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

AMANDA F. CUNHA[1*], ALLEN G. COLLINS[2] and ANTONIO C. MARQUES[1]

[1]*Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, Travessa 14, 101, 05508-090, São Paulo, Brazil*
[2]*National Systematics Laboratory, National Marine Fisheries Service (NMFS), National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA*

Species delimitation in marine taxa is often problematic given large intraspecific variation. Based on extensive, recently published genetic sampling from specimens of the hydrozoan families Campanulariidae, Clytiidae and Obeliidae, we evaluate morphological variation in this group, correlating morphometric and phylogenetic patterns for species delimitation. Several species of Campanulariidae are confidently delimited based on differences in size (e.g. *Bonneviella* species, *Tulpa tulipifera* and *Rhizocaulus verticillatus*), while others are re-identified 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 delimit the species *Clytia linearis*, *C. elsaeoswaldae* and *C. noliformis* from others. However, few characters reliably differentiate the clades associated with the nominal species *C. gracilis* and *C. hemisphaerica*. In Obeliidae, *Obelia geniculata* is distinctive in its higher perisarc thickness, and corroborated as a widely distributed species. *Obelia longissima* and clades refered to *O. dichotoma* are subtly distinguished, showing a few differences in size and branching of colonies. The taxonomic implications of these results are discussed. With a few exceptions, species can be delimited based on morphometric patterns, once morphological variation is compared.

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

## 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 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 for 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 have been long considered to widen dispersal capabilities of such species (Ralph, 1961; Cornelius, 1981a, 1992a;

*Corresponding author. E-mail: amanfcunha@gmail.com

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 have shown 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 different samples from, usually distant, localities often are likely to represent separate lineages (Schuchert, 2014; Postaire *et al.*, 2017a, b; Boissin *et al.*, 2018). Molecular studies have also revealed a need for major changes in the classification of the group at several taxonomic levels (Collins *et al.*, 2004, 2006, 2008; Cartwright *et al.*, 2008; Leclère *et al.*, 2009; Maronna *et al.*, 2016; Moura *et al.*, 2018), allowing the description of new species (e.g. Schierwater & Ender, 2000; Cunha *et al.*, 2015) and revalidations of former synonyms (e.g. Schuchert, 2005; Miglietta *et al.*, 2007, 2009; Lindner *et al.*, 2011; Moura *et al.*, 2012; Cunha *et al.*, 2015).

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

Several morphological characters used in traditional diagnoses have proven to be uninformative for delimiting species and genera in these families (Cunha *et al.*, 2017). Besides information from the cnidome (Östman, 1982a, 1999; Lindner & Migotto, 2001) and life cycles (Lindner & Migotto, 2002; Lindner *et al.*, 2011; Zhou *et al.*, 2013; He *et al.*, 2015), morphometric data are also a promising tool for delimiting species boundaries in the group (e.g. Cunha *et al.*, 2015), especially if the range of variation of morphological characters is investigated (Cunha *et al.*, 2016).

This study aims to evaluate patterns of morphological variation correlated with species delimitation in the suborder Proboscoida (*sensu* Maronna *et al.*, 2016). Morphometric patterns of nearly all specimens included in a previous phylogeny (Cunha *et al.*, 2017) were analysed based on their phylogenetic relationships, integrating morphological, morphometric and molecular data for the delimitation of species of Campanulariidae, Clytiidae and Obeliidae.

## MATERIAL AND METHODS

### TAXONOMIC SAMPLING

Specimens used in this study are the same vouchers that were included in the molecular phylogenetic analysis by Cunha *et al.* (2017), with a few exceptions (Supporting Information, Table S1). Therefore, materials used for DNA analyses were also used in the morphometric analyses whenever possible, and the results of the two studies can thus be directly compared. Also, vouchers of previously published sequences, deposited in the National Museum of Natural History, Smithsonian Institution (USNM) (Govindarajan *et al.*, 2006; Lindner *et al.*, 2011), Muséum d'Histoire Naturelle de Genève (MHNG) (Leclère *et al.*, 2009) and Museu de Zoologia da Universidade de São Paulo (MZUSP) (Cunha *et al.*, 2015) were studied. Additional type and non-type materials from these and other museum collections (see Supporting Information, Table S1) were studied, enhancing taxon sampling and comparisons to delimit specific lineages.

In total, we analysed morphometric data for 291 specimens of the suborder Proboscoida, comprising 16 species of Campanulariidae (and all currently accepted genera, cf. Schuchert, 2019), 16 species of Clytiidae (and one out of two accepted genera) and 14 species of Obeliidae (covering all accepted genera). In the analysis we tried to include as many individuals of each species as possible, but this was determined by the number of sequences available for each species, because it is important to have a direct comparison between morphometric data and molecular lineages. In some cases, only one individual representing the species was measured [e.g. *Clytia paulensis* (Vanhöffen, 1910)], whereas in other cases up to 26 different individuals were included for comparison [e.g. *Orthopyxis sargassicola* (Nutting, 1915)]. Additionally, some collection lots had two to three polyps of the same colony (individual) measured, allowing for intracolony comparisons (see Supporting Information, Table S1).

## MORPHOLOGICAL AND MORPHOMETRIC ANALYSES

We studied morphological characters of the polyps of species of Proboscoida, in accordance with the previous phylogeny of the group (Cunha *et al.*, 2017). We were not able to study vouchers of published sequences that came from medusae (Zhou *et al.*, 2013; Laakmann & Holst, 2014; He *et al.*, 2015). However, their original publications, as well as some additional studies, provided important information on medusa characters that improved the discussion (e.g. Lindner & Migotto, 2002; Lindner *et al.*, 2011; Zhou *et al.*, 2013; Laakmann & Holst, 2014; He *et al.*, 2015).

Morphological characters were initially chosen based on measurements of polyps of Proboscoida reported in species descriptions that have been considered informative for species delimitation (e.g. Millard, 1975; Cornelius, 1982, 1990, 1995; Calder, 1991; Migotto, 1996; Lindner & Migotto, 2002; Lindner *et al.*, 2011). Based on our previous experience with the genus *Orthopyxis* (Cunha *et al.*, 2015) and morphological variation in Proboscoida (Cunha *et al.*, 2016), further characters were added to the analysis to capture more of the interspecific variation, specially regarding size and shape of hydrothecae and gonothecae, as well as the thickness of the perisarc (by measuring the diameter and thickness in three different positions; 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 analysed 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 and 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; for all abbreviations, see Table 1) 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 *Bonneviella ingens* Nutting, 1915, *B. regia* (Nutting, 1901), *B. superba* Nutting, 1915 and *Tulpa tulipifera* (Allman, 1888) from other Campanulariidae, based on their larger hydrothecae and pedicels (Fig. 1A, C). Similarly, *Rhizocaulus verticillatus* (Linnaeus, 1758) can be distinguished from *Campanularia* and *Orthopyxis* by its larger hydrothecae and trophosome (Fig. 1D, E). Differences in size are not only informative for delimiting different genera, but are considerably variable among *Bonneviella* species (Supporting Information, Table S2). The dimensions of the specimens of *B. regia* (USNM 1106181; Govindarajan *et al.*, 2006) are congruent with the type material of this species, while measurements of the unidentified specimens (*Bonneviella* sp.2 and sp.4; Govindarajan *et al.*, 2006) are closer to type material of the other species examined (Supporting Information, Table S2). *Bonneviella* sp.2 (USNM 1106182), here re-identified as *B. superba*, and *B. grandis* (Allman, 1876) are among the species with larger hydrothecae and trophosome, while *Bonneviella* sp.4 (USNM 1106187), here re-identified as *B. ingens*, have hydrothecae and trophosome almost half the size of the three previous species (Supporting Information, Table S2; Fig. 2A–C).

Perisarc thickness, as well as the number and height of hydrothecal cusps, separate several species within Campanulariidae (Fig. 1B). *Silicularia rosea* Meyen, 1834 is clearly distinct from *Bonneviella*, *Campanularia*, *R. verticillatus* and *Tulpa* due to its thicker perisarc (Figs 1C, 2D). In contrast, species of *Campanularia* can hardly be differentiated by any of the characters included in the analysis, because they have similar morphological patterns (Fig. 1D). The exception is *C. hincksii* Alder, 1856, slightly set apart from the remaining *Campanularia* by its taller hydrothecal cusps (HCMax, HCMin; Fig. 1D), a character that shows little or no overlap among the species when intraspecific variation is considered (Fig. 3B). However, the remaining characters do not show this pattern (Fig. 3A, C, D).

Perisarc thickness is also informative for separating *Orthopyxis* from species of *Campanularia*, although morphological variation may attenuate this difference. Several specimens of *O. sargassicola* and *O. crenata* (Hartlaub, 1901) group together with *Campanularia*, because of their thinner perisarc and presence of hydrothecal cusps, compared to the remaining species of *Orthopyxis* (Fig. 1E; Supporting Information, Fig. S1C). Although *O. crenata* and *O. sargassicola* have a thicker perisarc on average,

**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 |
| 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 |
| TLT | Total Length of Trophosome |

their range of variation may indeed overlap with *Campanularia* (Fig. 4A). Species of *Campanularia* have, on average, a thinner perisarc in comparison to most other *Orthopyxis* (except for *O. mianzani* Cunha *et al.*, 2015; Fig. 4B), and when there is overlap in the range of variation of perisarc thickness, these taxa
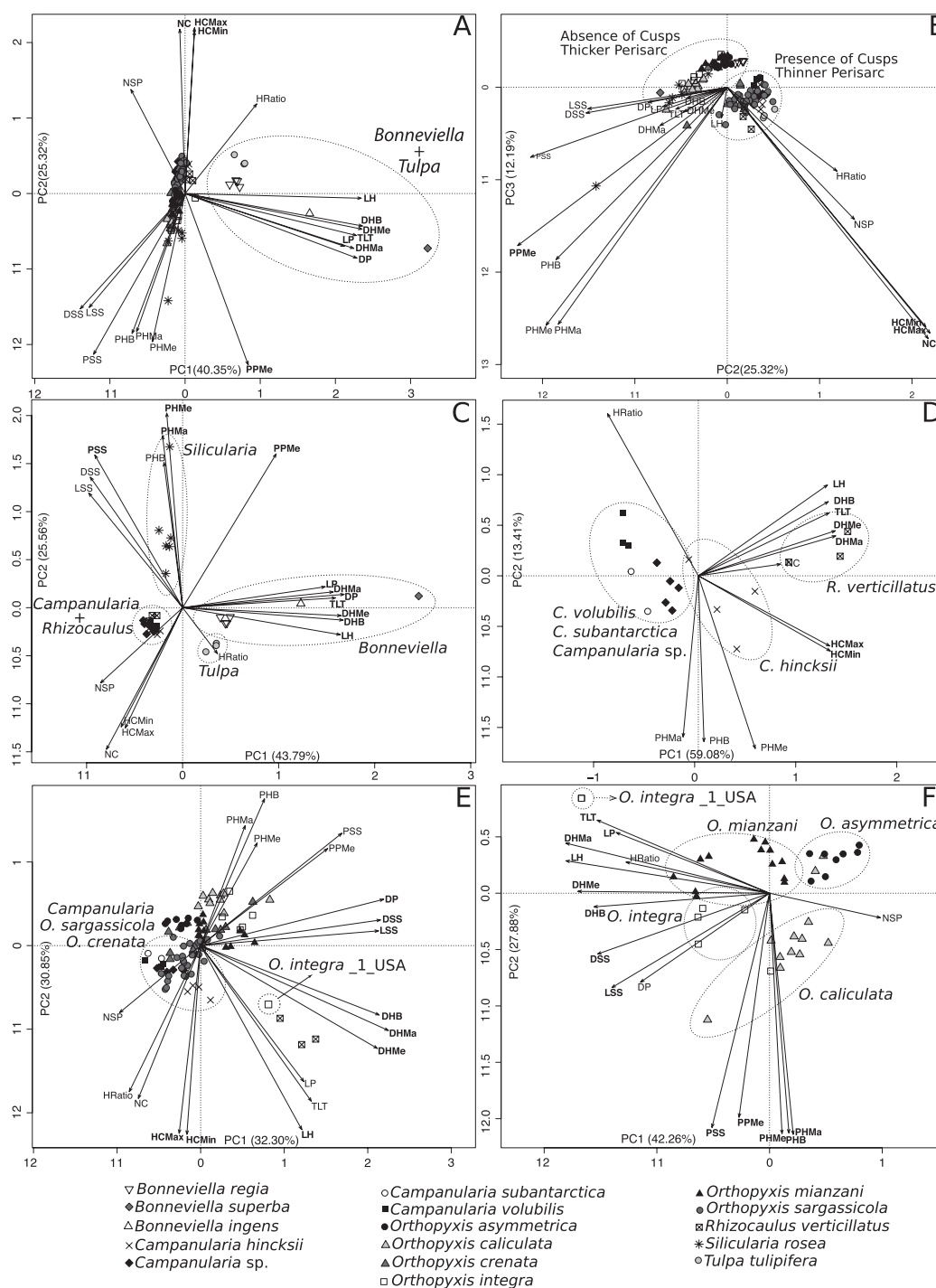
**Figure 1.** Distance biplots of the Principal Component Analysis (PCA) comprising data for Campanulariidae. A, first and second principal components (PCs) of the PCA with the complete dataset; B, second and third PCs of the PCA with the complete dataset; C, first and second PCs of the PCA without the 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).
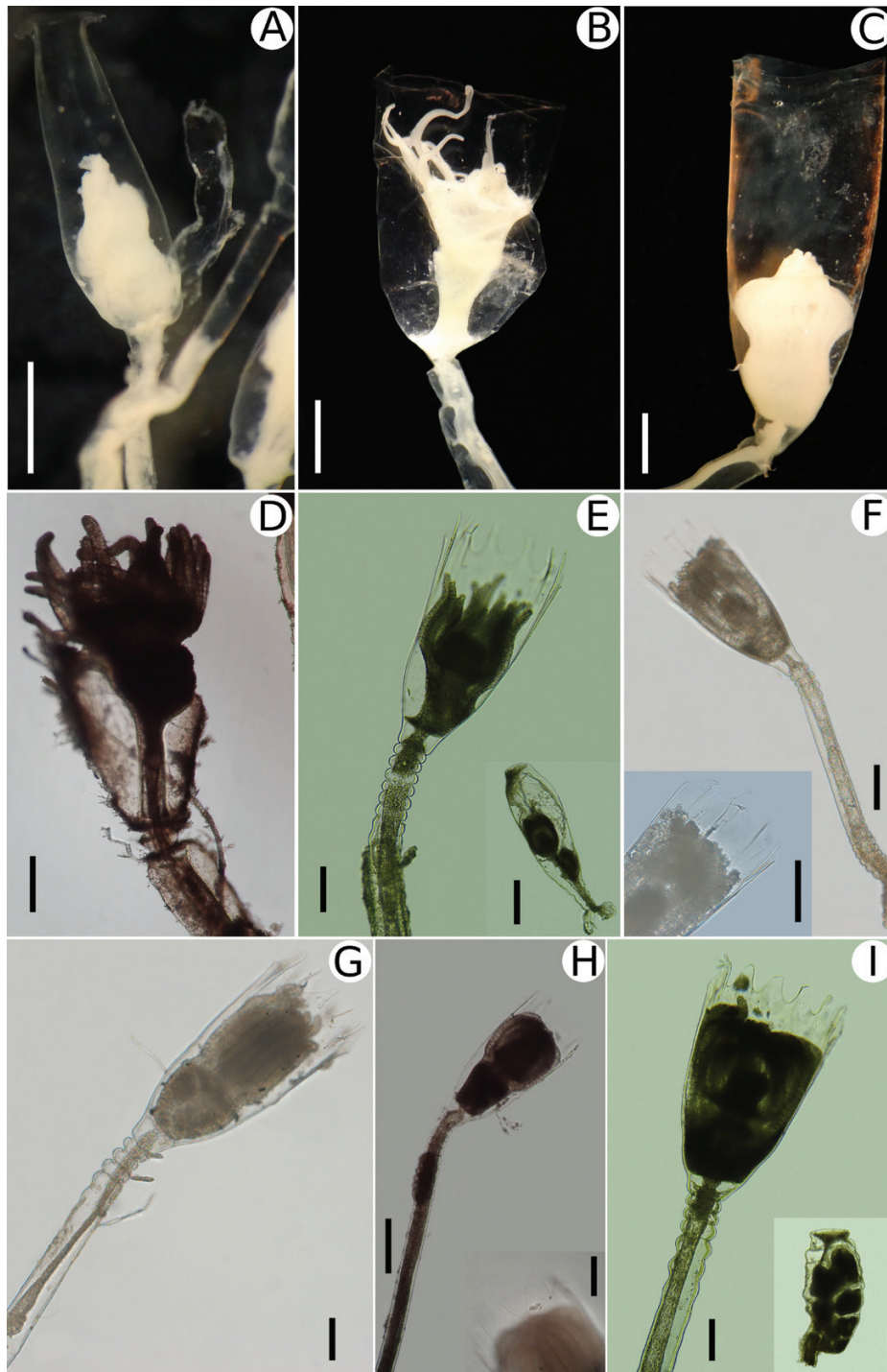
**Figure 2.** General morphology of species of Campanulariidae and Clytiidae. A, *Bonneviella regia* (USNM 1106181); B, *Bonneviella superba* (USNM 1106182); C, *Bonneviella ingens* (USNM 1106187); D, *Silicularia rosea* (PT11_ARG); E, *Clytia* cf. *gracilis* sp.1 (EL32_SLV), with gonotheca; F, *Clytia* 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; F (both), G, H (cusps), I (trophosome) = 100 μm; D, E (both), H (trophosome), I (gonotheca) = 200 μm.
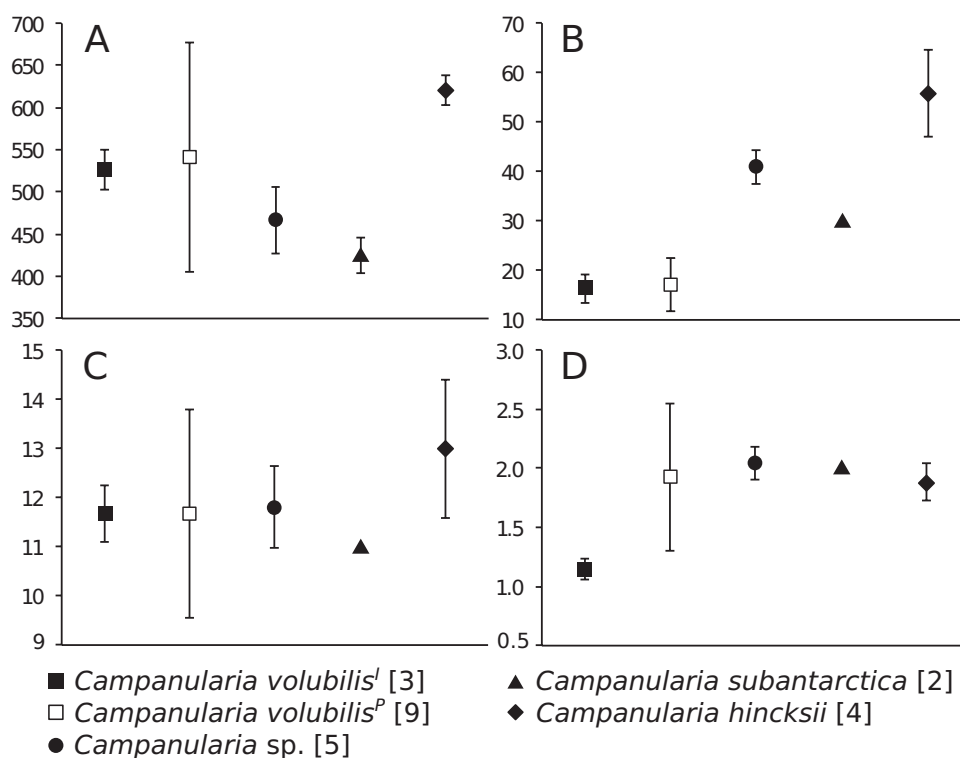
**Figure 3.** Mean ± standard deviation of morphometric data for *Campanularia*. Morphological variation in *C. volubilis* is presented as intracolony ([I]) and population variation ([P], ZMUC and USNM 29217; see Supporting Information, Table S1) for comparison. A, length of hydrothecae (LH, μm); B, maximum height of hydrothecal cusps (HCMax, μm); C, number of hydrothecal cusps (NC); D, length:diameter ratio of hydrotheca (HRatio). Brackets = [number of specimens measured].

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 (Fig. 1F): *Orthopyxis mianzani* has larger polyps with larger hydrothecae and a thinner perisarc; *O. asymmetrica* (Stechow, 1919) (see re-identified materials in Table 2) have shorter polyps and hydrothecae, with thinner perisarcs; *O. caliculata* (Hincks, 1853) has shorter polyps and hydrothecae, but a thicker perisarc; and *O. integra* (MacGillivray, 1842) (see re-identified 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. asymmetrica* and *O. caliculata*, *O. caliculata* and *O. integra*; 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* (Clark, 1876) and *O. integra*_ IT by Govindarajan *et al.* (2006) and Cunha *et al.* (2017) are congruent with that of *O. asymmetrica*. 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* (Supporting Information, Table S3).

Additional principal components were evaluated, but they do not show clear patterns of differentiation among species (Supporting Information, Fig. S1). A PCA including only data from specimens with gonothecae separated *S. rosea* because of its longer gonothecae, and *Orthopyxis* and *Bonneviella* because of 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* (Thorneley, 1900) and some specimens of *C. elsaeoswaldae* Stechow, 1914, *C.*
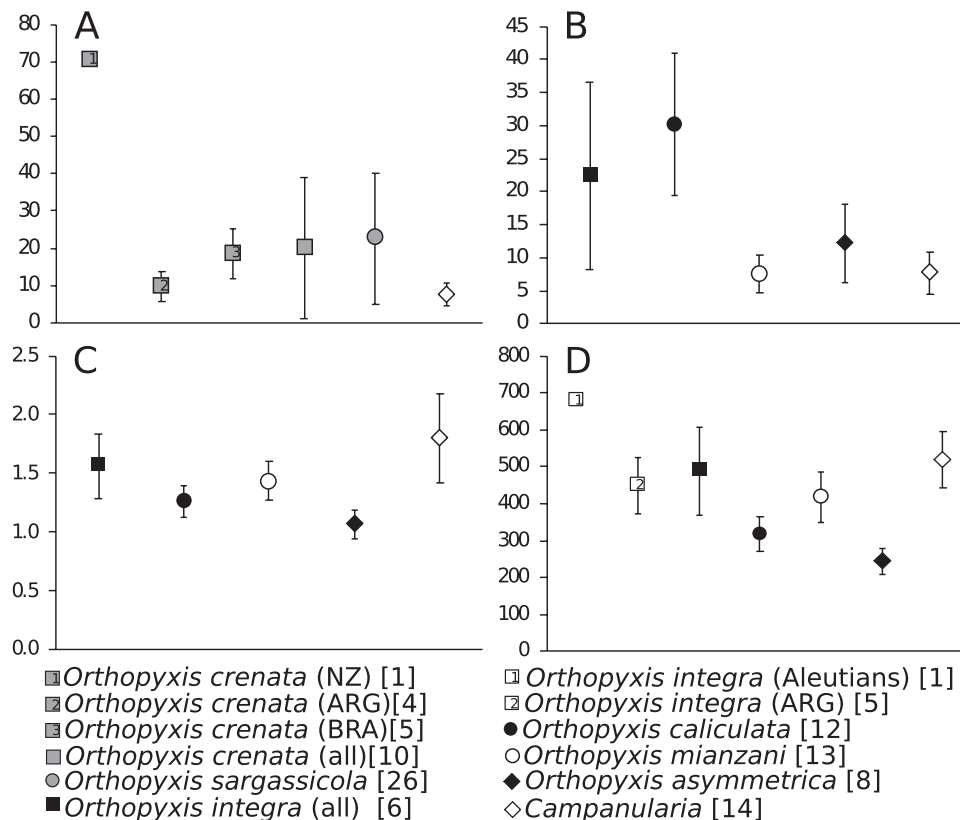
**Figure 4.** Mean ± standard deviation of morphometric data for *Orthopyxis*, including a comparison with species of *Campanularia* (i.e. *C. subantarctica*, *C. hincksii* and *Campanularia* sp.; Supporting Information, Table S1). Morphological variation in *O. crenata* and *O. integra* is presented separately for some populations and combined ('all'), for comparison. Data for specimens of *O. crenata* from New Zealand, Argentina and Brazil are represented with numbers 1 to 3, respectively. Similarly, data for specimens of *O. integra* from the Aleutian Islands and Argentina are represented with number 1 and 2, respectively. A, B, maximum perisarc thickness of hydrotheca at medial portion (PHMe, μm); C, length:diameter ratio of hydrotheca (HRatio); D, length of hydrotheca (LH, μm). Brackets = [number of specimens measured].

cf. *gracilis* (Sars, 1850) sp.1 and *C*. cf. *hemisphaerica* (Linnaeus, 1767) sp.1 from the remaining Clytiidae (Fig. 5A). 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 become evident (Fig. 5C, D). *Clytia linearis* is distinguished by its longer hydrothecae and cusps (LH, HCMax, HCmin; Fig. 5C, D), although the range of variation of cusp height overlaps with those of other species (Fig. 6A, B). Likewise, *C. elsaeoswaldae* is separated by the larger hydrothecal diameter (DHMa, DHMe, DHB, DBC; Fig. 5A, 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. 6C). Further comparisons show that *C. elsaeoswaldae* has a thicker diaphragm on average than *C. linearis*, as well as specimens of *C*. cf. *gracilis* and *C*. cf. *hemisphaerica* (Fig. 6D). 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. 5A, 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* (McCrady, 1859) because of their thicker perisarc, and *Clytia* sp.1, *C*. cf. *gracilis* sp.5 and *C. paulensis* because of their more cylindrical hydrothecae (Figs 5A, 6E, F). Although evident when directly compared among these species, differences in HRatio are not evident in all PCAs, probably because of the slight variation shown by the remaining species of *Clytia* (Fig. 6F).

Specimens of *C*. cf. *gracilis*, although not clearly individualized, can be set apart from each other when compared as a group: *C*. cf. *gracilis* sp.B, *C*. cf. *gracilis* sp.1 and sp.2 have larger hydrothecae and pedicels (LH, DHMa, DHMe, DHB, DP) with higher and more numerous cusps (NC, HCMax, HCMin), while *C*. cf. *gracilis* sp.3 and sp.4 have, in general, lower values for those characters (Fig. 5E, F). If measurements

**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 analysed in this study

| Taxon | Specimen(s) (see Supporting Information, 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.4 (USNM 1106187) | yes | Size and shape of hydrotheca | Campanulariidae |
| *Bonneviella regia* (Nutting, 1901) | USNM 1106181 | yes | Size of hydrotheca | Campanulariidae |
| *Bonneviella superba* Nutting, 1915 | *Bonneviella* sp.2 (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* Millard, 1971 | MZUSP 2639, 2643 | yes | Distinctive morphometric characters not found | - |
| *Campanularia* sp. | MZUSP 2641–42, 2761 | yes | Distinctive morphometric characters not found | - |
| *Campanularia volubilis* (Linnaeus, 1758) | USNM 1106166 | yes | Distinctive morphometric characters not 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, 1901) | MZUSP 2551, 2560, 2598, 2601, 2633, 3359, *Orthopyxis* sp. (MZUSP 2644); *Orthopyxis integra*_NZ (USNM 1106163) | yes | Number and height of hydrothecal cusps (but may eventually present even hydrothecal rim) | other species of *Orthopyxis*, except for *O. sargassicola* |

**Table 2.** Continued

| Taxon | Specimen(s) (see Supporting Information, Table S1) | Monophyletic? (Cunha et al., 2017) | Morphometric diagnostic characters | Morphometric characters are distinctive when compared to |
|---|---|---|---|---|
| *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* (Nutting, 1915) | MZUSP 2593–97, 2599–2600, 2602–03, 2605–11, 2617–20, 2627–2630, 2632, 4597 | yes | Number and height of hydrothecal cusps | other species of *Orthopyxis*, 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, 1888) | MZUSP 3366 | yes | Size of hydrotheca | Campanulariidae |
| **Infraorder Obeliida Maronna *et al*., 2016** | | yes | | |
| 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 | MZUSP 2762-65; USNM 1078725, 1078728 | yes | Diameter of hydrotheca, thickness of diaphragm | *Clytia* cf. *gracilis* and *Clytia* cf. *hemisphaerica* (diameter); Clytiidae (diaphragm) |
| *Clytia* cf. *gracilis* (Sars, 1850) sp.1 | MZUSP 2768–70, 2772, 2773 | 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.2 | 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 | 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 | 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) |

**Table 2.** Continued

| Taxon | Specimen(s) (see Supporting Information, Table S1) | Monophyletic? (Cunha et al., 2017) | Morphometric diagnostic characters | Morphometric characters are distinctive when compared to |
|---|---|---|---|---|
| *Clytia* cf. *gracilis* sp.5 | 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* (Linnaeus, 1767) sp.1 | MZUSP 2786–89[3] | yes | Distinctive morphometric characters not found | - |
| *Clytia* cf. *hemisphaerica* sp.2 | MZUSP 2790–95; USNM 1106186 | yes | Distinctive morphometric characters not found | - |
| *Clytia* cf. *hemisphaerica* sp.3 | USNM 1420636, 1420659, 1420673 | yes | Distinctive morphometric characters not found | - |
| *Clytia linearis* (Thorneley, 1900) | MZUSP 2796; USNM 1078729 | yes | Length of hydrotheca | Clytiidae |
| *Clytia noliformis* (McCrady, 1859) | MZUSP 2797–98; USNM 1078720 | yes | Perisarc thickness | Clytiidae, except for *Clytia* sp.2 |
| *Clytia paulensis* (Vanhöffen, 1910) | 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, 1859) | MZUSP 2802–03; USNM 1106154 | 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 *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 1106177 | yes | Distinctive morphometric characters not found | - |
| *Laomedea flexuosa* Alder, 1857 | MZUSP 2816; USNM 1106190, 1106192 | yes | Diameter of hydrotheca and pedicel | Obeliidae |

**Table 2.** Continued

| Taxon | Specimen(s) (see Supporting Information, Table S1) | Monophyletic? (Cunha et al., 2017) | Morphometric diagnostic characters | Morphometric characters are distinctive when compared to |
|---|---|---|---|---|
| **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* (Linnaeus, 1758) sp.1 | MZUSP 3336–40, 3344–45 | yes | Distinctive morphometric characters not found | - |
| *Obelia* cf. *dichotoma* sp.2 | MZUSP 3335, 3342–43; USNM 1106156 | yes | Distinctive morphometric characters not found | - |
| *Obelia* cf. *dichotoma* sp.3 | 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 | MZUSP 3341, 3346 | yes | Branching of erect colonies, total length of trophosome | *Obelia* cf. *dichotoma* sp.1 and sp.2 |
| *Obelia geniculata* (Linnaeus, 1758) | MZUSP 3347–51; USNM 1106165, 1106176, 1106179 | yes | Perisarc thickness | Obeliidae |
| *Obelia longissima* (Pallas, 1766) | MZUSP 3352–55; USNM 1106153, 1106173, 1106189, 1106191 | yes | Branching of erect colonies, total length of trophosome, length of internodes and hydrotheca, height (shape) of hydrothecal cusps | Obeliidae, except some specimens of *Obelia* cf. *dichotoma* (branching, total length); some 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 re-identifications.
[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 re-identifications.
[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 re-identifications.
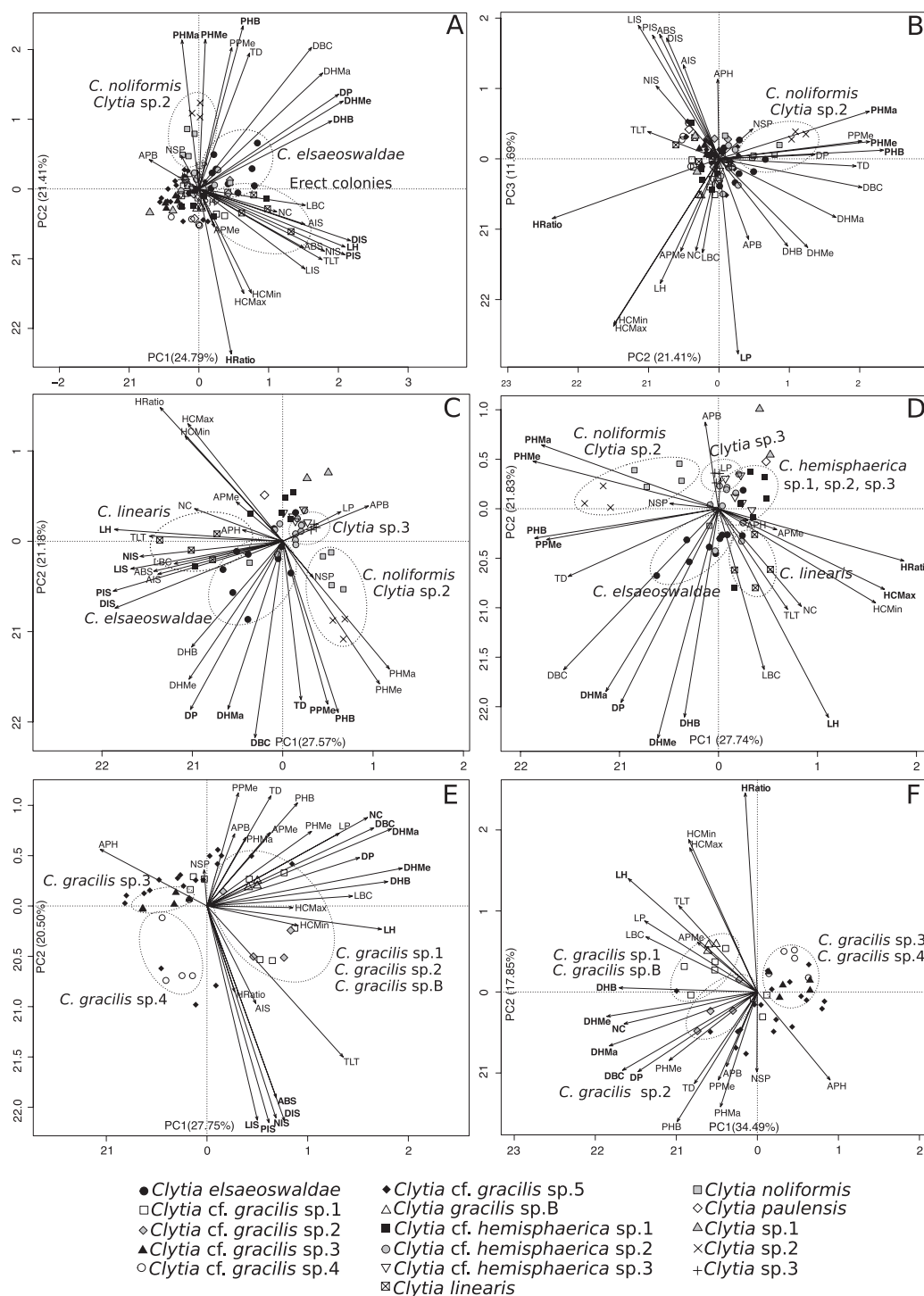
**Figure 5.** Distance biplots of Principal Component Analysis (PCA) comprising data for Clytiidae. A, first and second principal components (PCs) of the PCA with the complete dataset; B, second and third PCs of the PCA with the complete dataset; C, first and second PCs of the PCA without *Clytia* cf. *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).
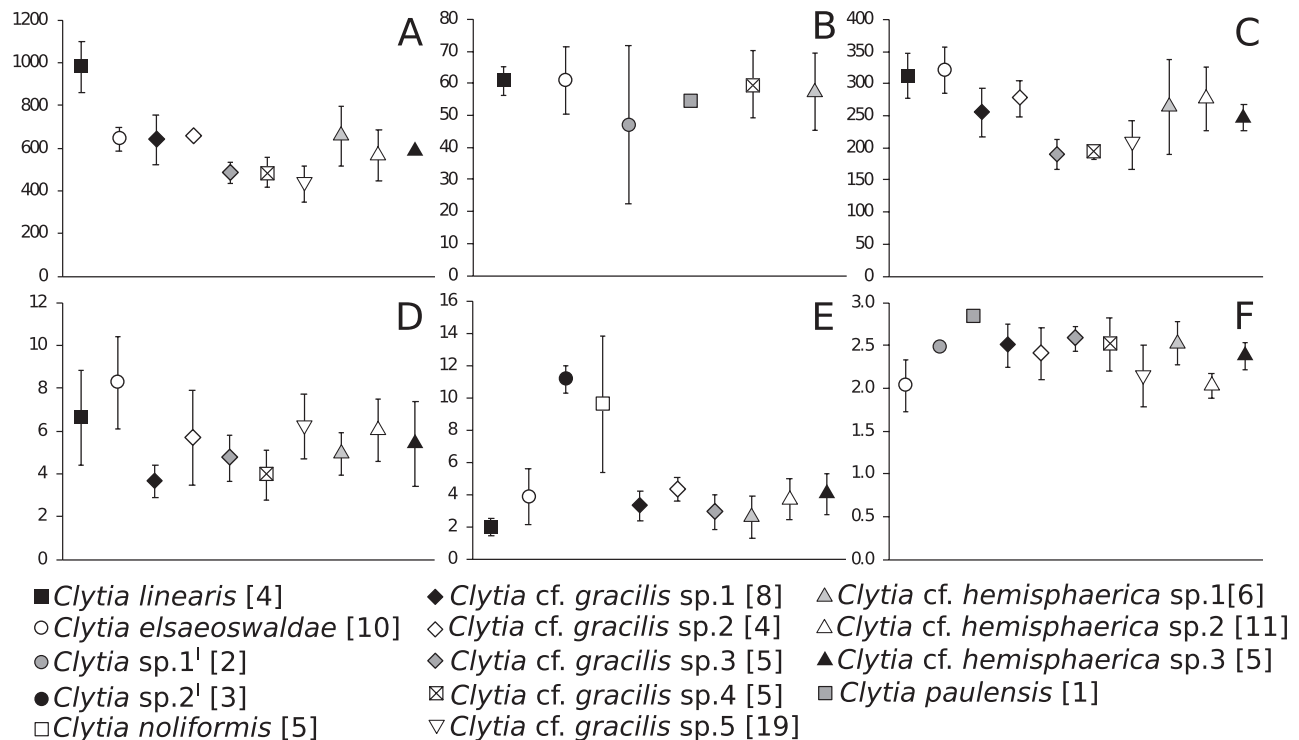
■ *Clytia linearis* [4]
○ *Clytia elsaeoswaldae* [10]
● *Clytia* sp.1ᴵ [2]
● *Clytia* sp.2ᴵ [3]
□ *Clytia noliformis* [5]
◆ *Clytia* cf. *gracilis* sp.1 [8]
◇ *Clytia* cf. *gracilis* sp.2 [4]
◆ *Clytia* cf. *gracilis* sp.3 [5]
⊠ *Clytia* cf. *gracilis* sp.4 [5]
▽ *Clytia* cf. *gracilis* sp.5 [19]
△ *Clytia* cf. *hemisphaerica* sp.1[6]
△ *Clytia* cf. *hemisphaerica* sp.2 [11]
▲ *Clytia* cf. *hemisphaerica* sp.3 [5]
■ *Clytia paulensis* [1]

**Figure 6.** 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].

related to erect colonies are excluded from the analysis (LIS, PIS, NIS, DIS), *C.* cf. *gracilis* sp.1 and *C.* cf. *gracilis* sp.B can be further separated from *C.* cf. *gracilis* sp.2 by the length (LH) and length:diameter ratio of the hydrotheca (HRatio; Fig. 5F), although these differences are too small to be informative and delimit lineages. Specimens of *C.* cf. *gracilis* sp.5 spread along the four quadrants of the graph because of their high variation in the characters examined (Fig. 5E, F). Additional comparisons with literature descriptions show that morphological variation is pronounced in the presumably typical *C. gracilis*, and the lineages analysed here could fit one or more descriptions (Supporting Information, Table S4).

Specimens of *C.* cf. *hemisphaerica* are not separated by any of the morphological measurements, showing intermediate values for most of the characters evaluated (Fig. 5A–D; Supporting Information, Fig. S2). Characters that are important to differentiate other species of *Clytia* are uninformative for lineages of *C.* cf. *hemisphaerica*, especially because of their wide range of variation and extensive overlap. This variability is also seen when descriptions from the

literature are compared (Supporting Information, Table S5; Supporting Information, Fig. S3).

Additional PCAs, including characters from the gonotheca, show less conspicuous patterns of differentiation among species (Supporting Information, Fig. S2). *Clytia hummelincki* (Leloup, 1935) has been shown to not be part of Clytiidae in previous phylogenetic analysis (Cunha *et al.*, 2017) and, therefore, was not included in the PCAs with this family.

FAMILY OBELIIDAE

Patterns of morphological variation in Obeliidae are mostly congruent among the different datasets examined (Fig. 7). Considering all species, perisarc thickness (PHMA, PHMe, PHB, PPMe, TD) explains most of the data variation, separating *Obelia geniculata* (Linnaeus, 1758) by its thicker perisarc (Fig. 7A, B). This character also set apart *O. geniculata* from the remaining species when only the genus *Obelia* is considered (Fig. 7C). In addition, *Obelia geniculata* has the widest range of variation of perisarc
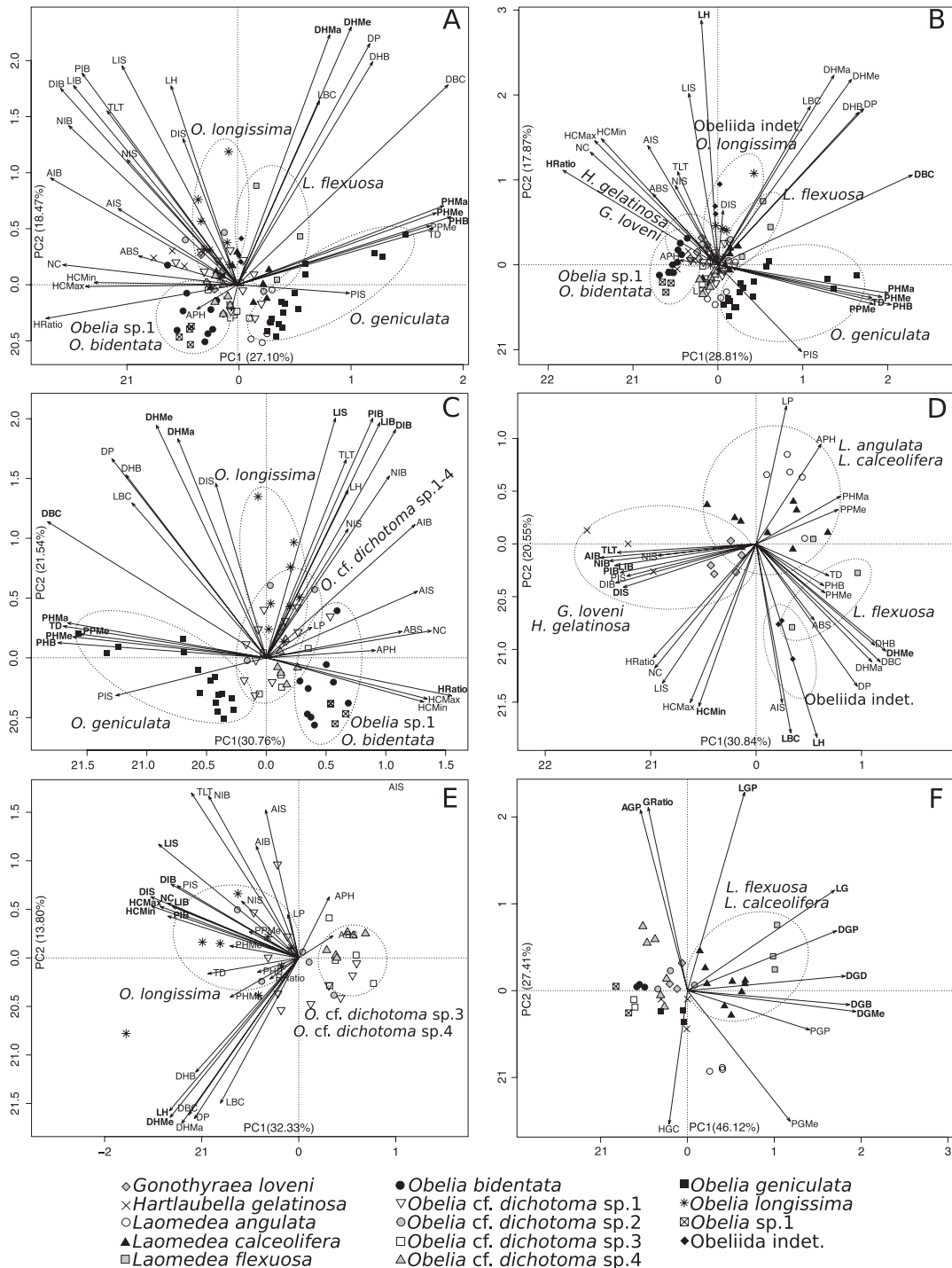
**Figure 7.** Distance biplots of the Principal Component Analysis (PCA) comprising data for the family Obeliidae. A, first and second principal components (PCs) of the PCA with the complete dataset; B, first and second PCs of the PCA with the complete dataset, excluding measurements related to second-order branches of erect colonies (NIB, DIB, AIB, LIB, PIB); C, first and second PCs of the PCA with species of *Obelia* only; D, first and second PCs of the PCA without species of *Obelia*; E, first and second PCs of the PCA with lineages of *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).

thickness, when *Laomedea* and *Obelia* are compared (Fig. 8A). For the remaining genera, perisarc thickness does not notably contribute to the differentiation of the species, because of its extensive overlap (Fig. 8A). Measurements of diameter (DHMa, DHMe, DHB, DBC, DP) explain another direction of variation of the data, and mainly differentiate *L. flexuosa* Alder, 1857 from the remaining Obeliidae by its broader hydrothecae (Figs 7A, B, D, 8B). Species of *Laomedea* also show a wide range of variation and overlap in pedicel length (LP; Fig. 8C), but their pedicels are on average longer than in *Obelia*.

*Obelia longissima* (Pallas, 1766) is distinguished from the remaining Obeliidae by its larger measurements of first- and second-order branches (LIS, DIS, NIS, LIB, DIB, NIB; Fig. 7A–C). It also has a wider range of variation in the hydrothecal length compared to the remaining species, and it cannot be distinguished based on this character because of the extensive overlap with other species (Fig. 8D). Erect and branched colonies also differentiate *Gonothyraea loveni* (Allman, 1859) and *Hartlaubella gelatinosa* (Pallas, 1766), although to a lesser extent; this pattern is clearly observed when *Obelia* is excluded from the analysis (Fig. 7D). These species, together with

*O. bidentata* Clark, 1875 and *Obelia* sp.1, also differ from the remaining Obeliidae in their more cylindrical hydrothecae (higher values of HRatio) and taller hydrothecal cusps (Figs 7B–D, 8E, F). The exception is Obeliida *indet*., which has the tallest hydrothecal cusps compared to all other species (Fig. 8F). In general, Obeliida *indet*. has similar morphometric patterns to *O. longissima*, mostly related to the presence of erect colonies and hydrothecal length (Fig. 7B, D). The hydrotheca is typically longer in Obeliida *indet*., but morphological variation attenuates this difference (Fig. 8D).

It is evident from most of the analyses that lineages of *Obelia* cf. *dichotoma* (Linnaeus, 1758) are not distinguished from each other by any of the measurements, showing intermediate values for all characters evaluated (Fig. 7A–C, E). Many specimens of *O. longissima* cannot be distinguished from the lineages of *O.* cf. *dichotoma*, and although some are differentiated by their larger erect and branched colonies, variations in these characters prevent a clear separation of these species (Fig. 9A). *Obelia longissima* also has longer hydrothecae and taller hydrothecal cusps on average, but their range of variation overlap among species (Fig. 9B, D). *Obelia* cf.
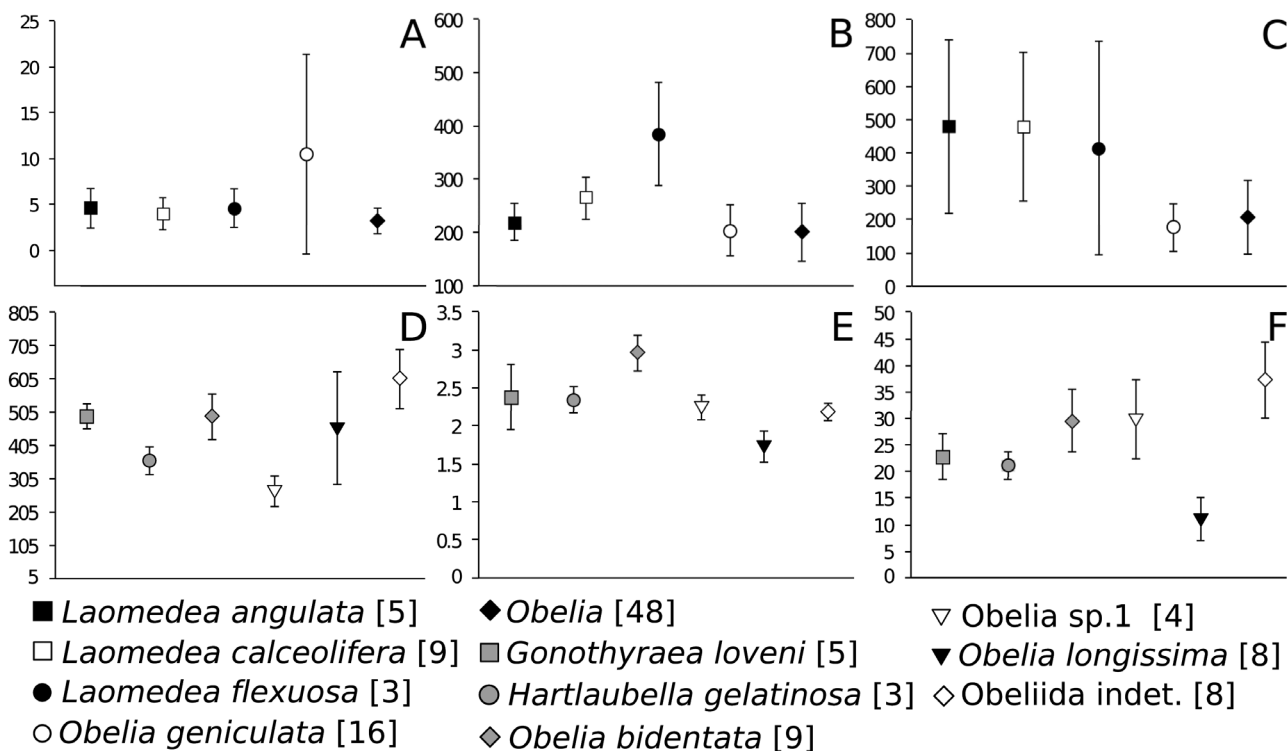


**Figure 8.** Mean ± standard deviation of morphometric data for Obeliidae. Data for the genus *Obelia* comprises all species included in this study, except *O. geniculata*. A, maximum hydrothecal perisarc thickness at margin (PHMa, µm); B, maximum hydrothecal diameter at margin (DHMa, µm); C, length 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].

*dichotoma* sp.3 and *O.* cf. *dichotoma* sp.4 are grouped together and slightly separated from the remaining species of *Obelia*, probably because of their smaller and less branched colonies, but no further patterns of differentiation are seen among these lineages (Fig. 7E). Indeed, when compared to literature descriptions, the size and branching of colonies seem to be among the few characters that could fairly differentiate some of the lineages of *O.* cf. *dichotoma*, which are similar to the descriptions of other nominal species (Supporting Information, Table S6).

Characters related to the gonothecae do not differentiate the species of *Obelia*, but species of *Laomedea* can be distinguished by their larger gonothecae (LG, DGD, DGMe, DGB, DGP; Fig. 7F). Additional PCAs do not show further patterns of differentiation among Obeliidae (Supporting Information, Fig. S4).

## 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 are included [see *Campanularia volubilis* (Linnaeus, 1758); Fig. 3]. However, we also demonstrate 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 for delimiting lineages within Proboscoida.

### SIZE DIFFERENCES IN CAMPANULARIIDAE

In Campanulariidae, the length and diameter of the trophosome, pedicels and hydrothecae can reliably distinguish *Bonneviella*, *R. verticillatus* and *T. tulipifera* from genera *Campanularia*, *Orthopyxis* and *Silicularia*, which in turn can be characterized by differences in perisarc thickness. Indeed, several species of *Bonneviella* were originally assigned to *Campanularia* and distinguished by their 'enormous' size or 'immense' hydrothecae (as *Campanularia grandis* in Allman, 1876; as *C. regia* in Nutting, 1901). Later, the pre-oral cavity on the hypostome of these
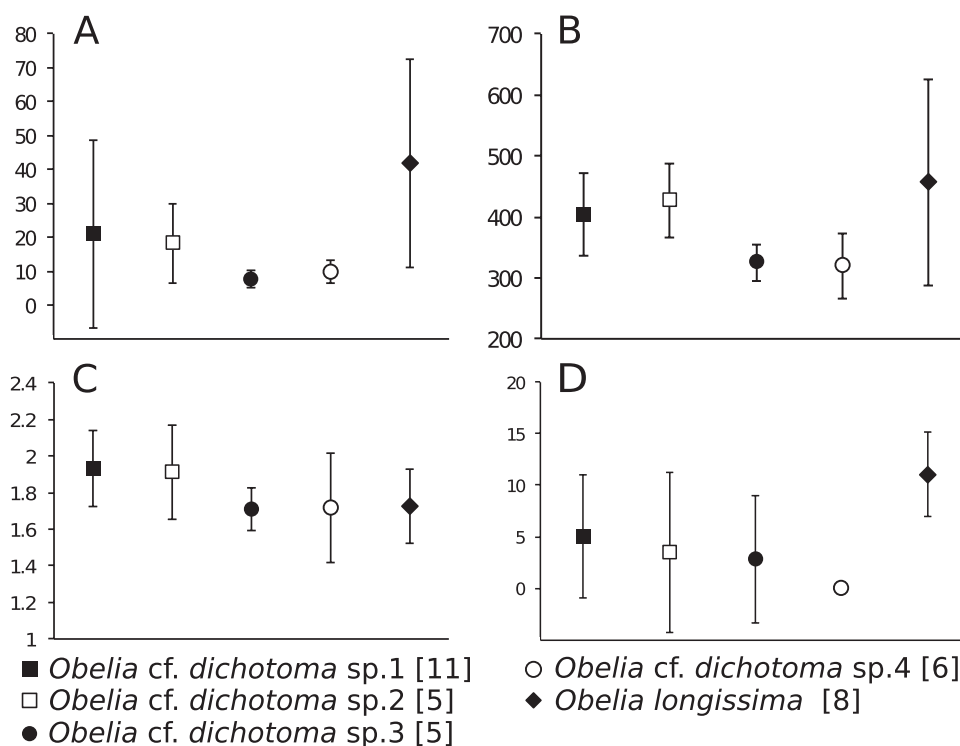


**Figure 9.** Mean ± standard deviation of morphometric data for the lineages identified as *Obelia* cf. *dichotoma*. A, total length of the trophosome (TLT, mm); B, length of the hydrotheca (LH, μm); C, length:diameter ratio of the hydrotheca (HRatio); D, maximum height of hydrothecal cusps (HCMax, μm). Brackets = [number of specimens/colonies measured].

species was considered the main diagnostic character of the group (Bonneviellidae, Broch, 1909; Nutting, 1915). *Tulpa tulipifera* and *Rhizocaulus verticillatus* 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). However, the generic value of these characters has been questioned by some authors, especially given the similarities in the hydrothecae and gonothecae between *Campanularia volubilis* 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 confirms that they differ consistently in size, which should also be considered in 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 the next section 'Trends in perisarc thickness and size/shape of Hydrothecae' for further discussion).

Because of the considerable interspecific variation in *Bonneviella*, differences in size may also be informative for delimiting the species examined in this study. As pointed out by Nutting (1915), *Bonneviella regia* can be differentiated from *B. grandis* by the shape of their gonothecae and the noticeably smaller hydrothecae of *B. regia* (Supporting Information, Table S2). *Bonneviella superba* has the largest hydrothecae among *Bonneviella* species, while hydrothecae in *Bonneviella ingens* are intermediate in size, but considerably different in shape from those of *B. superba* (Nutting, 1915; Naumov, 1969). The morphometric patterns of the type material supports 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). However, this is a tentative identification, 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.

The clade comprising *Bonneviella*, *C. volubilis* and *R. verticillatus* may represent a local radiation, and it is necessary to examine additional material from other localities (Govindarajan *et al.*, 2006).

Although *C. volubilis* is not differentiated from other *Campanularia* species based on characters related to size, both *R. verticillatus* and *Bonneviella* are characterized by their larger size (Fig. 1A, D) and all their records come from the Aleutian Islands (Supporting Information, Table S1). *Rhizocaulus verticillatus* was originally recorded from Cumberland, England, UK (Cornelius, 1981, 1982) and is known for its arctic–boreal distribution (Antsulevich, 1992; Calder, 2003; Schuchert, 2001; Stepanjants *et al.*, 2006; Ronowicz, 2007). Species of *Bonneviella* were originally (and have been subsequently) recorded in arctic and subarctic regions (type localities for *B. grandis*, *B. ingens*, *B. regia* and *B. superba* are Prince William Sound, Tsugaru Strait, Simushir Island and the Bering Sea, respectively; Nutting, 1901, 1915; Broch, 1910; Kramp, 1913; Naumov, 1969; Yamada, 1969; Schuchert, 2001). Even though these genera have a close phylogenetic relationship (Govindarajan *et al.*, 2006; Cunha *et al.*, 2017), their large size may be related to their occurrence in colder waters, a relationship previously described for other species of Proboscoida [e.g. *Obelia geniculata*, *Orthopyxis integra*, *Silicularia bilabiata* (Coughtrey, 1875); Ralph & Thomson, 1968; Ralph, 1957; Naumov, 1969]. The same is seen in *T. tulipifera*, which was originally recorded from Heard Island in Antarctica (Allman, 1888; Stechow, 1921) and has a Kerguelen–Patagonian distribution (Peña Cantero & García Carrascosa, 1999; Soto Àngel & Peña Cantero, 2015), indicating that its larger size is probably a case of convergence. Nevertheless, further comparisons with additional material from different populations are essential to evaluate the intraspecific range of variation of these characters and their relationship to the species geographic distribution.

TRENDS IN PERISARC THICKNESS AND SIZE/SHAPE OF HYDROTHECAE

Our results show that perisarc thickness is among the most variable of characters (e.g. Millard, 1975; Cornelius, 1982, 1995; Cunha *et al.*, 2015), but yet most informative for delimiting *Campanularia*, *Orthopyxis* and *Silicularia*. Besides the unique bilaterally symmetrical hydrothecae of *Silicularia*, a conspicuous character for delimiting 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* is widely distributed in antarctic and subantarctic waters, and was considered synonymous with *S. bilabiata* (Vervoort & Watson, 2003), a species shown by Ralph (1956, 1957) to have wide intraspecific variation and to comprise of several nominal species in *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). 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 we studied have an oblique hydrothecal aperture (Fig. 2D), as is typical of *S. rosea* (Vervoort & Watson, 2003; Galea *et al.*, 2014), but the hydrothecae of specimens from New Zealand were smaller (398.5 µm on average) than in Argentinean specimens (790.4 µm). 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 specimens from New Zealand 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, California, 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 making a taxonomic decision regarding *Campanularia* until more, and unequivocal, material of the type species from the type locality is available. Presently, a possible conclusion derived from our results would be to merge *Bonneviella* and *Rhizocaulus* with *Campanularia*, but this decision is contra-indicated by several morphological differences between 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* 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* 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 accepted mainly based on the gonophore producing a reduced medusa (medusoid; Agassiz, 1862; Cornelius, 1995). Our analysis shows 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 within the range of variation of *O. sargassicola* and *O. crenata*, because the perisarcs in these two *Orthopyxis* species vary from thin to thick, and their hydrothecae from campanulate to cylindrical (Vervoort & Watson, 2003; Cunha *et al.*, 2015, 2016). *Campanularia* and *Orthopyxis* can be reliably delimited based on these characters if their ranges of variation are evaluated, especially when there is overlap between the different species.

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

Distinct lineages of *Orthopyxis* with the traditional morphological diagnostic characters of *O. integra* were shown to be delimited by the degree of perisarc thickening and the size and shape of the hydrothecae (Cunha *et al.*, 2015). Our results corroborate these patterns and further attest that the clade comprising the specimen of *O. integra* from the Aleutian Islands ('*Orthopyxis integra*_1_USA', USNM 1106184; see: Cunha *et al.*, 2017 and Supporting Information, Table S1) with spirally grooved gonothecae (Fig. 10A) has morphological patterns that are commonly regarded as distinctive for *O. integra*, such as larger and more cylindrical hydrothecae (Nutting, 1915; Bale, 1934; Hirohito, 1995; Calder *et al.*, 2014). Although we cannot verify the presence of spirally grooved gonothecae in Argentinean specimens ('Campanulariidae sp. *indet.*' and '*O. integra*_PT20'; see Supporting Information, Table S1), they are here regarded as *O. integra*
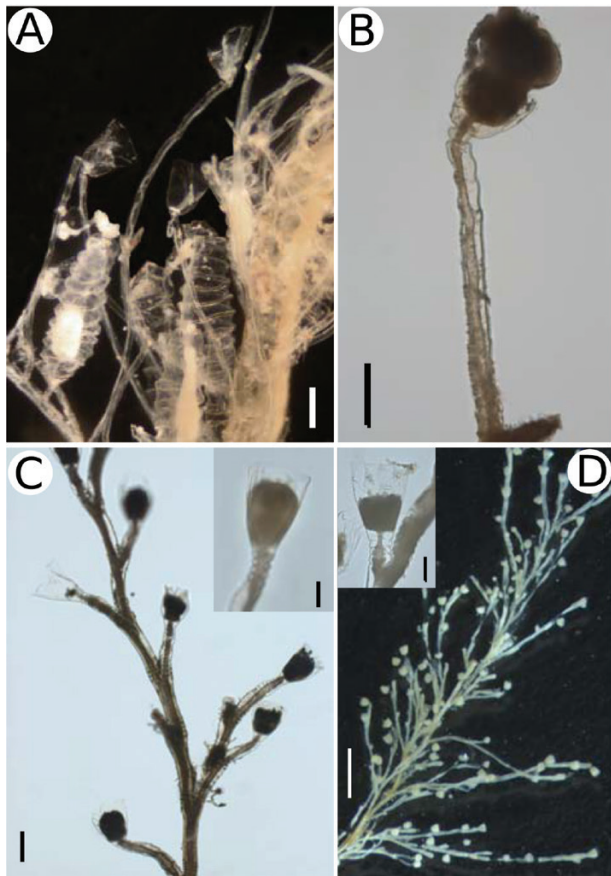
**Figure 10.** A, *Orthopyxis integra*_1_USA (USNM 1106184), with gonothecae; B, *Orthopyxis asymmetrica* (EL02_SLV); C, *Obelia* cf. *dichotoma* sp.3 (PAF07_BRA), with detail of hydrotheca; D, *Obelia* cf. *dichotoma* sp.4 (Site 1.1_USA), with detail of hydrotheca. Scales: A, F = 500 µm; B, D (gonotheca) = 300 µm; C, D (trophosome), C (colony), E = 200 µm; C, D (hydrotheca) = 100 µm; D (colony) = 1 mm. For specimens and codes see Supporting Information, Table S1.

given their morphological and phylogenetic patterns (Table 2), contradicting the hypothesis that this species does not occur in the south-western Atlantic (Cunha *et al.*, 2015). Also, perisarc thickness can be variable in *O. integra*, showing extensive overlap with *O. caliculata* (Fig. 4B).

In addition to *O. integra*, our analysis also shows 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 1F, 10B). Although their perisarc is not as thick as described by Stechow (1919), we believe

that these specimens should be assigned to *Orthopyxis asymmetrica*, 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 species (see Table 2 for re-identifications).

## 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* 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 show that the range of intraspecific variation of the size of the hydrothecae in *C. linearis* does not overlap with those of other species (Fig. 6A), and this character can be useful to delimit the species.

*Clytia elsaeoswaldae* was also shown to be a distinct clade (Lindner *et al.*, 2011; Cunha *et al.*, 2017). It is differentiated from *C. gracilis* and *C. hemisphaerica* 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. 6C). 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. 6C).

*Clytia noliformis* 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 described in the literature (but see: Calder, 1991), can also be used to delimit this species (Fig. 6E).

Similarly, *Clytia paulensis* is regarded as distinctive because of the shape of its hydrothecal cusps (Millard, 1975; Cornelius, 1982, 1995), but we note that the species also has a more cylindrical hydrotheca in comparison with some other members of *Clytia* (HRatio; Fig. 6F). The length:diameter ratio of the hydrothecae of *C. paulensis* is known to be variable, ranging from 1.5 to 4.0 in different populations (Millard, 1966; Cornelius, 1982). Because we were able to study the intracolony variation of only one specimen of *C. paulensis*, this character should be considered with caution for the delimitation of the species.

Molecular analyses of *C. gracilis* resulted in several cryptic lineages in previous studies (Govindarajan *et al.*, 2006; Lindner *et al.*, 2011; Cunha *et al.*, 2017). The polyp of *C. gracilis* is distinguished from *C. hemisphaerica* mainly by the inclined and pointed triangular cusps and the smooth gonothecae, contrasting with the non-inclined, rounded cusps and the spirally ribbed gonothecae in *C. hemisphaerica* (Calder, 1991; Cornelius, 1995). However, we found that the height, number and shape of the hydrothecal cusps vary within the different lineages of *C. gracilis*, as do the hydrothecal length and length:diameter ratio (Figs 2E–G, 11). The same variation was found among specimens of *C. gracilis* described in the literature from presumably different populations (Vervoort, 1959; Calder, 1991; Cornelius, 1995; Schuchert, 2001; Peña Cantero & García Carrascosa, 2002), and the lineages analysed herein could fit into one or more of these descriptions (Supporting Information, Table S4). This emphasizes the difficulties in correlating the morphometric patterns of these lineages with the type of *C. gracilis*, especially considering that its original description was based on two species, currently *C. gracilis* and *Gonothyraea loveni* (Sars, 1850, 1857; cf. Cornelius, 1982; Cornelius & Östman, 1986; Calder, 1991). Although a lectotype of *C. gracilis* was designated by Cornelius (1982: 94), it was based on the original illustration provided by Sars (1857), and information on its diagnostic characters remains subjective and incomplete. For a proper delimitation of the type species, it is essential to obtain specimens of *C. gracilis* from one of the type localities (Lofoten or Finnmark, Norway; Sars, 1850, 1857; Calder, 1991) and correlate their phylogenetic (molecular) and morphometric patterns to the cryptic lineages. The delimitation of a neotype would also be beneficial, since the type series seems to be based on original illustrations (cf. Cornelius, 1982; Cornelius & Östman, 1986).

*Clytia hemisphaerica* also comprises several cryptic lineages (Cunha *et al.*, 2017). We were unable to differentiate these by their morphometric patterns (Supporting Information, Fig. S4), although all lineages have diagnostic characters that are generally attributed to polyps of *C. hemisphaerica* (Fig. 2H, I; Calder, 1991; Cornelius, 1995). They also fit into one or more published descriptions, impeding the delimitation and identification of characters from the type of *C. hemisphaerica* (Supporting Information, Table S5), which was recorded from 'Belgian seas' (cf. Linnaeus, 1767; Cornelius, 1982). The three lineages of *C. hemisphaerica* analysed in this study are geographically structured, comprising specimens from Belize, the United States and the Mediterranean/North Sea, and forming a clade in most of the concatenated phylogenies (Cunha *et al.*, 2017; Supporting Information, Table S1). These results raise doubts as to whether *C. hemisphaerica* should indeed be considered a species complex, or a species with pronounced population subdivisions (see: Schuchert, 2014; Postaire *et al.*, 2017b).

Recently, two new species of *Clytia* were described from China, together with information on their life cycles and nematocysts (Zhou *et al.*, 2013; He *et al.*, 2015). *Clytia xiamenensis* Zhou *et al.*, 2013 was shown to be closely related to *C. hemisphaerica*, also clustering with specimens of *C.* cf. *gracilis* sp.A from the USA (Lindner *et al.*, 2011; Zhou *et al.*, 2013). This pattern was corroborated by Cunha *et al.* (2017), although in their study additional specimens of *C. hemisphaerica* from the USA clustered with *C. xiamenensis* [see 16S phylogenies in Cunha *et al.* (2017)]. Originally, the hydroid of *C. xiamenensis* was differentiated from *C. hemisphaerica* by its pointed and inclined hydrothecal cups, and its smaller B-type microbasic mastigophores (Zhou *et al.*, 2013). However, we show that specimens of *C. hemisphaerica* from the same clade (*C.* cf. *hemisphaerica* sp.1; see Supporting Information, Table S1) do not have inclined hydrothecal cusps (Fig. 2H), even though their cusps are not as rounded as those of *C.* cf. *hemisphaerica* sp.2 (compare with Fig. 2I). Indeed, inclined cusps can be variable in some species (e.g. *C. gracilis*), and the definition of the shape of hydrothecal cusps does not seem reliable for differentiating between *C. hemisphaerica* and *C. xiamenensis*. We lack information on the nematocysts and life cycle of these specimens, which may support the separation of the species, as suggested by Zhou *et al.* (2013). However, it is important that the diagnostic characters of the type of *C. hemisphaerica* are clearly defined before the two species can be confidently differentiated. This would involve the analysis of specimens of *C. hemisphaerica* from the type locality, and the comparison of their phylogenetic and morphometric patterns, as well as life cycle and nematocysts, with those of the clade comprising *C. xiamenensis*. If this clade indeed proves to be distinct from the other lineages, then specimens from the USA should be assigned to *C. xiamenensis*.

Similarly, *Clytia gulangensis* He & Zheng, 2015 (He *et al.*, 2015) clustered with specimens of *C. gracilis* from Brazil (*C.* cf. *gracilis* sp.5; Supporting Information, Table S1) in the phylogenetic analysis of Cunha *et al.* (2017). Brazilian specimens do not have all the diagnostic characters of *C. gulangensis*, at least in the polyp stage, because some specimens have non-inclined hydrothecal cusps and smaller hydrothecae, with a length:diameter ratio near 2 (Supporting Information, Table S4; Fig. 2E–G). In fact, the shape of the hydrothecal cusps shows broad variation among the different Brazilian specimens (Fig. 11). He *et al.* (2015) differentiated the polyp of *C. gracilis* from *C. gulangensis* based on the presence of asymmetric and inclined cusps (tilted, cf. Schuchert, 2003) in *C. gracilis*. However, some Brazilian specimens clustering with *C. gulangensis* had asymmetric and inclined cusps (Fig. 11B, 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* (Sars, 1850)
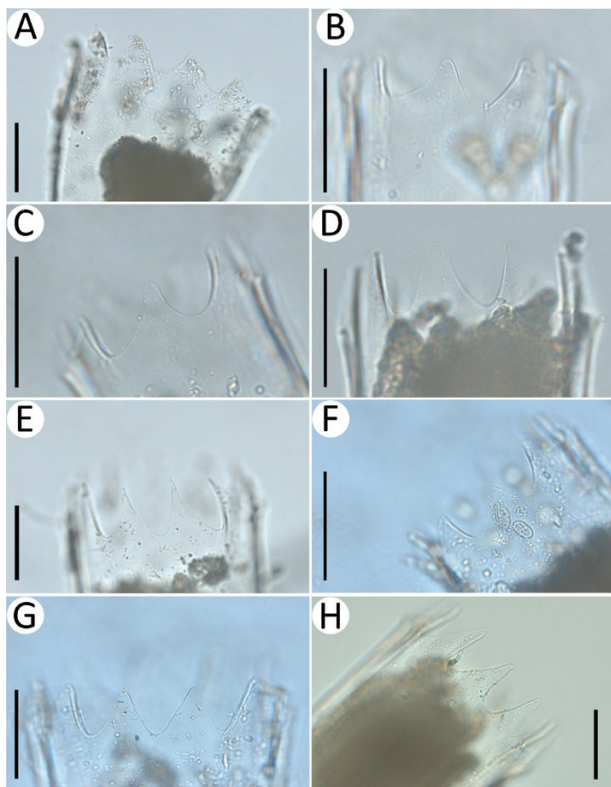


**Figure 11.** Variation in the shape of hydrothecal cusps of *Clytia* cf. *gracilis* sp.5. A, B, specimens from Fortaleza, Brazil (CE2_BRA, CE5_BRA); C, D, specimens from Cascavel, Brazil (CE1_BRA, CE3_BRA); E, F, specimens from São Luís do Maranhão, Brazil (MAP01_BRA, MAP11_BRA); G, specimen from Trairi, Brazil (T1_BRA); H, specimen from Salinópolis, Brazil (PAF03_BRA). Scale: 100 μm.

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* 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. 8A), 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. 8B). 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* Hincks, 1861 and *L. calceolifera* (Hincks, 1861) 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 can be confidently distinguished from *Obelia* based on their longer pedicels (Fig. 8C), 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* 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 *O. dichotoma* [taken as conspecific with *O. spherulina* Péron & Lesueur, 1810, the type species of *Obelia* (Cornelius, 1975, 1982)]. However, this action is presently premature, because there is no sequence of *O. dichotoma* from its type locality (south-western England; Cornelius, 1975), and the delimitation of this species is unclear (see section 'Morphometric Patterns of Obelia dichotoma and Obelia longissima').

ERECT COLONIES AND DIFFERENCES IN SHAPE AND NUMBER OF HYDROTHECAL CUSPS

The species *G. loveni*, *H. gelatinosa* and *Obelia 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* can also be differentiated by its paired branches that are successively arranged at right angles on opposite sides of the polysiphonic main stem (Cornelius, 1995). However, this feature is also present in large colonies of *O. bidentata* (Cornelius, 1995), which has contributed to some confusion in the past (Cornelius, 1982, 1990). *Hartlaubella gelatinosa* and *G. loveni* can be differentiated from *O. bidentata* by the shape and number of cusps, which are taller and more numerous in the latter (Fig. 8F). *Obelia bidentata* also has a more cylindrical hydrotheca than *H. gelatinosa* and *G. loveni* (Fig. 8E).

*Obelia bidentata* is assumed to have broad intraspecific variation, particularly in erect colonies, which vary from small and monosiphonic to large and polysiphonic; and in the shape of the hydrothecal cusps, with deep or shallow embayments (Cornelius, 1975, 1982, 1990, 1995; Millard, 1975; Mammen, 1965; Calder, 1991). This variation led to some dispute on the validity of several nominal species that have been frequently synonymized with *O. bidentata*, basically due to misinterpretation of intra- or interspecific variations (e.g. *Obelia austrogeorgiae* Jäderholm, 1904, *O. longicyatha* Allman, 1877; Cornelius, 1975, 1982; Calder, 1991). Calder (2013) recently regarded *O. oxydentata* Stechow, 1914 as a valid species based on the smaller size of the monosiphonic colonies from the tropical and subtropical western Atlantic (<1 cm high). In our study, we found that small (0.3–1.0 cm high) monosiphonic colonies and large (>6 cm high) polysiphonic colonies (USNM 1106185, from the North

Sea) are related in nearly all topologies analysed in previous molecular studies (Govindarajan *et al.*, 2006; Cunha *et al.*, 2017), partially contradicting the idea that these variations could indicate interspecific differences (see: Calder, 2017). However, as pointed out by Cunha *et al.* (2017), *O. bidentata* exhibits intraspecific genetic distances that are comparable to interspecific distances in other clades, and this could be evidence of either extensive population differentiation or the occurrence of a species complex (as in *C. hemisphaerica*, see above).

Obeliida *indet.* was ambiguously positioned at the base of Obeliidae and Clytiidae plus Obeliidae in the phylogenetic analysis of Cunha *et al.* (2017) (also see Fig. 12). In that study, this species was tentatively assigned to *Clytia stolonifera* Blackburn, 1938. We show that it can be differenciated from the remaining Obeliidae by its longer hydrothecae and taller hydrothecal cusps (Table 2). However, the inclusion and comparison of more specimens is necessary to confirm this identification and ascertain if this species should be considered in the genus *Clytia* or *Obelia*.

MORPHOMETRIC PATTERNS OF *OBELIA DICHOTOMA* AND *OBELIA LONGISSIMA*

Differences in size, branching patterns, tanning of the main stem and the shapes of the hydrothecae and hydrothecal rim have long been used to distinguish *Obelia dichotoma* and *O. longissima* (Alder, 1857; Hincks, 1868; Nutting, 1915; Kramp, 1935). Currently, besides the differences in their nematocysts (Östman, 1982a), *O. longissima* is characterized by having predominantly monosiphonic colonies with usually longer stems and branches roughly uniform in length, and a dark and flexuous main stem. *Obelia dichotoma*, on the other hand, has polysiphonic stems in older colonies, with branches often nearly as long as the main stem, giving the colony a bushy appearance (Östman, 1987; Cornelius, 1990, 1995; Schuchert, 2001; Calder, 2012). Additionally, the hydrotheca in *O. dichotoma* is often polygonal in cross-section, with an even to crenate rim; while the hydrotheca in *O. longissima* is round with the rim castellate to sinuous (Cornelius, 1990, 1995). The hydrothecal diaphragm varies from transverse to oblique in both species (Cornelius, 1990, 1995). Previous molecular studies showed that *O. dichotoma* comprises several cryptic lineages (Cunha *et al.*, 2017), and *O. longissima* was corroborated as a monophyletic and widely distributed species (Govindarajan *et al.*, 2006; Cunha *et al.*, 2017). Our results reveal that some characters support the separation of the species (Supporting Information, Table S6), viz. (1)
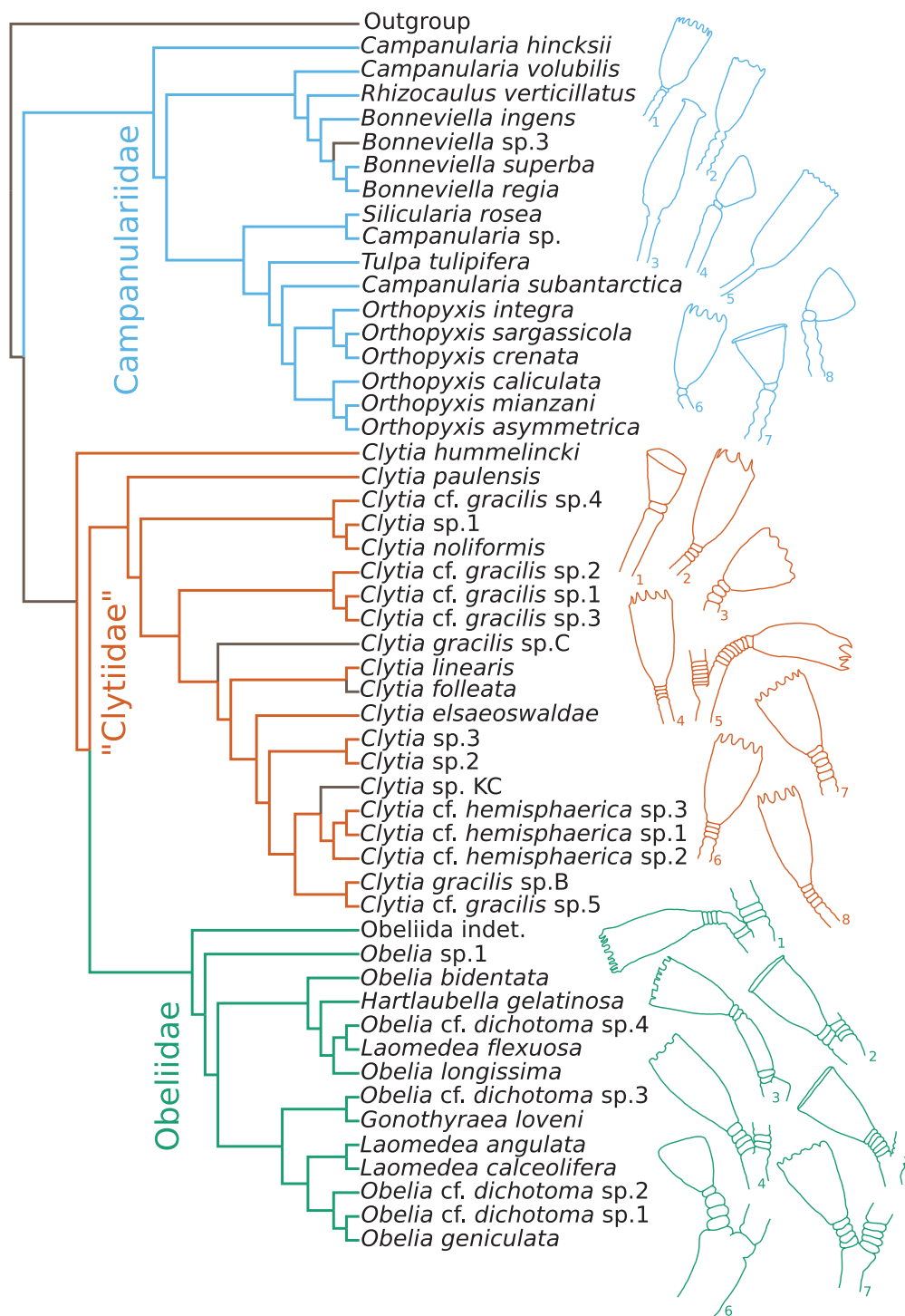
**Figure 12.** Phylogenetic hypothesis of Proboscoida based on the Maximum Likelihood phylogeny of Cunha *et al.* (2017: fig. 2) therein), including the re-identifications proposed in this study. Branches in grey indicate lineages not analysed in this study. Specimens' codes (also see Supporting Information, Table S1): Campanulariidae – 1, *Campanularia hincksii* (IT); 2, *C. volubilis* (USNM 29217); 3, *Bonneviella regia*; 4, *Silicularia rosea* (PT11); 5, *Tulpa tulipifera* (PT18); 6, *Orthopyxis sargassicola* (PTY1); 7, *O. caliculata* (PAB3); 8, *O. asymmetrica* (EL04); Clytiidae – 1, *Clytia hummelincki* (CBC42); 2, *C.* cf. *gracilis* sp.4 (CBC20); 3, *C. noliformis* (SP3); 4, *Clytia* sp.1 (IT13); 5, *C. linearis* (PY10); 6, *C.* cf. *hemisphaerica* sp.2 (EL06); 7, *C. elsaeoswaldae* (Me26); 8, *C.* cf. *gracilis* sp.5 (PAF03); Obeliidae – 1, *Obelia bidentata* (MAR02); 2, *O.* cf. *dichotoma* sp.4 (UR6); 3, *Hartlaubella gelatinosa* (PT16); 4, *Gonothyraea loveni* (SWM03); 5, *Laomedea calceolifera* (ROW03); 6, *Obelia geniculata* (UNH01); 7, *O.* cf. *dichotoma* sp.2 (MMA03). Outlines not to scale.

size of the colony, with *O. longissima* usually larger than species of *O.* cf. *dichotoma*, although some lineages of the latter exceeded the former in the number of branches; (2) length of internodes, longer on average in *O. longissima* but with some overlap with lineages of *O.* cf. *dichotoma*; (3) hydrothecal length, usually longer in *O. longissima* but with some overlap with species of *O.* cf. *dichotoma*; and (4) shape of the hydrothecal rim, varying from smooth to crenate in all lineages of *O.* cf. *dichotoma*, and invariably sinuous in *O. longissima*. Morphological variation may obscure some of these differences, but colonies of *O. longissima* can be reliably delimited by these characters when intraspecific variation is considered.

Contrastingly, cryptic lineages of *O.* cf. *dichotoma* do not show morphometric differences, presenting extensive variation and overlap in their characters (Fig. 9). Although *O.* cf. *dichotoma* sp.3 and sp.4 could be distinguished from the remaining lineages by their smaller and less branched colonies (Fig. 9A; Supporting Information, Table S6), in some cases colonies vary from unbranched to branched within the same lineage, indicating that these characters vary intra- and interspecifically. This also partially contradicts the idea that the amount of branching of the colonies could support the validation of former synonyms of *O. dichotoma* (e.g. *Obelia griffini* Calkins, 1899, *O. hyalina* Clarke, 1879; see Calder, 2013; Calder *et al.*, 2014), although their size and the shape of the hydrothecae are probably distinctive. For instance, Calder (2013) showed that colonies of *O. hyalina* are usually small and occur in tropical and warm-temperate waters. We found that all specimens of Brazilian *O.* cf. *dichotoma* are also small (~4–11 mm) and have few branches, although some have a slightly crenate hydrothecal rim (*O.* cf. *dichotoma* sp.3; Fig. 10C; Supporting Information, Table S6), in contrast to the even hydrothecal rim of *O. hyalina* (Clarke, 1879; Calder, 2013). Similarly, all specimens of *O.* cf. *dichotoma* sp.4 have rounded hydrothecae in cross-section and an even hydrothecal rim (Fig. 10D; Supporting Information, Table S6), in accordance with the diagnostic characters of *O. griffini*, recently revalidated by Calder *et al.* (2014). Although these identifications are tentative and need further confirmation, our results can support the revalidation of former synonyms of *O. dichotoma* to accommodate these cryptic lineages. Better knowledge of the nematocysts of these lineages might be particularly important for their corroboration, especially given that $I_D$ and $I_d$-type isorhizae are diagnostic for *O. dichotoma* and assumed to be invariably present in the species (Östman, 1982a, 1987; Cornelius, 1990).

## CONCLUSIONS

Our study demonstrates the usefulness of morphometric data to delimit species in Proboscoida. We show that morphometric characters related to size, perisarc thickness, shape of hydrothecae and hydrothecal cusps may contribute to the delimitation of several species, although in some cases (e.g. *Campanularia* spp., *Clytia gracilis*, *Clytia hemisphaerica*, *Laomedea* spp. and *Obelia dichotoma*), morphometric differences are masked by intraspecific variation (see summary in Table 2 and phylogenetic hypothesis with the species re-identified in this study in Fig. 12). Considering that our study is limited to the hydroid stage, extending this approach to investigate characters of the medusa stage and nematocysts is promising, and may shed light on some of the remaining difficult cases. However, some attention and specific procedures should be taken into consideration for this taxonomic approach. Even though many marine groups have wide intraspecific variation, consistent differences in morphometric patterns may be uncovered once this variation is comparatively investigated. This might be difficult to persue at first, without access to data from different populations and morphological characters. However, this problem will be gradually overcome once taxonomic descriptions that include morphometric characters and their amplitude of variation become more frequently linked to molecular data of voucher specimens. Morphometric characters are usually easy to obtain with the aid of compound or stereomicroscopes and digital cameras, and in most cases they will be more informative for the identification if considered in conjunction with other discrete diagnostic characters, as well as information on genetic differentiation of populations.

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

## ACKNOWLEDGEMENTS

## REFERENCES

**Agassiz L. 1862.** *Contributions to the natural history of the United States of America. Second monograph*. Boston: Little, Brown and Company.

**Alder J. 1857.** A catalogue of the zoophytes of Northumberland and Durham. *Transactions of the Tyneside Naturalists' Field Club* **3:** 93–162.

**Allman G. 1876.** Diagnoses of new genera and species of Hydroida. *Journal of the Linnean Society, Zoology* **12:** 251–284.

**Allman G. 1888.** Report of the Hydroida dredged by H.M.S. Challenger during the years 1873–1876. Part II. The Tubularinae, Corymorphinae, Campanularinae, Sertularinae, and Thalamophora. *Report on the Scientific Results of the Voyage of H.M.S. Challenger During the Years 1873–76* **23:** 1–90.

**Altuna A. 1994.** Descripcion de *Clytia linearis* (Thornely, 1899) (Cnidaria, Hydrozoa) y su variabilidad en la Costa Vasca; consideraciones biocenologicas, biogeograficas y ecologicas sobre la especie. *Kobie (Serie Ciencias Naturales), Bilbao* **22:** 59–66.

**Antsulevich AE. 1992.** Observations on the hydroid fauna of the Kurile Islands. *Scientia Marina* **56:** 213–216.

**Bale WM. 1934.** Note on *Campanularia integra* and *Orthopyxis caliculata*. *Proceedings of the Linnean Society of New South Wales* **59:** 273–275.

**Barroso R**, **Klautau M**, **Solé-Cava AM**, **Paiva PC. 2010.** *Eurythoe complanata* (Polychaeta: Amphinomidae), the 'cosmopolitan' fireworm, consists of at least three cryptic species. *Marine Biology* **157:** 69–80.

**Bell JJ**, **Barnes DKA. 2000.** The influences of bathymetry and flow regime upon the morphology of sublittoral sponge communities. *Journal of the Marine Biological Association of the UK* **80:** 707–718.

**Blanco O. 1967.** Estudio critico sobre las especies del genero *Silicularia* Meyen 1834. *Revista del Museo de La Plata* **9:** 217–241.

**Borcard D**, **Gillet F**, **Legendre P. 2011.** *Numerical ecology with R*. New York: Springer.

**Boero F**, **Bouillon J. 1993.** Zoogeography and life cycle patterns of Mediterranean hydromedusae (Cnidaria). *Biological Journal of the Linnean Society* **48:** 239–266.

**Boero F**, **Bouillon J**, **Piraino S. 1996.** Classification and phylogeny in the Hydroidomedusae (Hydrozoa, Cnidaria). *Scientia Marina* **60:** 17–33.

**Boissin E**, **Hoareau TB**, **Postaire B**, **Gravier-Bonnet N**, **Bourmaud CA-F. 2018.** Cryptic diversity, low connectivity and suspected human-mediated dispersal among 17 widespread Indo-Pacific hydroid species of the south-western Indian Ocean. *Journal of Biogeography* **45:** 2104–2117.

**Bouillon J**, **Medel MD**, **Pagès F**, **Gili JM**, **Boero F**, **Gravili C. 2004.** Fauna of the Mediterranean Hydrozoa. *Scientia Marina* **68:** 5–438.

**Broch H. 1909.** Hydroiduntersuchungen II. Zur Kenntnis der Gattungen *Bonneviella* und *Lictorella*. *Nyt Magazin for Naturvidenskaberne* **47:** 195–205.

**Broch H. 1910.** Die Hydroiden der arktischen Meere. *Fauna Arctica* **5:** 128–247.

**Bruno JF**, **Edmunds PJ. 1997.** Clonal variation for phenotypic plasticity in the coral *Madracis mirabilis*. *Ecology* **78:** 2177–2190.

**Budd AF**, **Romano SL**, **Smith ND**, **Barbeitos MS. 2010.** Rethinking the phylogeny of scleractinian corals: a review of morphological and molecular data. *Integrative and Comparative Biology* **50:** 411–427.

**Calder DR. 1991.** Shallow-water hydroids of Bermuda: the Thecatae, exclusive of Plumularioidea. *Life Science Contributions Royal Ontario Museum* **154:** 1–140.

**Calder DR. 1993.** Local distribution and biogeography of the hydroids (Cnidaria) of Bermuda. *Caribbean Journal of Science* **29:** 61–74.

**Calder DR. 2003.** Subtidal hydroids (Cnidaria) of Northumberland Strait, Atlantic Canada, with observations on their life cycles and distributions. *Canadian Field-Naturalist* **117:** 555–564.

**Calder DR. 2012.** On a collection of hydroids (Cnidaria, Hydrozoa, Hydroidolina) from the wests coast of Sweden, with a checklist of species from the region. *Zootaxa* **3171:** 1–77.

**Calder DR. 2013.** Some shallow-water hydroids (Cnidaria: Hydrozoa) from the central east coast of Florida, USA. *Zootaxa* **3648:** 1–72.

**Calder DR. 2017.** Additions to the hydroids (Cnidaria, Hydrozoa) of the Bay of Fundy, northeastern North America, with a checklist of species reported from the region. *Zootaxa* **4256:** 1–86.

**Calder D**, **Choong H**, **Carlton J**, **Chapman J**, **Miller J**, **Geller J. 2014.** Hydroids (Cnidaria: Hydrozoa) from Japanese tsunami marine debris washing ashore in the northwestern United States. *Aquatic Invasions* **9:** 425–440.

**Cartwright P**, **Evans NM**, **Dunn CW**, **Marques AC**, **Miglietta MP**, **Schuchert P**, **Collins AG. 2008.** Phylogenetics of Hydroidolina (Hydrozoa: Cnidaria). *Journal of the Marine Biological Association of the United Kingdom* **88:** 1663.

**Clarke S. 1879.** Report on the Hydroida collected during the exploration of the Gulf Stream and Gulf of Mexico by Alexander Agassiz, 1877–78. *Bulletin of the Museum of Comparative Zoölogy at Harvard College* **5:** 239–252.

**Collins AG**, **Winkelmann S**, **Hadrys H**, **Schierwater B. 2004.** Phylogeny of Capitata and Corynidae (Cnidaria, Hydrozoa) in light of mitochondrial 16S rDNA data. *Zoologica Scripta* **34:** 91–99.

**Collins A**, **Schuchert P**, **Marques A**, **Jankowski T**, **Medina M**, **Schierwater B. 2006.** Medusozoan phylogeny and character evolution clarified by new large and small subunit rDNA data and an assessment of the utility of phylogenetic mixture models. *Systematic Biology* **55:** 97–115.

**Collins AG**, **Bentlage B**, **Lindner A**, **Lindsay D**, **Haddock SHD**, **Jarms G**, **Norenburg JL**, **Jankowski T**, **Cartwright P. 2008.** Phylogenetics of Trachylina (Cnidaria: Hydrozoa) with new insights on the evolution of some problematical taxa. *Journal of the Marine Biological Association of the United Kingdom* **88:** 1673–1685.

**Cornelius PFS. 1975.** The hydroid species of *Obelia* (Coelenterata, Hydrozoa: Campanulariidae), with notes on the medusa stage. *Bulletin of the British Museum (Natural History)* **5:** 251–293.

**Cornelius PFS. 1981a.** Life cycle, dispersal and distribution among the Hydroida. *Porcupine Newsletter* **2:** 47–50.

**Cornelius PFS. 1981b.** *Clytia* Lamouroux, 1812, *Laomedea* Lamouroux, 1812, and *Campanularia* Lamarck, 1816 (Coelenterata, Hydroida): proposed designations of type species by use of the plenary powers, and comments on related genera. *Bulletin of Zoological Nomenclature* **38:** 208–220.

**Cornelius PFS. 1982.** Hydroids and medusae of the family Campanulariidae recorded from the eastern North Atlantic, with a world synopsis of genera. *Bulletin of the British Museum (Natural History)* **42:** 37–148.

**Cornelius PFS. 1990.** European *Obelia* (Cnidaria, Hydroida): systematics and identification. *Journal of Natural History* **24:** 535–578.

**Cornelius PFS. 1992a.** Medusa loss in leptolid Hydrozoa (Cnidaria), hydroid rafting, and abbreviated life-cycles among their remote-island faunae: an interim review. *Scientia Marina* **56:** 245–261.

**Cornelius PFS. 1992b.** The Azores hydroid fauna and its origin, with discussions of rafting and medusa suppression. *Arquipelago, Life and Earth Sciences* **10:** 75–99.

**Cornelius PFS. 1995.** North-west European thecate hydroids and their medusae. Part 2. Sertulariidae to Campanulariidae. *Synopses of the British Fauna New Series* **50:** 1–386.

**Cornelius PFS. 1999.** A changing taxonomic paradigm: studies on *Obelia* and some other Campanulariidae (Cnidaria: Hydrozoa). *Zoosystematica Rossica* **1:** 5–16.

**Cornelius PFS**, **Östman C. 1986.** On the names of two species of the genus *Clytia* Lamouroux, 1812 (Cnidaria, Hydrozoa) common in western Europe. *Bulletin of Zoological Nomenclature* **43:** 163–170.

**Cunha AF**, **Genzano GN**, **Marques AC. 2015.** Reassessment of morphological diagnostic characters and species boundaries requires taxonomical changes for the genus *Orthopyxis* L. Agassiz, 1862 (Campanulariidae, Hydrozoa) and some related campanulariids. *PLoS ONE* **10:** e0117553.

**Cunha AF**, **Maronna MM**, **Marques AC. 2016.** Variability on microevolutionary and macroevolutionary scales: a review on patterns of morphological variation in Cnidaria Medusozoa. *Organisms Diversity & Evolution* **16:** 431–442.

**Cunha AF**, **Collins AG**, **Marques AC. 2017.** Phylogenetic relationships of Proboscoida Broch, 1910 (Cnidaria, Hydrozoa): are traditional morphological diagnostic characters relevant for the delimitation of lineages at the species, genus, and family levels? *Molecular Phylogenetics and Evolution* **106:** 118–135.

**Debiasse MB**, **Hellberg ME. 2015.** Discordance between morphological and molecular species boundaries among Caribbean species of the reef sponge *Callyspongia*. *Ecology and Evolution* **5:** 663–675.

**El Beshbeeshy M**, **Jarms G. 2011.** Thekate Hydroiden von patagonischen Schelf (Cnidaria, Hydrozoa, Thecata). *Verhandlungen des Naturwischenschaftlichen Vereins in Hamburg* **46:** 19–233.

**Forsman ZH**, **Barshis DJ**, **Hunter CL**, **Toonen RJ. 2009.** Shape-shifting corals: molecular markers show morphology is evolutionarily plastic in *Porites*. *BMC Evolutionary Biology* **9:** 45.

**Forsman ZH**, **Concepcion GT**, **Haverkort RD**, **Shaw RW**, **Maragos JE**, **Toonen RJ. 2010.** Ecomorph or endangered coral? DNA and microstructure reveal Hawaiian species complexes: *Montipora dilatata/flabellata/turgescens* & *M. patula/verrilli*. *PLoS ONE* **5:** e15021.

**Fukami H**, **Budd AF**, **Paulay G**, **Solé-Cava A**, **Allen Chen C**, **Iwao K**, **Knowlton N. 2004.** Conventional taxonomy obscures deep divergence between Pacific and Atlantic corals. *Nature* **427:** 832–835.

**Fukami H**, **Chen CA**, **Budd AF**, **Collins A**, **Wallace C**, **Chuang YY**, **Chen C**, **Dai CF**, **Iwao K**, **Sheppard C**, **Knowlton N. 2008.** Mitochondrial and nuclear genes suggest that stony corals are monophyletic but most families of stony corals are not (order Scleractinia, class Anthozoa, phylum Cnidaria). *PLoS ONE* **3:** e3222.

**Galea HR. 2010.** Notes on a small collection of thecate hydroids (Cnidaria: Hydrozoa) from Tristan da Cunha, south Atlantic. *Zootaxa* **18:** 1–18.

**Galea HR**, **Häussermann V**, **Försterra G. 2009.** New additions to the hydroids (Cnidaria: Hydrozoa) from the fjords region of southern Chile. *Zootaxa* **2019:** 1–28.

**Galea HR**, **Schories D**, **Försterra G**, **Häussermann V. 2014.** New species and new records of hydroids (Cnidaria: Hydrozoa) from Chile. *Zootaxa* **3852:** 1.

**Govindarajan AF**, **Boero F**, **Halanych KM. 2006.** Phylogenetic analysis with multiple markers indicates repeated loss of the adult medusa stage in Campanulariidae (Hydrozoa, Cnidaria). *Molecular Phylogenetics and Evolution* **38:** 820–834.

**Govindarajan AF**, **Halanych KM**, **Cunningham CW. 2005.** Mitochondrial evolution and phylogeography in the hydrozoan *Obelia geniculata* (Cnidaria). *Marine Biology* **146:** 213–222.

**Hartlaub C. 1901.** Hydroiden aus dem Stillen Ocean. Ergebnisse einer Reise nach dem Pacific (Schauinsland 1896–97). *Zoologische Jahrbücher, Systematik* **14:** 349–379.

**He J**, **Zheng L**, **Zhang W**, **Lin Y**, **Cao W. 2015.** Morphology and molecular analyses of a new *Clytia* species (Cnidaria: Hydrozoa: Campanulariidae) from the East China Sea. *Journal of the Marine Biological Association of the United Kingdom* **95:** 289–300.

**Hincks T. 1868.** *A history of the British hydroid zoophytes*. London: John van Voorst.

**Hirohito ES. 1995.** *The hydroids of Sagami Bay II. Thecata*. Tokyo: Publications of the Biological Laboratory Imperial Household.

**ICZN (International Commission on Zoological Nomenclature). 1985.** Opinion 1345. *Laomedea flexuosa* Alder, 1857, *Sertularia volubilis* Linnaeus, 1758 and *Campanularia johnstoni* Alder, 1856 designated as type species of *Laomedea* Lamouroux, 1812, *Campanularia* Lamarck, 1816 and *Clytia* Lamouroux, 1812 (Coelenterata, Hydroida) respectively. *Bulletin of Zoological Nomenclature* **42:** 271–273.

**Kaandorp JA. 1999.** Morphological analysis of growth forms of branching marine sessile organisms along environmental gradients. *Marine Biology* **134:** 295–306.

**Kawauchi GY**, **Giribet G. 2014.** *Sipunculus nudus* Linnaeus, 1766 (Sipuncula): cosmopolitan or a group of pseudo-cryptic species? An integrated molecular and morphological approach. *Marine Ecology* **35:** 478–491.

**Klautau M**, **Russo CAM**, **Lazoski C**, **Boury-Esnault N**, **Thorpe JP**, **Sole-Cava AM. 1999.** Does cosmopolitanism result from overconservative systematics? A case study using the marine sponge *Chondrilla nucula*. *Evolution* **53:** 1414.

**Kramp PL. 1913.** Hydroids collected by the 'Tjalfe' Expedition to the west coast of Greenland in 1908 and 1909. *Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening i Kjøbenhavn* **65:** 1–36.

**Kramp PL. 1935.** Polypdyr (Coelenterata). I. Ferskvandspolypper og Goplepolypper. *Danmarks Fauna* **41:** 1–207.

**Laakmann S**, **Holst S. 2014.** Emphasizing the diversity of North Sea hydromedusae by combined morphological and molecular methods. *Journal of Plankton Research* **36:** 64–76.

**Leclère L**, **Schuchert P**, **Cruaud C**, **Couloux A**, **Manuel M. 2009.** Molecular phylogenetics of thecata (Hydrozoa, Cnidaria) reveals long-term maintenance of life history traits despite high frequency of recent character changes. *Systematic Biology* **58:** 509–526.

**Legendre P**, **Legendre L. 1998.** *Numerical ecology*. Amsterdam: Elsevier Science.

**Lindner A**, **Calder DR. 2000.** *Campanularia noliformis* McCrady, 1859 (currently *Clytia noliformis*; Cnidaria, Hydrozoa): proposed conservation of the specific name by the designation of a neotype. *Bulletin of Zoological Nomenclature* **57:** 140–143.

**Lindner A**, **Migotto AE. 2001.** *Merotrichous isorhiza*, a nematocyst new to the Campanulariidae (Cnidaria: Hydrozoa), and its relevance for the classification of Cnidae. *Proceedings of the Biological Society of Washington* **114:** 825–832.

**Lindner A**, **Migotto AE. 2002.** The life cycle of *Clytia linearis* and *Clytia noliformis*: metagenic campanulariids (Cnidaria: Hydrozoa) with contrasting polyp and medusa stages. *Journal of the Marine Biological Association of the UK* **82:** 541–553.

**Lindner A**, **Govindarajan AF**, **Migotto AE. 2011.** Cryptic species, life cycles, and the phylogeny of *Clytia* (Cnidaria: Hydrozoa: Campanulariidae). *Zootaxa* **36:** 23–36.

**Linnaeus C. 1758.** *Systema naturae per regna tria naturae, secundum clases, ordines, genera, species, cum characteribus, differentiis, synonymis, locis*. Stockholm: L. Salvius.

**Mammen T. 1965.** On a collection of hydroids from South India. II. Suborder Thecata. *Journal of the Marine Biological Association of India* **7:** 1–57.

**Maronna MM**, **Miranda TP**, **Peña Cantero ÁL**, **Barbeitos MS**, **Marques AC. 2016.** Towards a phylogenetic classification of Leptothecata (Cnidaria, Hydrozoa). *Scientific Reports* **6:** 18075.

**Medel MD**, **Vervoort W. 2000.** Atlantic Haleciidae and Campanulariidae (Hydrozoa, Cnidaria) collected during the CANCAP and Mauritania-II expeditions of the National Museum of Natural History, Leiden, The Netherlands. *Zoologische Verhandelingen, Leiden* **330:** 1–68.

**Meroz-Fine E**, **Brickner I**, **Loya Y**, **Ilan M. 2003.** The hydrozoan coral *Millepora dichotoma*: speciation or phenotypic plasticity? *Marine Biology* **143:** 1175–1183.

**Miglietta MP**, **Piraino S**, **Kubota S**, **Schuchert P. 2007.** Species in the genus *Turritopsis* (Cnidaria, Hydrozoa): a molecular evaluation. *Journal of Zoological Systematics and Evolutionary Research* **45:** 11–19.

**Miglietta MP**, **Schuchert P**, **Cunningham CW. 2009.** Reconciling genealogical and morphological species in a worldwide study of the family Hydractiniidae (Cnidaria, Hydrozoa). *Zoologica Scripta* **38:** 403–430.

**Miglietta MP**, **Odegard D**, **Faure B**, **Faucci A. 2015.** Barcoding techniques help tracking the evolutionary history of the introduced species *Pennaria disticha* (Hydrozoa, Cnidaria). *PLoS ONE* **10:** e0144762.

**Migotto AE. 1996.** Benthic shallow-water hydroids (Cnidaria, hydrozoa) of the coast of São Sebastião, Brazil, including a checklist of Brazilian hydroids. *Zoologische Verhandelingen* **306:** 1–125.

**Millard NAH. 1966.** The Hydrozoa of the south and west coasts of South Africa. Part III. The Gymnoblastea and small families of Calyptoblastea. *Annals of the South African Museum* **48:** 427–487.

**Millard NAH. 1971.** Hydrozoa. In: Bakker MZ, Winterbotton JM, Dyer RA, eds. *Marion and Prince Edward Islands*. Cape Town: Balkema, 396–408.

**Millard NAH. 1975.** Monograph on the Hydroida of southern Africa. *Annals of the South African Museum* **68:** 1–513.

**Moura CJ**, **Harris DJ**, **Cunha MR**, **Rogers AD. 2008.** DNA barcoding reveals cryptic diversity in marine hydroids (Cnidaria, Hydrozoa) from coastal and deep-sea environments. *Zoologica Scripta* **37:** 93–108.

**Moura CJ**, **Cunha MR**, **Porteiro FM**, **Yesson C**, **Rogers AD. 2012.** Evolution of *Nemertesia* hydroids (Cnidaria: Hydrozoa, Plumulariidae) from the shallow and deep waters of the NE Atlantic and western Mediterranean. *Zoologica Scripta* **41:** 79–96.

**Moura CJ**, **Lessios H**, **Cortés J**, **Nizinski M**, **Reed J**, **Santos RS**, **Collins AG. 2018.** Hundreds of genetic barcodes of the species-rich hydroid superfamily Plumularioidea (Cnidaria, Medusozoa) provide a guide toward more reliable taxonomy. *Scientific Reports* **8:** 17986.

**Naumov DV. 1969.** *Hydroids and hydromedusae of the USSR*. Jerusalem: Israel Program for Scientific Translations.

**Nutting CC. 1901.** Papers from the Harriman Alaska Expedition. *Proceedings of the Washington Academy of Sciences* **3:** 157–216.

**Nutting CC. 1915.** *American hydroids. Part III. The Campanulariidae and the Bonneviellidae*. Washington, DC: Government Printing Office.

**Oksanen J**, **Guillaume Blanchet F**, **Kindt R**, **Legendre P**, **Minchin PR**, **O'Hara RB**, **Simpson GL**, **Solymos P**, **Henry M**, **Stevens H**, **Wagner H. 2015.** *Vegan: community ecology package*. R package version 2.3-0. Available at http://CRAN.R-project.org/package=vegan (date last accessed, 08 December 2019).

**Östman C. 1982a.** Nematocysts and taxonomy in *Laomedea*, *Gonothyraea* and *Obelia* (Hydrozoa, Campanulariidae). *Zoologica Scripta* **11:** 227–241.

**Östman C. 1982b.** Isoenzymes and taxonomy in scandinavian hydroids (Cnidaria, Campanulariidae). *Zoologica Scripta* **11:** 155–163.

**Östman C. 1987.** New techniques and old problems in hydrozoan systematics. In: Bouillon J, Boero F, Cicogna F, Cornelius PFS, eds. *Modern trends in the systematics, ecology, and evolution of hydroids and hydromedusae*. Oxford: Clarendon Press, 67–82.

**Östman C. 1999.** Nematocysts and their value as taxonomic parameters within the Campanulariidae (Hydrozoa). A review based on light and scanning electron microscopy. *Zoosystematica Rossica* **1:** 17–28.

**Östman C**, **Piraino S**, **Roca I. 1987.** Nematocysts comparisons between some Mediterranean and Scandinavian campanulariids (Cnidaria, Hydrozoa). In: Bouillon J, Boero F, Cicogna F, Cornelius PFS, eds. *Modern trends in the systematics, ecology and evolution of hydroids and hydromedusae*. Oxford: Clarendon Press, 299–310.

**Peña Cantero AL**, **García Carrascosa AM. 1999.** Biogeographical distribution of the benthic thecate hydroids collected during the Spanish 'Antártida 8611' expedition and comparison between Antarctic and Magellan benthic hydroid faunas. *Scientia Marina* **63:** 209–218.

**Peña Cantero AL**, **García Carrascosa AM. 2002.** The benthic hydroid fauna of the Chafarinas Islands (Alborán Sea, western Mediterranean). *Zoologische Verhandelingen* **337:** 1–180.

**Pérez-Barros P**, **Confalonieri VA**, **Paschke K**, **Lovrich GA. 2015.** Incongruence between molecular and morphological characters in the southern king crabs *Lithodes santolla* and *Lithodes confundens* (Decapoda: Anomura). *Polar Biology* **38:** 2097–2107.

**Piraino S**, **Morri C. 1990.** Zonation and ecology of epiphytic hydroids in a Mediterranean coastal lagoon: the 'Stagnone' of Marsala (north-west Sicily). *Marine Ecology* **11:** 43–60.

**Postaire B**, **Magalon H**, **Bourmaud CAF**, **Gravier-Bonnet N**, **Bruggemann JH. 2016.** Phylogenetic relationships within Aglaopheniidae (Cnidaria, Hydrozoa) reveal unexpected generic diversity. *Zoologica Scripta* **45:** 103–114.

**Postaire B**, **Gélin P**, **Bruggemann JH**, **Magalon H. 2017a.** One species for one island? Unexpected diversity and weak connectivity in a widely distributed tropical hydrozoan. *Heredity* **118:** 385–394.

**Postaire B**, **Gélin P**, **Bruggemann JH**, **Pratlong M**, **Magalon H. 2017b.** Population differentiation or species formation across the Indian and the Pacific Oceans? An example from the brooding marine hydrozoan *Macrorhynchia phoenicea*. *Ecology and Evolution* **7:** 8170–8186.

**Prada C**, **Schizas NV**, **Yoshioka PM. 2008.** Phenotypic plasticity or speciation? A case from a clonal marine organism. *BMC Evolutionary Biology* **8:** 47.

**Prada C**, **DeBiasse MB**, **Neigel JE**, **Yednock B**, **Stake JL**, **Forsman ZH**, **Baums IB**, **Hellberg ME. 2014.** Genetic species delineation among branching Caribbean *Porites* corals. *Coral Reefs* **33:** 1019–1030.

**R Core Team**. **2019.** *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Available at: http://www.R-project.org (date last accessed, 08 December 2019).

**Ralph PM. 1956.** Variation in *Obelia geniculata* (Lennaeus, 1758) and *Silicularia bilabiata* (Coughtrey, 1875) (Hydroida, F. Campanulariidae). *Transactions of the Royal Society of New Zealand* **84:** 279–296.

**Ralph PM. 1957.** New Zealand thecate hydroids. Part I. Campanulariidae and Campanulinidae. *Transactions of the Royal Society of New Zealand* **84:** 811–854.

**Ralph PM. 1961.** New Zealand thecate hydroids. Part V. The distribution of the New Zealand thecate hydroids. *Transactions of the Royal Society of New Zealand* **1:** 103–111.

**Ralph PM**, **Thomson HG. 1968.** Seasonal changes in growth of the erect stem of *Obelia geniculata* in Wellington Harbour, New Zealand. *Zoology Publications from Victoria University of Wellington* **44:** 1–21.

**Rees W**, **Thursfield S. 1965.** The hydroid collections of James Ritchie. *Proceedings of the Royal Society of Edinburg, Section B (Biology)* **69:** 34–220.

**Rees WJ**, **Vervoort W. 1987.** Hydroids from the John Murray expedition to the Indian Ocean, with revisory notes on *Hydrodendron*, *Abietinella*, *Cryptolaria* and *Zygophylax* (Cnidaria: Hydrozoa). *Zoologische Verhandelingen, Leiden* **237:** 1–209.

**Ronowicz M. 2007.** Benthic hydroids (Cnidaria: Hydrozoa) from Svalbard waters – biodiversity and distribution. *Journal of the Marine Biological Association of the UK* **87:** 1089–1094.

**Sars M. 1850.** Beretning om en i sommeren 1849 foretagen zoologisk reise i Lofoten og Finmarken. *Nyt Magazin for Naturvidenskaberne* **6:** 121–211.

**Sars M. 1857.** Bidrag til kundskaben om Middlehavets littoral-fauna, reisebemaerkninger fra Italien. *Nyt Magazin for Naturvidenskaberne* **9:** 110–164.

**Schierwater B**, **Ender A. 2000.** *Sarsia marii* n. sp. (Hydrozoa, Anthomedusae) and the use of 16S rDNA sequences for unpuzzling systematic relationships in Hydrozoa. *Scientia Marina* **64:** 117–122.

**Schneider Ca**, **Rasband WS**, **Eliceiri KW. 2012.** NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* **9:** 671–675.

**Schuchert P. 2001.** Hydroids of Greenland and Iceland (Cnidaria, Hydrozoa). *Meddelelser om Grønland, Bioscience* **53:** 1–184.

**Schuchert P. 2003.** Hydroids (Cnidaria, Hydrozoa) of the Danish expedition to the Kei Islands. *Steenstrupia* **27:** 137–256.

**Schuchert P. 2005.** Species boundaries in the hydrozoan genus *Coryne*. *Molecular Phylogenetics and Evolution* **36:** 194–199.

**Schuchert P. 2014.** High genetic diversity in the hydroid *Plumularia setacea*: a multitude of cryptic species or extensive population subdivision? *Molecular Phylogenetics and Evolution* **76:** 1–9.

**Schuchert P. 2019.** Campanulariidae Jonhston, 1936. In: Schuchert P, ed. *World Hydrozoa Database*. Accessed through: World Register of Marine Species Available at: http://www.marinespecies.org/aphia.php?p=taxdetails&id=1606 (accessed 27 October 2019).

**Soto Àngel JJ**, **Peña Cantero AL. 2015.** On the benthic hydroids from the Scotia Arc (Southern Ocean): new insights into their biodiversity, ecology and biogeography. *Polar Biology* **38:** 983–1007.

**Stechow E. 1919.** Zur Kenntnis der Hydroidenfauna des Mittelmeeres, Amerikas und anderer Gebiete, nebst Angaben über einige Kirchenpauer'sche Typen von Plumulariden. Zoologische Jahrbücher. *Abteilung für Systematik, Geographie und Biologie der Tiere* **42:** 1–172.

**Stechow E. 1920.** Neue Ergebnisse auf dem Gebiete der Hydroidenforschung. *Sitzungsberichte der Gesellschaft für Morphologie und Physiologie in München* **31:** 9–45.

**Stechow E. 1921.** Neue Genera und Species von Hydrozoen und anderen Everebraten. *Archiv für Naturgeschichte* **87:** 248–265.

**Stefani F**, **Benzoni F**, **Yang SY**, **Pichon M**, **Galli P**, **Chen CA. 2011.** Comparison of morphological and genetic analyses reveals cryptic divergence and morphological plasticity in Stylophora (Cnidaria, Scleractinia). *Coral Reefs* **30:** 1033–1049.

**Stepanjants S. 1998.** *Obelia* (Cnidaria, Medusozoa, Hydrozoa): phenomenon, aspects of investigations, perspectives for utilization. *Oceanography and Marine Biology: an Annual Review* **36:** 179–215.

**Stepanjants SD**, **Cortese G**, **Kruglikova SB**, **Bjørklund KR. 2006.** A review of bipolarity concepts: history and examples from Radiolaria and Medusozoa (Cnidaria). *Marine Biology Research* **2:** 200–241.

**Todd PA. 2008.** Morphological plasticity in scleractinian corals. *Biological Reviews* **83:** 315–337.

**Trussell GC. 1996.** Phenotypic plasticity in an intertidal snail: the role of a common crab predator. *Evolution* **50:** 448.

**Vervoort W. 1959.** The Hydroida of the tropical west coast of Africa. *Atlantide Report* **5:** 211–325.

**Vervoort W. 1972.** Hydroids from the Theta, Vema and Yelcho cruises of the Lamont-Doherty Geological Observatory. *Zoologische Verhandelingen* **120:** 1–247.

**Vervoort W**, **Watson JE. 2003.** *The marine fauna of New Zealand: Leptothecata (Cnidaria:Hydrozoa) (Thecate Hydroids)*. Wellington: NIWA (National Institute of Water and Atmospheric Research).

**Willette DA**, **Iñiguez AR**, **Kupriyanova EK**, **Starger CJ**, **Varman T**, **Toha AH**, **Maralit BA**, **Barber PH. 2015.** Christmas tree worms of Indo-Pacific coral reefs: untangling the *Spirobranchus corniculatus* (Grube, 1862) complex. *Coral Reefs* **34:** 899–904.

**Yamada M. 1969.** Notes on Japanese species of *Bonneviella* (Hydrozoa). *Bulletin of the Marine Biological Association of Asamushi* **13:** 241–245.

**Yoshioka PM. 1982.** Predator-induced polymorphism in the bryozoan *Membranipora membranacea* (L.). *Journal of Experimental Marine Biology and Ecology* **61:** 233–242.

**Zhou K**, **Zheng L**, **He J**, **Lin Y**, **Cao W**, **Zhang W. 2013.** Detection of a new *Clytia* species (Cnidaria: Hydrozoa: Campanulariidae) with DNA barcoding and life cycle analyses. *Journal of the Marine Biological Association of the United Kingdom* **93:** 2075–2088.

# SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

**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 lineages of *Clytia* cf. *gracilis*; C, second and third PCs of the PCA without *C.* cf. *gracilis* and measurements related to internodes of erect colonies; D, first and second PCs of the PCA with lineages of *C.* cf. *hemisphaerica*, but without measurements related to internodes of erect colonies; E, second and third PCs of the PCA with lineages of *C.* cf. *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.** 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].

**Figure S4.** 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, PIB); C, second and third PCs of the PCA without species of the genus *Obelia*; D, second and third PCs of the PCA with species of the genus *Obelia* only; E, second and third PCs of the PCA with lineages of O. cf. *dichotoma* and O. *longissima*; F, second and third 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).

**Table S1.** Materials analyzed in this study. The symbol * indicates materials that were reidentified in this study (see Table 2). Specimens in bold indicate samples from which intracolony measurements were taken. Vouchers and specimen codes are in accordance with Cunha *et al*. (2017), unless not included in that study. USNM, National Museum of Natural History, Smithsonian Institution, USA; MZUSP, Museu de Zoologia da Universidade de São Paulo, Brazil; ZMUC, Zoological Museum, Natural History Museum of Denmark; MHNG INVE, Muséum d'Histoire Naturelle de Genève, Switzerland; BMNH, Natural History Museum, United Kingdom.

**Table S2.** Comparison among different species of *Bonneviella* [mean ± standard error (range)]. Specimens in bold indicate measurements taken from type materials deposited at the National Museum of Natural History, Smithsonian Institution. Numbers in brackets indicate total number of specimens examined. Morphometric data for B. *grandis* were based on the literature. The symbol "-" indicates lack of the structure to be measured (e.g. gonothecae, pedicel).

**Table S3.** Comparison among different species of *Orthopyxis* [mean±standard error (range)]. Specimens in bold indicate measurements taken from type materials deposited at the National Museum of Natural History, Smithsonian Institution (USNM), and the Natural History Museum, United Kingdom (BMNH). Number in brackets indicate total number of specimens examined. Morphometric data for the species O. *asymmetrica* and O. *angulata* are based on the literature. Symbol "-" indicates lack of the structure to be measured (e.g. gonotheca, pedicel), or lack of information from the literature.

**Table S4.** Comparison among lineages identified as C. cf. *gracilis* [mean±standard error (range)] and descriptions from the literature. Number in brackets indicates total number of specimens examined. The symbol "-" indicates lack of the structure to be measured (e.g. gonothecae, pedicel) or lack of information from the literature.

**Table S5.** Comparison among lineages identified as *Clytia* cf. *hemisphaerica* [mean±standard error (range)] and descriptions from the literature. Number in brackets indicate total number of specimens examined. The symbol "-" indicates lack of the structure to be measured (e.g. gonothecae, pedicel) or lack of information from the literature.

**Table S6.** Comparison among lineages identified as *Obelia* cf. *dichotoma* and O. *longissima* [mean±standard error (range)], and literature descriptions. Number in brackets indicates total number of specimens examined. The symbol "-" indicates lack of the structure to be measured (e.g. gonothecae) or lack of information from the literature.