis* (Fig. 7D-F), though not clearly individualized, can be set apart
242 from each other when compared as a group: *C.* cf. *gracilis* sp.B , *C.* cf. *gracilis* sp.1 and sp.2
243 have larger hydrothecae and pedicels (LH, DHMa, DHMe, DHB, DP) with higher and more
244 numerous cusps (NC, HCMax, HCMin), while *C.* cf. *gracilis* sp.3 and sp.4 have, in general,
245 lower values for those characters (Fig. 6E-F). If measurements related to erect colonies are
246 excluded from the analysis (LIS, PIS, NIS, DIS), *C.* cf. *gracilis* sp.1 and *C.* cf. *gracilis* sp.B
247 can be further separated from *C.* cf. *gracilis* sp.2 by the length (LH) and length:diameter ratio
248 of the hydrotheca (HRatio, Fig. 6F), although these differences are too small to be informative
249 and delimit lineages. Specimens of *C.* cf. *gracilis* sp.5 spread along the four quadrants of the
250 graph because of their high variation in the characters examined (Figure 6E-F). Additional
251 comparisons with literature descriptions show that morphological variation is pronounced in
252 the presumably typical *C. gracilis*, and the lineages analyzed here could fit one or more
253 descriptions (Supporting Information, Table S4).

254 Species of *C.* cf. *hemisphaerica* are not separated by any of the morphological
255 measurements, showing intermediate values for most of the characters evaluated (Fig. 6A-D,
256 Supporting Information, Fig. S2). Characters that are important to differentiate other species
257 of *Clytia* are uninformative for lineages of *C.* cf. *hemisphaerica*, especially because of their
258 wide range of variation and extensive overlap. This variability is also seen when descriptions
259 from the literature are compared (Supporting Information, Table S5 and Fig. S4).

260 Additional PCAs, including characters from the gonotheca, show less conspicuous
261 patterns of differentiation among species (Supporting Information, Fig. S2). *Clytia*
262 *hummelincki* has been shown to not be part of Clytiidae in previous phylogenetic analysis
263 (Cunha *et al.*, 2017), and, therefore, was not included in the PCAs with this family.

264

265 Family Obeliidae

266 Patterns of morphological variation in Obeliidae are mostly congruent among the
267 different datasets examined (Fig. 10). Considering all species, perisarc thickness (PHMA,
268 PHMe, PHB, PPMe, TD) explains most of the data variation, separating *Obelia geniculata* by
269 its thicker perisarc (Figs. 10A-B). This character also set apart *O. geniculata* from the
270 remaining species when only the genus *Obelia* is considered (Fig. 10C). In addition, *Obelia*
271 *geniculata* has the widest range of variation of perisarc thickness, when *Laomedea* and *Obelia*
272 are compared (Fig. 12A). For the remaining genera, perisarc thickness does not notably
273 contribute to the differentiation of the species, because of its extensive overlap (Fig. 12A).
274 Measurements of diameter (DHMa, DHMe, DHB, DBC, DP) explain another direction of
275 variation of the data, and mainly differentiate *L. flexuosa* from the remaining Obeliidae by its
276 broader hydrothecae (Figs. 10A-B, D, 12B). Species of *Laomedea* also show a wide range of
277 variation and overlap in pedicel length (LP, Fig. 12C), but their pedicels are on average longer
278 than in *Obelia*.

279 *Obelia longissima* is distinguished from the remaining Obeliidae by its larger
280 measurements of first- and second-order branches (LIS, DIS, NIS, LIB, DIB, NIB, Figs. 10A-
281 C). It also has a wider range of variation in the hydrothecal length compared to the remaining
282 species, and it cannot be distinguished based on this character because of the extensive overlap
283 with other species (Fig. 12D). Erect and branched colonies also differentiate *Hartlaubella*
284 *gelatinosa* and *Gonothyraea loveni*, though to a lesser extent; this pattern is clearly observed
285 when *Obelia* is excluded from the analysis (Fig. 10D). These species, together with *O.*
286 *bidentata* and *Obelia* sp.1, also differ from the remaining Obeliidae in their more cylindrical
287 hydrothecae (higher values of HRatio) and taller hydrothecal cusps (Figs. 10B-D, 12F). The
288 exception is Obeliida indet., which has the tallest hydrothecal cusps when all these species are

289 compared (Fig. 12F). In general, Obeliida indet. has similar morphometric patterns to *O.*
290 *longissima*, mostly related to the presence of erect colonies and hydrothecal length (Fig. 10B,
291 D). The hydrotheca is typically longer in Obeliida indet., but morphological variation
292 attenuates this difference (Fig. 12D).

293 It is evident from most of the analyses that lineages of *Obelia* cf. *dichotoma* are not
294 distinguished from each other by any of the measurements, showing intermediate values for all
295 characters evaluated (Figs. 10A-C, E). Many specimens of *O. longissima* cannot be
296 distinguished from the lineages of *O.* cf. *dichotoma* as well, and although some are
297 differentiated by their larger erect and branched colonies, variations in these characters prevent
298 a complete separation of the species (Fig. 13A). *Obelia longissima* also has longer hydrothecae
299 and taller hydrothecal cusps on average, but their range of variation overlap among the species
300 (Fig. 13B, D). *Obelia* cf. *dichotoma* sp.3 and *O.* cf. *dichotoma* sp.4 are grouped together and
301 slightly separated from the remaining species of *Obelia*, probably because of their smaller and
302 less branched colonies, but no further patterns of differentiation are seen among these lineages
303 (Figs. 10E). Indeed, when compared to literature descriptions, the size and branching of
304 colonies seem to be among the few characters that could fairly differentiate some of the
305 lineages of *O.* cf. *dichotoma*, which are similar to the descriptions of other nominal species
306 (Supporting Information, Table S6).

307 Characters related to the gonothecae do not differentiate the species of *Obelia*, but
308 species of *Laomedea* can be distinguished by their larger gonothecae (LG, DGD, DGMe, DGB,
309 DGP, Fig. 10F). Additional PCAs do not show further patterns of differentiation among
310 Obeliidae (Supporting Information, Fig. S3).

311

312 **Discussion**

At first glance, morphometric patterns in the suborder Proboscoida are not discriminative, and most species would be indistinguishable. Indeed, several characters that have been historically considered as variable (e.g., colony size, perisarc thickness, height of hydrothecal cusps; Ralph, 1956; Cornelius, 1975, 1982; Millard, 1975) were corroborated as such in our current analysis, especially when different populations were included (see *Campanularia volubilis*, Fig. 3). However, we also demonstrated the existence of consistent morphological patterns when characters are investigated at different levels of comparison and their range of variation is fully considered in the analysis. Below, we discuss the main morphometric patterns observed, and how they can be informative to delimit lineages within Proboscoida.

Size differences in Campanulariidae

In Campanulariidae, the length and diameter of the trophosome, pedicels, and hydrothecae can reliably distinguish *Bonneviella*, *T. tulipifera*, and *R. verticillatus* from the genera *Campanularia*, *Silicularia*, and *Orthopyxis*, which in turn can be characterized by differences in perisarc thickness (see below). Indeed, several species of *Bonneviella* Broch, 1909 were originally assigned to *Campanularia* Lamarck, 1816, and distinguished by their "enormous" size or "immense" hydrothecae (Allman, 1876, as *Campanularia grandis*; Nutting, 1901, as *C. regia*). Later, the pre-oral cavity on the hypostome of these species was considered the main diagnostic character of the group (Bonneviellidae, Broch, 1909; Nutting, 1915). *Tulpa tulipifera* (Allman, 1888) and *Rhizocaulus verticillatus* (Linnaeus, 1758) were also originally assigned to *Campanularia* (Linnaeus, 1758; Allman, 1888), and subsequently defined as separate genera based on differences in hydrothecal size and shape, and the presence of polysiphonic colonies, respectively (Stechow, 1920, 1921). The generic value of these characters, however, has been questioned by some authors, especially given the similarities in

the hydrothecae and gonothecae between *Campanularia volubilis* (Linnaeus, 1758) and *R. verticillatus* (Rees & Thursfield, 1965; Boero *et al.*, 1996, but see Cornelius, 1982: 57, 1999). The phylogenetic relationships of these species support their separation (Cunha *et al.*, 2017), and our current analysis confirmed that they differ consistently in size, which should also be considered for their delimitation. *Tulpa tulipifera*, in addition to size, can be differentiated from *Campanularia* species by the absence of a subhydrothecal spherule (Vervoort, 1972; El Beshbeeshy & Jarms, 2011). However, conclusions as to whether these differences should be considered at the genus or species level must rely on future taxonomic decisions regarding the genus *Campanularia*, especially because it is not monophyletic (see next section for further discussion).

Because of the considerable interspecific variation in *Bonneviella*, differences in size may also be informative to delimit the species examined in this study. As pointed out by Nutting (1915), *Bonneviella regia* (Nutting, 1901) can be differentiated from *Bonneviella grandis* (Allman, 1876) by the shapes of their gonothecae and the noticeably smaller hydrothecae of *B. regia* (Supporting Information, Table S2). *Bonneviella superba* Nutting, 1915 has the largest hydrothecae among *Bonneviella* species, while hydrothecae in *Bonneviella ingens* Nutting, 1915 are intermediate in size, but considerably different in shape from those of *B. superba* (Nutting, 1915; Naumov, 1969). The morphometric patterns of the type materials support the hypothesis that the vouchers of *Bonneviella* sp. (USNM 1106182 and 1108187, Govindarajan *et al.*, 2006) are close to *B. superba* and *B. ingens*, respectively (Supporting Information, Table S2). This is a tentative identification, however, because both materials lack reproductive structures. Also, intraspecific variation in *Bonneviella* was not investigated because of the small number of specimens studied (*B. regia*: N=3, *B. superba* and *B. ingens*: N=1), making it difficult to determine whether the range of variation of these characters could overlap among the species examined.

363 The clade comprising *C. volubilis*, *R. verticillatus*, and *Bonneviella* may represent a local
364 radiation, and it is necessary to examine additional material from other localities (Govindarajan
365 *et al.*, 2006). Although *C. volubilis* was not differentiated from any other *Campanularia* species
366 based on characters related to size, both *R. verticillatus* and *Bonneviella* were characterized by
367 their larger size (Fig. 1A, D), and all their records come from the Aleutians (Supporting
368 Information, Table S1). *Rhizocaulus verticillatus* was originally recorded from Cumberland,
369 England (Cornelius, 1981, 1982), and is known for its arctic-boreal distribution (Antsulevich,
370 1992; Calder, 2003; Schuchert, 2001; Stepanjants *et al.*, 2006; Ronowicz, 2007). Species of
371 *Bonneviella* were originally and have been subsequently recorded in arctic and subarctic
372 regions (type localities for *B. regia*, *B. grandis*, *B. ingens* and *B. superba* are Prince William
373 Sound, Tsugaru Strait, Simushir Island, and Bering Sea, respectively; Broch, 1910; Kramp,
374 1913; Nutting, 1901, 1915; Naumov, 1969; Yamada, 1969; Schuchert, 2001). Even though
375 these genera have a close phylogenetic relationship (Govindarajan *et al.*, 2006; Cunha *et al.*,
376 2017), their large size may be related to their occurrence in colder waters, a relationship
377 previously described for other species of Proboscoida (e.g., *Obelia geniculata*, *Silicularia*
378 *bilabiata*, *Orthopyxis integra*; Ralph & Thomson, 1956; Ralph, 1957; Naumov, 1969). The
379 same occurs with *T. tulipifera*, which was originally recorded from Heard Island in Antarctica
380 (Allman, 1888; Stechow, 1921) and has a Kerguelen-Patagonian distribution (Peña Cantero &
381 García Carrascosa, 1999; Soto Àngel & Peña Cantero, 2015), indicating that its larger size is
382 probably a convergence. Nevertheless, further comparisons with additional material from
383 different populations are essential to evaluate the intraspecific range of variation of these
384 characters and their relationship to the species geographic distribution.
385
386 Trends in perisarc thickness and size/shape of hydrothecae

Our results show that perisarc thickness is among the most variable characters (e.g., Millard, 1975; Cornelius, 1982, 1995; Cunha *et al.*, 2015), but yet most informative to delimit *Silicularia*, *Campanularia*, and *Orthopyxis*. Besides the unique bilaterally symmetrical hydrothecae of *Silicularia* Meyen, 1834, a conspicuous character to delimit the genus (Ralph, 1956, 1957; Blanco, 1967), *S. rosea* can also be delimited by the comparatively thicker perisarc of its hydrothecae and pedicels. *Silicularia rosea* Meyen, 1834 is widely distributed in antarctic and subantarctic waters, and was considered synonymous with *S. bilabiata* (Coughtrey, 1875) (Vervoort & Watson, 2003), a species shown by Ralph (1956, 1957) to have wide intraspecific variation and comprise several nominal species within *Silicularia*. A previous molecular analysis of nuclear and mitochondrial genes showed that specimens of *S. rosea* from Argentina and New Zealand were closely related (Cunha *et al.* 2017), and we found similar morphological patterns among these specimens (Fig. 1, "*Silicularia rosea*" and "*Silicularia rosea* NZ1"). All these lines of evidence indicate that *S. rosea* is a widely distributed species, although Galea *et al.* (2014) recently assigned previous records of *S. rosea* from Chile (Galea *et al.*, 2009) and Tristan da Cunha (Galea, 2010) to *S. bilabiata* and *S. hemisphaerica* (Allman, 1888), respectively. All specimens that we studied had an oblique hydrothecal aperture (Fig. 2H) as is typical of *S. rosea* (Vervoort & Watson, 2003; Galea *et al.*, 2014), but the hydrothecae of specimens from New Zealand were smaller (398.5µm on average) than in Argentinean specimens (790.4µm). These differences are similar to those reported by Galea *et al.* (2014, =length raised wall) for *S. rosea* and *S. hemisphaerica*. However, considering the absence of gonothecae in New Zealand specimens and their close phylogenetic relationship with specimens from Argentina, which could indicate intraspecific variations, it is essential to evaluate additional material to corroborate these proposals.

Campanularia, on the other hand, was not found to be monophyletic in previous molecular analyses (Cunha *et al.*, 2017). *Campanularia volubilis* (type locality Brighton,

England, Cornelius 1981, 1982) is the type species of the genus (Cornelius, 1981b, ICZN 1985), but the clade comprising this species is hypothesized to represent a local radiation (Govindarajan *et al.*, 2006), as discussed above. In addition, the specimens included in the phylogenetic analysis come from Monterey, USA (Govindarajan *et al.*, 2006; Cunha *et al.*, 2017), and can not be assumed to represent the type species. For this reason, we refrain from any taxonomic decision regarding *Campanularia* until more and unequivocal material of the type species is available. Presently, a possible conclusion derived from the results would be to merge *Bonneviella* and *Rhizocaulus* into *Campanularia*, but this decision is contraindicated by the several morphological differences among these genera. Although not monophyletic, all species of *Campanularia* have similar morphological patterns, and most of their similarities could be considered symplesiomorphic character states. Also, differences in size of the hydrothecae between *C. hincksii* Alder, 1856 and *C. volubilis* can be masked by intraspecific variation (see Cornelius, 1982, 1995), especially when different populations are evaluated (Fig. 3). Species included in this study can only be reliably delimited by their gonothecae (Millard, 1971, 1975; Cornelius, 1982, 1995), although the height of the hydrothecal cusps in *C. hincksii* might also be distinctive.

Orthopyxis L. Agassiz, 1862 is a monophyletic genus (Cunha *et al.*, 2017), and despite several past taxonomic disputes as to whether it should be considered a synonym of *Campanularia* (Millard, 1975; Cornelius, 1982, 1995; Hirohito, 1995; Bouillon *et al.*, 2004), *Orthopyxis* was considered valid mainly based on the gonophore producing a reduced medusa (medusoid, Agassiz, 1862; Cornelius, 1995). Our analysis showed that *Orthopyxis* could also be distinguished from *Campanularia* based on trophosomal characters, such as perisarc thickness and length:diameter ratio of hydrothecae. However, *Campanularia* may fall into the range of variation of *O. sargassicola* (Nutting, 1915) and *O. crenata* (Hartlaub, 1901), because the perisarcs in these two *Orthopyxis* species vary from thin to thick, and their hydrothecae

437 from campanulate to cylindrical (Vervoort & Watson, 2003; Cunha *et al.*, 2015, 2016).
438 *Campanularia* and *Orthopyxis* can be reliably delimited based on these characters if their
439 ranges of variation are evaluated, especially when there is overlap between the different
440 species.

441 Indeed, variation in *O. crenata* is conspicuous. In molecular phylogenies, specimens of
442 *O. crenata* from New Zealand clustered with unidentified *Orthopyxis* specimens from
443 Argentina (see 16S and COI phylogenies, Cunha *et al.*, 2017). This clade forms a monophyletic
444 group with specimens of *O. crenata* from Brazil (concatenated phylogenies, Cunha *et al.*,
445 2017). Our results showed that, despite their affinities, specimens from New Zealand and
446 Argentina show clear differences in the perisarc thickness (Fig. 4A), as well as size and shape
447 of the hydrothecae in comparison with *O. crenata* from Brazil. However, the close
448 phylogenetic relationship with *O. crenata* from New Zealand, the type locality of the species
449 (Hartlaub, 1901; Vervoort & Watson, 2003), led us to consider these morphological differences
450 as intraspecific variations, also because they are commonly reported for this species (Ralph,
451 1957; Millard, 1975; Cornelius, 1982; Vervoort & Watson, 2003; Galea *et al.*, 2009). This
452 decision, however, may be changed in the future, with additional evidence from morphology,
453 ecology and genetics/genomics.

454 Distinct lineages of *Orthopyxis* with the traditional morphological diagnostic characters
455 of *O. integra* (MacGillivray, 1852) were shown to be delimited by the degree of perisarc
456 thickening and the size and shape of the hydrothecae (Cunha *et al.*, 2015). Our results
457 corroborate these patterns, and further attest that the clade comprising the specimen of *O.*
458 *integra* from the Aleutian Islands ("*Orthopyxis integra*_1_USA", USNM 1106184, see Cunha
459 *et al.*, 2017 and Supporting Information, Table S1), with spirally grooved gonothecae (Fig.
460 5A), has morphological patterns that are commonly regarded as distinctive for *O. integra*
461 (MacGillivray, 1842), such as larger and more cylindrical hydrothecae (Nutting, 1915; Bale,

1934; Hirohito, 1995; Calder *et al.*, 2014). Although we could not verify the presence of spirally grooved gonothecae in the Argentinean specimens ("Campanulariidae sp. indet." and "*O. integra*_PT20", see Supporting Information, Table S1), they are here regarded as *O. integra* given their morphological and phylogenetic patterns (Table 2), contradicting the hypothesis that this species does not occur in the southwestern Atlantic (Cunha *et al.*, 2015). Also, although the perisarc is rather thin in the Aleutian *O. integra*, the Argentinean specimens show that the perisarc thickness can be variable in this species, and may overlap with *O. caliculata* (Fig. 4B).

In addition to *O. integra*, our analysis also showed that Mediterranean specimens identified as *O. integra*_IT, *O. everta* and *Orthopyxis* sp.1 by Govindarajan *et al.* (2006) and Cunha *et al.,* (2017), and that form a clade in the molecular phylogeny of the group (Cunha *et al.*, 2017), have similar morphological patterns and can be delimited by their shorter hydrothecae and thinner perisarc, in comparison to other *Orthopyxis* species (Figs 1, 5). Although their perisarc is not as thick as described by Stechow (1919), we believe that these specimens should be assigned to *Orthopyxis asymmetrica* Stechow, 1919, a species commonly reported in the Mediterranean (Piraino & Morri, 1990; Peña Cantero & García Carrascosa, 2002; Bouillon *et al.*, 2004). Even though this species was proposed to be a synonym of *O. integra* (e.g., Cornelius, 1982; Östman *et al.*, 1987), our findings support *O. asymmetrica* as a distinct and valid species (see Table 2 for reidentifications).

Morphometric patterns in the delimitation of *Clytia* species

With some exceptions, several species of *Clytia* have morphometric differences congruent with their phylogenetic patterns (Cunha *et al.*, 2017). *Clytia linearis*, for instance, is monophyletic in all phylogenetic analyses (Cunha *et al.*, 2017), with consistent morphometric patterns shared by the specimens, corroborating it as a widely distributed species (Rees &

Vervoort, 1987; Medel & Vervoort, 2000). Classically, *C. linearis* (Thornely, 1900) is distinguished by the hydrothecal inward folds (cf. Calder, 1991; Lindner & Migotto, 2002; Schuchert, 2003). However, this species can also be differentiated from other members of *Clytia* by its erect colonies and the size of the hydrothecae, even though its "deep" hydrothecae, frequently mentioned in descriptions, are also commonly reported as variable in size (e.g., Cornelius, 1982; Altuna, 1994). Our analyses showed that the range of intraspecific variation of the size of the hydrothecae in *C. linearis* does not overlap with those of other species (Fig. 8A), and this character can also be useful to delimit the species.

Clytia elsaeoswaldae Stechow, 1914 was also shown to be a distinct, monophyletic lineage (Lindner *et al.*, 2011; Cunha *et al.*, 2017). It is differentiated from *C. gracilis* (M. Sars, 1850) and *C. hemisphaerica* (Linnaeus, 1767) by its occasional polysiphonic colonies, inclined hydrothecal cusps, and smooth gonothecae growing exclusively on the hydrorhiza of the polyps, and by its smaller medusae (Lindner *et al.*, 2011). The morphometric patterns of *C. elsaeoswaldae* shown in this study further support its delimitation, since it can be differentiated from species of *C.* cf. *gracilis* and, to a lesser extent, *C.* cf. *hemisphaerica* by its hydrothecal diameter (Fig. 8C). The rounded basal portion of the hydrothecae (cf. Lindner *et al.*, 2011) seems to be another distinctive character of the species, probably related to its broader hydrothecae. However, some specimens of *C.* cf. *hemisphaerica* fall into its range of variation (Fig. 8C).

Clytia noliformis (McCrady, 1859) has been confounded with *C. hemisphaerica*, but it was considered distinct from the latter by several authors (e.g., Östman *et al.*, 1987; Calder, 1991; Lindner & Calder, 2000). The shape of the hydrothecae and gonothecae, as well as the distinct annulations (= subhydrothecal spherules) and the presence of merotrichous isorhizae (a unique type of nematocyst) differentiate *C. noliformis* from its congeners (Calder, 1991; Linder & Migotto, 2001, 2002). We found that the perisarc thickness, a character rarely

512 described in the literature (but see Calder, 1991), can also be used to delimit this species (Fig.
513 8E).

514 Similarly, *Clytia paulensis* (Vanhöffen, 1910) is regarded as distinctive because of the
515 shape of its hydrothecal cusps (Millard, 1975; Cornelius, 1982, 1995), but we noted that the
516 species also has a more cylindrical hydrotheca in comparison with some other members of
517 *Clytia* (HRatio, Fig. 8F). The length:diameter ratio of the hydrothecae of *C. paulensis* is known
518 to be variable, though, ranging from 1.5 to 4 in different populations (Millard, 1966; Cornelius,
519 1982). Since we were able to study the intracolony variation of only one specimen of *C.*
520 *paulensis*, this character should be considered with caution for the delimitation of the species.

521 Molecular analyses of *C. gracilis* resulted in several cryptic lineages in previous studies
522 (Govindarajan *et al.*, 2006; Lindner *et al.*, 2011; Cunha *et al.*, 2017). The polyp of *C. gracilis*
523 is distinguished from *C. hemisphaerica* mainly by the inclined and pointed triangular cusps
524 and the smooth gonothecae, contrasting with the non-inclined, rounded cusps and the spirally
525 ribbed gonothecae in *C. hemisphaerica* (Calder, 1991; Cornelius, 1995). We found, however,
526 that the height, number and shape of the hydrothecal cusps vary within the different lineages
527 of *C. gracilis*, as do the hydrothecal length and length:diameter ratio (Figs 7D-F, 14). The same
528 variations are found among specimens of *C. gracilis* described in the literature from
529 presumably different populations (Vervoort, 1959; Calder, 1991; Cornelius, 1995; Schuchert,
530 2001; Peña Cantero & García Carrascosa, 2002), and the lineages analyzed herein could fit into
531 one or more of these descriptions (Supporting Information, Table S4). This emphasizes the
532 difficulties in correlating the morphometric patterns of these lineages with the type of *C.*
533 *gracilis*, especially considering that its original description was based on two species, currently
534 *C. gracilis* and *Gonothyraea loveni* (Allman, 1859) (M. Sars, 1850, 1857; cf. Cornelius, 1982;
535 Cornelius & Östman, 1986; Calder, 1991). Although a lectotype of *C. gracilis* was designated
536 by Cornelius (1982: 94), it was based on the original illustration provided by M. Sars (1857),

537 and information on its diagnostic characters remains subjective and incomplete. For a sound
538 delimitation of the type species, it is now essential to obtain specimens of *C. gracilis* from the
539 type locality (Lofoten and Finnmark, Norway; Sars, 1850, 1857; Calder, 1991) and correlate
540 their phylogenetic (molecular) and morphometric patterns to the cryptic lineages. The
541 delimitation of a neotype would also be beneficial, since the type series seems to be based on
542 original illustrations (cf. Cornelius, 1982; Cornelius & Östman, 1986).

543 *Clytia hemisphaerica* also comprises several cryptic lineages (Cunha *et al.*, 2017). We
544 were unable to differentiate them by their morphometric patterns (Supporting Information, Fig.
545 S4), although all lineages have the diagnostic characters that are generally attributed to polyps
546 of *C. hemisphaerica* (Fig. 7G-H; Calder, 1991; Cornelius, 1995). They also fit into one or more
547 published descriptions, impeding the delimitation and identification of characters from the type
548 of *C. hemisphaerica* (Supporting Information, Table S5), which was recorded from "Belgian
549 seas" (cf. Linnaeus, 1767; Cornelius, 1982). The three lineages of *C. hemisphaerica* analyzed
550 in this study were geographically structured, comprising specimens from Belize, the United
551 States, and the Mediterranean/North Sea, and forming a monophyletic group in most of the
552 concatenated phylogenies (Cunha *et al.*, 2017, Supporting Information, Table S1). These
553 results raise doubts as to whether *C. hemisphaerica* should indeed be considered a species
554 complex, or a species with pronounced population subdivisions (see Schuchert, 2014; Postaire
555 *et al.*, 2017).

556 Recently, two new species of *Clytia* were described from China, together with
557 information on their life cycles and nematocysts (Zhou *et al.*, 2013; He *et al.*, 2015). *Clytia*
558 *xiamenensis* Zhou *et al.*, 2013 was shown to be closely related to *C. hemisphaerica*, also
559 clustering with specimens of *C.* cf. *gracilis* sp.A from the USA (Lindner *et al.*, 2011; Zhou *et*
560 *al.*, 2013). This pattern was corroborated by Cunha *et al.* (2017), although in their study
561 additional specimens of *C. hemisphaerica* from the USA clustered with *C. xiamenensis* (see

562 16S phylogenies, Cunha *et al.*, 2017). Originally, the hydroid of *C. xiamenensis* was
563 differentiated from *C. hemisphaerica* by its pointed and inclined hydrothecal cups, as well as
564 its smaller B-type microbasic mastigophores (Zhou *et al.*, 2013). We showed, however, that
565 specimens of *C. hemisphaerica* from the same clade (*C.* cf. *hemisphaerica* sp.1, see Supporting
566 Information, Table S1) do not have inclined hydrothecal cusps (Fig. 7G), even though their
567 cusps are not as rounded as those of *C.* cf. *hemisphaerica* sp.2 (compare with Fig. 7H). Indeed,
568 inclined cusps can be variable in some species (*C. gracilis*, see below), and the definition of
569 the shape of hydrothecal cusps does not seem reliable to differentiate *C. hemisphaerica* and *C.*
570 *xiamenensis*. We lack information on the nematocysts and life cycle of these specimens, which
571 may support the separation of the species, as suggested by Zhou *et al.* (2013). However, it is
572 important that the diagnostic characters of the type of *C. hemisphaerica* are clearly defined
573 before the two species can be confidently differentiated. This would envolve the analysis of
574 specimens of *C. hemisphaerica* from the type locality, and the comparison of their phylogenetic
575 and morphometric patterns, as well as life cycle and nematocysts with those of the clade
576 comprising *C. xiamenensis*. If this clade indeed proves to be distinct from the other lineages,
577 then specimens from the USA should be assigned to *C. xiamenensis*.
578 Similarly, *Clytia gulangensis* He & Zheng, 2015 (He *et al.*, 2015) clustered with
579 specimens of *C. gracilis* from Brazil (*C.* cf. *gracilis* sp.5, Supporting Information, Table S1)
580 in the phylogenetic analysis of Cunha *et al.* (2017). Brazilian specimens do not have all the
581 diagnostic characters of *C. gulangensis*, at least in the polyp stage, because some specimens
582 have non-inclined hydrothecal cusps and smaller hydrothecae, with a length:diameter ratio near
583 two (Supporting Information, Table S4, Fig. 7D-F). In fact, the shape of the hydrothecal cusps
584 showed wide variation among the different Brazilian specimens (Fig. 14). He *et al.*, (2015)
585 differentiated the polyp of *C. gracilis* from *C. gulangensis* based on the presence of asymmetric
586 and inclined cusps (tilted, cf. Schuchert, 2003) in *C. gracilis*; however, some Brazilian

specimens clustering with *C. gulangensis* had asymmetric and inclined cusps (Fig. 14B, C, E). Therefore, we conclude that the polyps of *C. gulangensis* cannot be confidently delimited from those of *C. gracilis* until the diagnostic characters of *C. gracilis* (M. Sars, 1850) are reliably determined. Nevertheless, information on the nematocysts and life cycle is still lacking for Brazilian specimens, and these characters may prove to be distinctive for *C. gulangensis* (cf. He *et al.*, 2015).

Size and perisarc thickness differences in Obeliidae

One of the main variations found among species of Obeliidae was related to perisarc thickness, setting apart *O. geniculata* from all its congeners, as well as the remaining Obeliidae. Indeed, *O. geniculata* (Linnaeus, 1758) is a relatively easy species to identify because of its characteristic asymmetrical thickening of the internodes (Cornelius, 1975, 1990, 1995; Schuchert, 2001; Calder, 2012). Our study shows that the range of variation of perisarc thickness in *O. geniculata* is the widest among the Obeliidae (Fig. 12A), corroborating several literature descriptions that reported colonies with thin to strongly thickened perisarc (e.g., Millard, 1975; Migotto, 1996; Vervoort & Watson, 2003; Calder, 2013). Although *O. geniculata* has been suggested to represent a complex of cryptic species (Govindarajan *et al.*, 2005), molecular phylogenies including mitochondrial and nuclear markers supported its monophyly (Govindarajan *et al.*, 2006; Cunha *et al.*, 2017), showing low intraspecific distances when compared to other species of *Obelia* (see Cunha *et al.*, 2017). Similarly, our study corroborates the perisarc thickness as its distinctive character, and the nematocysts were also shown to be diagnostic (Östman, 1982a, 1999). These results indicate that there is currently little support for the delimitation of distinct species within its molecular lineages, and *O. geniculata* could be considered a widely distributed species.

Laomedea flexuosa was differentiated from the remaining members of Obeliidae by the diameter of its hydrothecae and pedicels (Fig. 12B). Indeed, this species is frequently described with a robust hydrotheca, having its length nearly equal to its width (Cornelius, 1982, 1995). *Laomedea flexuosa* was also distinguished from other members of Obeliidae by its isoenzyme patterns and nematocysts, further supporting its delimitation (Östman, 1982a, b). *Laomedea angulata* and *L. calceolifera*, on the other hand, do not show clear patterns of differentiation, except for the shape and position of their gonothecae, probably the most conspicuous character for their delimitation (cf. Cornelius, 1982). All species of *Laomedea* included in our analysis could be confidently distinguished from *Obelia* based on their longer pedicels (Fig. 12C), even though the genus did not prove to be monophyletic in previous molecular phylogenies (Govindarajan *et al.*, 2006; Cunha *et al.*, 2017). Because *L. flexuosa* (Alder, 1857) is the type species of the genus *Laomedea* (Cornelius 1981b, ICZN 1985), the best decision at present would be to assign *L. calceolifera* and *L. angulata* to *Obelia*, if the clade comprising all these species (Cunha *et al.*, 2017) contains the type species of *O. dichotoma* (Linnaeus, 1758) (taken as conspecific with *O. spherulina* Péron & Lesueur, 1810, the type species of *Obelia* Péron & Lesueur, 1810 (Cornelius, 1975, 1982)). However, this action is presently premature because there is no sequence of *O. dichotoma* from its type locality (southwestern England, Cornelius, 1975), and the delimitation of this species is unclear (see below).

Erect colonies and differences in shape and number of hydrothecal cusps

The species *G. loveni, H. gelatinosa* and *O. longissima*, the last to a greater extent, are separated from the remaining Obeliidae by their typically erect, branched colonies (Cornelius, 1982, 1990, 1995). *Hartlaubella* Poche, 1914 is distinguished from *Obelia* by its fixed gonophores (free medusa in *Obelia*; Cornelius, 1990; Boero *et al.*, 1996; Stepanjants, 1998), and *H. gelatinosa* (Pallas, 1766) can also be differentiated by its paired branches that are

636 successively arranged at right angles on opposite sides of the polysiphonic main stem
637 (Cornelius, 1995). However, this feature is also present in large colonies of *O. bidentata* Clark,
638 1875 (Cornelius, 1995), which has contributed to some confusion in the past (Cornelius, 1982,
639 1990). *Hartlaubella gelatinosa* and *G. loveni* can be differentiated from *O. bidentata* by the
640 shape and number of cusps, which are taller and more numerous in the latter (Fig. 12F). *Obelia*
641 *bidentata* also has a more cylindrical hydrotheca than *H. gelatinosa* and *G. loveni* (Fig. 12E).
642 *Obelia bidentata* is assumed to have wide intraspecific variation, particularly in erect
643 colonies, which vary from small and monosiphonic to large and polysiphonic; and in the shape
644 of the hydrothecal cusps, with deep or shallow embayments (Cornelius, 1975, 1982, 1990,
645 1995; Millard, 1975; Mammen, 1965; Calder, 1991). This variation led to some dispute on the
646 validity of several nominal species that have been frequently synonymized with *O. bidentata*,
647 basically due to misinterpretation of intra- or interspecific variations (e.g., *Obelia longicyatha*
648 Allman, 1877, *Obelia austrogeorgiae* Jäderholm, 1904; Cornelius, 1975, 1982; Calder, 1991).
649 Calder (2013) recently regarded *O. oxydentata* Stechow, 1914 as a valid species based on the
650 smaller size of the monosiphonic colonies from the tropical and subtropical western Atlantic
651 (<1 cm high). In our study, we found that small (0.3-1 cm high) monosiphonic colonies and
652 large (>6 cm high) polysiphonic colonies (USNM 1106185, from the North Sea) are related in
653 nearly all topologies analyzed in previous molecular studies (Govindarajan *et al.*, 2006; Cunha
654 *et al.*, 2017), partially contradicting the idea that these variations could indicate interspecific
655 differences (see Calder, 2017). However, as pointed out by Cunha *et al.* (2017), *O. bidentata*
656 exhibits intraspecific genetic distances that are comparable to interspecific distances in other
657 clades, and this could be evidence of either extensive population differentiation or the
658 occurrence of a species complex (as in *C. hemisphaerica*, see above).
659 Obeliida indet. was ambiguously positioned at the base of Obeliidae and Clytiidae plus
660 Obeliidae in the phylogenetic analysis of Cunha *et al.* (2017). In that study, this species was

661 tentatively assigned to *Clytia stolonifera* Blackburn, 1938. We show that it can be
662 differenciated from the remaining Obeliidae by its longer hydrothecae and taller hydrothecal
663 cusps (Table 2). However, the inclusion and comparison of more specimens is necessary to
664 confirm this identification and ascertain if this species should be considered in the genus *Clytia*
665 or *Obelia*.
666
667 Morphometric patterns of *Obelia dichotoma* and *O. longissima*
668 Differences in size, branching patterns, tanning of the main stem, and the shapes of the
669 hydrothecae and hydrothecal rim have long been used to distinguish *Obelia longissima* (Pallas,
670 1766) and *O. dichotoma* (Linnaeus, 1758) (Alder, 1857; Hincks, 1868; Nutting, 1915; Kramp,
671 1935). Currently, besides the differences in their nematocysts (Östman, 1982a), *O. longissima*
672 is characterized by having predominantly monosiphonic colonies with usually longer stems
673 and branches roughly uniform in length, as well as a dark and flexuous main stem. *Obelia*
674 *dichotoma*, on the other hand, has polysiphonic stems in older colonies, with branches often
675 nearly as long as the main stem, giving the colony a bushy appearance (Östman, 1987;
676 Cornelius, 1990, 1995; Schuchert, 2001; Calder, 2012). Additionally, the hydrotheca in *O.*
677 *dichotoma* is often polygonal in cross-section, with an even to crenate rim; while the
678 hydrotheca in *O. longissima* is round with the rim castellate to sinuous (Cornelius, 1990, 1995).
679 The hydrothecal diaphragm varies from transverse to oblique in both species (Cornelius, 1990,
680 1995). Previous molecular studies showed that *O. dichotoma* comprises several cryptic
681 lineages (Cunha *et al.*, 2017), and *O. longissima* was corroborated as a monophyletic and
682 widely distributed species (Govindarajan *et al.*, 2006; Cunha *et al.*, 2017). Our results revealed
683 that some characters support the separation of the species (Supporting Information, Table S6),
684 viz. (1) size of the colony, with *O. longissima* usually larger than species of *O.* cf. *dichotoma*,
685 although some lineages of the latter exceeded the former in the number of branches; (2) length

686 of internodes, longer on average in *O. longissima* but with some overlap with lineages of *O.*
687 cf. *dichotoma*; (3) hydrothecal length, usually longer in *O. longissima* but with some overlap
688 with species of *O.* cf. *dichotoma*; (4) shape of the hydrothecal rim, varying from smooth to
689 crenate in all lineages of *O.* cf. *dichotoma*, and invariably sinuous in *O. longissima*.
690 Morphological variation may obscure some of these differences, but colonies of *O. longissima*
691 can be reliably delimited by these characters when intraspecific variation is considered.
692 Contrastingly, cryptic lineages of *O.* cf. *dichotoma* do not show morphometric
693 differences, presenting extensive variation and overlap in their characters (Fig. 13). Although
694 *O.* cf. *dichotoma* sp.3 and sp.4 could be distinguished from the remaining lineages by their
695 smaller and less branched colonies (Fig. 13A, Supporting Information, Table S6), in some
696 cases colonies varied from unbranched to branched within the same lineage, indicating that
697 these characters vary intra- and interspecifically. This also partially contradicts the idea that
698 the amount of branching of the colonies could support the validation of former synonyms of
699 *O. dichotoma* (e.g., *Obelia hyalina* Clarke, 1879, *Obelia griffini* Calkins, 1899; see Calder,
700 2013; Calder *et al.*, 2014), although their size and the shape of the hydrothecae are probably
701 distinctive. For instance, Calder (2013) showed that colonies of *O. hyalina* are usually small
702 and occur in tropical and warm-temperate waters. We found that all specimens of Brazilian *O.*
703 cf. *dichotoma* are also small (~4-11 mm) and have few branches, although some have a slightly
704 crenate hydrothecal rim (*O.* cf. *dichotoma* sp.3, Fig. 11K, Supporting Information, Table S6),
705 in contrast to the even hydrothecal rim of *O. hyalina* (Clarke, 1879; Calder, 2013). Similarly,
706 all specimens of *O.* cf. *dichotoma* sp.4 have rounded hydrothecae in cross section and an even
707 hydrothecal rim (Fig. 11L, Supporting Information, Table S6), in accordance with the
708 diagnostic characters of *O. griffini*, recently revalidated by Calder *et al.* (2014). Although these
709 identifications are tentative and need further confirmation, our results could support the
710 revalidation of former synonyms of *O. dichotoma* to accommodate these cryptic lineages.

711 Better knowledge of the nematocysts of these lineages might be particularly important for their
712 corroboration, especially given that I_D and I_d-type isorhizae are diagnostic for *O. dichotoma*
713 and assumed to be invariably present in the species (Östman, 1982a, 1987; Cornelius, 1990).
714
715 **Conclusions**
716 This study demonstrates the usefulness of morphometric data to delimit species in
717 Proboscoida. We showed that morphometric characters related to size, perisarc thickness,
718 shape of hydrothecae, and hydrothecal cusps may contribute to the delimitation of several
719 species, although in some cases (e.g., *Campanularia* spp., *Clytia gracilis*, *Clytia*
720 *hemisphaerica*, *Laomedea* spp., *Obelia dichotoma*), morphometric differences are masked by
721 intraspecific variation (see summary in Table 2 and phylogenetic hypothesis with the species
722 reidentified in this study in Fig. 15). Considering that our study was limited to the hydroid
723 stage, extending this approach to investigate characters of the medusa stage and nematocysts
724 is promising, and may shed light on some of the remaining difficult cases. However, some
725 attention and specific procedures should be taken into consideration for this taxonomic
726 approach. Even though many marine groups have wide intraspecific variation, consistent
727 differences in morphometric patterns may be uncovered once this variation is comparatively
728 investigated. This might be difficult to persue at first, without access to data from different
729 populations and morphological characters. However, this problem will be gradually overcome
730 once taxonomic descriptions that include morphometric characters and their amplitude of
731 variation are more often linked to molecular data of voucher specimens. Morphometric
732 characters are usually simple to obtain with the aid of compound or stereo microscopes and
733 digital cameras, and in most cases they will be more informative for the identification if
734 considered in conjunction with other discrete diagnostic characters, as well as information on
735 genetic differentiation of populations.

736 Thorough investigations using morphometric data for voucher specimens and molecular
737 trees, complemented by broader inferences in population morphological and morphometric
738 variation, will improve delimitations of species and, as a corollary, result in more complete and
739 precise taxonomic descriptions that allow for accurate identifications. This approach will
740 directly impact our current knowledge on Hydrozoa (as well as Medusozoa and other marine
741 taxa), refining our assessments of marine species diversity.
742
743 **References**

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Figure Legends

Figure 1. Distance biplots of the Principal Component Analysis (PCA) comprising data for Campanulariidae. A. First and second principal components (PCs) of the PCA with the complete dataset; B. Second and third PCs of the PCA with the complete dataset; C. First and second PCs of the PCA without the genus *Orthopyxis*; D. First and second PCs of the PCA with *Campanularia* and *Rhizocaulus*; E. First and second PCs of the PCA with *Campanularia* and *Orthopyxis*; F. First and second PCs of the PCA with *Orthopyxis*, but excluding *O. sargassicola* and *O. crenata*. In E and F, position of the specimen *Orthopyxis integra*_1_USA is shown (see Supporting Information, Table S1). Numbers in parentheses indicate percentages of variation explained by each principal component. Abbreviations of morphometric variables as in Table 1, and those in bold indicate measurements that were correlated with each principal component (Pearson correlation >0.7 and <-0.7).

Figure 2. General morphology of species of Campanulariidae and Clytiidae. A. *Bonneviella regia* (USNM 1106181); B. *Bonneviella superba* (USNM 1106182); C. *Bonneviella ingens* (USNM 1106187); D. *Silicularia rosea* (PT11_ARG); E. *Clytia* cf. *gracilis* sp.1 (EL32_SLV), with gonotheca; F. *Clytia* cf. *gracilis* sp.3 (EL05_SLV), with detail of hydrothecal cusps; G. *Clytia* cf. *gracilis* sp.5 (PAF03_BRA); H. *Clytia* cf. *hemisphaerica* sp.1 (FLT03_USA), with detail of hydrothecal cusps; I. *Clytia* cf. *hemisphaerica* sp.2 (EL06_SLV), with gonotheca. Scales: A, C = 1 mm; B = 2mm; = 300 μm; F (both), G, H (cusps), I (trophosome) = 100 μm; D, E (both), H (trophosome), I (gonotheca) = 200 μm.

Figure 3. Mean ± standard deviation of morphometric data for *Campanularia*. Morphological variation in *C. volubilis* is presented as intracolony (^{I}) and population variation (^{P}, ZMUC and USNM 29217, see Table S1) for comparison. A. Length of hydrothecae (LH, μm); B.

1152 Maximum height of hydrothecal cusps (HCMax, μm); C. Number of hydrothecal cusps (NC);
1153 D. Length:diameter ratio of hydrotheca (HRatio). Brackets = [number of specimens measured].
1154
1155 Figure 4. Mean ± standard deviation of morphometrica data for *Orthopyxis*, including a
1156 comparison with species of *Campanularia* (ie., *C. subantarctica*, *C. hincksii* and
1157 *Campanularia* sp., Supporting Information, Table S1). Morphological variation in *O. crenata*
1158 and *O. integra* is presented separately for some populations and combined ("all"), for
1159 comparison. Data for specimens of *O. crenata* from New Zealand, Argentina and Brazil are
1160 represented with numbers 1 to 3, respectively. Similarly, data for specimens of *O. integra* from
1161 the Aleutian Islands and Argentina are represented with number 1 and 2, respectively. A, B.
1162 Maximum perisarc thickness of hydrotheca at medial portion (PHMe, μm); C. Length:diameter
1163 ratio of hydrotheca (HRatio); D. Length of hydrotheca (LH, μm). Brackets = [number of
1164 specimens measured].
1165
1166 Figure 5. A. *Orthopyxis integra*_1_USA (USNM 1106184), with gonothecae; B. *Orthopyxis*
1167 *integra* (PT20_ARG); B.; C. *Orthopyxis caliculata* (PAB4_BRA, MZUSP 2554), with
1168 gonotheca; D. *Orthopyxis mianzani* (FOB7_BRA, MZUSP 2580), with gonotheca; E.
1169 *Orthopyxis asymmetrica* (EL02_SLV); F. Gonothecae of *Orthopyxis asymmetrica*
1170 (EL02_SLV). Scales: A, F = 500 μm; B, D (gonotheca) = 300 μm; C, D (trophosome), E = 200
1171 μm. For specimens and codes see Supporting Information, Table S1.
1172
1173 Figure 6. Distance biplots of Principal Component Analysis (PCA) comprising data for
1174 Clytiidae. A. First and second principal components (PCs) of the PCA with the complete
1175 dataset; B. Second and third PCs of the PCA with the complete dataset; C. First and second
1176 PCs of the PCA without *Clytia* cf. *gracilis* lineages; D. First and second PCs of the PCA

without *C.* cf. *gracilis* lineages and measurements related to internodes of erect colonies (NIS, LIS, AIS, PIS, DIS); E. First and second PCs of the PCA with lineages of *C.* cf. *gracilis*; F. First and second PCs of the PCA with lineages of *C.* cf. *gracilis*, excluding measurements related to internodes of erect colonies (NIS, LIS, AIS, PIS, DIS). Numbers in parentheses indicate percentages of variation explained by each principal component. Abbreviations of morphometric variables as in Table 1, and those in bold indicate measurements that were correlated with each principal component (Pearson correlation >0.7 and <-0.7).

Figure 8. Mean ± standard deviation of morphometric data for *Clytia* species. Data for *Clytia* sp.1 and sp.2 refers to intracolony (^{I}) variation. A. Length of the hydrotheca (LH, μm); B. Maximum height of hydrothecal cusps (HCMax, μm); C. Maximum diameter of hydrotheca at medial portion (DHMe, μm); D. Thickness of diaphragm (TD, μm); E. Maximum hydrothecal perisarc thickness at margin (PHMa, μm); F. Length:diameter ratio of hydrotheca (HRatio). Brackets = [number of specimens measured].

Figure 10. Distance biplots of the Principal Component Analysis (PCA) comprising data for the family Obeliidae. A. First and second principal components (PCs) of the PCA with the complete dataset; B. First and second PCs of the PCA with the complete dataset, excluding measurements related to second-order branches of erect colonies (NIB, DIB, AIB, LIB); C. First and second PCs of the PCA with *Obelia* only; D. First and second PCs of the PCA without *Obelia* and measurements related to second-order branches of erect colonies (NIB, DIB, AIB, LIB); E. First and second PCs of the PCA with *O.* cf. *dichotoma* and *O. longissima*; F. First and second PCs of the PCA with measurements of the gonothecae. Numbers in parentheses indicate percentages of variation explained by each principal component. Abbreviations of

morphometric variables as in Table 1, and those in bold indicate measurements that were correlated with each principal component (Pearson correlation >0.7 and <-0.7).

Figure 11. A. *Obelia geniculata* (BZ5_BRA); B. *Laomedea flexuosa* (RYE02_USA), with gonothecae; C, D. *Obelia longissima* (GFP04_USA), with detail of hydrotheca (C); E. *Obelia bidentata* (MAP10_BRA), with gonothecae; F. *Obelia bidentata* (USNM 1106185); G. *Hartlaubella gelatinosa* (PT14_ARG); H. *Hartlaubella gelatinosa* (PT16_ARG), with gonotheca; I. *Obelia* cf. *dichotoma* sp.1 (PIM01_USA), with detail of hydrotheca; J. *Obelia* cf. *dichotoma* sp.2 (PT2_ARG), with detail of hydrotheca; K. *Obelia* cf. *dichotoma* sp.3 (PAF07_BRA), with detail of hydrotheca; L. *Obelia* cf. *dichotoma* sp.4 (Site 1.1_USA), with detail of hydrotheca. Scales: A, B (both), E, K (colony) = 200 μm; C, H (both), I-L (all hydrotheca) = 100 μm; D, F, G = 2 mm; I, J, L (all colony) = 1 mm.

Figure 12. Mean ± standard deviation of morphometric data for Obeliidae. Data for the genus *Obelia* comprises all species included in this study, except *O. geniculata*. A. Maximum hydrothecal perisarc thickness at margin (PHMa, μm); B. Maximum hydrothecal diameter at margin (DHMa, μm); C. Length of pedicel (LP, μm); D. Length of the hydrotheca (LH, μm); E. Length:diameter ratio of the hydrotheca (HRatio); F. Maximum height of hydrothecal cusps (HCMax, μm). Brackets = [number of specimens/colonies measured].

Figure 13. Mean ± standard deviation of morphometric data for the lineages identified as *Obelia* cf. *dichotoma.* A. Total length of the trophosome (TLT, mm); B. Length of the hydrotheca (LH, μm); C. Length:diameter ratio of the hydrotheca (HRatio); D. Maximum height of hydrothecal cusps (HCMax, μm). Brackets = [number of specimens/colonies measured].

Figure 14. Variation in the shape of hydrothecal cusps of *Clytia* cf. *gracilis* sp.5. A, B. Specimens from Fortaleza, Brazil (CE2_BRA, CE5_BRA); C, D. Specimens from Cascavel, Brazil (CE1_BRA, CE3_BRA); E, F. Specimens from São Luís do Maranhão, Brazil (MAP01_BRA, MAP11_BRA); G. Specimen from Trairi, Brazil (T1_BRA); H. Specimen from Salinópolis, Brazil (PAF03_BRA). Scale: 100 μm.

Figure 15. Phylogenetic hypothesis of Proboscoida based on the Maximum Likelihood phylogeny of Cunha *et al.* (2017, Fig. 2 therein), including the reidentifications proposed in this study. Branches in grey indicate lineages not analyzed in this study.

Figure S1. Distance biplots of the Principal Component Analysis (PCA) comprising data for Campanulariidae. A. Second and third principal components (PCs) of the PCA without the genus *Orthopyxis*; B. Second and third PCs of the PCA with *Campanularia* and *Orthopyxis*; C. First and second PCs of the PCA including only *Orthopyxis*; D. Second and third PCs of the PCA with *Orthopyxis*; E. Second and third PCs of the PCA with *Orthopyxis,* but excluding *O. sargassicola* and *O. crenata*; F. First and second PCs of the PCA with measurements of the gonothecae. Numbers in parentheses indicate percentages of variation explained by each principal component. Abbreviations of morphometric variables as in Table 1, and those in bold indicate measurements that were correlated with each principal component (Pearson correlation >0.7 and <-0.7).

Figure S2. Distance biplots of the Principal Component Analysis (PCA) comprising data for Clytiinae. A. First and second principal components (PCs) of the PCA with the complete dataset, and without measurements related to internodes of erect colonies (NIS, LIS, AIS, PIS,

DIS, ABS); B. Second and third PCs of the PCA without *Clytia gracilis*; C. Second and third PCs of the PCA without *C. gracilis* and measurements related to internodes of erect colonies; D. First and second PCs of the PCA with *C. hemisphaerica*, but without measurements related to internodes of erect colonies; E. Second and third PCs of the PCA with *C. gracilis*; F. First and second PCs of the PCA with measurements of the gonothecae. Numbers in parentheses indicate percentages of variation explained by each principal component. Abbreviations of morphometric variables as in Table 1, and those in bold indicate measurements that were correlated with each principal component (Pearson correlation >0.7 and <-0.7).

Figure S3. Distance biplots of the Principal Component Analysis (PCA) comprising data for Obeliidae. A. Second and third principal components (PCs) of the PCA with the complete dataset; B. Second and third PCs of the PCA with the complete dataset, but excluding measurements related to second-order branches of erect colonies (NIB, DIB, AIB, LIB); C. Second and third PCs of the PCA without the genus *Obelia*; D. Second and third PCs of the PCA with the genus *Obelia* only; E. Second and third PCs of the PCA with *O. dichotoma* and *O. longissima*; F. First and second PCs of the PCA with *O. geniculata*. Numbers in parentheses indicate percentages of variation explained by each principal component. Abbreviations of morphometric variables as in Table 1, and those in bold indicate measurements that were correlated with each principal component (Pearson correlation >0.7 and <-0.7).

Figure S4. Mean ± standard deviation of morphometric data for species identified as *Clytia* cf. *hemisphaerica*. A. Length of the hydrotheca (LH, μm); B. Length:diameter ratio of hydrotheca (HRatio, μm); C. Number of hydrothecal cusps (NC); D. Maximum height of hydrothecal cusps (HCMax, μm). Brackets = [number of specimens/colonies measured].

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1278 Table 1. Measurements included in the morphometric analysis (codes are in alphabetical order).

Code	Measurement
AG	Number of Gonothecal Annuli
AGP	Number of Annuli of Gonothecal Pedicel
AIB	Maximum Number of Annuli of the Internodes of Side Branches
AIS	Maximum Number of Annuli of the Internodes of Main Stem
APB	Number of Pedicel Annuli at Base
APH	Number of Pedicel Annuli below Hydrotheca
APMe	Number of Pedicel Annuli at Medial Portion
DBC	Diameter of Hydrothecal Basal Chamber (at diaphragm)
DGB	Maximum Gonothecal Diameter at Base
DGD	Maximum Gonothecal Diameter at Distal Portion
DGMe	Maximum Gonothecal Diameter at Medial Portion
DGP	Maximum Diameter of Gonothecal Pedicel at Medial Portion
DHB	Maximum Hydrothecal Diameter at Base
DHMa	Maximum Hydrothecal Diameter at Margin
DHMe	Maximum Hydrothecal Diameter at Medial Portion
DIB	Maximum Diameter of Internode of Side Branches at Medial Portion
DIS	Maximum Diameter of Internode of Main Stem at Medial Portion
DP	Maximum Diameter of Pedicel at Medial Portion
DSS	Maximun Diameter of Subhydrothecal Spherule
GRatio	Length:Diameter (at medial portion) Ratio of Gonotheca
HCMax	Maximum Height of Hydrothecal Cusps
HCMin	Minimum Height of Hydrothecal Cusps
HGC	Height of Gonothecal Collar

Code	Measurement
HRatio	Length:Diameter (at medial portion) Ratio of Hydrotheca
LBC	Length of Hydrothecal Basal Chamber
LG	Length of Gonotheca
LGP	Length of Gonothecal Pedicel
LH	Length of Hydrotheca
LIB	Length of Internode of Side Branches
LIS	Length of Internode of Main Stem
LP	Length of Pedicel
LSS	Length of Subhydrothecal Spherule
NC	Number of Hydrothecal Cusps
NIB	Maximum Number of Internodes of Side Branches
NIS	Total Number of Internodes of Main Stem
NSG	Number of Gonothecal Sinuosities (crenations)
NSP	Maximum Number of Pedicel Sinuosities (crenations)
PGMe	Maximum Gonothecal Perisarc Thickness at Medial Portion
PGP	Maximum Perisarc Thickness of Gonothecal Pedicel at Medial Portion
PHB	Maximum Hydrothecal Perisarc Thickness at Base
PHMa	Maximum Hydrothecal Perisarc Thickness at Margin
PHMe	Maximum Hydrothecal Perisarc Thickness at Medial Portion
PIB	Maximum Perisarc Thickness of Internode of Side Branches at Medial Portion
PIS	Maximum Perisarc Thickness of Internode of Main Stem at Medial Portion
PPMe	Maximum Perisarc Thickness of Pedicel at Median Portion
PSS	Maximum Perisarc Thickness of Subhydrothecal Spherule
TD	Thickness of Diaphragm

Code	Measurement
TLT	Total Length of Trophosome

Table 2. Summary of species delimited in this study and their morphometric characters. This symbol * indicate groups that were monophyletic in most, but not all of the phylogenies in Cunha *et al.* (2017). The species *Orthopyxis integra* (MacGillivray, 1842) is not monophyletic in its traditional sense (see text). The genera *Rhizocaulus*, *Tulpa*, *Gonothyraea* and *Hartlaubella* were represented by only one species, therefore their monophyletism needs confirmation (Cunha *et al.*, 2017). When referring to family or genus, comparative conclusions on distinctive morphometric characters are limited to the species analyzed in this study.

Taxon	**Specimen(s) (see Table S1)**	**Monophyletic? (Cunha *et al.*, 2017)**	**Morphometric diagnostic characters**	**Morphometric characters are distinctive when compared to**
Infraorder Campanulariida Bouillon, 1984		yes		
Family Campanulariidae Johnston, 1836		yes		
Genus *Bonneviella* Broch, 1909		yes*	Total length of the trophosome, length of the pedicel and hydrotheca	Campanulariidae
Bonneviella ingens Nutting, 1915	*Bonneviella* sp. (USNM 1106187)	yes	Size and shape of hydrotheca	Campanulariidae

Taxon	**Specimen(s) (see Table S1)**	**Monophyletic? (Cunha *et al.*, 2017)**	**Morphometric diagnostic characters**	**Morphometric characters are distinctive when compared to**
Bonneviella regia (Nutting, 1901)	USNM 1106181	yes	Size of hydrotheca	Campanulariidae
Bonneviella superba Nutting, 1915	*Bonneviella* sp. (USNM 1106182)	yes	Size of hydrotheca (the largest in *Bonneviella*)	Campanulariidae
Genus *Campanularia* Lamarck, 1816		**no**	Perisarc thickness, length and length:diameter ratio of hydrotheca	*Orthopyxis*, except for some specimens of *O. sargassicola* and *O. crenata*
Campanularia hincksii Alder, 1856	MZUSP 2759-60; USNM 1106157	yes	Height of hydrothecal cusps	other species of *Campanularia*
Campanularia subantarctica	MZUSP 2639, 2643	yes	Distinctive morphometric characters not found	-
Campanularia sp.	MZUSP 2641-42, 2761	yes	Distinctive morphometric characters not found	-
Campanularia volubilis	USNM 1106166	yes	Distinctive morphometric characters not	-

Taxon	Specimen(s) (see Table S1)	Monophyletic? (Cunha *et al.*, 2017)	Morphometric diagnostic characters	Morphometric characters are distinctive when compared to
			found	
Genus *Orthopyxis* L. Agassiz, 1862		yes*	Perisarc thickness, length and length:diameter ratio of hydrotheca	*Campanularia*
Orthopyxis asymmetrica Stechow, 1919	*Orthopyxis* sp.1, *Orthopyxis everta*, *Orthopyxis integra*_IT (MZUSP 3360-63; USNM 1106159-80)	yes	Length of hydrotheca and pedicel, perisarc thickness, length:diameter ratio of hydrothecal basal chamber	other species of *Orthopyxis*
Orthopyxis caliculata (Hincks, 1853)	MZUSP 2612-15, 2550, 2552, 2554, 2556, 2563, 2565, 4177, 4265	yes	Length of hydrotheca and pedicel, perisarc thickness	other species of *Orthopyxis*
Orthopyxis crenata (Hartlaub,	MZUSP 2551, 2560,	yes	Number and height of hydrothecal cusps	other species of *Orthopyxis*,

Taxon	Specimen(s) (see Table S1)	Monophyletic? (Cunha *et al.*, 2017)	Morphometric diagnostic characters	Morphometric characters are distinctive when compared to
1901)	2598, 2601, 2633, 3359, *Orthopyxis* sp. (MZUSP 2644); *Orthopyxis integra*_NZ (USNM 1106163)		(but may eventually present even hydrothecal rim)	except for *O. sargassicola*
Orthopyxis integra (MacGillivray, 1842)	MZUSP 3358, USNM 1106184, Campanulariidae sp. indet. (MZUSP 2638, 2640)	yes	Length of hydrotheca and pedicel, perisarc thickness, length:diameter ratio of hydrotheca	other species of *Orthopyxis*
Orthopyxis mianzani Cunha, Genzano & Marques, 2015	MZUSP 2559, 2570-80; USNM 1259970	yes	Length of hydrotheca and pedicel, perisarc thickness	other species of *Orthopyxis*
Orthopyxis sargassicola	MZUSP 2593-97,	yes	Number and height of hydrothecal cusps	other species of *Orthopyxis,*

Taxon	Specimen(s) (see Table S1)	Monophyletic? (Cunha *et al.*, 2017)	Morphometric diagnostic characters	Morphometric characters are distinctive when compared to
(Nutting, 1915)	2599-2600, 2602-03, 2605-11, 2617-20, 2627-2630, 2632, 4597			except for *O. crenata*
Genus *Rhizocaulus* Stechow, 1919		yes*		
Rhizocaulus verticillatus (Linnaeus, 1758)	USNM 1106183	yes	Total length of trophosome, length of hydrotheca	*Campanularia* and *Orthopyxis*
Genus *Silicularia* Meyen, 1834		yes		
Silicularia rosea Meyen, 1834	MZUSP 3365, 3364; USNM 1106164	yes	Perisarc thickness	Campanulariidae, except for *Orthopyxis*
Genus *Tulpa* Stechow, 1921		yes*		
Tulpa tulipifera (Allman,	MZUSP 3366	yes	Size of hydrotheca	Campanulariidae

Taxon	Specimen(s) (see Table S1)	Monophyletic? (Cunha *et al.*, 2017)	Morphometric diagnostic characters	Morphometric characters are distinctive when compared to
1888)				
Infraorder Obeliida		yes		
Maronna *et al.*, 2016				
Obeliida indet.	USNM 1420685, 1420678	yes	Height of hydrothecal cusps, length of hydrothecae	Obeliidae, except for *O. longissima* (length of hydrothecae)
Family Clytiidae Cockerell, 1911		**no**		
Genus *Clytia* Lamouroux, 1812		**no**		
Clytia elsaeoswaldae Stechow, 1914	LEM PM18, PM36, Me26, CB19; USNM 1078725, 1078728	yes	Diameter of hydrotheca, thickness of diaphragm	*Clytia* cf. *gracilis* and *Clytia* cf. *hemisphaerica* (diameter); Clytiidae (diaphragm)
Clytia cf. *gracilis* sp.1	*Clytia gracilis* I (MZUSP 2768-70,	yes	Length and diameter of hydrotheca and pedicel, number and height of	*Clytia* cf. *gracilis* sp.3 and sp.4

Taxon	Specimen(s) (see Table S1)	Monophyletic? (Cunha *et al.*, 2017)	Morphometric diagnostic characters	Morphometric characters are distinctive when compared to
	2772, 2773)		hydrothecal cusps	
Clytia cf. *gracilis* sp.2	*Clytia gracilis* II (MZUSP 2785); *Clytia gracilis* sp.D (USNM 1106152)	yes	Length and diameter of hydrotheca and pedicel, number and height of hydrothecal cusps	*Clytia* cf. *gracilis* sp.3 and sp.4
Clytia cf. *gracilis* sp.3	*Clytia gracilis* III (MZUSP 2766, 2767, 2771)[1]	yes	Length and diameter of hydrotheca and pedicel, number and height of hydrothecal cusps	*Clytia* cf. *gracilis* sp.1, sp.2 and sp.B
Clytia cf. *gracilis* sp.4	*Clytia gracilis* IV (USNM 1420648, 1420655, 1420660)	yes	Length and diameter of hydrotheca and pedicel, number and height of hydrothecal cusps, length:diameter ratio of hydrotheca	*Clytia* cf. *gracilis* sp.1, sp.2 and sp.B (length, diameter, number and height of cusps); Clytiidae, except for remaining *C.* cf. *gracilis* and *C.* cf. *hemisphaerica* (ratio)

Taxon	**Specimen(s) (see Table S1)**	**Monophyletic? (Cunha *et al.*, 2017)**	**Morphometric diagnostic characters**	**Morphometric characters are distinctive when compared to**
Clytia cf. *gracilis* sp.5	*Clytia gracilis* V (MZUSP 2774-84)[2]	yes	Distinctive morphometric characters not found	-
Clytia cf. *gracilis* sp.B	USNM 1078730	yes	Length and diameter of hydrotheca and pedicel, number and height of hydrothecal cusps	*Clytia* cf. *gracilis* sp.3 and sp.4
Clytia cf. *hemisphaerica* sp.1	*Clytia hemisphaerica* I (MZUSP 2786-89)[3]	yes	Distinctive morphometric characters not found	-
Clytia cf. *hemisphaerica* sp.2	*Clytia hemisphaerica* II (MZUSP 2790-95; USNM 1106186)	yes	Distinctive morphometric characters not found	-
Clytia cf. *hemisphaerica* sp.3	*Clytia hemisphaerica* III (USNM 1420636, 1420659, 1420673)	yes	Distinctive morphometric characters not found	-
Clytia linearis	MZUSP 2796;	yes	Length of hydrotheca	Clytiidae

Taxon	Specimen(s) (see Table S1)	Monophyletic? (Cunha *et al.*, 2017)	Morphometric diagnostic characters	Morphometric characters are distinctive when compared to
	USNM 1078729			
Clytia noliformis	MZUSP 2797-98; USNM 1078720	yes	Perisarc thickness	Clytiidae, except for *Clytia* sp.2
Clytia paulensis	USNM 1106158	yes	Length:diameter ratio of hydrotheca	Clytiidae, except for *C.* cf. *gracilis*
Clytia sp.1	MZUSP 2799	yes	Length:diameter ratio of hydrotheca	Clytiidae, except for *C.* cf. *gracilis* and *C.* cf. *hemisphaerica*
Clytia sp.2	MZUSP 2800	yes	Perisarc thickness	Clytiidae, except for *C. noliformis*
Clytia sp.3	MZUSP 2801	yes	Length of pedicel, number of pedicel annuli at base	Clytiidae, except for *C.* cf. *gracilis* and *C.* cf. *hemisphaerica*
Family Obeliidae Haeckel, 1879		yes		
Genus *Gonothyraea* Allman, 1864		yes*		
Gonothyraea loveni (Allman,	MZUSP 2802-03;	yes	Branching of erect colonies,	Obeliidae, except for *Obelia*

Taxon	Specimen(s) (see Table S1)	Monophyletic? (Cunha *et al.*, 2017)	Morphometric diagnostic characters	Morphometric characters are distinctive when compared to
1859)	USNM 1106154		length:diameter ratio of hydrotheca, height of hydrothecal cusps	(branching); *O. bidentata* (ratio and cusps)
Genus *Hartlaubella* Poche, 1914		yes*		
Hartlaubella gelatinosa (Pallas, 1766)	MZUSP 2804-06	yes	Branching of erect colonies, length:diameter ratio of hydrotheca, height of hydrothecal cusps	Obeliidae, except for *Obelia* (branching); *O. bidentata* (ratio and cusps)
Genus *Laomedea* Lamouroux, 1812		**no**	Length of pedicel and gonotheca	*Obelia* (pedicel); Obeliidae (gonotheca)
Laomedea angulata Hincks, 1861	MZUSP 2807-08	yes	Distinctive morphometric characters not found	-
Laomedea calceolifera (Hincks, 1861)	MZUSP 2810, 2812-15; MHNG INVE 37296; USNM	yes	Distinctive morphometric characters not found	-

Taxon	Specimen(s) (see Table S1)	Monophyletic? (Cunha *et al.*, 2017)	Morphometric diagnostic characters	Morphometric characters are distinctive when compared to
	1106177			
Laomedea flexuosa Alder, 1857	MZUSP 2816; USNM 1106190, 1106192	yes	Diameter of hydrotheca and pedicel	Obeliidae
Genus *Obelia* Péron & Lesueur, 1810		**no**		
Obelia bidentata Clark, 1875	MZUSP 2817-2818; USNM 1106162, 1106185, 1420668	yes	Length:diameter ratio of hydrotheca, number and height of hydrothecal cusps	Obeliidae (ratio); *G. loveni* and *H. gelatinosa* (cusps)
Obelia cf. *dichotoma* sp.1	*Obelia dichotoma* I (MZUSP 3336-40, 3344-45)	yes	Distinctive morphometric characters not found	-
Obelia cf. *dichotoma* sp.2	*Obelia dichotoma* II (MZUSP 3335, 3342-	yes	Distinctive morphometric characters not found	-

Taxon	Specimen(s) (see Table S1)	Monophyletic? (Cunha *et al.*, 2017)	Morphometric diagnostic characters	Morphometric characters are distinctive when compared to
	43; USNM 1106156)			
Obelia cf. *dichotoma* sp.3	*Obelia dichotoma* III (MZUSP 2819-20, 3334)	yes	Branching of erect colonies, total length of trophosome	*Obelia* cf. *dichotoma* sp.1 and sp.2
Obelia cf. *dichotoma* sp.4	*Obelia dichotoma* IV (MZUSP 3341, 3346)	yes	Branching of erect colonies, total length of trophosome	*Obelia* cf. *dichotoma* sp.1 and sp.2
Obelia geniculata (Linnaeus, 1758)	*Obelia geniculata* I, II, III, IV (MZUSP 3347-51; USNM 1106165, 1106176, 1106179)	yes	Perisarc thickness	Obeliidae
Obelia longissima (Pallas, 1766)	MZUSP 3352-55; USNM 1106153, 1106173, 1106189,	yes	Branching of erect colonies, total length of trophosome, length of internodes and hydrotheca, height (shape) of	Obeliidae, except some specimens of *Obelia* cf. *dichotoma* (branching, total length); some

Taxon	Specimen(s) (see Table S1)	Monophyletic? (Cunha *et al.*, 2017)	Morphometric diagnostic characters	Morphometric characters are distinctive when compared to
	1106191		hydrothecal cusps	specimens of *O.* cf. *dichotoma* (all remaining characters)
Obelia sp.1	MZUSP 3356-57	yes	Length:diameter ratio of hydrotheca, length of hydrotheca, height of hydrothecal cusps	*O. bidentata* (ratio and length); Obeliidae, except for *O. bidentata* and Obeliida indet. (cusps)

[1]Specimens identified as *Clytia sp.* from He *et al.* (2015) clustered with specimens of *Clytia* cf. *gracilis* sp.3 in the phylogeny of Cunha *et al.* (2017), and should be referred to that species. However, since we were not able to study the morphology of these specimens, they were not considered in the proposed reidentifications.

[2]Specimens identified as *Clytia gulangensis* from He *et al.* (2015) clustered with specimens of *Clytia* cf. *gracilis* sp.5 in the phylogeny of Cunha *et al.* (2017) (see discussion). Since we were not able to study the morphology of these specimens, they were not considered in the proposed reidentifications.

[3]Specimens identified as *Clytia gracilis* sp.A from Lindner *et al.* (2011) clustered with specimens of *Clytia* cf. *hemisphaerica* sp.1 in the phylogeny of Cunha *et al.* (2017), and should be referred to that species. Specimens identified as *Clytia xiamenensis* from Zhou *et al.* (2013) also clustered

with *Clytia* cf. *hemisphaerica* sp.1, but these results are only based on 16S sequences (see Cunha *et al.*, 2017), and should be confirmed. Since we were not able to study the morphology of these specimens, they were not considered in the proposed reidentifications.