Amanda Ferreira Cunha

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From morphological variability to taxonomic diversity in Proboscoida (Cnidaria, Hydrozoa): phylogenetic and morphometric inferences for lineages delimitation

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Orientador: Prof. Dr. Antonio Carlos Marques

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Prof. Dr. Antonio Carlos Marques

Dedicatória

Aos meus pais e irmã, pelo apoio em
todos os momentos

"Again, we have many slight differences which may be called individual diferences, such as are known frequently to appear in the offspring from the same parents, or which may be presumed to have thus arisen, from being frequently observed in the individuals of the same species inhabiting the same confined locality. No one supposes that all individuals of the same species are cast in the very same mould. These individual differences are highly important for us, as they afford material for natural selection to accumulate (...)"

Charles Darwin, *On the origin of species*, 1859: 45.

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Índice

Introdução Geral

A variabilidade morfológica intraespecífica acentuada é um aspecto microevolutivo comum em vários grupos marinhos, principalmente em organismos sésseis coloniais (e.g., Okamura & Partridge 1999, em Bryozoa; Bell & Barnes 2000, em Porifera; Griffith & Newberry 2008, em Hydrozoa; Bruno & Edmunds 1997; Todd 2008, em Anthozoa). O crescimento modular permite a esses organismos a capacidade de uma rápida exploração dos recursos disponíveis e menor risco de morte da colônia, principalmente pelo seu grande poder regenerativo (Hughes & Cancino 1985; Hughes 1989). A plasticidade fenotípica expressa em organismos coloniais advém destas características, permitindo que as colônias ocupem ambientes heterogêneos, ajustando-se às condições ambientais locais (Hughes 1989; Marfenin 1997). Além disso, essa plasticidade não se limita às diversificações morfológicas da colônia, mas se expressa também em padrões fisiológicos, reprodutivos e comportamentais (e.g., Chornesky 1983; West et al. 1993; Lesser et al. 1994).

Vários fatores físicos estão relacionados à variabilidade morfológica em organismos coloniais marinhos, como hidrodinamismo (Kaandorp 1999; Okamura & Partridge 1999; Griffith & Newberry 2008), luminosidade (Helmuth et al. 1997), profundidade (West et al. 1993; Bell & Barnes 2000), latitude e temperatura da água (Ralph 1956; Chen et al. 2011). Entretanto, o avanço das análises moleculares tem mostrado que, em muitos casos, a presumida variabilidade morfológica intraespecífica é resultado da diferenciação genética de linhagens, e várias espécies consideradas "amplamente distribuídas" apresentam populações geneticamente estruturadas que, na realidade, correspondem a espécies crípticas (Klautau et al. 1999; Fukami et al. 2004; Prada et al. 2008; Schmidt-Roach et al. 2013). Por outro lado, também é possível encontrar espécies que são distintas morfologicamente mas não geneticamente (e.g., Forsman et al. 2010; Pizón et al. 2013; Prada et al. 2014), demonstrando que a plasticidade fenotípica de caracteres morfológicos pode também ser erroneamente interpretada como variação interespecífica. De fato, no nível taxonômico, a determinação dos padrões de variação morfológica intra e interespecíficos não é simples, já que a variabilidade apresentada pelas espécies leva à sobreposição de seus caracteres diagnósticos, dificultando a definição dos caracteres relevantes para delimitação interespecífica.

Dentre os Cnidaria Medusozoa, os padrões de variabilidade não se limitam ao estágio de pólipo, mas também se expressam nas medusas (Dawson 2005; Gershwin 1999; Miranda

et al. 2009) e nos próprios ciclos de vida, que podem variar intra e interespecificamente (Boero et al. 1992; Bavestrello et al. 2000; Holst et al. 2007; Miranda et al. 2010). Por apresentarem diferentes níveis de variação morfológica, historicamente os taxonomistas enfrentam dificuldades de interpretação dos caracteres diagnósticos nesse grupo, levando a muitos problemas taxonômicos (para alguns exemplos, veja Dawson 2005; Miglietta et al. 2009). Vários estudos moleculares têm mostrado incongruências entre as classificações tradicionais e as relações filogenéticas de muitas espécies, gêneros e famílias (e.g., Schuchert 2005, 2014; Miglietta et al. 2007, 2009; Bentlage et al. 2010; Moura et al. 2011a, b, 2012a, b), assim como ordens e subordens (e.g., Leclére et al. 2009; Maronna et al. 2016). Apesar disso, em Medusozoa, são raros os estudos que correlacionam os padrões de variabilidade morfológica de caracteres diagnósticos com os padrões filogenéticos das espécies (e.g., Dawson 2003, 2005; Moura et al. 2011b; Cunha et al. 2015), e por isso muitos dos limites interespecíficos ainda não são claros. Abordagens relacionando a amplitude de variação de caracteres morfológicos em diferentes níveis taxonômicos e a variação genética associada podem fornecer informações relevantes de delimitações taxonômicas em grupos com morfologia variável.

Os hidroides historicamente associados à família Campanulariidae Johnston, 1836 (Hydrozoa, Leptothecata) se destacam pela ampla variação morfológica apresentada pelas espécies e as dificuldades taxonômicas associadas. A família tradicionalmente compreende de 11 a 13 gêneros (dependendo da proposta taxonômica) arranjados em três subfamílias (Cornelius 1982, 1995; Calder 1991). Por apresentarem o hipostômio em forma de trompete, em oposição ao hipostômio cônico da maioria dos demais Leptothecata, a família foi incluída na ordem Proboscoida Broch, 1910, junto com Bonneviellidae Broch, 1909 e Phialuciidae Kramp, 1955 (Broch 1910; Bouillon 1985). Entretanto, Leclére et al. (2009) mostraram que as ordens Conica Broch, 1910 e Proboscoida, no seu sentido tradicional, não são monofiléticas, e propuseram as ordens Macrocolonia e Statocysta com base nas relações filogenéticas de Leptothecata. Mais recentemente, Maronna et al. (2016) propuseram uma nova classificação filogenética para Leptothecata, redefinindo Proboscoida como uma subordem dentro de Statocysta, e dividindo os Campanulariidae (no seu sentido tradicional) em três famílias, Campanulariidae Johnston, 1836, Clytiidae Cockerell, 1911 e Obeliidae Haeckel, 1879, com escopo semelhante à divisão clássica em subfamílias. Phialuciidae não foi amostrada nesse estudo, e por isso sua inclusão dentro dos Proboscoida ainda precisa ser confirmada.

Apesar dos avanços nas propostas de classificação do grupo nos níveis de subordem e família, pouco se avançou sobre as suas relações filogenéticas no nível de espécies e gêneros. Estudos com Campanulariidae demonstram que a família, no seu sentido tradicional, não é monofilética, assim como alguns gêneros (e.g., *Obelia* Perón & Lesueur, 1810, *Laomedea* Lamouroux, 1812) e espécies (e.g., *Orthopyxis integra* (MacGillivray, 1842), *Clytia gracilis* (Sars, 1850), Govindarajan et al. 2005, 2006; Lindner et al. 2011). A revisão dos caracteres morfológicos em alguns casos, levou à revalidação de antigos sinônimos e descrição de novas espécies (Lindner et al. 2011; Zhou et al. 2013; Cunha et al. 2015; He et al. 2015), porém ainda há muitas inconsistências na interpretação dos caracteres diagnósticos no grupo, e seus padrões de variabilidade morfológica são raramente investigados (e.g., Cunha et al. 2015).

Objetivos Gerais

Este estudo teve como objetivos (1) sintetizar o conhecimento sobre os padrões de variação morfológica em Medusozoa, avaliando sua influência na taxonomia e diversidade do grupo; (2) avaliar os níveis taxonômicos em que os padrões de variabilidade morfológica são informativos para a delimitação interespecífica em Proboscoida (c.f. Maronna et al. 2016), (3) testando as diferentes classificações propostas para o grupo, principalmente em relação aos caracteres diagnósticos classicamente associados às suas espécies e gêneros; e (4) adequando o uso destes padrões de diferenciação à sua história filogenética, através da correlação das variações morfológicas e morfométricas à delimitação de linhagens.

Nesse sentido, o Capítulo 1 é uma revisão crítica dos padrões de variabilidade morfológica em Medusozoa, avaliando como as diferentes interpretações dos níveis de variação podem influenciar a compreensão sobre os padrões de diversificação no grupo. Diferentes níveis de variação morfológica são considerados e revistos para o grupo, a partir de dados publicados e não-publicados, e a diversidade críptica em Hydrozoa é estimada a partir de sequências de DNA disponíveis no GenBank.

O Capítulo 2 trata das relações filogenéticas de Proboscoida, a partir de dados moleculares. A hipótese filogenética proposta é comparada com classificações tradicionais da família Campanulariidae, assim como a nova classificação proposta por Maronna et al. (2016), avaliando a congruência e relevância dos caracteres diagnósticos propostos, em diferentes níveis taxonômicos.

O Capítulo 3 avalia os padrões de variação morfológica em Proboscoida, correlacionando-os à delimitação de espécies no grupo. Para isso, os padrões morfométricos

da maioria dos espécimes incluídos na hipótese filogenética do capítulo anterior são avaliados e comparados entre si com base nas suas relações filogenéticas. Além disso, materiais tipo e *vouchers* de sequências de DNA depositados no GenBank foram estudados, contribuindo para uma ampla revisão dos caracteres morfológicos do grupo e sua amplitude de variação.

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Capítulo 1

Variability on micro- and macroevolutionary scales: a review of the patterns of morphological variation in Cnidaria Medusozoa

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Abstract

The members of Cnidaria Medusozoa are known for their wide morphological variation, which is expressed on many different levels, especially in different phases of the life cycle. Difficulties in interpreting morphological variations have posed many taxonomic problems, since intraspecific morphological variations are often misinterpreted as interspecific variations, and vice-versa, hampering species delimitation. This study reassessed the patterns of morphological variation in the Medusozoa, to evaluate how different interpretations of the levels of variation may influence the understanding of the patterns of diversification in the group. Additionally, we provide an estimate of the cryptic diversity in the Hydrozoa, based on COI sequences deposited in GenBank. Morphological variations frequently overlap between micro- and macroevolutionary scales, contributing to misinterpretations of the different levels of variation. In addition, most of the cryptic diversity described for the Medusozoa is a result of previously overlooked morphological differences, and there is still great potential for discovering cryptic lineages in the Hydrozoa. We provide evidence that the number of species in the Medusozoa is misestimated, and emphasize the necessity of examining different levels of morphological variations when studying species boundaries, in order to avoid generalizations and misinterpretations of morphological characters.

Resumo

Os Cnidaria Medusozoa são conhecidos pela sua ampla variabilidade morfológica, a qual é expressa em diferentes níveis, especialmente quando diferentes fases do ciclo de vida são consideradas (pólipo ou medusa). Dificuldades na interpretação dos caracteres morfólogicos gera muitos problemas taxonômicos, já que variações morfológicas intraespecíficas são frequentemente interpretadas de forma errônea como variações interespecíficas, e vice-versa, prejudicando a delimitação das espécies. Este estudo revisa os

padrões de variação morfológica em Medusozoa baseado em resultados publicados e não publicados, para discutir como diferentes interpretações dos níveis de variação podem influenciar a compreensão dos padrões de diversificação biológica no grupo. Além disso, calculamos uma estimativa da diversidade críptica em Hydrozoa, baseada em sequências haplotípicas de COI depositadas no Genbank. Três principais níveis de variação morfológica são considerados: variação microevolutiva (intraespecífica), variação macroevolutiva (interespecífcia) e ausência de variação (espécies crípticas/*sibling species*). É mostrado que as variações morfológicas frequentemente se sobrepõe entre os níveis micro e macroevolutivos, contribuindo para erros nas interpretações dos diferentes níveis de variação. Além disso, mostramos que a maioria da diversidade críptica descrita para Medusozoa é resultado da presença de diferenças morfológicas previamente não consideradas, e ainda existe um grande potencial para a descoberta de linhagens crípticas em Hydrozoa, especialmente se táxons com diferentes ciclos de vida forem considerados. Nós fornecemos importantes evidências de que o número de espécies em Medusozoa não está corretamente estimado, e enfatizamos a necessidade de se examinar diferentes níveis de variação morfológica para a delimitação de espécies, de forma a evitar generalizações e erros de interpretação dos caracteres morfológicos.

Introduction

Following the fundamental work of Darwin (1859), understanding the expression of variation in nature has become essential for the study of evolution, since variation is the basis for evolutionary change. The interpretation of variation, however, has changed in recent years to incorporate phenotypic (developmental) plasticity, in addition to genetic mutations, as an important driver of evolutionary change (West-Eberhard 1989, 2003, 2005; Price et al. 2003; Schlichting 2004; Pigliucci 2007; Pfennig et al. 2010). A major concept is that selection acts on phenotypes and, consequently, phenotypic variation is selectable variation, whether or not it is initially associated with genetic variation (West-Eberhard 1989, 2003, 2005). Phenotypic plasticity can, therefore, contribute to micro- and macroevolutionary processes, emphasizing the importance of the study of variation at different evolutionary levels.

Cnidaria are known for their great morphological variation (e.g. De Weerdt 1981; Silveira and Migotto 1991; Dawson 2005a; Griffith and Newberry 2008; Forsman et al. 2009; Menezes et al. 2013; Ong et al. 2013). Modular growth, characteristic of the polyp stage of many cnidarians, enables a wide variability of colony form through increased regenerative capacity and varying growth rates, branching, number of hydranths, and annulations,

contributing to morphological variation in response to differences in environmental conditions (Hughes 1989; Gili and Hughes 1995; Marfenin 1997). The expression of alternative life cycle stages in Medusozoa (Marques & Collins, 2004) is another important source of variation in this group. At the microevolutionary level, intraspecific variation in life cycles (e.g. Stefani 1959; Bouillon et al. 1991) has been suggested to have a genetic basis, by means of a switch mechanism (West-Eberhard 1986) responsible for the expression of alternative phenotypes in accordance with environmental cues (Boero and Sàra 1987: 137; Boero and Bouillon 1989; Cornelius 1990a; Boero et al. 1997; Bavestrello et al. 2000). At the macroevolutionary level, consecutive suppression and re-expression of the medusa stage during the evolutionary history of the group account for the remarkable diversity of life cycles of Medusozoa (e.g. Holst et al. 2007; Miranda et al. 2010; Straehler-Pohl and Jarms 2011), especially Hydrozoa (Boero and Sàra 1987; Boero et al. 1992; Cornelius 1992; Boero and Bouillon 1993, 1994; Piraino et al. 1996). The origin of a medusa in the lineage leading to the Medusozoa extends the levels of variation in this group, and reiterates the characteristic modular developmental basis of Cnidaria, contributing to its widely known phenotypic plasticity (see West-Eberhard 2003; Table 1).

Difficulties in interpreting the morphological variations of Medusozoa have led to many taxonomic problems and are still a source of disagreement among taxonomists on the importance of morphological characters used to diagnose species (e.g. Boschma 1948; De Weerdt 1984 [Hydrozoa, Milleporidae]; Gershwin 2001; Dawson 2003 [Scyphozoa, *Aurelia*]; Cornelius 1982, 1990b; Cunha et al. 2015 [Hydrozoa, Campanulariidae]; Hirano 1997; Miranda et al. 2009 [Staurozoa, *Haliclystus*]; Miglietta et al. 2009 [Hydrozoa, Hydractiniidae]). These disagreements occur mainly because intraspecific morphological variations are often misinterpreted as interspecific variations or vice-versa, and consequently, the diversity of the group is frequently misestimated. In many taxa that show some degree of developmental plasticity, intraspecific variation of adaptive traits often parallels interspecific variation (e.g. Badyaev and Foresman 2000; West-Eberhard 2003; Gomez-Mestre and Buchholz 2006). For taxonomy, the definition of species' diagnostic characters may be confounded by these factors if different levels of variation are not initially considered. For this reason, the variation of morphological diagnostic characters should be carefully examined, and generalizations should be treated with caution.

Considering the phenotypic plasticity of Medusozoa and its importance in the evolutionary history of the group, this study reassessed patterns of morphological variation

shown by Medusozoa, in order to evaluate how different interpretations of the levels of morphological variation may influence the understanding of the patterns of diversification of the group. We offer an overview of the different levels of morphological variation in Medusozoa based on information from the literature, as well as published and unpublished results (Online Resource 1, Supporting Information) with morphological and genetic data. In addition, we compiled information on COI haplotype sequences deposited in GenBank from several species of Hydrozoa (Online Resource 2, Supporting Information), in order to provide an estimate of their cryptic diversity.

Microevolutionary morphological variation: intraspecific variation

Intraspecific variation is the variation found within a species, including not only variation between conspecific populations, but also individual variation, such as ontogenetic variation and polymorphisms (see Mayr 1973). Intraspecific variation may result from phenotypic plasticity when more than one phenotypic alternative is produced in response to environmental factors (West-Eberhard 1989, 2003). Genotype-specific alternatives are usually referred to polymorphisms (Mayr 1973; West-Eberhard 1989). The term polymorphism, however, has been used in many different contexts, sometimes including aspects of phenotypic plasticity (see Clark 1976 and discussion by West-Eberhard 2003: 378). In the case of marine colonial organisms, polymorphisms are defined as discontinuous variations in the morphology of zooids within a colony (Boardman and Cheetham 1973; Harvell 1994). They are considered an important evolutionary innovation in the Hydrozoa, which evolved independently in multiple lineages within the Hydroidolina (Cartwright and Nawrocki 2010; Maronna et al. 2016).

The extent of functional specialization of polyps varies among species, but generally involves feeding (gastrozooids), reproduction (gonozooids) and defense (dactylozooids) (Millard 1975; Bouillon et al. 2004; Mills et al. 2007). Additional types of polymorphisms are found in particular groups (e.g. Namikawa et al. 1992; Gravier-Bonnet, 2004, 2008), reaching their highest complexity in siphonophores (Pugh 1999; Bouillon et al. 2004; Dunn and Wagner 2006). Being distinctive features, many polymorphisms (and their absence) are important diagnostic characters (e.g. Calder 1988; Boero et al. 1998; Boero et al. 2000; Schuchert 2008 [Hydractiniidae, Milleporidae, Porpitidae, Zancleidae]; Calder 1997; Gravier-Bonnet 2004 [Plumulariidae]). However, their use as diagnostic characters of supraspecific taxa may cause some taxonomic inconsistencies, since their occurrence varies among species (e.g. *Clava* and the Hydractiniidae, Boero et al. 1998; Schuchert 2004).

Another source of morphological variation is related to ontogeny. Many species, especially in the medusa stage, have been described based on ontogenetic differences (Mayer 1910; Bouillon and Boero 2000). This type of variation may be responsible for considerable differences in bell size and shape or in the number of tentacles and statocysts, and for the appearance or disappearance of morphological characters during development (Russell 1953; Zamponi and Girola 1989; Cornelius 1990b; Lindner and Migotto 2002; Widmer 2004). In the polyp stage of colonial species, such as many hydrozoans, ontogenetic variation is responsible for changes in zooid morphology during its development (Boardman and Cheetham 1973). Variations in internode length, number and orientation of branches are typical ontogenetic changes found in polyps within a colony, particularly in species with upright colonies (e.g. Cornelius 1975, 1990b; Kosevich 2006). Developmental changes may continue throughout the colony's life because of its characteristic modular growth, and may affect many levels of colony organization (Hughes 1989, 2005; Marfenin 1997). Differences associated with colony development (growth and senescence), such as hydranth budding and stolonal growth, branching and regression are usually referred to astogeny (Boardman and Cheetham 1973; Hughes 1989), and may be responsible for spatial and temporal changes in colony morphology (Braverman 1974; McFadden et al. 1984; Vogt et al., 2011). Although they appear during the course of development, variations in colony morphology may also result from variations in physiological processes (Dudgeon and Buss 1996; Vogt et al. 2008; Bumann and Buss 2008) and environmental factors (e.g. Vogt et al. 2011; Miglietta and Cunningham 2012).

Intraspecific variation may be triggered by many different factors, especially in different populations. Notably, individual morphological variation may sometimes parallel variations found among populations. For instance, experiments with replicated and transplanted colonies of *Millepora* spp. and *Bougainvillia muscus* (Allman, 1863) have shown that colony growth patterns change from branched forms to more robust, solid forms with variations in water flow (De Weerdt 1981; Griffith and Newberry 2008). Similar morphological variations are found among populations living in habitats with contrasting water-movement conditions (Kaandorp 1999). In addition, in the family Campanulariidae Johnston, 1836, variations in colony size, perisarc thickness, length of the hydrotheca and gonotheca, and number of branches and annulations are found among populations subjected to contrasting water flow, temperature and substrate type (Naumov 1969; Ralph 1956; Cornelius 1975, 1982, 1990b; Linder and Migotto 2002). Many of these variations, however,

may also occur within a single colony (Fig. 1). Although the amplitude of variation of morphological characters at the colony level is not the same as at the population level (Fig. 2), there may be an overlap of morphological variation produced by different levels of intraspecific variation.

Assessing morphological variations between populations may be a difficult task since it involves the presupposition that populations are conspecific, when they might, in fact, represent different species. Although many studies have reported intraspecific morphological variation in the Medusozoa, only a few of them have attempted to assess the identity of the populations studied, using phylogenetic inferences or other methods for detecting reproductive isolation (e.g. Dawson 2005a; Galea and Lèclere 2007). For instance, many studies have reported variations in the symmetry of medusae (Scyphozoa and Hydrozoa, Navas-Pereira 1984; Gershwin 1999; Silva et al. 2003; Nogueira Jr. and Haddad 2006) and stauromedusae (Zagal 2008). Although these variations are known to originate at the clonemate level in *Aurelia* (Gershwin 1999), and may also be a response to variation in physical factors (e.g. temperature and salinity, Zamponi and Genzano 1989), the underlying causes of these variations are still unclear, and they may be different depending on the group and populations studied. As a result, studies of morphological variation may end in questioning the taxonomic affinities of the populations sampled (e.g. Bolton and Graham 2004). Considering the complicated taxonomic history of many groups within the Medusozoa, it is not always easy to delimit conspecific individuals or populations based on morphological characters alone. Individuals from different species may frequently be regarded as conspecific, particularly since intraspecific morphological variation may extend from individuals to populations. Obviously, in order to be confident of the taxonomic level investigated, it is important to know whether one is dealing with intra- or interspecific variations.

Macroevolutionary morphological variation: interspecific variation

Difficulties related to variations in morphological characters have led taxonomists to search for additional characters that could contribute to species delimitation. Characters of the cnidome in many hydroid species (e.g. Östman 1982, 1987; Marques 1995, 1996; Morandini and Marques 2010), as well as ecological and behavioral patterns in medusae (e.g. Dawson and Martin 2001; Dawson 2005b) have contributed to the diagnosis of species in these groups, but the difficulties in assessing and describing these characters have limited their use in species delimitation. Advances in molecular techniques, however, have

introduced many new approaches for studying species relationships, and have improved our understanding of the evolutionary history and diversification of the Medusozoa (Collins et al. 2006; Leclère et al. 2007; Leclère et al. 2009; Cartwright and Nawrocki 2010; Kayal et al. 2013; Cunha et al. 2015).

The inclusion of molecular data in taxonomic studies, especially those with marine taxa, led to the discovery of high genetic diversity in different groups (see Knowlton 1993, 2000), challenging the idea that marine species have wide geographic ranges and high rates of gene flow among distant populations (Palumbi 1992, 1994). In addition, the molecular-based diagnosis of species, introduced under the assumptions of DNA barcoding (e.g. Hebert et al. 2003), has contributed to a rapid assessment and increased knowledge of biodiversity (Hajibabaei et al. 2007; Vernooy et al. 2010), but also generated many criticisms (see Moritz and Cicero 2004; Will and Rubinoff 2004). Species delimitation, however, still remains difficult, and many studies adopt integrative approaches, recognizing species based not only on genetic divergence, but also on additional characters such as morphological, ecological and behavioral, that could contribute evidence for species boundaries (Dayrat 2005; Padial et al. 2010).

The combination of morphological and molecular data for studying species boundaries in the Medusozoa contributed to the re-evaluation of several morphological diagnostic characters, leading to the description of many new species (e.g. Schierwater and Ender 2000; Collins and Daly 2005; Bayha and Dawson 2010; Collins et al. 2011; Cunha et al. 2015) and the revalidation of formerly synonymized species (e.g. Dawson 2003, 2005c; Schuchert 2005; Miglietta et al. 2007, 2009; Fritz et al. 2009; Lindner et al. 2011; Moura et al. 2012). Indeed, reassessment of morphological characters is showing that many 'species' previously considered cosmopolitan are in fact geographically isolated lineages, which often can be delimited morphologically (Dawson 2003; Miglietta et al. 2007; Bentlage et al. 2010). This means that the underestimation of species diversity in the Medusozoa, in most cases, results from misinterpretations of species diagnostic characters, which may explain the historical splitting and lumping of species that have been common in several groups within the Medusozoa. The paucity of morphological characters and poor descriptions in some groups, as well as the wide morphological variation in others, have certainly contributed to these misinterpretations.

Morphological variation can be misleading when there is an overlap between intra- and interspecific variations. Considering the phenotypic plasticity in colony form shown by

species of *Millepora* in different water-movement conditions (e.g. De Weerdt 1981; Kaandorp 1999, see previous section), molecular and morphological data proved that this variation is also interspecific, and resulted in the delimitation of two different lineages based on colony growth form (Meroz-Fine et al. 2003). Moreover, branched and unbranched forms of species of *Aglaophenia*, commonly thought to be a result of phenotypic plasticity (e.g. Andrade and Migotto 1999), were shown to represent different species in the North Atlantic (Thorpe et al. 1992). It is clear from these findings that interspecific variations may easily be misinterpreted as intraspecific variation.

Once again, the family Campanulariidae is a good example of the historical splitting and lumping of species owing to misinterpretations of morphological characters. The validity of the genus *Orthopyxis* L. Agassiz, 1862, for instance, is a frequent source of disagreement among taxonomists, since the perisarc thickness, regarded by some authors as one of the diagnostic characters of the genus (Calder 1991; Cornelius 1995; Bouillon et al. 2004), is also thought to be phenotypically plastic (Millard 1975; Galea et al. 2009). This common belief prevents the use of perisarc thickness as a diagnostic character in *Orthopyxis*, although it may have taxonomic value for delimiting other species of the family (e.g. *Obelia geniculata* (Linnaeus, 1758), Cornelius 1975). Molecular and morphological data clearly show that intraspecific variation in perisarc thickness occurs, but the perisarc also shows interspecific variation, which makes it a reliable character for species delimitation within *Orthopyxis* (Cunha et al. 2015). Additionally, this approach supported the validity of the species *Orthopyxis caliculata* (Hincks, 1853) (Cunha et al. 2015), which was long regarded as a synonym of the widespread species *Orthopyxis integra* (MacGillivray, 1842) (Cornelius 1982, 1995). The evidence that characters previously regarded as intraspecifically variable may be diagnostic of different species in *Orthopyxis*, support the idea that *O. integra* might not have as wide a geographic range as presently thought, and that the morphological variation assumed for this species is overestimated (e.g. shape of the gonotheca; see Cornelius 1995; Cunha et al. 2015).

Macroevolutionary phylogenetic signal with no morphological variation: cryptic species

The existence of species that are morphologically indistinguishable has always intrigued taxonomists. These species were originally termed ‘sibling species’ and defined as “sympatric forms which are morphologically very similar or indistinguishable, but which posses specific biological characteristics and are reproductively isolated” (Mayr 1964: 200). This morphological indistinctness, however, may prove to be a result of previously

overlooked morphological differences (Mayr 1976). This may explain the majority of the cryptic diversity found among the Medusozoa (e.g., *Aurelia*, Dawson and Jacobs 2001; Schroth et al. 2002; Dawson 2003; *Nemertesia*, Moura et al. 2008, 2012; *Acryptolaria*, *Lafoea*, Moura et al. 2011), although in some cases a reassessment of morphological characters has not proved useful for delimiting species (e.g., *Cassiopea*, Holland et al. 2004; *Stylactaria*, Miglietta et al. 2009; *Cryptolaria pectinata*, Moura et al. 2011).

The absence of morphological variation between species is particularly common in marine environments (see Knowlton 1993, 2000), where chemical signaling plays a crucial role in species interactions (see Zimmar and Butman 2000; Hay 2009). Chemical signaling, however, does not necessarily result in correlated morphological differences, contributing to the occurrence of cryptic species (Knowlton 1993; Bickford et al. 2006). Therefore, the apparent similarity of marine cryptic lineages may be a result of symplesiomorphies of morphological characters. This seems rarely to be the case in the Medusozoa, since the majority of cryptic species discovered could be delimited by morphological characters once these were investigated (e.g., *Turritopsis*, Miglietta et al. 2007; *Nemertesia*, Moura et al. 2012, see previous section).

Current estimates of the total global species richness indicate that the Hydrozoa as a group has an increasing rate of species discovery and a high proportion of cryptic species, probably due to the paucity of morphological diagnostic characters (Appeltans et al. 2012). Indeed, our estimate showed that the amount of cryptic diversity within nominal species of Hydrozoa is significant (Fig. 3, Online Resource II). We fitted a Linear Model (LM) including geographical distance and life-cycle strategy as explanatory variables in relation to genetic distance among haplotypes. The LM showed an overall significant fit (R^2_{adj}=27.2%, $p<0.001$, Fig. 3). The positive association between geographical and genetic distances ($F=21.0417$, $p<0.01$) indicated that many species of Hydrozoa may contain cryptic lineages, especially if samples from different geographical locations are considered. Schuchert (2014), for instance, showed that *Plumularia setacea* is a species complex that is mostly composed of geographically circumscribed lineages, and the same is true for other species of hydrozoans (e.g. *Obelia geniculata*, Govindarajan, Halanych & Cunningham, 2005; *Clytia gracilis* and *Obelia dichotoma*, A.F. Cunha pers. obs.).

Additionally, the occurrence of species complexes in the Hydrozoa has frequently been associated with limited dispersal abilities of species that lack a long-lasting pelagic phase (Moura et al. 2011, 2012; Schuchert 2014). Hydrozoans with holopelagic life-cycle

stages were shown to be more widely distributed and have lower species richness than benthic and meroplanktonic species (Gibbons et al. 2009), corroborating the prediction that a relatively short period in the plankton is associated with limited dispersal (Palumbi 1992; Bradbury et al. 2008). Following these predictions, we also found a significant relationship when considering different life-cycle strategies, with meroplanktonic/benthic species showing higher genetic distance between haplotypes than did holoplanktonic species ($F=4.5027$, $p>0.0379$, Fig. 3). This is evidence that benthic/meroplanktonic taxa have potential for the discovery of cryptic lineages even over short geographical distances, probably because of their limited dispersal ability. Studies investigating species boundaries among taxa with different life-cycle strategies are important to corroborate this hypothesis. Nevertheless, our results provide evidence that the number of species in the Hydrozoa, and probably in all the Medusozoa, is underestimated. Increased sampling, integrative approaches and careful investigations of morphological variations will inevitably uncover this hidden species diversity.

Conclusions

It is clear from the above discussion that morphological characters have many different levels of variation, or may not vary at all in some cases (summarized in Fig. 4). When morphological variation is present, it may frequently overlap between micro- and macroevolutionary scales, hampering their use at different hierarchical and inclusive taxonomic levels. The widespread morphological variation of the Medusozoa, as well as the frequent overlapping between intra- and interspecific variation, suggest that phenotypic plasticity may play an important role in the diversification of the group (see West-Eberhard 1989; Pfennig et al. 2010). However, whether alternative phenotypes are, indeed, environmentally induced or are genetically controlled is an important question (see Schwander and Leimar 2011), which needs further investigation. Nevertheless, at the taxonomic level, morphological variation leads to misinterpretations of diagnostic characters and difficulties in species delimitation. Importantly, however, the level of variation and amount of overlap may be different depending on the group studied and its general biology and life history. In order to minimize the possibility of misinterpretations of morphological characters, generalizations should be avoided, and morphological variation should be interpreted within the context of each taxon, taking into account its phylogenetic relationships and evolutionary history.

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Table 1. Phenotypic plasticity in Cnidaria Medusozoa.

Types of variation	Description	Environmental cue	Referências
Life cycle	Intraspecific variation in life cycle (polyp/medusa/resting stages production) in response to environmental factors	Temperature, salinity, light exposure, food availability, unfavorable environmental conditions	e.g., Stefani 1959; Boero and Sarà, 1987; Bouillon et al. 1991; Schierwater and Hadrys 1998; Ma and Purcell 2005; Kawamura and Kubota 2008 [Hydrozoa]; Brewer and Feingold 1991; Purcell 2007; Stampar et al. 2008; Holst and Jarms 2010; Nawroth et al. 2010; Purcell et al. 1999, 2012; Thein et al. 2013 [Scyphozoa]; Courtney and Seymour, 2013 [Cubozoa]; Piraino et al. 2004 [Cnidaria]
Medusa stage			
- Population dynamics	Variations in growth and reproduction of conspecific populations in response to environmental factors	Temperature, food availability	e.g., Lucas 2001 [Scyphozoa]
- Intraspecific morphological variation	Morphological variations among conspecific populations	Predation pressure, hydrodynamics	e.g., Dawson 2005a [Scyphozoa]
Polyp stage			
- Population dynamics	Variations in growth and reproduction of conspecific populations in response to environmental factors	Substrate type	e.g., Hughes 1986; Van Winkle et al. 2000 [Hydrozoa]
- Intraspecific morphological variations	Individual morphological variation and morphological variations among conspecific populations	Hydrodynamics, temperature, food availability, substrate type	e.g., De Weerdt 1981; Andrade and Migotto 1999; Meroz-Fine et al. 2003; Bumman and Buss 2008; Griffith and Newberry 2008 [Hydrozoa]

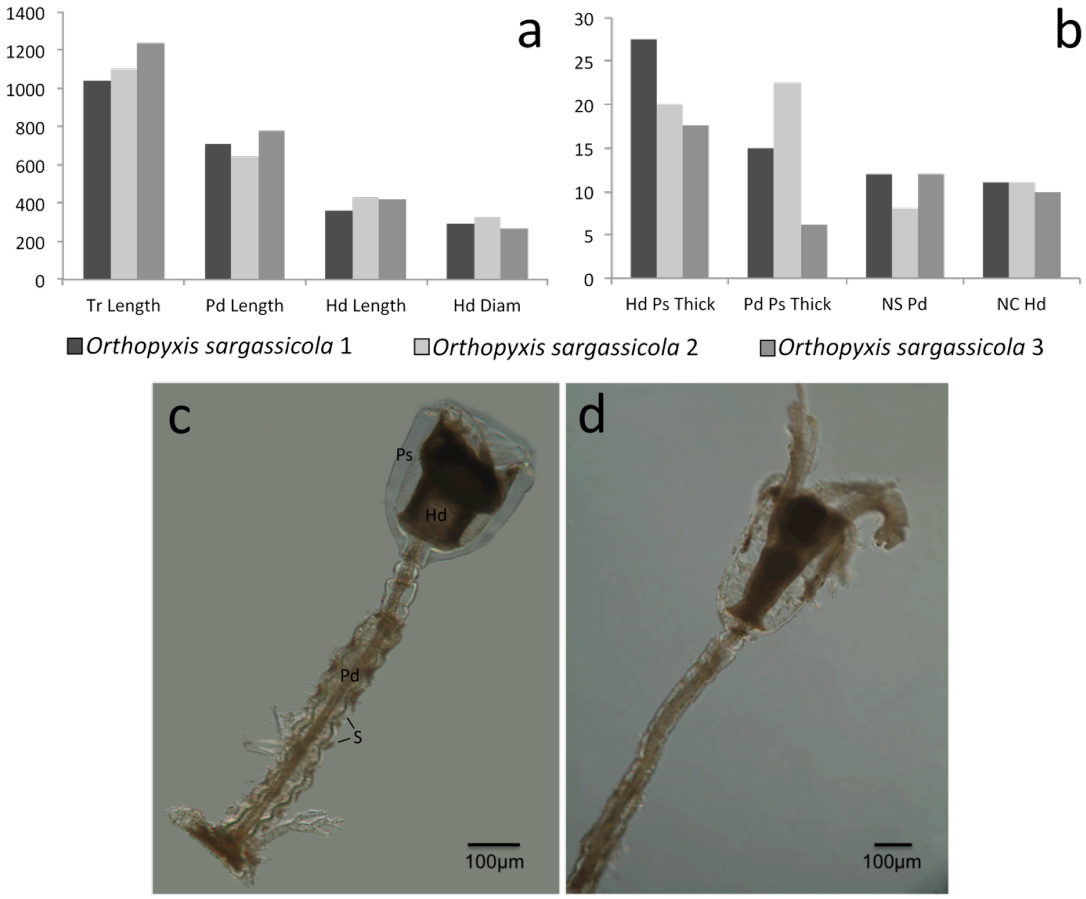

Fig. 1 Intracolony variation in *Orthopyxis sargassicola* (Nutting, 1915), based on three polyps randomly sampled from a single colony (MZUSP4079, see Online Resource I). a. Measurements (in µm) of total length of trophosome (Tr), length of pedicel (Pd), hydrothecal length (Hd) and diameter at margin (Diam); b. Measurements (in µm) of maximum perisarc (Ps) thickness (Thick) of hydrotheca and pedicel at medial portion, as well as maximum number of sinuosities (NS) in pedicel and number of hydrothecal cusps (NC); c, d. Polyps of *O. sargassicola* from a single colony (both polyps are at the same position of maximum perisarc thickness). Note the differences in size and shape of the pedicels (Pd) and hydrotheca (Hd), as well as the perisarc thickness (Ps) and sinuosities of the pedicel (S).

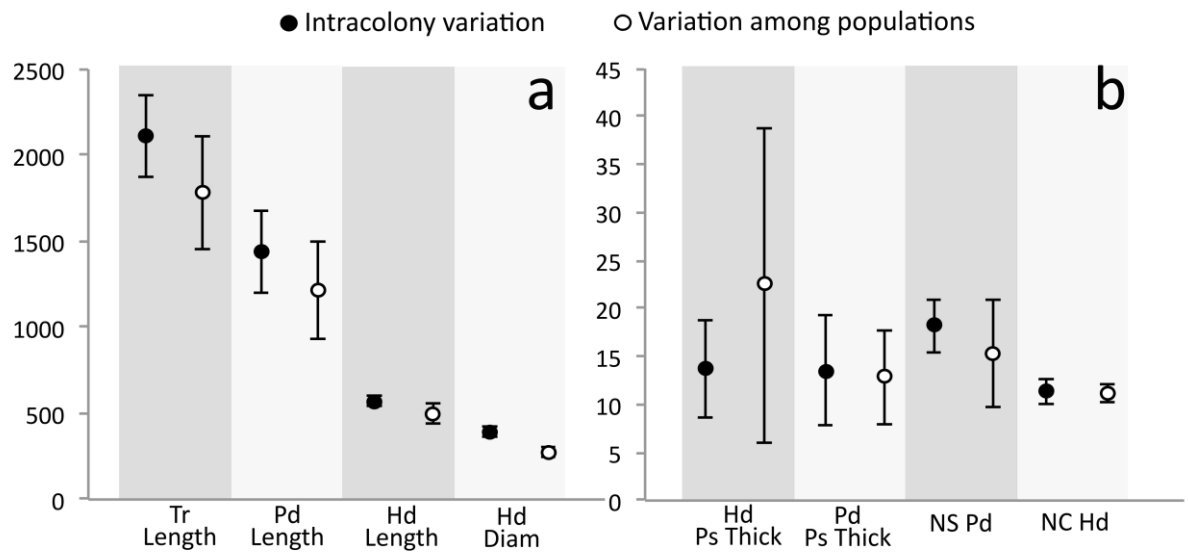

Fig. 2 Mean values (μm) (± standard deviation) of (a) total length of trophosome (Tr), length of pedicel (Pd), hydrothecal length (Hd) and diameter at margin (Diam); and (b) maximum perisarc (Ps) thickness (Thick) of hydrotheca and pedicel at medial portion, maximum number of sinuosities in pedicel (NS) and number of hydrothecal cusps (NC). Measurements were taken from polyps of the same colony of *Orthopyxis sargassicola* (intracolony variation, MZUSP4161, see Online Resource I) and from polyps of different populations of the same species from the states of São Paulo, Espírito Santo, Rio de Janeiro and Santa Catarina, Brazil (variation among populations, Online Resource I).

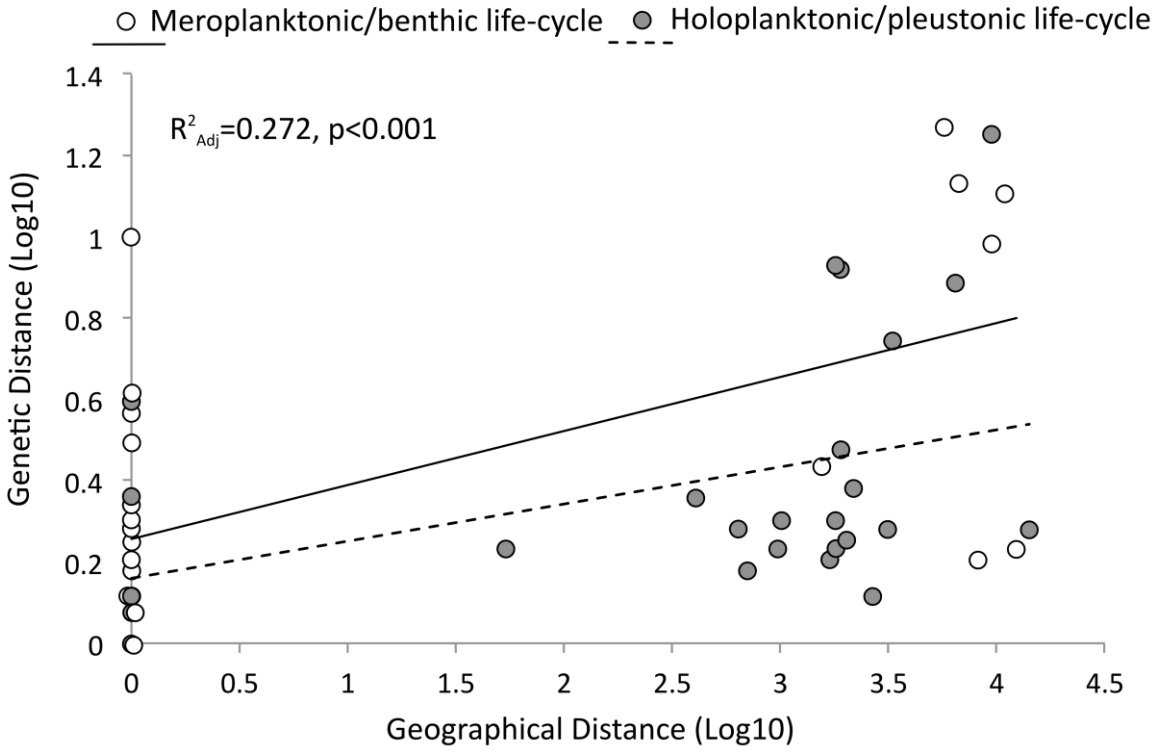

Fig. 3 A Linear Model (LM) showing the relationship between haplotype genetic distance (n≥2) and geographical distance (Km) of specimens with COI sequences deposited in GenBank, in accordance with their life-cycle strategy (holoplanktonic/pleustonic or benthic/meroplanktonic). Both geographical distance and life-cycle strategy were included as explanatory variables. Continuous variables were log10(x+1) transformed to meet the assumptions of the LM. Similarity was calculated using BLAST (Altschul et al. 1990) by pairwise comparisons of all haplotypes of a single species, and the inverse of the minimum value of similarity recorded was used as its genetic distance. The geographical distance was calculated based on geographical information provided with the metadata for the sequences. When geographical coordinates were not provided, they were estimated based on the name of the sampling location.

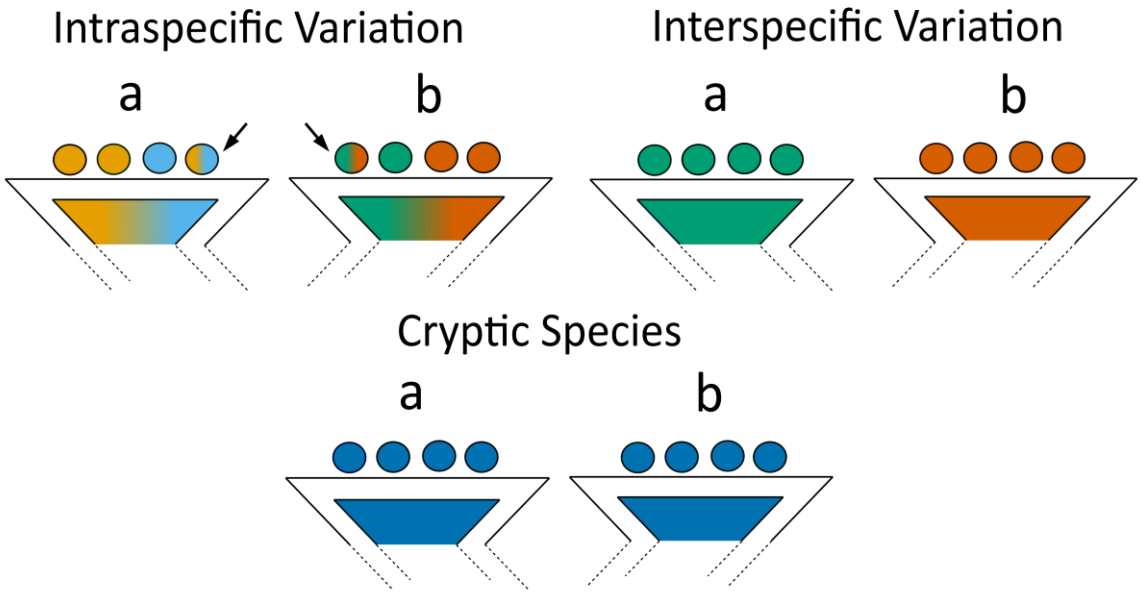

Fig. 4 Schematic summary of levels of morphological variation found in medusozoans, including the absence of variation (cryptic species). Clades indicate different lineages, colors and shades represent the phenotype in current time, and the circles represent individuals. Note that there is individual variation (arrows) and it can parallel intraspecific variation. The same occurs with interspecific variation, which can parallel intraspecific variation in b.

Supporting Information

Online Resource 1. Specimens of *Orthopyxis sargassicola* (Nutting, 1905) measured in this study, and their respective morphometric data. Intracolony variation was evaluated for these (*) specimens. MZUSP=Museu de Zoologia da Universidade de São Paulo.

Specimen	Locality	Geographical Coordinates	Total Length of the Trophoso-me	Length of Pedicel	Length of Hydro-teca	Diameter of Hydrothe-ca at Margin	Maximum Perisarc Thickness of the Hydrotheca at Medial Portion	Maximum Perisarc Thickness of the Pedicel at Medial Portion	Maximum Number of Sinuosities in the Pedicel	Number of Hydro-thecal Cusps
MZUSP4079*	Bombas, Bombinhas, Santa Catarina (SC), Brazil	27°07.874'S 48°30.817'W	1040	710	365	290	27.5	15	12	11
			1100	640	430	330	20	22.5	8	11
			1240	780	420	265	17.5	6.25	12	10
			1120	800	340	285	22.5	27.5	9	11
			1120	680	325	265	22.5	17.5	8	11
MZUSP4161*	Trapiche, Bombinhas, SC, Brazil	27°08.795'S 48°28.871'W	2060	1500	560	385	17.5	22.5	17	12
			2380	1610	620	420	13.75	12.5	17	13
			2240	1680	540	425	20	15	18	10
			1740	1090	560	375	10	7.5	16	10
			2160	1320	590	355	7.5	10	23	12
MZUSP4597	Campeche Island, Florianópolis, SC, Brazil	27°41'27"S 48°27'51"W	1460	900	540	320	7.5	12.5	14	11
MZUSP4665			2180	1600	550	290	5	7.5	3	11
MZUSP2605	Paraty, Rio de Janeiro (RJ), Brazil	Specific coordinates unknown	2220	1400	530	275	3.75	7.5	20	11
MZUSP2606			1620	1120	470	235	2.5	6.25	4	10
MZUSP2607			1640	1110	500	260	2.5	7.5	5	11
MZUSP2608			2360	1690	575	260	2.5	5	3	11
MZUSP2609			2080	1520	520	315	37.5	8.75	10	14
MZUSP2610	Ratos Island, Paraty, RJ, Brazil	23°11.640'S 44°36.408'W	1860	1050	570	270	7.5	12.5	11	11

Specimen	Locality	Geographical Coordinates	Total Length of the Trophoso-me	Length of Pedicel	Length of Hydro-teca	Diameter of Hydrothe-ca at Margin	Maximum Perisarc Thickness of the Hydrotheca at Medial Portion	Maximum Perisarc Thickness of the Pedicel at Medial Portion	Maximum Number of Sinuosities in the Pedicel	Number of Hydro-thecal Cusps
MZUSP2611	Meros Island, Paraty, RJ, Brazil	23°11.264'S 44°34.635'W	1720	1200	480	270	3.75	7.5	13	11
MZUSP2617	Padres, Aracruz, Espírito Santo (ES), Brazil	19º55.941'S 40º07.327'W	2280	1300	590	300	11.25	12.5	19	10
MZUSP2618			2100	1560	530	325	2.5	10	21	10
MZUSP2619			1720	1250	440	375	50	22.5	14	11
MZUSP2620			1680	1110	480	355	70	17.5	17	10
MZUSP2624			2640	2110	550	375	32.5	15	21	12
MZUSP2627			1780	1300	430	300	30	10	20	11
MZUSP2628			1720	1260	460	315	27.5	22.5	19	11
MZUSP2632			1980	1390	540	345	37.5	15	22	11
MZUSP2629	Formosa, Aracruz, ES, Brazil	Specific coordinates unknown	1640	1180	430	290	22.5	12.5	19	10
MZUSP2630			1580	1180	450	315	27.5	12.5	17	12
MZUSP2594	Lázaro, Ubatuba, São Paulo (SP), Brazil	23°30'32.64"S 45°08'18.52"W	1160	630	460	310	32.5	5	9	11
MZUSP2595			1760	1300	450	340	10	12.5	17	11
MZUSP2596			1680	1220	470	315	42.5	15	17	10
MZUSP2597			1200	810	380	255	40	15	16	10
MZUSP2599			1520	1040	500	335	15	12.5	17	13
MZUSP2600			1500	1060	640	285	27.5	12.5	16	10
MZUSP2602			1460	950	460	350	32.5	20	13	12
MZUSP2603			1840	1340	460	320	15	15	18	12
MZUSP2593	Preta, São Sebastião, São Paulo, Brazil	Specific coordinates unknown	1600	1090	450	305	27.5	10	18	12

Specimen	Locality	Geographical Coordinates	Total Length of the Trophoso-me	Length of Pedicel	Length of Hydro-teca	Diameter of Hydrothe-ca at Margin	Maximum Perisarc Thickness of the Hydrotheca at Medial Portion	Maximum Perisarc Thickness of the Pedicel at Medial Portion	Maximum Number of Sinuosities in the Pedicel	Number of Hydro-thecal Cusps
MZUSP1319	Massaguaçu Island, Caraguatatuba, SP, Brazil	23°25'S 44°49'W	1660	1150	490	350	25	22.5	13	12
MZUSP0256	Sino, Ilhabela, SP, Brazil	23°44.800'S 45°20.948'W	1560	1000	560	285	2.5	10	16	11
MZUSP0183	Barequeçaba, São Sebastião, SP, Brazil	23°49.888'S 45°25.915'W	2260	840	450	345	32.5	17.5	25	13

Online Resource 2. GenBank data (up to January 2013) and additional information for estimation of Hydrozoa cryptic diversity (Fig. 3). Names of species are in accordance with the original information provided for the sequences in GenBank, although we are aware that misidentifications may occur. When no geographical coordinate was provided with the species record, the coordinates were estimated based on the name of the sampling location, in order to calculate the geographical distance. Species were classified in accordance with their life-cycle strategy (holoplanktonic/pleustonic or benthic/meroplanktonic) based on information from the literature.

COI Sequences (Genbank accession numbers)	**Species**	**Number of Haplotypes**	**Number of geographical locations**	**Similarity (%)**	**Distance (Km)**	**Life Cycle**
GQ119939, HM053518	*Abylopsis tetragona*	2	2	99.1	14115	Holoplanktonic
JQ716190-JQ716193, JQ716195-JQ716197	*Aequorea australis*	7	1	97.3	0	Meroplanktonic
JQ716175-JQ716177	*Aequorea conica*	3	1	98.8	0	Meroplanktonic
JQ716182, JQ716184, JQ716181, JQ716056, JQ716185	*Aequorea papillata*	5	1	99.0	0	Meroplanktonic
GQ119940-GQ119943, AY937363	*Agalma elegans*	5[a]	2	98.0	1900	Holoplanktonic
GQ119944, GQ119945, GQ119947, GQ119948	*Agalma okeni*	4[a]	1	99.1	637	Holoplanktonic
FJ602534, FJ602535, GQ120073	*Aglantha digitale*	3	3	95.5	3257	Holoplanktonic
JQ716085, JQ716057	*Amphinema dinema*	2[a]	1	99.8	0	Meroplanktonic
GQ119956, AY937364	*Athorybia rosacea*	2	1	99.3	1796	Holoplanktonic
GQ119959, GQ119960	*Bassia bassensis*	2	1	99.5	706	Holoplanktonic
JQ716118, JQ716117, JQ716119	*Blackfordia polytentaculata*	3[a]	1	99.4	0	Meroplanktonic

COI Sequences (Genbank accession numbers)	Species	Number of Haplotypes	Number of geographical locations	Similarity (%)	Distance (Km)	Life Cycle
JQ716112, JQ716113, JQ716115, JQ716116	*Blackfordia virginica*	4[a]	1	99.4	0	Meroplanktonic
JX121578, GU812438	*Candelabrum cocksii*	2[b]	1	100.0	0	Benthic
AY789898, AY789899, AY789901, DQ068054-DQ068056	*Clytia gracilis*	6	4	82.4	5697	Meroplanktonic
AY789902, HM053515, HM053517	*Clytia hemisphaerica*	3	1	91.4	9391	Meroplanktonic
AY789894, AY789895	*Clytia hummelincki*	2	2	99.4	8248	Meroplanktonic
JQ716206-JQ716209	*Clytia* sp. KC JRH-2012	4[b]	1	100.0	0	Meroplanktonic
JQ716198-JQ716205	*Clytia* sp. n. JRH-2012	7[b]	1	99.0	0	Meroplanktonic
GQ120076, GQ120077	*Colobonema sericeum*	2	1	99.2	2038	Holoplanktonic
JQ716061-JQ716064	*Corymorpha verrucosa*	4[b]	1	99.5	0	Meroplanktonic
GQ119967-GQ119970	*Diphyes bojani*	4	1	98.7	0	Holoplanktonic
GQ119971, GQ119973, AY937367	*Diphyes dispar*	3	2	99.1	3165	Holoplanktonic
JQ716152-JQ716155	*Eirene brevistylus*	4[b]	1	99.2	0	Meroplanktonic
FJ418658, HM053525-HM053527, JQ716138, JQ716140-JQ716142	*Eirene ceylonensis*	8[b]	1	91.1	0	Meroplanktonic
JQ716148-JQ716151, FJ418659	*Eirene hexanemalis*	5[b]	1	97.9	0	Meroplanktonic
JQ716129, JQ716128, JQ716131, FJ418660	*Eirene kambara*	4[b]	1	99.2	0	Meroplanktonic
JQ716136, JQ716134, JQ716132, JQ716135, FJ418662	*Eirene menoni*	5[b]	1	99.7	0	Meroplanktonic

COI Sequences (Genbank accession numbers)	Species	Number of Haplotypes	Number of geographical locations	Similarity (%)	Distance (Km)	Life Cycle
FJ418656, JQ716144-JQ716146	*Eirene pyramidalis*	4[b]	1	99.8	0	Meroplanktonic
GQ119977-GQ119980	*Eudoxoides mitra*	4	1	98.7	405	Holoplanktonic
GQ119981-GQ119983	*Eudoxoides spiralis*	3	1	99.3	962	Holoplanktonic
JQ716171, JQ716172, JQ716174, FJ418666	*Eugymnanthea japonica*	4[b]	1	99.5	0	Meroplanktonic
FJ602537-FJ602539	*Euphysa flammea*	3	1	99.0	1011	Holoplanktonic
AB458447-AB458461, AB458463-AB458468, AB458470, AB458473-AB458492, AB458494-AB458502	*Eutima japonica*	49	1	96.9	0	Meroplanktonic
JQ716158-JQ716160, FJ418664	*Eutima krampi*	4[b]	1	99.0	0	Meroplanktonic
FJ418663, JQ716156, JQ716157	*Eutima levuka*	3[b]	1	99.4	0	Meroplanktonic
GQ119990, GQ119992, GQ120047	*Halistemma amphytridis*	3[a]	1	99.7	2649	Holoplanktonic
JQ716163-JQ716165, FJ418667	*Helgicirrha brevistyla*	4[b]	1	99.0	0	Meroplanktonic
JQ716161, JQ716162, FJ418665	*Helgicirrha malayensis*	3[b]	1	99.5	0	Meroplanktonic
GQ119993-GQ119998, HM053521	*Hippopodius hippopus*	7	2	93.3	6405	Holoplanktonic
JQ716120, JQ716121	*Laodicea undulata*	2[b]	1	99.4	0	Meroplanktonic

COI Sequences (Genbank accession numbers)	Species	Number of Haplotypes	Number of geographical locations	Similarity (%)	Distance (Km)	Life Cycle
AY789910-AY789912	*Laomedea flexuosa*	3	1	98.3	1545	Benthic
GQ120001, GQ120066	*Lensia campanella*	2	1	97.1	0	Holoplanktonic
GQ120004-GQ120007	*Lensia fowleri*	4	1	99.3	52	Holoplanktonic
GQ120017, GQ120016	*Lilyopsis rosea*	2	1	99.8	0	Holoplanktonic
JQ716067, JQ716065	*Liriope tetraphylla*	2[b]	1	100.0	0	Holoplanktonic
JQ716109-JQ716111	*Malagazzia carolinae*	3[b]	1	99.7	0	Meroplanktonic
AY937373, GQ120022, JQ716068-JQ716071	*Nanomia bijuga*	6	3	83.1	9431	Holoplanktonic
AY789904, AY789905	*Obelia bidentata*	2	1	87.5	6691	Meroplanktonic
AY789906-AY789909	*Obelia longissima*	4	3	99.3	12325	Meroplanktonic
AY789884-AY789887	*Orthopyxis integra*	4	2	88.3	11000	Meroplanktonic
FJ602531-FJ602533	*Paragotoea bathybia*	3	1	99.7	0	Holoplanktonic
GQ120033, GQ120032, AY937374	*Physalia physalis*	3	1	99.0	1795	Pleustonic
JQ716077-JQ716081	*Proboscidactyla ornata*	5[b]	1	99.4	0	Meroplanktonic
FJ602541, FJ602540	*Rathkea octopunctata*	2	1	100.0	0	Meroplanktonic
GQ120038-GQ120040	*Rhizophysa eysenhardti*	3	1	99.4	1675	Holoplanktonic
AY937377, GQ120041	*Rhizophysa filiformis*	2	2	92.7	1875	Holoplanktonic
GQ120043-GQ120045	*Rosacea* sp. 1 BO-2009	3	1	92.5	1804	Holoplanktonic
HQ603190-HQ603197	*Stylaster californicus*	8	1	99.1	0	Benthic
HQ718594, HQ718600, JQ716090-JQ716108	*Sugiura chengshanense*	21[b]	2	99.0	0	Meroplanktonic
GQ120048-GQ120050, AY937378	*Sulculeolaria quadrivalvis*	4	1	98.6	2169	Holoplanktonic
JQ716123-JQ716127	*Tiaricodon coeruleus*	5[b]	1	99.0	0	Meroplanktonic
JQ716166-JQ716170	*Tima formosa*	5[b]	1	99.0	0	Meroplanktonic

COI Sequences (Genbank accession numbers)	Species	Number of Haplotypes	Number of geographical locations	Similarity (%)	Distance (Km)	Life Cycle
JQ716082-JQ716083	*Turritopsis nutricula*	3[b]	1	99.7	0	Meroplanktonic
HM053528-HM053531, HM053543	*Varitentacula yantaiensis*	5[b]	1	99.8	0	Holoplanktonic

[a] At least one haplotype without specific location.

[b] Haplotypes were assumed to belong to the same location, based on geographical information provided with the metadata for the sequences ("lat_lon")

Capítulo 2

Phylogenetic relationships of Proboscoida (Cnidaria, Hydrozoa): are the traditional morphological diagnostic characters relevant for the delimitation of lineages at the species, genus, and family levels?

A.F. Cunha, A.G. Collins, A.C. Marques

Abstract

The overlapping of morphological characters variation leads to their misinterpretation as relevant diagnostic characters for the delimitation of different lineages. This is the case of Campanulariidae, a family known for its wide morphological variation and complicated taxonomic history. We inferred the phylogenetic relationships of Campanulariidae based on molecular data, testing the relevance of the diagnostic characters associated to the traditional classification of the family, as well as the recently proposed phylogenetic classification of the suborder Proboscoida. Campanulariidae is monophyletic only if including *Bonneviella* and excluding *Billardia* from its scope. Most of its subfamilies and genera also needs a revision of their scope, since the traditional classification is not consistent with most of our results. On the other hand, the phylogenetic classification of Proboscoida is congruent with our findings, but some variations occur. *Campanularia* and *Clytia* are not monophyletic, and species with *Obelia*-like medusae do not form a monophyletic group, as well as species with fixed gonophores, indicating these characters are not relevant for the diagnosis of different genera. Finally, *Orthopyxis integra*, *Clytia gracilis*, and *Obelia dichotoma* are not monophyletic, suggesting that most of their current diagnostic characters are not informative for their delimitation. Several diagnostic characters in this group need to be reassessed, as well as their patterns of morphological variation, in order to have a consistent taxonomic and phylogenetic framework for the classification of Campanulariidae.

Resumo

A variação de caracteres morfológicos leva à sua sobreposição e consequentemente, a erros de interpretação sobre a sua relevância como caracteres diagnósticos de diferentes linhagens. Esse é o caso da família Campanulariidae, a qual é conhecida pela sua ampla variação morfológica e complexa história taxonômica. Neste estudo, nós avaliamos as relações filogenéticas dessa família com base em dados moleculares, testando a relevância

dos caracteres diagnósticos tradicionalmente usados na sua classificação taxonômica, assim como a classificação filogenética da subordem Proboscoida recentemente proposta. A família Campanulariidae não é monofilética pela inclusão de *Bonneviella* e exclusão de *Billardia*. Da mesma forma, uma revisão do escopo da maioria das subfamílias e gêneros é necessária, já que a classificação atual não está de acordo com a maioria das filogenias apresentadas neste estudo. Por outro lado, a classificação filogenética de Proboscoida é congruente com nossos resultados, embora com algumas variações. *Campanularia* e *Clytia* não são monofiléticos, e as espécies com medusa do tipo "*Obelia*" não formam um grupo monofilético, assim como as espécies com gonóforos fixos, indicando que esses caracteres não são relevantes para a diagnose dos diferentes gêneros. Finalmente, as espécies *Orthopyxis integra*, *Clytia gracilis* e *Obelia dichotoma* não são monofiléticas, sugerindo que a maioria dos seus caracteres diagnósticos não são informativos para sua delimitação. Vários caracteres diagnósticos desse grupo precisam ser revisados, assim como seus padrões de variação morfológica, de forma a constituir uma sólida estrutura filogenética e taxonômica para a classificação da família Campanulariidae.

Introduction

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

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

The family Campanulariidae Johnston, 1836 (Cnidaria, Hydrozoa) is known for the wide morphological variability of its species, which, in addition, have simple and similar morphological diagnostic characters (cf. Cornelius 1982). Both conditions resulted in a complicated taxonomic history, with recurrent disagreements among taxonomists on the relevance of the morphological characters used to diagnose and delimit genera and species (Nutting 1915; Millard 1975; Cornelius 1982, 1995b; Calder 1991). At the species level, the validity and scope of some taxa are frequently questioned (e.g., *Obelia longissima*, Cornelius 1975, 1990; *O. dichotoma*, Calder 2013; Calder et al. 2014; *Orthopyxis integra*, Cunha et al. 2015), while others were described as potentially cryptic (e.g., *O. integra*, *Obelia geniculata*, *Clytia gracilis*, Govindarajan et al. 2005, 2006; Lindner et al. 2011). At the genus level, several generic divisions were considered doubtful (e.g., *Orthopyxis* and *Campanularia*, Millard 1975, Schuchert 2001; *Laomedea*, *Hartlaubella*, *Gastroblasta*, *Tulpa*, *Rhizocaulus*, Boero et al. 1996), as well as some nominal genera (*Orthonia*, *Eucalix*, Cornelius 1982, Calder 1991). Finally, at the suprageneric level, molecular studies with representatives of Campanulariidae have shown a disputable monophyly of the family (Govindarajan et al. 2006; Peña Cantero et al. 2010), and even its phylogenetic placement among Leptothecata was questioned (Collins 2000; Leclère et al. 2009). This scenario has posed the question of whether the classification of Campanulariidae is based on relevant diagnostic characters that reliably reflect its evolutionary patterns.

Campanulariid hydroids are traditionally known for their stolonal or upright colonies, campanulate hydrothecae and trumpet-shaped hypostomes (Millard 1975; Cornelius 1982; Bouillon 1985; Calder 1991). The family conventionally comprises 11 genera (up to 13 if different taxonomic proposals are considered, cf. Cornelius 1982), divided into three subfamilies, Campanulariinae Johnston, 1836, Clytiinae Cockerell, 1911, and Obeliinae Haeckel, 1879 (cf. Cornelius 1982, 1995). A recent and comprehensive phylogenetic inference of Lepthothecata, however proposed a new classification for the order Proboscoida Broch, 1910, dividing campanulariids into two infraorders, viz., Campanulariida Buillon, 1984 and Obeliida Maronna et al. 2016, and three families, viz., Campanulariidae, Clytiidae and Obeliidae (Maronna et al. 2016), with similar scope to the former subfamilies division. Although originally included in Proboscoida (Bouillon 1985), Phialuciidae was not covered by their analysis, and its inclusion in this order still needs confirmation (Maronna et al. 2016).

The muddled taxonomical history of Campanulariidae prevents an indisputable estimation of the number of valid species, although WoRMS (World Register of Marine

Species) accounts approximately 150 species (Schuchert 2015). Despite the lack of precision, campanulariids are frequently among the richest and dominant groups in marine epibenthic communities (e.g., Llobet et al. 1991; Watson 1992; Calder 1995; Gravier-Bonnet 1999; Migotto et al. 2001; Cunha and Jacobucci 2010; Fernandez et al. 2014, 2015), and their medusae are commonly reported in the plankton (e.g., Segura-Puertas and Damas-Romero 1997; Palma et al. 2014; Laakmann and Holst 2014; Nagata et al. 2014), occasionally in large populations (Genzano et al. 2008). Despite of the richness, abundance, and ubiquituousness of the campanulariids, the basic knowledge on its phylogenetic relationships and taxonomy is still highly deficient.

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

Material and methods

Taxonomic sampling

Sequence data of the family Campanulariidae were obtained during this study and from published works, comprising several localities (Tables 1-2). Most of the sequences assigned to Campanulariidae and available in Genbank were considered in the analysis, including those from Collins (2002), Collins et al. (2004, 2006), Govindarajan et al. (2006), Evans et al. (2008), Leclére et al. (2009), Ortman et al. (2010), Peña Cantero et al. (2010), Lindner et al. (2011), Zhou et al. (2013), Laakmann and Holst (2014) and He et al. (2015). GenBank sequences from closely related taxa, such as species of Campanulinidae, Eirenidae, Mitrocomidae, Lovenellidae and Phialellidae, were included as outgroups. Sequences obtained during this study are deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank/).

Specimens used in this study were primarily identified based on traditional morphological diagnostic characters, in accordance with previous studies (Vervoort 1972; Millard 1971, 1975; Cornelius 1975, 1990, 1982, 1995b; Calder 1991; Schuchert 2001; Vervoort and Watson 2003; Bouillon et al. 2004). Voucher specimens were deposited in the

Museu de Zoologia da Universidade de São Paulo, Brazil (MZUSP) and in the National Museum of Natural History, Smithsonian Institution, United States of America (USNM). Vouchers from previously published sequences were studied whenever possible. This study comprises representatives of the three traditionally recognized subfamilies (cf. Cornelius 1982; Calder 1991), as well as all accepted genera, with the exception of *Gastroblasta* Keller 1883. *Orthonia* Stechow, 1923 and *Eucalix* Stechow, 1921 have a doubtful taxonomic status, and are not considered in this analysis. In addition, *Billardia* Totton, 1930 is assigned to Campanulariidae by many authors (Ralph 1957; Bouillon 1985; Vervoort and Watson 2003), but previous phylogenetic analysis have consistently placed it outside Campanulariidae (Govindarajan et al. 2006; Leclére et al. 2009; Peña Cantero et al. 2010), and, therefore, its was not included in this analysis. In comparison to a recent phylogenetic study (Maronna et al. 2016), we have included data comprising the three families proposed (Campanulariidae, Clytiidae, and Obelliidae), with a substantial increase in the number of sampled taxa within each group.

Molecular data

Samples were processed in the Laboratory of Molecular Evolution (University of São Paulo) and in the Laboratories of Analytical Biology (National Museum of Natural History). DNA was extracted either with Agencourt DNAdvance (Beckman Coulter, Beverly, MA, USA) or DNeasy (QUIAGEN, Valencia, CA, USA) extraction kits following the manufacturer's protocol. Mitochondrial genes 16S and COI and nearly complete sequences of nuclear 18S and 28S genes were obtained using standard PCR and sequencing primers (Table S1).

PCRs were performed either in a total volume of 25µl (with 75mM Tris-HCL (pH 8.8 at 25^{o}C), 20mM $(NH_4)SO_2$, 2.5mM $MgCl_2$, 0.26U/µl Taq polymerase (Thermo Fisher Scientific, Waltham, MA, USA), 0.2mM dNTP and 0.4µM primers), 20µl (with 1x Phusion Buffer, 0.02U/µl Taq polymerase Phusion (FinnZymes, Thermo Fisher Scientific, Waltham, MA, USA), 1.1mM $MgCl_2$, 0.2mM dNTP, 0.4µM primers) or 10µl (with 10x NH_4 Buffer, 3mM $MgCl_2$, 0.05U/µl Biolase Taq polymerase (Bioline, London, UK), 0.1mM dNTP, 0.5mM bovine serum albumin (BSA), 0.3µM primers). Dimethyl sulfoxide (DMSO) was included in some PCRs for amplification of nuclear genes (1.25µl for 25µl reactions, 0.5µl for 10µl reactions). Subsequent steps were either conducted as described in Cunha et al. (2015), or by the following procedure: PCR products were purified with ExoSapIT (Affymetrix, Santa Clara, CA, USA), and used in cycle sequencing reactions with Big Dye

Terminator v3.1 kit (Applied Biosystems, Foster City, CA, USA) and diluted primers from PCR (0.03μM). Cycle sequencing products were purified with Sephadex G-50 (Sigma-Aldrich, Buchs, Switzerland) and sequenced on an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Both strands were sequenced for all samples.

Sequences were assembled and edited using Geneious v. 7.1 (Biomatters, Auckland, New Zealand), and compared with those deposited in GenBank using the Basic Local Alignment Search Tool (BLAST, Altschul et al. 1990) to confirm genes and species of interest. Sequences were aligned using MAFFT (Katoh et al. 2002), implemented in Geneious R7, and missing ends were removed from the alignments using GBlocks (Castresana 2000), implemented in SeaView (Gouy et al. 2010), with default settings for a less stringent selection.

Phylogenetic analysis

Phylogenetic analyses were performed on (a) individual markers and (b) the entire combined dataset (16S+COI+18S+28S). This last dataset was analyzed based on (b1) taxa with sequences available for at least 3 markers, and (b2) taxa with sequences available for all 4 markers. In the first case (b1), absent fragments in the alignment were coded as missing data. The datasets were analyzed based on parsimony (P) and maximum likelihood (ML) criteria. Parsimony analyses were performed in PAUP* v4b10 (Swofford 2002), with heuristic searches with 1,000 replicates of random-addition-sequence, saving up to 100 trees per replicate, and branch-swapping by TBR (Tree Bisection-Reconnection). Gaps were treated as fifth state. For 16S and COI alignments, 10,000 replicates of random-addition-sequence were performed to improve search. Branch support was estimated with bootstrap based on 1,000 replicates (with 10 replicates of random-addition-sequence). Bremer support was calculated using the script available in TNT (through constrained searches for each of the groups, Goloboff et al. 2008). ML analyses were performed in GARLI v2.01 (Zwickl 2006) and consisted of 100 replicates searches with taxa randomly added to the starting tree. Additionally, concatenated datasets were analyzed as different partitions, corresponding to each gene. Models of molecular evolution for each dataset were chosen using jModeltest v2.1.7 (Guindon and Gascuel 2003; Darriba et al. 2012), with the Akaike Informarion Criterion (AIC, Table 3). Uncorrected p-distances for COI and 16S were calculated using PAUP* v4b10.

Results

All single gene phylogenies agree in the lineages identified on less inclusive levels, although there is lack of resolution and/or support for relationships among higher lineages, specially concerning genus and subfamily or family levels. Resolution and support enhances with the concatenation of all genes. The combined dataset including taxa with sequences for at least three genes (hereafter Dataset 1) comprises 181 taxa (Figures 1-2, Table 3), while the combined dataset including taxa with sequences for all four genes (hereafter Dataset 2) has only 80 taxa (Figures S1-S2, Table 3). The topologies generated from both datasets are highly congruent, and minor contradictions usually occur among weakly supported groups. Therefore, we base our conclusions largely on the results from Dataset 1, because its inclusiveness allows a broader discussion. 16S phylogenies are presented for further discussions on specific lineages (Figures 3-4), and the remaining single gene phylogenies are remarked when necessary (Figures S3-8).

Family and subfamily levels

Topologies derived from the combined dataset show three main well supported groups, which nearly agree with the three traditionally recognized subfamilies, as well as the change of their status to family level (Figures 1-2). However, some variations occur. The P topology derived from Dataset 1 have the species *Clytia hummelincki*, *C. paulensis* and *C. stolonifera* ambiguously placed at the base of the Clytiidae+Obeliidae clade (Infraorder Obeliida Maronna et al. 2016) (Figure 1), while the ML topology shows *C. hummelincki* placed outside Clytiidae+Obeliidae, with high support (Boostrap=100; Figure 2). Both topologies derived from Dataset 2 (Figures S1-S2) are congruent with the last scenario. Additionally, sequences of *Bonneviella* (Bonneviellidae) are placed within Campanulariidae, and are closely related to *Campanularia volubilis* and *Rhizocaulus verticilatus* (Figures 1-2; cf. original publication by Govindarajan et al. 2006). Three main clades are also recovered in single gene phylogenies, with the exception of COI (P topology) and 18S, in which some of the recently proposed families and traditional subfamilies are not monophyletic (Figures S3-6). This scenario, however, has low support. Also, most of the single gene phylogenies have outgroup representatives placed within the ingroup, but this is never well supported (Figures 3-4, S3-S8).

Genus level

Six out of 10 genera included in the analysis were recovered as monophyletic in the concatenated phylogenies (Figures 1-2, S1-S2). *Tulpa* and *Rhizocaulus*, however, are only

represented by one species, and their monophyly still need further confirmation. Although *Orthopyxis* was not recovered monophyletic in the P topology derived from Dataset 2 (Figure S1), it is monophyletic in accordance with topologies derived from Dataset 1 (Figures 1-2), which are more informative at the genus level. Similarly, *Silicularia* was recovered as monophyletic in the concatenated phylogenies, while *Campanularia* is clearly not monophyletic (Figures 1-2). *Bonneviella* was not recovered as monophyletic in the P topology of Dataset 1, but this scenario is weakly supported (Figure 1).

Clytia is not monophyletic because three of its species are ambiguously placed: *C. hummelincki* (always placed outside *Clytia* in concatenated phylogenies; Figures 1-2, S1-2), *C. stolonifera* (placed at the base of Obeliidae in the ML topology of Dataset 1; Figure 2), and *C. paulensis* (placed at the base of *Clytia* in the ML topology of Dataset 1, but its placement is unresolved in the P topology; Figure 1). There is no congruence and little support for the position of the three species within *Clytia* in single gene phylogenies, although *C. paulensis* and *C. stolonifera* are frequently placed inside *Clytia* and Obeliidae, respectively (Figures 2-3, S3-S8).

Regarding Obellidae, only *Gonothyraea* and *Hartlaubella* were recovered as monophyletic, with high bootstrap and bremer supports in most of the phylogenies, including those of individual genes in which these lineages were sampled (Figures 1-2, S1-8). *Laomedea* is not monophyletic because *L. flexuosa* resulted outside the main *Laomedea* clade (*L. angulata* + *L. calceolifera*) (Figures 1-2). *Obelia* is also not monophyletic, since many of its lineages are more closely related to different genera then to *Obelia* itself. It is important to note that species of *Obelia* are distributed into four different, well supported and rather distant (considering branch lengths in the ML topology, Figure 2) monophyletic clades: *Gonothyraea+Obelia* (clade S), *Obelia+Laomedea* (clade AA), *Obelia bidentata* (clade Z), *Obelia+Laomedea+Hartlaubella* (clade AC) (Figures 1-4). Relationships among these clades vary between ML and P topologies derived from Dataset 1 (Figures 1-2), and only P topologies recover clade S at the base of Obeliidae. However, both ML and P topologies derived from Dataset 2 (Figures S1-S2) also place clade S at the base of the group, giving further support to this hypothesis.

Species level

Lineages at the species level are highly congruent among single gene and concatenated phylogenies (Figures 1-4, S1-S8). In Campanulariidae, *Silicularia rosea* is formed by one clade from New Zealand (B) and another from Argentina (C), which are not

monophyletic in 16S topologies (Figures 3-4). The maximal intra-clade distance is 7.76% for clade B, and 0.99% for clade C, and they have a minimum inter-clade distance of 6.37% (Figure 5, clade D). The species is monophyletic, though, in concatenated phylogenies (Figures 1-2). Similarly, *Orthopyxis integra* was recovered in three different and relatively distant clades, indicating it is a cryptic species, as previously suggested (Govindarajan et al. 2006). One of these clades is closely related to *O. crenata*, suggesting that misidentifications might have occurred (Clade H, Figure 1-4). Indeed, specimens of *O. integra* and *O. crenata* from clade H (see Figures 3-4, S3-S4) have low intra-clade distances (Figure 5), and they form a monophyletic group with another *O. crenata* clade (G) in concatenated phylogenies (Figures 1-2, clade I). Specimens identified as *O. integra* also cluster with *O. everta* and *Orthopyxis* sp.1 (clade J, Figures 1-4), with low intra-clade distances (Figure 5).

Several lineages were recovered in Clytiidae, seven of them identified as *Clytia gracilis*, but not monophyletic (Figures 1-2). Although these lineages seem to be geographically structured, one species identified as *Clytia* sp. from China falls into *C. gracilis* clade from Slovenia (clade K, Figures 1-4), and *C. gulangensis*, also from China, clusters with specimens of *C. gracilis* from Brazil (clade N, Figures 1-4). In both cases, intra-clade distances indicate close affinities within each group (Figure 5). Also, specimens of *C. gracilis* from the Mediterranean split into two closely related clades (K and L, minimum inter-clade distances are 9.65% for COI and 6.60% for 16S, Figure 5), which are monophyletic in concatenated phylogenies, but not in individual 16S and COI (clade M, Figures 1-4, S3-4). Additionally, specimens of *C. gracilis* also fall within a clade (O) comprising *C. hemisphaerica* and the recently described *C. xiamenensis* (Figures 3-4). Intra-clade distances also indicate close affinities between these specimens (Figure 5). Finally, *C. hemisphaerica* split into two main reciprocally monophyletic clades (O and P, inter-clade distances in Figure 5).

Four lineages corresponding to *Obelia dichotoma* were recovered in the Obeliidae (Figures 1-4). Clade R is formed exclusively by Brazilian specimens, and is closely related to the species *Gonothyraea loveni* in the concatenated phylogenies (Figures 1-2, clade S). Clade T and U are closely related to each other and to the species *O. geniculata*, with which they form a monophyletic group (W, intra and inter-clade distances in Figure 5). These two clades also seem to be geographically structured, although USA specimens of *O. dichotoma* fall within the Mediterranen clade (T). Specimens from the USA and Uruguay are also present in a fourth *O. dichotoma* clade (X), which is more closely related to the species *Laomedea flexuosa* and *O. longissima* (clade Y, Figures 1-4). *Obelia geniculata* is formed by three

different clades, unambiguously monophyletic in most of the phylogenies (Figures 1-4, S1-S8). Its low inter-clades distances (clade V, Figure 5) further supports the hypothesis of a unique monophyletic lineage. Finally, *O*. *bidentata* forms a monophyletic clade in nearly all topologies (except for COI, Figures 1-4, S1-S8), but its intra-clade distances are comparable to inter-clade distances of other lineages (clade Z, Figure 5).

Discussion

The molecular phylogeny of the family Campanulariidae (in its traditional sense) obtained in this study is incompatible with many current morphology-based taxonomic hypotheses at family, genus and species level. Some traditional morphological diagnostic characters are not informative for the delimitation of campanulariid species and genera. The phylogenetic relationships presented herein are congruent with previous molecular studies (Govindarajan et al. 2006; Zhou et al. 2013; He et al. 2015; Cunha et al. 2015), although we provide further evidence due to increased taxon sampling. We found that mitochondrial markers (16S and COI) were informative for delimitation of lineages at the species level, supporting their use as barcoding genes (e.g., *Obelia* and some *Clytia* medusae, Laakmann and Holst 2014; He et al. 2015).

Although the 16S is also considered useful for inferring relationships among hydrozoan lineages at less inclusive levels (e.g., Moura et al. 2008; Peña Cantero et al. 2010; Zhou et al. 2013; Calder et al. 2015; Cunha et al. 2015), our analysis demonstrated that 16S phylogenies may show inconsistencies with combined nuclear and mitochondrial genes phylogenies, always at nodes with little support (Figures 3-4). For instance, specimens of *Silicularia rosea* (clade D) and *Clytia gracilis* (clade M) present high intra-clade distances (Figure 5) and are not recovered as monophyletic in 16S topologies, but are well-supported monophyletic lineages in combined nuclear and mitochondrial genes phylogenies (Figures 1-2). Therefore, while 16S results could be interpreted as evidence for cryptic species, the use of more conserved, nuclear markers may indicate population subdivision, a conclusion more consistent with current taxonomic classification (see Schuchert 2014). Nuclear markers (18S and 28S) were more informative at the genus and family levels, even though Obeliidae was not recovered as monophyletic in the 18S analysis (Govindarajan et al. 2006, as subfamily Obeliinae; this study, Figures S5-S6). In this particular case, signal from the 28S proved to be more informative, increasing resolution and support at this level of the tree.

Delimiting Campanulariidae and its subfamilies

The family Campanulariidae is monophyletic if *Bonneviella* is included and *Billardia* excluded from its scope (Govindarajan et al. 2006; Penã Cantero et al. 2010; this study). However, there is not a consensus about the taxonomic affinities of *Bonneviella* and *Billardia*, probably because their phylogenetic relationships are not congruent with former morphological studies. Bonneviellidae and *Bonneviella* were erected by Broch (1909) based on a pre-oral cavity present in the hydranth, formed by the projection of the tentacular base into the gastrovascular cavity (viz., veloid, Broch 1909; also Naumov 1969; Yamada 1969; Schuchert 2001). Later, *Bonneviella* was included in Campanulariidae based on putative developmental similarities of its hypostome (Broch 1918). Although *Bonneviella* was also suggested to have affinities with Lafoeidae due to its aggregation of gonothecae, similar to some lafoeid coppinia (Stechow 1923; Cornelius 1995b: 221; Marques et al. 2006), Bonneviellidae was reinstated as valid and placed among Proboscoida Broch, 1910 again based on the hypostome shape (Bouillon 1984, 1985). Our analysis supports *Bonneviella* in Campanulariidae, as proposed by Broch (1918). Further studies with species of *Bonneviella* will be decisive to corroborate their similarities with other campanulariids concerning its hypostome development. Anyhow, the pre-oral cavity is evidently a diagnostic character of the genus.

Differently, *Billardia* was originally included in Campanulariidae (Totton 1930), disregarding disputable affinities with *Hincksella* and Lafoeidae (Totton 1930; Millard 1975; Cornelius 1982). Molecular studies confirmed this hypothesis, showing that the genus is more closely related to species of Hebellidae (*Scandia gigas*, Peña Cantero et al. 2010; Moura et al. 2011c). The campanulate hydrotheca with a large but completely retractable hydranth (Vervoort 1972; Vervoort and Watson 2003) may explain the initial inclusion of *Billardia* among Campanulariidae. However, based on its phylogenetic relationships, these characters are probably plesiomorphic in the Leptothecata and should not be regarded as diagnostic of Campanulariidae, at least if other characters are not present for a reliable identification.

The three main monophyletic groups obtained with our phylogenetic analysis are congruent with both the traditional taxonomy, which divides the family Campanulariidae in three subfamilies (cf. Cornelius 1982; Calder 1991), and the phylogenetic classification of Proboscoida (cf. Maronna et al. 2016). However, considering the traditional taxonomy, only Obeliinae is close to its original scope (*Clytia stolonifera* is frequently placed at the base of the group, but with little support, see Figures 1-4, as Obeliidae). Campanulariinae, characterized by a subhydrothecal spherule and annular perisarc thickening at the hydrothecal

base, but lacking a true diaphragm (Cornelius 1982; Calder 1991; Bouillon 1985), is monophyletic if *Tulpa* and *Bonneviella* are included, even though these genera do not have subhydrothecal spherules (Stechow 1921; Vervoort 1972; Schuchert 2001; Vervoort and Watson 2003). The annular perisarc thickening occur in *Tulpa* and, although not an universal feature (i.e., absent in *Bonneviella*), it would be the best approximation of morphological character to delimit the subfamily (cf. Boero et al. 1996), because it is not present in non-Campanulariinae taxa.

Clytiinae becomes monophyletic if *Clytia stolonifera* and *Clytia hummelincki* are excluded from the subfamily. This scenario, however, is ambiguously supported by our results. The subhydrothecal spherule of *C. hummelincki*, a character commonly associated with Campanulariinae, as well as a diaphragm and medusae with tentacle bulbs, characteristic of Clytiinae, led Cornelius (1982) to regard this species with uncertain taxonomic affinities, although he followed Millard (1966) and kept the species in Clytiinae based on characters of the medusa stage. Govindarajan et al. (2006, concatenated phylogeny) recovered this species at the base of the Clytiinae, and concluded that the subhydrothecal spherule is plesiomorphic of campanulariids in general. We found congruent results (Figures 1-2), but considering that this character is not present in non-campanulariid taxa, the hypothesis of convergence (Cornelius 1982: 83) can not be discarded based on the present phylogeny.

The ambiguous placement of several *Clytia* (e.g., *C. stolonifera*, *C. hummelincki*, *C. paulensis*, Figures 1-2) within Clytiidae+Obellidae (or subfamilies Clytiinae and Obeliinae, in the traditional sense) suggests these groups have close taxonomic affinities. In fact, the two subfamilies are only differentiated based on medusa characters, since their polyps are mainly characterized by a true hydrothecal diaphragm (Cornelius 1982; Calder 1991). Following previous authors, Boero et al. (1996) suggested that Clytiinae and Obellinae should be merged, considering that lineages in Obellinae that lost their medusa stage (e.g., *Laomedea*, *Hartlaubella*) can not be differentiated from *Clytia* exclusively based on hydroid characters. Indeed, establishing diagnostic characters of Obelliinae based on medusa characters is problematic, because most of its genera do not produce free medusae.

It is clear that a reassessment of the traditional subfamily division, as well as the scope and diagnostic characters of Campanulariidae is necessary, especially considering that there are few characters which are unique among Campanulariidae, and the presence of a campanulate/bell-shaped hydrotheca is probably a simplesiomorphy. The phylogenetic classification by Maronna et al. (2016), therefore, significantly improves the taxonomy of the group by elevating to family level the three main monophyletic groups that comprise

campanulariid hydroids (former subfamilies), most of them with unique and conspicuous diagnostic characters. In addition, the new infraorder proposed (=Obeliida Maronna et al. 2016), comprising the families Clytiidae and Obeliidae, is congruent with the morphological affinities between these groups, specially concerning the hydroid stage. However, as already discussed, Clytiidae and Obeliidae are not unequivocally supported as monophyletic in this study, and their classification as two distinct families still needs futher confirmation.

Generic limits in Campanulariidae

Gonophore morphology have long been considered to have generic value for Campanulariidae, although in Hydrozoa much have been debated on whether species with different types of gonophores (fixed sporosacs or free medusae) should be assigned to separate genera (Levinsen 1893; Kramp 1935; Rees 1957; Petersen 1990). Campanulariidae remarkably has gonophores varying from fixed sporosacs, released or retained medusoids, and meconidia to free medusae, including the singular medusae of *Obelia* (Cornelius 1990; Boero et al. 1996). Even though the occurrence of gonophore reduction, from free medusae to fixed gonophores, was hypothesized to reflect phylogenetic patterns in the family (Boero and Sàra 1987), subsequent studies showed that taxonomical classification based on types of gonophores does not result in monophyletic genera, because medusa reduction can happen multiple times within the same genus (Petersen 1990; Cunningham and Buss 1993). Following these ideas, *Laomedea*, *Clytia*, and *Obelia* were though not to be monophyletic (Boero et al. 1996), and this hypothesis was indeed corroborated by molecular studies (Govindarajan et al. 2006; this study). Our phylogenies shows that species with *Obelia*-like medusae do not form a monophyletic group, as well as those species with fixed gonophores, indicating these characters are irrelevant to diagnose the different genera.

Even if we consider some of the main classifications proposed along the last 100 years, there are no or few classifications in which the scope of *Campanularia*, *Clytia*, *Obelia*, and *Laomedea* could be considered monophyletic based on our phylogenetic analyses (Table 4, Figures 1-4). In contrast, *Orthopyxis*, *Silicularia*, *Gonothyraea*, and *Hartlaubella* are consistent with most of the proposed classifications (Table 4). These inconsistencies and variation occur because most of the classifications separate genera based on the type of gonophore, and conspicuous morphological diagnostic characters are absent in some groups.

Campanularia, the most problematic genus of Campanulariinae, resulted as polyphyletic, and its current diagnostic characters are symplesiomorphies (e.g., stolonal colonies, campanulate hydrotheca, annular perisarc thickening, subhydrothecal spherule,

fixed sporosacs; Ralph 1957; Cornelius 1982; Bouillon 1985; Calder 1991). *Orthopyxis* can be differentiated from *Campanularia* by the occasional presence of thickened hydrothecal walls and medusoid features, whereas *Silicularia* may be differentiated from all remaining genera by the bilaterally symmetrical hydrotheca, in which the hydranth can not fully retract (Nutting 1915; Ralph 1957; Bouillon 1985; Vervoort and Watson 2003). The resulted topologies show that these characters are informative for the delimitation of genera in Campanulariinae. More samples of *Tulpa* and *Rhizocaulus* are needed to test the relevance of their diagnostic characters, though some authors suggested their inclusion into *Campanularia* (Boero et al. 1996; Bouillon et al. 2004), but this arrangement would also result in non-monophyletic groups.

Clytia is monophyletic only in classifications disregarding the subhydrothecal spherule as part of the diagnostic characters of the genus, which exclude *C. hummelincki* (Nutting 1915; Ralph 1957; Hirohito 1995). In spite of that, traditional diagnostic characters of the hydroids (e.g., stolonal or erect colonies and true hydrothecal diaphragm; Millard 1975; Cornelius 1982, 1995b; Bouillon 1985; Calder 1991; Bouillon et al. 2004) are not entirely relevant to delimit the genus, because they are shared with Obeliinae species. Characters of the medusa stage, on the other hand, are important diagnostic features for *Clytia*, and they support the inclusion of *C. hummelincki* in this genus (Gravili et al. 2008), although additional studies on *C. hummelincki* and *C. stolonifera* are crucial to ascertain their phylogenetic relationships. The basal position of *C. stolonifera* in Obeliinae suggests closer affinities with this group, but its gonossome is still not fully described (Blackburn 1938; Millard and Bouillon 1973, as *C. latitheca*; Watson 2005).

Obeliinae is the most problematic group of the family, including nearly all types of gonophores, but this variation is clearly not informative to delimit genera. *Gonothyraea* might be the only exception, considering that meconidia are exclusive in that genus, and therefore it is regarded as monophyletic in most of the classifications proposed (Nutting, 1915; Ralph 1957; Millard 1975; Cornelius 1982, 1995b; Bouillon 1985; Table 4). *Hartlaubella* is also monophyletic in most classifications because of its polysiphonic colonies, clearly differentiating this genus from *Laomedea*, although both have gonophores as fixed sporosacs (Cornelius 1982, 1995b; Bouillon 1985; Bouillon et al. 2004). Trophossomal charcaters, however, are irrelevant for the delimitation of *Laomedea* and *Obelia*, both described as presenting erect, sympodial colonies, with a true hydrothecal diaphragm (Nutting 1915; Millard 1975; Cornelius 1975, 1982; Calder 1991; Bouillon 1985; Bouillon et al. 2004). Indeed, *Obelia* would become monophyletic only by the inclusion of

Laomedea, *Hartlaubella*, and *Gonothyraea*, similar to what was proposed by Naumov (1969). In this sense, there are no conspicuous or unambiguous morphological characters, neither from hydroids or medusae, that support *Obelia* or *Laomedea* as monophyletic genera. The reassessment of their scope and morphological diagnostic characters is critical to reflect the phylogenetic patterns of the family.

Species boundaries in Campanulariidae

At least three campanulariid species are not monophyletic and represent cryptic lineages: *Orthopyxis integra*, *Clytia gracilis*, and *Obelia dichotoma*. Indeed, some of these species were shown to be polyphyletic in previous molecular studies (Govindarajan et al. 2005, 2006; Lindner et al. 2011; Cunha et al. 2015). Several other species showed signs of population subdivision (*Silicularia rosea*, *Orthopyxis crenata*, *Clytia hemisphaerica*, *Obelia geniculata*), although they resulted as monophyletic in the concatenated analysis (Figures 1-2). Also, most of them have identification problems related to their wide morphological variability and/or lack of conspicuous diagnostic characters (Ralph 1957; Cornelius 1982, 1995b). These problems contribute to misinterpretations between intra and interspecific variations, leading to the discovery of cryptic species that are frequently a result of previously overlooked morphological differences (e.g., Lindner et al. 2011; Cunha et al. 2015; also see Cunha et al. 2016).

Cornelius (1982, 1995b) included more than 15 nominal species in the synonymy of *Orthopyxis integra*, a species believed to be cosmopolitan and to comprise several different morphotypes as a result of its wide morphological variability (e.g., thickened to unthickened hydrothecal walls, sinuous to smooth pedicels, smooth to completely spirally grooved gonotheca). Govindarajan et al. (2006) were the first to show that this species comprised several cryptic lineages, and Cunha et al. (2015) remarked that much of the variation within *O. integra* was overestimated, attributing part of its former morphotypes to two different species (*O. caliculata* (Hincks, 1853) and *O. mianzani* Cunha, Genzano & Marques, 2015). The "true" *O. integra* was assigned to the morphotype with a spirally grooved gonotheca (Cunha et al. 2015: 21). Following these ideas, we argue that the clade comprising the specimen of *O. integra* from the Aleutian Islands (*O.integra*_1_USA =*O.integra*(AK), Govindarajan et al. 2006) probably corresponds to the "true" *O. integra*, which comprises specimens from the USA, Iceland, and Argentina (see 16S phylogenies, Figure 3-4). The specimen of *O. integra* from New Zealand (*O.integra*_NZ, Govindarajan et al. 2006) clusters with a specimen of *O. crenata* also from New Zealand, its type locality (Hartlaub 1901,

Vervoort & Watson 2003; see 16S phylogenies, Figures 3-4), and with an unidentified specimen from Argentina (*O*.sp._Co1_ARG, Cunha et al. 2015), believed to have close affinities with *O*. *crenata* (Cunha et al. 2015). These evidences suggest that *O*.*integra*_NZ is a misidentification, also because this clade clusters with specimens of *O*. *crenata* from Brazil. The variation of the hydrothecal cusps of *O*. *crenata* may lead to confusion with *O*. *integra* for the occasional occurrence of even hydrothecal margins among its specimens (Ralph 1957; Millard 1975). Finally, the specimen of *O*. *integra* from Italy (*O*.*integra*_IT, Govindarajan et al. 2006), clusters with specimens of *O*. *everta* and *Orthopyxis* sp. 1, all from the Mediterranean (Italy and Slovenia, Table 1 and 2), and they are clearly separated from the "true" *O*. *integra* (clade F, Figures 1-4). This suggests the clade comprising *O*.*integra*_IT corresponds to a different species occurring in the Mediterranean Sea (e.g., *O*. *everta* or *O*. *asymmetrica*; cf. Peña Cantero and García Carrascosa 2002; Bouillon et al. 2004).

Similarly to *O*. *integra*, *Clytia gracilis* is also considered to be widely distributed (Calder 1991; Cornelius 1995b), and was for long regarded as conspecific with *C*. *hemisphaerica*, based on the variation of its hydrothecal cusps and gonothecal shape (Ralph 1957, as *C*. *johnstoni*; Millard 1966; Cornelius 1982). Several subsequent studies, however, found consistent differences among characters of the hydranths and nematocysts of the two species, and demonstrated that many trophosomal characters previously regarded as intraspecific variations were actually diagnostic at the species level (Östman 1979; Cornelius 1987a,b, 1995b). Unfortunately, based on our molecular phylogenies, we are unable to assign any of the lineages to the "true" *C*. *gracilis*, and further samples are necessary to ascertain which of the morphotypes comprised by these lineages should correspond to that species. Anyhow, it is clear that this species might still comprise morphological variations erroneously interpreted as intraspecific. Recently, the location of the insertion of the gonotheca (hydrorhiza or stem) was shown to differentiate *C*. *gracilis* from *C*. *elsaeoswaldae* (Lindner et al. 2011), which is corroborated as a distinct, monophyletic lineage in this study (Figures 1-4). The shape of the hydrothecal cusps and gonothecae of the polyps, as well as size, shape of gonads and number of tentacles of the medusae, were considered diagnostic for two new species of *Clytia*, *C*. *xiamenensis* and *C*. *gulangensis*, differentiating them from *C*. *hemisphaerica* and *C*. *gracilis*, their presumed closest congeners (Zhou et al. 2013; He et al. 2015; see Figures 1-4). The fact that *C*. *gulangensis* clusters with specimens identified as *C*. *gracilis* from Brazil, and *C*. *xiamenensis* clusters with specimens identified as *C*. *hemisphaerica* from the USA (as well as *C*. cf. *gracilis*_sp.A, Lindner et al. 2011; Figures 1-4) suggests that morphological differences between these species are still unclear, and it is

necessary a detailed analysis to test the relevance of diagnostic characters for the delimitation of these lineages. Although the shape of hydrothecal cusps and gonothecae were traditionally used to differentiate *C. gracilis* and *C. hemisphaerica*, these characters are not informative for the delimitation of these species, considering the phylogenies presented herein.

A similar situation occurs among Obeliinae, in which *Obelia dichotoma* is not monophyletic (Figures 1-4). This would be expected, considering that several diagnostic characters of species of *Obelia* are frequently reported as intraspecifically variable, hampering species identification (Cornelius 1975, 1982, 1990, 1995b). Cornelius (1975) was the first to conduct a formal revision of the genus, in which he regarded several diagnostic characters of the polyp and medusa of distinct species as intraspecific variations, lumping more than 80 nominal species of *Obelia* into three (*O. bidentata*, *O. geniculata*, and *O. dichotoma*). Among these characters, colony size, branching, shape of the hydrothecal rim and number of annulations in the pedicels of the polyps, as well as number of tentacles and position of the gonads on the medusae were shown to be variable, and correlated with changes in environmental factors (Ralph 1956; Ralph and Thomson 1968; Hughes 1980; Kubota 1981). Later, Östman (1982a,b) showed consistent differences in the nematocysts types and isoenzymes patterns among *O. dichotoma* and *O. longissima*, regarding both species valid, and was followed by Cornelius (1990, 1995b), who also corroborated the validity of the species based on characters of the hydranths (Cornelius 1987b). Currently, the four species are separated based solely on polypoid characters, since medusa characters do not seem to be reliable for their morphological distinction (Cornelius 1975, 1990, 1995b). Diagnostic characters of *O. bidentata* and *O. geniculata* are usually conspicuous and were corroborated to delimit monophyletic lineages, but there is still much confusion in the separation of the other two species, due to the variability of their diagnostic characters (Cornelius 1990, 1995b). *Obelia dichotoma* is mainly distinguished from *O. longissima* by its branching patterns and shape of the hydrothecal rim, but our analysis shows these characters are not informative for the delimitation of the species. There is a misinterpretation of the patterns of variation of its diagnostic characters, and detailed analyses are needed, especially if we consider that intraspecific variations usually parallel interspecific variations in morphologically variable species (Cunha et al. 2016). In fact, further discriminations of these characters have recently corroborated the revalidation of former synonyms of *O. dichotoma* (Calder 2013, Calder et al. 2014), and this might also prove to be the case for the cryptic lineages of *O. dichotoma* presented in this study.

Conclusions

We corroborated previous results and presented novel evidences for the phylogenetic relationships within the family Campanulariidae. Considering the traditional morphological diagnostic characters of the family and the phylogenetic patterns presented in this study, the scope of the family Campanulariidae should be changed, as well as the scope of its subfamilies and, in this sense, the phylogenetic classification recently proposed by Maronna et al. (2016) improves the taxonomy of the group, but the relationships between Clytiidae and Obeliidae taxa still remain with open questions. Additionally, generic limits will only reflect phylogenetic patterns if different types of gonophores are disregarded as generic charcaters, especially within Obeliidae. Finally, diagnostic characters of several species have to be reassessed based on a detailed study of their patterns of morphological variation. Further morphological studies will be very important to establish a solid taxonomic and phylogenetic framework for the classification of Proboscoida, contributing to a broader discussion on morphological variation and species delimitation, particularly in extensively variable groups.

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Table 1. Codes, sampling sites, museum vouchers and GenBank accession numbers for the sequences obtained during this study. LEM = Laboratory of Marine Evolution, University of São Paulo, Brazil.

Code in tree	Species	Locality	Geographic coordinate	Voucher	GenBank Acession Numbers				Reference
					16S	COI	18S	28S	
IT10_IT	*Campanularia hincksii*	Italy	not available	LEM		-			This study
IT14_IT	*Campanularia hincksii*	Italy	not available	LEM		-		-	This study
PT10_ARG	*Campanularia* sp.	Punta Cuevas, San Juliàn, Argentina	not available	LEM				-	This study
SJ2_ARG	*Campanularia subantarctica*	La Mina, Puerto San Julián, Argentina	49°09.413'S 67°37.987'W	MZUSP 2639	KM405574	KM405569		-	Cunha et al. (2015); this study
PM36_BRA	*Clytia elsaeoswaldae*	Palmas Island, Brazil	not available	LEM					This study
CB19_BRA	*Clytia elsaeoswaldae*	Cabras Island, Ilhabela, Brazil	not available	LEM					This study
PM18_BRA	*Clytia elsaeoswaldae*	Palmas Island, Brazil	not available	LEM			-	-	This study
Me26_BRA	*Clytia elsaeoswaldae*	Mel Island, Brazil	25°33'22.12"S 48°17'55.36"W	LEM			-	-	This study
EL05_SLV	*Clytia gracilis*	Mund Bay, Piran, Slovenia	not available	LEM					This study
EL14_SLV	*Clytia gracilis*	Strunjan, Piran, Slovenia	45°32'13.26"N 13°36'5.09"E	LEM					This study
EL15_SLV	*Clytia gracilis*	Strunjan, Piran, Slovenia	45°32'13.26"N 13°36'5.09"E	LEM					This study
EL31_SLV	*Clytia gracilis*	Slovenia	45°35'28.20"N 13°41'59.18"E	LEM					This study
EL32_SLV	*Clytia gracilis*	Slovenia	45°35'28.20"N 13°41'59.18"E	LEM					This study
EL38_SLV	*Clytia gracilis*	Piran, Slovenia	45°31'49"N 13°34'03"E	LEM					This study
IT12_IT	*Clytia gracilis*	Italy	not available	LEM					This study
IT13_IT	*Clytia gracilis*	Italy	not available	LEM		-			This study
CBC13_BLZ	*Clytia gracilis*	Twin Cays, Belize	16°49'41.52"N 88°6'3.71"W	LEM					This study

Code in tree	Species	Locality	Geographic coordinate	Voucher	GenBank Acession Numbers				Reference
					16S	COI	18S	28S	
CBC20_BLZ	*Clytia gracilis*	Carry Bow Cay, Belize	16°48'28.80"N 88°3'46.80"W	LEM			-	-	This study
CBC26_BLZ	*Clytia gracilis*	Twin Cays Fisheries Dock, Belize	16°49'24.61"N 88°6'21.56"W	LEM			-	-	This study
MAP01_BRA	*Clytia gracilis*	Panaquatira, São Luís do Maranhão, Brazil	02°29.904'S 44°01.433'W	LEM					This study
MAP11_BRA	*Clytia gracilis*	Panaquatira, São Luís do Maranhão, Brazil	02° 29.904'S 44° 01.433'W	LEM			-	-	This study
PAF03_BRA	*Clytia gracilis*	Farol Velho, Salinópolis, Brazil	0°35.460'S 47°19.487'W	LEM					This study
T1_BRA	*Clytia gracilis*	Flexeiras, Trairí, Brazil	03°13.020'S 39°16.026'W	LEM					This study
T5_BRA	*Clytia gracilis*	Flexeiras, Trairí, Brazil	03°13.333'S 39°15.015'W	LEM					This study
T6_BRA	*Clytia gracilis*	Flexeiras, Trairí, Brazil	03°13.333'S 39°15.015'W	LEM			-	-	This study
CE1_BRA	*Clytia gracilis*	Náutico, Fortaleza, Brazil	not available	LEM				-	This study
CE2_BRA	*Clytia gracilis*	Caponga, Cascavel, Brazil	04°02.348'S 38°11.572'W	LEM			-	-	This study
CE3_BRA	*Clytia gracilis*	Náutico, Fortaleza, Brazil	not available	LEM			-	-	This study
CE5_BRA	*Clytia gracilis*	Caponga, Cascavel, Brazil	04°02.348'S 38°11.572'W	LEM			-	-	This study
Me24_BRA	*Clytia gracilis*	Mel Island, Brazil	25°34'22.58"S 48°18'32.77"W	LEM				-	This study
PT9_ARG	*Clytia gracilis*	Punta Cuevas, San Juliàn, Argentina	not available	LEM				-	This study
FLT03_USA	*Clytia hemisphaerica*	Westport, USA	41°30'46.57"N 71°04'35.28"W	LEM					This study
HCM04_USA	*Clytia hemisphaerica*	Salem, USA	42°31'19"N 70°52'56"W	LEM				-	This study
MMA05_USA	*Clytia hemisphaerica*	Bourne, USA	41°44'23"N 70° 37'27"W	LEM				-	This study

Code in tree	Species	Locality	Geographic coordinate	Voucher	GenBank Acession Numbers				Reference
					16S	COI	18S	28S	
PTJ01_USA	*Clytia hemisphaerica*	Point Judith, Rhode Island, USA	41°23'15.72"N 71°31'1.56"W	LEM	-			-	This study
EL06_SLV	*Clytia hemisphaerica*	Mund Bay, Piran, Slovenia	not available	LEM					This study
EL08_SLV	*Clytia hemisphaerica*	Mund Bay, Piran, Slovenia	not available	LEM					This study
EL12_SLV	*Clytia hemisphaerica*	Strunjan, Piran, Slovenia	45°32'13.26"N 13°36'5.09"E	LEM					This study
EL20_SLV	*Clytia hemisphaerica*	Strunjan, Piran, Slovenia	45°32'13.26"N 13°36'5.09"E	LEM					This study
EL28_CRO	*Clytia hemisphaerica*	Croacia	not available	LEM					This study
EL35_SLV	*Clytia hemisphaerica*	Slovenia	45°35'28.20"N 13°41'59.18"E	LEM					This study
CBC1_BLZ	*Clytia hemisphaerica*	Carrie Bow Cay, Belize	not available	LEM			-	-	This study
CBC25_BLZ	*Clytia hemisphaerica*	Twin Cays Fisheries Dock, Belize	-88.10599; 16.823503	LEM					This study
CBC40.1_BLZ	*Clytia hemisphaerica*	Cuda Cut, Twin Cays, Belize	not available	LEM			-	-	This study
CBC42_BLZ	*Clytia hummelincki*	Cuda Cut, Twin Cays, Belize	not available	LEM					This study
PY10_BRA	*Clytia linearis*	Paraty, Brazil	not available	LEM		-			This study
SP3_BRA	*Clytia noliformis*	Barão Tefé Island, São Pedro and São Paulo Archipelago, Brazil	not available	LEM					This study
SP9_BRA	*Clytia noliformis*	Barão Tefé Island, São Pedro and São Paulo Archipelago, Brazil	not available	LEM					This study
SP1_BRA	*Clytia* sp.1	Boca da Enseada, São Pedro and São Paulo Archipelago, Brazil	not available	LEM					This study
CE4_BRA	*Clytia* sp.2	Caponga, Cascavel, Brazil	04°02.348'S 38°11.572'W	LEM					This study
NAT05_BRA	*Clytia* sp.3	Natal, Rio Grande do Norte	not available	LEM					This study
CBC45_BLZ	*Clytia stolonifera*	Cuda Cut, Twin Cays, Belize	not available	LEM		-			This study
CBC40.2_BLZ	*Clytia stolonifera*	Cuda Cut, Twin Cays, Belize	not available	LEM		-	-	-	This study

Code in tree	Species	Locality	Geographic coordinate	Voucher	GenBank Acession Numbers				Reference
					16S	COI	18S	28S	
BPM03_USA	*Gonothyraea loveni*	Plymouth, USA	not available	LEM					This study
SWM03_USA	*Gonothyraea loveni*	Sandwich, USA	41°46'13"N 70°30'13"W	LEM				-	This study
PT14_ARG	*Hartlaubella gelatinosa*	Río Gallegos, Argentina	not available	LEM				-	This study
PT16_ARG	*Hartlaubella gelatinosa*	Río Gallegos, Argentina	not available	LEM				-	This study
PT13_ARG	*Hartlaubella gelatinosa*	Río Gallegos, Argentina	not available	LEM		-	-	-	This study
EL40_SLV	*Laomedea angulata*	Piran, Slovenia	45°30'54"N 13°34'46"E	LEM					This study
EL50_SLV	*Laomedea angulata*	Mund Bay, Piran, Slovenia	not available	LEM					This study
IT11_IT	*Laomedea angulata*	Italy	not available	LEM					This study
FTA01_USA	*Laomedea calceolifera*	Newport, USA	41°28'41" 71°20 '08"W	LEM					This study
GFP01_USA	*Laomedea calceolifera*	Gloucester, USA	42°36'54.44"N 70°39'1.36"W	LEM					This study
HRM06_USA	*Laomedea calceolifera*	Hampton, USA	42°32'24"N 70°29'42"W	LEM				-	This study
MMA06_USA	*Laomedea calceolifera*	Bourne, USA	41°44'23"N 70° 37'27"W	LEM				-	This study
ROW03_USA	*Laomedea calceolifera*	Boston, USA	42°21'25" N 71°02'27" W	LEM				-	This study
RYE02_USA	*Laomedea flexuosa*	Rye, USA	42°58'36.37" N 70°45'56.06" W	LEM		-		-	This study
CBC35_BLZ	*Obelia bidentata*	Cuda Cut, Twin Cays, Belize	not available	LEM					This study
MAR02_BRA	*Obelia bidentata*	Raposa Channel, São Luís do Maranhão, Brazil	02° 25.629'S 44° 04.198'W	LEM					This study
MAP10_BRA	*Obelia bidentata*	Panaquatira, São Luís do Maranhão, Brazil	02° 29.904'S 44° 01.433'W	LEM			-	-	This study
PAF09_BRA	*Obelia dichotoma*	Farol Velho, Salinópolis, Brazil	0°35.460'S 47°19.487'W	LEM					This study

Code in tree	Species	Locality	Geographic coordinate	Voucher	GenBank Acession Numbers				Reference
					16S	COI	18S	28S	
MA03_BRA	*Obelia dichotoma*	Calhau, São Luís Maranhão, Brazil	2°28.786'S 44°14.573'W	LEM					This study
PAF07_BRA	*Obelia dichotoma*	Farol Velho, Salinópolis, Brazil	0°35.460'S 47°19.487'W	LEM					This study
MMA03_USA	*Obelia dichotoma*	Bourne, USA	41°44'23"N 70° 37'27"W	LEM				-	This study
FLT04_USA	*Obelia dichotoma*	Westport, USA	41°30'46.57"N 71°04'35.28"W	LEM				-	This study
PIM01_USA	*Obelia dichotoma*	New Bedfort, USA	41°38'41.42"N 70°55'50.99"W	LEM				-	This study
PIM02_USA	*Obelia dichotoma*	New Bedfort, USA	41°38'41.42"N 70°55'50.98"W	LEM					This study
PTJ03_USA	*Obelia dichotoma*	Point Judith, Rhode Island, USA	41°23'15.72"N 71°31'1.56"W	LEM					This study
ROW04_USA	*Obelia dichotoma*	Boston, USA	42°21'25" N 71°02'27" W	LEM					This study
S1.1_USA	*Obelia dichotoma*	Providence, USA	not available	LEM					This study
EL30_SLV	*Obelia dichotoma*	Slovenia	45°35'28.20"N 13°41'59.18"E	LEM					This study
PT2_ARG	*Obelia dichotoma*	Punta Cuevas, San Juliàn, Argentina	not available	LEM			-	-	This study
PT3_ARG	*Obelia dichotoma*	Punta Cuevas, San Juliàn, Argentina	not available	LEM				-	This study
UR1_URG	*Obelia dichotoma*	Rocha, Uruguay	34° 39.140'S 54° 08.495'W	LEM					This study
UR6_URG	*Obelia dichotoma*	Rocha, Uruguay	34° 39.247'S 54° 08.610'W	LEM					This study
BSF05_USA	*Obelia geniculata*	South Freeport, USA	43°49'17.29"N 70°6'28.51"W	LEM		-		-	This study
BZ5_BRA	*Obelia geniculata*	João Gonçalves, Búzios, Brazil	not available	LEM					This study
EL23_SLV	*Obelia geniculata*	Mund Bay, Piran, Slovenia	not available	LEM					This study

Code in tree	Species	Locality	Geographic coordinate	Voucher	GenBank Acession Numbers				Reference
					16S	COI	18S	28S	
PT5_ARG	*Obelia geniculata*	Punta Cuevas, San Juliàn, Argentina	not available	LEM				-	This study
UNH01_USA	*Obelia geniculata*	New Castle, USA	43°04'20.21"N 70°42'56.65"W	LEM		-		-	This study
PT1_ARG	*Obelia longissima*	San Julián, Argentina	not available	LEM				-	This study
GFP04_USA	*Obelia longissima*	Gloucester, USA	not available	LEM					This study
HRM05_USA	*Obelia longissima*	Hampton, USA	not available	LEM					This study
MMA04_USA	*Obelia longissima*	Bourne, USA	41°44'23"N 70° 37'27"W	LEM				-	This study
T2_BRA	*Obelia* sp.1	Flexeiras, Trairí, Brazil	03°13.020'S 39°16.026'W	LEM					This study
PAF08_BRA	*Obelia* sp.1	Farol Velho, Salinópolis, Brazil	0°35.460'S 47°19.487'W	LEM		-	-	-	This study
AB_BRA	*Orthopyxis caliculata*	Armação, Penha, Brazil	26°47'S 48°37'W	MZUSP 2565	KM405578	KM405567		-	Cunha et al. (2015); this study
JGB3_BRA	*Orthopyxis caliculata*	João Gonçalves, Búzios, Brazil	not available	MZUSP 2614	KM405584	KM405565			Cunha et al. (2015); this study
CB_BRA	*Orthopyxis crenata*	Caponga, Cascavel, Brazil	04°02.348'S 38°11.572'W	MZUSP 2633	KM405590				Cunha et al. (2015); this study
PAB2_BRA	*Orthopyxis crenata*	Paciência, Penha, Brazil	26°46'38"S 48°36'10"W	MZUSP 2551	KM405593	KM405559			Cunha et al. (2015); this study
PT20_ARG	*Orthopyxis integra*	San Julián, Argentina	not available	LEM				-	This study
PT19_ARG	*Orthopyxis crenata*	Comodoro Rivadavia, Argentina	not available	LEM	-			-	This study
MB1_BRA	*Orthopyxis mianzani*	Mel Island, Brazil	25°33'22.12"S 48°17'55.36"W	MZUSP 2570	KM405603	KM405549			Cunha et al. (2015); this study

Code in tree	Species	Locality	Geographic coordinate	Voucher	GenBank Acession Numbers				Reference
					16S	COI	18S	28S	
PAB6_BRA	*Orthopyxis mianzani*	Paciência, Penha, Brazil	26°46'38"S 48°36'10"W	MZUSP 2559	KM405607	KM405545			Cunha et al. (2015); this study
PTY2_BRA	*Orthopyxis sargassicola*	Paraty, Brazil	not available	MZUSP 2606	KM405629	KM405523			Cunha et al. (2015); this study
LB9_BRA	*Orthopyxis sargassicola*	Lázaro, Ubatuba, Brazil	23°30'32.64"S 45°08'18.52"W	MZUSP 2602	KM405618	KM405534		-	Cunha et al. (2015); this study
PB1_BRA	*Orthopyxis sargassicola*	Padres, Aracruz, Brazil	19°55.941'S 40°07.327'W	MZUSP 2617	KM405622	KM405531		-	Cunha et al. (2015); this study
Co1_ARG	*Orthopyxis* sp.	Caleta Olivia, Argentina	46º25.539'S 67º31.183'W	MZUSP 2644	KM405635				Cunha et al. (2015); this study
EL02_SLV	*Orthopyxis* sp.1	Mund Bay, Piran, Slovenia	not available	LEM					This study
EL04_SLV	*Orthopyxis* sp.1	Mund Bay, Piran, Slovenia	not available	LEM					This study
EL16_SLV	*Orthopyxis* sp.1	Strunjan, Piran, Slovenia	45°32'13.26"N 13°36'5.09"E	LEM					This study
EL52_SLV	*Orthopyxis* sp.1	Strunjan, Piran, Slovenia	45°32'13.26"N 13°36'5.09"E	LEM					This study
PT11_ARG	*Silicularia rosea*	Río Deseado, San Julián, Argentina	not available	LEM					This study
RG4_ARG	*Silicularia rosea*	Río Grande, Cabo Santo Domingo, Argentina	53°41.330'S 67°50.673'W	MZUSP 2645	KM405636	-			Cunha et al. (2015); this study
PT8_ARG	*Silicularia rosea*	San Julián, Argentina	not available	LEM		-		-	This study
PT18_ARG	*Tulpa tulipifera*	Patagonia, Argentina	not available	LEM				-	This study
OUTGROUPS									
U10_URG	*Eucheilota* sp.	Uruguay	not available	LEM					This study
U11_URG	*Eucheilota* sp.	Uruguay	not available	LEM					This study

Table 2. Codes, sampling sites and GenBank accession numbers for published sequences included in the analysis. * Chimeric sequence.

Code in tree	Species	Locality	GenBank Acession Numbers				Reference
			16S	COI	18S	28S	
USA	*Bonneviella regia*	Aleutians, USA	AY789805	AY789890	AY789740	-	Govindarajan et al. (2006)
USA	*Bonneviella* sp.2	Aleutians, USA	AY789806	AY789891	AY789741	-	Govindarajan et al. (2006)
USA	*Bonneviella* sp.3	Aleutians, USA	AY789807	AY789892	AY789742	-	Govindarajan et al. (2006)
USA	*Bonneviella* sp.4	Aleutians, USA	AY789808	AY789893	AY789743	-	Govindarajan et al. (2006)
IT	*Campanularia hincksii*	Otranto, Italy	AY789794	AY789882	AY789729		Govindarajan et al. (2006)
SJ4_ARG	*Campanularia* sp.	La Mina, Puerto San Julián, Argentina	KM405572	KM405571	KM454908		Cunha et al. (2015)
SJ5_ARG	*Campanularia* sp.	La Mina, Puerto San Julián, Argentina	KM405573	KM405570	KM454909		Cunha et al. (2015)
AN	*Campanularia* sp.	Low Island, Antarctica	FN424118	-	-	-	Penã Cantero et al. (2010)
SJ6_ARG	*Campanularia subantarctica*	La Mina, Puerto San Julián, Argentina	KM405575	KM405568	KM454911		Cunha et al. (2015)
USA	*Campanularia volubilis*	Monterey, USA	AY789804	AY789889	AY789739	-	Govindarajan et al. (2006); Lindner et al. (2011)
SJ1_ARG	*Campanulariidae* sp. indet.	La Mina, Puerto San Julián, Argentina	KM405576	-	KM454912		Cunha et al. (2015)
SJ3_ARG	*Campanulariidae* sp. indet.	La Mina, Puerto San Julián, Argentina	KM405577	-	KM454913		Cunha et al. (2015)
MA_USA	*Clytia* cf. *gracilis* sp.A	Woods Hole, USA	AY789812	AY789900	AY789751	-	Govindarajan et al. (2006); Lindner et al. (2011)
ME_USA	*Clytia* cf. *gracilis* sp.A	Maine, USA	DQ068061	DQ068054	DQ068051	-	Lindner et al. (2011)
BRA	*Clytia* cf. *gracilis* sp.B	São Sebastião, Brazil	DQ068062	DQ068055	DQ068052	-	Lindner et al. (2011)
USA	*Clytia* cf. *gracilis* sp.B	Beaufort, USA	AY789813	AY789901	AY789752	-	Govindarajan et al. (2006); Lindner et al. (2011)
BRA	*Clytia* cf. *gracilis* sp.C	São Sebastião, Brazil	DQ068063	DQ068056	DQ068053	-	Govindarajan et al. (2006); Lindner et al. (2011)
USA	*Clytia* cf. *gracilis* sp.D	Georges Bank, USA	AY789811	AY789899	AY789750	-	Govindarajan et al. (2006); Lindner et al. (2011)
1_BRA	*Clytia elsaeoswaldae*	São Sebastião, Brazil	DQ064793	DQ064800	DQ064796	-	Govindarajan et al. (2006); Lindner et al. (2011)

Code in tree	Species	Locality	GenBank Acession Numbers				Reference
			16S	COI	18S	28S	
2_BRA	*Clytia elsaeoswaldae*	São Sebastião, Brazil	DQ068064	-	-	-	Lindner et al. (2011)
1_CHI	*Clytia folleata*	China	-	JQ716211	-	-	Zhou et al. (2013)
2-6_CHI	*Clytia folleata*	China	JQ716051-55	KF962081-85	KF962213-17	-	Zhou et al. (2013); He et al. (2015)
IT	*Clytia gracilis*	Italy	AY346364	AY789898	AY789749	-	Govindarajan et al. (2006)
XMCG1-15_CHI	*Clytia gulangensis*	Xiamen Bay, China	KF962425-39	KF962086-2100	KF962218-32	KF962318-32	He et al. (2015)
NS	*Clytia hemisphaerica*	North Sea	AY789814	AY789902	AY789753	-	Govindarajan et al. (2006); Lindner et al. (2011)
FR	*Clytia hemisphaerica*	Villefranche-sur-mer, France	-	-	FJ550601	FJ550457	Leclère et al. (2009)
IT	*Clytia hummelincki*	S. Caterina, Italy	AY346363	AY789895	AY789745	-	Govindarajan et al. (2006); Lindner et al. (2011)
SA	*Clytia hummelincki*	South Africa	AY789809	AY789894	AY789744	-	Govindarajan et al. (2006)
-	*Clytia languida*	no precise information	-	GQ120064-65	-	-	Ortman et al. (2010)
USA	*Clytia linearis*	Beaufort, USA	AY789810	AY789897	AY789748	-	Govindarajan et al. (2006); Lindner et al. (2011)
IT	*Clytia linearis*	Torre Inserraglio, Italy	AY346362	-	AY789747	-	Govindarajan et al. (2006); Lindner et al. (2011)
BRA	*Clytia linearis*	São Sebastião, Brazil	DQ064791	-	DQ064794	-	Govindarajan et al. (2006); Lindner et al. (2011)
1_BRA	*Clytia noliformis*	São Sebastião, Brazil	DQ064792	-	DQ064795	-	Govindarajan et al. (2006); Lindner et al. (2011)
2_BRA	*Clytia noliformis*	São Sebastião, Brazil	-	-	EU272554	EU272611	Evans et al. (2008)
IT	*Clytia paulensis*	Otranto, Italy	AY346361	AY789896	AY789746	-	Govindarajan et al. (2006); Lindner et al. (2011)
XMCL1-3_CHI	*Clytia* sp.	China	KF962440-42	KF962101-3	KF962233-35	-	He et al. (2015)
KC1-5_CHI	*Clytia* sp.	China	JQ716046-50	JQ716206-10	KF962238-47	-	He et al. (2015)
AGC_USA	*Clytia* sp.	California, USA	AY512519	-	AF358074	-	Collins (2002); Collins et al. (2004)

Code in tree	Species	Locality	GenBank Acession Numbers				Reference
			16S	COI	18S	28S	
USA	*Clytia* sp.*	California, USA	AY800195	AY789903	AF358074	-	Collins et al. (2004); Govindarajan et al. (2006)
1-15_HR	*Clytia* sp.1	Helgoland Roads, North Sea	-	KC439960-74	-	-	Laakmann and Holst (2014)
1-4_HR	*Clytia* sp.2	Helgoland Roads, North Sea	-	KC439975-78	-	-	Laakmann and Holst (2014)
1-8_CHI	*Clytia xiamenensis*	Xiamen Bay, China	JQ716037-44	JQ716198-205	-	-	Zhou et al. (2013)
IC	*Gonothyraea loveni*	Sandgerdi, Iceland	FJ550480	-	FJ550547	FJ550404	Leclère et al. (2009)
USA	*Gonothyraea loveni*	Dennis, USA	AY789826	-	AY789765	-	Govindarajan et al. (2006)
FR	*Gonothyraea loveni*	Roscoff, France	AY789827	-	AY789766	-	Govindarajan et al. (2006)
FR	*Laomedea calceolifera*	Herquemoulin, France	FJ550504	-	FJ550590	FJ550447	Leclère et al. (2009)
USA	*Laomedea calceolifera*	Woods Hole, USA	AY789829	AY789914	AY789768	-	Govindarajan et al. (2006)
FR	*Laomedea flexuosa*	Roscoff, France	AY789823	AY789910	AY789762	-	Govindarajan et al. (2006)
IC	*Laomedea flexuosa*	Iceland	AY789824	AY789911	AY789763	-	Govindarajan et al. (2006)
WS	*Laomedea flexuosa*	White Sea	AY789825	AY789912	AY789764	-	Govindarajan et al. (2006)
USA	*Laomedea inornata*	Friday Harbor, USA	AY789822	-	AY789761	-	Govindarajan et al. (2006)
USA	*Obelia bidentata*	Beaufort, USA	AY789815	AY789904	AY789754	-	Govindarajan et al. (2006)
FR	*Obelia bidentata*	Utah Beach, France	FJ550503	-	FJ550589	FJ550446	Leclère et al. (2009)
NS	*Obelia bidentata*	North Sea	AY789816	AY789905	AY789755	-	Govindarajan et al. (2006)
IT	*Obelia dichotoma*	Otranto, Italy	AY789828	AY789913	AY789767	-	Govindarajan et al. (2006)
FR	*Obelia geniculata*	Roscoff, France	AY530359	AY530410	AY789769	-	Govindarajan et al. (2006)
NB_CAN	*Obelia geniculata*	New Brunswick, Canada	AY530344	AY530395	AY789770	-	Govindarajan et al. (2006)
IC	*Obelia geniculata*	Sandgerdi, Iceland	FJ550481	-	FJ550548	FJ550405	Leclère et al. (2009)
JP	*Obelia geniculata*	Japan	AY530335	AY530386	AY789771	-	Govindarajan et al. (2006)
NZ	*Obelia geniculata*	New Zealand	AY530378	AY530429	AY789772	-	Govindarajan et al. (2006)
NZ	*Obelia longissima*	Dunedin, New Zealand	AY789817	AY789906	AY789756	-	Govindarajan et al. (2006)
AN	*Obelia longissima*	Antarctic Peninsula	AY789821	AY789909	AY789760	-	Govindarajan et al. (2006)
IC	*Obelia longissima*	Sandgerdi, Iceland	AY789820	AY789908	AY789759	-	Govindarajan et al. (2006)

Code in tree	Species	Locality	GenBank Acession Numbers				Reference
			16S	COI	18S	28S	
WS	*Obelia longissima*	White Sea	AY789819	AY789907	AY789758	-	Govindarajan et al. (2006)
USA	*Obelia longissima*	Ryders Cove, USA	AY789818	-	AY789757	-	Govindarajan et al. (2006)
1-11_HR	*Obelia* sp.1	Helgoland Roads, North Sea	-	KC439979-89	-	-	Laakmann and Holst (2014)
1-5_HR	*Obelia* sp.2	Helgoland Roads, North Sea	-	KC439990-94	-	-	Laakmann and Holst (2014)
1-9_HR	*Obelia* sp.3	Helgoland Roads, North Sea	-	KC439995-440003	-	-	Laakmann and Holst (2014)
JGB1_BRA	*Orthopyxis caliculata*	João Gonçalves, Búzios, Brazil	KM405582	-	KM454918	-	Cunha et al. (2015)
JGB2_BRA	*Orthopyxis caliculata*	João Gonçalves, Búzios, Brazil	KM405583	-	KM454919	-	Cunha et al. (2015)
JGB4_BRA	*Orthopyxis caliculata*	João Gonçalves, Búzios, Brazil	KM405585	-	KM454921	-	Cunha et al. (2015)
PAB1_BRA	*Orthopyxis caliculata*	Paciência, Penha, Brazil	KM405586	KM405564	KM454922	-	Cunha et al. (2015)
PAB3_BRA	*Orthopyxis caliculata*	Paciência, Penha, Brazil	KM405587	KM405563	KM454923	-	Cunha et al. (2015)
PAB4_BRA	*Orthopyxis caliculata*	Paciência, Penha, Brazil	KM405588	KM405562	KM454924	-	Cunha et al. (2015)
PAB5_BRA	*Orthopyxis caliculata*	Paciência, Penha, Brazil	KM405589	KM405561	KM454925	-	Cunha et al. (2015)
GB_BRA	*Orthopyxis caliculata*	Grande Beach, Penha, Brazil	KM405581	KM405566	KM454917	-	Cunha et al. (2015)
BB_BRA	*Orthopyxis caliculata*	Bombas Beach, Bombinhas, Brazil	KM405579	-	KM454915	-	Cunha et al. (2015)
COB_BRA	*Orthopyxis caliculata*	Conceição, Bombinhas, Brazil	KM405580	-	KM454916	-	Cunha et al. (2015)
LB5_BRA	*Orthopyxis crenata*	Lázaro, Ubatuba, Brazil	KM405591	-	KM454927	-	Cunha et al. (2015)
LB8_BRA	*Orthopyxis crenata*	Lázaro, Ubatuba, Brazil	KM405592	-	KM454928	-	Cunha et al. (2015)
PAB7_BRA	*Orthopyxis crenata*	Paciência, Penha, Brazil	KM405594	KM405558	KM454931	-	Cunha et al. (2015)
LG_BRA	*Orthopyxis crenata*	Prainha, Laguna, Brazil	-	KM405560	KM454929	-	Cunha et al. (2015)
NZ	*Orthopyxis crenata*	Wellington, New Zealand	FJ550466	-	-	FJ550383	Leclère et al. (2009)
IT	*Orthopyxis everta*	Torre del Serpe, Italy	AY789793	AY789881	AY789728	-	Govindarajan et al. (2006)
IT	*Orthopyxis integra*	Italy	AY789799	AY789884	AY789734	-	Govindarajan et al. (2006)
1_USA	*Orthopyxis integra*	Aleutians, USA	AY789800	AY789885	AY789735	-	Govindarajan et al. (2006)

Code in tree	Species	Locality	GenBank Acession Numbers				Reference
			16S	COI	18S	28S	
2_USA	*Orthopyxis integra*	Friday Harbor, USA	AY789798	-	AY789733	-	Govindarajan et al. (2006)
IC	*Orthopyxis integra*	Sandgerdi, Iceland	AY789802	AY789887	AY789737	-	Govindarajan et al. (2006)
NZ	*Orthopyxis integra*	New Zealand	AY789801	AY789886	AY789736	-	Govindarajan et al. (2006)
USA	*Orthopyxis integra* CA sp.1	Monterey, USA	AY789796	-	AY789731	-	Govindarajan et al. (2006)
USA	*Orthopyxis integra* CA sp.2	Monterey, USA	AY789797	-	AY789732	-	Govindarajan et al. (2006)
MB2_BRA	*Orthopyxis mianzani*	Mel Island, Brazil	KM405603	KM405549	KM454939	-	Cunha et al. (2015)
MB3_BRA	*Orthopyxis mianzani*	Mel Island, Brazil	KM405604	KM405548	KM454940	-	Cunha et al. (2015)
MB4_BRA	*Orthopyxis mianzani*	Mel Island, Brazil	KM405605	KM405547	KM454941	-	Cunha et al. (2015)
MB5_BRA	*Orthopyxis mianzani*	Mel Island, Brazil	KM405606	KM405546	KM454942	-	Cunha et al. (2015)
FOB1_BRA	*Orthopyxis mianzani*	Mel Island, Brazil	KM405595	KM405557	KM454932	-	Cunha et al. (2015)
FOB2_BRA	*Orthopyxis mianzani*	Mel Island, Brazil	KM405596	KM405556	KM454933	-	Cunha et al. (2015)
FOB3_BRA	*Orthopyxis mianzani*	Mel Island, Brazil	KM405597	KM405555	KM454934	-	Cunha et al. (2015)
FOB4_BRA	*Orthopyxis mianzani*	Mel Island, Brazil	KM405598	KM405554	KM454935	-	Cunha et al. (2015)
FOB5_BRA	*Orthopyxis mianzani*	Mel Island, Brazil	KM405599	KM405553	KM454936	-	Cunha et al. (2015)
FOB6_BRA	*Orthopyxis mianzani*	Mel Island, Brazil	KM405600	KM405552	KM454937	-	Cunha et al. (2015)
FOB7_BRA	*Orthopyxis mianzani*	Mel Island, Brazil	KM405601	KM405551	-	-	Cunha et al. (2015)
BRA	*Orthopyxis sargassicola*	São Sebastião, Brazil	AY789795	AY789883	AY789730	-	Govindarajan et al. (2006); Lindner et al. (2011)
FB1_BRA	*Orthopyxis sargassicola*	Formosa, Aracruz, Brazil	KM405610	KM405542	KM454946	-	Cunha et al. (2015)
FB2_BRA	*Orthopyxis sargassicola*	Formosa, Aracruz, Brazil	KM405611	KM405541	-	-	Cunha et al. (2015)
PB2_BRA	*Orthopyxis sargassicola*	Padres, Aracruz, Brazil	KM405623	KM405530	KM454958	-	Cunha et al. (2015)
PB3_BRA	*Orthopyxis sargassicola*	Padres, Aracruz, Brazil	KM405624	KM405529	KM454959	-	Cunha et al. (2015)
PB4_BRA	*Orthopyxis sargassicola*	Padres, Aracruz, Brazil	KM405625	KM405528	KM454960	-	Cunha et al. (2015)
PB5_BRA	*Orthopyxis sargassicola*	Padres, Aracruz, Brazil	KM405626	KM405527	KM454961	-	Cunha et al. (2015)
PB6_BRA	*Orthopyxis sargassicola*	Padres, Aracruz, Brazil	KM405627	KM405526	KM454962	-	Cunha et al. (2015)
PB7_BRA	*Orthopyxis sargassicola*	Padres, Aracruz, Brazil	-	KM405525	KM454963	-	Cunha et al. (2015)

Code in tree	Species	Locality	GenBank Acession Numbers				Reference
			16S	COI	18S	28S	
PTY1_BRA	*Orthopyxis sargassicola*	Paraty, Brazil	KM405628	KM405524	KM454964	-	Cunha et al. (2015)
PTY3_BRA	*Orthopyxis sargassicola*	Paraty, Brazil	KM405630	KM405522	KM454966	-	Cunha et al. (2015)
PTY4_BRA	*Orthopyxis sargassicola*	Paraty, Brazil	KM405631	KM405521	KM454967	-	Cunha et al. (2015)
PTY5_BRA	*Orthopyxis sargassicola*	Paraty, Brazil	KM405632	KM405520	KM454968	-	Cunha et al. (2015)
RI_BRA	*Orthopyxis sargassicola*	Ratos Island, Paraty, Brazil	KM405633	KM405519	KM454969	-	Cunha et al. (2015)
MI_BRA	*Orthopyxis sargassicola*	Meros Island, Paraty, Brazil	KM405621	KM405532	KM454956	-	Cunha et al. (2015)
LB1_BRA	*Orthopyxis sargassicola*	Lázaro, Ubatuba, Brazil	KM405612	KM405540	KM454947	-	Cunha et al. (2015)
LB2_BRA	*Orthopyxis sargassicola*	Lázaro, Ubatuba, Brazil	KM405613	KM405539	KM454948	-	Cunha et al. (2015)
LB3_BRA	*Orthopyxis sargassicola*	Lázaro, Ubatuba, Brazil	KM405614	KM405538	KM454949	-	Cunha et al. (2015)
LB4_BRA	*Orthopyxis sargassicola*	Lázaro, Ubatuba, Brazil	KM405615	KM405537	KM454950	-	Cunha et al. (2015)
LB6_BRA	*Orthopyxis sargassicola*	Lázaro, Ubatuba, Brazil	KM405616	KM405536	KM454951	-	Cunha et al. (2015)
LB7_BRA	*Orthopyxis sargassicola*	Lázaro, Ubatuba, Brazil	KM405617	KM405535	KM454952	-	Cunha et al. (2015)
LB10_BRA	*Orthopyxis sargassicola*	Lázaro, Ubatuba, Brazil	KM405619	-	KM454954	-	Cunha et al. (2015)
LB11_BRA	*Orthopyxis sargassicola*	Lázaro, Ubatuba, Brazil	KM405620	KM405533	KM454955	-	Cunha et al. (2015)
SS_BRA	*Orthopyxis sargassicola*	Preta Beach, São Sebastião, Brazil	KM405634	KM405518	KM454970	-	Cunha et al. (2015)
CI1_BRA	*Orthopyxis sargassicola*	Campeche Island, Florianópolis, Brazil	KM405608	KM405544	KM454944	-	Cunha et al. (2015)
CI2_BRA	*Orthopyxis sargassicola*	Campeche Island, Florianópolis, Brazil	KM405609	KM405543	KM454945	-	Cunha et al. (2015)
USA	*Rhizocaulus verticillatus*	Aleutians, USA	AY789803	AY789888	AY789738	-	Govindarajan et al. (2006); Lindner et al. (2011)
1_NZ	*Silicularia rosea*	Bay of Islands, New Zealand	AY789792	-	AY789727	-	Govindarajan et al. (2006)
2_NZ	*Silicularia rosea*	Wellington, New Zealand	FJ550482	-	FJ550549	FJ550406	Leclère et al. (2009)
OUTGROUPS							
FR	*Calycella syringa*	Roscoff, France	FJ550460	-	FJ550519	FJ550372	Leclère et al. (2009)
USA	*Calycella syringa*	Woods Hole, USA	AY789833	AY789916	AY789776	-	Govindarajan et al. (2006)
-	*Mitrocomella niwai*	Devonport, New Zealand	FJ550473	-	FJ550536	FJ550392	Leclère et al. (2009)
-	*Phialella quadrata*	Whangaparoa, New Zealand	FJ550474	-	FJ550537	FJ550393	Leclère et al. (2009)

Code in tree	Species	Locality	GenBank Acession Numbers				Reference
			16S	COI	18S	28S	
-	*Eugymnanthea inquilina*	Taranto, Italy	AY789832	AY789915	AY789775	-	Govindarajan et al. (2006)
-	*Eucheilota maculata*	Luc-sur-mer, France	FJ550501	-	FJ550587	FJ550444	Leclère et al. (2009)
-	*Eirene viridula*	Luc-sur-mer, France	FJ550502	-	FJ550588	FJ550445	Leclère et al. (2009)
-	*Blackfordia virginica*	Northern California, USA	AY512516	-	AF358078	AY920800	Collins (2002); Collins et al. (2004); Collins et al. (2006)
-	*Aequorea aequorea*	Woods Hole, USA	AY512518	-	AF358076	EU305505	Collins (2002); Collins et al. (2004); Cartwright et al. (2008)
-	*Aequorea victoria*	not available	EU305469	-	AF358077	AY920799	Collins (2002); Collins et al. (2006); Cartwright et al. (2008)
-	*Opercularella pumila*	Woods Hole, USA	AY789834	-	AY789777	-	Govindarajan et al. (2006)
-	*Lovenella gracilis*	Wildwood Crest, USA	AY789830	-	AY789773	-	Govindarajan et al. (2006)
-	*Eucheilota bakeri*	California, USA	AY789831	-	AY789774	-	Govindarajan et al. (2006)
-	*Tiaropsidium kelsey*	not available	-	-	AF358079	-	Govindarajan et al. (2006)

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

	16S+COI+18S+28S											
	Dataset 1				Dataset 2				16S	COI	18S	28S
	16S	COI	18S	28S	16S	COI	18S	28S				
Number of taxa	181				80				220	202	182	88
Total number of characters	627	669	1829	3396	632	680	1891	3391	641	665	1840	3443
Number of informative characters (ML)	234	263	412	904	191	248	332	835	247	262	422	918
Model of nucleotide evolution (ML)	TPM3uf+I+G	GTR+I+G	TIM1+I+G	GTR+I+G	TPM2uf+I+G	TIM2+I+G	TrN+I+G	TIM2+I+G	GTR+I+G	GTR+I+G	TIM1+I+G	GTR+I+G
Log Likelihood (ML)	-64219.9805				-42777.3438				-11165.2990	-13700.0579	-13659.9397	-26737.0506
Number of informative characters (P)	1954				1736				264	262	467	1010
Number of most parsimonious trees (P)	725				8				182263	4000	16424	9
Minimum length (P)	13092				7922				2409	3082	2449	5501

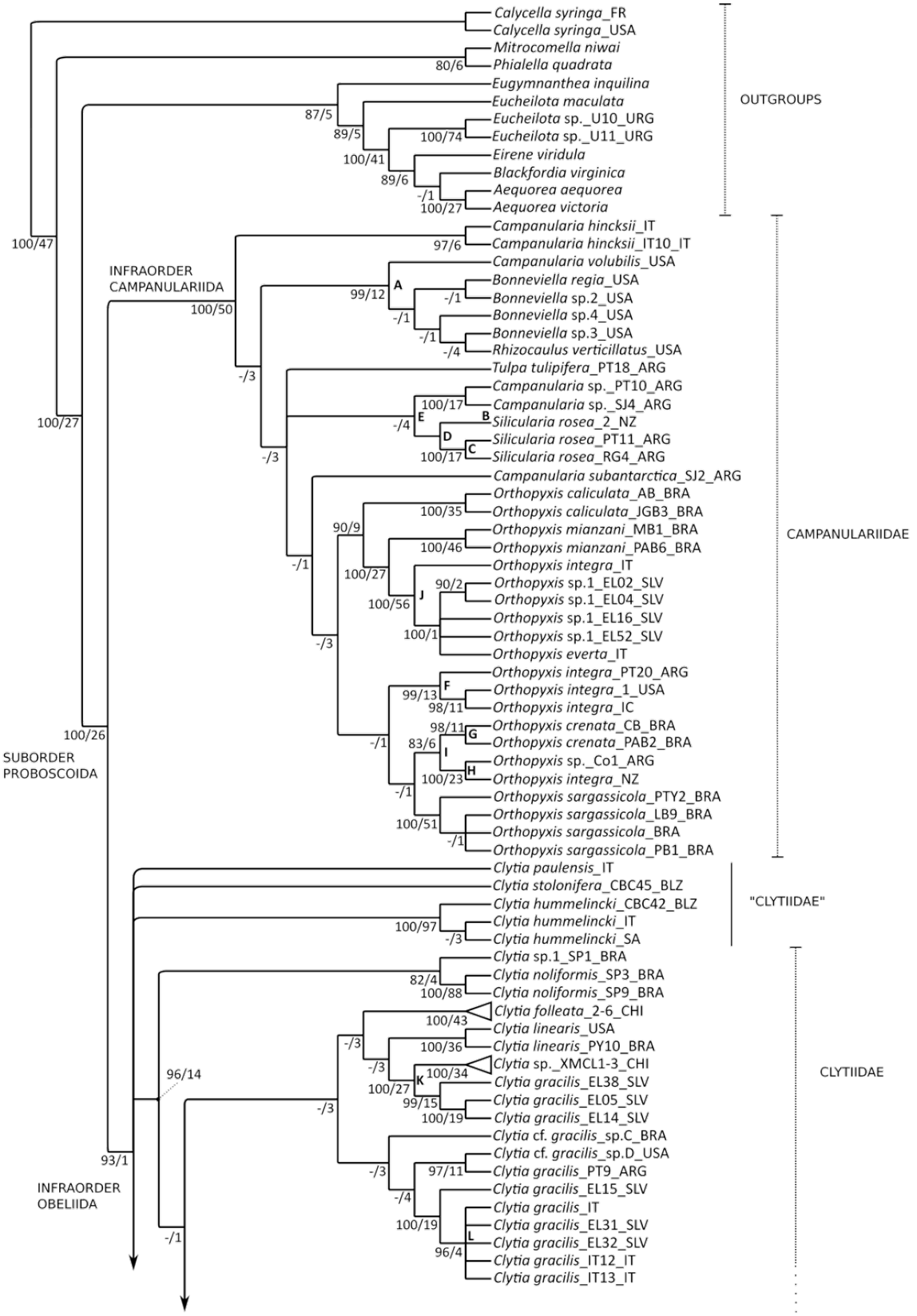

Figure 1 (continues on next page). Strict consensus of 725 most parsimonious trees based on 16S, COI, 18S and 28S data. Only taxa with sequences for at least 3 genes (Dataset 1) were analyzed. Bootstrap/Bremer supports shown for each node. Nodes without numbers (-) indicate support below 70 (Boostrap). Abbreviations in accordance with Tables 1-2.

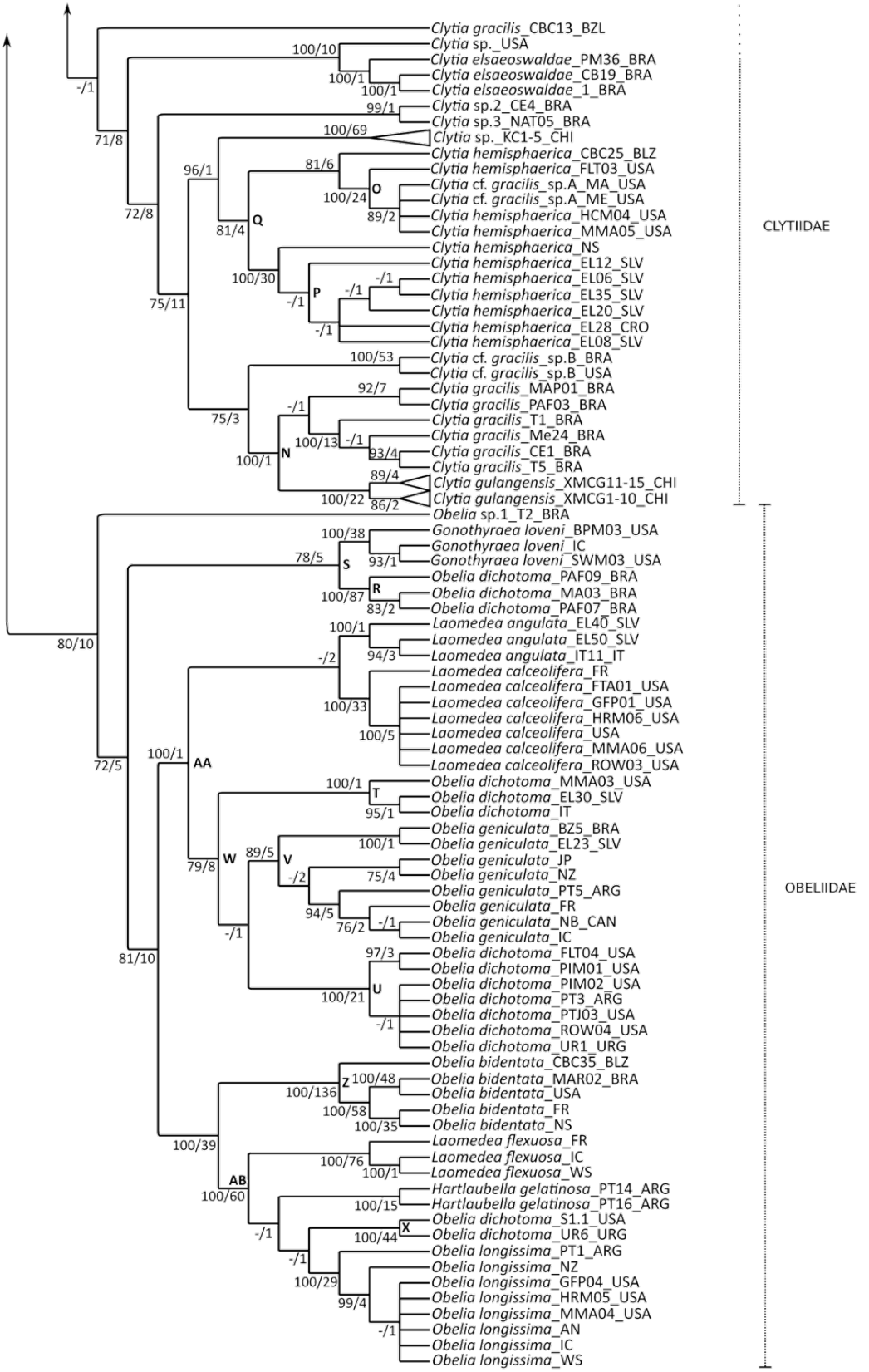

Figure 1 (cont.). Strict consensus of 725 most parsimonious trees based on 16S, COI, 18S and 28S data. Only taxa with sequences for at least 3 genes (Dataset 1) were analyzed. Bootstrap/Bremer supports shown for each node. Nodes without numbers (-) indicate support below 70 (Boostrap). Abbreviations in accordance with Tables 1-2.

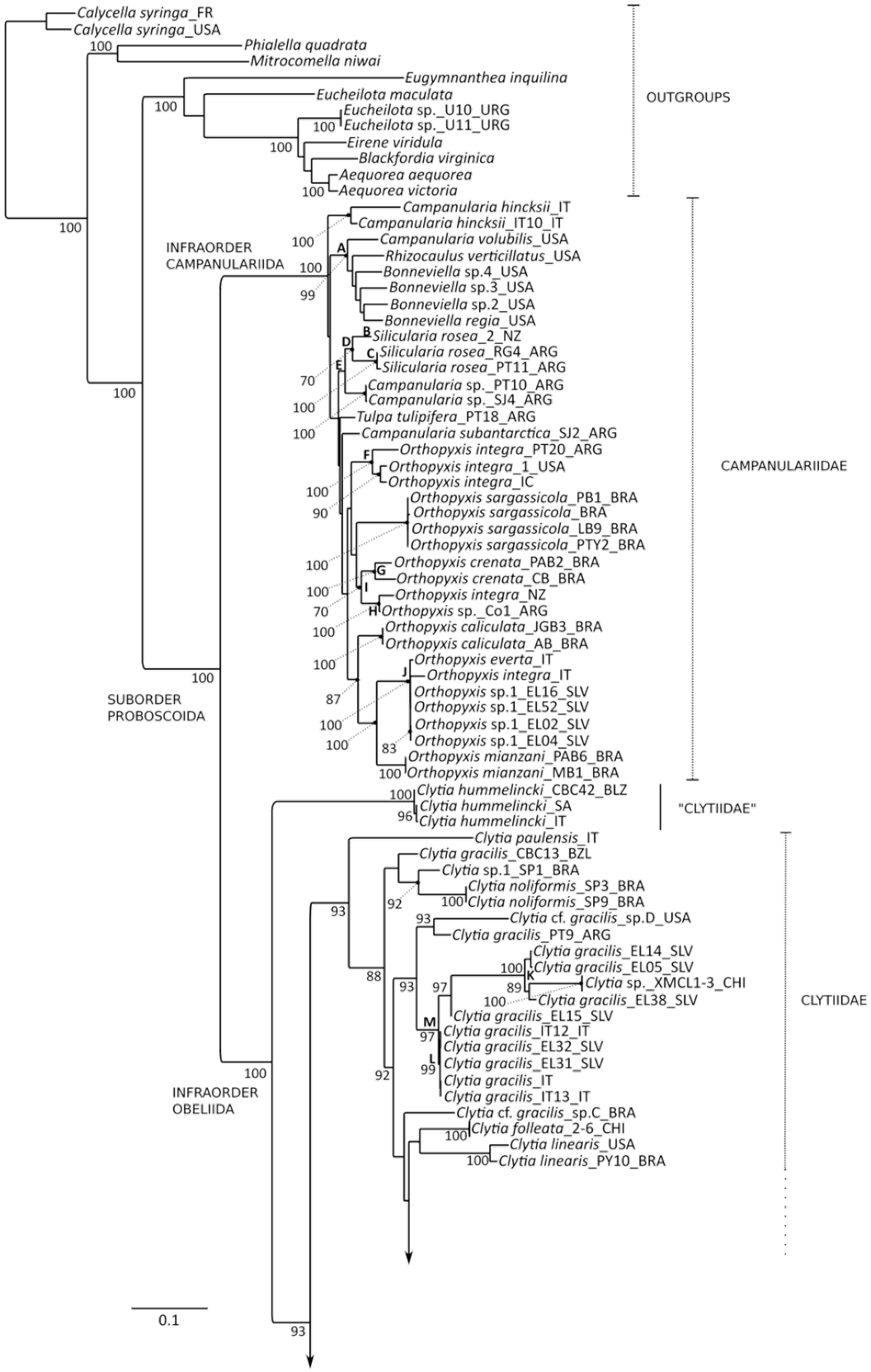

Figure 2 (continues on next page). Maximum likelihood tree based on 16S, COI, 18S and 28S data. Only taxa with sequences for at least 3 genes (Dataset 1) were analyzed. Bootstrap support shown for each node. Nodes without numbers (-) indicate support below 70. Abbreviations in accordance with Tables 1-2.

Figure 2 (cont.). Maximum likelihood tree based on 16S, COI, 18S and 28S data. Only taxa with sequences for at least 3 genes (Dataset 1) were analyzed. Bootstrap support shown for each node. Nodes without numbers (-) indicate support below 70. Abbreviations in accordance with Tables 1-2.

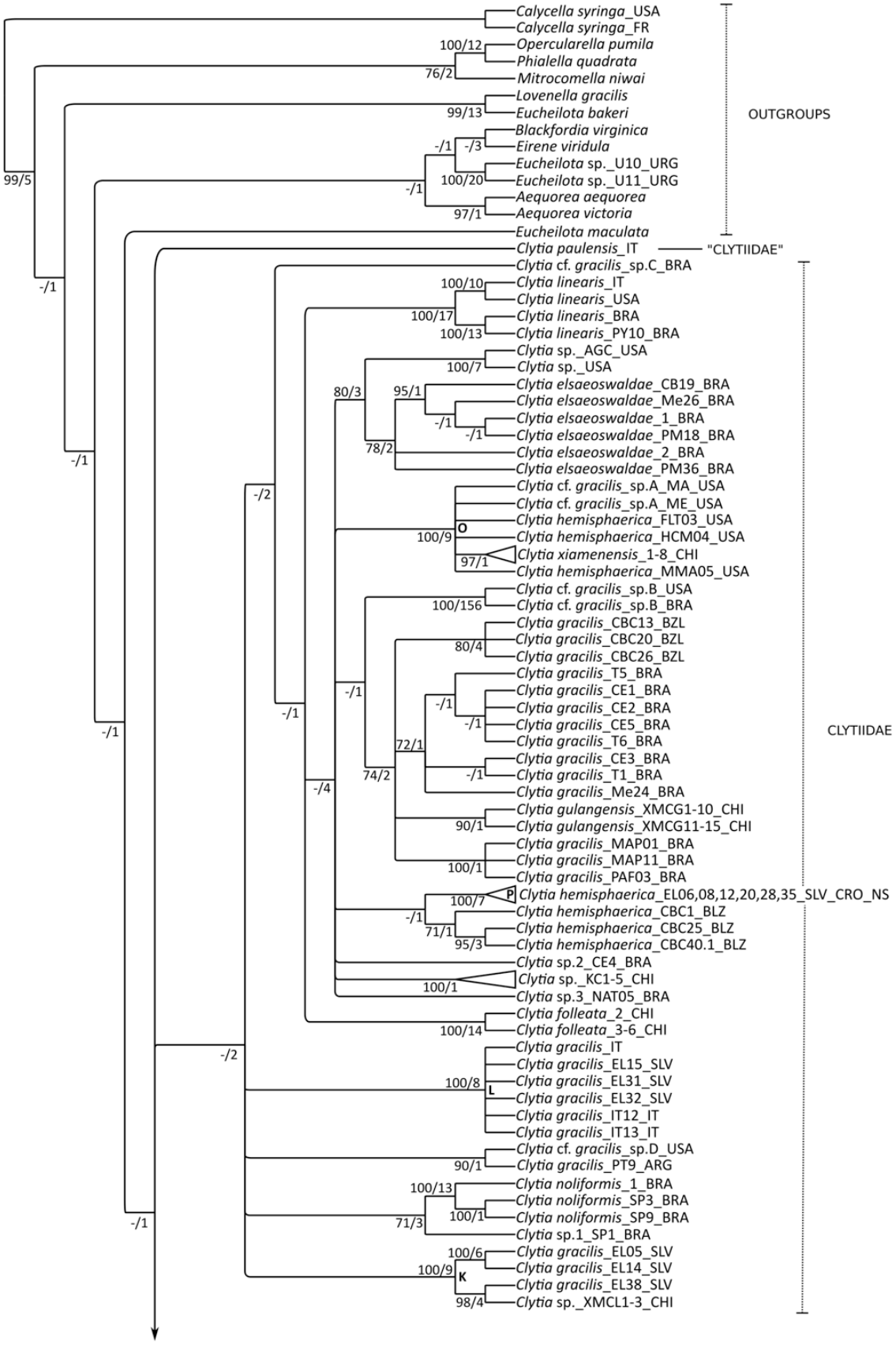

Figure 3 (continues on next page). Strict consensus of 182,263 most parsimonious trees based on 16S data. Bootstrap/Bremer supports shown for each node. Nodes without numbers (-) indicate support below 70 (Boostrap). Abbreviations in accordance with Tables 1-2.

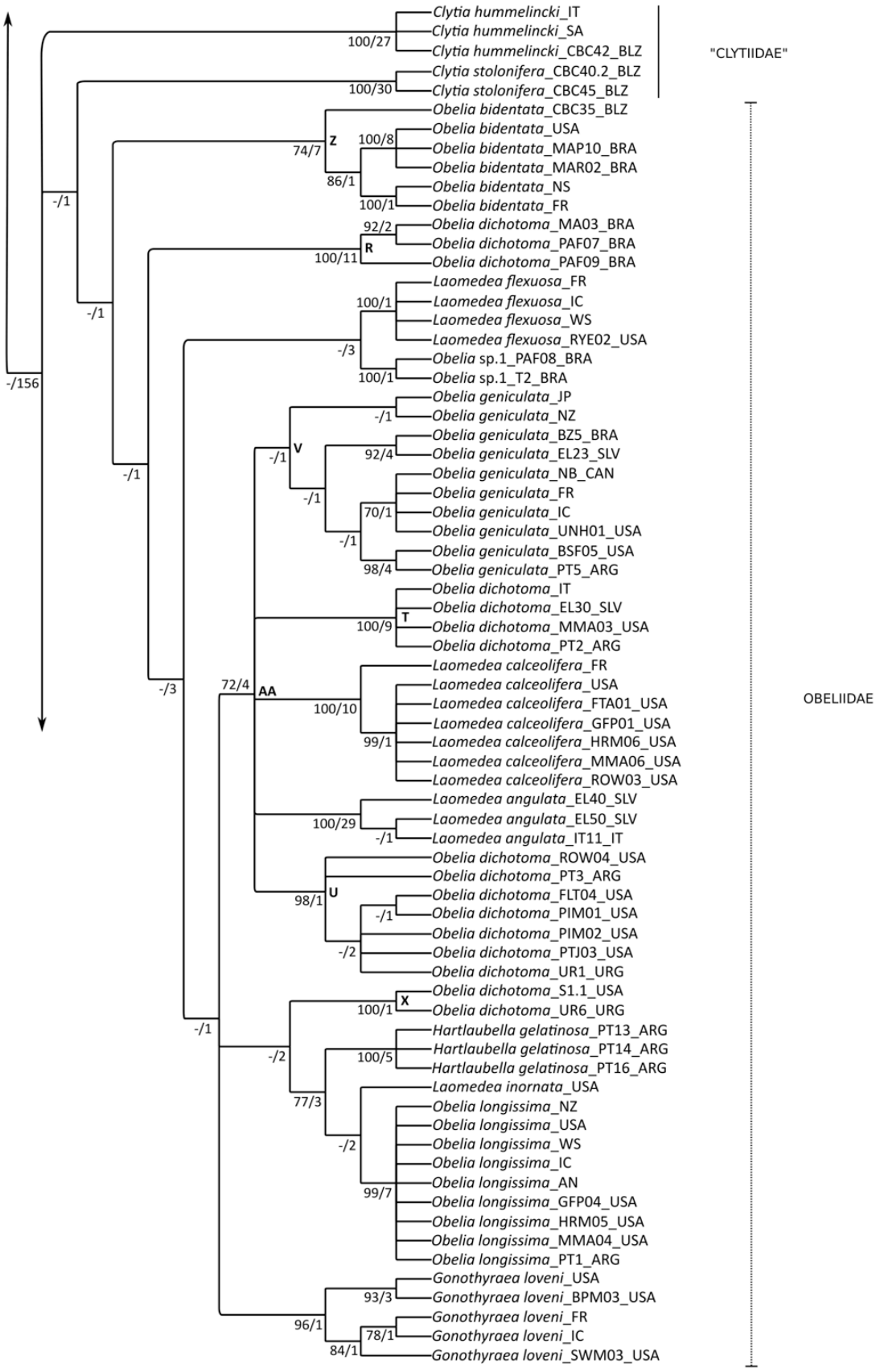

Figure 3 (continues on next page). Strict consensus of 182,263 most parsimonious trees based on 16S data. Bootstrap/Bremer supports shown for each node. Nodes without numbers (-) indicate support below 70 (Boostrap). Abbreviations in accordance with Tables 1-2.

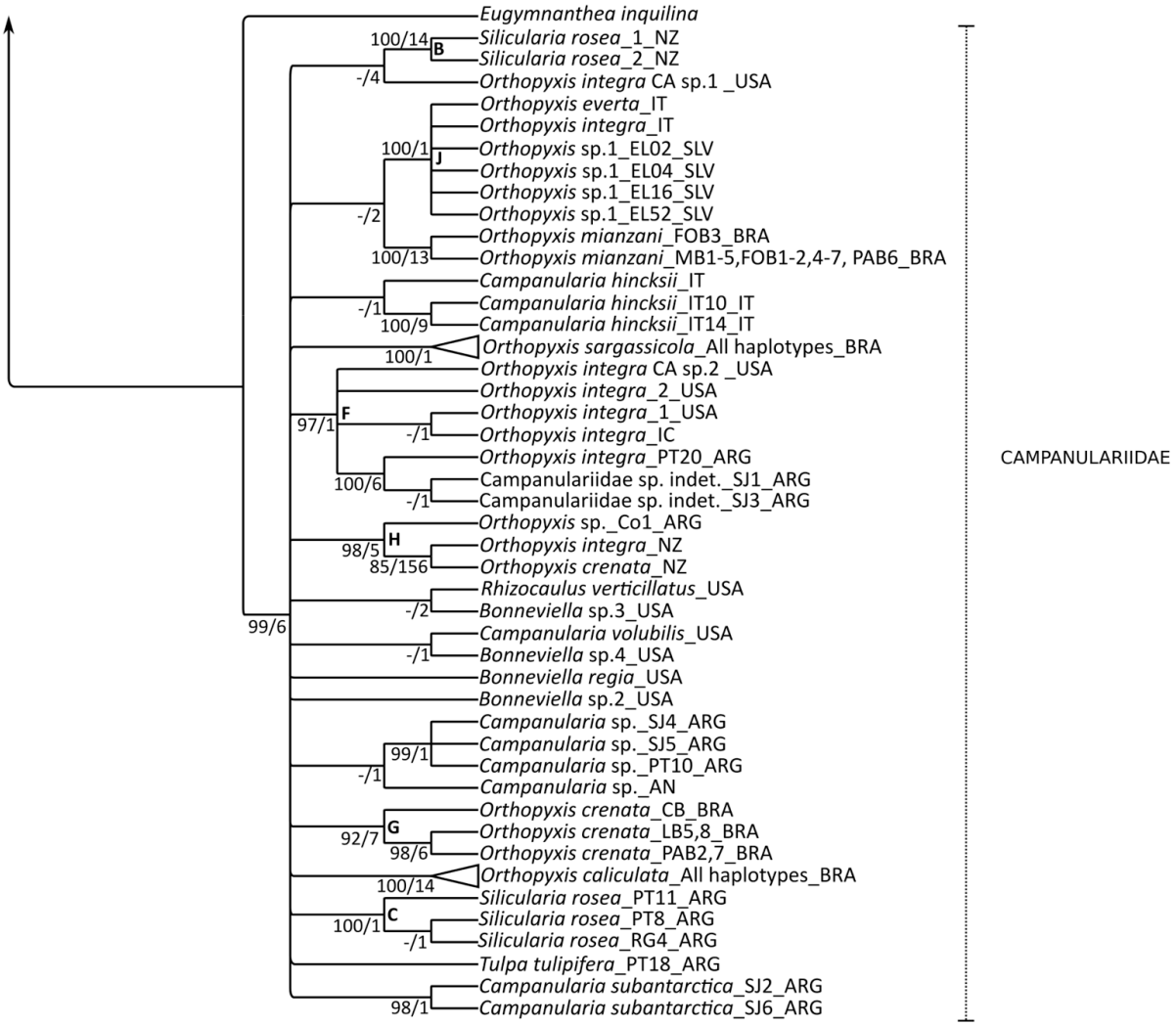

Figure 3 (cont.). Strict consensus of 182,263 most parsimonious trees based on 16S data. Bootstrap/Bremer supports shown for each node. Nodes without numbers (-) indicate support below 70 (Boostrap). Abbreviations in accordance with Tables 1-2.

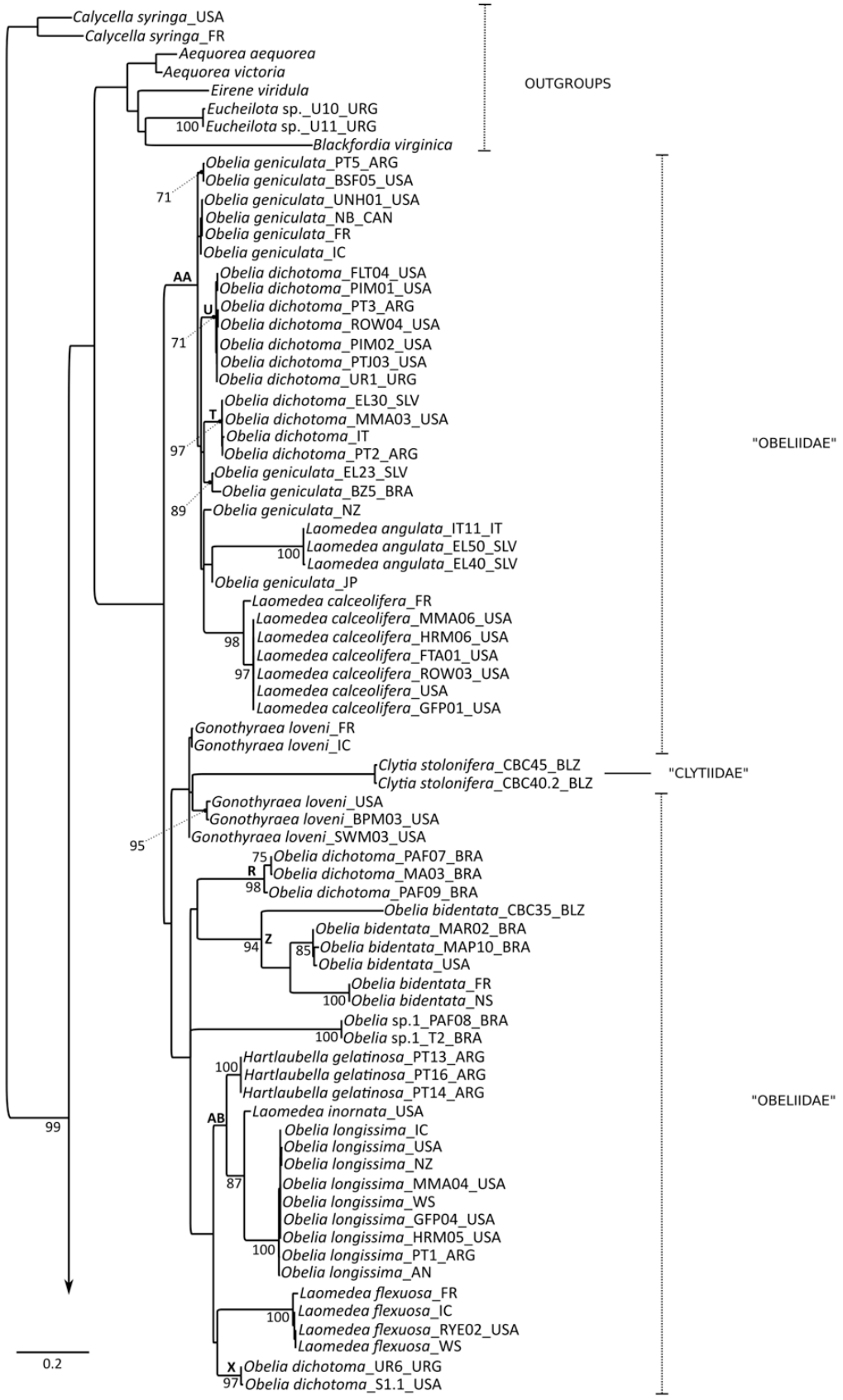

Figure 4 (continues on next page). Maximum likelihood tree based on 16S data. Bootstrap support shown for each node. Nodes without numbers (-) indicate support below 70. Abbreviations in accordance with Tables 1-2.

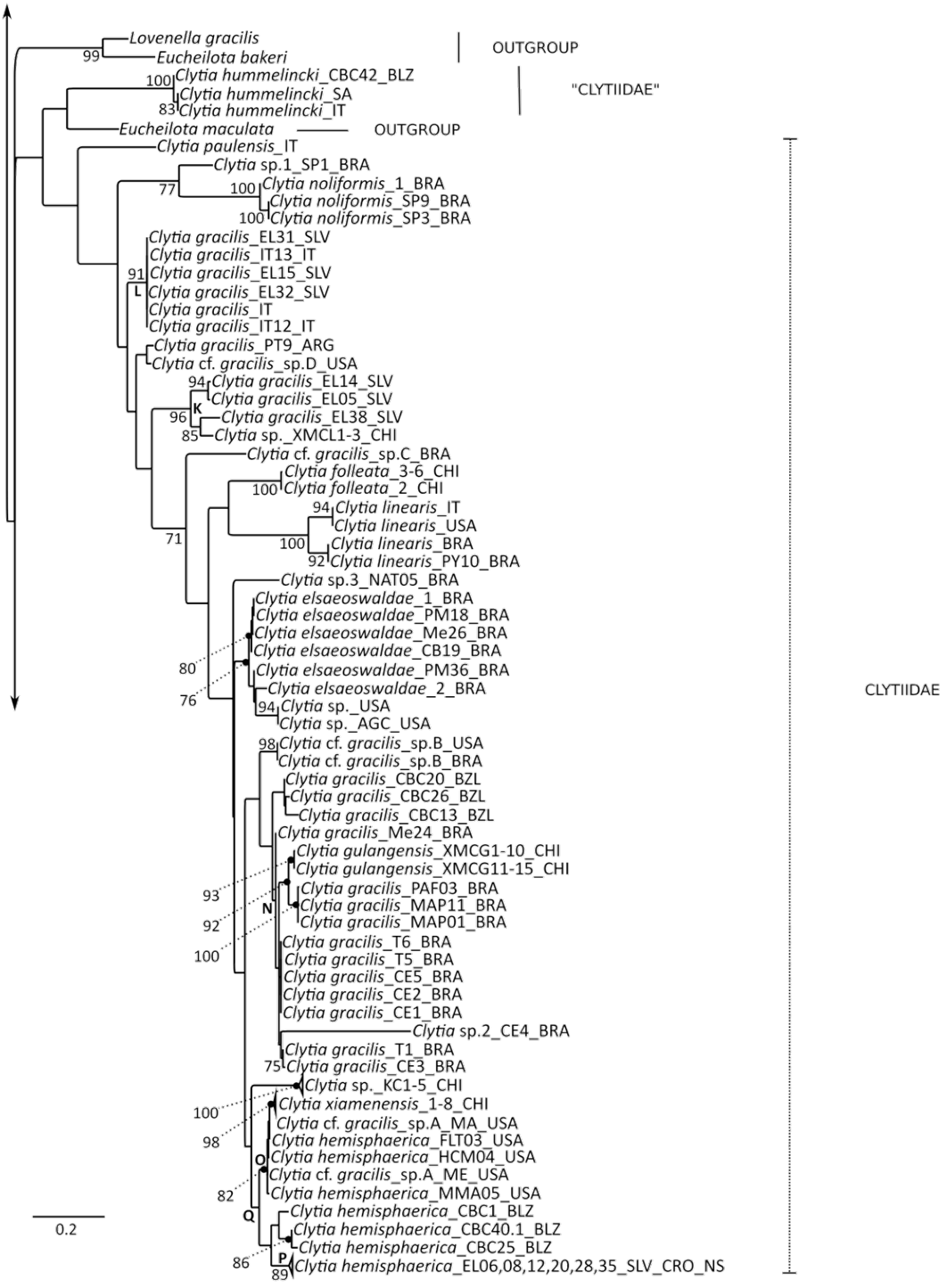

Figure 4 (continues on next page). Maximum likelihood tree based on 16S data. Bootstrap support shown for each node. Nodes without numbers (-) indicate support below 70. Abbreviations in accordance with Tables 1-2.

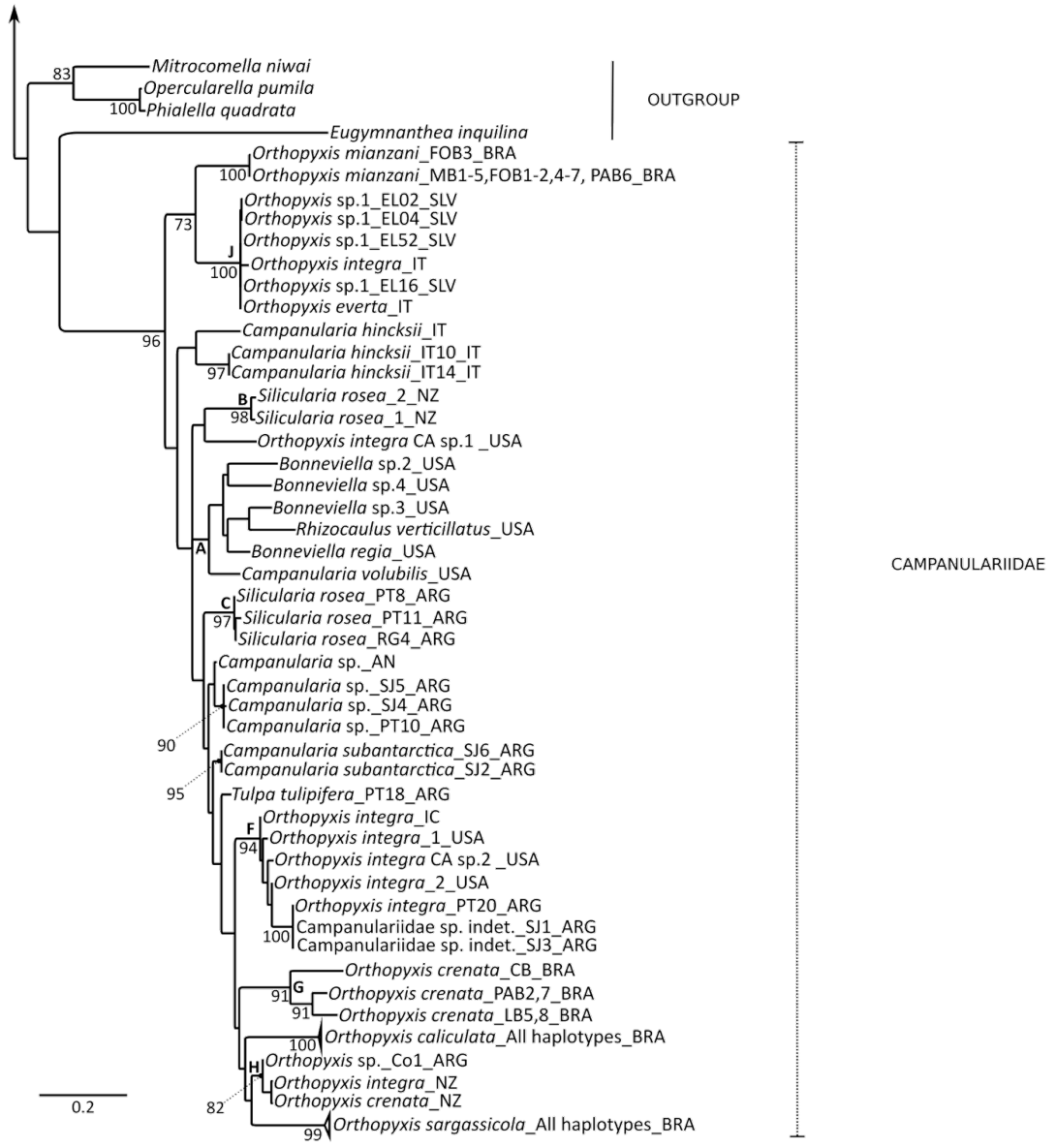

Figure 4 (cont.). Maximum likelihood tree based on 16S data. Bootstrap support shown for each node. Nodes without numbers (-) indicate support below 70. Abbreviations in accordance with Tables 1-2.

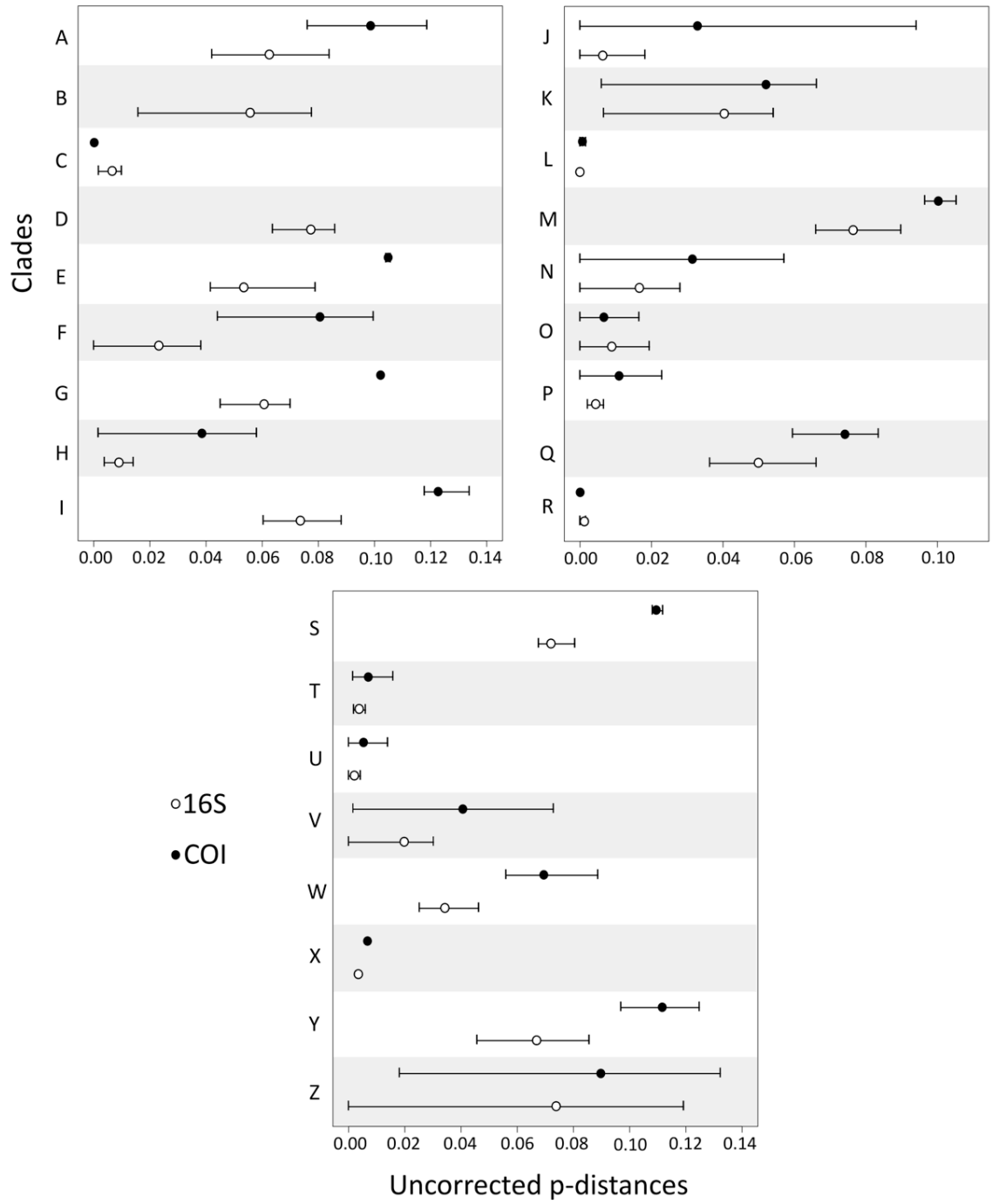

Figure 5. Intra and inter-clade uncorrected p distances based on 16S and COI data. Mean distances with minimum and maximum values are presented for each clade. Letters are in accordance with clades shown in Figures 1-4, S1-S4).

Table 4. Comparison between the resulted phylogenetic topologies (Figures 1-2) and genera proposed in previous classifications of Campanulariidae. Black cells – monophyletic in the topologies; white cells – non monophyletic in the topologies; * – synonymized genera; grey striped cells – genera not included in the corresponding classification. Numbers on the left of each cell indicate total number of species described; numbers on the right indicate total number of synonymized species. [A]as *Obelaria* Hartlaub, 1987; [B]as *Verticillina* Naumov, 1960; [C]as *Eulaomedea* Broch, 1909; [D]Bouillon (1985) provided diagnosis for all genera but did not describe any species; [E]more synonyms in Cornelius (1982); [F]more synonyms in Cornelius (1975) and (1982); [G]Hirohito (1995) only mentions one species for this genus, *Tulpa* (*Campanularia*) *speciosa*, which was not originally included in the genus; [H]the diagnosis of the genera were inferred based on diagnoses of the species, and the grey cell indicate the inference of monophyly is unclear; [I]no synonyms are provided in this study.

	Nutting (1915)			Ralph (1957)			Naumov (1969)			Millard (1975)			Cornelius (1975)			Cornelius (1982)		
Campanularia Lamarck, 1816	35		12	2		0	10		4	9		5				3		9
Orthopyxis L. Agassiz, 1862	5		2	4		1		*			*					2		23
Silicularia Meyen, 1834	6		3	1		7												
Tulpa Stechow, 1921		*		1		0												
Rhizocaulus Stechow, 1919		*					2[B]		0							1		1
Clytia Lamouroux, 1812	13		2	2		1		*		7		5				7		37
Obelia Péron & Lesueur, 1810	17		6	6		1	8		1	3		0	3		+80	3		+80
Laomedea Lamouroux, 1812		*						*		1[C]		0				5		8
Gonothyraea Allman, 1864	4		1	1		1		*		1		0				1		1
Hartlaubella Poche, 1914	1[A]		3		*			*								1		1

	Calder 1991			Bouillon (1985)[D]		Cornelius (1995)			Hirohito (1995)			Vervoort and Watson (2003)[H]			Bouillon et al. (2004)[I]	
Campanularia Lamarck, 1816	1		8			2		1[E]	9		1	2		2	3	
Orthopyxis L. Agassiz, 1862	1		1			1		2[E]		*		4		6	5	
Silicularia Meyen, 1834												1		11		
Tulpa Stechow, 1921									1[G]		0	1		0		
Rhizocaulus Stechow, 1919						1		0		*						
Clytia Lamouroux, 1812	6		40			4		3	9		2	7		1	10	
Obelia Péron & Lesueur, 1810	2		50			4		2[F]	4		5	7		5	4	
Laomedea Lamouroux, 1812						5		6		*					5	
Gonothyraea Allman, 1864						1		1		*		1		1	1	
Hartlaubella Poche, 1914						1		0				1		0	1	

Supplementary Material

Table S1. Primers and PCR conditions for DNA amplification.

Genes	Fragment size (approx.)	Primers	Reference	Primers Sequence (5' - 3')	PCR conditions
16S	640 bp	C&B1	Cunningham and Buss (1993)	TCGACTGTTTACCAAAAACATAGC	Init. Denat.: 94°C, 3min; 5 cycles: 94°C, 30sec; 45°C, 50sec; 72°C, 1min; 30 cycles: 95°C, 30sec; 50°C, 45sec; 72°C, 1 min; Fin. Ext.: 72°C, 7min; 10°C
		HYD_16SRN	This study	GTA GAT AGA AAC CTT CCT GTC T	
		rnl_f_jl	Designed by J. Lawley	GAC TGT TTA CCA AAG ACA TAG C	Init. Denat.: 95°C, 3min; 40 cycles: 95°C, 30seg; 52°C, 30sec; 72°C, 45sec; Fin. Ext.: 72°C, 10min; 10°C
		rnl_r_jl	Designed by J. Lawley	AAG ATA GAA ACC TTC CTG TC	
COI	680 bp	LCO1490	Folmer et al. (1994)	GGTCAACAAATCATAAAGATATTGG	Init. Denat.: 94°C, 2min; 10 cycles: 94°C, 30sec; 48°C, 1min; 72°C, 1min20sec; 25 cycles: 94°C, 30sec; 50°C, 40sec; 72°C, 1min20sec; Fin. Ext.: 72°C, 7min; 10°C
		HCO2198	Folmer et al. (1994)	TAAACTTCAGGGTGACCAAAAAATCA	
18S	1800 bp	A (F)	Medlin et al. (1988)	AAC CTG GTT GAT CCT GCC AGT	Init. Denat.: 94°C, 3min; 35 cycles: 95°C, 30sec; 57°C, 45sec; 72°C, 1min; Fin. Ext.: 72°C, 7min; 4°C
		L (R)	Apakupakul et al. (1999)	CCA ACT ACG AGC TTT TTA ACT G	
		C (F)	Apakupakul et al. (1999)	CGG TAA TTC CAG CTC CAA TAG	
		Y (R)	Apakupakul et al. (1999)	CAG ACA AAT CGC TCC ACC AAC	
		O (F)	Apakupakul et al. (1999)	AAG GGC ACC ACC AGG AGT GGA G	
		B (R)	Medlin et al. (1988)	TGA TCC TTC CGC AGG TTC ACC T	
	1600 bp	AF_cnidarian	Designed by R. Wilson	GTG GYA ATT CTA GAG CTA ATA CAT GCG	Init. Denat.: 95°C, 3min; 35 cycles: 95°C, 30sec; 50°C, 30sec; 72°C, 2min30sec; Fin. Ext.: 72°C, 7min; 8°C
		1800R18S	Redmond et al. 2007	GTT CAC CTA CYG AAA CCT TGT T	
		C_new cnidarian	Designed by R. Wilson	CAG CCG CGG TAA TTC CAG C	Internal primers used in cycle sequencing reactions
		O_new cnidarian	Designed by R. Wilson	GGT CCA GAC ATA GTA AGG ATT G	
		L_new cnidarian	Designed by R. Wilson	CCT RTT CCA TTA TTC CAT GCT C	
		R1_cnidarian	Designed by R. Wilson	CGG AAT TAA CCA GAC AAA TC	
28S	3200bp	F97	Evans et al. (2008)	CCY YAG TAA CGG CGA GT	Init. Denat.: 95°C, 3min; 35 cycles: 95°C, 30sec; 57°C, 30sec; 72°C, 2min30sec; Fin. Ext.: 72°C, 7min; 8°C
		R2084	Evans et al. (2008)	AGA GCC AAT CCT TTT CC	
		F1383	Evans et al. (2008)	GGA CGG TGG CCA TGG AAG T	
		R3264	Medina et al. (2001)	TTC YGA CTT AGA GGC GTT CGA	
		F635	Medina et al. (2001)	CCG TCT TGA AAC ACG GAC C	Internal primers used in cycle sequencing reactions

Genes	Fragment size (approx.)	Primers	Reference	Primers Sequence (5' - 3')	PCR conditions
		F1379	Medina et al. (2001)	GAC AGC AGG ACG GTG GYC ATG G	
		F1586	Medina et al. (2001)	GTG CAG ATC TTG GTD GNA GTA GCA AAT ATT C	
		F2076	Medina et al. (2001)	TAA CYT CGG GAW AAG GAT TGG CTC	
		F2766	Medina et al. (2001)	AGT TTG GCT GGG GCG GYA CA	
		R635	Medina et al. (2001)	GGT CCG TGT TTC AAG ACG G	
		R1411	Medina et al. (2001)	GTT GTT ACA CAC TCC TTA GCG G	
		R2084	Evans et al. (2008)	Same as above	
		R2800	Voigt et al. (2004)	GAG CTY RCC TTA GGA CAC CTG C	

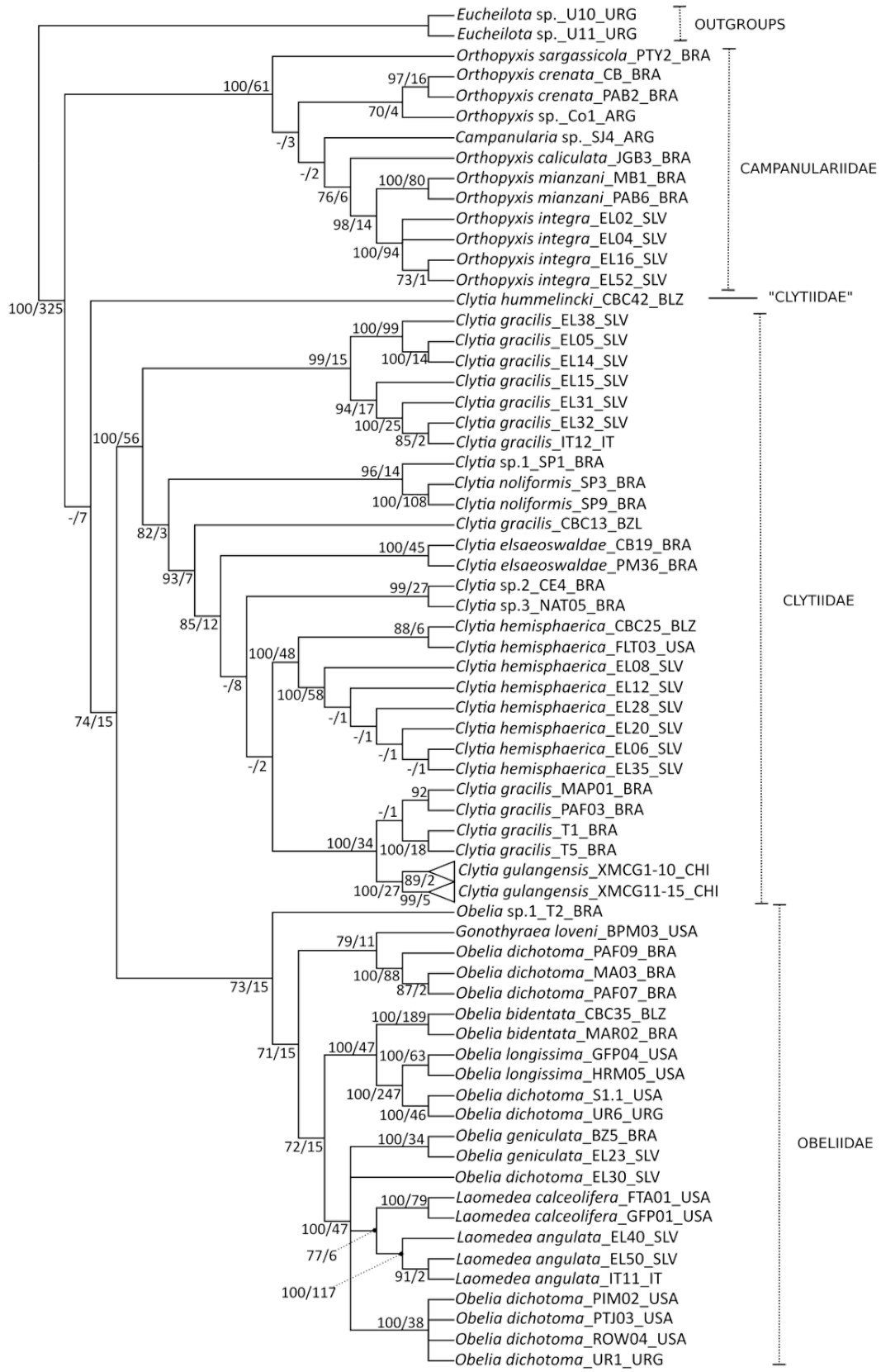

Figure S1. Strict consensus of 8 most parsimonious trees based on 16S, COI, 18S and 28S data. Only taxa with sequences for all 4 genes (Dataset 1) were analyzed. Bootstrap/Bremer supports shown for each node. Nodes without numbers (-) indicate support below 70 (Boostrap). Abbreviations in accordance with Tables 1-2.

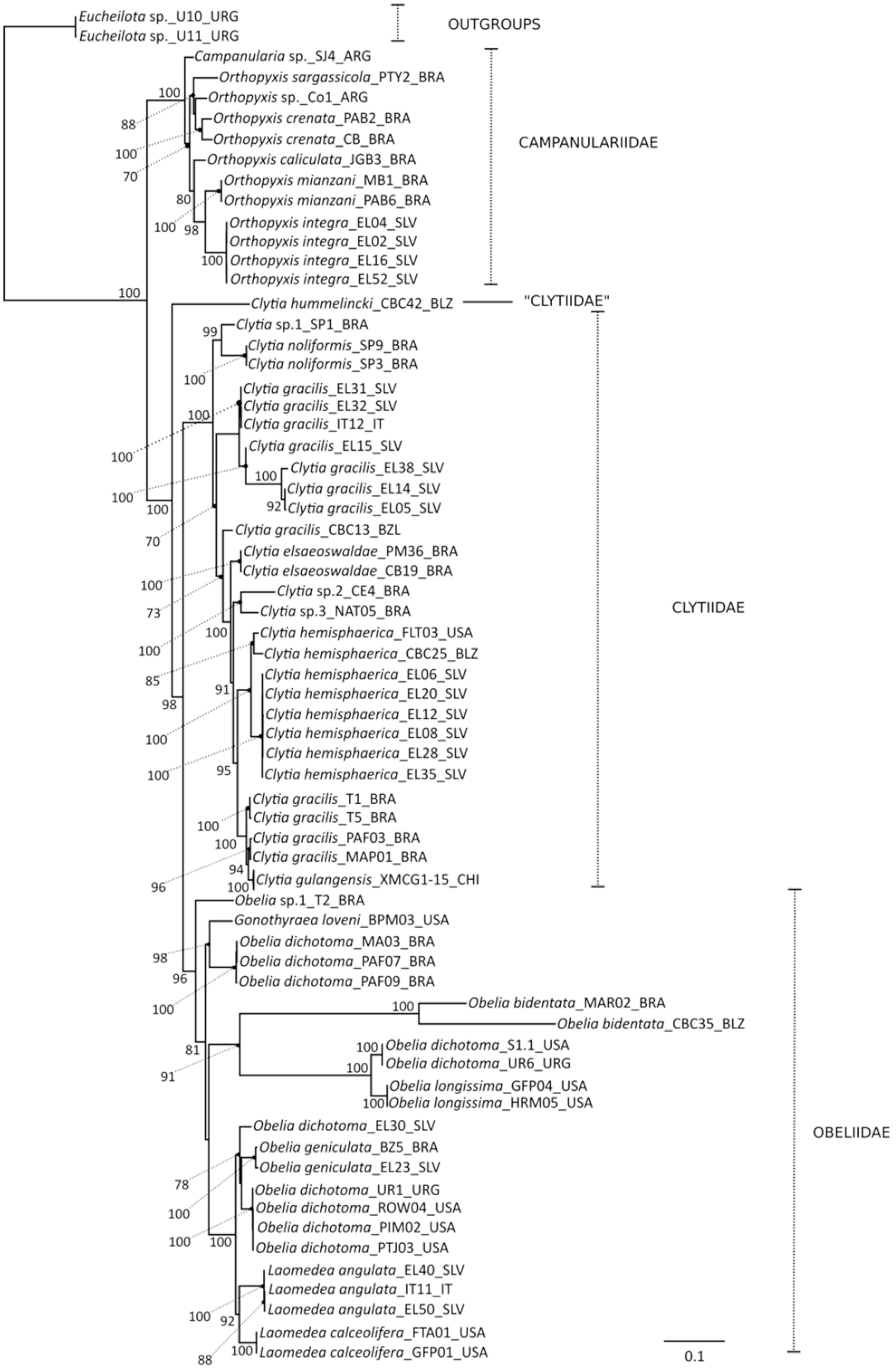

Figure S2. Maximum likelihood tree based on 16S, COI, 18S and 28S data. Only taxa with sequences for all 4 genes (Dataset 1) were included in this analysis. Bootstrap supports shown for each node. Nodes without numbers (-) indicate support below 70. Abbreviations in accordance with Tables 1-2.

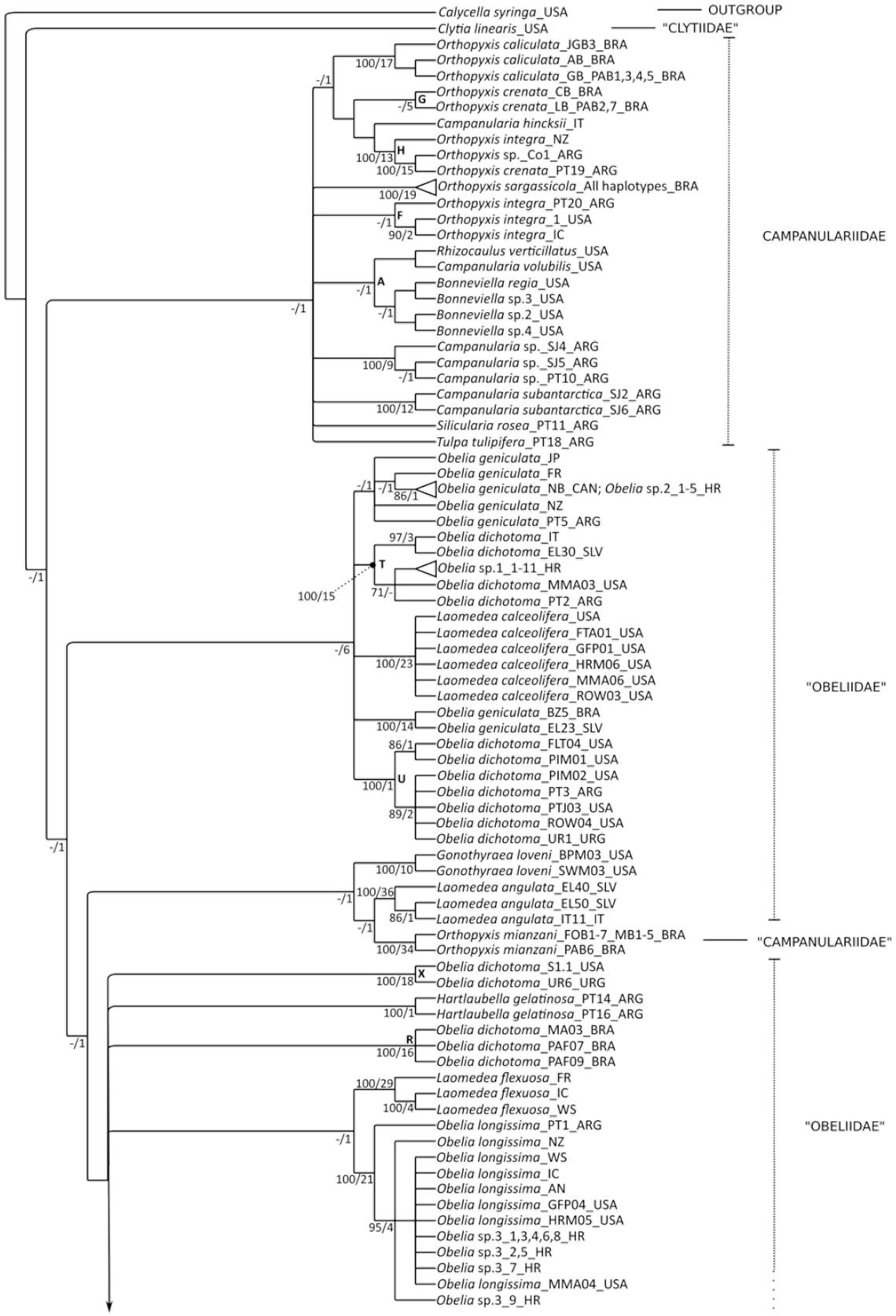

Figure S3 (continues on next page). Strict consensus of 4,000 most parsimonious trees based on COI data. Bootstrap/Bremer supports shown for each node. Nodes without numbers (-) indicate support below 70 (Boostrap). Abbreviations in accordance with Tables 1-2.

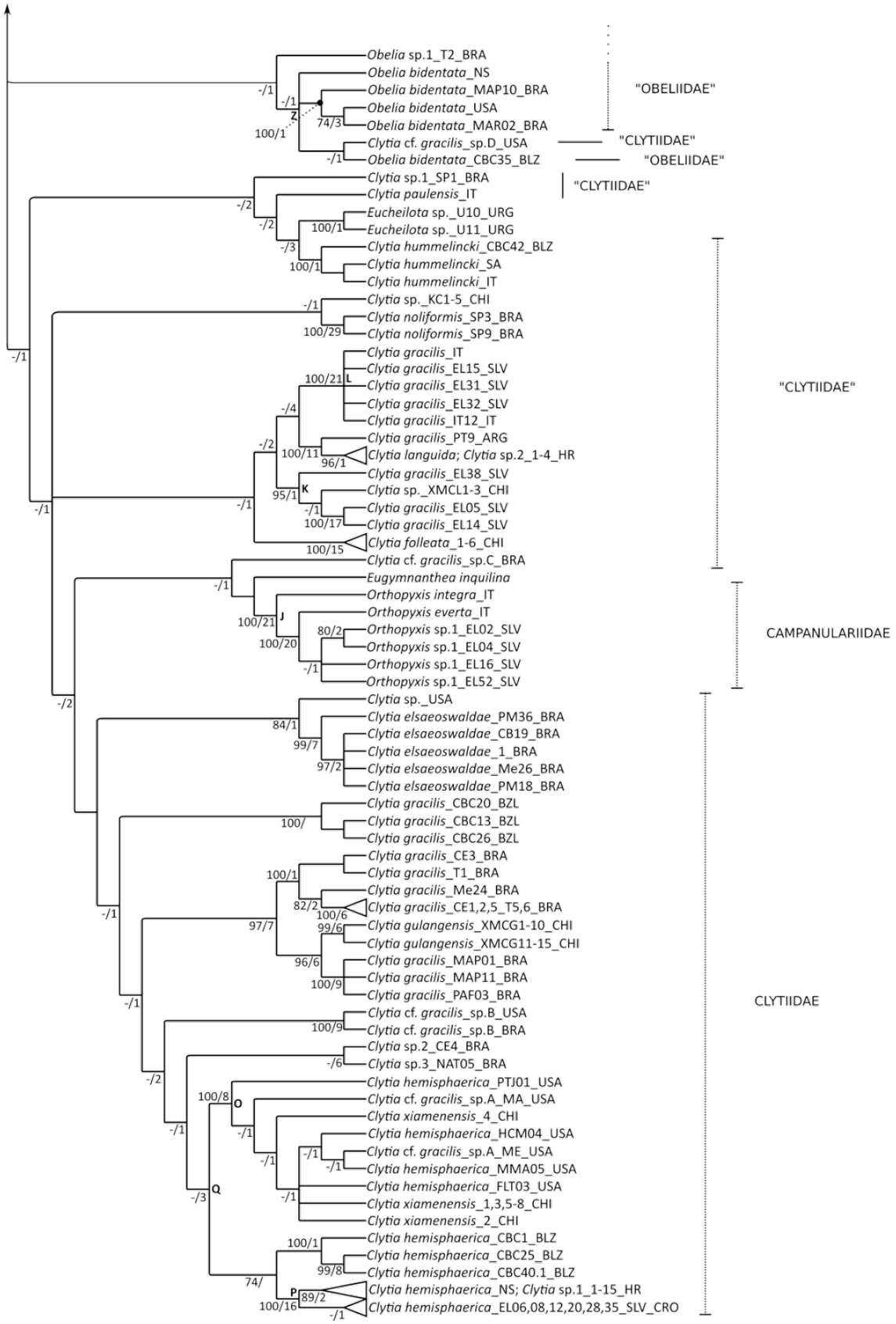

Figure S3 (cont.). Strict consensus of 4,000 most parsimonious trees based on COI data. Bootstrap/Bremer supports shown for each node. Nodes without numbers (-) indicate support below 70 (Boostrap). Abbreviations in accordance with Tables 1-2.

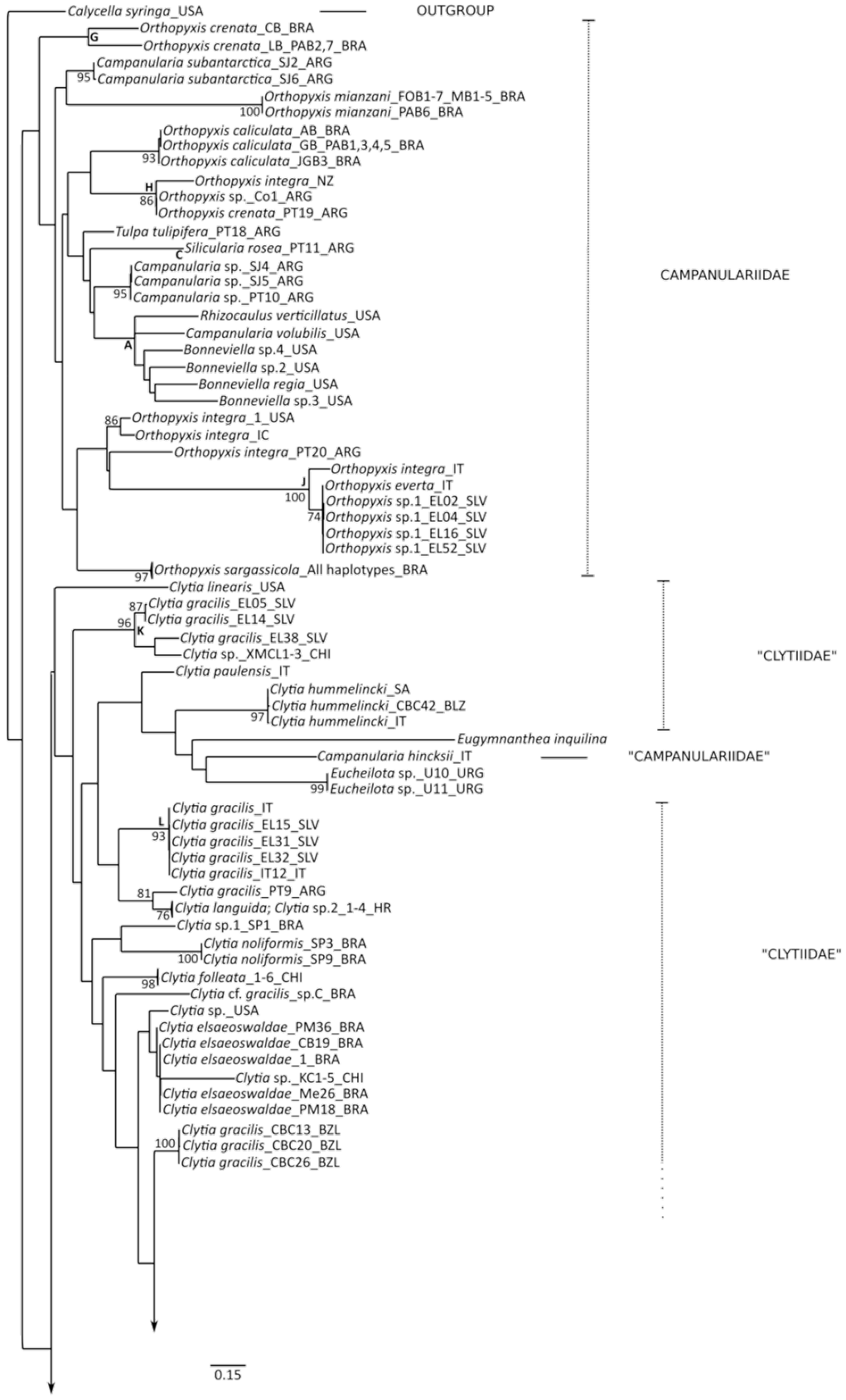

Figure S4 (continues on next page). Maximum likelihood tree based on COI data. Bootstrap supports shown for each node. Nodes without numbers (-) indicate support below 70. Abbreviations in accordance with Tables 1-2.

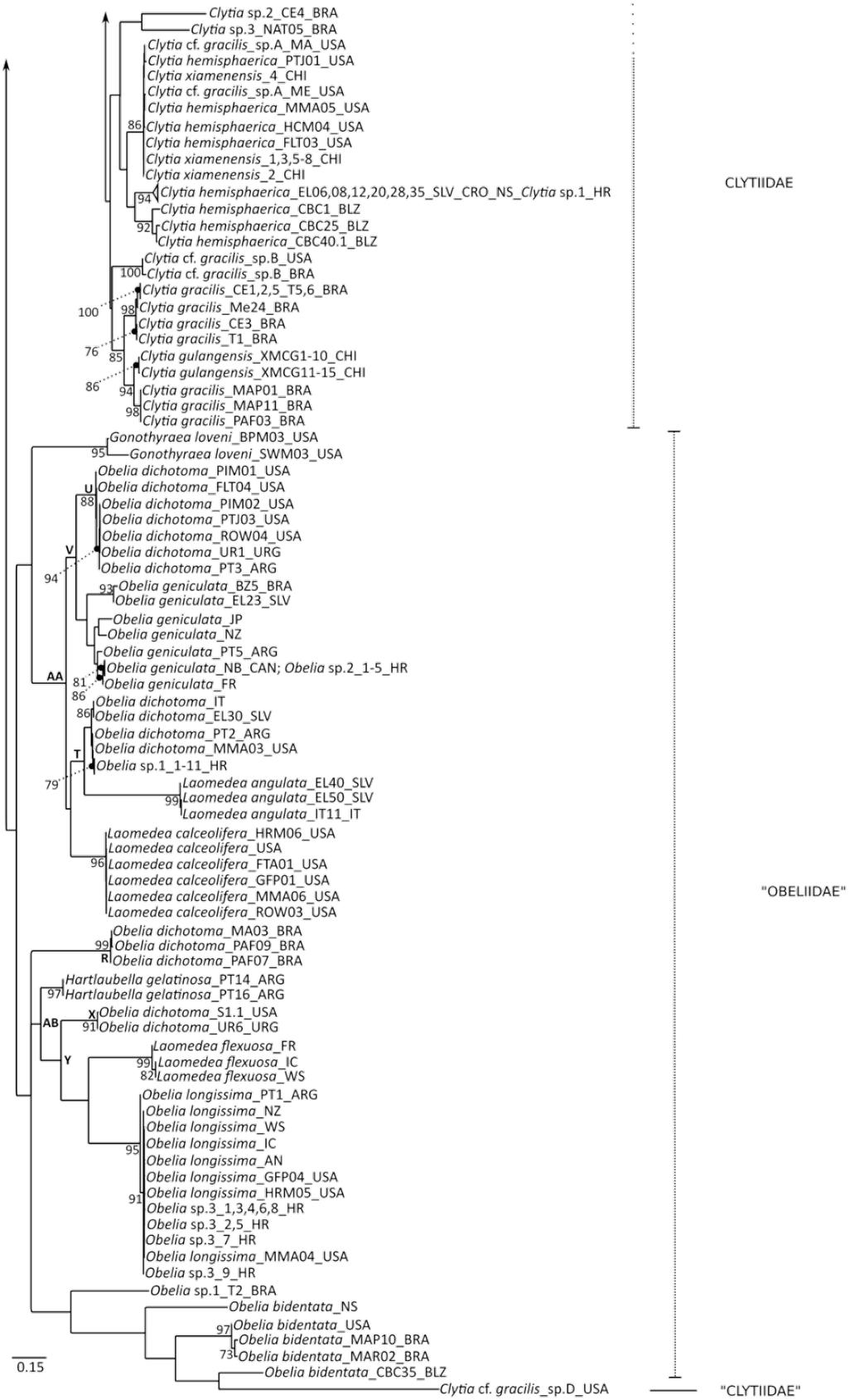

Figure S4 (cont.). Maximum likelihood tree based on COI data. Bootstrap supports shown for each node. Nodes without numbers (-) indicate support below 70. Abbreviations in accordance with Tables 1-2.

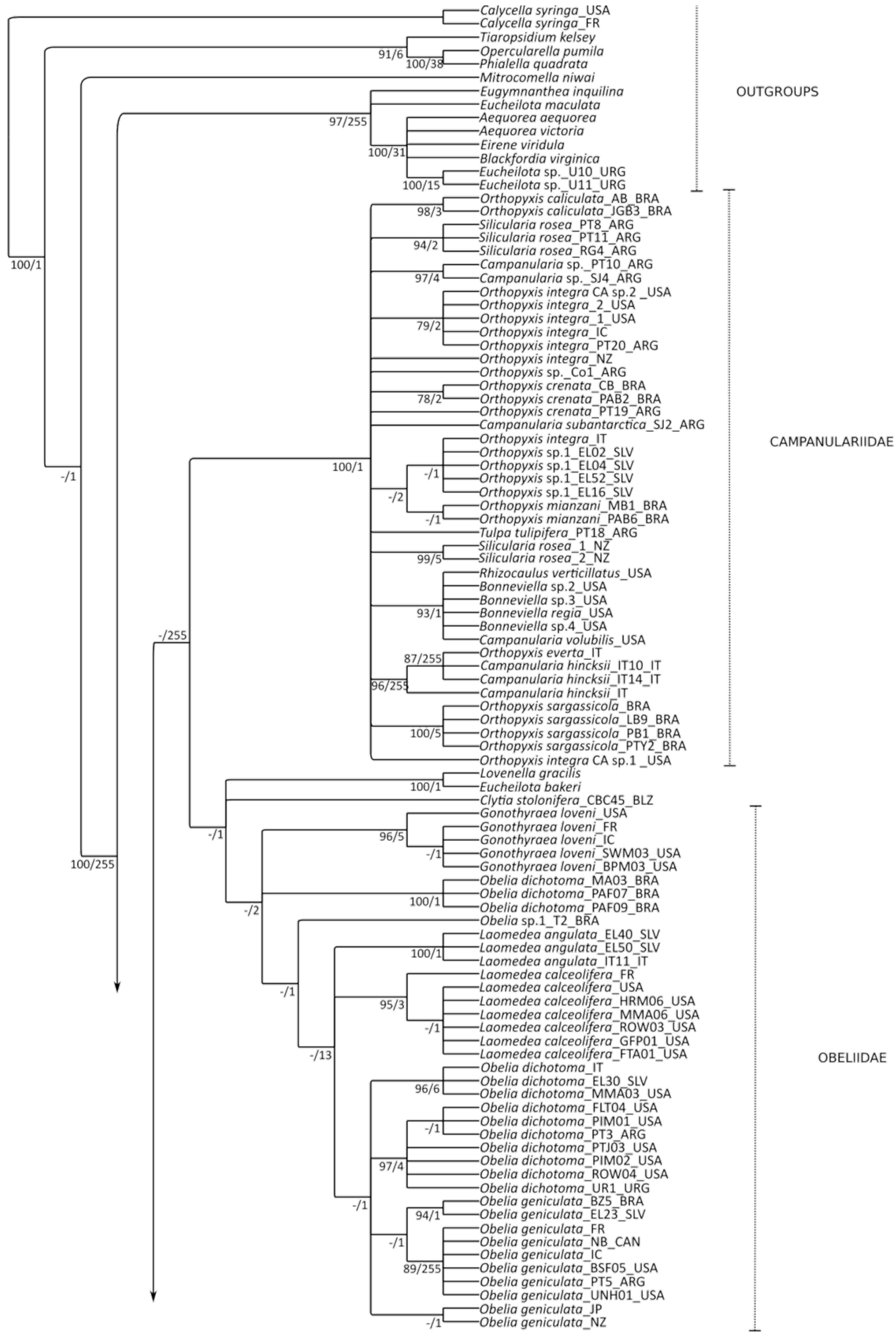

Figure S5 (continues on next page). Strict consensus of 16,424 most parsimonious trees based on 18S data. Bootstrap/Bremer supports are shown for each node. Nodes without numbers indicate support below 70 (Boostrap). Abbreviations in accordance with tables 1 and 2.

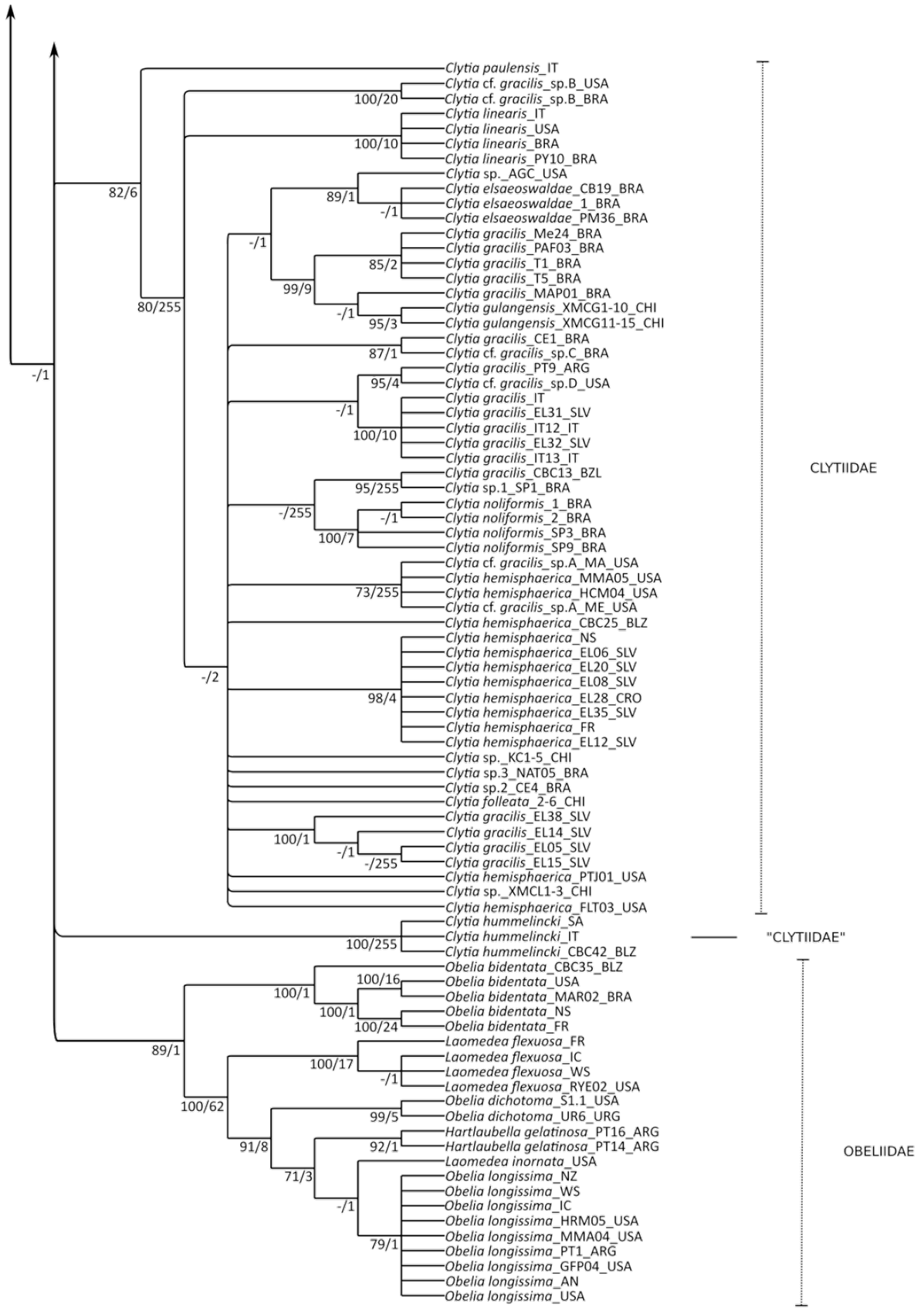

Figure S5 (cont.). Strict consensus of 16,424 most parsimonious trees based on 18S data. Bootstrap/Bremer supports shown for each node. Nodes without numbers (-) indicate support below 70 (Boostrap). Abbreviations in accordance with Tables 1-2.

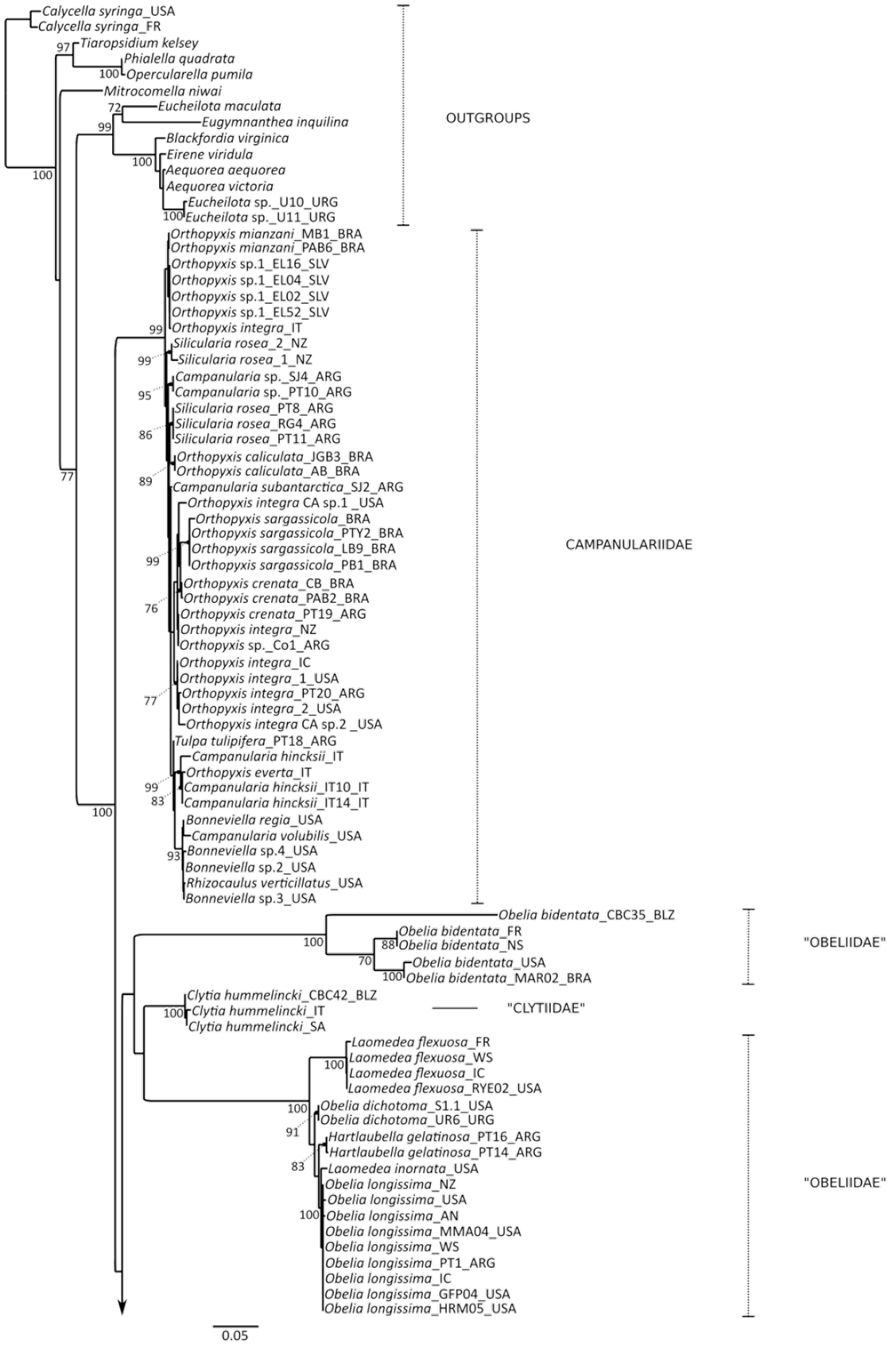

Figure S6 (continues on next page). Maximum likelihood tree based on 18S data. Bootstrap supports shown for each node. Nodes without numbers (-) indicate support below 70. Abbreviations in accordance with Tables 1-2.

Figure S6 (cont.). Maximum likelihood tree based on 18S data. Bootstrap supports shown for each node. Nodes without numbers (-) indicate support below 70. Abbreviations in accordance with Tables 1-2.

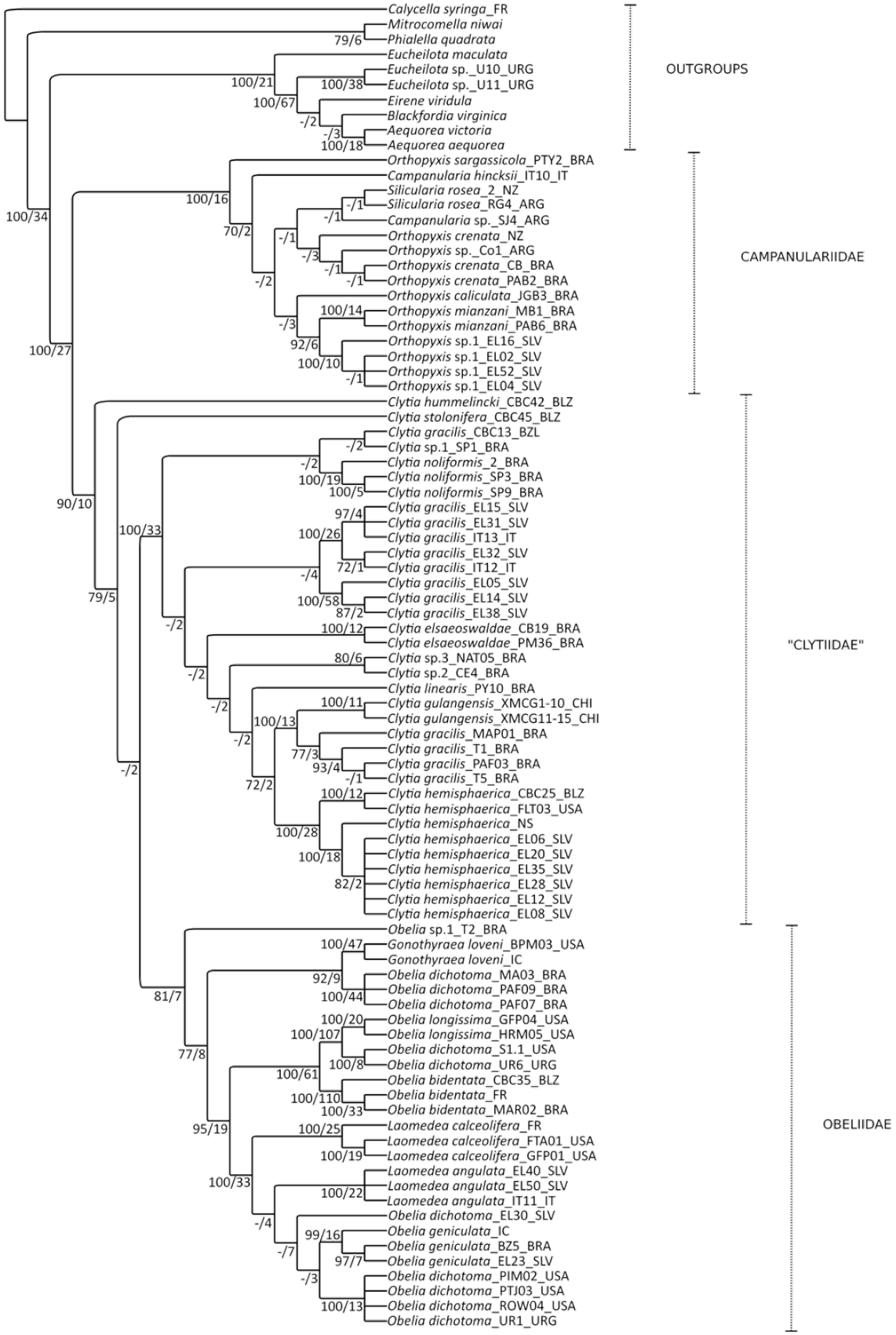

Figure S7. Strict consensus of 9 most parsimonious trees based on 28S data. Bootstrap/Bremer supports shown for each node. Nodes without numbers (-) indicate support below 70 (Boostrap). Abbreviations in accordance with Tables 1-2.

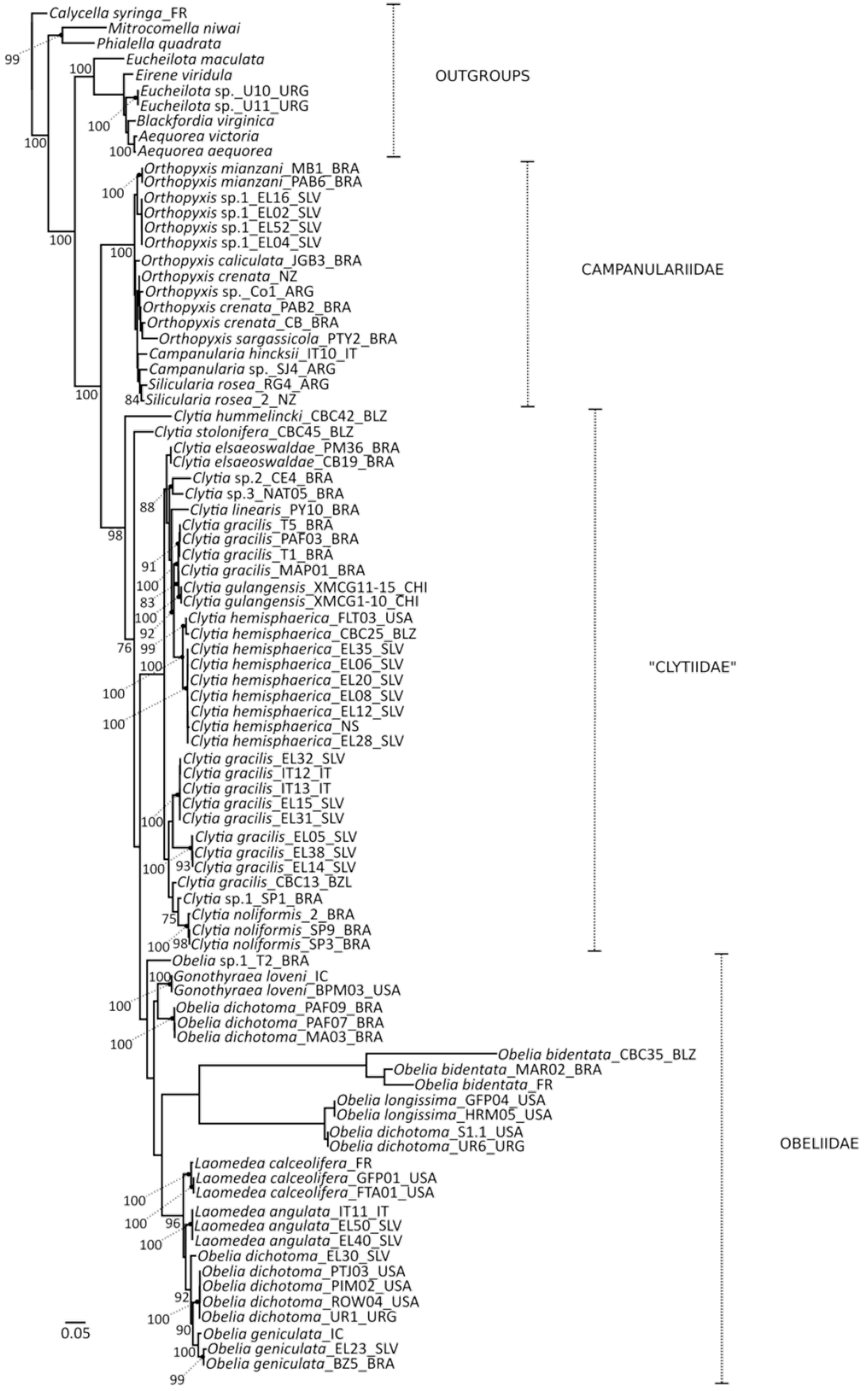

Figure S8. Maximum likelihood tree based on 28S data. Bootstrap supports shown for each node. Nodes without numbers (-) indicate support below 70. Abbreviations in accordance with Tables 1-2.

Capítulo 3

When morphometry meets taxonomy – morphological variation and species boundaries in Proboscoida (Cnidaria, Hydroza)

A.F. Cunha, A.G. Collins, A.C. Marques

Abstract

Species delimitation in marine taxa is often problematical given the wide intraspecific variation. Based on the phylogenetic relationships of the families Campanulariidae, Clytiidae and Obeliidae given in a previous study, we evaluated the morphological variation in this group, correlating morphometric patterns with species delimitation. In the Campanulariidae, differences in size were most important for the differentiation of *Bonneviella*, *Tulpa tulipifera* and *Rhizocaulus verticillatus*, while perisarc thickness reliably distinguished *Silicularia*, *Orthopyxis* and *Campanularia*. In the Clytiidae, the length and diameter of hydrothecae, height of hydrothecal cusps and perisarc thickness separated the species *Clytia linearis*, *C. elsaeoswaldae* and *C. noliformis*, among others. However, few characters reliably differentiated the lineages related to *C. gracilis* and *C. hemisphaerica*. In the Obeliidae, *Obelia geniculata* is distinctive for its higher perisarc thickness, while the diameter of the hydrothecae and length of pedicels may differentiate some species of *Laomedea*. *Obelia longissima* and lineages related to *O. dichotoma* were subtly distinguished by their morphometric patterns, showing a few differences in size and branching of colonies. The taxonomic implications of these results are broadly discussed. With a few exceptions, several species could be delimited based on their morphometric patterns, once morphological variation was investigated in a comparative manner. Surely, species delimitation in all the Hydrozoa will profit from studies that combine phylogenetic and morphometric patterns.

Resumo

A delimitação de espécies em táxons marinhos é frequentemente problemática devido a ampla variabilidade morfológica apresentada pelas espécies. Com base nas relações filogenéticas das famílias Campanulariidae Johnston, 1836, Clytiidae Cockerell, 1911 e Obeliidae Haeckel, 1879 apresentadas num estudo prévio, nós avaliamos a variação morfológica nesse grupo, correlacionando padrões morfométricos à delimitação de espécies. Nos Campanulariidae, diferenças de tamanho foram mais importantes para a diferenciação de

Bonneviella, *Tulpa tulipifera* e *Rhizocaulus verticillatus*, enquanto a espessura do perisarco diferencia espécies de *Silicularia*, *Orthopyxis* e *Campanularia*. Nos Clytiidae, comprimento e diâmetro da hidroteca, altura das cúspides e espessura do perissarco separa as espécies *Clytia linearis*, *C. elsaeoswaldae*, e *C. noliformis*, entre outras. Entretanto, poucos caracteres diferenciam de forma confiável as linhages relacionadas a *C. gracilis* e *C. hemisphaerica*. Nos Obeliidae, a espécie *Obelia geniculata* se distingue pelo seu perisarco mais espresso, enquanto o diâmetro da hidroteca e comprimento dos pedículos pode diferenciar algumas espécies de *Laomedea*. *Obelia longissima* e linhagens relacionadas a *O. dichotoma* não são claramente separadas pelos seus padrões morfométricos, mostrando algumas diferenças no tamanho e ramificação das colônias. As implicações taxonômicas desses resultados são discutidas amplamente. Com algumas poucas exceções, várias espécies foram delimitadas com base nos seus padrões morfométricos, uma vez que a variação morfológica foi investigada de uma forma comparativa. Certamente, a delimitação de espécies em Hydrozoa se beneficiará com estudos que combinem padrões filogenéticos e morfométricos.

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). Molecular approaches have helped to resolve incongruencies between molecular and morphological data, and many traditional characters considered to be diagnostic are usually uninformative (Forsman et al. 2009, 2010; Fukami et al. 2004, 2008; Budd et al. 2010; Pérez-Barros et al. 2015; DeBiasse & Hellberg 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; Willete et al. 2015). Contemporary studies use integrative approaches as taxonomic standards for species delimitation, but delimiting a species remains far from simple because population-level variation may commonly be mistaken as interspecific variation or vice-versa, and these patterns are often not easy to differentiate (e.g., Meroz-Fine et al. 2003; Prada et al. 2008; Forsman et al. 2010; Stefani et al. 2011; see also Schuchert 2014; Cunha et al. 2016a).

Species delimitation in the Hydrozoa involves similar problems (reviewed by Cunha et al. 2016a). A planktonic medusa stage and hydroid rafting widen the dispersal capabilities

of the species (Ralph 1961; Cornelius 1981a, 1992a; Boero & Bouillon 1993; Calder 1993), theoretically enhancing gene flow and supporting the traditional view that most hydrozoan species have nearly cosmopolitan distributions, and the group is considered to be one of the most widespread taxa among marine invertebrates (Cornelius 1981a, 1992b). However, molecular studies are showing that genetic diversity in Hydrozoa is higher than previously assumed (Schuchert 2005, 2014; Miglietta et al. 2007, 2009; Moura et al. 2008, 2011a, b, 2012; Postaire et al. 2016), and that samples from different, usually distant, localities likely represent their own lineages (Schuchert 2014; Cunha et al. 2016a). Molecular studies also revealed the 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), allowing the description of new species (e.g., Schierwater & Ender 2000; Cunha et al. 2015) as well as 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 object of important and recent taxonomic changes. Because of the supposed 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 the species and genera, and many nominal species were either split or lumped excessively (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; Linder et al. 2011; Cunha et al. 2015). Additionally, their phylogenetic relationships and extensive morphological diversity have shown that campanulariids can be included in three families within the Proboscoida Broch, 1910: Campanulariidae Johnston, 1836, Clytiidae Cockerell, 1911, and Obeliidae Haeckel, 1879 (cf. Maronna et al. 2016).

Several traditional morphological diagnostic characters have proven to be uninformative to delimit species and genera in these families (Cunha et al. 2016b). 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 promising to delimit 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. 2016a).

This study aimed 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. 2016b) were analyzed based on their phylogenetic relationships, integrating morphological, morphometric and molecular delimitation of species of Campanulariidae, Clytiidae and Obeliidae.

Material and Methods

Taxonomic sampling

The specimens used in this study are the same vouchers that were included in the molecular analysis by Cunha et al. (2016b), with a few exceptions (Table 1). Therefore, materials used for DNA analyses were also used in morphometric analyses whenever possible, and the results of the two studies can be directly compared. Also, vouchers of previously published sequences, deposited in the National Museum of Natural History (USNM), Smithsonian Institution (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 Table 1) were studied, enhancing taxon sampling and comparisons to delimit specific lineages.

Morphological and morphometric analyses

We studied morphological characters of the polyps of species of Proboscoida, similarly to the previous phylogeny of the group (Cunha et al. 2016b). Exceptions were a few medusae (Zhou et al. 2013; He et al. 2015; Laakmann & Holst 2014) that we were not able to study. 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; He et al. 2015; Laakmann & Holst 2014). 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. Finally, several trophosomal characters were studied, based on species descriptions (e.g., Millard 1975; Cornelius 1982, 1995; Calder

1991; Migotto 1996; Lindner & Migotto 2002; Lindner et al. 2011), but additional characters were also included (Table 2).

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). Colonies from different species were evaluated, as well as from different populations of a single species. Additionally, some collection lots had two to three polyps of the same colony measured, allowing intracolony comparisons (see Table 1 for further details). Morphometric data were analyzed with a Principal Component Analysis (PCA, see Legendre & Legendre 1998; Borcard et al. 2011) using the *vegan* package (Oksanen et al. 2015) for the R programming language (R Core Team 2015). The PCA was conducted on a correlation matrix, and distance biplots were generated for a graphical view of the results. The analysis comprised different levels of comparison within each family, including the complete dataset as well as subsets of data, in order to have a more detailed investigation of patterns of morphological variation in these groups.

Results

Family Campanulariidae

The PCA with all species shows that several measurements of length and diameter (LH, DHMa, DHMe, DHB, LP, TLT) are responsible for the largest amount of variation in the data (PC1), while the presence of cusps (NC, HCMax, HCMin) and the perisarc thickness (PHMe, PPMe, PSS) explain another direction of high variation among species (PC2, Figure 1A, B). Differences in size separate *Tulpa tulipifera*, *Bonneviella* sp., and *B. regia* from other Campanulariidae, based on their larger hydrothecae and pedicels (Figure 1A, C; Figure 2). Similarly, *Rhizocaulus verticillatus* can be distinguished from *Campanularia* and *Orthopyxis* by its larger hydrothecae and trophosome (Figure 1D, E), especially when these characters are directly compared among the species (Figure 3). Differences in size are not only informative to delimit different genera, but are considerably variable among *Bonneviella* species (Table 3, Figure 2). The dimensions of the specimens of *B. regia* (USNM 1106181) are congruent with the type material of this species, while measurements of the unidentified specimens (*Bonneviella* sp. USNM 1106182 and 1106187) are closer to type materials of other species examined (Table 3). *Bonneviella* sp. (USNM 1106182), *B. superba* and *B. grandis* are among the species with larger hydrothecae and trophosome, while *Bonneviella* sp. (USNM 1106187) and *B. ingens* have hydrothecae and trophosome almost half the size of the three previous species (Table 3, Figure 3).

Perisarc thickness, as well as the number and height of hydrothecal cusps, separate several species within Campanulariidae (Figure 1B). *Silicularia rosea* is clearly distinct from *Campanularia*, *R. verticillatus*, *Tulpa* and *Bonneviella* due to its thicker perisarc (Figure 1C, 4E-F). Species of *Campanularia*, in contrast, can hardly be differentiated by any of the characters included in the analysis, since they have similar morphological patterns (Figure 1D, 4A-D). The exception is *C. hincksii*, slightly set apart from the remaining *Campanularia* by its taller hydrothecal cusps (Figure 1D), a character that shows little or no overlap among the species when intraspecific variation is considered (Figure 5B). The remaining characters, however, do not show this pattern (Figure 5A, C-D).

Perisarc thickness is also informative to separate *Orthopyxis* from *Campanularia*, although morphological variation may attenuate this difference. Several specimens of *O. sargassicola* and *O. crenata* group together with *Campanularia* because of their thinner perisarc and presence of hydrothecal cusps, compared to the remaining species of *Orthopyxis* (Figure 1E, S1C). Indeed, although *O. sargassicola* and *O. crenata* have a thicker perisarc on average, their range of variation may overlap with *Campanularia* (Figure 6A). Species of *Campanularia* have a thinner perisarc in comparison to most other *Orthopyxis* (except for *O. mianzani*, Figure 7A), but when there is overlap in the range of variation of perisarc thickness, these taxa can be confidently distinguished by the hydrothecal length and length:diameter ratio (Figure 7B, C). However, this is not true when *Campanularia* is compared to *O. sargassicola* and *O. crenata*, because they show similar patterns of size and shape of hydrothecae (Figure 6B, C). It is difficult to differentiate *O. sargassicola* and *O. crenata* based on the characters included in this analysis (Figure 1E, 6). They show noticeable differences only in the hydrothecal length, which is shorter in *O. crenata* (Figure 6B).

When considering only species of *Orthopyxis* without hydrothecal cusps, the variation in size and perisarc thickness distinguish all individual lineages (Figure 1F, 7, 8): *Orthopyxis mianzani* has larger polyps with larger hydrothecae and a thinner perisarc; *Orthopyxis* sp.1, *O. integra*_IT and *O. everta* have shorter polyps and hydrothecae, with thinner perisarcs; *O. caliculata* has shorter polyps and hydrothecae, but a thicker perisarc; and *O. integra* and Campanulariidae sp. indet. have larger polyps and hydrothecae, with thicker perisarcs. *Orthopyxis integra*_1_USA is distinguished by its larger hydrothecae and pedicels (Figure 1E-F, 7A-B). However, variation occurs in all species, and some may overlap in their ranges, sometimes contradicting the separation of the lineages (e.g., *O. caliculata* and *Orthopyxis* sp.1, *O. integra* and *O. caliculata*, see Figure 1F, 7). Additional comparisons with type

species and descriptions from the literature (Table 4) show that the morphological patterns of the specimens identified as *Orthopyxis* sp.1, *O. everta* and *O. integra*_IT are congruent with that of *O. asymmetrica* (Stechow, 1919a). 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, 1919a), and *O. caliculata* (Hincks, 1853) (Table 4).

Additional principal components were evaluated, but they did not show clear patterns of differentiation among species (Figure S1). A PCA including only data from specimens with gonothecae separated *S. rosea* for its longer gonothecae, as well as *Orthopyxis* and *Bonneviella* for their broader gonothecae (Figure S1F).

Family Clytiidae

When all species of *Clytia* are compared, the PCA shows that most of the variation (PC1) is related to the presence of erect colonies, and the number, length, diameter, and perisarc thickness of the internodes (NIS, LIS, DIS, PIS) separate *Clytia linearis* and *C. stolonifera*, as well as some specimens of *C. elsaeoswaldae*, *C. gracilis*, and *C. hemisphaerica* (Figure 9A). However, when data for *C. gracilis* and measurements related to internodes are excluded from the analysis, further morphological patterns among species with erect colonies appear (Figure 9C, D). *Clytia linearis* is distinguished by its longer hydrothecae and cusps (LH, HCMax, HCmin, Figure 9C, D, 10A), although the range of variation of the cusp height overlaps with those of other species (Figure 11A, C). Likewise, *C. elsaeoswaldae* is separated by the larger hydrothecal diameter (DHMa, DHMe, DBC, Figure 9C, D, 10B), but this character is more informative when compared to lineages of *C. gracilis* and *C. hemisphaerica*, with which it shows less overlap (Figure 11B). Further comparisons show that *C. stolonifera* has lower hydrothecal cusps (Figure 10C, 11C) and *C. elsaeoswaldae* has a thicker diaphragm on average than *C. linearis*, *C. gracilis* and *C. hemisphaerica* (Figure 11D). However, the morphological variation is high and certainly attenuates these differences, leading to large overlaps among species.

The second direction accounting for most variation (PC2, Figure 9A, B) is related to perisarc thickness (PHMa, PHMe, PHB, PPMe) and length:diameter ratio of the hydrotheca (HRatio). It sets apart *Clytia* sp.2 and *Clytia noliformis* for their thicker perisarc, and *Clytia* sp.1, *C. gracilis* IV and *C. paulensis* for their more cylindrical hydrothecae (Figure 9A,B, 10). Although evident when directly compared among these species (Figure 12B), differences in HRatio are not evident in all PCAs, probably because of the slight variation shown by the

remaining species of *Clytia* (Figures 14E, 15B). *Clytia hummelincki* and *Clytia* sp.3 can be separated in a few PCAs by their longer and more annulated pedicels (Figure 9C, D), but this pattern is not clear when the ranges of variation of different species are considered, because of their overlapping (Figure 12C).

Lineages of *C. gracilis* (Figure 13A-F), though not clearly individualized, can be set apart from each other when compared as a group: *C. gracilis*_sp.B_USA, *C. gracilis* I and II have larger hydrothecae and pedicels (LH, DHMa, DHMe, DHB, DP) with higher and more numerous cusps (NC, HCMax, HCMin), while *C. gracilis* III and IV have, in general, lower values for those characters (Figure 9E, 14A-D). If measurements related to erect colonies are excluded from the analysis (LIS, PIS, NIS, DIS, ABS), *C. gracilis* I and *C. gracilis*_sp.B_USA can be further separated from *C. gracilis* II by the length (LH) and length:diameter ratio of the hydrotheca (HRatio, Figure 9F), although these differences are too small to be informative and delimit lineages (Figure 14A, E). Specimens of *C. gracilis* V spread along the four quadrants of the graph because of their high variation in the characters examined (Figure 9E, F), which can be also observed by comparing their range of variation with other lineages (Figure 14). Additional comparisons with literature descriptions show that morphological variation is pronounced in the presumably typical *C. gracilis*, and the lineages analyzed here could fit one or more descriptions (Table 5).

Lineages of *C. hemisphaerica* (Figure 13G-I), similarly to *C. gracilis*, are not separated by any of the morphological measurements, showing intermediate values for most of the characters evaluated (Figure 9A-D, S2). Characters that are important to differentiate other species of *Clytia* are uninformative for lineages of *C. hemisphaerica*, especially because of their wide range of variation and extensive overlap (Figure 15). This variability is also seen when descriptions from the literature are compared (Table 6).

Additional PCAs, including characters from the gonotheca, show less conspicuous patterns of differentiation among species (Figure S2A-F).

Family Obeliidae

Patterns of morphological variation in Obeliidae are mostly congruent among the different datasets examined (Figure 16A-F). Considering all species, perisarc thickness (PHMA, PHMe, PHB, PPMe, TD) explains most of the data variation, separating *Obelia geniculata* by its thicker perisarc (Figure 16A, B, Figure 17B). This character set apart *O. geniculata* from the remaining species in an analysis carried out exclusively with the genus *Obelia* (Figure 16C). *Obelia geniculata* also has the widest range of variation of perisarc

thickness, when *Laomedea* and *Obelia* are compared (Figure 18A, B). For the remaining genera, perisarc thickness does not notably contribute to the differentiation of the species, because of its extensive overlap (Figure 18A, B). Measurements of diameter (DHMa, DHMe, DHB, DBC, DP) explain another direction of variation of the data, and mainly differentiate *L. flexuosa* from the remaining Obeliidae by its broader hydrothecae (Figures 16A-B, D, 17C, 18C). Species of *Laomedea* also show a wide range of variation and overlap in pedicel length (LP, Figures 17, 18D), but their pedicels are generally longer than in *Obelia*.

Obelia longissima is distinguished from the remaining Obeliidae by its larger measurements of first- and second-order branches (LIS, DIS, NIS, LIB, DIB, NIB, Figures 16A-C, 17G-H). 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 (Figure 19A). Erect and branched colonies also differentiate *Hartlaubella gelatinosa* and *Gonothyraea loveni*, though to a lesser extent; this pattern is clearly observed when *Obelia* is excluded from the analysis (Figure 16D). These species, together with *O. bidentata* and *Obelia* sp.1, also differ from the remaining Obeliidae in their more cylindrical hydrothecae (higher values of HRatio, Figures 17, 19B) and taller hydrothecal cusps (Figures 16B-D, 19C), although *G. loveni* and *H. gelatinosa* have fewer cusps than *Obelia* (Figure 19D).

It is evident from most of the analyses that lineages of *Obelia dichotoma* are not distinguished from each other by any of the measurements, showing intermediate values for all characters evaluated (Figure 16A-C, E). Many specimens of *O. longissima* cannot be distinguished from the lineages of *O. dichotoma* as well, and although some are differentiated by their larger erect and branched colonies, variations in these characters prevent a complete separation of the species (Figure 20A). *Obelia longissima* also has longer hydrothecae and taller hydrothecal cusps on average, but their range of variation overlap among the species (Figure 20B, D). *Obelia dichotoma* III and *O. dichotoma* IV are grouped together and slightly separated from the remaining lineages of *Obelia*, probably because of their smaller and less branched colonies, but no further patterns of differentiation are seen among these lineages (Figure 16E, 21). Indeed, when compared to literature descriptions, the size and branching of colonies seem to be the only characters that could fairly differentiate some of the lineages of *O. dichotoma*, which are similar to the descriptions of other nominal species (Table 7).

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, Figure 16F). Additional PCAs do not show further patterns of differentiation among Obeliidae (Figure S3).

Discussion

At first glance, morphometric patterns in the suborder Proboscoida are not discriminative, and most species would be indistinguishable due to their presumed wide morphological variation. 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 more, different populations were included (see *Campanularia volubilis*, Figure 5). However, we also demonstrated the existence of consistent morphological patterns when characters are investigated at different levels of comparison and their range of variation is fully considered in the analysis. In some cases, refined morphometric analysis may further support the delimitation of lineages, and some characters can be used together with previously known diagnostic ones; in other cases, morphometric patterns show previously overlooked but consistent differences between lineages. In a few instances, the characters investigated do not contribute to separate species. We discuss the main morphometric patterns observed at different levels of comparison, and how they can be informative to delimit lineages within Proboscoida.

Bonneviella, *Tulpa tulipifera* and *Rhizocaulus verticillatus*: differences in size

In Campanulariidae, the length and diameter of the trophosome, pedicels, and hydrothecae can reliably distinguish *Bonneviella*, *T. tulipifera*, and *R. verticillatus* from the genera *Campanularia*, *Silicularia*, and *Orthopyxis*, which in turn can be characterized by differences in perisarc thickness (see below). Indeed, several species of *Bonneviella* Broch, 1909 were originally assigned to *Campanularia* Lamarck, 1816, and distinguished by their "enormous" size or "immense" hydrothecae (Allman 1876, as *Campanularia grandis*; Nutting 1901, as *C. regia*). Later, the pre-oral cavity on the hypostome of these species was considered the main diagnostic character of the group (Bonneviellidae, Broch 1909; Nutting 1915). *Tulpa tulipifera* (Allman, 1888) and *Rhizocaulus verticillatus* (Linnaeus, 1758) were also originally assigned to *Campanularia* (Linnaeus 1758; Allman 1888), and subsequently defined as separate genera based on differences in hydrothecal size and shape, and the presence of polysiphonic colonies, respectively (Stechow 1919b, 1921). The generic value of these characters, however, has been questioned by some authors, especially given the similarities in the hydrothecae and gonothecae between *Campanularia volubilis* (Linnaeus, 1758) and *R. verticillatus* (Rees and Thursfield 1965; Boero et al. 1996, but see Cornelius

1982: 57; 1999). The phylogenetic relationships of these species support their separation (Cunha et al. 2016b), and our current analysis confirmed that they differ consistently in size, which should also be considered for their delimitation. *Tulpa tulipifera*, in addition to size, can be differentiated from *Campanularia* species by its subhydrothecal spherule (Vervoort 1972; El Beshbeeshy & Jarms 2011). However, conclusions as to whether these differences should be considered at the genus or species level must rely on future taxonomic decisions regarding the genus *Campanularia*, especially because it is not monophyletic (see next section for further discussion).

Because of the considerable interspecific variation in *Bonneviella* (Figure 3), differences in size may also be informative to delimit the species examined in this study. As pointed out by Nutting (1915), *Bonneviella regia* (Nutting, 1901) can be differentiated from *Bonneviella grandis* (Allman, 1876) by the shapes of their gonothecae and the noticeably smaller hydrothecae of *B. regia* (Table 3). *Bonneviella superba* Nutting, 1915 has the largest hydrothecae among *Bonneviella* species, while hydrothecae in *Bonneviella ingens* Nutting, 1915 are intermediate in size, but considerably different in shape from those of *B. superba* (Nutting 1915; Naumov 1969). Although we were not able to directly assign the vouchers of *Bonneviella* sp. (USNM 1106182 and 1108187) to unidentified specimens obtained by Govindarajan et al. (2006), the morphometric patterns of the type materials support the hypothesis that they are close to *B. superba* and *B. ingens*, respectively (Table 3). This is a tentative identification, however, because both materials lack reproductive structures. Also, intraspecific variation in *Bonneviella* was not investigated because of the small number of specimens studied, making it difficult to determine whether the range of variation of these characters could overlap among the species examined (Cunha et al. 2016a).

The clade comprising *C. volubilis*, *R. verticillatus*, and *Bonneviella* may represent a local radiation, and it is necessary to examine additional material from other localities (Govindarajan et al. 2006). Although *C. volubilis* was not differentiated from any other *Campanularia* species based on characters related to size, both *R. verticillatus* and *Bonneviella* were characterized by their larger size (Figure 3), and all their records come from the Aleutians (Table 2). Indeed, *R. verticillatus* is known for its arctic-boreal distribution (Antsulevich 1992; Calder 2003; Schuchert 2001; Ronowicz 2007; Stepanjants et al. 2006), while species of *Bonneviella* have been recorded in arctic and subarctic regions (Broch 1910; Kramp 1913; Nutting 1915; Naumov 1969; Yamada 1969; Schuchert 2001). Even though these genera have a close phylogenetic relationship (Govindarajan et al. 2006; Cunha et al. 2016b), 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*, *Silicularia bilabiata*, *Orthopyxis integra*; Ralph & Thompson 1956; Ralph 1957; Naumov, 1969). The same occurs with *T. tulipifera*, which has a Kerguelen-Patagonian distribution (Peña Cantero & García Carrascosa 1999; Soto Àngel & Peña Cantero 2015), indicating that its larger size is probably a 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 distribution.

Silicularia, *Campanularia* and *Orthopyxis*: trends in perisarc thickness and size/shape of hydrothecae

Our results show that perisarc thickness is among the most variable (e.g., Millard 1975; Cornelius 1982, 1995; Cunha et al. 2015), but yet most informative characters to delimit *Silicularia*, *Campanularia*, and *Orthopyxis*. Besides the unique bilaterally symmetrical hydrothecae of *Silicularia* Meyen, 1834, a conspicuous character to delimit the genus (Ralph 1956, 1957; Blanco 1967), *S. rosea* can also be delimited by the comparatively thicker perisarc of its hydrothecae and pedicels. *Silicularia rosea* Meyen, 1834 is widely distributed in antarctic and subantarctic waters, and was considered synonymous with *S. bilabiata* (Coughtrey, 1875) (Vervoort & Watson 2003), a species shown by Ralph (1956, 1957) to have wide intraspecific variation and comprise several nominal species within *Silicularia*. A previous molecular analysis of nuclear and mitochondrial genes showed that specimens of *S. rosea* from Argentina and New Zealand were monophyletic (Cunha et al. 2016b), and we found similar morphological patterns among these specimens (Figure 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, based on the position of the hydrothecal rim and size differences. All specimens that we studied had an oblique hydrothecal aperture (Figure 4F), and they were not separated by differences in size. Therefore, 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. 2016b). *Campanularia volubilis* 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. For this reason, we refrain from any taxonomic decision regarding *Campanularia* until more and unequivocal

material of the type species is available. Presently, a possible conclusion derived from the results would be to merge *Bonneviella* and *Rhizocaulus* into *Campanularia*, but this decision is contraindicated by the several morphological differences among these genera. Although not monophyletic, all species of *Campanularia* have similar morphological patterns, and most of their similarities could be considered symplesiomorphic character states. Also, differences in size of the hydrothecae between *C. hincksii* Alder, 1856 and *C. volubilis* can be masked by intraspecific variation (see Cornelius 1982, 1995), especially when different populations are evaluated (Figure 5). 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* seems to be distinctive.

Orthopyxis L. Agassiz, 1862 is a monophyletic genus (Cunha et al. 2016b), 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* is valid mainly based on the gonophore producing a small medusa (medusoid, Agassiz 1862; Cornelius 1995). Our analysis showed that *Orthopyxis* could also be distinguished from *Campanularia* based on trophosomal characters, such as perisarc thickness and length:diameter ratio of hydrothecae. However, *Campanularia* may fall into the range of variation of *O. sargassicola* (Nutting, 1915) and *O. crenata* (Hartlaub, 1901), because the perisarcs in these two *Orthopyxis* species vary from thin to thick, and their hydrothecae from campanulate to cylindrical (Vervoort & Watson 2003; Cunha 2011; Cunha et al. 2015, 2016a). It is important, therefore, to evaluate the range of variation of the specimens before these genera can be reliably delimited.

Indeed, variation in *O. crenata* is conspicuous. The specimen from New Zealand assigned to *O. integra* (USNM 1106163, Table 1) is a misidentification (see Cunha et al. 2016b), since it has hydrothecae with a smooth or crenate rim, typical of *O. crenata* (Millard 1975: 205). In molecular phylogenies (Cunha et al. 2016b), this specimen clustered with another specimen of *O. crenata* from New Zealand and unidentified *Orthopyxis* specimens from Argentina (see 16S and COI phylogenies), and this clade forms a monophyletic group with specimens of *O. crenata* from Brazil (concatenated phylogenies). Our results showed that, despite their affinities, unidentified specimens show clear differences in the perisarc thickness, size and shape of the hydrothecae in comparison with *O. crenata* from Brazil (Figure 6). However, the close phylogenetic relationship with *O. crenata* from New Zealand, the type locality of the species (Hartlaub 1901; Vervoort & Watson 2003), led 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). Therefore we regard the Brazilian and unidentified Argentinean specimens as *O. crenata*.

Distinct lineages of *Orthopyxis* with the traditional morphological diagnostic characters of *O. integra* (MacGillivray, 1852) were shown to be delimited by the degree of perisarc thickening and the size and shape of the hydrothecae (Cunha et al. 2015). Also, *O. caliculata* (Hincks, 1853) and *O. mianzani* Cunha, Genzano & Marques, 2015 were distinguished from the typical *O. integra* by their smooth gonothecae, contrasting with the spirally grooved gonothecae of the latter species (Cunha et al. 2015). Our results corroborate these patterns, and further attest that the clade comprising the Aleutian specimen of *O. integra* (USNM 1106184), with spirally grooved gonothecae (Figure 8B), has morphological patterns that are commonly regarded as distinctive for the typical *O. integra*, such as larger and more cylindrical hydrothecae (Nutting 1915; Bale 1934; Hirohito 1995; Calder et al. 2014). Although we could not verify the presence of spirally grooved gonothecae in the Argentinean specimens (Campanulariidae sp. indet. and *O. integra*_PT20), they are here regarded as *O. integra* given their morphological and phylogenetic patterns, contradicting the hypothesis that this species does not occur in the southwestern Atlantic (Cunha et al. 2015). Also, although the perisarc is rather thin in the Aleutian *O. integra*, the Argentinean specimens show that the perisarc thickness can be variable in this species, and may overlap with *O. caliculata* (Figure 7A).

In addition to *O. integra*, our analysis also showed that Mediterranean specimens identified as *O. integra*_IT, *O. everta* and *Orthopyxis* sp.1, which form a distinct clade in the molecular phylogeny of the group (Cunha et al. 2016b), have similar morphological patterns and can be delimited by their shorter hydrothecae and thinner perisarc, in comparison to other *Orthopyxis* species (Figures 1, 7). Although their perisarc is not as thick as described by Stechow (1919a), we believe that these specimens are close to *Orthopyxis asymmetrica* Stechow, 1919a, a species commonly reported in the Mediterranean (Piraino & Morri 1990; Peña Cantero & García Carrascosa 2002; Bouillon et al. 2004). Even though this species was proposed to be a synonym of *O. integra* (e.g., Cornelius 1982; Östman et al. 1987), our findings support *O. asymmetrica* as a distinct and valid species.

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. 2016b). *Clytia linearis*, for instance,

is monophyletic in all phylogenetic analyses (Cunha et al. 2016b), with consistent morphometric patterns shared by the specimens, corroborating it as a widely distributed species (Rees & Vervoort 1987; Medel & Vervoort 2000). Classically, *C. linearis* (Thornely, 1900) is distinguished by the hydrothecal inward folds (cf. Calder 1991; Lindner & Migotto 2002; Schuchert 2003). However, this species can also be differentiated from other members of *Clytia* by its erect colonies and the size of the hydrothecae (Figure 10A), even though its "deep" hydrothecae, frequently mentioned in descriptions, are also commonly reported as variable in size (e.g., Cornelius 1982; Altuna 1994). Our analyses showed that the range of intraspecific variation of the size of the hydrothecae in *C. linearis* does not overlap with those of other species (Figure 11A), and this character can also be useful to delimit the species.

Clytia elsaeoswaldae Stechow, 1914 was also shown to be a distinct, monophyletic lineage (Lindner et al. 2011; Cunha et al. 2016b). It is differentiated from *C. gracilis* (M. Sars, 1850) and *C. hemisphaerica* (Linnaeus, 1767), its closest congeners (Cornelius 1982; Calder 1991; Lindner et al. 2011), 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 *C. gracilis* and, to a lesser extent, from *C. hemisphaerica* by its hydrothecal diameter (Figures 10B, 11B). 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. hemisphaerica* fall into its range of variation (cf. Figures 10B and 13H; also 11B).

Clytia noliformis (McCrady, 1859) has been confounded with *C. hemisphaerica*, but it was considered distinct from the latter by several authors (e.g., Östman et al. 1987; Calder 1991; Lindner & Calder 2000). The shape of the hydrothecae and gonothecae, as well as the distinct annulations (= subhydrothecal spherules) and the presence of merotrichous isorhizae (a unique type of nematocyst) differentiate *C. noliformis* from its congeners (Calder 1991; Linder & Migotto 2001, 2002). We found that the perisarc thickness, a character rarely described in the literature (but see Calder 1991), can also be used to delimit this species (Figures 10 and 12).

Similarly, *Clytia paulensis* (Vanhöffen, 1910) is regarded as distinctive because of the shape of its hydrothecal cusps (Millard 1975; Cornelius 1982, 1995), but we noted that the species also has a more cylindrical hydrotheca in comparison with some other members of *Clytia* (Hratio, Figure 12B). The length:diameter ratio of the hydrothecae of *C. paulensis* is

known to be variable, though, ranging from 1.5 to 4 in different populations (Millard 1966; Cornelius 1982). Since 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.

Clytia stolonifera and *C. hummelincki* were ambiguously positioned at the base of Obeliidae and Clytiidae+Obeliidae, respectively (Cunha et al. 2016b). These results, strictly taken, suggest that these species should not be assigned to Clytiidae. However, in this study we compare *C. stolonifera* and *C. hummelincki* with other species of *Clytia* because of their similar morphology. Both species have a diaphragm, and *C. hummelincki* (Leloup, 1935) has a medusa typical of a member of *Clytia* Lamouroux, 1812 (Gravili et al. 2008). Similarly, medusa buds with marginal bulbs were described for a nominal species synonymous with *C. stolonifera* Blackburn, 1938 (*Clytia latitheca*, Millard & Bouillon 1973; Watson 2005), suggesting that it also possesses the typical characters of *Clytia* medusae. Additionally, *Clytia stolonifera* has the typical branching of species of *Clytia*, with each branch arising from an upward-curved apophysis (Watson 2005; Figure 10C). Our results showed that *C. stolonifera* was separated from other species of *Clytia* because of its highly branched colonies, and *C. hummelincki* was differentiated by its longer pedicels, a character frequently mentioned in descriptions (e.g., Millard 1966, 1975; Cornelius 1982). Nevertheless, the morphological affinities of both species with *Clytia* are not supported by the molecular analysis (Cunha et al. 2016b), and the study of more specimens from different localities is necessary to determine the morphological and molecular relationships among these species, Clytiidae and Obeliidae.

Unidentified specimens (*Clytia* sp.1, *Clytia* sp.2 and *Clytia* sp.3) do not show clear phylogenetic (Cunha et al. 2016b) and morphometric patterns for their delimitation, and although they could be differentiated from other species by variations in some characters (e.g., length:diameter ratio of hydrotheca, perisarc thickness, length of pedicel), these differences are not distinctive for their identification.

Clytia gracilis and *Clytia hemisphaerica*: extensive genetic diversity, but few or no morphometric differences

Molecular analyses of *C. gracilis* resulted in several cryptic lineages in previous studies (Govindarajan et al. 2006; Lindner et al. 2011; Cunha et al. 2016b), suggesting that it is not a widespread species as commonly assumed (e.g., Calder 1991; Cornelius 1995; Peña Cantero & García Carrascosa 2002), and its current diagnostic characters are uninformative to delimit the species. 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). Our study showed, however, 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 (Figure 14). The same variations are 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; Table 5), and the lineages analyzed herein could fit into one or more of these descriptions (Table 5). This emphasizes the difficulties in correlating the morphometric patterns of these lineages with the typical *C*. *gracilis*, especially considering that its original description was based on two species, currently *C*. *gracilis* and *Gonothyraea loveni* (Allman, 1859) (M. Sars 1850, 1857; cf. Cornelius 1982; Calder 1991). Although a lectotype of *C*. *gracilis* was designated by Cornelius (1982: 94), it was based on the original illustration provided by M. Sars (1857), and information on the diagnostic characters of the typical *C*. *gracilis* remains subjective and incomplete.

Clytia hemisphaerica also comprises several cryptic lineages (Cunha et al. 2016b). We were unable to differentiate them by their morphometric patterns (Figure 15), although all lineages have the diagnostic characters that are generally attributed to polyps of *C*. *hemisphaerica* (see above and Calder 1991; Cornelius 1995). They also fit one or more published descriptions, impeding the delimitation and identification of the typical *C*. *hemisphaerica* (Table 6). The three lineages of *C*. *hemisphaerica* analyzed in this study were geographically structured, comprising specimens from Belize, the United States, and the Mediterranean/North Sea (Cunha et al. 2016b, Table 1), and forming a monophyletic group in most of the concatenated phylogenies (Cunha et al. 2016b). 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).

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. (2016b), although in their study additional specimens of *C*. *hemisphaerica* from the USA clustered with *C*. *xiamenensis* (see 16S phylogenies, Cunha et al. 2016b). Originally, the hydroid of *C*. *xiamenensis* was differentiated from *C*. *hemisphaerica* by its pointed and inclined hydrothecal cups, as well as

its smaller B-type microbasic mastigophores (Zhou et al. 2013). We showed, however, that specimens of *C. hemisphaerica* from the same clade (*C. hemisphaerica* I, see Table 1; Cunha et al. 2016b) do not have pointed and inclined hydrothecal cusps (Figure 13G), even though their cusps are not as rounded as those of *C. hemisphaerica* II (compare with Figure 13H). Indeed, inclined cusps can be variable in some species (*C. gracilis*, see below), and the definition of the shape of hydrothecal cusps is somewhat subjective. These characters do not seem reliable to differentiate *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). If this proves to be the case, then all specimens from the USA identified here as *C. hemisphaerica* should be assigned to *C. xiamenensis*.

Similarly, *Clytia gulangensis* He & Zheng, 2015 (He et al. 2015) clustered with specimens of *C. gracilis* from Brazil (Cunha et al. 2016b; *C. gracilis* V, Table 1). 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 two (Table 5, Figure 13). In fact, the shape of the hydrothecal cusps showed wide variation among the different Brazilian specimens (Figure 22). 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 (Figures 22B, E). In addition, as discussed above, the diagnostic characters of the typical *C. gracilis* are unclear, and several descriptions of the species do not mention this particular shape of the hydrothecal cusps (e.g., Calder 1991; Cornelius 1995; Schuchert 2001). Therefore, we believe that *C. gulangensis* cannot be confidently delimited from *C. gracilis* until the diagnostic characters of typical *C. gracilis* 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).

Obelia geniculata and *Laomedea*: differences in sizes of hydrothecae and pedicels, and perisarc thickness

One of the main variations found among species of Obeliidae was related to perisarc thickness, setting apart *O. geniculata* from all its congeners, as well as the remaining Obeliidae. Indeed, *O. geniculata* (Linnaeus, 1758) is a relatively easy species to identify because of its characteristic asymmetrical thickening of the internodes (Cornelius 1975, 1990,

1995; Schuchert 2001; Calder 2012). Our study shows that the range of variation of perisarc thickness in *O. geniculata* is the widest among the Obeliidae (Figure 18A, B), 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. 2016b), suggesting that these variations are intraspecific. Indeed, the intraspecific variation in perisarc thickness, colony height, hydrothecal length, branching, and number of annulations on the pedicels of *O. geniculata* is extensive (Ralph 1956; Cornelius 1975; Millard 1975; Ralph & Thompson 1986). Our study, following Cunha et al. (2016b), supported *O. geniculata* as a true widely distributed species, and corroborated the perisarc thickness as its distinctive character. The nematocysts were also shown to be diagnostic (Östman 1982a, 1999).

Laomedea flexuosa is differentiated from the remaining members of Obeliidae by the diameter of its hydrothecae and pedicels (Figure 18C). Indeed, this species is frequently described with a robust hydrotheca, having its length nearly equal to its width (Cornelius 1982, 1995). *Laomedea flexuosa* is also distinguished from other members of Obeliidae by its isoenzyme patterns and nematocysts, further supporting its delimitation (Östman 1982a, b). *Laomedea angulata* and *L. calceolifera*, on the other hand, do not show clear patterns of differentiation, except for the shape and position of their gonothecae, probably the most conspicuous character for their delimitation (cf. Cornelius 1982). All species of *Laomedea* included in our analysis could be confidently distinguished from *Obelia* based on their longer pedicels (Figure 18D), even though the genus did not prove to be monophyletic in previous molecular phylogenies (Govindarajan et al. 2006; Cunha et al. 2016b). Because *L. flexuosa* (Alder, 1857) is the type species of the genus *Laomedea* (Cornelius 1981b, ICZN 1985), the best decision at present would be to assign *L. calceolifera* and *L. angulata* to *Obelia*, if the clade comprising all these species (Cunha et al. 2016b) contains the typical *O. dichotoma* (Linnaeus, 1758) (taken as conspecific with *O. spherulina* Péron & Lesueur, 1810, the type species of *Obelia* Péron & Lesueur, 1810 (Cornelius 1975, 1982)). However, this decision is presently premature because there is no sequence of *O. dichotoma* from its type locality (southwestern England, Cornelius 1975), and the delimitation of this species is unclear (see below).

Large erect colonies and differences in shape and number of hydrothecal cusps: *Gonothyraea*, *Hartlaubella*, *Obelia bidentata* and *O. longissima*

The species *G. loveni, H. gelatinosa* and *O. longissima*, the last to a greater extent, are separated from the remaining Obeliidae by their typically erect, branched colonies (Cornelius 1982, 1990, 1995). *Hartlaubella* Poche, 1914 is distinguished from *Obelia* by its fixed gonophores (free medusa in *Obelia*; Cornelius 1990; Boero et al. 1996; Stepanjants 1998), and *H. gelatinosa* (Pallas, 1766) can also be differentiated by its paired branches that are successively arranged at right angles on opposite sides of the polysiphonic main stem (Cornelius 1995; Figure 17K). However, this feature is also present in large colonies of *O. bidentata* Clark, 1875 (Cornelius 1995, Figure 17F), which has contributed to some confusion in the past (Cornelius 1982; 1990). Actually, the two species are phylogenetically distinct, although closely related (Cunha et al. 2016b). *Hartlaubella gelatinosa* and *G. loveni* can be differentiated from *O. bidentata* by the shape and number of the cusps, which are taller and more numerous in the latter (Figure 19C, D). *Obelia bidentata* also has a more cylindrical hydrotheca than *H. gelatinosa* and *G. loveni* (Figure 19B).

Obelia bidentata is assumed to have wide 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 longicyatha* Allman, 1877, *Obelia austrogeorgiae* Jäderholm, 1904; Cornelius 1975, 1982; Calder 1991). Calder (2013) have 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, however, we found that small (0.3-1 cm high) monosiphonic colonies and large (>6 cm high) polysiphonic colonies (USNM 1106185, from the North Sea) are related in nearly all topologies analyzed in previous molecular studies (Govindarajan et al. 2006; Cunha et al. 2016b). Therefore, differences in colony size should probably be regarded as intraspecific variation. On the other hand, *Obelia* sp.1 has similar morphometric patterns to *O. bidentata*, but they are not closely related (Cunha et al. 2016b). If variations in these species are compared, hydrothecal length and number and height of cusps may be regarded as interspecific variations, which would support the revalidation of previous synonyms of *O. bidentata* (Figure 19). However, the small number of specimens, lack of reproductive structures, and inconstant phylogenetic placement

of *Obelia* sp.1 prevent us from reaching definite conclusions, and identification beyond the genus level is presently not possible.

Obelia dichotoma and *O. longissima*: morphometric patterns partially delimiting species

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 longissima* (Pallas, 1766) and *O. dichotoma* (Linnaeus, 1758) (Alder 1857; Hincks 1868; Nutting 1915; Kramp 1935). However, the extensive variation in most of these characters (cf. Ralph 1957; Vervoort 1972; Millard 1975) also supported the hypothesis that *O. longissima* was a synonym of *O. dichotoma*, and that their morphological differences were intraspecific variations (Cornelius 1975). The species were subsequently revalidated by differences in isoenzyme patterns and nematocysts (Östman 1982a, b), and both were morphologically redescribed with their differences emphasized (Cornelius 1987, 1990, 1995).

Currently, besides the differences in their nematocysts (Östman 1982a, b), *O. longissima* is characterized by having predominantly monosiphonic colonies with usually longer stems and branches roughly uniform in length, as well as 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). Previous molecular studies showed that *O. dichotoma* comprises several cryptic lineages (Cunha et al. 2016b), and *O. longissima* was corroborated as a monophyletic and widely distributed species (Govindarajan et al. 2006; Cunha et al. 2016b). Our results revealed that some characters support the separation of the species (Table 6), viz. (1) size of the colony, with *O. longissima* usually larger than *O. 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. dichotoma*; (3) hydrothecal length, usually longer in *O. longissima* but with some overlap with *O. dichotoma*; (4) shape of the hydrothecal rim, varying from smooth to crenate in all lineages of *O. dichotoma*, and invariably sinuous in *O. longissima*. Morphological variation may obscure some of these differences, but colonies of *O. longissima* can be delimited by these characters, once the intraspecific variation is evaluated.

Contrastingly, cryptic lineages of *O*. *dichotoma* do not show morphometric differences, presenting extensive variation and overlap in their characters (Figures 20, 21). Although *O*. *dichotoma* III and IV could be distinguished from the remaining lineages by their smaller and less branched colonies (Figure 20, Table 6), in some cases colonies varied 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 hyalina* Clarke, 1879, *Obelia griffini* Calkins, 1899; 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*. *dichotoma* are also small (~4-11 mm) and have few branches, although some have a slightly crenate hydrothecal rim (*O*. *dichotoma* III, see Table 6, Figure 21), in contrast to the even hydrothecal rim of *O*. *hyalina* (Clarke 1879; Calder 2013). Similarly, all specimens of *O*. *dichotoma* IV have rounded hydrothecae in cross section and an even hydrothecal rim (Table 6, Figure 21), 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 could 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 are invariably present in the species (Östman 1982a, 1987; Cornelius 1990).

Conclusions

Wide morphological variation is an inherent characteristic present in all Hydrozoa (as well as Medusozoa, Cunha et al. 2016a), and its correct interpretation is crucial to understand the taxonomy and diversity of the group. Detailed investigations on the variation of specific diagnostic characters will result in sound delimitations of species and, as a corollary, in more complete and precise taxonomic descriptions. The discovery of cryptic species is highly likely (Cunha et al. 2016a).

Our study supported the usefulness of morphometric data to delimit species in Proboscoida (see taxonomic summary, Table 8), but some attention and specific procedures should be taken into consideration for this taxonomic approach. Even though this group has wide intraspecific variation, consistent differences in morphometric patterns may be

uncovered once this variation is comparatively investigated. Characters related to size, perisarc thickness, shape of hydrothecae, and hydrothecal cusps are the most important in the delimitation of most species, although in some cases (e.g., *Campanularia* spp., *Clytia gracilis*, *Clytia hemisphaerica*, *Laomedea* spp., *Obelia dichotoma*), morphometric differences are masked by intraspecific variation. Considering that our study was 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.

Studies on Hydrozoa, as well as Medusozoa, will benefit from analyses combining or at least contrasting phylogenetic and morphometric data, especially when the ranges of variation of morphological characters are compared and investigated in detail. Thorough investigations using morphometric data for voucher specimens and molecular trees, complemented by broader inferences in population morphological and morphometric variation, will directly impact our current knowledge on Hydrozoa (as well as on Medusozoa and other marine taxa). This approach will establish well grounded valid species, refining our assessments of marine species diversity.

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Table 1. Species included in this study. The symbol * indicates vouchers that, due to insuficient or contradictory information, were tentatively assigned to the specimens in the phylogeny. Specimens in bold indicate samples from which intracolony measurements were taken. The codes are in accordance with the phylogeny by Cunha et al. (2016b). 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; LEM = Laboratory of Marine Evolution, São Paulo, Brazil.

Species	**Locality**	**Voucher**	**Code** (as in Cunha et al. 2016b)
Bonneviella ingens	Simushir Island, Japan	USNM 34576 (type)	not included
Bonneviella regia	Aleutians, USA	USNM 1106181	USA
Bonneviella regia	Prince William Sound, USA	USNM 71390 (type)	not included
Bonneviella suberba	Bering Sea	USNM 3480	not included
Bonneviella sp.	Aleutians, USA	USNM 1106182*, 1106187*	USA
Campanularia hincksii	Italy	LEM	**IT10**, IT14_IT
Campanularia hincksii	Otranto, Italy	USNM 1106157	IT
Campanularia sp.	Punta Cuevas, San Julián, Argentina	LEM	**PT10_ARG**
Campanularia sp.	La Mina, Puerto San Julián, Argentina	MZUSP 2641, 2642	SJ4, SJ5_ARG
Campanularia subantarctica	La Mina, Puerto San Julián, Argentina	MZUSP 2639, 2643	SJ2, SJ6_ARG
Campanularia volubilis	Monterey, USA	USNM 1106166	**USA**
Campanularia volubilis	Casco Bay, USA	USNM 29127	not included
Campanularia volubilis	Greenland	ZMUC	not included
Clytia elsaeoswaldae	Palmas Island, Brazil	LEM	PM18, PM36_BRA
Clytia elsaeoswaldae	Mel Island, Brazil	LEM	**Me26_BRA**
Clytia elsaeoswaldae	Cabras Island, Ilhabela, Brazil	LEM	**CB19_BRA**
Clytia elsaeoswaldae	São Sebastião, Brazil	USNM 1078725, 1078728	1, 2_BRA
Clytia gracilis III	Mund Bay, Piran, Slovenia	LEM	**EL05_SLV**

Species	**Locality**	**Voucher**	**Code** (as in Cunha et al. 2016b)
Clytia gracilis III	Strunjan, Piran, Slovenia	LEM	EL14_SLV
Clytia gracilis III	Piran, Slovenia	LEM	EL38_SLV
Clytia gracilis I	Strunjan, Piran, Slovenia	LEM	EL15_SLV
Clytia gracilis I	Slovenia	LEM	EL31, **EL32**_SLV
Clytia gracilis I	Italy	LEM	IT12, **IT13**_IT
Clytia gracilis II	Punta Cuevas, San Julián, Argentina	LEM	**PT9_ARG**
Clytia gracilis II	Georges Bank, USA	USNM 1106152	USA
Clytia gracilis IV	Twin Cays, Belize	LEM	CBC13_BLZ
Clytia gracilis IV	Carry Bow Cay, Belize	LEM	CBC20_BLZ
Clytia gracilis IV	Twin Cays Fisheries Dock, Belize	LEM	**CBC26**_BLZ
Clytia gracilis V	Mel Island, Brazil	LEM	Me24_BRA
Clytia gracilis V	Farol Velho, Salinópolis, Brazil	LEM	PAF03_BRA
Clytia gracilis V	Panaquatira, São Luís do Maranhão, Brazil	LEM	**MAP01**, MAP11_BRA
Clytia gracilis V	Flexeiras, Trairí, Brazil	LEM	T1, T5, T6_BRA
Clytia gracilis V	Náutico, Fortaleza, Brazil	LEM	CE1, **CE3**_BRA
Clytia gracilis V	Caponga, Cascavel, Brazil	LEM	**CE2**, CE5_BRA
Clytia gracilis sp.B_USA	Beaufort, USA	USNM 1078730	**USA**
Clytia hemisphaerica III	Carrie Bow Cay, Belize	LEM	**CBC1_BLZ**
Clytia hemisphaerica III	Twin Cays Fisheries Dock, Belize	LEM	CBC25_BLZ
Clytia hemisphaerica III	Cuda Cut, Twin Cays, Belize	LEM	CBCB40.1_BLZ
Clytia hemisphaerica II	Mund Bay, Piran, Slovenia	LEM	**EL06**, EL08_SLV
Clytia hemisphaerica II	Strunjan, Piran, Slovenia	LEM	EL12, EL20_SLV
Clytia hemisphaerica II	Slovenia	LEM	EL35_SLV
Clytia hemisphaerica II	Croacia	LEM	EL28_CRO
Clytia hemisphaerica II	North Sea	USNM 1106186	NS
Clytia hemisphaerica I	Westport, USA	LEM	**FLT03_USA**
Clytia hemisphaerica I	Salem, USA	LEM	HCM04_USA
Clytia hemisphaerica I	Bourne, USA	LEM	MMA05_USA
Clytia hemisphaerica I	Point Judith, Rhode Island, USA	LEM	PTJ01_USA

Species	**Locality**	**Voucher**	**Code** (as in Cunha et al. 2016b)
Clytia hummelincki	Cuda Cut, Twin Cays, Belize	LEM	**CBC42_BLZ**
Clytia linearis	Paraty, Brazil	LEM	**PY10_BRA**
Clytia linearis	Beaufort, USA	USNM 1078729	USA
Clytia noliformis	Barão Tefé Island, São Pedro and São Paulo Archipelago, Brazil	LEM	SP3, **SP9**_BRA
Clytia noliformis	São Sebastião, Brazil	USNM 1078720*	1_BRA
Clytia paulensis	Otranto, Italy	USNM 1106158	IT
Clytia sp.1	Boca da Enseada, São Pedro and São Paulo Archipelago, Brazil	LEM	**SP1_BRA**
Clytia sp.2	Caponga, Cascavel, Brazil	LEM	**CE4_BRA**
Clytia sp.3	Natal, Brazil	LEM	**NAT05_BRA**
Clytia stolonifera	Cuda Cut, Twin Cays, Belize	LEM	**CBC40.2**, CBC45_BLZ
Gonothyraea loveni	Dennis, USA	USNM 1106154	USA
Gonothyraea loveni	Plymouth, USA	LEM	BPM03_USA
Gonothyraea loveni	Sandwich, USA	LEM	**SWM03_USA**
Hartlaubella gelatinosa	Río Gallegos, Argentina	LEM	PT13, PT14, PT16_ARG
Laomedea angulata	Piran, Slovenia	LEM	**EL40**, EL50_SLV
Laomedea calceolifera	Bourne, USA	LEM	MMA06_USA
Laomedea calceolifera	Boston, USA	LEM	**ROW03_USA**
Laomedea calceolifera	Gloucester, USA	LEM	GFP01_USA
Laomedea calceolifera	Hampton, USA	LEM	HRM06_USA
Laomedea calceolifera	Newport, USA	LEM	FTA01_USA
Laomedea calceolifera	Herquemoulin, Normandie, France	MHNG INVE 37296	FR
Laomedea calceolifera	Woods Hole, USA	USNM 1106177	USA
Laomedea flexuosa	Rye, USA	LEM	RYE02_USA
Laomedea flexuosa	Sandgerdi, Iceland	USNM 1106190	IC
Laomedea flexuosa	White Sea, Russia	USNM 1106192	WS
Laomedea inornata	Friday Harbor, USA	USNM 1106170	USA

Species	**Locality**	**Voucher**	**Code** (as in Cunha et al. 2016b)
Obelia bidentata	Cuda Cut, Twin Cays, Belize	LEM	**CBC35_BLZ**
Obelia bidentata	Raposa Channel, São Luís do Maranhão, Brazil	LEM	MAR02_BRA
Obelia bidentata	Panaquatira, São Luís do Maranhão, Brazil	LEM	MAP10_BRA
Obelia bidentata	North Sea, Denmark	USNM 1106185	NS
Obelia bidentata	Beaufort, USA	USNM 1106162	USA
Obelia dichotoma I	Westport, USA	LEM	FLT04_USA
Obelia dichotoma I	New Bedfort, USA	LEM	PIM01, **PIM02**_USA
Obelia dichotoma I	Boston, USA	LEM	ROW04_USA
Obelia dichotoma I	Punta Cuevas, San Julián, Argentina	LEM	PT3_USA
Obelia dichotoma I	Point Judith, Rhode Island, USA	LEM	PTJ03_USA
Obelia dichotoma I	Rocha, Uruguay	LEM	**UR1_URG**
Obelia dichotoma II	Slovenia	LEM	EL30_SLV
Obelia dichotoma II	Bourne, USA	LEM	MMA03_USA
Obelia dichotoma II	Punta Cuevas, San Julián, Argentina	LEM	**PT2_USA**
Obelia dichotoma II	Otranto, Italy	USNM 1106156	IT
Obelia dichotoma III	Farol Velho, Salinópolis, Brazil	LEM	PAF07, **PAF09**_BRA
Obelia dichotoma III	Calhau, São Luís do Maranhão, Brazil	LEM	MA03_BRA
Obelia dichotoma IV	Providence, USA	LEM	**Site1.1_USA**
Obelia dichotoma IV	Rocha, Uruguay	LEM	**UR6_URG**
Obelia geniculata I	South Freeport, USA	LEM	BSF05_USA
Obelia geniculata I	Punta Cuevas, San Julián, Argentina	LEM	**PT5_ARG**
Obelia geniculata II	New Castle, New Hampshire, USA	LEM	**UNH01_USA**
Obelia geniculata II	New Brunswick, Canada	USNM 1106176	NB_CAN
Obelia geniculata III	João Gonçalves, Búzios, Brazil	LEM	**BZ5_BRA**
Obelia geniculata III	Mund Bay, Piran, Slovenia	LEM	**EL23_SLV**
Obelia geniculata IV	Misaki, Sagami Bay, Japan	USNM 1106179	JP
Obelia geniculata IV	North Island, Wellington, New Zealand	USNM 1106165	NZ
Obelia longissima	Bourne, USA	LEM	MMA04_USA

Species	**Locality**	**Voucher**	**Code** (as in Cunha et al. 2016b)
Obelia longissima	Gloucester, USA	LEM	GFP04_USA
Obelia longissima	Hampton, USA	LEM	HRM05_USA
Obelia longissima	San Julián, Argentina	LEM	PT1_ARG
Obelia longissima	Antarctic Peninsula	USNM 1106173	AN
Obelia longissima	Sandgerdi, Iceland	USNM 1106189	IC
Obelia longissima	Ryders Cove, USA	USNM 1106153	USA
Obelia longissima	White Sea, Russia	USNM 1106191	WS
Obelia sp.1	Farol Velho, Salinópolis, Brazil	LEM	PAF08_BRA
Obelia sp.1	Flexeiras, Trairí, Brazil	LEM	**T2_BRA**
Orthopyxis caliculata	João Gonçalves, Búzios, Brazil	MZUSP 2612-15	JGB1-4_BRA
Orthopyxis caliculata	Penha, Brazil	MZUSP 2563, 2565	AB, GB_BRA
Orthopyxis caliculata	Bombinhas, Brazil	MZUSP 4177, 4265	BB, COB_BRA
Orthopyxis caliculata	Paciência, Penha, Brazil	MZUSP 2550, 2552, 2554, 2556	PAB1, PAB3, PAB4, PAB5_BRA
Orthopyxis caliculata	Kinsale, Ireland	BMNH 1853.4.7.16 (type)	not included
Orthopyxis compressa	Shumagin Islands, USA	USNM 4408 (type)	not included
Orthopyxis crenata	Caponga, Cascavel, Brazil	MZUSP 2633	CB_BRA
Orthopyxis crenata	Paciência, Penha, Brazil	MZUSP 2551, 2560	PAB2, PAB7_BRA
Orthopyxis crenata	Lázaro, Ubatuba, Brazil	MZUSP 2598, 2601	LB5, LB8_BRA
Campanulariidae sp. indet.	La Mina, Puerto San Julián, Argentina	MZUSP 2638, 2640	SJ1, SJ3_ARG
Orthopyxis integra	San Julián, Argentina	LEM	**PT20_ARG**
*Orthopyxis integra*_1_USA	Aleutians, USA	USNM 1106184	1_USA
Orthopyxis integra CA sp.1	Monterey, USA	USNM 1106167*	USA
Orthopyxis mianzani	Mel Island, Brazil	MZUSP 2570-80, USNM 1259970	MB1-5, FOB1-7_BRA
Orthopyxis mianzani	Paciência, Penha, Brazil	MZUSP 2559	PAB6_BRA

Species	**Locality**	**Voucher**	**Code** (as in Cunha et al. 2016b)
Orthopyxis sargassicola	Aracruz, Brazil	MZUSP 2617-20, 2627-2630, 2632	FB1-2, PB2-7_BRA
Orthopyxis sargassicola	Paraty, Brazil	MZUSP 2605-09	PTY1-5_BRA
Orthopyxis sargassicola	Ratos Island, Paraty, Brazil	MZUSP 2610	RI_BRA
Orthopyxis sargassicola	Meros Island, Paraty, Brazil	MZUSP 2611	MI_BRA
Orthopyxis sargassicola	Lázaro, Ubatuba, Brazil	MZUSP 2594-97, 2599-2600, 2602-03	LB1-5, LB6-7, LB9-10_BRA
Orthopyxis sargassicola	São Sebastião, Brazil	MZUSP 2593	SS_BRA
Orthopyxis sargassicola	Campeche Island, Florianópolis, Brazil	MZUSP 4597	CI1_BRA
Orthopyxis sp. 1	Piran, Slovenia	LEM	**EL02**, EL04, EL16, EL52_SLV
Orthopyxis everta	Torre del Serpe, Italy	USNM 1106159	IT
*Orthopyxis integra*_IT	Italy	USNM 1106180	IT
Orthopyxis sp.	Comodoro Rivadavia, Argentina	LEM	**PT19_ARG**
Orthopyxis sp.	Caleta Olivia, Argentina	MZUSP 2644	Co1_ARG
*Orthopyxis integra*_NZ	New Zealand	USNM 1106163	NZ
Rhizocaulus verticillatus	Aleutians, USA	USNM 1106183	**USA**
Silicularia rosea	San Julián, Argentina	LEM	**PT8**, PT11_ARG
*Silicularia rosea*_1_NZ	Bay of Islands, New Zealand	USNM 1106164	1_NZ
Tulpa tulipifera	Patagonia, Argentina	LEM	PT18_ARG

Table 2. Measurements included in the morphometric analysis.

Code	Measurement
TLT	Total Length of Trophosome
TD	Thickness of Diaphragm
DBC	Diameter of Hydrothecal Basal Chamber (at diaphragm)
LBC	Length of Hydrothecal Basal Chamber
HRatio	Length:Diameter (at medial portion) Ratio of Hydrotheca
LH	Length of Hydrotheca
DHMa	Maximum Hydrothecal Diameter at Margin
DHMe	Maximum Hydrothecal Diameter at Medial Portion
DHB	Maximum Hydrothecal Diameter at Base
PHMa	Maximum Hydrothecal Perisarc Thickness at Margin
PHMe	Maximum Hydrothecal Perisarc Thickness at Medial Portion
PHB	Maximum Hydrothecal Perisarc Thickness at Base
NC	Number of Hydrothecal Cusps
HCMax	Maximum Height of Hydrothecal Cusps
HCMin	Minimum Height of Hydrothecal Cusps
DSS	Maximun Diameter of Subhydrothecal Spherule
LSS	Length of Subhydrothecal Spherule
PSS	Maximum Perisarc Thickness of Subhydrothecal Spherule
LP	Length of Pedicel
DP	Maximum Diameter of Pedicel at Medial Portion
NSP	Maximum Number of Pedicel Sinuosities (crenations)
APB	Number of Pedicel Annuli at Base
APH	Number of Pedicel Annuli below Hydrotheca
APMe	Number of Pedicel Annuli at Medial Portion
PPMe	Maximum Perisarc Thickness of Pedicel at Median Portion
GRatio	Length:Diameter (at medial portion) Ratio of Gonotheca
LG	Length of Gonotheca
DGD	Maximum Gonothecal Diameter at Distal Portion
DGMe	Maximum Gonothecal Diameter at Medial Portion
DGB	Maximum Gonothecal Diameter at Base
NSG	Number of Gonothecal Sinuosities (crenations)
AG	Number of Gonothecal Annuli
HGC	Height of Gonothecal Collar
PGMe	Maximum Gonothecal Perisarc Thickness at Medial Portion
LGP	Length of Gonothecal Pedicel
DGP	Maximum Diameter of Gonothecal Pedicel at Medial Portion
PGP	Maximum Perisarc Thickness of Gonothecal Pedicel at Medial Portion
AGP	Number of Annuli of Gonothecal Pedicel
NIS	Total Number of Internodes of Main Stem
LIS	Length of Internode of Main Stem
DIS	Maximum Diameter of Internode of Main Stem at Medial Portion

Code	Measurement
PIS	Maximum Perisarc Thickness of Internode of Main Stem at Medial Portion
AIS	Maximum Number of Annuli of the Internodes of Main Stem
NIB	Maximum Number of Internodes of Side Branches
LIB	Length of Internode of Side Branches
DIB	Maximum Diameter of Internode of Side Branches at Medial Portion
PIB	Maximum Perisarc Thickness of Internode of Side Branches at Medial Portion
AIB	Maximum Number of Annuli of the Internodes of Side Branches
ABS	Number of Annuli at the Base of Main Stem

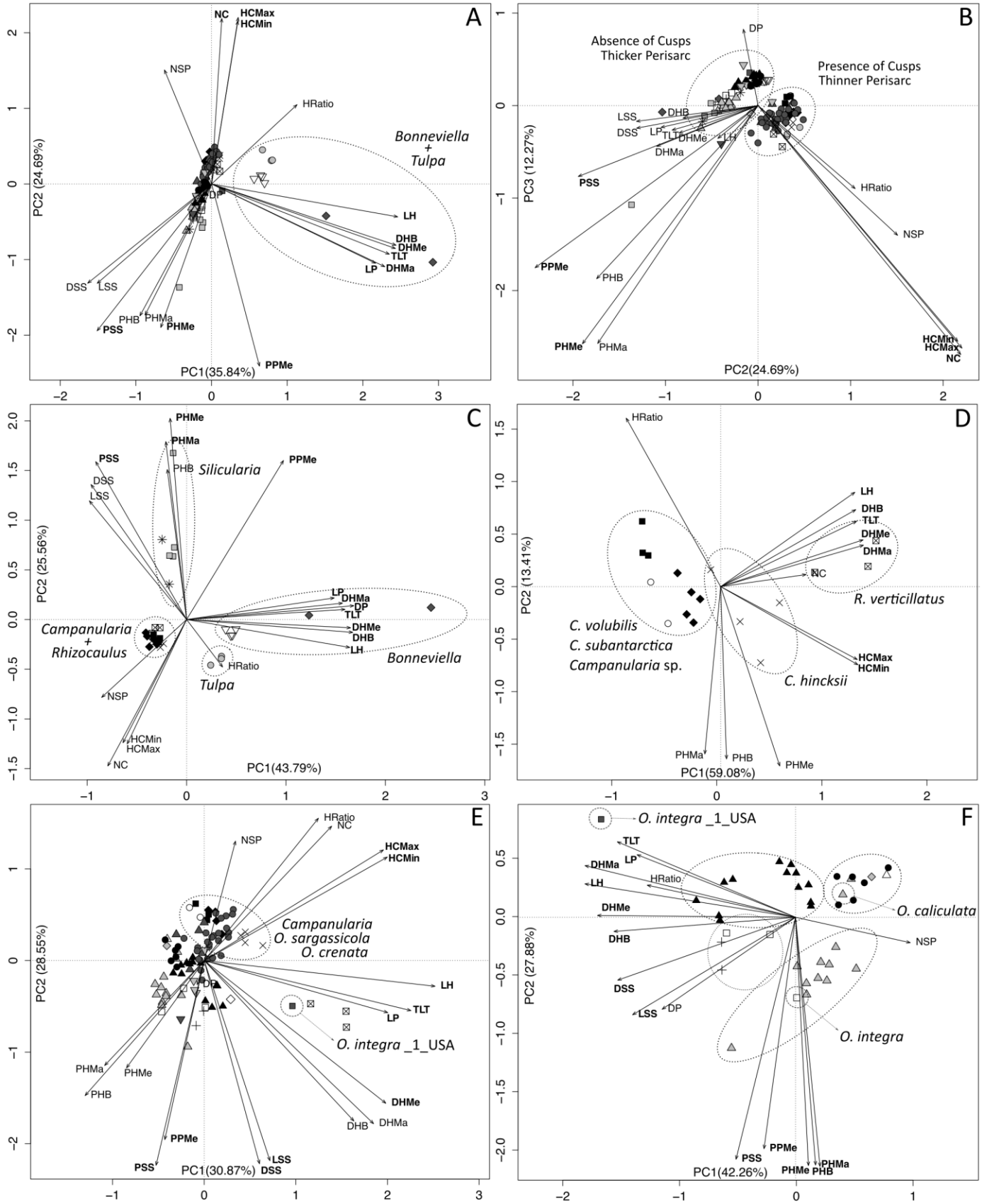
A
NC
HCMax
HCMin
NSP
HRatio
Bonneviella
+
Tulpa
LH
DHB
DHMe
TLT
LP
DHMa
DSS
LSS
PHB
PHMa
PSS
PHMe
PPMe
PC1(35.84%)
PC2 (24.69%)
B
Absence of Cusps
Thicker Perisarc
Presence of Cusps
Thinner Perisarc
DP
LSS
DHB
DSS
LP
TLT
DHMe
DHMa
LH
PSS
HRatio
NSP
PPMe
PHB
PHMe
PHMa
HCMin
HCMax
NC
PC2(24.69%)
PC3 (12.27%)
C
PHMe
PHMa
PSS
PHB
Silicularia
PPMe
DSS
LSS
LP
DHMa
DP
TLT
DHMe
DHB
LH
Campanularia
+
Rhizocaulus
HRatio
Bonneviella
Tulpa
NSP
HCMin
HCMax
NC
PC1(43.79%)
PC2 (25.56%)
D
HRatio
LH
DHB
TLT
DHMe
DHMa
NC
R. verticillatus
C. volubilis
C. subantarctica
Campanularia sp.
HCMax
HCMin
C. hincksii
PHMa
PHB
PHMe
PC1(59.08%)
PC2 (13.41%)
E
HRatio
NC
NSP
HCMax
HCMin
Campanularia
O. sargassicola
O. crenata
LH
TLT
LP
O. integra _1_USA
PHMa
PHMe
PHB
DHMe
DHB
DHMa
PPMe
PSS
LSS
DSS
PC1(30.87%)
PC2 (28.55%)
F
O. integra _1_USA
TLT
LP
DHMa
LH
HRatio
DHMe
DHB
O. caliculata
NSP
DSS
LSS
DP
O. integra
PSS
PPMe
PHMe
PHB
PHMa
PC1(42.26%)
PC2 (27.88%)
Bonneviella regia
Bonneviella sp.
Campanularia hincksii
Campanularia sp.
Campanularia subantarctica
Campanularia volubilis
Campanulariidae sp. indet.
Orthopyxis caliculata
Orthopyxis crenata
Orthopyxis everta
Orthopyxis integra
Orthopyxis integra CA sp.1
Orthopyxis integra_IT
Orthopyxis integra_1_USA
Orthopyxis integra_NZ
Orthopyxis mianzani
Orthopyxis sargassicola
Orthopyxis sp.
Orthopyxis sp.1
Rhizocaulus verticillatus
Silicularia rosea
Silicularia rosea_NZ1
Tulpa tulipifera

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*. Numbers in parentheses indicate percentages of variation explained by each principal component. Abbreviations of morphometric variables as in Table 2, and those in bold indicate measurements that were correlated with each principal component (Pearson correlation >0.7 and <-0.7).

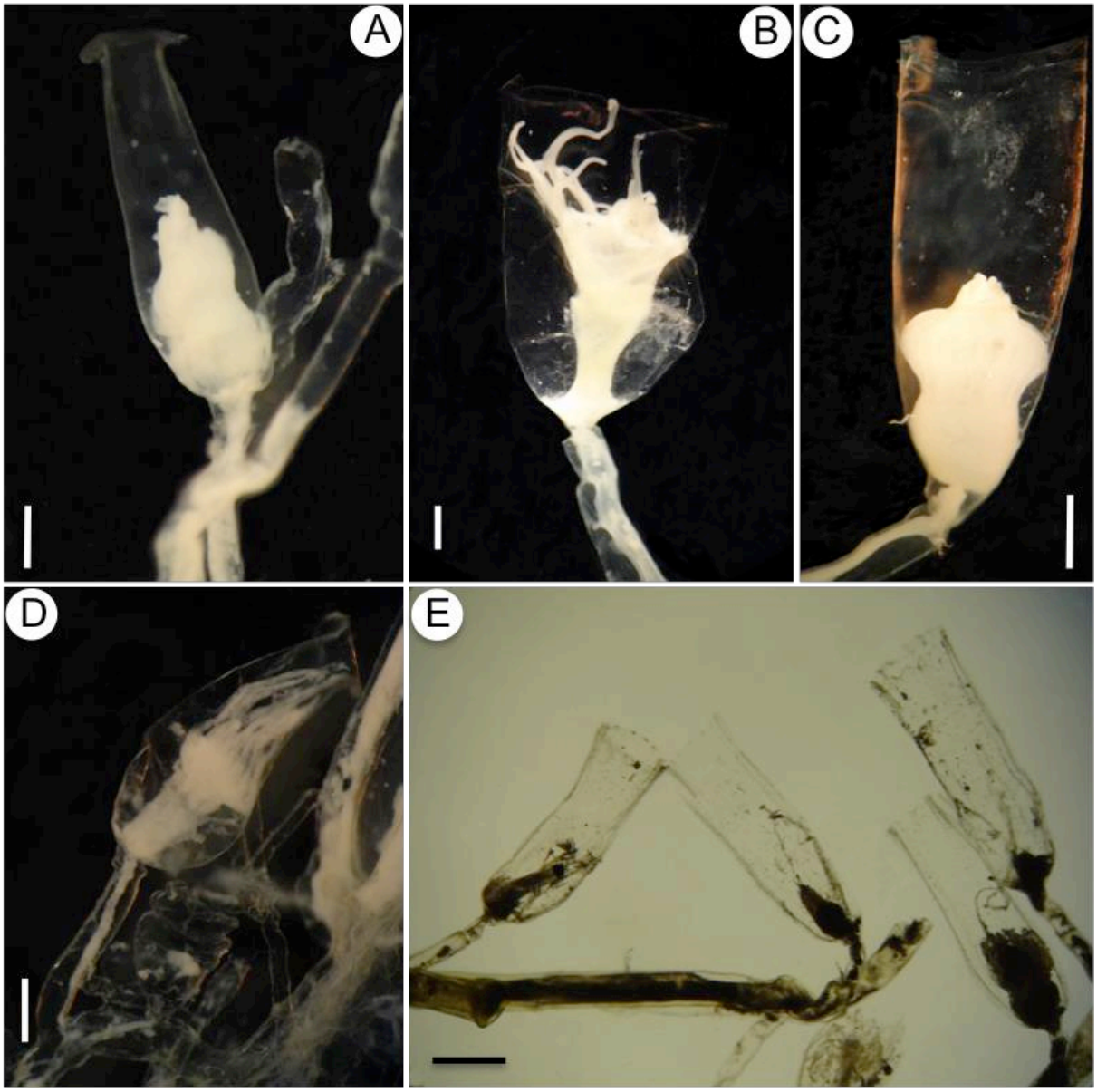

Figure 2. A. *Bonneviella regia* (USNM 1106181), hydrotheca; B. *Bonneviella* sp. (USNM 1106182), hydrotheca; C. *Bonneviella* sp. (USNM 1106187), hydrotheca; D. *Bonneviella regia* (USNM 1106181), hydrotheca and gonotheca; E. *Tulpa tulipifera* (PT18_ARG), colony and hydrothecae. Scales: A, D, E = 500 μm; B, C = 1 mm.

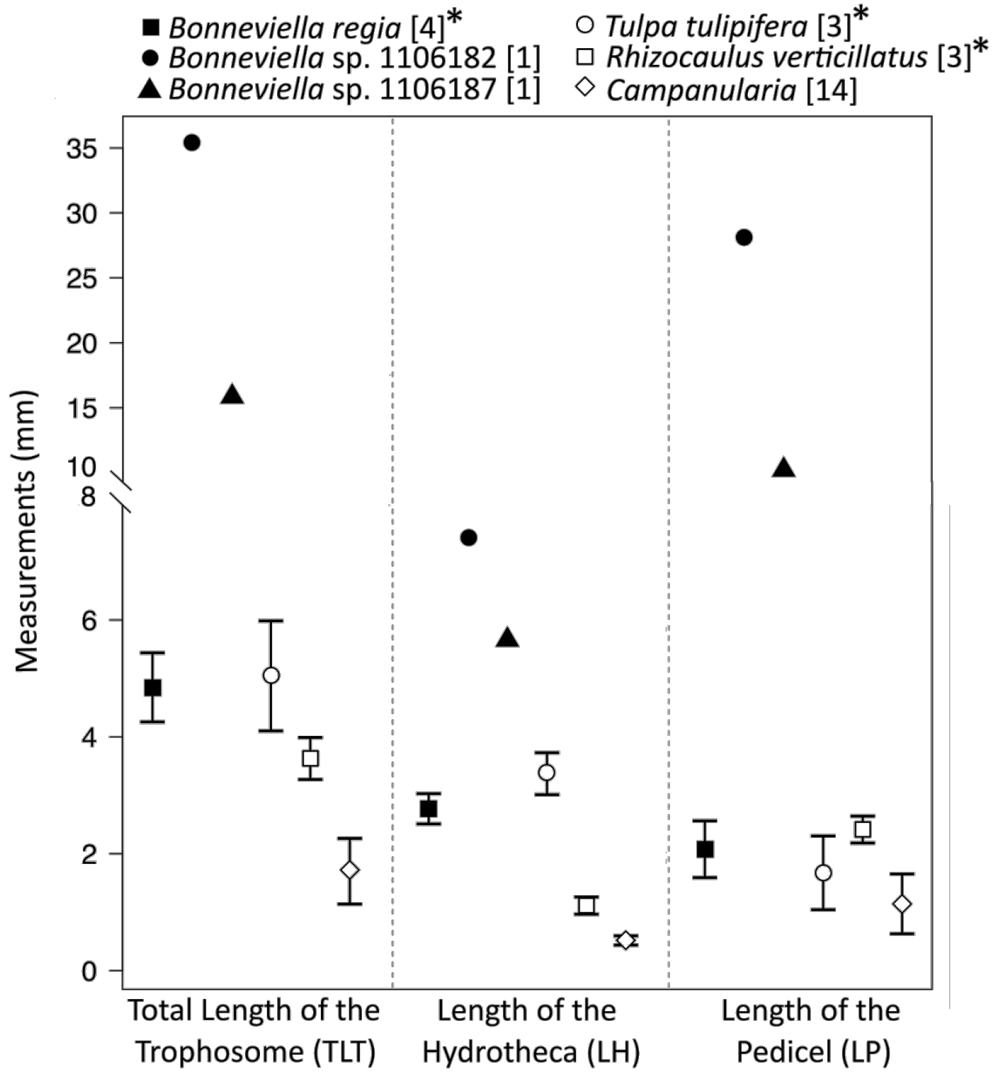

Figure 3. Mean ± standard deviation (mm) of morphometric data for *Bonneviella regia*, *Bonneviella* sp., *Tulpa tulipifera*, *Rhizocaulus verticillatus*, and all four species of *Campanularia* analyzed in this study. * = indicates intracolony variation; brackets = [number of specimens/colonies measured].

Table 3. 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).

Measures (mm)	***Bonneviella regia* USNM 71390 [2]**	*Bonneviella regia* USNM 1106181 [4]	*Bonneviella* sp. USNM 1106182 [1]	*Bonneviella* sp. USNM 1106187 [1]	***Bonneviella ingens* USNM 34576 [2]**	***Bonneviella superba* USNM 3480 [1]**	*Bonneviella grandis* [Schuchert, 2001]
Total Length of Trophosome	-	4.8476±0.2956 (4.0132-5.3944)	35.4123	15.8825	10.1459±1.4820 (8.6638-11.6280)	24.4778	-
Hydrotheca							
Length	2.5790±0.4149 (2.1642-2.9939)	2.7700±0.1295 (2.4487-3.0061)	7.4064	5.6638	3.9850±0.8077 (3.1774-4.7927)	9.8829	7.0
Diameter at margin	0.8298±0.0677 (0.7621-0.8975)	0.5407±0.1182 (0.3300-0.8499)	4.7948	2.5493	2.3789±0.0975 (2.2814-2.4764)	2.8841	2.5
Length:Diameter Ratio	3.2729±0.0284 (3.2446-3.3013)	3.1023±0.1522 (2.8369-3.4191)	1.7263	2.3124	1.8832±0.3385 (1.5446-2.2217)	3.4267	2.8
Pedicel							
Length	-	2.0776±0.2428 (1.5646-2.7209)	28.0060	0.8149	6.1608±2.2897 (3.8711-8.4506)	14.5949	-
Diameter at Medial Portion	-	0.2574±0.0038 (0.2502-0.2650)	10.2187	0.4744	0.6383±0.0811 (0.5571-0.7194)	0.6796	-
Gonotheca							
Length	-	1.1538±0.0462 (1.0884-1.2192) [2]	-	-	-	5.9855	6.0-8.0
Maximum Diameter	-	0.7048±0.0057 (0.6967-0.7129) [2]	-	-	-	1.61487	2.5
Shape	-	Cylindrical, transversely ribbed	-	-	-	Cylindrical, transversely ribbed	Oblong ellipsoid, longitudinally ribbed

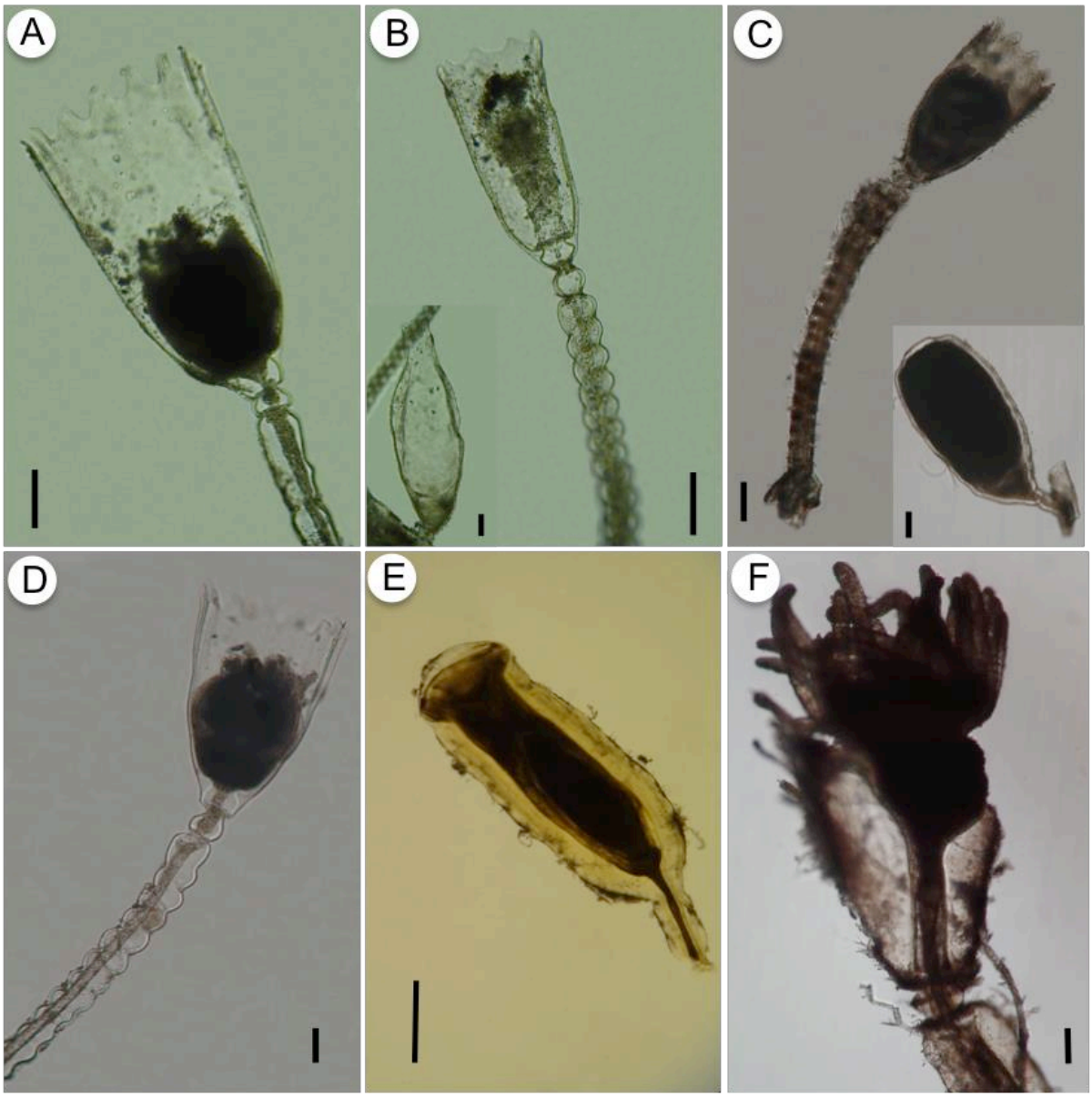

Figure 4. A. *Campanularia hincksii* (IT14_IT), hydrotheca; B. *Campanularia volubilis* (USNM 29127), hydrotheca, pedicel, and gonotheca; C. *Campanularia subantarctica* (SJ2_ARG), hydrotheca, pedicel, and gonotheca; D. *Campanularia* sp. (SJ4_ARG), hydrotheca with pedicel; E. *Silicularia rosea* (PT11_ARG), gonotheca; F. *Silicularia rosea* (PT11_ARG), hydrotheca. Scales – A, B (both), C (both), D, F = 100 μm; E = 500 μm.

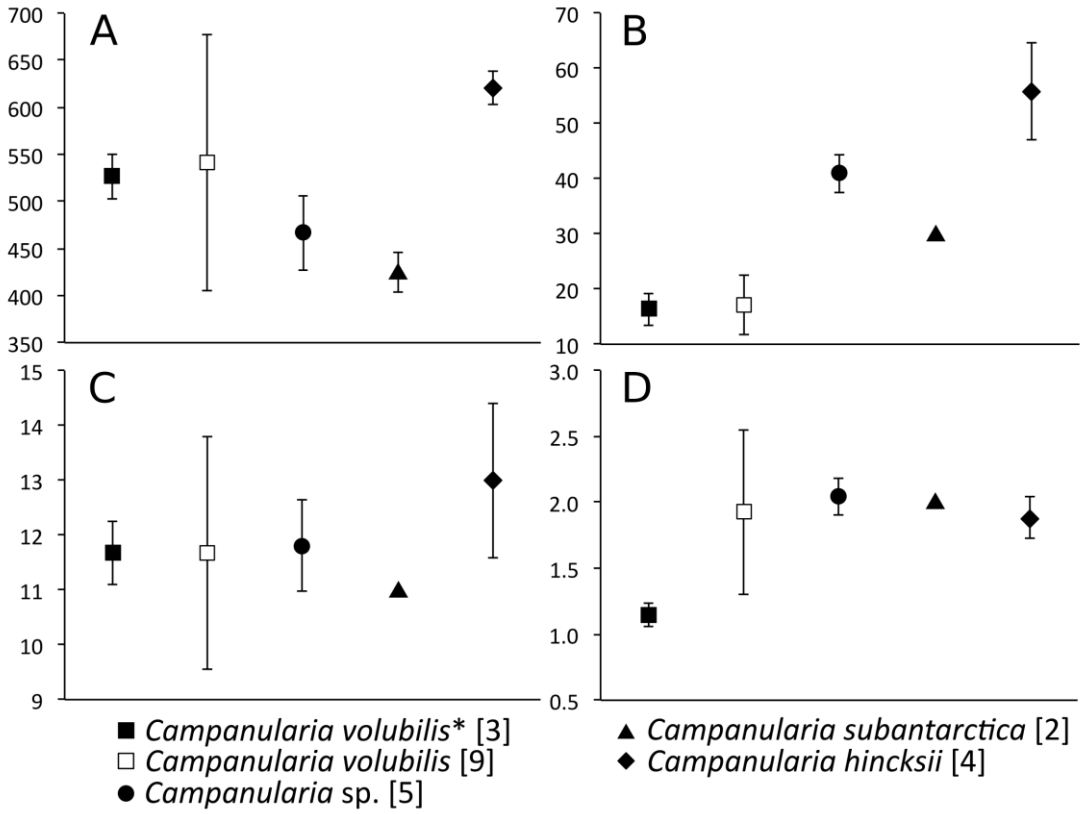

Figure 5. Mean ± standard deviation (µm) of morphometric data for *Campanularia*. Note that the amplitude of variation changes when specimens from different populations are considered (*C. volubilis*, ZMUC and USNM 29217). A. Length of hydrothecae (LH); B. Maximum height of hydrothecal cusps (HCMax); C. Number of hydrothecal cusps (NC); D. Length:diameter ratio of hydrotheca (HRatio). * = indicates intracolony variation; brackets = [number of specimens/colonies measured].

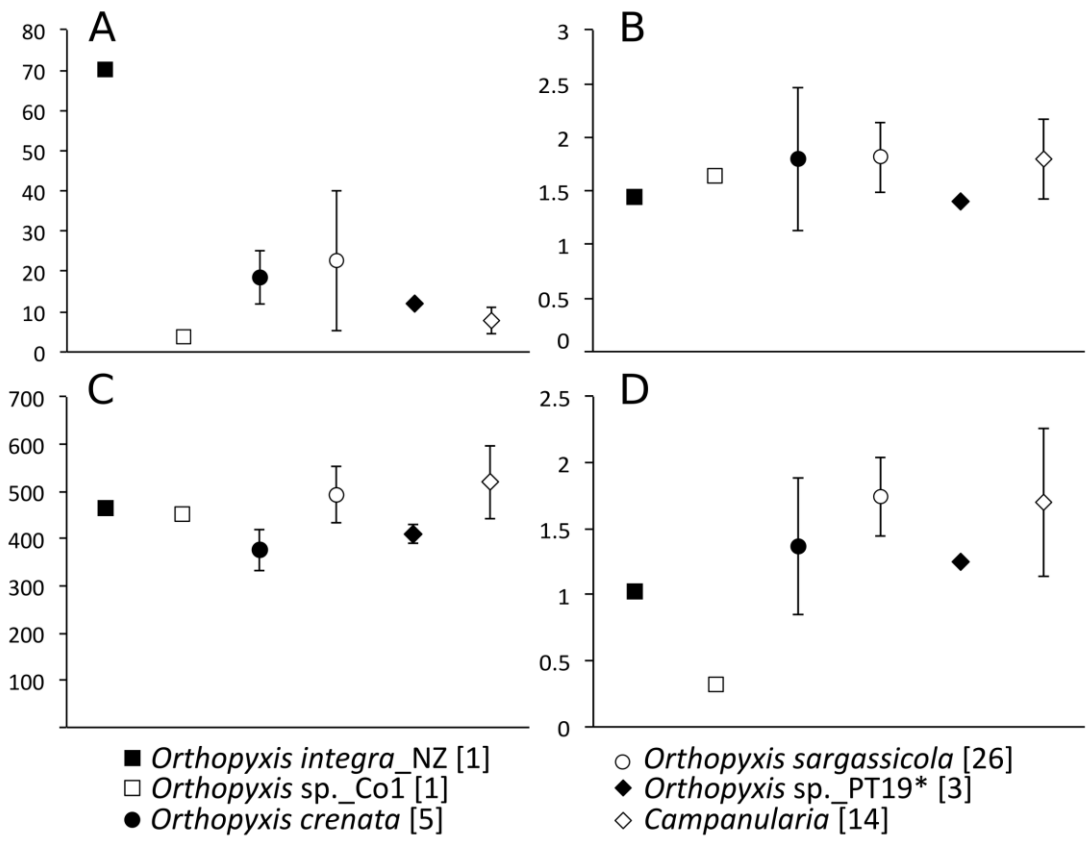

Figure 6. Mean ± standard deviation of morphometric data for *Orthopyxis sargassicola*, *Orthopyxis crenata* and the lineages related to *O*. *crenata*, as well as a comparison with *Campanularia*. A. Maximum perisarc thickness of hydrotheca at medial portion (PHMe, μm); B. Length:diameter ratio of the hydrotheca (HRatio, μm); C. Length of the hydrotheca (LH, μm); D. Total length of trophosome (TLT, mm). * = indicates intracolony variation; brackets = [number of specimens/colonies measured].

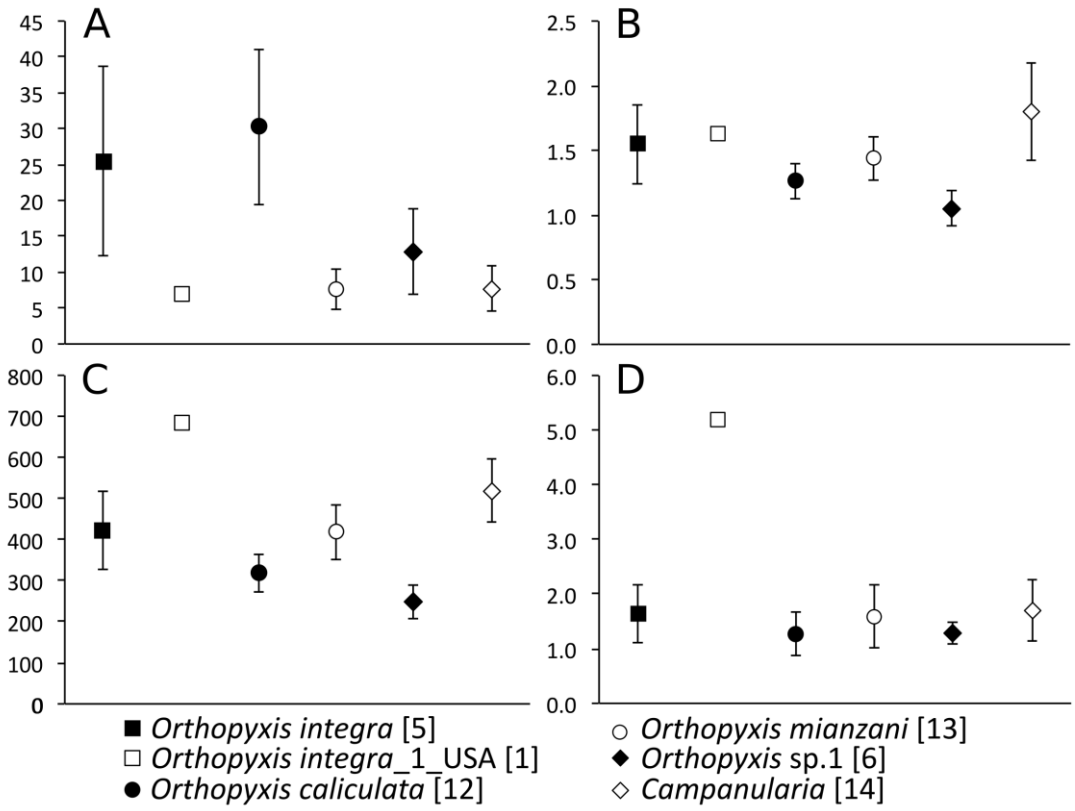

Figure 7. Mean ± standard deviation of morphometric data for *Orthopyxis integra*, *Orthopyxis caliculata*, *Orthopyxis mianzani*, *Orthopyxis* sp.1, and a comparison with the genus *Campanularia*. Campanulariidae sp. indet. was included among the specimens of *O. integra* due to their close phylogenetic relationship (Cunha et al. 2016b). A. Maximum perisarc thickness of hydrotheca at medial portion (PHMe, μm); B. Length:diameter ratio of hydrotheca (HRatio, μm); C. Length of the hydrotheca (LH, μm); D. Total length of the trophosome (TLT, mm). Brackets = [number of specimens/colonies measured].

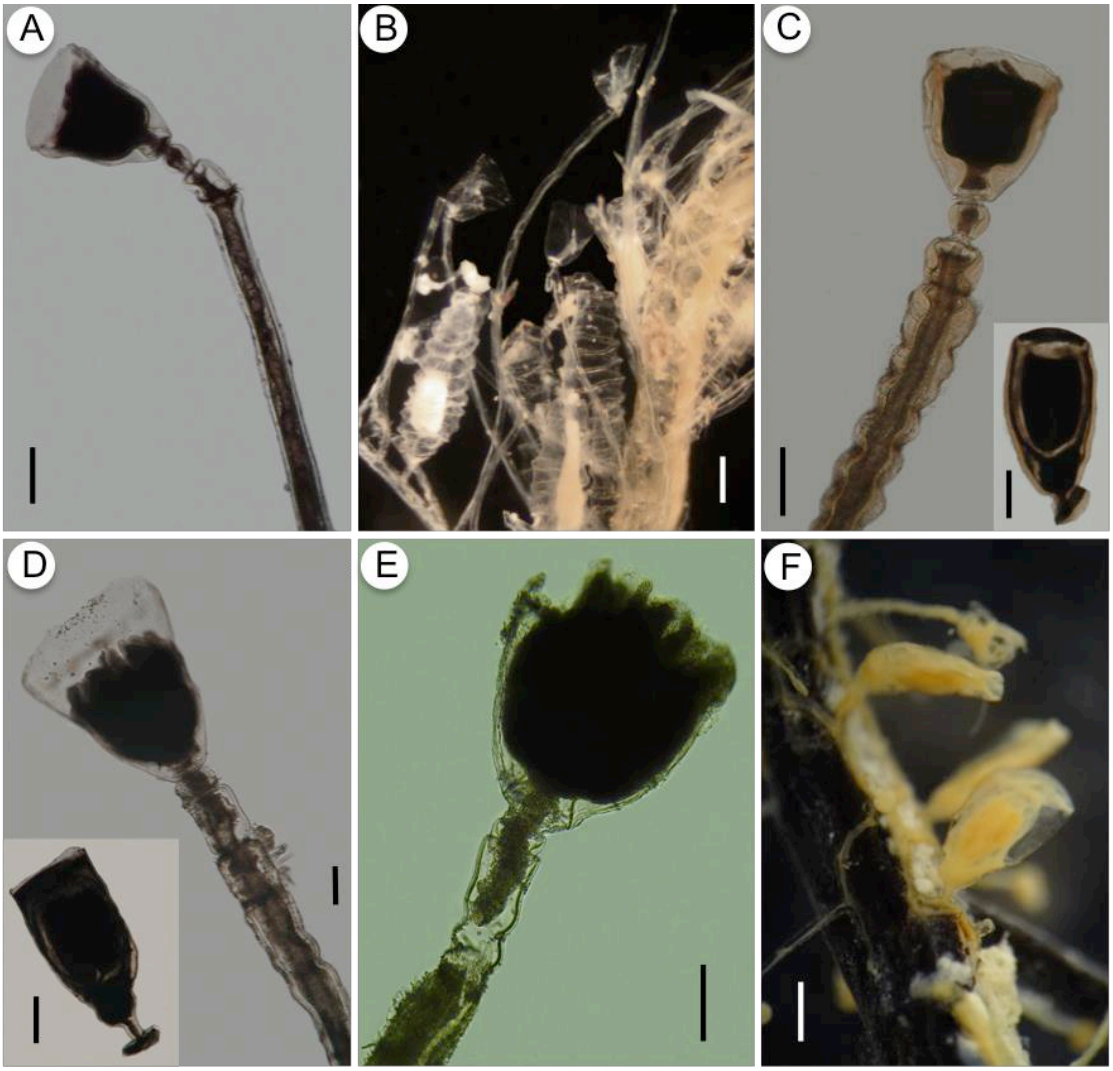

Figure 8. A. *Orthopyxis integra* (PT20_ARG), hydrotheca and pedicel; B. *Orthopyxis integra*_1_USA (USNM 1106184), colony with gonothecae; C. *Orthopyxis caliculata* (PAB4_BRA, MZUSP 2554), hydrotheca, pedicel, and gonotheca; D. *Orthopyxis mianzani* (FOB7_BRA, MZUSP 2580), hydrotheca, pedicel, and gonotheca; E. *Orthopyxis* sp.1 (EL02_SLV), hydrotheca and pedicel; F. *Orthopyxis* sp.1 (EL02_SLV), gonothecae on substrate. Scales – A, C (hydrotheca and pedicel) = 200 μm; B, C (gonotheca), D (gonotheca), F = 500 μm; D (hydrotheca and pedicel), E = 100 μm.

Table 4. 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.

Measures (μm)	*Orthopyxis* sp.1 [6]	*Orthopyxis everta* USNM 1106159 [1]	*Orthopyxis integra*_IT USNM 1106180 [1]	*Orthopyxis asymmetrica* [Stechow1919a; Peña-Cantero & Carcía-Carrascosa 2002]	*Orthopyxis angulata* [Bale 1914; Watson 2005]	***Orthopyxis compressa* USNM 4408 [3]**	***Orthopyxis caliculata* NHM-UK 1853.4.7.16 [3]**
Total Length of Trophosome	1284.41±80.46 (1054.67-1573.61)	1038.97	886.58	1000-1200	-	2696.53±699.11 (1334.95-3652.68)	1001.65±34.66 (933.33-1045.92)
Hydrotheca							
Length	248.15±16.56 (184.77-296.94)	237.02	228.03	336	435-593	673.99±61.55 (551.26-743.59)	273.14±2.51 (268.31-276.76)
Diameter at margin	270.47±14.12 (213.15-314.34)	262.56	246.9	320	270-370	417.64±34.14 (362.07-479.79)	223.62±6.53 (215.09-236.45)
Length:Diameter Ratio	1.05±0.06 (0.8851-1.2739)	1.15	1.09	1.05	-	1.61±0.08 (1.52-1.77)	1.22±0.04 (1.16-1.29)
Maximum Perisarc Thickness at Medial Portion	12.84±2.44 (7.94-24.26)	15.36	4.96	-	-	24.12±10.62 (11.21-45.17)	29.03±1.72 (25.60-31.01)
Length:Diameter Ratio of the Basal Chamber	0.64±0.07 (0.5475-0.6739)	1.07	0.89	-	-	1.37±0.21 (0.95-1.63)	0.86±0.03 (0.80-0.90)
Pedicel							
Length	990.81±85.85 (721.10-1310.71)	762.73	615.55	-	686-2900	2050.28±686.95 (726.54-3030.73)	664.60±31.18 (603.53-706.04)

Measures (μm)	*Orthopyxis* sp.1 [6]	*Orthopyxis everta* USNM 1106159 [1]	*Orthopyxis integra*_IT USNM 1106180 [1]	*Orthopyxis asymmetrica* [Stechow1919a; Peña-Cantero & Carcía-Carrascosa 2002]	*Orthopyxis angulata* [Bale 1914; Watson 2005]	***Orthopyxis compressa* USNM 4408 [3]**	***Orthopyxis caliculata* NHM-UK 1853.4.7.16 [3]**
Diameter at Medial Portion	84.97±2.95 (73.53-94.79)	61.97	90.75	-		133.80±17.69 (107.88-167.63)	87.22±9.13 (69.69-100.42)
Maximum Perisarc Thickness at Medial Portion	10.71±1.18 (7.52-14.5)	6.09	14.42	-		14.84±5.46 (9.34-25.75)	18.46±5.80 (10.80-29.83)
Gonotheca							
Length	1242.49±218.36 (536.22-1912.54) [5]	1052.19	-	-	1176-1333	1528.11±58.32 (1411.90-1594.76)	-
Maximum Diameter	917.14±102.64 (681.94-1300.00) [5]	492.18	-	-	882-980	1312.18±5.86 (1300.84-1320.45)	-

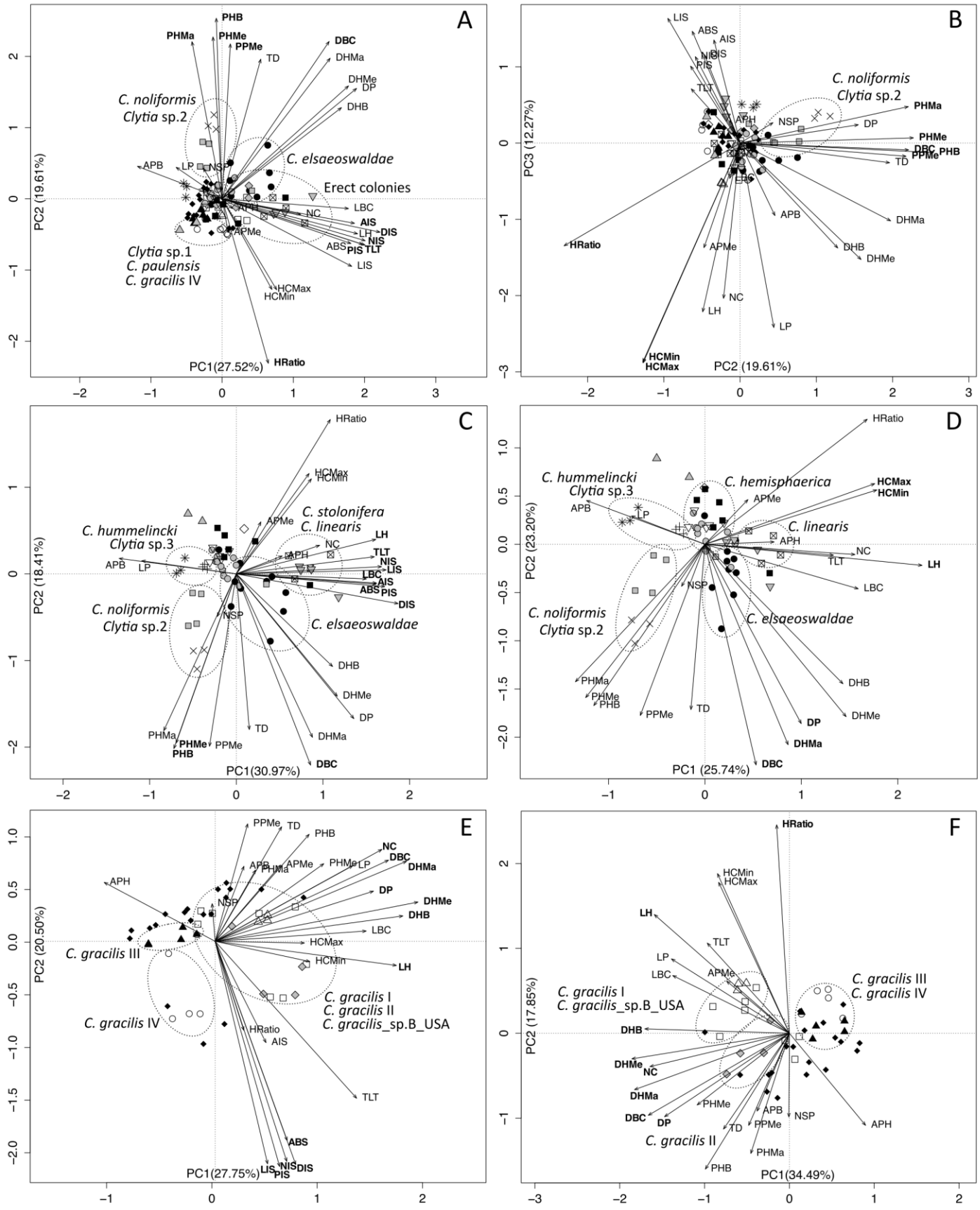
A
PC1(27.52%)
PC2 (19.61%)
C. noliformis
Clytia sp.2
C. elsaeoswaldae
Erect colonies
Clytia sp.1
C. paulensis
C. gracilis IV
B
PC2 (19.61%)
PC3 (12.27%)
C. noliformis
Clytia sp.2
C
PC1(30.97%)
PC2 (18.41%)
C. hummelincki
Clytia sp.3
C. stolonifera
C. linearis
C. noliformis
Clytia sp.2
C. elsaeoswaldae
D
PC1 (25.74%)
PC2 (23.20%)
C. hummelincki
Clytia sp.3
C. hemisphaerica
C. linearis
C. noliformis
Clytia sp.2
C. elsaeoswaldae
E
PC1(27.75%)
PC2 (20.50%)
C. gracilis III
C. gracilis IV
C. gracilis I
C. gracilis II
C. gracilis_sp.B_USA
F
PC1(34.49%)
PC2 (17.85%)
C. gracilis I
C. gracilis_sp.B_USA
C. gracilis III
C. gracilis IV
C. gracilis II
Clytia elsaeoswaldae
Clytia gracilis I
Clytia gracilis II
Clytia gracilis III
Clytia gracilis IV
Clytia gracilis V
Clytia gracilis_sp.B_USA
Clytia hemisphaerica I
Clytia hemisphaerica II
Clytia hemisphaerica III
Clytia hummelincki
Clytia linearis
Clytia noliformis
Clytia paulensis
Clytia sp.1
Clytia sp.2
Clytia sp.3
Clytia stolonifera

Figure 9. 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 gracilis*; D. First and second PCs of the PCA without *C. gracilis* and measurements related to internodes of erect colonies (NIS, LIS, AIS, PIS, DIS, ABS); E. First and second PCs of the PCA with *C. gracilis*; F. First and second PCs of the PCA with *C. gracilis*, excluding measurements related to internodes of erect colonies (NIS, LIS, AIS, PIS, DIS, ABS). Numbers in parentheses indicate percentages of variation explained by each principal component. Abbreviations of morphometric variables as in Table 2, and those in bold indicate measurements that were correlated with each principal component (Pearson correlation >0.7 and <-0.7).

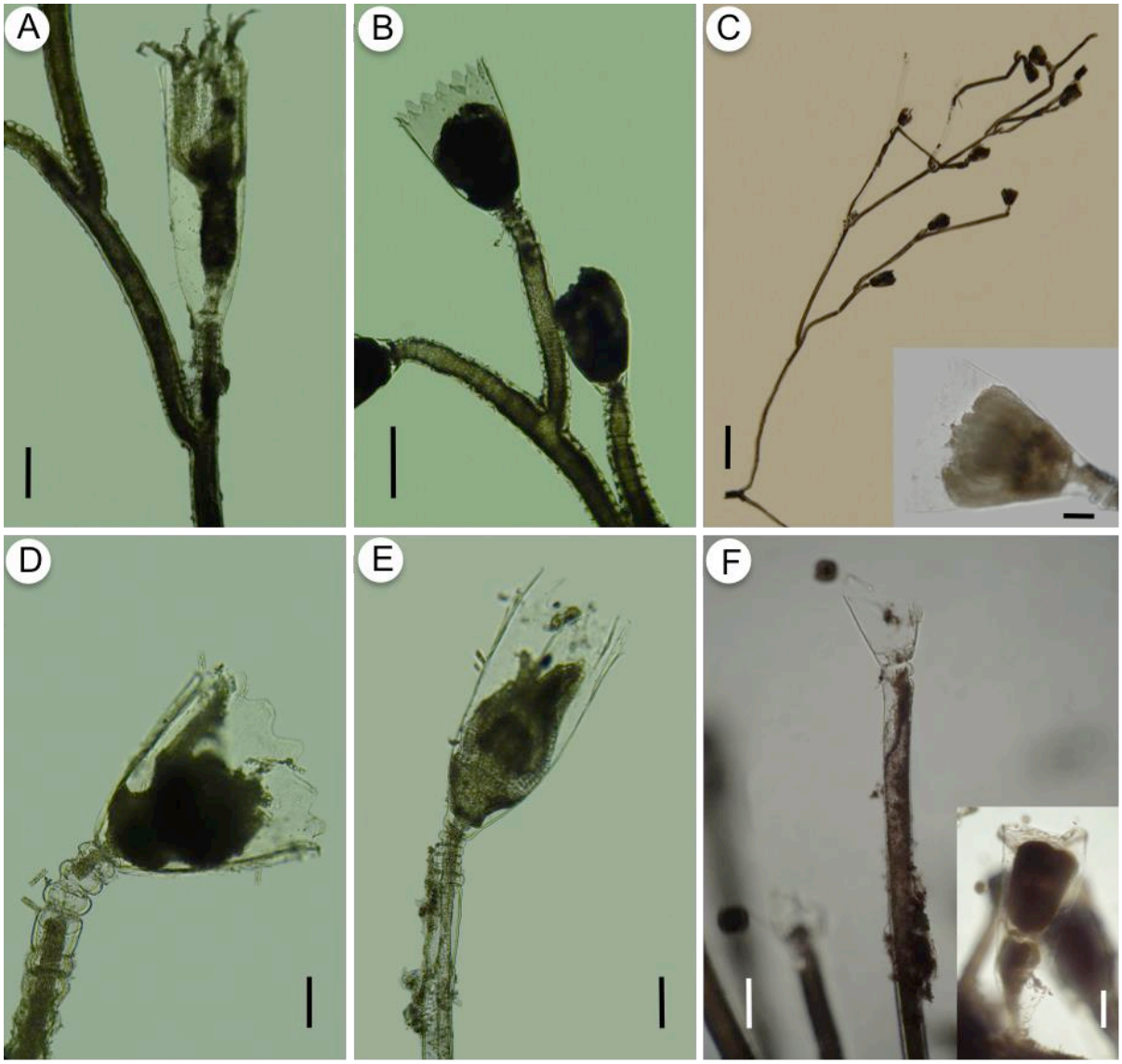

Figure 10. A. *Clytia linearis* (PY10_BRA), hydrotheca; B. *Clytia elsaeoswaldae* (Me26_BRA), branching in erect colony; C. *Clytia stolonifera* (CBC45_BLZ), erect colony, hydrotheca; D. *Clytia noliformis* (SP3_BRA), hydrotheca; E. *Clytia* sp.1 (SP1_BRA), hydrotheca; F. *Clytia hummelincki* (CBC42_BLZ), hydrotheca, pedicel, and gonotheca. Scales: A = 500 µm; B = 300 µm; C (colony) = 1 mm, C, D, E (hydrotheca), F (gonotheca) = 100 µm; F (hydrotheca and pedicel) = 200 µm.

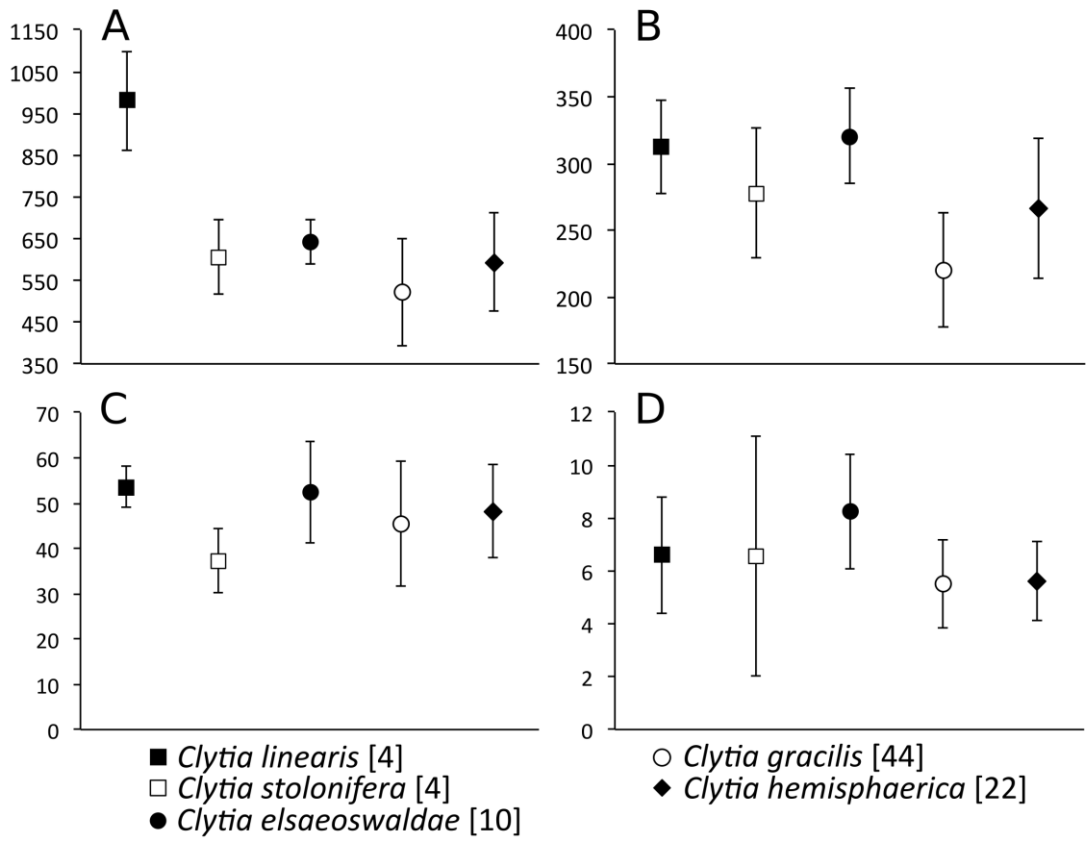

Figure 11. Mean ± standard deviation of morphometric data for *Clytia linearis*, *Clytia stolonifera*, *Clytia elsaeoswaldae*, and all lineages identified as *Clytia gracilis* and *Clytia hemisphaerica*. A. Length of the hydrotheca (LH, µm); B. Maximum diameter of hydrotheca at medial portion (DHMe, µm); C. Maximum height of hydrothecal cusps (HCMax, µm); D. Thickness of diaphragm (TD, µm). Brackets = [number of specimens/colonies measured].

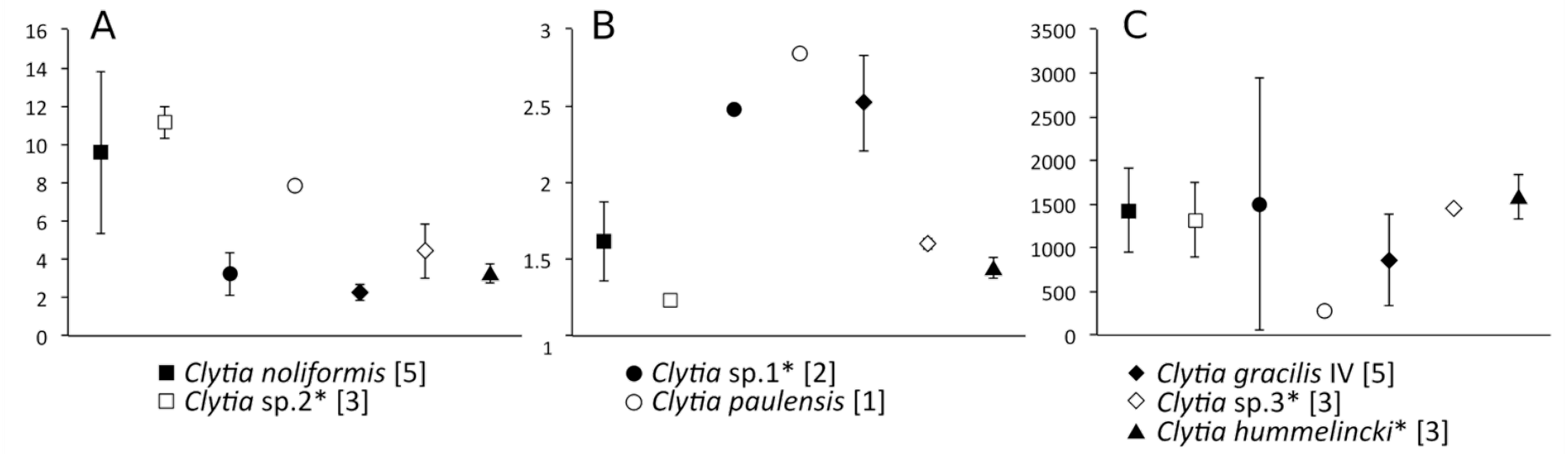

Figure 12. Mean ± standard deviation of morphometric data for *Clytia noliformis*, *Clytia* sp.1, *Clytia* sp.2, *Clytia* sp.3, *Clytia paulensis*, *Clytia gracilis* IV and *Clytia hummelincki*. A. Maximum hydrothecal perisarc thickness at margin (PHMa, μm); B. Length:diameter ratio of hydrotheca (HRatio, μm); C. Length of pedicel (LP, μm). * = indicates intracolony variation; brackets = [number of specimens/colonies measured].

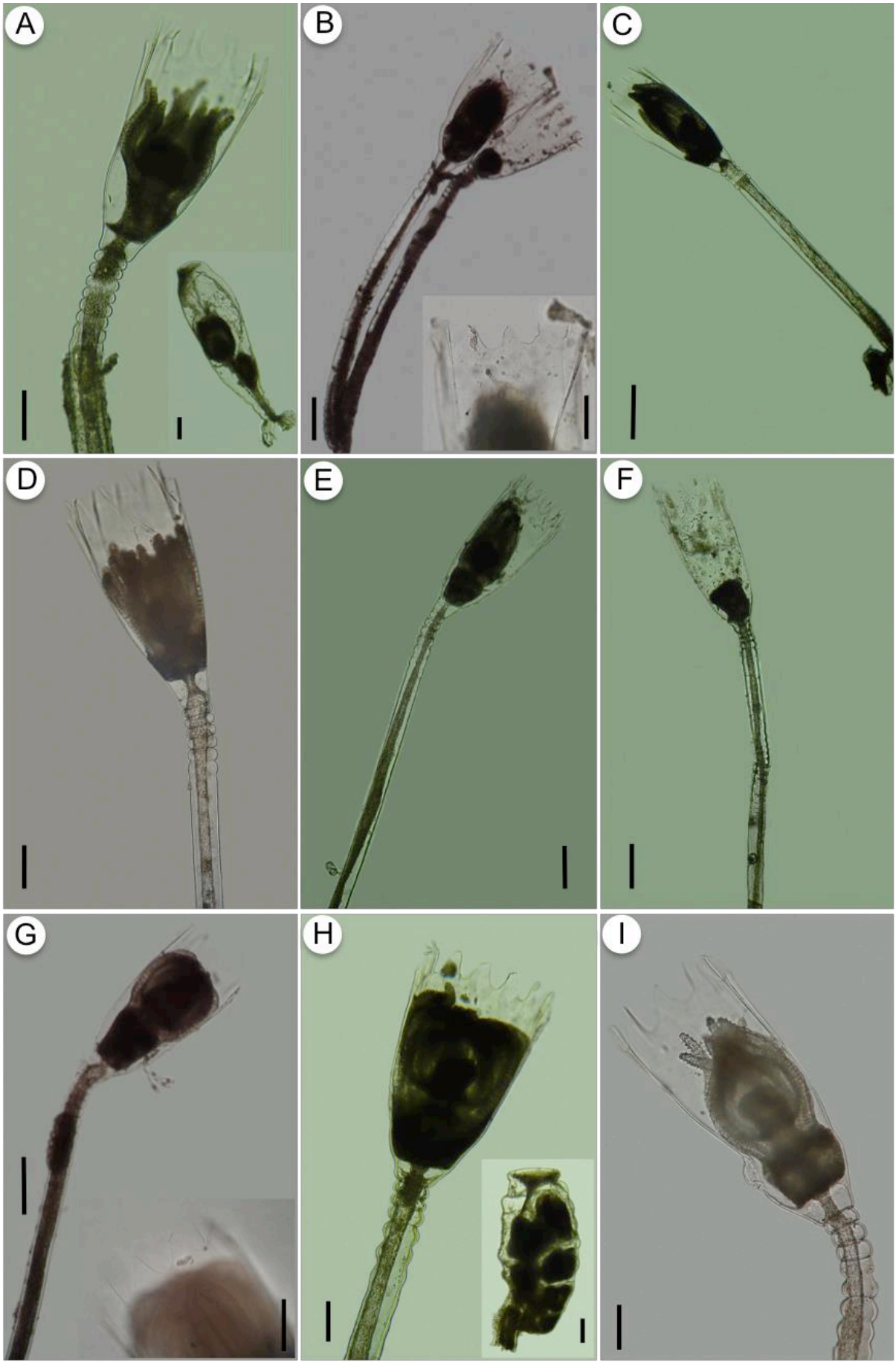
A
B
C
D
E
F
G
H
I

Figure 13. A. *Clytia gracilis* I (EL32_SLV), hydrotheca and gonotheca; B. *Clytia gracilis* II (PT9_ARG), branching in erect colony, and hydrothecal cusps; C. *Clytia gracilis* III (EL05_SLV), trophosome; D. *Clytia gracilis* IV (CBC13_BLZ), hydrotheca; E. *Clytia gracilis* V (PAF03_BRA), trophosome; F. *Clytia gracilis* sp.B_USA (USNM 1078730), trophosome; G. *Clytia hemisphaerica* I (FLT03_USA), hydrotheca, and hydrothecal cusps; H. *Clytia hemisphaerica* II (EL06_SLV), hydrotheca, and gonotheca; I. *Clytia hemisphaerica* III (CBC25_BLZ), hydrotheca. Scales: A (both), B (cusps), D, G (both), H (both), I = 100 μm; B (colony), C, E, F = 200 μm.

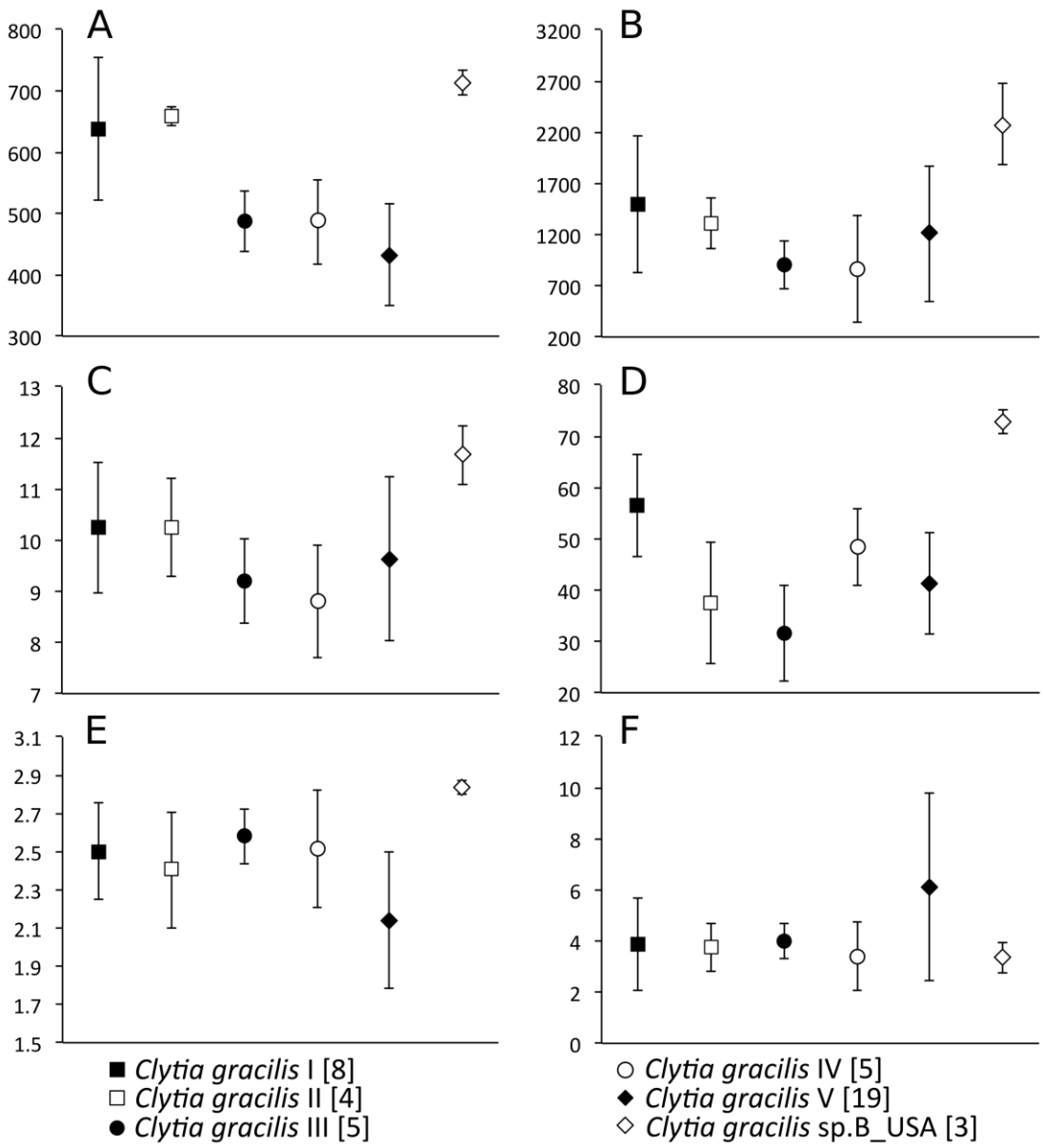

Figure 14. Mean ± standard deviation of morphometric data for lineages identified as *Clytia gracilis*. A. Length of the hydrotheca (LH, µm); B. Length of pedicel (LP, µm); C. Number of hydrothecal cusps (NC); D. Maximum height of hydrothecal cusps (HCMax, µm); E. Length:diameter ratio of hydrotheca (HRatio, µm); F. Number of annuli of the pedicel below the hydrotheca (APH, µm). Brackets = [number of specimens/colonies measured].

Table 5. Comparison among lineages identified as *C. 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.

Measures (μm)	*Clytia gracilis*_sp.B_USA [3]	*Clytia gracilis* I [8]	*Clytia gracilis* II [4]	*Clytia gracilis* III [5]	*Clytia gracilis* IV [5]	*Clytia gracilis* V	*Clytia gulangensis* [He et al. 2015]	*Clytia gracilis* [Calder 1991]	*Clytia gracilis* [Cornelius 1995]	*Clytia gracilis* [Schuchert 2001]
Colony	Stolonal	Stolonal or erect	Erect or planktonic	Stolonal	Stolonal or erect	Stolonal	Stolonal or erect	Stolonal or erect	Erect	Erect
Total Length of Trophosome	2990.86±218.55 (2741.86-3426.48)	2500.15±361.81 (973.26-3864.64)	3695.33±504.25 (2315.12-4543.49)	1393.55±126.63 (1004.20-1721.53)	2423.75±211.27 (1866.44-2930.02)	2053.73±281.65 (1115.65-5187.18)	-	up to 11000	up to 20000	up to 2000
Hydrotheca										
Length	713.77±11.81 (690.36-728.16)	638.37±41.21 (487.44-791.12)	658.92±7.75 (648.72-681.58)	487.44±28.64 (443.85-558.93)	486.39±30.92 (369.70-547.92)	431.96±18.99 (320.56-729.91)	530-1020	736-932	500-900	1000-1200
Diameter (Maximum or at Margin)	284.51±2.64 (279.30-287.91)	284.53±9.76 (248.28-327.05)	301.53±17.63 (261.45-338.07)	210.43±21.82 (174.36-260.24)	218.70±9.31 (194.57-251.66)	244.56±11.54 (178.54-352.18)	180-330	391-522	300-400	400
Length:Diameter Ratio	2.83±0.02 (2.80-2.87)	2.5±0.09 (2.21-2.93)	2.40±0.15 (2.13-2.83)	2.58±0.08 (2.35-2.70)	2.51±0.14 (2.08-2.88)	2.14±0.08 (1.57-2.64)	2.94-3.09	-	-	2.5-3.0
Hydrothecal Cusps										
Number	11.66±0.33 (11-12)	10.25±0.45 (9-12)	10.25±0.48 (9-11)	9.2±0.48 (8-10)	8.8±0.49 (8-10)	9.63±0.37 (7-12)	8-12	12-15	8-12	10-12
Maximum Height of Cusps	83.61±1.54 (81.36-86.57)	67.16±3.70 (48.62-81.64)	39.65±5.99 (24.07-50.25)	38.02±4.56 (27.59-48.69)	59.75±4.72 (48.33-72.03)	49.01±2.46 (29.96-70.82)	-	-	-	-
Inclined	yes	yes, 1 specimen	no	yes, 1 specimen	yes	yes, 4 specimens	yes	no[A]	yes	yes
Pedicel										
Length	2277.07±230.35 (2013.70-2736.11)	1500.11±237.12 (485.83-2466.46)	1307.50±121.55 (1071.29-1633.54)	906.11±137.21 (560.35-1162.59)	860.76±233.54 (344.24-1489.87)	1209.32±152.16 (613.03-3646.93)	up to 5900	500-3500	2000	-

Measures (μm)	*Clytia gracilis*_sp.B_USA [3]	*Clytia gracilis* I [8]	*Clytia gracilis* II [4]	*Clytia gracilis* III [5]	*Clytia gracilis* IV [5]	*Clytia gracilis* V	*Clytia gulangensis* [He et al. 2015]	*Clytia gracilis* [Calder 1991]	*Clytia gracilis* [Cornelius 1995]	*Clytia gracilis* [Schuchert 2001]
Diameter (Maximum or at Medial Portion)	70.52±1.40 (67.87-71.02)	85.27±3.41 (97.18-85.27)	82.00±1.96 (78.55-87.62)	58.24±2.48 (53.73-63.14)	55.21±3.60 (45.80-64.27)	69.33±3.17 (46.72-96.07)	60-100	103-145		-
Gonotheca		Smooth	Smooth			Smooth	Smooth	Smooth	Smooth	Smooth
Length	-	681.98±14.03 (625.85-681.98) [2]	1377.5 [1]	-	-	434.91±99.34 (268.86-612.43)	790-900	1000	1100-1800	1500
Maximum Diameter	-	262.46±7.49 (247.49-277.44) [2]	282.5 [1]	-	-	219.44±13.25 (203.60-245.75)	260-290	425	400-600	550
Growing from	-	Hydrorhiza	Branches	-	-	Hydrorhiza	Hydrorhiza, pedicels, branches	Hydrorhiza	-	-
Locality	United States	Italy, Slovenia	Argentina, United States	Slovenia	Belize	Brazil	Xiamen Bay, China	Bermuda	North-west Europe	Iceland

[A]Not mentioned in the text, but the cusps are not included in the illustrations (Vervoort 1959, Fig. 55b, c; Calder 1991, Fig. 31).

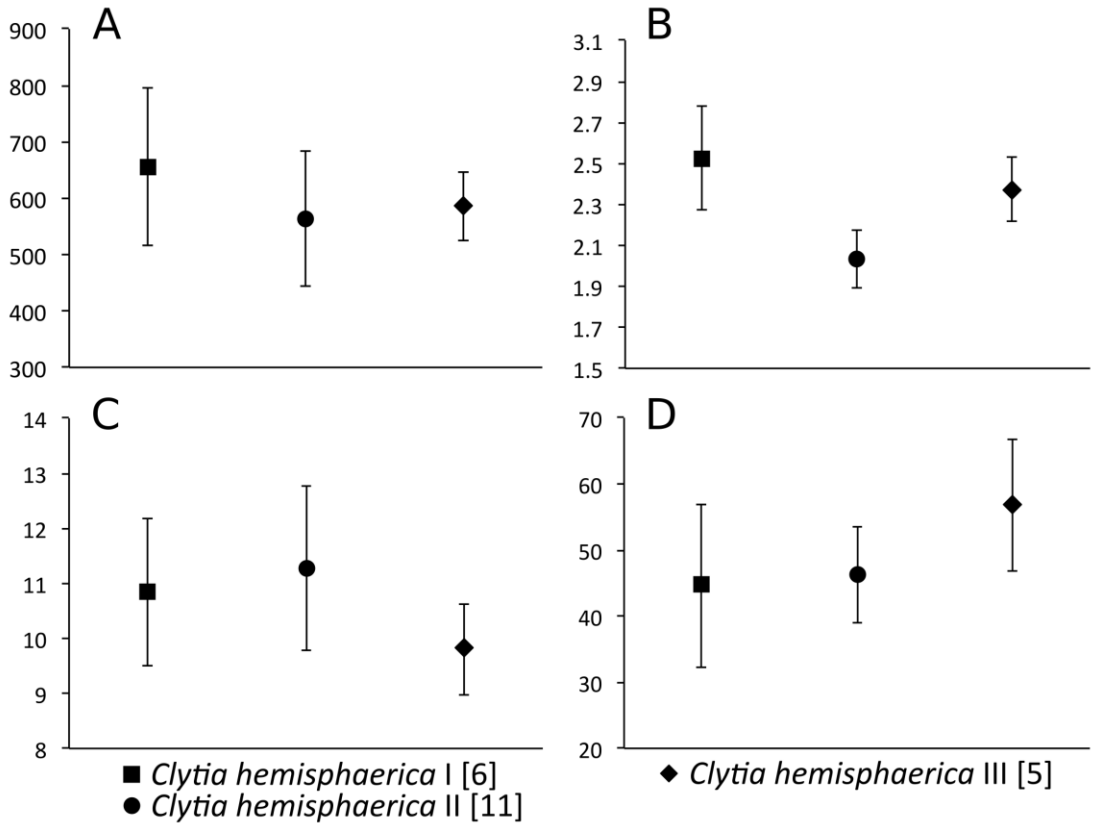

Figure 15. Mean ± standard deviation of morphometric data for lineages identified as *Clytia hemisphaerica*. A. Length of the hydrotheca (LH, μm); B. Length:diameter ratio of hydrotheca (HRatio, μm); C. Number of hydrothecal cusps (NC); D. Maximum height of hydrothecal cusps (HCMax, μm). Brackets = [number of specimens/colonies measured].

Table 6. Comparison among lineages identified as *Clytia 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.

Measures (μm)	*Clytia hemisphaerica* I [6]	*Clytia hemisphaerica* II [11]	*Clytia hemisphaerica* III [5]	*Clytia xiamenensis* [Zhou et al. 2013]	*Clytia hemisphaerica* [Calder 1991]	*Clytia hemisphaerica* [Cornelius 1995]	*Clytia hemisphaerica* [Peña Cantero & García Carrascosa 2002]
Colony	Stolonal or erect	Stolonal	Stolonal	Stolonal, rarely erect	Stolonal, ocasionally erect	Stolonal or erect	-
Total Length of Trophosome	4040.44±979.97 (1782.27-7734.57)	1698.03±210.02 (898.94-3375.35)	2071.67±220.30 (1569.02-2890.12)	-	-	20000	-
Hydrotheca							
Length	655.93±57.00 (474.90-861.70)	563.59±36.00 (440.56-814.05)	585.95±27.27 (521.40-660.51)	260-470	596-926	400-650	400-808
Diameter (Maximum or at Margin)	275.08±31.23 (186.30-404.28)	294.64±14.36 (193.17-346)	274.19±9.93 (244.83-305.49)	140-230	234-394	200-350	176-400
Length:Diameter Ratio	2.52±0.10 (2.17-2.85)	2.03±0.04 (1.70-2.20)	2.37±0.07 (2.18-2.59)	1.5-2.5	-	-	-
Hydrothecal Cusps							
Number	10.83±0.54 (9-13)	11.27±0.45 (10-15)	9.8±0.37 (9-11)	6-12	10-14	8-14	8-14
Maximum Height of Cusps	57.4±4.88 (39.85-70.26)	55.24±1.78 (43.29-66.00)	65.11±6.19 (46.10-81.38)	-	-	-	-
Inclined	no	no	no	yes	yes[A]	no[B]	no[B]
Pedicel							
Length	1583.02±357.03 (533.05-3122.36)	1134.47±176.17 (446.39-2561.30)	1485.72±203.81 (1047.62-2260.33)	260-1500	600-800	2200	-
Diameter (Maximum or at Medial Portion)	72.26±4.95 (59.43-93.91)	75.92±2.49 (60.43-93.38)	69.83±1.88 (64.26-73.90)	-	64-83	-	-
Gonotheca	Transverselly ribbed	Transverselly ribbed	Transverselly ribbed	Undulated walls	With distinct spiral ribs	Deeply concertinared walls, but smooth in some specimens	Transverselly ribbed[C]

Measures (µm)	*Clytia hemisphaerica* I [6]	*Clytia hemisphaerica* II [11]	*Clytia hemisphaerica* III [5]	*Clytia xiamenensis* [Zhou et al. 2013]	*Clytia hemisphaerica* [Calder 1991]	*Clytia hemisphaerica* [Cornelius 1995]	*Clytia hemisphaerica* [Peña Cantero & García Carrascosa 2002]
Length	796.85±54.77 (742.08-851.62) [2]	664.57±110.51 (422.88-901.12) [8]	669.98±24.26 (610.12-705.08)	890-1400	750	900-1200	-
Maximum Diameter	300.47±117.39 (183.08-417.86) [2]	281.34±33.59 (204.84-344.02) [8]	259.06±9.11 (240.87-284.35)	200-300	350	450-600	-
Growing from	Hydrorhiza	Hydrorhiza and pedicels	Hydrorhiza	Hydrorhiza, pedicels or branches	Hydrorhiza	-	-
Locality	United States	Slovenia, North Sea	Belize	Xiamen Bay, China	Bermuda	North-west Europe	Western Mediterranean

[A]According to Calder (1991), the cusps are "often somewhat skewed" (page 59, Fig. 32).

[B]Not mentioned in the text, but the cusps are not inclided in the illustrations (Cornelius 1995, Fig. 57; Peña Cantero & García Carrascosa 2002, Fig. 28C).

[C]As seen in the illustration (Peña Cantero & García Carrascosa 2002, Fig. 28C, D).

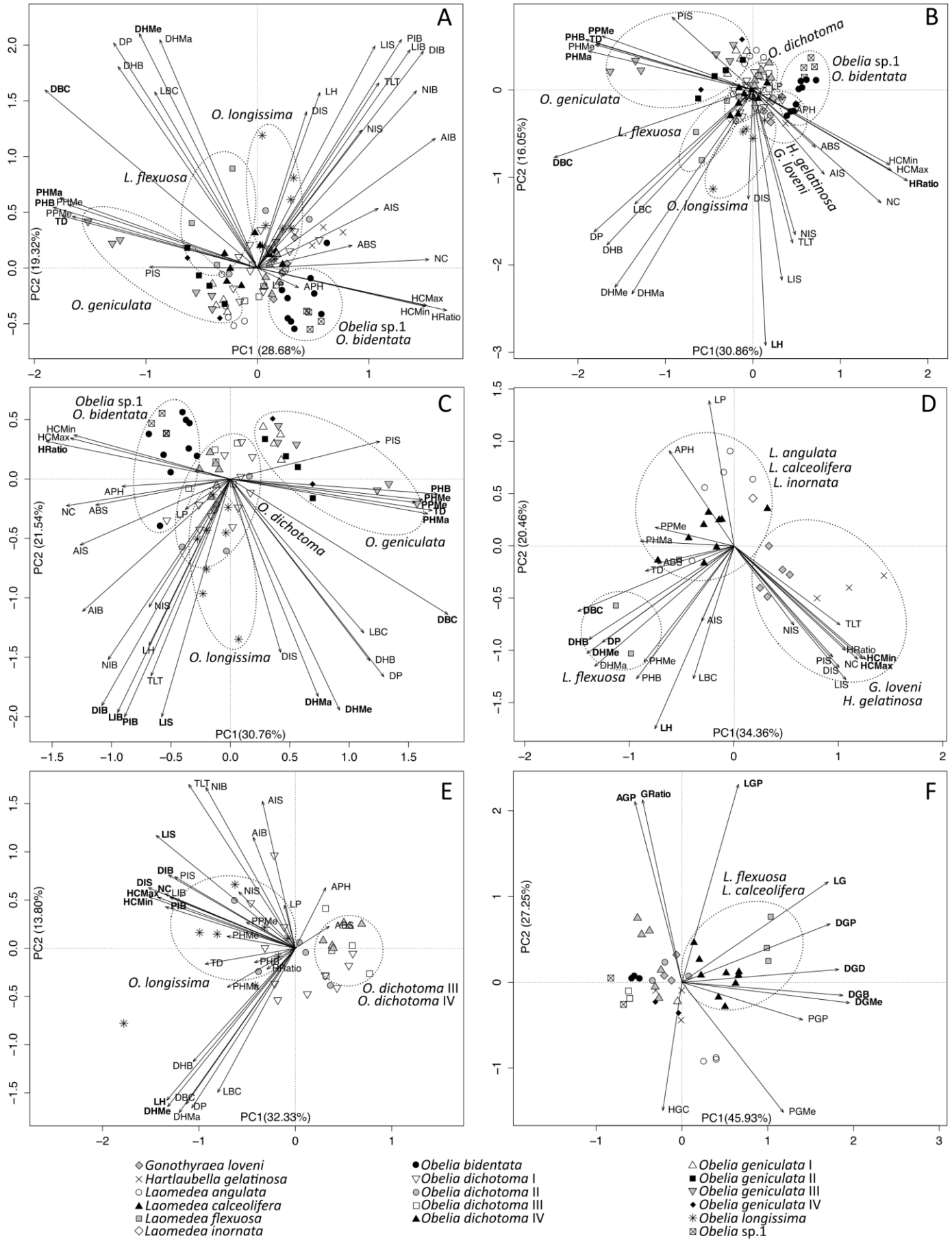
A
B
C
D
E
F
PC1 (28.68%)
PC2 (19.32%)
PC1(30.86%)
PC2 (16.05%)
PC1(30.76%)
PC2 (21.54%)
PC1(34.36%)
PC2 (20.46%)
PC1(32.33%)
PC2 (13.80%)
PC1(45.93%)
PC2 (27.25%)
O. longissima
L. flexuosa
O. geniculata
Obelia sp.1
O. bidentata
O. dichotoma
H. gelatinosa
G. loveni
L. angulata
L. calceolifera
L. inornata
O. dichotoma III
O. dichotoma IV
Gonothyraea loveni
Hartlaubella gelatinosa
Laomedea angulata
Laomedea calceolifera
Laomedea flexuosa
Laomedea inornata
Obelia bidentata
Obelia dichotoma I
Obelia dichotoma II
Obelia dichotoma III
Obelia dichotoma IV
Obelia geniculata I
Obelia geniculata II
Obelia geniculata III
Obelia geniculata IV
Obelia longissima
Obelia sp.1

Figure 16. Distance biplots of the Principal Component Analysis (PCA) comprising data for the family Obeliidae. A. First and second principal components (PCs) of the PCA with the complete dataset; B. First and second PCs of the PCA with the complete dataset, excluding measurements related to second-order branches of erect colonies (NIB, DIB, AIB, LIB); C. First and second PCs of the PCA with *Obelia* only; D. First and second PCs of the PCA without *Obelia*; E. First and second PCs of the PCA with *O. 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 2, and those in bold indicate measurements that were correlated with each principal component (Pearson correlation >0.7 and <-0.7).

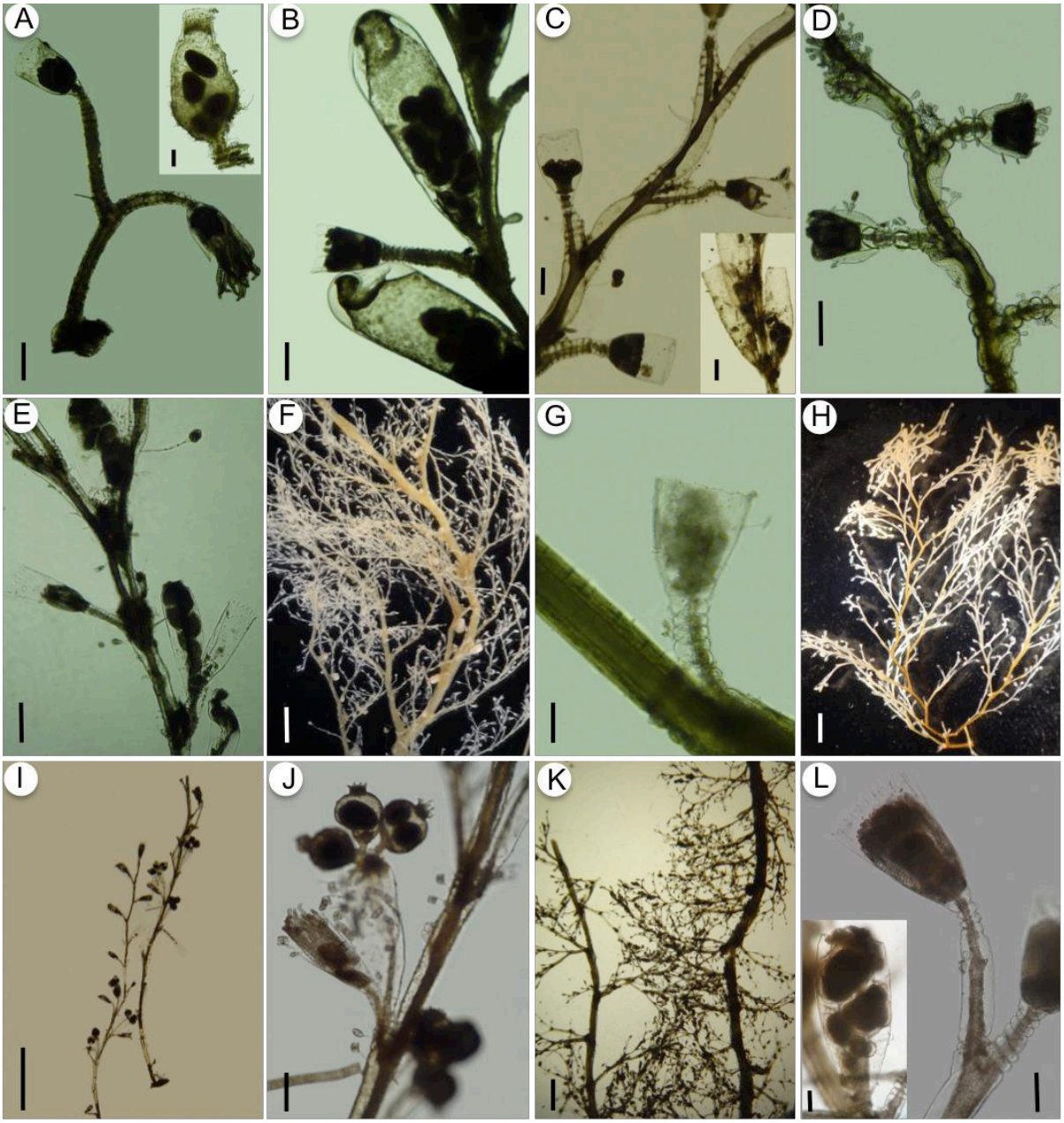

Figure 17. A. *Laomedea angulata* (EL40, EL50_SLV), trophosome and gonotheca; B. *Laomedea calceolifera* (ROW03_USA), hydrotheca and gonothecae; C. *Laomedea flexuosa* (RYE02_USA), trophosome and gonothecae (scale 200 μm); D. *Obelia geniculata* (BZ5_BRA), trophosome; E. *Obelia bidentata* (MAP10_BRA), trophosome with gonothecae; F. *Obelia bidentata* (USNM 1106185), colony (scale 2 mm); G, H. *Obelia longissima* (GFP04_USA), hydrotheca (G) and colony (H), scale 2 mm); I, J. *Gonothyraea loveni* (SWM03_USA), colony (I), hydrotheca and gonotheca with meconidia (J); K, L. *Hartlaubella gelatinosa* (PT16_ARG), colony (K), hydrotheca and gonotheca (L). Scales: A (trophosome), B, D, E, J = 200 μm; A (gonotheca), C (gonotheca), G, L = 100 μm; C (trophosome) = 500 μm; F, H, I, K = 2 mm.

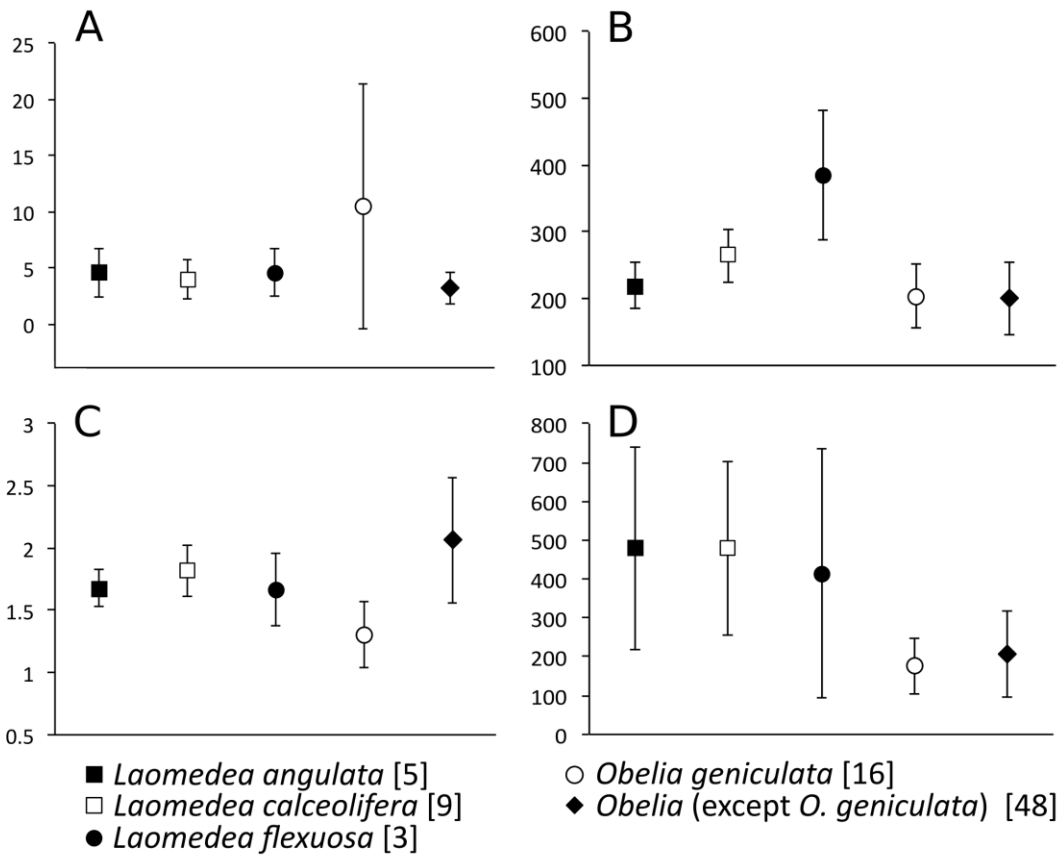

Figure 18. Mean ± standard deviation of morphometric data for *Laomedea angulata*, *Laomedea calceolifera*, *Laomedea flexuosa*, *Obelia geniculata*, and all species of *Obelia* except for *Obelia geniculata*. A. Maximum hydrothecal perisarc thickness at margin (PHMa, μm); B. Maximum perisarc thickness of pedicel at medial portion (PPMe, μm); C. Maximum hydrothecal diameter at margin (DHMa, μm); D. Length of pedicel (LP, μm). Brackets = [number of specimens/colonies measured].

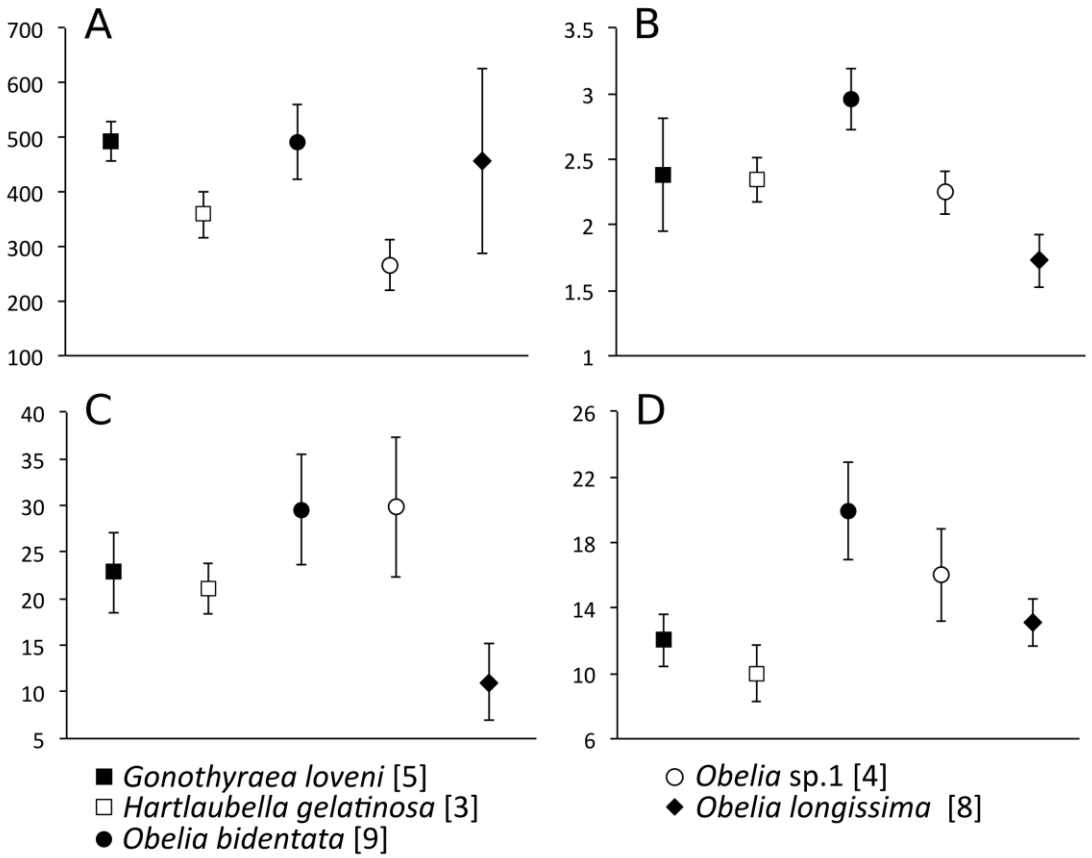

Figure 19. Mean ± standard deviation of morphometric data for *Gonothyraea loveni*, *Hartlaubella gelatinosa*, *Obelia bidentata*, *Obelia* sp.1 and *Obelia longissima*. A. Length of the hydrotheca (LH, μm); B. Length:diameter ratio of the hydrotheca (HRatio, μm); C. Maximum height of hydrothecal cusps (HCMax, μm); D. Number of hydrothecal cusps (NC, μm). Brackets = [number of specimens/colonies measured].

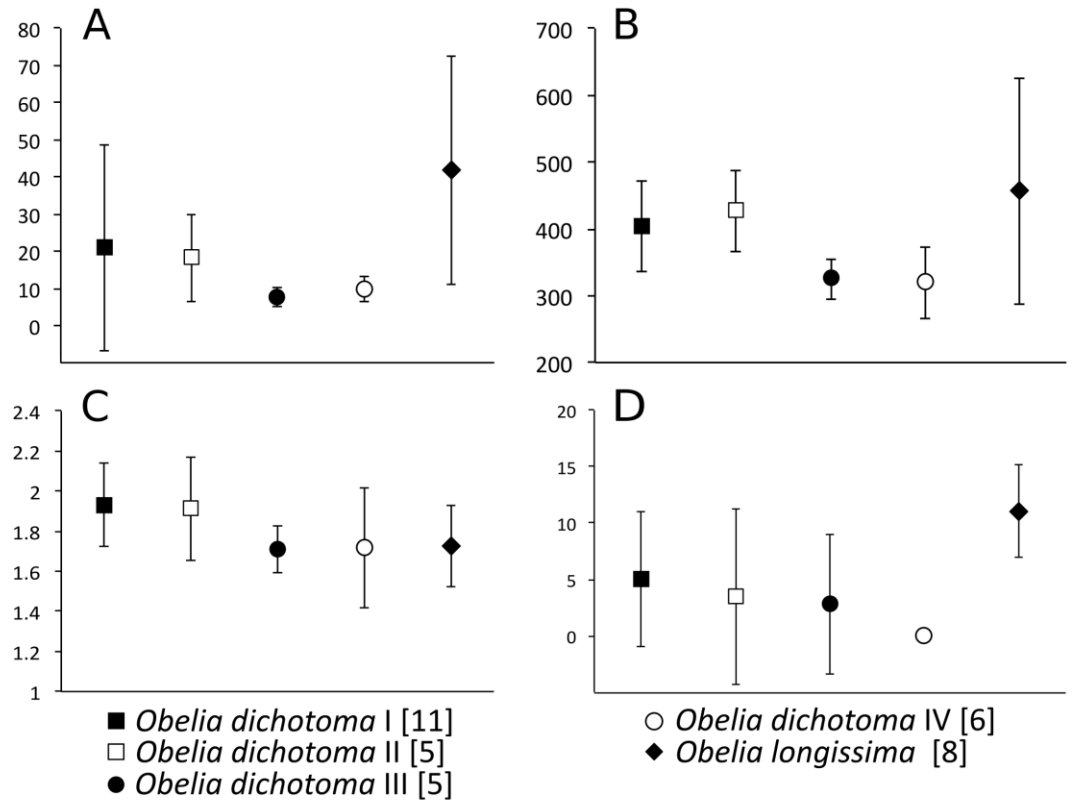

Figure 20. Mean ± standard deviation of morphometric data for the lineages identified as *Obelia dichotoma*. A. Total length of the trophosome (TLT, mm); B. Length of the hydrotheca (LH, μm); C. Length:diameter ratio of the hydrotheca (HRatio, μm); D. Maximum height of hydrothecal cusps (HCMax, μm). Brackets = [number of specimens/colonies measured].

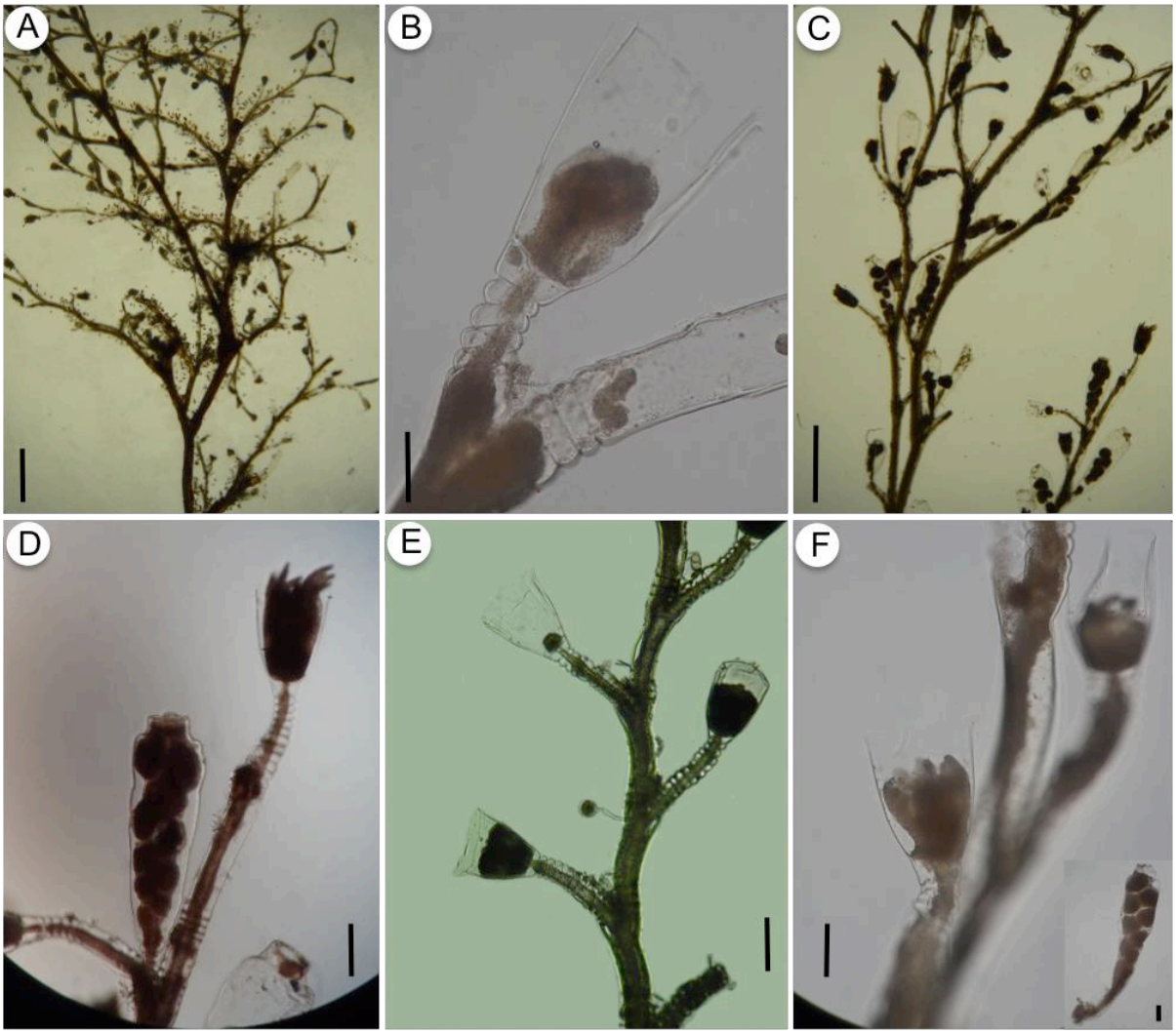

Figure 21. A, B. *Obelia dichotoma* I (PIM01_USA), colony (A) and hydrotheca (B); C, D. *Obelia dichotoma* II (PT2_ARG), colony (C) and hydrotheca (D); E. *Obelia dichotoma* III (PAF07_BRA), trophosome; F. *Obelia dichotoma* IV (Site 1.1_USA), hydrotheca and gonotheca. Scale: A, C = 1 mm; B, D, F (both) = 100 μm; E = 200 μm.

Table 7. Comparison among lineages identified as *Obelia 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.

Measures (μm)	*Obelia dichotoma* I [11]	*Obelia dichotoma* II [5]	*Obelia dichotoma* III [5]	*Obelia dichotoma* IV [6]	*Obelia longissima* [8]	*Obelia hyalina* [Vannucci 1949; Calder 2013]	*Obelia griffini* [Calkins 1899]	*Obelia dichotoma* [Calder 1991]	*Obelia dichotoma* [Cornelius 1995]
Colony	Unbranched to 6th-order branched, monosiphonic	Unbranched to 3rd-order branched, monosiphonic	Unbranched to 1st-order branched, monosiphonic	Up to 2nd-order branched, monosiphonic	Up to 4th-order branched, monosiphonic	Monosiphonic	Branched, monosi-phonic[B]	Unbranched to branched, monosiphonic	Mono to polysiphonic
Total Length of Trophosome (mm)	20.86±83.14 (35.70-90)	18.18±52.15 (8.69- 36.97)	7.81±1.18 (3.95-11.15)	9.89±1.38 (5.61-13.31)	41.66±10.84 (15.07-105)	15-20	25-50	21	50-350
Length of Internode of Main Stem	1129.80±233.73 (440.89-2709.04)	1261.81±208.01 (884.75-2066.44)	553.87±45.61 (445.98-718.64)	577.68±37.92 (464.04-715.20)	1725.41±237.24 (689.84-2549.04)		-	-	up to 2000
Hydrotheca									
Length	404.53±20.62 (331.48-542.29)	426.27±26.88 (359.92-517.745)	324.64±13.33 (294.42-372.82)	319.78±22.00 (253.15-390.78)	456.83±59.88 (338.17-844.59)	315-390	250-350	219-359	300-400
Diameter (Maximum or at Margin)	211.36±12.09 (164.01-286.37)	223.2182±5.60 (211.22-237.92)	191.22±11.02 (156.49-219.47)	187.44±8.08 (158.94-214.76)	261.41±26.25 (206.20-422.73)	190-270	-	210-317	200-250
Length:Diameter Ratio	1.93±0.06 (1.96-2.02)	1.91±0.12 (1.53-2.00)	1.71±0.05 (1.59-1.88)	1.71±0.12 (1.29-2.15)	1.73±0.07 (1.56-2.10)	-	-	-	-
Diaphragm	transverse to oblique	transverse to oblique	transverse to oblique	transverse to oblique	transverse to oblique	oblique[A]	transverse[B]	oblique	transverse to oblique
Hydrothecal Margin	even to crenate	even to crenate	even to crenate	even	sinuous	even	even	even	even to crenate
Number of cusps	5.27±1.84 (0-13)	2.80±2.80 (0-14)	2.80±2.80 (0-14)	-	13.12±0.51 (11-15)	-	-	-	-
Maximum Height of Cusps	5.05±1.81 (0-15.35)	3.48±3.48 (0-17.42)	2.76±2.76 (0-13.81)	-	11.05±1.45 (6.98-19.95)	-	-	-	-
Pedicel									

Measures (μm)	*Obelia dichotoma* I [11]	*Obelia dichotoma* II [5]	*Obelia dichotoma* III [5]	*Obelia dichotoma* IV [6]	*Obelia longissima* [8]	*Obelia hyalina* [Vannucci 1949; Calder 2013]	*Obelia griffini* [Calkins 1899]	*Obelia dichotoma* [Calder 1991]	*Obelia dichotoma* [Cornelius 1995]
Length	152.63±14.07 (101.60-241.03)	296.43±61.51 (128.65-457.85)	262.57±54.57 (122.08-445.59)	145.47±33.69 (71.54- 266.94)	227.17±38.97 (112.35-465.95)	160-900	-	-	200-400 (up to 700)
Diameter (Maximum or at Medial Portion)	89.3±4.08 (70.70-114.47)	88.65±3.10 (78.52-95.94)	81.52±1.72 (76.88-86.72)	76.17±2.55 (70.22-84.55)	95.76±5.20 (78.94-126.39)	-	-	-	-
Gonotheca									
Length	-	896.64±128.37 (666.02-1109.65) [3]	516.53±68.17 (448.36-584.70) [2]	711.39±93.68 (429.58-980.49)	-	390-430	800-1000	708-885	800-1050
Maximum Diameter	-	262.86±16.18 (233.53-289.35) [3]	157.66±9.41 (148.25-167.07) [2]	185.12±11.65 (148.20-231.27)	-	190-220	250-300	233-294	230-290
Locality	United States, Uruguay, Argentina	Italy, Slovenia, United States, Argentina	Brazil	United States, Argentina	Iceland, United States, White Sea, Argentina, Antarctica	Brazil, United States	Puget Sound, United States	Bermuda	North West Europe

[A]Not mentioned in the species description, taken from the illustrations (Clarke 1879; Calder 2013).

[B]Not clearly mentioned in the text, taken from the illustration (Calkins 1899).

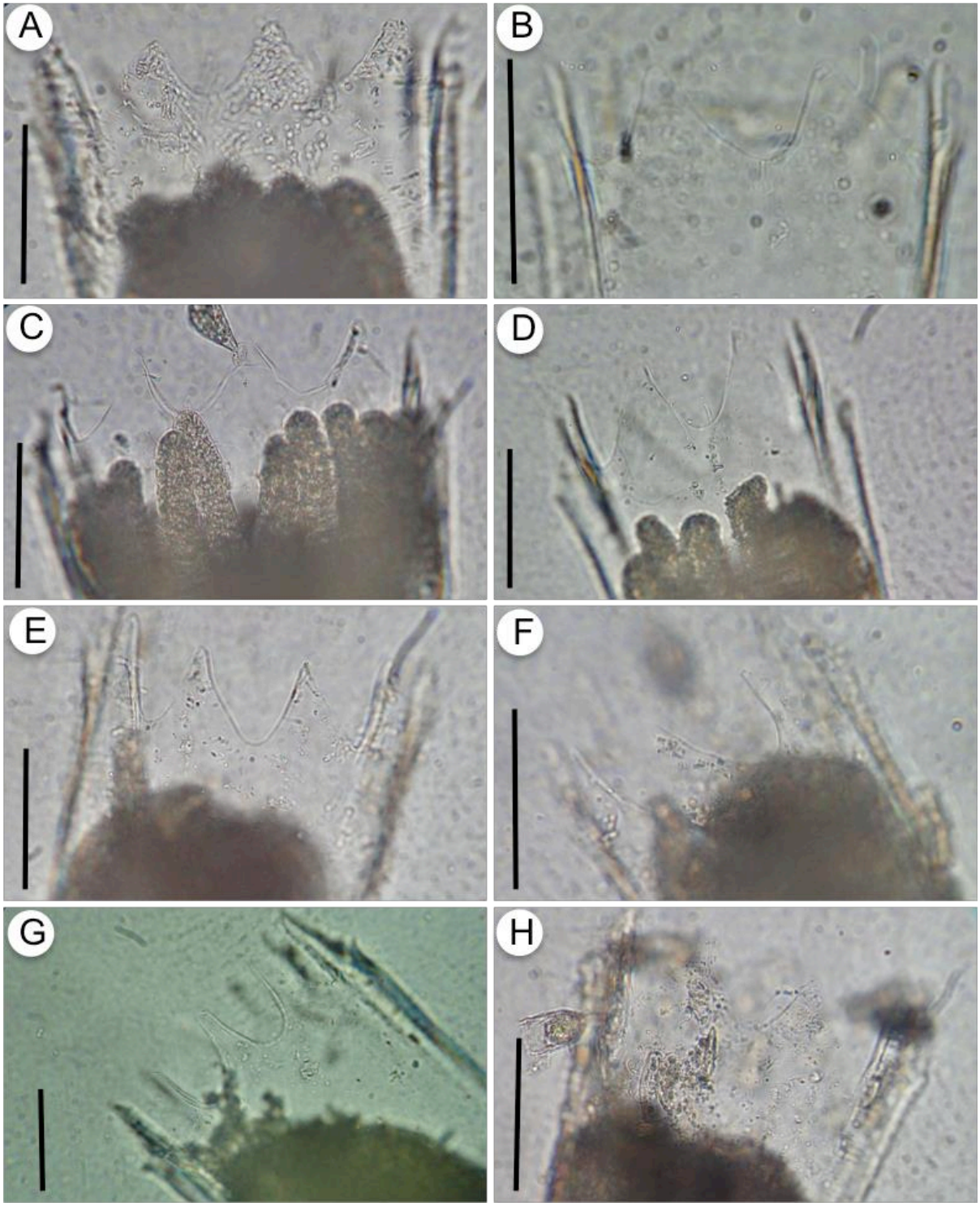

Figure 22. Variation in the shape of hydrothecal cusps of *Clytia gracilis* V. A, C. Specimens from Fortaleza, Brazil (CE2_BRA, CE5_BRA); B, D. Specimens from Cascavel, Brazil (CE1_BRA, CE3_BRA); E. Specimen from Trairi, Brazil (T1_BRA); F, H. Specimens from São Luís do Maranhão, Brazil (MAP01_BRA, MAP11_BRA); G. Specimen from Salinópolis, Brazil (PAF03_BRA). Scale: 100 µm.

Table 8. Taxonomic summary of specific and supraspecific taxa of Proboscoida delimited in this study, together with their morphometric characters. LEM = Laboratory of Marine Evolution, São Paulo, Brazil; MHNG INVE = Muséum d'Histoire Naturelle de Genève, Switzerland; MZUSP = Museu de Zoologia da Universidade de São Paulo, Brazil; USNM = National Museum of Natural History, Smithsonian Institution, USA.

Taxon	Specimen(s) reidentified (see Table 1)	Monophyletic? (Cunha et al. 2016b)	Morfometric diagnostic characters	Morphometric characters are distinctive when compared to[D]
Family Campanulariidae Johnston, 1836		yes		
Genus *Bonneviella* Broch, 1909		yes[A]	Total length of trophosome, length of pedicel and hydrotheca	Campanulariidae
Bonneviella ingens Nutting, 1915	USNM 1106187 (tentative)	yes	Size and shape of hydrotheca	Campanulariidae
Bonneviella regia (Nutting, 1901)	USNM 1106181	yes	Size of hydrotheca	Campanulariidae
Bonneviella superba Nutting, 1915	USNM 1106182 (tentative)	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	LEM IT10, IT14; USNM 1106157	yes	Height of hydrothecal cusps	Other species of *Campanularia*
Campanularia subantarctica	MZUSP 2639, 2643	yes	No distinctive morphometric characters	-
Campanularia sp.	MZUSP 2641, 2642	yes	No distinctive morphometric characters	-
Campanularia volubilis	USNM 1106166	yes	No distinctive morphometric characters	-
Genus *Orthopyxis* L. Agassiz, 1862		yes[A]	Perisarc thickness, length and length:diameter ratio of hydrotheca	*Campanularia*

Taxon	Specimen(s) reidentified (see Table 1)	Monophyletic? (Cunha et al. 2016b)	Morfometric diagnostic characters	Morphometric characters are distinctive when compared to[D]
Orthopyxis asymmetrica Stechow, 1919	LEM EL02, EL04, EL16, EL52; 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 2612-2615, 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, 2644; LEM PT19; USNM 1106163	yes	Number and height of hydrothecal cusps (but may eventually present even hydrothecal rim)	Other species of *Orthopyxis*, except for *O. sargassicola*[E]
Orthopyxis integra (MacGillivray, 1842)	MZUSP 2638, 2640; LEM PT20; USNM 1106184	yes[B]	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[C]		
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	LEM PT8, PT11; USNM 1106164	yes	Perisarc thickness	Campanulariidae, except for *Orthopyxis*

Taxon	Specimen(s) reidentified (see Table 1)	Monophyletic? (Cunha et al. 2016b)	Morfometric diagnostic characters	Morphometric characters are distinctive when compared to[D]
Genus *Tulpa* Stechow, 1921		yes[C]		
Tulpa tulipifera (Allman, 1888)	LEM PT18	yes	Size of hydrotheca	Campanulariidae
Family Clytiidae Cockerell, 1911		**no**		
Genus *Clytia* Lamouroux, 1812		**no**		
Clytia elsaeoswaldae Stechow, 1914	LEM PM18, PM36, Me26, CB19; USNM 1078725, 1078728	yes	Diameter of hydrotheca, thickness of diaphragm	*Clytia* cf. *gracilis* and *Clytia* cf. *hemisphaerica* (diameter); Clytiidae (diaphragm)
Clytia cf. *gracilis* sp.1	LEM EL15, EL31, EL32, IT12, IT13	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	LEM PT9; 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	LEM EL05, EL14, EL38	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	LEM CBC13, CBC20, CBC26	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)
Clytia cf. *gracilis* sp.5	LEM Me24, PAF03, MAP01, MAP11, CE1, CE2, CE3, CE5, T1, T5, T6	yes	No distinctive morphometric characters	-

Taxon	Specimen(s) reidentified (see Table 1)	Monophyletic? (Cunha et al. 2016b)	Morfometric diagnostic characters	Morphometric characters are distinctive when compared to[D]
Clytia cf. *gracilis* sp.B	USNM 1078730	yes	Length and diameter of hydrotheca and pedicel, number and height of hydrothecal cusps	*Clytia* cf. *gracilis* sp.3 and sp.4
Clytia cf. *hemisphaerica* sp.1	LEM FLT03, HCM04, MMA05, PTJ01	yes	No distinctive morphometric characters	-
Clytia cf. *hemisphaerica* sp.2	LEM EL06, EL08, EL12, EL20, EL28, EL35; USNM 1106186	yes	No distinctive morphometric characters	-
Clytia cf. *hemisphaerica* sp.3	LEM CBC1, CBC25, CBC40.1	yes	No distinctive morphometric characters	-
Clytia hummelincki	LEM CBC42	yes	Length of pedicel, number of pedicel annuli at base	Clytiidae, except for *C.* cf. *gracilis* and *C.* cf. *hemisphaerica*
Clytia linearis	LEM PY10; USNM 1078729	yes	Length of hydrotheca, height of hydrothecal cusps	Clytiidae (length); Clytiidae, except for *C. elsaeoswaldae*, *C.* cf. *gracilis* and *C.* cf. *hemisphaerica* (height of cusps)
Clytia noliformis	LEM SP3, SP9; USNM 1078720	yes	Perisarc thickness	Clytiidae, except for *Clytia* sp.2
Clytia paulensis	USNM 1106158	yes	Length:diameter ratio of hydrotheca	Clytiidae, except for *C.* cf. *gracilis* and *C.* cf. *hemisphaerica*
Clytia sp.1	LEM SP1	yes	Length:diameter ratio of hydrotheca	Clytiidae, except for *C.* cf. *gracilis* and *C.* cf. *hemisphaerica*

Taxon	Specimen(s) reidentified (see Table 1)	Monophyletic? (Cunha et al. 2016b)	Morfometric diagnostic characters	Morphometric characters are distinctive when compared to[D]
Clytia sp.2	LEM CE4	yes	Perisarc thickness	Clytiidae, except for *C. noliformis*
Clytia sp.3	LEM NAT05	yes	Length of pedicel, number of pedicel annuli at base	Clytiidae, except for *C.* cf. *gracilis* and *C.* cf. *hemisphaerica*
Clytia stolonifera Blackburn, 1938	LEM CBC 40.2, CBC45	yes	Height of hydrothecal cusps, branching of erect colonies	Clytiidae
Family Obeliidae Haeckel, 1879		yes		
Genus *Gonothyraea* Allman, 1864		yes[C]		
Gonothyraea loveni (Allman, 1859)	LEM BPM03, SWM03; USNM 1106154	yes	Branching of erect colonies, length:diameter ratio of hydrotheca, height of hydrothecal cusps	Obeliidae, except for *Obelia* (branching); Obeliidae, except for *O.* sp1 (ratio); Obeliidae (cusps)
Genus *Hartlaubella* Poche, 1914		yes[C]		
Hartlaubella gelatinosa (Pallas, 1766)	LEM PT13, PT14, PT16	yes	Branching of erect colonies, length:diameter ratio of hydrotheca, height of hydrothecal cusps	Obeliidae, except for *Obelia* (branching); Obeliidae, except for *O.* sp1 (ratio); Obeliidae (cusps)
Genus *Laomedea* Lamouroux, 1812		**no**	Length of pedicel and gonotheca	*Obelia* (pedicel); Obeliidae (gonotheca)
Laomedea angulata Hincks, 1861	LEM EL40, EL50	yes	No distinctive morphometric characters	-
Laomedea calceolifera (Hincks, 1861)	LEM MMA06, ROW03, GFP01, HRM06, FTA01; MHNG INVE 37296; USNM 1106177	yes	No distinctive morphometric characters	-

Taxon	Specimen(s) reidentified (see Table 1)	Monophyletic? (Cunha et al. 2016b)	Morfometric diagnostic characters	Morphometric characters are distinctive when compared to[D]
Laomedea flexuosa Alder, 1857	LEM RYE02; USNM 1106190, 1106192	yes	Diameter of hydrotheca and pedicel	Obeliidae
Genus *Obelia* Péron & Lesueur, 1810		**no**		
Obelia bidentata Clark, 1875	LEM CBC35, MAR02, MAP10; USNM 1106162, 1106185	yes	Length:diameter ratio of hydrotheca, number and height of hydrothecal cusps, length of hydrotheca	Obeliidae (ratio); Obeliidae, except for *Obelia* sp.1 (number and height of cusps); *Obelia* sp.1 (length)
Obelia cf. *dichotoma* sp.1	LEM FLT04, PIM01, PIM02, ROW04, PT3, PTJ03, UR1	yes	No distinctive morphometric characters	-
Obelia cf. *dichotoma* sp.2	LEM EL30, MMA03, PT2; USNM 1106156	yes	No distinctive morphometric characters	-
Obelia cf. *dichotoma* sp.3	LEM PAF03, PAF09, MA03	yes	Branching of erect colonies, total length of trophosome	Remaining *Obelia* cf. *dichotoma*
Obelia cf. *dichotoma* sp.4	LEM Site1.1, UR6	yes	Branching of erect colonies, total length of trophosome	Remaining *Obelia* cf. *dichotoma*
Obelia geniculata (Linnaeus, 1758)	LEM BSF05, PT5, UNH01, BZ5, EL23; USNM 1106165, 1106176, 1106179	yes	Perisarc thickness	Obeliidae

Taxon	Specimen(s) reidentified (see Table 1)	Monophyletic? (Cunha et al. 2016b)	Morfometric diagnostic characters	Morphometric characters are distinctive when compared to[D]
Obelia longissima (Pallas, 1766)	LEM MMA04, GFP04, HRM05, PT1; 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	LEM PAF08, T2	yes	Length:diameter ratio of hydrotheca, height of hydrothecal cusps, length of hydrotheca	Obeliidae, except for *G. loveni* and *H. gelatinosa* (ratio); Obeliidae, except for *O. bidentata* (height of cusps); *O. bidentata* (length)

[A]In most of the phylogenies (see Cunha et al. 2016b).
[B]Not monophyletic in its traditional sense (see text).
[C]These taxa are represented by only one species, therefore their monophyletism needs confirmation (see Cunha et al. 2016b).
[D]When referring to family or genus, this conclusion is limited to the species analyzed in this study.
[E]Specimens with even hydrothecal rim may be difficult to differentiated from additional species of *Orthopyxis* (e.g., *O. integra*).

Supplementary Material

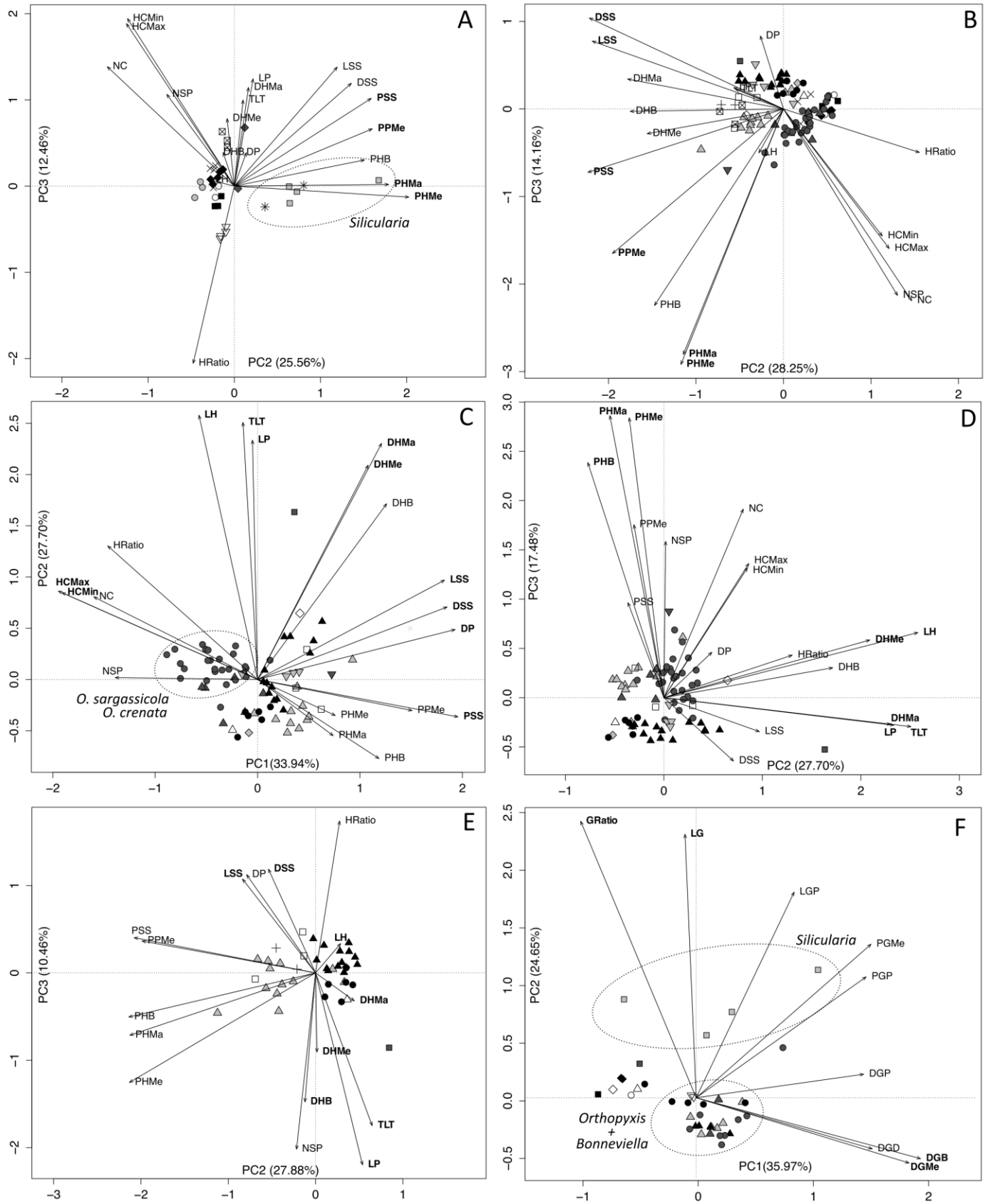

Figure S1. Distance biplots of the Principal Component Analysis (PCA) comprising data for Campanulariidae. A. Second and third principal components (PCs) of the PCA without the genus *Orthopyxis*; B. Second and third PCs of the PCA with *Campanularia* and *Orthopyxis*; C. First and second PCs of the 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 2, and those in bold indicate measurements that were correlated with each principal component (Pearson correlation >0.7 and <-0.7).

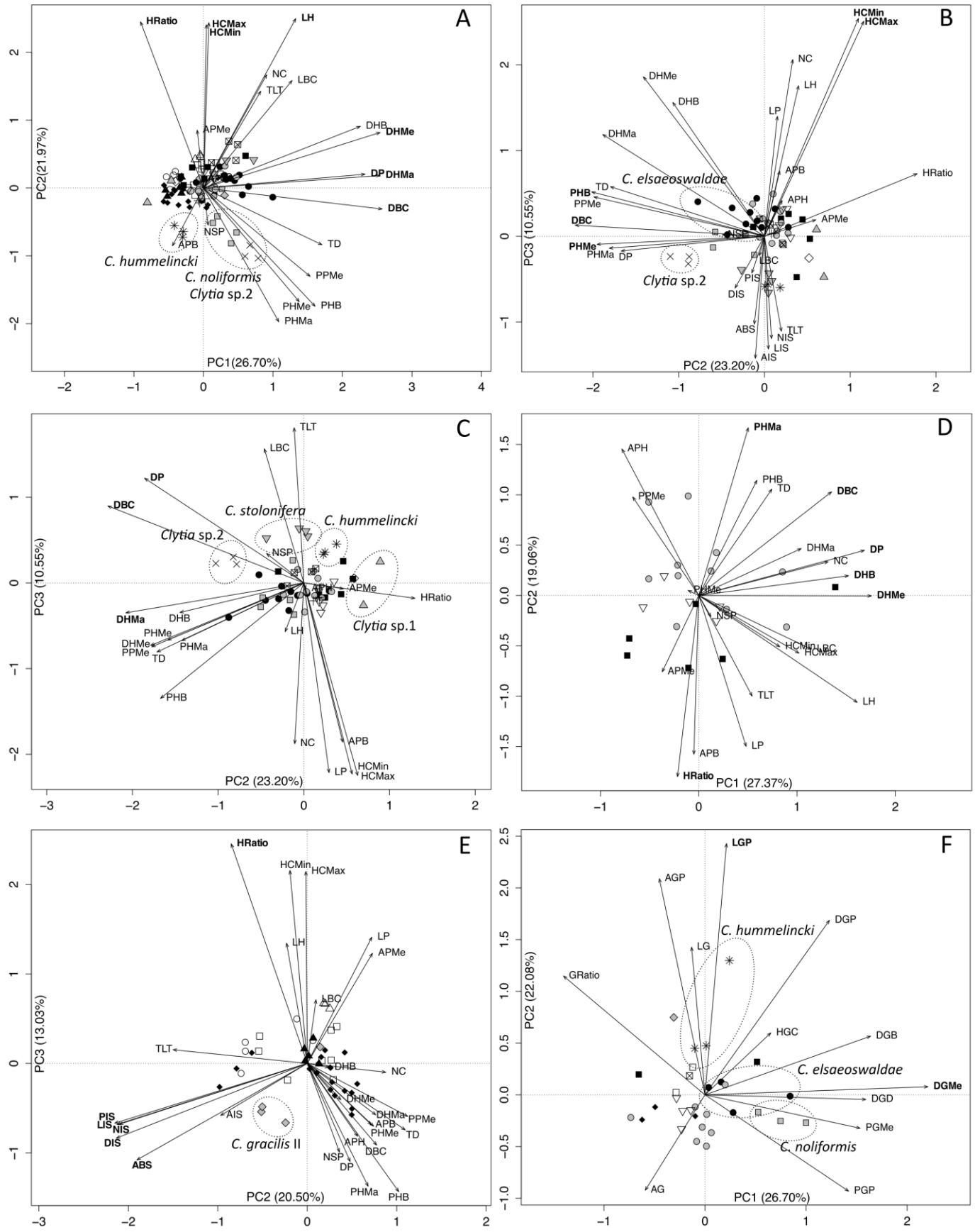
A
HRatio
HCMax
HCMin
LH
NC
LBC
TLT
APMe
DHB
DHMe
DP
DHMa
DBC
NSP
APB
TD
PPMe
PHMe
PHB
PHMa
C. hummelincki
C. noliformis
Clytia sp.2
PC1(26.70%)
PC2(21.97%)
B
HCMin
HCMax
NC
DHMe
LH
DHB
LP
DHMa
APB
HRatio
C. elsaeoswaldae
PHB
TD
APH
PPMe
APMe
DBC
PHMe
PHMa
DP
LBC
Clytia sp.2
PIS
DIS
ABS
TLT
NIS
LIS
AIS
PC2 (23.20%)
PC3 (10.55%)
C
TLT
LBC
DP
DBC
C. stolonifera
C. hummelincki
Clytia sp.2
NSP
APMe
HRatio
DHMa
DHB
PHMe
DHMe
PPMe
TD
PHMa
LH
Clytia sp.1
PHB
NC
APB
LP
HCMin
HCMax
PC2 (23.20%)
PC3 (10.55%)
D
PHMa
APH
PHB
TD
PPMe
DBC
DHMa
DP
NC
DHB
PPMe
DHMe
NSP
HCMin
LBC
HCMax
APMe
TLT
LH
LP
APB
HRatio
PC1 (27.37%)
PC2 (19.06%)
E
HRatio
HCMin
HCMax
LH
LP
APMe
LBC
TLT
DHB
NC
DHMe
DHMa
APB
PPMe
PHMe
TD
PIS
LIS
NIS
DIS
AIS
C. gracilis II
APH
DBC
NSP
DP
ABS
PHMa
PHB
PC2 (20.50%)
PC3 (13.03%)
F
LGP
AGP
C. hummelincki
DGP
LG
GRatio
HGC
DGB
C. elsaeoswaldae
DGMe
DGD
PGMe
C. noliformis
AG
PGP
PC1 (26.70%)
PC2 (22.08%)
Clytia elsaeoswaldae
Clytia gracilis I
Clytia gracilis II
Clytia gracilis III
Clytia gracilis IV
Clytia gracilis V
Clytia gracilis_sp.B_USA
Clytia hemisphaerica I
Clytia hemisphaerica II
Clytia hemisphaerica III
Clytia hummelincki
Clytia linearis
Clytia noliformis
Clytia paulensis
Clytia sp.1
Clytia sp.2
Clytia sp.3
Clytia stolonifera

Figure S2. Distance biplots of the Principal Component Analysis (PCA) comprising data for Clytiinae. A. First and second principal components (PCs) of the PCA with the complete dataset, and without measurements related to internodes of erect colonies (NIS, LIS, AIS, PIS, DIS, ABS); B. Second and third PCs of the PCA without *Clytia gracilis*; C. Second and third PCs of the PCA without *C. gracilis* and measurements related to internodes of erect colonies; D. First and second PCs of the PCA with *C. hemisphaerica*, but without measurements related to internodes of erect colonies; E. Second and third PCs of the PCA with *C. gracilis*; F. First and second PCs of the PCA with measurements of the gonothecae. Numbers in parentheses indicate percentages of variation explained by each principal component. Abbreviations of morphometric variables as in Table 2, and those in bold indicate measurements that were correlated with each principal component (Pearson correlation >0.7 and <-0.7).

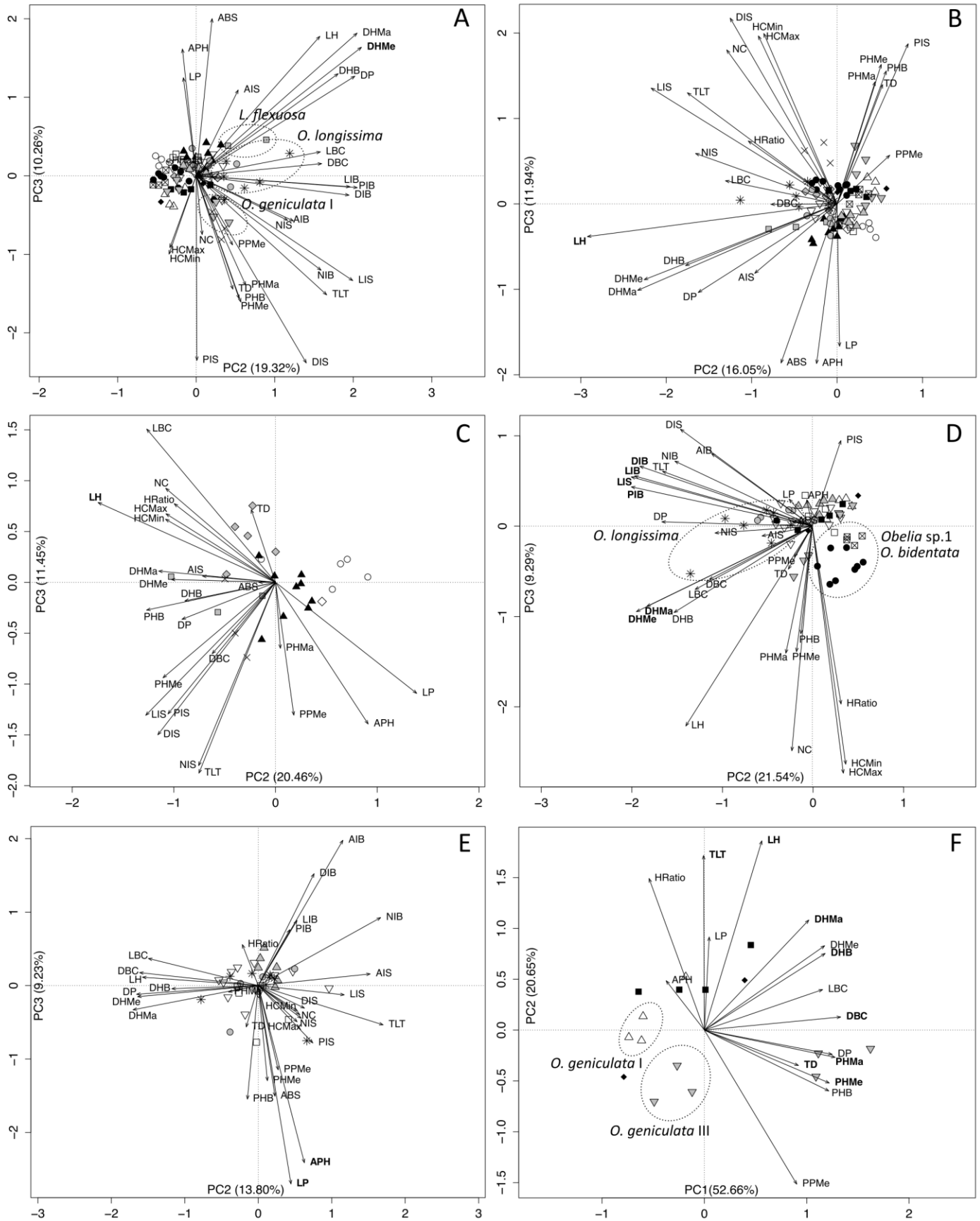
A
ABS
APH
LP
AIS
LH
DHMa
DHMe
DHB
DP
L. flexuosa
O. longissima
LBC
DBC
LIB
PIB
DIB
O. geniculata I
AIB
NIS
NC
PPMe
HCMax
HCMin
NIB
LIS
TD
PHMa
PHB
PHMe
TLT
PIS
DIS
PC3 (10.26%)
PC2 (19.32%)
B
DIS
HCMin
HCMax
NC
PIS
PHMe
PHB
PHMa
TD
LIS
TLT
HRatio
NIS
PPMe
LBC
DBC
LH
DHB
DHMe
DHMa
AIS
DP
LP
ABS
APH
PC3 (11.94%)
PC2 (16.05%)
C
LBC
NC
LH
HRatio
HCMax
HCMin
TD
DHMa
AIS
DHMe
ABS
DHB
PHB
DP
PHMa
DBC
PHMe
LP
LIS
PIS
PPMe
APH
DIS
NIS
TLT
PC3 (11.45%)
PC2 (20.46%)
D
DIS
AIB
PIS
NIB
DIB
TLT
LIB
LIS
PIB
LP
APH
DP
NIS
AIS
O. longissima
Obelia sp.1
O. bidentata
PPMe
TD
DBC
LBC
DHMa
DHMe
DHB
PHB
PHMa
PHMe
HRatio
LH
NC
HCMin
HCMax
PC3 (9.29%)
PC2 (21.54%)
E
AIB
DIB
NIB
LIB
PIB
HRatio
LBC
AIS
DBC
LH
DHB
LIS
DP
DHMe
DHMa
HCMin
DIS
NC
NIS
TLT
TD
HCMax
PIS
PPMe
PHMe
PHB
ABS
APH
LP
PC3 (9.23%)
PC2 (13.80%)
F
LH
TLT
HRatio
DHMa
LP
DHMe
DHB
APH
LBC
DBC
DP
PHMa
TD
PHMe
PHB
O. geniculata I
O. geniculata III
PPMe
PC2 (20.65%)
PC1(52.66%)
Gonothyraea loveni
Hartlaubella gelatinosa
Laomedea angulata
Laomedea calceolifera
Laomedea flexuosa
Laomedea inornata
Obelia bidentata
Obelia dichotoma I
Obelia dichotoma II
Obelia dichotoma III
Obelia dichotoma IV
Obelia geniculata I
Obelia geniculata II
Obelia geniculata III
Obelia geniculata IV
Obelia longissima
Obelia sp.1

Figure S3. Distance biplots of the Principal Component Analysis (PCA) comprising data for Obeliidae. A. Second and third principal components (PCs) of the PCA with the complete dataset; B. Second and third PCs of the PCA with the complete dataset, but excluding measurements related to second-order branches of erect colonies (NIB, DIB, AIB, LIB); C. Second and third PCs of the PCA without the genus *Obelia*; D. Second and third PCs of the PCA with the genus *Obelia* only; E. Second and third PCs of the PCA with *O*. *dichotoma* and *O*. *longissima*; F. First and second PCs of the PCA with *O*. *geniculata*. Numbers in parentheses indicate percentages of variation explained by each principal component. Abbreviations of morphometric variables as in Table 2, and those in bold indicate measurements that were correlated with each principal component (Pearson correlation >0.7 and <-0.7).

Discussão Geral e Conclusões

Cnidaria é reconhecido por sua variabilidade morfológica, com implicação direta na interpretação sobre a diversidade do grupo e, consequentemente, sobre sua taxonomia. Estudos avaliando a amplitude de variação de caracteres diagnósticos são comuns em Anthozoa (e.g., Kim et al. 2004; Prada et al. 2008; Stefani et al. 2008), mas essa abordagem é rara em Medusozoa, embora sua utilidade tenha sido discutida (Dawson 2003, 2005, para Scyphozoa). O estudo que apresentamos acrescenta evidências novas e importantes sobre os padrões de variação morfológica em Medusozoa, demonstrando a utilidade de se avaliar a amplitude de variação de caracteres diagnósticos, em associação com dados moleculares, para lidar com as dificuldades associadas à delimitação de espécies no grupo.

A revisão dos diferentes níveis de variação morfológica em Medusozoa deixou claro que variações intraespecíficas e interespecíficas estão frequentemente correlacionadas, contribuindo para que haja sobreposição dos caracteres morfológicos entre os diferentes níveis, e gerando problemas taxonômicos. Tomemos um exemplo: um caráter, como a espessura do perissarco em *Orthopyxis* (Cunha et al. 2015, 2016), é variável no nível intraespecífico, quando se compara pólipos de uma mesma colônia (variação individual) ou pólipos de diferentes colônias (variação populacional), mas também varia no nível interespecífico, em que diferentes espécies também podem ser separadas pelo grau de espessura do perissarco. Esse padrão torna-se um problema para a taxonomia quando os caracteres morfológicos não são testados de forma independente, já que existe um grande potencial para erros de interpretação. De fato, estudos moleculares têm demonstrado a existência de espécies crípticas em Medusozoa, sendo que algumas delas puderam ser delimitadas morfologicamente investigando-se seus caracteres diagnósticos em um contexto filogenético (e.g., Dawson 2003, 2005; Schuchert 2005; Miglietta et al. 2007, 2009; Moura et al. 2012). Nossa estimativa demonstrou que, ao menos em Hydrozoa, há um grande potencial para a descoberta de espécies crípticas, e por consequência, o conhecimento atual sobre a diversidade específica no grupo ainda é limitado.

Hidroides da subordem Proboscoida Broch, 1910 (*sensu* Maronna et al. 2016) são um exemplo importante do impacto que os padrões de variação morfológica têm sobre a taxonomia do grupo. Govindarajan et al. (2006) foram os primeiros a estudar as relações filogenéticas da família Campanulariidae Johnston, 1836 (no seu sentido tradicional, cf. Cornelius 1982), evidenciando o não monofiletismo da família, de alguns gêneros (*Obelia,*

Laomedea) e espécies (*Orthopyxis integra*, *Clytia gracilis*). Ao ampliarmos significativamente o número de táxons das análises, demonstramos também o não monofiletismo de *Campanularia* e *Clytia*, assim como da subfamília Clytiinae, se consideradas em seu contexto tradicional. Em relação às espécies, estudos anteriores com *Clytia* e *Orthopyxis* contribuíram para a revisão de caracteres morfológicos e delimitação interespecífica nesses grupos (Lindner et al. 2011; Zhou et al. 2013; Cunha et al. 2015; He et al. 2015). Nosso estudo amplia os níveis de comparação entre as linhagens, corroborando a validade das espécies *Clytia elsaeoswaldae* Stechow, 1914, *Orthopyxis caliculata* (Hincks, 1853) e *Orthopyxis mianzani* Cunha, Genzano & Marques 2015 (Lindner et al. 2011; Cunha et al. 2015), e o não monofiletismo de *Clytia gracilis* (cf. Lindner et al. 2011), que compreende um complexo de espécies maior que o considerado até aqui. Entretanto, *Clytia xiamenensis* Zhou et al., 2013 e *Clytia gulangensis* He & Zheng, 2015, recentemente descritas (Zhou et al. 2013; He et al. 2015), resultaram proximamente relacionadas a linhagens de *C. hemisphaerica* dos EUA e *C. gracilis* do Brasil, respectivamente, demonstrando que seus limites interespecíficos não estão claros.

A análise de dados moleculares contribuiu para o estudo das relações filogenéticas entre espécies, gêneros e famílias de Proboscoida, e também para a revisão dos caracteres morfológicos diagnósticos tradicionalmente associados a essas linhagens. Com a inclusão de *Bonneviella* Broch, 1909 e exclusão de *Billardia* Totton, 1930, o hipostômio em forma de trompete deixa de ser uma característica compartilhada por todos os Campanulariidae, já que está ausente em *Bonneviella*, que apresenta o "veloid" e cavidade pré-oral (Broch 1909). Igualmente, a hidroteca em forma de campânula é uma provável simplesiomorfia, já ocorre externamente a Campanulariidae (e.g. *Billardia*, Vervoort & Watson 2003). Esses resultados corroboram a classificação proposta por Maronna et al. (2016), que considera no nível de família os três principais grupos que tradicionalmente compõe os Campanulariidae (subfamílias Campanulariinae Johnston 1836, Clytiinae Cockerell 1911 e Obeliinae Haeckel, 1879, Cornelius 1982; Govindarajan et al. 2006). Em relação aos gêneros, nossos resultados sugerem a necessidade de revisão dos caracteres morfológicos, principalmente em Obeliidae, cujos gêneros são historicamente diagnosticados pelo grau de redução da medusa (Cornelius 1982; Boero & Sarà 1987). Esse caráter não é decisivo para a taxonomia, pois resulta em grupos não monofiléticos. Da mesma forma, muitas espécies não são corroboradas de maneira inequívoca pelos seus caracteres diagnósticos (e.g., *Clytia gracilis*, *Obelia dichotoma*), que necessitam ser revistos. Em alguns casos, os padrões filogenéticos

apresentados não foram congruentes com as afinidades taxonômicas tradicionalmente conhecidas para algumas espécies (e.g., *C. hummelincki*, *C. stolonifera*).

As dificuldades associadas à delimitação de gêneros e espécies em Proboscoida relacionam-se, portanto, à ampla variação morfológica intraespecífica e sobreposição entre os diferentes níveis de variação. Esse cenário requer diferentes abordagens na investigação dos limites interespecíficos nesse grupo. Demonstramos, a partir da análise comparada da amplitude de variação dos caracteres, que várias espécies e gêneros possuem diferenças consistentes nos padrões morfométricos de caracteres morfológicos. Nossos resultados contribuem para a delimitação de várias espécies, evidenciando caracteres morfométricos que deram suporte à sua diferenciação. Dentre eles, podemos destacar: (a) variações de tamanho (comprimento e diâmetro das hidrotecas e pedículos) em espécies de *Bonneviella*, *Rhizocaulus verticillatus*, *Tulpa tulipifera*, algumas linhagens de *Clytia* cf. *gracilis*, *C. elsaeoswaldae*, *Laomedea flexuosa*; (b) variações de espessura do perissarco no gênero *Campanularia* e em espécies de *Orthopyxis*, *Silicularia rosea*, *C. noliformis*, *Obelia geniculata*; (c) variações no número e altura das cúspides da hidroteca em *Orthopyxis sargassicola*, *Orthopyxis crenata*, *C. linearis*, *C. stolonifera*, *Obelia bidentata*; e (d) variações em caracteres relacionadas às ramificações de colônias eretas em *Gonothyraea loveni*, *Hartlaubella gelatinosa*, *Obelia longissima* e algumas linhagens de *Obelia* cf. *dichotoma*.

Esses resultados demonstram o potencial existente na investigação dos padrões de variabilidade morfológica para a taxonomia dos Proboscoida. Fica claro que as variações morfológicas podem oferecer dados consistentes para a delimitação de espécies, e a aplicação dessa abordagem em outros grupos de Hydrozoa e Medusozoa é igualmente promissora. É importante, porém, que os caracteres morfológicos sejam estudados em associação aos padrões filogenéticos do grupo em questão, para que os níveis das variações morfológicas sejam corretamente interpretados. Dessa forma, esperamos que essa abordagem seja testada e amplamente utilizada em outros táxons marinhos, oferecendo novas bases para a delimitação de espécies em grupos com morfologia variável e contribuindo para o conhecimento sobre a diversidade de espécies no ambiente marinho.

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Resumo

A variabilidade morfológica é comum em vários táxons marinhos, e os membros de Cnidaria Medusozoa se destacam por expressar a variabilidade em diferentes níveis, especialmente considerando as diferentes fases do ciclo de vida. Entretanto, muitos problemas taxonômicos surgem a partir das dificuldades em interpretar os níveis de variação, já que variações intraespecíficas muitas vezes são interpretadas de forma imprecisa como interespecíficas, e vice-versa. Neste estudo, revisamos os padrões de variação morfológica em Cnidaria Medusozoa, avaliando sua influência na taxonomia e diversidade do grupo. Seguindo essa abordagem, investigamos as relações filogenéticas da subordem Proboscoida, testando a relevância dos caracteres morfológicos diagnósticos tradicionais para a delimitação de linhagens em vários níveis taxonômicos. Além disso, avaliamos os seus padrões de variação morfológica, contrastando dados morfométricos e filogenéticos. Ficou claro que a variação intraespecífica em Medusozoa está frequentemente correlacionada com a variação interespecífica, e existe sobreposição entre os diferentes níveis. Igualmente, mostramos que a diversidade de espécies em Medusozoa está imprecisamente estimada, e existe ainda um grande potencial para a descoberta de espécies crípticas em Hydrozoa. Isso foi comprovado em Proboscoida, já que seus padrões filogenéticos mostraram que vários grupos não são monofiléticos, incluindo a família Clytiidae, os gêneros *Campanularia*, *Clytia*, *Obelia* e *Laomedea*, e as espécies *Orthopyxis integra*, *Clytia gracilis* e *Obelia dichotoma*. Da mesma forma, vários caracteres diagnósticos tradicionais resultaram não informativos para a delimitação de espécies e gêneros. Por outro lado, encontramos padrões morfométricos consistentes entre caracteres investigados em diferentes níveis de comparação. Dentre eles, tamanho e forma da hidroteca, espessura do perissarco, assim como número e altura das cúspides da hidroteca corroboraram a delimitação de várias linhagens. Nosso estudo demonstrou a importância das análises que combinam dados morfométricos e filogenéticos, especialmente quando a amplitude de variação dos caracteres morfológicos é detalhadamente comparada e investigada. Estudos em Hydrozoa, assim como Medusozoa e outros táxons marinhos se beneficiarão dessa abordagem, estabelecendo espécies válidas bem fundamentadas, e aprimorando nossas estimativas sobre a diversidade de espécies no ambiente marinho.

Abstract

Morphological variability is common in several marine taxa, and members of Cnidaria Medusozoa are noticeable for expressing variability in many different levels, especially in different phases of the life cycle. However, difficulties in interpreting the levels of variation have posed many taxonomic problems, since intraspecific variations are often misinterpreted as interspecific variations, and vice-versa. In this study, we reassessed patterns of morphological variation in Cnidaria Medusozoa to evaluate their influence on the taxonomy and diversity of the group. Following this approach, we investigated the phylogenetic relationships in the suborder Proboscoida, testing the relevance of traditional morphological diagnostic characters for delimiting lineages in several taxonomic levels. Also, we evaluated their patterns of morphological variation, contrasting morphometric and phylogenetic data. It is clear that in Medusozoa intraspecific variation often parallels interspecific variation, and there is overlap between the different levels. In addition, we show that species diversity in Medusozoa is probably misestimated, and there is still a great potential for the discovery of cryptic species in Hydrozoa. This is true for Proboscoida, since their phylogenetic patterns showed that several groups are not monophyletic, including the family Clytiidae, the genera *Campanularia*, *Clytia*, *Obelia* and *Laomedea*, and the species *Orthopyxis integra*, *Clytia gracilis* and *Obelia dichotoma*. Similarly, several traditional diagnostic characters were shown not be informative for the delimitation of species and genera. On the other hand, we found consistent morphometric patterns among characters investigated at different levels of comparison. Among them, size and shape of hydrotheca, perisarc thickness, as well as number and height of hydrothecal cusps, supported the delimitation of several lineages. Our results showed the importance of analyses combining phylogenetic and morphometric data, especially when the ranges of variation of morphological characters are compared and investigated in detail. Studies on Hydrozoa, as well as Medusozoa and other marine taxa will benefit from this approach, establishing well grounded valid species and refining our assessments of marine species diversity.