



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



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ABSTRACT

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

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1. 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; Miranda et al., 2016), 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 are inadequate (e.g., Fukami et al., 2004; Bo et al., 2012).

Among hydrozoan species, characters such as colony size, branching pattern, 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, often 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 species may result in 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,

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which, in addition, have simple and similar morphological characters (cf. Cornelius, 1982). Not surprisingly, Campanulariidae has 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 history.

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 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, 1995b). A recent and comprehensive phylogenetic inference of Leptothecata, however proposed a new classification for Proboscoida Broch, 1910, dividing campanulariids into two infraorders, viz., Campanulariida Bouillon, 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. Although originally included in Proboscoida (Bouillon, 1985), Phialuciidae was not covered by their analysis, and its inclusion in this suborder 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 the richness, abundance, and ubiquitousness of the campanulariids, the basic knowledge on their 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 relevance of diagnostic characters from traditional classifications over the last 100 years, at the family, 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).

2. Material and methods

2.1. Taxonomic sampling

Sequence data of the family Campanulariidae were obtained during this study and from published works, comprising several localities (Tables 1 and 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. (2005, 2006), Govindarajan et al. (2006), Evans et al. (2008), Leclère 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/>; Table 1).

Specimens included in this study were generally sampled at low tide, although a few colonies were collected at 20–30 m deep with scuba diving (*Clytia* sp.1 and *Clytia noliformis*, see Table 1). The hydroids occurred on a variety of substrates, including rocks, algae (mostly Phaeophyceae, such as kelps and *Sargassum* sp.), mussel shells, cirripeds, sponges, floating docks, *Rhizophora* sp. support roots, as well as other hydroids (e.g. *Ectopleura* sp., *Plumularia setacea*), and were preserved in 95–100% ethanol. Specimens 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 (see Table 2). This study comprises 35 nominal species, including representatives of the three traditionally recognized subfamilies of Campanulariidae (cf. Cornelius, 1982; Calder, 1991), as well as specimens of *Bonneviella* (family Bonneviellidae), which has fallen within Campanulariidae in previous studies (Govindarajan et al., 2006). In addition, all accepted genera were included, 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. Similarly, *Billardia* Totton, 1930 is assigned to Campanulariidae by many authors (Ralph, 1957; Bouillon, 1985; Vervoort and Watson, 2003), but previous phylogenetic analyses have consistently placed it well outside Campanulariidae (Govindarajan et al., 2006; Leclère et al., 2009; Peña Cantero et al., 2010), and, therefore, it was not included in this analysis. With respect to a recent phylogenetic study (Maronna et al., 2016), we have included data comprising the three families proposed (Campanulariidae, Clytiidae, and Obeliidae), with a substantial increase in the number of sampled taxa within each group.

2.2. 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 S11).

Table 1

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

Code in tree	Species	Locality	Geographic coordinate	Voucher	16S	COI	18S	28S
IT10_IT	<i>Campanularia hincksii</i>	Ancona, Italy	Not available	MZUSP2759	KX665304	–	KX665403	KX665500
IT14_IT	<i>Campanularia hincksii</i>	Paraggi, Italy	Not available	MZUSP2760	KX665308	–	KX665407	–
PT10_ARG	<i>Campanularia</i> sp.	Punta Cuevas, San Julián, Argentina	Not available	MZUSP2761	KX665335	KX665233	KX665434	–
SJ2_ARG	<i>Campanularia subantarctica</i>	La Mina, Puerto San Julián, Argentina	–49.1569 –67.6331	MZUSP 2639	KM405574*	KM405569*	KX665450	–
SJ4_ARG	<i>Campanularia</i> sp.	La Mina, Puerto San Julián, Argentina	–49.1569 –67.6331	MZUSP 2641	KM405572*	KM405571*	KX665154	KX665523
PM36_BRA	<i>Clytia elsaeoswaldae</i>	Palmas Island, Brazil	Not available	MZUSP2762	KX665328	KX665227	KX665428	KX665516
CB19_BRA	<i>Clytia elsaeoswaldae</i>	Cabras Island, Ilhabela, Brazil	Not available	MZUSP2763	KX665260	KX665163	KX665365	KX665466
PM18_BRA	<i>Clytia elsaeoswaldae</i>	Palmas Island, Brazil	Not available	MZUSP2764	KX665327	KX665226	–	–
Me26_BRA	<i>Clytia elsaeoswaldae</i>	Mel Island, Brazil	–25.5561 –48.2987	MZUSP2765	KX665315	KX665215	–	–
EL05_SLV	<i>Clytia gracilis</i>	Mund Bay, Piran, Slovenia	Not available	MZUSP2766	KX665278	KX665181	KX665377	KX665477
EL14_SLV	<i>Clytia gracilis</i>	Strunjan, Piran, Slovenia	45.5370 13.6014	MZUSP2767	KX665282	KX665185	KX665381	KX665481
EL15_SLV	<i>Clytia gracilis</i>	Strunjan, Piran, Slovenia	45.5370 13.6014	MZUSP2768	KX665283	KX665186	KX665382	KX665482
EL31_SLV	<i>Clytia gracilis</i>	Slovenia	45.5912 13.6998	MZUSP2769	KX665289	KX665192	KX665388	KX665488
EL32_SLV	<i>Clytia gracilis</i>	Slovenia	45.5912 13.6998	MZUSP2770	KX665290	KX665193	KX665389	KX665489
EL38_SLV	<i>Clytia gracilis</i>	Piran, Slovenia	45.5303 13.5675	MZUSP2771	KX665292	KX665195	KX665391	KX665491
IT12_IT	<i>Clytia gracilis</i>	Ancona, Italy	Not available	MZUSP2772	KX665306	KX665208	KX665405	KX665502
IT13_IT	<i>Clytia gracilis</i>	Ancona, Italy	Not available	MZUSP2773	KX665307	–	KX665406	KX665503
CBC13_BLZ	<i>Clytia gracilis</i>	Twin Cays, Belize	16.8282 –88.1073	USNM1420648	KX665262	KX665166	KX665367	KX665468
CBC20_BLZ	<i>Clytia gracilis</i>	Carrie Bow Cay, Belize	16.8080 –88.0630	USNM1420655	KX665263	KX665167	–	–
CBC26_BLZ	<i>Clytia gracilis</i>	Twin Cays Fisheries Dock, Belize	16.8235 –88.1060	USNM1420660	KX665265	KX665169	–	–
MAP01_BRA	<i>Clytia gracilis</i>	Panaquatira, São Luís do Maranhão, Brazil	–2.4984 –44.0239	MZUSP2774	KX665310	KX665210	KX665411	KX665506
MAP11_BRA	<i>Clytia gracilis</i>	Panaquatira, São Luís do Maranhão, Brazil	–2.4984 –44.0239	MZUSP2775	KX665312	KX665212	–	–
PAF03_BRA	<i>Clytia gracilis</i>	Farol Velho, Salinópolis, Brazil	–0.591 –47.3248	MZUSP2776	KX665321	KX665221	KX665422	KX665512
T1_BRA	<i>Clytia gracilis</i>	Flexeiras, Trairi, Brazil	–3.217 –39.2671	MZUSP2777	KX665352	KX665249	KX665456	KX665527
T5_BRA	<i>Clytia gracilis</i>	Flexeiras, Trairi, Brazil	–3.2222 –39.2502	MZUSP2778	KX665354	KX665251	KX665458	KX665529
T6_BRA	<i>Clytia gracilis</i>	Flexeiras, Trairi, Brazil	–3.2222 –39.2502	MZUSP2779	KX665355	KX665252	–	–
CE1_BRA	<i>Clytia gracilis</i>	Náutico, Fortaleza, Brazil	Not available	MZUSP2780	KX665271	KX665173	KX665372	–
CE2_BRA	<i>Clytia gracilis</i>	Caponga, Cascavel, Brazil	–4.0391 –38.1929	MZUSP2781	KX665272	KX665174	–	–
CE3_BRA	<i>Clytia gracilis</i>	Náutico, Fortaleza, Brazil	Not available	MZUSP2782	KX665273	KX665175	–	–
CE5_BRA	<i>Clytia gracilis</i>	Caponga, Cascavel, Brazil	–4.0391 –38.1929	MZUSP2783	KX665275	KX665177	–	–
Me24_BRA	<i>Clytia gracilis</i>	Mel Island, Brazil	–25.5729 –48.3091	MZUSP2784	KX665314	KX665214	KX665414	–
PT9_ARG	<i>Clytia gracilis</i>	Punta Cuevas, San Julián, Argentina	Not available	MZUSP2785	KX665334	KX665232	KX665433	–
FLT03_USA	<i>Clytia hemisphaerica</i>	Westport, USA	41.5129 –71.0765	MZUSP2786	KX665296	KX665199	KX665395	KX665495
HCM04_USA	<i>Clytia hemisphaerica</i>	Salem, USA	42.5219 –70.8822	MZUSP2787	KX665301	KX665204	KX665400	–
MMA05_USA	<i>Clytia hemisphaerica</i>	Bourne, USA	41.7397 –70.6242	MZUSP2788	KX665318	KX665218	KX665417	–
PTJ01_USA	<i>Clytia hemisphaerica</i>	Point Judith, Rhode Island, USA	41.3877 –71.5171	MZUSP2789	–	KX665240	KX665441	–
EL06_SLV	<i>Clytia hemisphaerica</i>	Mund Bay, Piran, Slovenia	Not available	MZUSP2790	KX665279	KX665182	KX665378	KX665478
EL08_SLV	<i>Clytia hemisphaerica</i>	Mund Bay, Piran, Slovenia	Not available	MZUSP2791	KX665280	KX665183	KX665379	KX665479
EL12_SLV	<i>Clytia hemisphaerica</i>	Strunjan, Piran, Slovenia	45.5370 13.6014	MZUSP2792	KX665281	KX665184	KX665380	KX665480
EL20_SLV	<i>Clytia hemisphaerica</i>	Strunjan, Piran, Slovenia	45.5370 13.6014	MZUSP2793	KX665285	KX665188	KX665384	KX665484
EL28_CRO	<i>Clytia hemisphaerica</i>	Croatia	Not available	MZUSP2794	KX665287	KX665190	KX665386	KX665486
EL35_SLV	<i>Clytia hemisphaerica</i>	Slovenia	45.5912 13.6998	MZUSP2795	KX665291	KX665194	KX665390	KX665490
CBC1_BLZ	<i>Clytia hemisphaerica</i>	Carrie Bow Cay, Belize	Not available	USNM1420636	KX665261	KX665165	–	–
CBC25_BLZ	<i>Clytia hemisphaerica</i>	Twin Cays Fisheries Dock, Belize	16.8235 –88.1060	USNM1420659	KX665264	KX665168	KX665368	KX665469
CBC40.1_BLZ	<i>Clytia hemisphaerica</i>	Cuda Cut, Twin Cays, Belize	Not available	USNM1420673	KX665267	KX665171	–	–
CBC42_BLZ	<i>Clytia hummelincki</i>	Cuda Cut, Twin Cays, Belize	Not available	USNM1420675	KX665269	KX665172	KX665370	KX665471
PY10_BRA	<i>Clytia linearis</i>	Paraty, Brazil	Not available	MZUSP2796	KX665343	–	KX665444	KX665519
SP3_BRA	<i>Clytia noliformis</i>	Barão Tefé Island, São Pedro and São Paulo Archipelago, Brazil	Not available	MZUSP2797	KX665349	KX665246	KX665453	KX665525
SP9_BRA	<i>Clytia noliformis</i>	Barão Tefé Island, São Pedro and São Paulo Archipelago, Brazil	Not available	MZUSP2798	KX665350	KX665247	KX665454	KX665526
SP1_BRA	<i>Clytia</i> sp.1	Boca da Enseada, São Pedro and São Paulo Archipelago, Brazil	Not available	MZUSP2799	KX665348	KX665245	KX665452	KX665524
CE4_BRA	<i>Clytia</i> sp.2	Caponga, Cascavel, Brazil	–4.0391 –38.1929	MZUSP2800	KX665274	KX665176	KX665373	KX665473
NAT05_BRA	<i>Clytia</i> sp.3	Jenipabú, Natal, Rio Grande do Norte	–5.70 –35.19	MZUSP2801	KX665320	KX665220	KX665419	KX665509
CBC45_BLZ	Obeliida indet.	Cuda Cut, Twin Cays, Belize	Not available	USNM1420678	KX665270	–	KX665371	KX665472
CBC40.2_BLZ	Obeliida indet.	Cuda Cut, Twin Cays, Belize	Not available	USNM1420685	KX665268	–	–	–

Table 1 (continued)

Code in tree	Species	Locality	Geographic coordinate	Voucher	16S	COI	18S	28S
BPM03_USA	<i>Gonothyrea loveni</i>	Plymouth, USA	Not available	MZUSP2802	KX665257	KX665161	KX665362	KX665464
SWM03_USA	<i>Gonothyrea loveni</i>	Sandwich, USA	41.7703 –70.5036	MZUSP2803	KX665351	KX665248	KX665455	–
PT13_ARG	<i>Hartlaubella gelatinosa</i>	Río Gallegos, Argentina	Not available	MZUSP2804	KX665337	–	–	–
PT14_ARG	<i>Hartlaubella gelatinosa</i>	Río Gallegos, Argentina	Not available	MZUSP2805	KX665338	KX665235	KX665436	–
PT16_ARG	<i>Hartlaubella gelatinosa</i>	Río Gallegos, Argentina	Not available	MZUSP2806	KX665339	KX665236	KX665437	–
EL40_SLV	<i>Laomedea angulata</i>	Piran, Slovenia	45.515 13.5794	MZUSP2807	KX665293	KX665196	KX665392	KX665492
EL50_SLV	<i>Laomedea angulata</i>	Mund Bay, Piran, Slovenia	Not available	MZUSP2808	KX665294	KX665197	KX665393	KX665493
IT11_IT	<i>Laomedea angulata</i>	Ancona, Italy	Not available	MZUSP2809	KX665305	KX665207	KX665404	KX665501
FTA01_USA	<i>Laomedea calceolifera</i>	Newport, USA	41.4780 –71.3355	MZUSP2810	KX665298	KX665201	KX665397	KX665496
GFP01_USA	<i>Laomedea calceolifera</i>	Gloucester, USA	42.6151 –70.6504	MZUSP2812	KX665299	KX665202	KX665398	KX665497
HRM06_USA	<i>Laomedea calceolifera</i>	Hampton, USA	42.54 –70.495	MZUSP2813	KX665303	KX665206	KX665402	–
MMA06_USA	<i>Laomedea calceolifera</i>	Bourne, USA	41.7397 –70.6242	MZUSP2814	KX665319	KX665219	KX665418	–
ROW03_USA	<i>Laomedea calceolifera</i>	Boston, USA	42.3569 –71.0408	MZUSP2815	KX665344	KX665242	KX665446	–
RYE02_USA	<i>Laomedea flexuosa</i>	Rye, USA	42.9768 –70.7656	MZUSP2816	KX665346	–	KX665448	–
CBC35_BLZ	<i>Obelia bidentata</i>	Cuda Cut, Twin Cays, Belize	Not available	USNM1420668	KX665266	KX665170	KX665369	KX665470
MAR02_BRA	<i>Obelia bidentata</i>	Raposa Channel, São Luís do Maranhão, Brazil	–2.4271 –44.0700	MZUSP2817	KX665313	KX665213	KX665412	KX665507
MAP10_BRA	<i>Obelia bidentata</i>	Panaquatira, São Luís do Maranhão, Brazil	–2.4984 –44.0239	MZUSP2818	KX665311	KX665211	–	–
PAF09_BRA	<i>Obelia dichotoma</i>	Farol Velho, Salinópolis, Brazil	–0.591 –47.3248	MZUSP2819	KX665324	KX665223	KX665424	KX665514
MA03_BRA	<i>Obelia dichotoma</i>	Calhau, São Luís do Maranhão, Brazil	–2.4798 –44.2429	MZUSP2820	KX665309	KX665209	KX665410	KX665505
PAF07_BRA	<i>Obelia dichotoma</i>	Farol Velho, Salinópolis, Brazil	–0.591 –47.3248	MZUSP3334	KX665322	KX665222	KX665423	KX665513
MMA03_USA	<i>Obelia dichotoma</i>	Bourne, USA	41.7397 –70.6242	MZUSP3335	KX665316	KX665216	KX665415	–
FLT04_USA	<i>Obelia dichotoma</i>	Westport, USA	41.5129 –71.0765	MZUSP3336	KX665297	KX665200	KX665396	–
PIM01_USA	<i>Obelia dichotoma</i>	New Bedford, USA	41.6579 –70.9308	MZUSP3337	KX665325	KX665224	KX665426	–
PIM02_USA	<i>Obelia dichotoma</i>	New Bedford, USA	41.6579 –70.9308	MZUSP3338	KX665326	KX665225	KX665427	KX665515
PTJ03_USA	<i>Obelia dichotoma</i>	Point Judith, Rhode Island, USA	41.3877 –71.5171	MZUSP3339	KX665342	KX665241	KX665442	KX665517
ROW04_USA	<i>Obelia dichotoma</i>	Boston, USA	42.3569 –71.0408	MZUSP3340	KX665345	KX665243	KX665447	KX665521
S1.1_USA	<i>Obelia dichotoma</i>	Providence, USA	Not available	MZUSP3341	KX665347	KX665244	KX665449	KX665522
EL30_SLV	<i>Obelia dichotoma</i>	Slovenia	45.5912 13.6998	MZUSP3342	KX665288	KX665191	KX665387	KX665487
PT2_ARG	<i>Obelia dichotoma</i>	Punta Cuevas, San Julián, Argentina	Not available	MZUSP3343	KX665330	KX665229	–	–
PT3_ARG	<i>Obelia dichotoma</i>	Punta Cuevas, San Julián, Argentina	Not available	MZUSP3344	KX665331	KX665230	KX665430	–
UR1_URG	<i>Obelia dichotoma</i>	Rocha, Uruguay	–34.6523 –54.1416	MZUSP3345	KX665359	KX665255	KX665462	KX665532
UR6_URG	<i>Obelia dichotoma</i>	Rocha, Uruguay	–34.6541 –54.1435	MZUSP3346	KX665360	KX665256	KX665463	KX665533
BSF05_USA	<i>Obelia geniculata</i>	South Freeport, USA	43.8215 –70.1079	MZUSP3347	KX665258	–	KX665363	–
BZ5_BRA	<i>Obelia geniculata</i>	João Gonçalves, Búzios, Brazil	Not available	MZUSP3348	KX665259	KX665162	KX665364	KX665465
EL23_SLV	<i>Obelia geniculata</i>	Mund Bay, Piran, Slovenia	Not available	MZUSP3349	KX665286	KX665189	KX665385	KX665485
PT5_ARG	<i>Obelia geniculata</i>	Punta Cuevas, San Julián, Argentina	Not available	MZUSP3350	KX665332	KX665231	KX665431	–
UNH01_USA	<i>Obelia geniculata</i>	New Castle, USA	43.0723 –70.7157	MZUSP3351	KX665358	–	KX665461	–
PT1_ARG	<i>Obelia longissima</i>	San Julián, Argentina	Not available	MZUSP3352	KX665329	KX665228	KX665429	–
GFP04_USA	<i>Obelia longissima</i>	Gloucester, USA	Not available	MZUSP3353	KX665300	KX665203	KX665399	KX665498
HRM05_USA	<i>Obelia longissima</i>	Hampton, USA	Not available	MZUSP3354	KX665302	KX665205	KX665401	KX665499
MMA04_USA	<i>Obelia longissima</i>	Bourne, USA	41.7397 –70.6242	MZUSP3355	KX665317	KX665217	KX665416	–
T2_BRA	<i>Obelia</i> sp.1	Flexeiras, Trairí, Brazil	–3.217 –39.2671	MZUSP3356	KX665353	KX665250	KX665457	KX665528
PAF08_BRA	<i>Obelia</i> sp.1	Farol Velho, Salinópolis, Brazil	–0.591 –47.3248	MZUSP3357	KX665323	–	–	–
AB_BRA	<i>Orthopyxis caliculata</i>	Armação, Penha, Brazil	–26.7833 –48.6167	MZUSP 2565	KM405578*	KM405567*	KX665361	–
JGB3_BRA	<i>Orthopyxis caliculata</i>	João Gonçalves, Búzios, Brazil	Not available	MZUSP 2614	KM405584*	KM405565*	KX665408	KX665504
CB_BRA	<i>Orthopyxis crenata</i>	Caponga, Cascavel, Brazil	–4.0391 –38.1929	MZUSP 2633	KM405590*	KX665164	KX665366	KX665467
PAB2_BRA	<i>Orthopyxis crenata</i>	Paciência, Penha, Brazil	–26.7772 –48.6028	MZUSP 2551	KM405593*	KM405559*	KX665420	KX665510
PT19_ARG	<i>Orthopyxis crenata</i>	Comodoro Rivadavia, Argentina	Not available	MZUSP3359	–	KX665238	KX665439	–
PT20_ARG	<i>Orthopyxis integra</i>	San Julián, Argentina	Not available	MZUSP3358	KX665341	KX665139	KX665440	–
MB1_BRA	<i>Orthopyxis mianzani</i>	Mel Island, Brazil	–25.5561 –48.2987	MZUSP 2570	KM405603*	KM405549*	KX665413	KX665508
PAB6_BRA	<i>Orthopyxis mianzani</i>	Paciência, Penha, Brazil	–26.7772 –48.6028	MZUSP 2559	KM405607*	KM405545*	KX665421	KX665511
PTY2_BRA	<i>Orthopyxis sargassicola</i>	Paraty, Brazil	Not available	MZUSP 2606	KM405629*	KM405523*	KX665443	KX665518
LB9_BRA	<i>Orthopyxis sargassicola</i>	Lázaro, Ubatuba, Brazil	–23.5091 –45.1385	MZUSP 2602	KM405618*	KM405534*	KX665409	–
PB1_BRA	<i>Orthopyxis sargassicola</i>	Padres, Aracruz, Brazil	–19.9323 –40.1221	MZUSP 2617	KM405622*	KM405531*	KX665425	–
Co1_ARG	<i>Orthopyxis</i> sp.	Caleta Olivia, Argentina	–46.4256 –67.5197	MZUSP 2644	KM405635*	KX665178	KX665374	KX665474
EL02_SLV	<i>Orthopyxis</i> sp.1	Mund Bay, Piran, Slovenia	Not available	MZUSP3360	KX665276	KX665179	KX665375	KX665475

Table 1 (continued)

Code in tree	Species	Locality	Geographic coordinate	Voucher	16S	COI	18S	28S
EL04_SLV	<i>Orthopyxis</i> sp.1	Mund Bay, Piran, Slovenia	Not available	MZUSP3361	KX665277	KX665180	KX665376	KX665476
EL16_SLV	<i>Orthopyxis</i> sp.1	Strunjan, Piran, Slovenia	45.5370 13.6014	MZUSP3362	KX665284	KX665187	KX665383	KX665483
EL52_SLV	<i>Orthopyxis</i> sp.1	Strunjan, Piran, Slovenia	45.5370 13.6014	MZUSP3363	KX665295	KX665198	KX665394	KX665494
PT11_ARG	<i>Silicularia rosea</i>	Río Deseado, San Julián, Argentina	Not available	MZUSP3364	KX665336	KX665234	KX665435	–
RG4_ARG	<i>Silicularia rosea</i>	Río Grande, Cabo Santo Domingo, Argentina	–53.6888 –67.8445	MZUSP 2645	KM405636*	–	KX665445	KX665520
PT8_ARG	<i>Silicularia rosea</i>	San Julián, Argentina	Not available	MZUSP3365	KX665333	–	KX665432	–
PT18_ARG	<i>Tulpa tulipifera</i>	Patagonia, Argentina	Not available	MZUSP3366	KX665340	KX665237	KX665438	–
<i>Outgroups</i>								
U10_URG	<i>Eucheilota</i> sp.	Uruguay	Not available	MZUSP3367	KX665356	KX665253	KX665459	KX665530
U11_URG	<i>Eucheilota</i> sp.	Uruguay	Not available	MZUSP3368	KX665357	KX665254	KX665460	KX665531

Table 2

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

Code in tree	Species	Locality	16S	COI	18S	28S	Reference
USA	<i>Bonneviella regia</i> *	Aleutians, USA	AY789805	AY789890	AY789740	–	Govindarajan et al. (2006)
USA	<i>Bonneviella</i> sp.2*	Aleutians, USA	AY789806	AY789891	AY789741	–	Govindarajan et al. (2006)
USA	<i>Bonneviella</i> sp.3	Aleutians, USA	AY789807	AY789892	AY789742	–	Govindarajan et al. (2006)
USA	<i>Bonneviella</i> sp.4*	Aleutians, USA	AY789808	AY789893	AY789743	–	Govindarajan et al. (2006)
IT	<i>Campanularia hincksii</i> *	Otranto, Italy	AY789794	AY789882	AY789729	–	Govindarajan et al. (2006)
SJ5_ARG	<i>Campanularia</i> sp.*	La Mina, Puerto San Julián, Argentina	KM405573	KM405570	–	–	Cunha et al. (2015)
AN	<i>Campanularia</i> sp.	Low Island, Antarctica	FN424118	–	–	–	Peña Cantero et al. (2010)
SJ6_ARG	<i>Campanularia subantarctica</i> *	La Mina, Puerto San Julián, Argentina	KM405575	KM405568	–	–	Cunha et al. (2015)
USA	<i>Campanularia volubilis</i> *	Monterey, USA	AY789804	AY789889	AY789739	–	Govindarajan et al. (2006), Lindner et al. (2011)
SJ1_ARG	<i>Campanulariidae</i> sp. indet.*	La Mina, Puerto San Julián, Argentina	KM405576	–	–	–	Cunha et al. (2015)
SJ3_ARG	<i>Campanulariidae</i> sp. indet.*	La Mina, Puerto San Julián, Argentina	KM405577	–	–	–	Cunha et al. (2015)
MA_USA	<i>Clytia cf. gracilis</i> sp. A	Woods Hole, USA	AY789812	AY789900	AY789751	–	Govindarajan et al. (2006), Lindner et al. (2011)
ME_USA	<i>Clytia cf. gracilis</i> sp.A	Maine, USA	DQ068061	DQ068054	DQ068051	–	Lindner et al. (2011)
BRA	<i>Clytia cf. gracilis</i> sp.B	São Sebastião, Brazil	DQ068062	DQ068055	DQ068052	–	Lindner et al. (2011)
USA	<i>Clytia cf. gracilis</i> sp.B*	Beaufort, USA	AY789813	AY789901	AY789752	–	Govindarajan et al. (2006), Lindner et al. (2011)
BRA	<i>Clytia cf. gracilis</i> sp.C	São Sebastião, Brazil	DQ068063	DQ068056	DQ068053	–	Govindarajan et al. (2006), Lindner et al. (2011)
USA	<i>Clytia cf. gracilis</i> sp.D	Georges Bank, USA	AY789811	AY789899	AY789750	–	Govindarajan et al. (2006), Lindner et al. (2011)
1_BRA	<i>Clytia elsaeoswaldae</i> *	São Sebastião, Brazil	DQ064793	DQ064800	DQ064796	–	Govindarajan et al. (2006), Lindner et al. (2011)
2_BRA	<i>Clytia elsaeoswaldae</i> *	São Sebastião, Brazil	DQ068064	–	–	–	Lindner et al. (2011)
1_CHI	<i>Clytia folleata</i>	China	–	JQ716211	–	–	Zhou et al. (2013)
2-6_CHI	<i>Clytia folleata</i>	China	JQ716051-55	KF962081-85	KF962213-17	–	Zhou et al. (2013), He et al. (2015)
IT	<i>Clytia gracilis</i>	Italy	AY346364	AY789898	AY789749	–	Govindarajan et al. (2006)
XMCG1-15_CHI	<i>Clytia gulangensis</i>	Xiamen Bay, China	KF962425-39	KF962086-2100	KF962218-32	KF962318-32	He et al. (2015)
NS	<i>Clytia hemisphaerica</i> *	North Sea	AY789814	AY789902	AY789753	–	Govindarajan et al. (2006), Lindner et al. (2011)
FR	<i>Clytia hemisphaerica</i>	Villefranche-sur-mer, France	–	–	FJ550601	FJ550457	Leclère et al. (2009)
IT	<i>Clytia hummelincki</i>	S. Caterina, Italy	AY346363	AY789895	AY789745	–	Govindarajan et al. (2006), Lindner et al. (2011)
SA	<i>Clytia hummelincki</i>	South Africa	AY789809	AY789894	AY789744	–	Govindarajan et al. (2006)
–	<i>Clytia languida</i>	No precise information	–	GQ120064-65	–	–	Ortman et al. (2010)
USA	<i>Clytia linearis</i> *	Beaufort, USA	AY789810	AY789897	AY789748	–	Govindarajan et al. (2006), Lindner et al. (2011)
IT	<i>Clytia linearis</i>	Torre Inserraglio, Italy	AY346362	–	AY789747	–	Govindarajan et al. (2006), Lindner et al. (2011)
BRA	<i>Clytia linearis</i>	São Sebastião, Brazil	DQ064791	–	DQ064794	–	Govindarajan et al. (2006), Lindner et al. (2011)
1_BRA	<i>Clytia noliformis</i> *	São Sebastião, Brazil	DQ064792	–	DQ064795	–	Govindarajan et al. (2006), Lindner et al. (2011)
2_BRA	<i>Clytia noliformis</i>	São Sebastião, Brazil	–	–	EU272554	EU272611	Evans et al. (2008)
IT	<i>Clytia paulensis</i> *	Otranto, Italy	AY346361	AY789896	AY789746	–	Govindarajan et al. (2006), Lindner et al. (2011)
XMCL1-3_CHI	<i>Clytia</i> sp.	China	KF962440-42	KF962101-3	KF962233-35	–	He et al. (2015)

Table 2 (continued)

Code in tree	Species	Locality	16S	COI	18S	28S	Reference
KC1-5_CHI	<i>Clytia</i> sp.	China	JQ716046-50	JQ716206-10	KF962238-47	-	He et al. (2015)
AGC_USA	<i>Clytia</i> sp.	California, USA	AY512519	-	AF358074	-	Collins (2002), Collins et al. (2005)
USA	<i>Clytia</i> sp.	California, USA	AY800195	AY789903	AF358074	-	Collins et al. (2005), Govindarajan et al. (2006)
1-15_HR	<i>Clytia</i> sp.1	Helgoland Roads, North Sea	-	KC439960-74	-	-	Laakmann and Holst (2014)
1-4_HR	<i>Clytia</i> sp.2	Helgoland Roads, North Sea	-	KC439975-78	-	-	Laakmann and Holst (2014)
1-8_CHI	<i>Clytia xiamenensis</i>	Xiamen Bay, China	JQ716037-44	JQ716198-205	-	-	Zhou et al. (2013)
IC	<i>Gonothyrea loveni</i>	Sandgerdi, Iceland	FJ550480	-	FJ550547	FJ550404	Leclère et al. (2009)
USA	<i>Gonothyrea loveni</i> *	Dennis, USA	AY789826	-	AY789765	-	Govindarajan et al. (2006)
FR	<i>Gonothyrea loveni</i>	Roscoff, France	AY789827	-	AY789766	-	Govindarajan et al. (2006)
FR	<i>Laomedea calceolifera</i> *	Herquemoulin, France	FJ550504	-	FJ550590	FJ550447	Leclère et al. (2009)
USA	<i>Laomedea calceolifera</i> *	Woods Hole, USA	AY789829	AY789914	AY789768	-	Govindarajan et al. (2006)
FR	<i>Laomedea flexuosa</i>	Roscoff, France	AY789823	AY789910	AY789762	-	Govindarajan et al. (2006)
IC	<i>Laomedea flexuosa</i> *	Iceland	AY789824	AY789911	AY789763	-	Govindarajan et al. (2006)
WS	<i>Laomedea flexuosa</i> *	White Sea	AY789825	AY789912	AY789764	-	Govindarajan et al. (2006)
USA	<i>Laomedea inornata</i> *	Friday Harbor, USA	AY789822	-	AY789761	-	Govindarajan et al. (2006)
USA	<i>Obelia bidentata</i> *	Beaufort, USA	AY789815	AY789904	AY789754	-	Govindarajan et al. (2006)
FR	<i>Obelia bidentata</i>	Utah Beach, France	FJ550503	-	FJ550589	FJ550446	Leclère et al. (2009)
NS	<i>Obelia bidentata</i> *	North Sea	AY789816	AY789905	AY789755	-	Govindarajan et al. (2006)
IT	<i>Obelia dichotoma</i> *	Otranto, Italy	AY789828	AY789913	AY789767	-	Govindarajan et al. (2006)
FR	<i>Obelia geniculata</i>	Roscoff, France	AY530359	AY530410	AY789769	-	Govindarajan et al. (2006)
NB_CAN	<i>Obelia geniculata</i> *	New Brunswick, Canada	AY530344	AY530395	AY789770	-	Govindarajan et al. (2006)
IC	<i>Obelia geniculata</i>	Sandgerdi, Iceland	FJ550481	-	FJ550548	FJ550405	Leclère et al. (2009)
JP	<i>Obelia geniculata</i> *	Japan	AY530335	AY530386	AY789771	-	Govindarajan et al. (2006)
NZ	<i>Obelia geniculata</i> *	New Zealand	AY530378	AY530429	AY789772	-	Govindarajan et al. (2006)
NZ	<i>Obelia longissima</i>	Dunedin, New Zealand	AY789817	AY789906	AY789756	-	Govindarajan et al. (2006)
AN	<i>Obelia longissima</i> *	Antarctic Peninsula	AY789821	AY789909	AY789760	-	Govindarajan et al. (2006)
IC	<i>Obelia longissima</i> *	Sandgerdi, Iceland	AY789820	AY789908	AY789759	-	Govindarajan et al. (2006)
WS	<i>Obelia longissima</i> *	White Sea	AY789819	AY789907	AY789758	-	Govindarajan et al. (2006)
USA	<i>Obelia longissima</i> *	Rydgers Cove, USA	AY789818	-	AY789757	-	Govindarajan et al. (2006)
1-11_HR	<i>Obelia</i> sp.1	Helgoland Roads, North Sea	-	KC439979-89	-	-	Laakmann and Holst (2014)
1-5_HR	<i>Obelia</i> sp.2	Helgoland Roads, North Sea	-	KC439990-94	-	-	Laakmann and Holst (2014)
1-9_HR	<i>Obelia</i> sp.3	Helgoland Roads, North Sea	-	KC439995-440003	-	-	Laakmann and Holst (2014)
JGB1_BRA	<i>Orthopyxis caliculata</i> *	João Gonçalves, Búzios, Brazil	KM405582	-	-	-	Cunha et al. (2015)
JGB2_BRA	<i>Orthopyxis caliculata</i> *	João Gonçalves, Búzios, Brazil	KM405583	-	-	-	Cunha et al. (2015)
JGB4_BRA	<i>Orthopyxis caliculata</i> *	João Gonçalves, Búzios, Brazil	KM405585	-	-	-	Cunha et al. (2015)
PAB1_BRA	<i>Orthopyxis caliculata</i> *	Paciência, Penha, Brazil	KM405586	KM405564	-	-	Cunha et al. (2015)
PAB3_BRA	<i>Orthopyxis caliculata</i> *	Paciência, Penha, Brazil	KM405587	KM405563	-	-	Cunha et al. (2015)
PAB4_BRA	<i>Orthopyxis caliculata</i> *	Paciência, Penha, Brazil	KM405588	KM405562	-	-	Cunha et al. (2015)
PAB5_BRA	<i>Orthopyxis caliculata</i> *	Paciência, Penha, Brazil	KM405589	KM405561	-	-	Cunha et al. (2015)
GB_BRA	<i>Orthopyxis caliculata</i> *	Grande Beach, Penha, Brazil	KM405581	KM405566	-	-	Cunha et al. (2015)
BB_BRA	<i>Orthopyxis caliculata</i> *	Bombas Beach, Bombinhas, Brazil	KM405579	-	-	-	Cunha et al. (2015)
COB_BRA	<i>Orthopyxis caliculata</i> *	Conceição, Bombinhas, Brazil	KM405580	-	-	-	Cunha et al. (2015)
LB5_BRA	<i>Orthopyxis crenata</i> *	Lázaro, Ubatuba, Brazil	KM405591	-	-	-	Cunha et al. (2015)
LB8_BRA	<i>Orthopyxis crenata</i> *	Lázaro, Ubatuba, Brazil	KM405592	-	-	-	Cunha et al. (2015)
PAB7_BRA	<i>Orthopyxis crenata</i> *	Paciência, Penha, Brazil	KM405594	KM405558	-	-	Cunha et al. (2015)
LG_BRA	<i>Orthopyxis crenata</i> *	Prainha, Laguna, Brazil	-	KM405560	-	-	Cunha et al. (2015)
NZ	<i>Orthopyxis crenata</i>	Wellington, New Zealand	FJ550466	-	-	FJ550383	Leclère et al. (2009)
IT	<i>Orthopyxis everta</i> ^A	Torre del Serpe, Italy	AY789793	AY789881	AY789728	-	Govindarajan et al. (2006)
IT	<i>Orthopyxis integra</i> ^B	Italy	AY789799	AY789884	AY789734	-	Govindarajan et al. (2006)
1_USA	<i>Orthopyxis integra</i>	Aleutians, USA	AY789800	AY789885	AY789735	-	Govindarajan et al. (2006)
2_USA	<i>Orthopyxis integra</i>	Friday Harbor, USA	AY789798	-	AY789733	-	Govindarajan et al. (2006)
IC	<i>Orthopyxis integra</i>	Sandgerdi, Iceland	AY789802	AY789887	AY789737	-	Govindarajan et al. (2006)
NZ	<i>Orthopyxis integra</i> ^C	New Zealand	AY789801	AY789886	AY789736	-	Govindarajan et al. (2006)
USA	<i>Orthopyxis integra</i> CA sp.1*	Monterey, USA	AY789796	-	AY789731	-	Govindarajan et al. (2006)
USA	<i>Orthopyxis integra</i> CA sp.2	Monterey, USA	AY789797	-	AY789732	-	Govindarajan et al. (2006)
MB2_BRA	<i>Orthopyxis mianzani</i> *	Mel Island, Brazil	KM405603	KM405549	-	-	Cunha et al. (2015)

Table 2 (continued)

Code in tree	Species	Locality	16S	COI	18S	28S	Reference
MB3_BRA	<i>Orthopyxis mianzani</i> *	Mel Island, Brazil	KM405604	KM405548	–	–	Cunha et al. (2015)
MB4_BRA	<i>Orthopyxis mianzani</i> *	Mel Island, Brazil	KM405605	KM405547	–	–	Cunha et al. (2015)
MB5_BRA	<i>Orthopyxis mianzani</i> *	Mel Island, Brazil	KM405606	KM405546	–	–	Cunha et al. (2015)
FOB1_BRA	<i>Orthopyxis mianzani</i> *	Mel Island, Brazil	KM405595	KM405557	–	–	Cunha et al. (2015)
FOB2_BRA	<i>Orthopyxis mianzani</i> *	Mel Island, Brazil	KM405596	KM405556	–	–	Cunha et al. (2015)
FOB3_BRA	<i>Orthopyxis mianzani</i> *	Mel Island, Brazil	KM405597	KM405555	–	–	Cunha et al. (2015)
FOB4_BRA	<i>Orthopyxis mianzani</i> *	Mel Island, Brazil	KM405598	KM405554	–	–	Cunha et al. (2015)
FOB5_BRA	<i>Orthopyxis mianzani</i> *	Mel Island, Brazil	KM405599	KM405553	–	–	Cunha et al. (2015)
FOB6_BRA	<i>Orthopyxis mianzani</i> *	Mel Island, Brazil	KM405600	KM405552	–	–	Cunha et al. (2015)
FOB7_BRA	<i>Orthopyxis mianzani</i> *	Mel Island, Brazil	KM405601	KM405551	–	–	Cunha et al. (2015)
BRA	<i>Orthopyxis sargassicola</i>	São Sebastião, Brazil	AY789795	AY789883	AY789730	–	Govindarajan et al. (2006), Lindner et al. (2011)
FB1_BRA	<i>Orthopyxis sargassicola</i> *	Formosa, Aracruz, Brazil	KM405610	KM405542	–	–	Cunha et al. (2015)
FB2_BRA	<i>Orthopyxis sargassicola</i> *	Formosa, Aracruz, Brazil	KM405611	KM405541	–	–	Cunha et al. (2015)
PB2_BRA	<i>Orthopyxis sargassicola</i> *	Padres, Aracruz, Brazil	KM405623	KM405530	–	–	Cunha et al. (2015)
PB3_BRA	<i>Orthopyxis sargassicola</i> *	Padres, Aracruz, Brazil	KM405624	KM405529	–	–	Cunha et al. (2015)
PB4_BRA	<i>Orthopyxis sargassicola</i> *	Padres, Aracruz, Brazil	KM405625	KM405528	–	–	Cunha et al. (2015)
PB5_BRA	<i>Orthopyxis sargassicola</i> *	Padres, Aracruz, Brazil	KM405626	KM405527	–	–	Cunha et al. (2015)
PB6_BRA	<i>Orthopyxis sargassicola</i> *	Padres, Aracruz, Brazil	KM405627	KM405526	–	–	Cunha et al. (2015)
PB7_BRA	<i>Orthopyxis sargassicola</i> *	Padres, Aracruz, Brazil	–	KM405525	–	–	Cunha et al. (2015)
PTY1_BRA	<i>Orthopyxis sargassicola</i> *	Paraty, Brazil	KM405628	KM405524	–	–	Cunha et al. (2015)
PTY3_BRA	<i>Orthopyxis sargassicola</i> *	Paraty, Brazil	KM405630	KM405522	–	–	Cunha et al. (2015)
PTY4_BRA	<i>Orthopyxis sargassicola</i> *	Paraty, Brazil	KM405631	KM405521	–	–	Cunha et al. (2015)
PTY5_BRA	<i>Orthopyxis sargassicola</i> *	Paraty, Brazil	KM405632	KM405520	–	–	Cunha et al. (2015)
RI_BRA	<i>Orthopyxis sargassicola</i> *	Ratos Island, Paraty, Brazil	KM405633	KM405519	–	–	Cunha et al. (2015)
MI_BRA	<i>Orthopyxis sargassicola</i> *	Meros Island, Paraty, Brazil	KM405621	KM405532	–	–	Cunha et al. (2015)
LB1_BRA	<i>Orthopyxis sargassicola</i> *	Lázaro, Ubatuba, Brazil	KM405612	KM405540	–	–	Cunha et al. (2015)
LB2_BRA	<i>Orthopyxis sargassicola</i> *	Lázaro, Ubatuba, Brazil	KM405613	KM405539	–	–	Cunha et al. (2015)
LB3_BRA	<i>Orthopyxis sargassicola</i> *	Lázaro, Ubatuba, Brazil	KM405614	KM405538	–	–	Cunha et al. (2015)
LB4_BRA	<i>Orthopyxis sargassicola</i> *	Lázaro, Ubatuba, Brazil	KM405615	KM405537	–	–	Cunha et al. (2015)
LB6_BRA	<i>Orthopyxis sargassicola</i> *	Lázaro, Ubatuba, Brazil	KM405616	KM405536	–	–	Cunha et al. (2015)
LB7_BRA	<i>Orthopyxis sargassicola</i> *	Lázaro, Ubatuba, Brazil	KM405617	KM405535	–	–	Cunha et al. (2015)
LB10_BRA	<i>Orthopyxis sargassicola</i> *	Lázaro, Ubatuba, Brazil	KM405619	–	–	–	Cunha et al. (2015)
LB11_BRA	<i>Orthopyxis sargassicola</i> *	Lázaro, Ubatuba, Brazil	KM405620	KM405533	–	–	Cunha et al. (2015)
SS_BRA	<i>Orthopyxis sargassicola</i> *	Preta Beach, São Sebastião, Brazil	KM405634	KM405518	–	–	Cunha et al. (2015)
CI1_BRA	<i>Orthopyxis sargassicola</i> *	Campeche Island, Florianópolis, Brazil	KM405608	KM405544	–	–	Cunha et al. (2015)
CI2_BRA	<i>Orthopyxis sargassicola</i> *	Campeche Island, Florianópolis, Brazil	KM405609	KM405543	–	–	Cunha et al. (2015)
USA	<i>Rhizocaulus verticillatus</i> *	Aleutians, USA	AY789803	AY789888	AY789738	–	Govindarajan et al. (2006), Lindner et al. (2011)
1_NZ	<i>Silicularia rosea</i> *	Bay of Islands, New Zealand	AY789792	–	AY789727	–	Govindarajan et al. (2006)
2_NZ	<i>Silicularia rosea</i>	Wellington, New Zealand	FJ550482	–	FJ550549	FJ550406	Leclère et al. (2009)
Outgroups							
FR	<i>Calycella syringa</i>	Roscoff, France	FJ550460	–	FJ550519	FJ550372	Leclère et al. (2009)
USA	<i>Calycella syringa</i>	Woods Hole, USA	AY789833	AY789916	AY789776	–	Govindarajan et al. (2006)
–	<i>Mitrocomella niwai</i>	Devonport, New Zealand	FJ550473	–	FJ550536	FJ550392	Leclère et al. (2009)
–	<i>Phialella quadrata</i>	Whangaparoa, New Zealand	FJ550474	–	FJ550537	FJ550393	Leclère et al. (2009)
–	<i>Eugymnanthea inquilina</i>	Taranto, Italy	AY789832	AY789915	AY789775	–	Govindarajan et al. (2006)
–	<i>Eucheilota maculata</i>	Luc-sur-mer, France	FJ550501	–	FJ550587	FJ550444	Leclère et al. (2009)
–	<i>Eirene viridula</i>	Luc-sur-mer, France	FJ550502	–	FJ550588	FJ550445	Leclère et al. (2009)
–	<i>Blackfordia virginica</i>	Northern California, USA	AY512516	–	AF358078	AY920800	Collins (2002), Collins et al. (2005, 2006)
–	<i>Aequorea aequorea</i>	Woods Hole, USA	AY512518	–	AF358076	EU305505	Collins (2002), Collins et al. (2005), Cartwright et al. (2008)
–	<i>Aequorea victoria</i>	Not available	EY305469	–	AF358077	AY920799	Collins (2002), Collins et al. (2006), Cartwright et al. (2008)
–	<i>Opercularella pumila</i>	Woods Hole, USA	AY789834	–	AY789777	–	Govindarajan et al. (2006)
–	<i>Lovenella gracilis</i>	Wildwood Crest, USA	AY789830	–	AY789773	–	Govindarajan et al. (2006)
–	<i>Eucheilota bakeri</i>	California, USA	AY789831	–	AY789774	–	Govindarajan et al. (2006)
–	<i>Tiaropsidium kelsey</i>	Not available	–	–	AF358079	–	Govindarajan et al. (2006)

^{A,B} These specimens were misidentified and most likely correspond to *Orthopyxis asymmetrica* Stechow, 1919 (accepted as *Campanularia breviscyphia* Sars, 1857 in WoRMS, Schuchert, 2015).

^C This specimen was misidentified and most likely correspond to *Orthopyxis crenata* (Hartlaub, 1901) (see Section 4.3 of the text for more details).

PCRs were performed either in a total volume of 25 μ l (with 75 mM Tris-HCl (pH 8.8 at 25 °C), 20 mM (NH₄)₂SO₄, 2.5 mM MgCl₂, 0.26 U/ μ l Taq polymerase (Thermo Fisher Scientific, Waltham, MA, USA), 0.2 mM dNTP and 0.4 μ M primers), 20 μ l (with 1x Phusion Buffer, 0.02U/ μ l Taq polymerase Phusion (FinnZymes, Thermo Fisher Scientific, Waltham, MA, USA), 1.1 mM MgCl₂, 0.2 mM dNTP, 0.4 μ M primers) or 10 μ l (with 10x NH₄ Buffer, 3 mM MgCl₂, 0.05U/ μ l Biolase Taq polymerase (Biolone, London, UK), 0.1 mM dNTP, 1x bovine serum albumin (BSA), 3 μ M primers). Dimethyl sulfoxide (DMSO) was included in some PCRs for amplification of nuclear genes (1.25 μ l for 25 μ l reactions, 0.5 μ l for 10 μ l 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 (0.03 μ M). 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 settings for a less stringent selection. Gaps within the contiguous sequences of 18S and 28S were coded as missing data in the alignments. For specimens with multiple sequences from the same locality, only unique haplotypes were included in the analysis (mainly present in single-gene phylogenies).

2.3. Phylogenetic analysis

Phylogenetic analyses were performed on (a) individual markers and (b) the concatenated dataset (16S + COI + 18S + 28S). This last dataset was analyzed based on (b1) taxa with sequences available for at least 3 markers (Dataset 1), and (b2) taxa with sequences available for all 4 markers (Dataset 2). In the first case (b1), absent fragments in the alignment were coded as missing data. The datasets were analyzed using parsimony (P), maximum likelihood (ML) and Bayesian (B) criteria. Parsimony analyses were performed in PAUP* v4b10 (Swofford, 2002), with heuristic searches with 1000 replicates of random-addition-sequence, saving up to 100 trees per replicate, and branch-swapping by TBR (Tree Bisection-Reconnection). Gaps were treated as a 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 1000 replicates (with 10 replicates of random-addition-sequence). ML analyses were performed in GARLI v2.01 (Zwickl, 2006) and consisted of 10 replicate searches with taxa randomly added to the starting tree. Branch support was estimated with Bootstrap based on 100 replicates. Bayesian analyses were performed using MrBayes 3.2 (Ronquist et al., 2012). For each dataset, two independent searches were run for 5,000,000 generations and trees were sampled every 1000 generations. For convergence diagnostics and calculation of posterior probabilities and branch lengths, the first 25% of the trees was discarded as burnin. For both ML and B, 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 Information Criterion (AIC, Table 3). For Bayesian analyses, a GTR + G + I model or partitioned GTR + G + I

models were used for the single-gene and concatenated datasets, respectively. Uncorrected p-distances for COI and 16S were calculated using PAUP* v4b10.

3. 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, especially at genus and family levels. Resolution and support enhances with the concatenation of all genes. The concatenated dataset including taxa with sequences for at least three genes (Dataset 1) comprises 181 taxa (Figs. 1 and 2, Table 3), while the combined dataset including taxa with sequences for all four genes (Dataset 2) has only 80 taxa (Figs. S1 and 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. Similarly, since ML and B topologies are nearly identical for all datasets, we only show the trees that resulted from ML analyses, with posterior probabilities plotted on these trees. The 16S phylogeny derived from ML analysis is presented for further discussions on specific lineages (Fig. 3), and the remaining single-gene phylogenies are remarked upon when relevant (Figs. S3–S9).

3.1. Family level

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 (Figs. 1 and 2). However, some discrepancies occur. The P topology derived from Dataset 1 has the species *Clytia hummelincki*, *C. paulensis* and *Obeliida* indet. ambiguously placed at the base of the Clytiidae + Obeliidae clade (Infraorder Obeliida Maronna et al., 2016) (Fig. 1), whereas the ML topology shows *C. hummelincki* placed outside Clytiidae + Obeliidae, with high support (Fig. 2) and *Obeliida* indet. at the base of Obeliidae. Both topologies derived from Dataset 2 (Figs. S1 and 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 verticillatus* (Figs. 1 and 2; 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 with negligible support (Figs. S4–S7). Also, most of the single-gene phylogenies have outgroup representatives placed within the ingroup, but this is never well supported (Figs. 3, S3–S9).

3.2. Genus level

Four of 11 genera included in the analysis were recovered as monophyletic in the concatenated phylogenies: *Silicularia*, *Orthopyxis*, *Gonothyraea*, and *Hartlaubella* (Figs. 1 and 2, S1 and S2). *Tulpa* and *Rhizocaulus* are only represented by one species, so their monophyly still needs testing. Although *Orthopyxis* was not recovered monophyletic in the P topology derived from Dataset 2 (Fig. S1), it is monophyletic in accordance with topologies derived from Dataset 1 (Figs. 1 and 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 (Figs. 1 and 2). *Bonneviella* was not recovered as monophyletic in the P topology of Dataset 1, but this scenario is weakly supported (Fig. 1).

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		1829	3396	632	680	1891	3391	641	665	1840	3443
Number of informative characters (ML)	234	669	412	904	191	248	332	835	247	262	422	918
Model of nucleotide evolution (ML)	TPM3uf + I	GTR + I	TIM1 + I	GTR + I	TPM2uf + I	TIM2 + I	TrN + I	TIM2 + I	GTR + I + G	GTR + I + G	TIM1 + I + G	GTR + I + G
Log likelihood (ML)	+ G	+ G	+ G	+ G	+ G	+ G	+ G	+ G	-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				182,263	4000	16,424	9
Minimum length (P)	13,092				7922				2409	3082	2449	5501

Clytia is not monophyletic because two of its species are ambiguously placed: *C. hummelincki* (always placed outside *Clytia* in concatenated phylogenies; Figs. 1 and 2, S1 and S2), 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; Fig. 1). There is no congruence and little support for the position of these species in single gene phylogenies, although *C. paulensis* is frequently placed inside *Clytia* (Figs. 2 and 3, S6 and S7). Similarly, specimens from Belize identified as *Obeliida* indet. (CBC40.2 and CBC45_BLZ) were ambiguously placed at the base of Clytiidae plus Obelliidae (P topology of Dataset 1; Fig. 1) or at the base of Obelliidae (ML topologies, Figs. 2 and 3), and this prevented us from reliably assigning the specimens to either *Clytia* or *Obelia*.

Regarding Obelliidae, only *Gonothyraea* and *Hartlaubella* were recovered as monophyletic, with high posterior probabilities and bootstrap supports in most of the phylogenies, including those of individual genes in which these lineages were sampled (Figs. 1 and 2, S4–S9). *Laomedea* is not monophyletic because *L. flexuosa* falls outside the main *Laomedea* clade (*L. angulata* + *L. calceolifera*) (Figs. 1 and 2). *Obelia* is also not monophyletic, since many of its lineages are more closely related to different genera than to other species of *Obelia*. 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, Fig. 2) monophyletic clades: *Gonothyraea* + *Obelia* (clade S), *Obelia* + *Laomedea* (clade AA), *Obelia bidentata* (clade Z), *Obelia* + *Laomedea flexuosa* + *Hartlaubella* (clade AB) (Figs. 1–3). Relationships among these clades vary between ML and P topologies derived from Dataset 1 (Figs. 1 and 2), and only P topologies recover clade S at the base of Obelliidae. However, both ML and P topologies derived from Dataset 2 (Figs. S1 and S2) also place clade S at the base of the group, giving further support to this hypothesis.

3.3. Species level

Lineages at the species level are highly congruent among single-gene and concatenated phylogenies (Figs. 1–3, S1–S9). 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 (Figs. 3, S3). The maximal intra-clade distance, considering the 16S, is 1.58% for clade B and 0.99% for clade C, and they have a minimum inter-clade distance of 6.37% (Fig. 4, clade D). The species is monophyletic, though, in concatenated phylogenies (Figs. 1 and 2, clade D). Similarly, *Orthopyxis integra* was recovered in three different and relatively distant clades. One of these clades is closely related to *O. crenata*, suggesting that misidentifications might have occurred (Clade H, Figs. 1–3, S4 and S5). Indeed, specimens of *O. integra* and *O. crenata* from clade H have low (<2%) intra-clade distances considering the 16S, and 3.85% intra-clade distances on average for COI (Fig. 4). In addition, they form a monophyletic group with another *O. crenata* clade (G) in concatenated phylogenies (Figs. 1 and 2, clade I). Specimens identified as *O. integra* also cluster with *O. everta* and *Orthopyxis* sp.1 (clade J, Figs. 1–3), with maximum intra-clade distances of 1.82% for 16S and 9.41% for COI (Fig. 4).

Numerous lineages were recovered in Clytiidae, including seven identified as *Clytia gracilis* that do not form a clade (Figs. 1 and 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, Figs. 1–3). This clade shows maximum intra-clade distances of 5.41% for 16S and 6.62% for COI (Fig. 4). Similarly, the species *Clytia gulangensis*, also from China, clusters with specimens of *C. gracilis* from Brazil (clade N, Figs. 1–3), with maximum intra-clade distances of 2.67% for 16S and 5.71% for COI (Fig. 4). Also, specimens of *C. gracilis* from the Mediterranean split into two closely related clades (K and L, minimum inter-

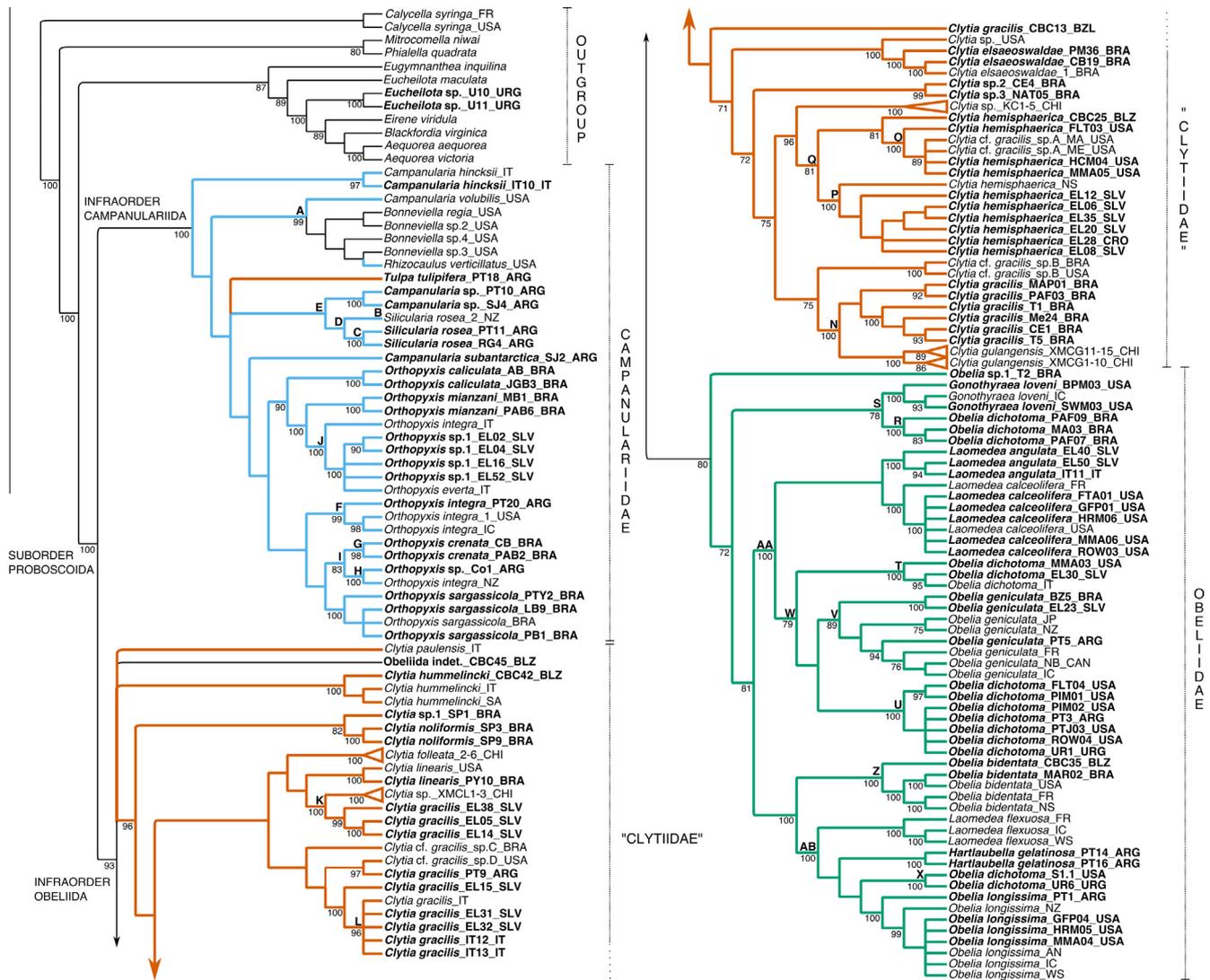


Fig. 1. Strict consensus of 725 most parsimonious trees based on 16S, COI, 18S and 28S data. Only taxa with sequences for at least 3 genes (Dataset 1) were analyzed. Bootstrap values are shown for each node. Nodes without numbers indicate support below 70%. Node letters in accordance with Fig. 4. Taxa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

clade distances are 6.60% for 16S and 9.65% for COI, Fig. 4, clade M), which are monophyletic in the ML concatenated phylogeny, but not in the P concatenated phylogeny, as well as 16S and COI topologies (Figs. 1–3, S3–S5). Additionally, specimens of *C. gracilis* also fall within a clade (O) comprising *C. hemisphaerica* and the recently described *C. xiamenensis* (Figs. 3 and 4). Intra-clade distances (<2%) indicate close affinities between these specimens (Fig. 4). Finally, *C. hemisphaerica* split into two main reciprocally monophyletic clades (O and P, inter-clade distances in Fig. 4, clade Q).

Four lineages corresponding to *Obelia dichotoma* were recovered in the Obeliidae (Figs. 1–3). Clade R is formed exclusively by Brazilian specimens, and is closely related to the species *Gonothyrea loveni* in the concatenated phylogenies (Figs. 1 and 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 Fig. 4). These two clades also seem to be geographically structured, although USA specimens of *O. dichotoma* fall within the Mediterranean 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, Figs. 1–3). *Obelia geniculata* lineages form three different clades, unambiguously monophyletic in most of the phylogenies (Figs. 1–3, S1–S9). They show intra-clade distances ranging from 0 to 3.02% for 16S and 0.16% to 7.29% for COI (clade V, Fig. 4). Finally, *O. bidentata* forms a monophyletic clade in nearly all topologies (except for COI, Figs. 1–3, S1–S9), but its intra-clade distances are comparable to inter-clade distances of other lineages (clade Z, Fig. 4).

4. 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 the family, genus and species levels. Some morphological diagnostic characters traditionally used for the delimitation of campanulariid species and genera are not informative. The phylogenetic relationships presented herein are largely congruent with previous

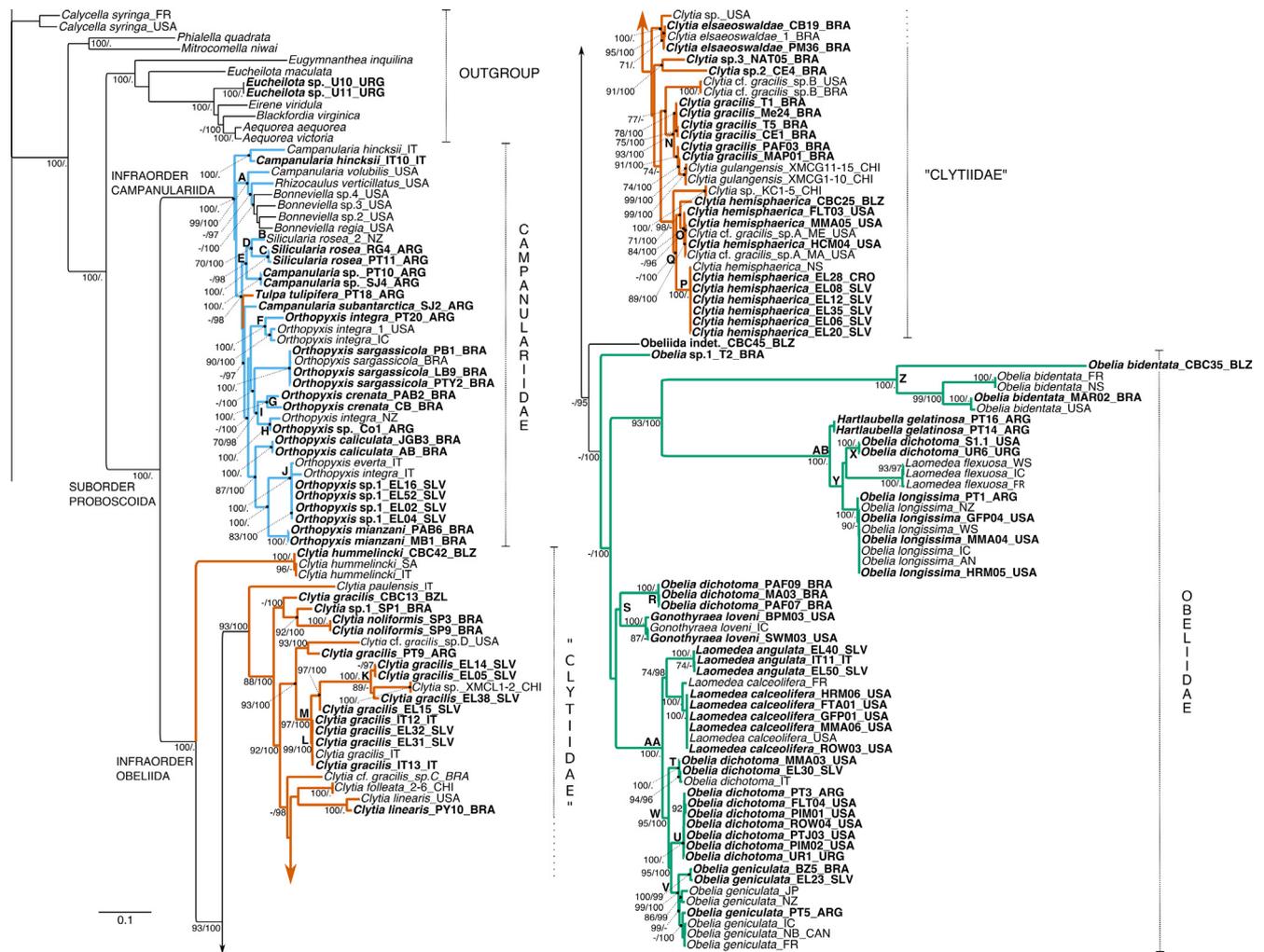


Fig. 2. Maximum likelihood tree based on 16S, COI, 18S and 28S data. Only taxa with sequences for at least 3 genes (Dataset 1) were analyzed. Bootstrap values/posterior probabilities (Bayesian analysis) are shown for each node, with dots (.) representing the same value for both measures. Nodes without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

molecular studies (Govindarajan et al., 2006; Zhou et al., 2013; He et al., 2015; Cunha et al., 2015), but our increased taxon sampling provides a more thorough test of prior hypotheses. 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 16S-only phylogenies show important inconsistencies with combined nuclear and mitochondrial genes phylogenies, always at nodes with little support (Figs. 3, S3). For instance, specimens of *Silicularia rosea* (clade D) and *Clytia gracilis* (clade M) present high intra-clade distances (Fig. 4) and are not recovered as monophyletic in 16S topologies, but are well-supported monophyletic lineages in combined nuclear and mitochondrial genes phylogenies (Figs. 1 and 2). Therefore, while 16S results could be interpreted as evidence for numerous cryptic species, the use of more conserved, nuclear markers may indicate the existence of great population subdivision within an otherwise morphologically cohesive species, which would reconcile more readily with current taxonomy (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, Figs. S6 and S7). In this particular case, signal from the 28S proved to be more informative, increasing resolution and support at this level of the tree.

4.1. Delimiting campanulariids at the family level

Phylogenetic relationships at the family level obtained from our analyses are congruent with the traditional taxonomy, which divides the family Campanulariidae in three subfamilies (cf. Cornelius, 1982; Calder, 1991), as well as with the phylogenetic classification of Proboscoida (cf. Maronna et al., 2016), in which the three main monophyletic groups that comprise campanulariids were elevated to families. This classification improves the taxonomy of the group when compared to the former subfamily division, especially considering that there are few characters that are unique to Campanulariidae (in its traditional sense), and the presence of a campanulate/bell-shaped hydrotheca is probably a symplesiomorphy of the group. In this sense, including the three clades in the family rank contributes to better communication by allowing for

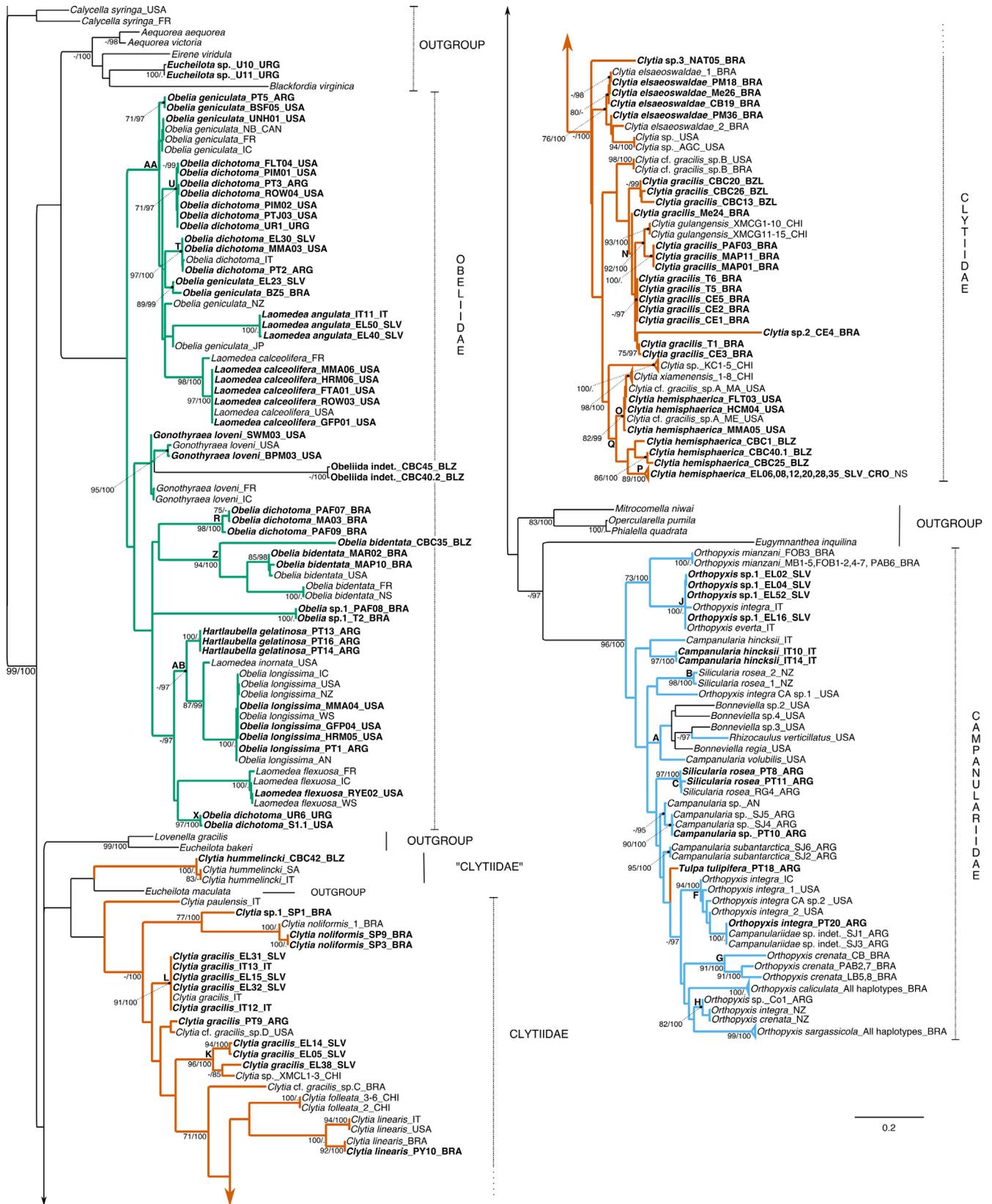


Fig. 3. Maximum likelihood tree based on 16S data. Branch colors, support values, and other notations are described in Fig. 2. For specimens codes and site abbreviations see Tables 1 and 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

more direct reference and assessment of the wide morphological diversity they exhibit. Our results further agree with the classification of Maronna et al. (2016) in their recovery of clades that comprise the infraorders Campanulariida and Obeliida, confirming the

close affinities between Bonneviellidae and Campanulariidae, as well as Clytiidae and Obeliidae. As a result, we follow Maronna et al.'s classification, pointing out its incongruencies when compared to the traditional taxonomy and our current results.

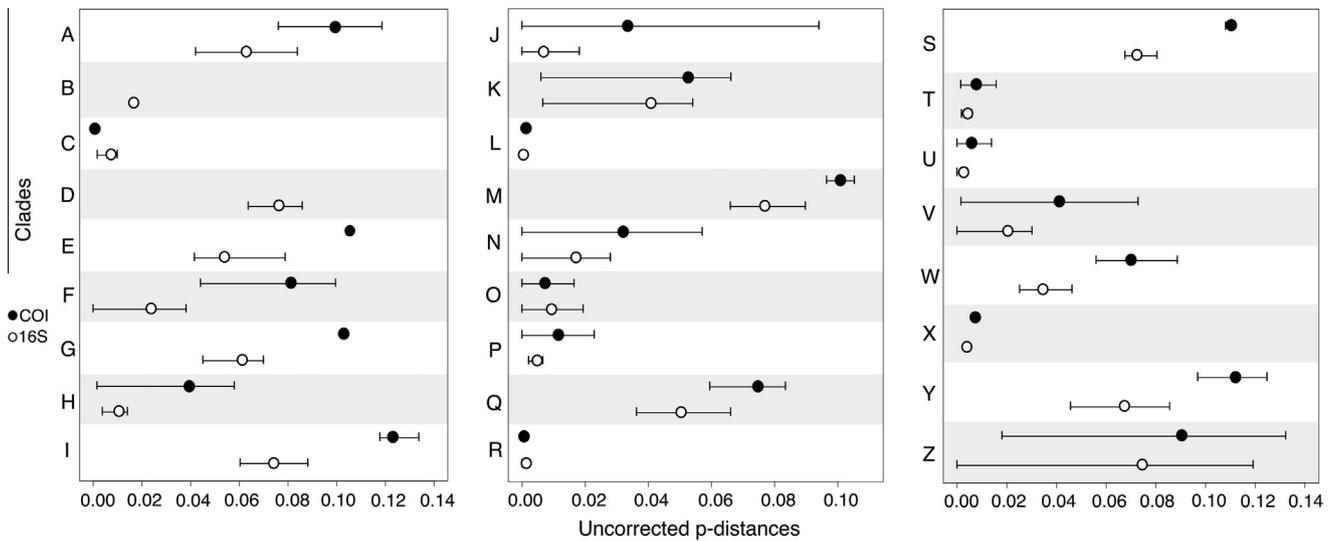


Fig. 4. Intra and inter-clade uncorrected p-distances based on 16S and COI data. Mean distances with minimum and maximum values are presented for each clade. Letters are in accordance with clades shown in Figs. 1–3, S3 and S4).

The family Campanulariidae is monophyletic in both its traditional sense and according to Maronna et al. (2016), so long as *Bonneviella* is included and *Billardia* excluded from its scope (Govindarajan et al., 2006; Peña 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 (see Broch, 1909, 1918; Cornelius, 1995b: 221; Marques et al., 2006 for *Bonneviella*; Totton, 1930; Vervoort, 1972; Vervoort and Watson, 2003 for *Billardia*). Our analysis supports *Bonneviella* in the Campanulariidae, and if further sampling continues to support monophyly of *Bonneviella* lineages, the presence of a pre-oral cavity in the hydranth (viz., veloid, Broch, 1909; Yamada, 1969; Schuchert, 2001) would be a likely synapomorphy and diagnostic character for the genus. *Billardia* is not part of the Campanulariidae (cf. Moura et al., 2011c), even though the campanulate hydrotheca with a large but completely retractable hydranth (Vervoort, 1972; Vervoort and Watson, 2003) may explain its initial inclusion in that family. However, based on its phylogenetic relationships, these characters are probably plesiomorphic in Leptothecata and should not be regarded as diagnostic of Proboscoidea, at least if other characters are not present for a reliable identification.

Although the three families comprising campanulariids were recovered in our analysis, only Obeliidae is close to its original scope (Obeliida indet. is frequently placed at the base of the group, but with little support, see Figs. 1–3). The family Campanulariidae, traditionally characterized by a subhydrothecal spherule and annular perisarc thickening at the hydrothecal base, but lacking a true diaphragm (see Cornelius, 1982; Calder, 1991; Bouillon, 1985, as subfamily Campanulariinae), 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). Annular perisarc thickening occurs in *Tulpa* and, although not universal (i.e., absent in *Bonneviella*), would appear to be the best available morphological character to delimit the family (Boero et al., 1996).

Clytiidae becomes monophyletic if *Clytia hummelincki* is excluded from the family. This scenario, however, is ambiguously supported by our results. The subhydrothecal spherule of *C. hummelincki*, a character commonly associated with Campanulariidae, as well as a diaphragm and medusae with tentacle bulbs, characteristic of Clytiidae, led Cornelius (1982) to regard this species as

having uncertain taxonomic affinities, although he followed Millard (1966) and kept the species in Clytiidae (as subfamily Clytiinae) based on characters of the medusa stage. Govindarajan et al. (2006, concatenated phylogeny) recovered this species at the base of the Clytiidae (as subfamily Clytiinae), and concluded that the subhydrothecal spherule is plesiomorphic of campanulariids in general. Given the phylogenies that resulted from our analysis (Figs. 1 and 2), the hypothesis of plesiomorphy is a possibility, as well as the hypothesis of convergence (Cornelius, 1982: 83), since it is also possible that the character originated at the base of Campanulariidae, and again at the lineage leading to *C. hummelincki*. Additional evidences are necessary to corroborate one of these hypotheses.

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

Specimens identified as Obeliida indet. (CBC40.2 and CBC45.BLZ) are similar to *Clytia stolonifera* Blackburn (1938) (Fig. S10), a species described from Australia (Blackburn, 1938; Watson, 2005), but recently recorded in Brazil by Fernandez et al. (2014, 2015). Although this species was originally considered in the genus *Clytia*, its gonophores have not been described, and our material lacks gonothecae as well. Considering the ambiguous phylogenetic position of these specimens, frequently placed at the base of Obelliidae, we conservatively considered them non-identified, at least until more information and specimens (particularly from the type locality) are available for a reliable identification of the species.

4.2. Generic limits in Proboscoida

As in other groups of Hydrozoa, gonophore morphology has long been used to distinguish genera of Campanulariidae (cf. Cornelius, 1982). Nevertheless, the topic is much debated and there are significant doubts that species with different types of gonophores (fixed sporosacs or free medusae) should be assigned to separate genera (Levensen, 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 Sarà, 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 thought not to be monophyletic (Boero et al., 1996), and these hypotheses were 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 that these characters are inappropriate for diagnosing different genera.

Even if we consider some of the main classifications proposed during 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 (Figs. 1–3, Table 4). In contrast, *Orthopyxis*, *Silicularia*, *Gonothyrea*, 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 Campanulariidae, appears to be polyphyletic, and its current diagnostic characters are symplesiomorphies of the family (e.g., stolonial colonies, campanulate hydrotheca, annular perisarc thickening, subhydrothecal spherule, fixed sporosacs; Ralph, 1957; Cornelius, 1982; Bouillon, 1985; Calder, 1991). Considering the phylogenetic patterns of *Campanularia* presented in this study, the definition of separate genera for the different clades would probably be the best taxonomic decision to establish monophyletic genera in Campanulariidae, other than including all species of the family, with a wide morphological diversity, into a large and single genus. There is little basis for this decision, however, at the moment, given that there are few morphological characters that could differentiate the new genera, and, most importantly, the typical *Campanularia* Lamouroux, 1812. In addition, *Campanularia volubilis*, the type species of the genus, appears to be more closely related to species of *Bonneviella* than to other *Campanularia* (Figs. 1–3), forming a clade that was thought to represent a local radiation (Govindarajan et al., 2006). In order to make sound taxonomic decisions, it is important that the phylogenetic relationships and morphological characters of more representatives of the typical *Campanularia* are studied. This will lead to a better characterization of *Campanularia*, contributing to the definition of new genera.

Considering the phylogenetic patterns of *Clytia* presented in this study, the only classifications that are congruent with a monophyletic genus are those that disregard the subhydrothecal spherule as part of the diagnostic characters of *Clytia*, which excludes *C. hummelincki* (Nutting, 1915; Ralph, 1957; Hirohito, 1995; Table 4). In spite of that, traditional diagnostic characters of the hydroids (e.g., stolonial 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 species of Obeliidae. Characters of the medusa stage, on the other hand, are important diagnostic features for *Clytia*, and they support the inclusion of *C. hummelincki* in the genus (Gravili et al., 2008). Nevertheless, additional studies on *C. hummelincki* are crucial to ascertain its precise phylogenetic positions.

Obeliidae is the most problematic family within Proboscoida, including nearly all types of gonophores, but this variation is clearly not informative to delimit genera. *Gonothyrea* might be the only exception, considering that meconidia are exclusive in that genus, and therefore it is regarded as distinct in most of the classifications proposed (Nutting, 1915; Ralph, 1957; Millard, 1975; Cornelius, 1982, 1995b; Bouillon, 1985; Table 4). *Hartlaubella* is considered a distinct genus 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). Trophosomal characters, 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 *Gonothyrea*, similar to what was proposed by Naumov (1969). In this sense, there are no conspicuous or unambiguous morphological characters, neither from hydroids nor 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.

4.3. Species boundaries in Proboscoida

At least three campanulariid species are not monophyletic and include 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 (Figs. 1 and 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 about 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). Sequences from type localities help to link the identification of clades as the typical species, and we tried to include them whenever possible, as well as comparisons with the presumably typical diagnostic morphological characters.

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,

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

	Nutting (1915)	Ralph (1957)	Naumov (1969)	Millard (1975)	Cornelius (1975)	Cornelius (1982)				
<i>Campanularia</i> Lamarck, 1816	35	12	2	0	10	4	9	5	3	9
<i>Orthopyxis</i> L. Agassiz, 1862	5	2	4	1	*			*	2	23
<i>Silicularia</i> Meyen, 1834	6	3	1	7						
<i>Tulpa</i> Stechow, 1921		*	1	0						
<i>Rhizocaulus</i> Stechow, 1919		*		2 ^B	0				1	1
<i>Clytia</i> Lamouroux, 1812	13	2	2	1				7	7	37
<i>Obelia</i> Péron & Lesueur, 1810	17	6	6	1	8	1	3	0	3	+80
<i>Laomedea</i> Lamouroux, 1812		*					1 ^C	0	5	8
<i>Gonothyrea</i> Allman, 1864	4	1	1	1				1	1	1
<i>Hartlaubella</i> 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	This study ^J			
<i>Campanularia</i> Lamarck, 1816	1	8	2	1 ^E	9	1	2	2	3	3
<i>Orthopyxis</i> L. Agassiz, 1862	1	1	1	2 ^E			4	6	5	5
<i>Silicularia</i> Meyen, 1834							1	11		
<i>Tulpa</i> Stechow, 1921				1 ^G	0		1	0		
<i>Rhizocaulus</i> Stechow, 1919			1	0						
<i>Clytia</i> Lamouroux, 1812	6	40	4	3	9	2	7	1	10	10
<i>Obelia</i> Péron & Lesueur, 1810	2	50	4	2 ^F	4	5	7	5	4	4
<i>Laomedea</i> Lamouroux, 1812			5	6					5	5
<i>Gonothyrea</i> Allman, 1864			1	1			1	1	1	1
<i>Hartlaubella</i> Poche, 1914			1	0			1	0	1	1

^A AS *Obelaria* Hartlaub, 1987; ^B as *Vorticellina* 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, 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; ^J *Tulpa* and *Rhizocaulus* are represented by only one specimen, and their monophyly needs to be confirmed.

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, Figs. 3, S3). 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, the type locality of *O. crenata* (Hartlaub, 1901; Vervoort and Watson, 2003; see 16S phylogenies, Figs. 3, S3), and with an unidentified specimen from Argentina (*Orthopyxis* sp._Co1_ARG, Cunha et al., 2015), believed to have close affinities with *O. crenata* (Cunha et al., 2015). This evidence suggests 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, Tables 1 and 2), and they are clearly separated from the “true” *O. integra* (clade F, Figs. 1–3). This suggests the clade comprising *O.integra*_IT corresponds to a different species occurring in the Mediterranean Sea (e.g., *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 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 more likely to be 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*, because none of our sequences come from the type locality of the species (i.e. Lofoten, Norway, Sars, 1850) or any close locality. This would give further support for the identification of the typical species, since the generally accepted concept of *C. gracilis* probably still comprises 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 (Figs. 1–3). 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 Figs. 1–3). 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; Figs. 1–3) suggests that morphological characters distinguishing these species as separate and valid are still unclear. 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 Obeliidae, in which *Obelia dichotoma* is not monophyletic (Figs. 1–3). 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 nematocyst types and isoenzyme patterns among *O. dichotoma* and *O. longissima*, regarding both species as 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. *Obelia dichotoma* is mainly distinguished from *O. longissima* by its branching patterns and shape of the hydrothecal rim (Cornelius, 1990, 1995b), but our analysis shows these characters are not informative for the delimitation of the species. 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.

5. Conclusions

Taxonomic investigation on Hydrozoa benefits from the inclusion of molecular data, as a matter of achieving a more complete understanding of species boundaries and the relevance of diagnostic characters. We corroborated previous results and presented novel evidence on the phylogenetic relationships within the suborder Proboscoida. Considering the traditional morphological diagnostic characters of the group and the phylogenetic patterns presented in this study, the scope of the family Campanulariidae (cf. Cornelius, 1982) 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), which raises the subfamilies to families, improves the taxonomy of the group. However, the relationships among taxa within Clytiidae and Obeliidae still remain open questions. Additionally, generic limits will only reflect phylogenetic patterns if different types of gonophores are disregarded as generic characters, especially within Obeliidae. Finally, diagnostic characters of several species have to be reassessed based on a detailed study of their patterns of morphological variation, including material from type localities whenever possible. Further morphological studies are essential 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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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2016.09.012>.

References

- Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. *J. Mol. Biol.* 215 (3), 403–410.
- Ardila, N.E., Giribet, G., Sánchez, J.A., 2012. A time-calibrated molecular phylogeny of the precious corals: reconciling discrepancies in the taxonomic classification and insights into their evolutionary history. *BMC Evol. Biol.* 12, 246.
- Benzoni, F., Stefani, F., Pichon, M., Galli, P., 2010. The name game: morpho-molecular species boundaries in the genus *Psammodora* (Cnidaria, Scleractinia). *Zool. J. Linn. Soc. – Lond.* 160, 421–456.
- Blackburn, M., 1938. Hydrozoa. The Sir Joseph Banks Islands. Reports of the Expedition of the McCoy Society for Field Investigation and Research. *Proc. R. Soc. Vic.* 50, 312–328.
- Bo, M., Bavestrello, G., Barucca, M., Makapedua, D.M., Poliseño, A., Forconi, M., Olmo, F., Canapa, A., 2012. Morphological and molecular characterization of the problematic whip black coral genus *Stichopathes* (Hexacorallia: Antipatharia) from Indonesia (North Sulawesi, Celebes Sea). *Zool. J. Linn. Soc. – Lond.* 166, 1–13.
- Boero, F., Sarà, M., 1987. Motile sexual stages and evolution of Leptomedusae (Cnidaria). *Boll. Zool.* 54, 131–139.
- Boero, J., Bouillon, J., Piraino, S., 1996. Classification and phylogeny in the Hydroidomedusae (Hydrozoa, Cnidaria). *Sci. Mar.* 60 (1), 17–33.
- Bouillon, J., 1985. Essai de classification des Hydromolypes-Hydroméduses (Hydrozoa-Cnidaria). *Indo-Malayan Zool.* 2, 29–243.
- Bouillon, J., Medel, M.D., Pagès, F., Gili, J.M., Boero, F., Gravili, C., 2004. Fauna of the Mediterranean Hydrozoa. *Sci. Mar.* 68 (Suppl. 2), 1–449.
- Broch, H., 1909. Hydroiduntersuchungen I. Tcephore Hydroiden von dem nördlichen Norwegen nebst Bemerkungen über die Variation und Artbegrenzung der nördischen *Laföea*-Arten. *Tromsø Museums Aarshefter* 29, 27–40.
- Broch, H., 1918. Hydroida (Part II). *Danish Ingolf-Exped 5* (7), 205.
- Bumann, D., Buss, L.W., 2008. Nutritional physiology and colony form in *Podocoryna carnea* (Cnidaria: Hydrozoa). *Invertebr. Biol.* 127 (4), 368–380.
- Calder, D.R., 1991. Shallow-water hydroids of Bermuda: the Thecatae, exclusive of Plumularioidea. *R. Ont. Mus. Life Sci. Contrib.* 154, 140.
- Calder, D.R., 1995. Hydroid assemblages on holopelagic *Sargassum* from the Sargasso Sea at Bermuda. *B. Mar. Sci.* 56 (2), 537–546.
- Calder, D.R., 1997. Shallow-water hydroids of Bermuda: superfamily Plumularioidea. *R. Ont. Mus. Life Sci. Contrib.* 161, 85.

- Calder, D.R., 2013. Some shallow-water hydroids (Cnidaria: Hydrozoa) from the central east coast of Florida, USA. *Zootaxa* 3648 (1), 001–072.
- Calder, D.R., Choong, H.H.C., Carlton, J.T., Chapman, J.W., Miller, J.A., Geller, J., 2014. Hydroids (Cnidaria: Hydrozoa) from Japanese tsunami marine debris washing ashore in the northwestern United States. *Aquat. Invasions* 9 (4), 425–440.
- Calder, D.R., Choong, H.H.C., McDaniel, N., 2015. *Similiclavula nivea* (Cnidaria: Hydrozoa: Similiclavidae): a new family, genus and species of athecate hydroid from the Pacific coast of North America. *J. Nat. Hist.* 49 (13–14), 735–753. <http://dx.doi.org/10.1080/00222933.2014.979261>.
- Cartwright, P., Evans, N.M., Dunn, C.W., Marques, A.C., Miglietta, M.P., Schuchert, P., Collins, A.G., 2008. Phylogenetics of Hydrozoidina (Hydrozoa: Cnidaria). *J. Mar. Biol. Assoc. UK* 88, 1663–1672.
- Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol. Biol. Evol.* 17 (4), 540–552.
- Collins, A.G., 2000. Towards understanding the phylogenetic history of Hydrozoa: hypothesis testing with 18S gene sequence data. *Sci. Mar.* 64 (supl. 1), 5–22.
- Collins, A.G., 2002. Phylogeny of Medusozoa and the evolution of cnidarians life cycles. *J. Evol. Biol.* 15, 418–432.
- Collins, A.G., Winkelmann, S., Hadrys, H., Schierwater, B., 2005. Phylogeny of Capitata (Cnidaria, Hydrozoa) and Corynidae (Capitata) in light of mitochondrial 16S rDNA data. *Zool. Scr.* 34, 91–99.
- Collins, A.G., Schuchert, P., Marques, A.C., Jankowski, T., Medina, M., Schierwater, B., 2006. Medusozoan phylogeny and character evolution clarified by new large and small subunit rDNA data and an assessment of the utility of phylogenetic mixture models. *Syst. Biol.* 55 (1), 97–115.
- Cornelius, P.F.S., 1975. The hydroid species of *Obelia* (Coelenterata, Hydrozoa: Campanulariidae), with notes on the medusa stage. *Bull. Br. Mus. Nat. Hist. Zool.* 28 (6), 249–293.
- Cornelius, P.F.S., 1982. Hydroids and medusae of the family Campanulariidae recorded from the eastern North Atlantic, with a world synopsis of genera. *Bull. Br. Mus. Nat. Hist. Zool.* 42 (2), 37–148.
- Cornelius, P.F.S., 1987a. The hydranths of *Clytia linearis* (Cnidaria, Hydrozoa) and related species. In: Bouillon, J., Boero, F., Cicogna, F., Cornelius, P.F.S. (Eds.), *Modern Trends in the Systematics, Ecology, and Evolution of Hydroids and Hydromedusae*. Clarendon Press, Oxford, pp. 291–297.
- Cornelius, P.F.S., 1987b. Taxonomic characters from the hydranths of thecate hydroids. In: Bouillon, J., Boero, F., Cicogna, F., Cornelius, P.F.S. (Eds.), *Modern Trends in the Systematics, Ecology, and Evolution of Hydroids and Hydromedusae*. Clarendon Press, Oxford, pp. 29–42.
- Cornelius, P.F.S., 1990. European *Obelia* (Cnidaria, Hydrozoa): systematics and identification. *J. Nat. Hist.* 24, 535–578.
- Cornelius, P.F.S., 1995a. North-West European Thecate hydroids and their medusae. Part 1. Introduction, laodiceidae to haleciidae. *Synopses Brit. Fauna (New Series)* 50 (2), 347.
- Cornelius, P.F.S., 1995b. North-West European Thecate hydroids and their medusae. Part 2. Sertulariidae to campanulariidae. *Synopses Brit. Fauna (New Series)* 50 (2), 386.
- Cunha, A.F., Jacobucci, G.B., 2010. Seasonal variation of epiphytic hydroids (Cnidaria: Hydrozoa) associated to a subtropical *Sargassum cymosum* (Phaeophyta: Fucales) bed. *Zoologia* 27 (6), 945–955.
- Cunha, A.F., Genzano, G.N., Marques, A.C., 2015. Reassessment of morphological diagnostic characters and species boundaries requires taxonomical changes for the genus *Orthopyxis* L. Agassiz, 1862 (Campanulariidae, Hydrozoa) and some related campanulariids. *PLoS ONE* 10 (2), e0117553. <http://dx.doi.org/10.1371/journal.pone.0117553>.
- Cunha, A.F., Maronna, M.M., Marques, A.C., 2016. Variability in the microevolutionary and macroevolutionary scales: a review on patterns of morphological variation in Cnidaria Medusozoa. *Org. Divers. Evol.* 16, 431–442. <http://dx.doi.org/10.1007/s13127-016-0276-4>.
- Cunningham, C.W., Buss, L.W., 1993. Molecular evidence for multiple episodes of paedomorphosis in the family Hydractiniidae. *Biochem. Syst. Ecol.* 21 (1), 57–69.
- Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. JModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods* 9 (8), 772.
- Evans, N.M., Lindner, A., Raikova, E.V., Collins, A.G., Cartwright, P., 2008. Phylogenetic placement of the enigmatic parasite, *Polypodium hydriforme*, within the Phylum Cnidaria. *BMC Evol. Biol.* 8, 139. <http://dx.doi.org/10.1186/1471-2148-8-139>.
- Fernandez, M.O., Navarrete, S.A., Marques, A.C., 2014. Temporal variation in richness and composition of recruits in a diverse cnidarian assemblage of subtropical Brazil. *J. Exp. Mar. Biol. Ecol.* 460, 144–152. <http://dx.doi.org/10.1016/j.jembe.2014.06.015>.
- Fernandez, M.O., Navarrete, S.A., Marques, A.C., 2015. A comparison of temporal turnover of species from benthic cnidarian assemblages in tropical and subtropical harbours. *Mar. Biol. Res.* 11 (5), 492–503. <http://dx.doi.org/10.1080/17451000.2014.955804>.
- Forsman, Z.H., Barshis, D.J., Hunter, C.L., Toonen, R.J., 2009. Shape-shifting corals: molecular markers show morphology is evolutionary plastic in *Porites*. *BMC Evol. Biol.* 9, 45. <http://dx.doi.org/10.1186/1471-2148-9-45>.
- Fukami, H., Budd, A.F., Paulay, G., Solé-Cava, A., Chen, C.A., Iwao, K., Knowlton, N., 2004. Conventional taxonomy obscures deep divergence between Pacific and Atlantic corals. *Nature* 427, 832–835.
- Genzano, G.N., Mianzan, H., Diaz-Briz, L., Rodriguez, C., 2008. On the occurrence of *Obelia* medusa blooms and empirical evidence of unusual massive accumulations of *Obelia* and *Amphisbetia* hydroids on the Argentina shoreline. *Lat. Am. J. Aquat. Res.* 36 (2), 301–307.
- Gouy, M., Guindon, S., Gascuel, O., 2010. SeaView Version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol. Biol. Evol.* 27 (2), 221–224.
- Govindarajan, A.F., Halanych, K.M., Cunningham, C.W., 2005. Mitochondrial evolution and phylogeography in the hydrozoan *Obelia geniculata* (Cnidaria). *Mar. Biol.* 146, 213–222.
- Govindarajan, A.F., Boero, F., Halanych, K.M., 2006. Phylogenetic analysis with multiple markers indicates repeated loss of the adult medusa stage in Campanulariidae (Hydrozoa, Cnidaria). *Mol. Phylogenet. Evol.* 38, 820–834.
- Gravier-Bonnet, N., 1999. *Obelia* and other campanulariids (Cnidaria, Hydrozoa) in seagrass beds of Madagascar (Indian Ocean). *Zoosyst. Rossica Suppl.* 1, 77–88.
- Gravili, C., D'Ambrosio, P., Di Camillo, C., Renna, G., Bouillon, J., Boero, F., 2008. *Clytia hummelincki* (Hydrozoidomedeae: Leptomedusae) in the Mediterranean Sea. *J. Mar. Biol. Assoc. UK* 88, 1547–1553.
- Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate phylogenies by Maximum Likelihood. *Syst. Biol.* 52 (5), 696–704.
- Gutiérrez-Rodríguez, C., Barbeitos, M.S., Sánchez, J.A., Lasker, H.R., 2009. Phylogeography and morphological variation of the branching octocoral *Pseudopterogorgia elisabethae*. *Mol. Phylogenet. Evol.* 50, 1–15.
- Hartlaub, C., 1901. Hydroiden aus dem Stillen ocean. *Ergebnisse einer Reise nach dem Pacific*. *Zool. Jahrb.* 14, 349–379.
- He, J., Zheng, L., Zhang, W., Lin, Y., Cao, W., 2015. Morphology and molecular analyses of a new *Clytia* species (Cnidaria: Hydrozoa: Campanulariidae) from the East China Sea. *J. Mar. Biol. Assoc. UK* 95 (2), 289–300. <http://dx.doi.org/10.1017/S0025315414000836>.
- Hirohito, E.S., 1995. The Hydroids of Sagami Bay II. Thecata. Publications of the Biological Laboratory Imperial Household, Tokyo, p. 355.
- Hughes, R.G., 1980. Current induced variations in the growth and morphology of hydroids. In: Tardent, P., Tardent, R. (Eds.), *Developmental and Cellular Biology of Coelenterates*. Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 179–184.
- Hughes, R.G., 1986. Differences in the growth, form and life history of *Plumularia setacea* (Ellis and Solander) (Hydrozoa: Plumulariidae) in two contrasting habitats. *P. Roy. Soc. Lond. B Bio.* 228 (1251), 113–125.
- Katoh, K., Misawa, K., K-i, Kuma, M., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* 30 (14), 3059–3066.
- Kim, E., Lasker, H.R., Coffroth, M.A., Kim, K., 2004. Morphological and genetic variation across reef habitats in a broadcast-spawning octocoral. *Hydrobiologia* 530 (531), 423–432.
- Kramp, P.L., 1935. Polypdyr (Coelenterata). I. Ferskvandspolypper og Goplepolypper. *Danmarks Fauna* 41, 1–207.
- Kubota, S., 1981. Life-history and taxonomy of an *Obelia* species (Hydrozoa: Campanulariidae) in Hokkaido, Japan. *J. Fac. Sci. Hokkaido Univ. Ser. VI, Zool.* 22 (4), 379–399.
- Laakmann, S., Holst, S., 2014. Emphasizing the diversity of North Sea hydromedusae by combined morphological and molecular methods. *J. Plankton Res.* 36 (1), 64–76. <http://dx.doi.org/10.1093/plankt/fbt078>.
- Leclère, L., Schuchert, P., Cruaud, C., Couloux, A., Manuel, M., 2009. Molecular phylogenetics of Thecata (Hydrozoa, Cnidaria) reveals long-term maintenance of life history traits despite high frequency of recent character changes. *Syst. Biol.* 58 (5), 509–526.
- Levinsen, G.M.R., 1893. Meduser, ctenophorer og hydroider fra Grønlands vestkyst, tilligemed bemaerkninger om hydroidernes systematik. *Vid. Medd. Naturh. Forh. København* 1892 (143–212), 215–220.
- Lindner, A., Govindarajan, A.F., Migotto, A.E., 2011. Cryptic species, life cycles, and the phylogeny of *Clytia* (Cnidaria: Hydrozoa: Campanulariidae). *Zootaxa* 2980, 23–36.
- Lobet, I., Gili, J.M., Hughes, R.G., 1991. Horizontal, vertical and seasonal distributions of epiphytic hydrozoa on the alga *Halimeda tuna* in the Northwestern Mediterranean Sea. *Mar. Biol.* 110 (1), 151–159.
- Maronna, M.M., Miranda, T.P., Peña Cantero, A.L., Barbeitos, M.S., Marques, A.C., 2016. Towards a phylogenetic classification of Leptothecata (Cnidaria, Hydrozoa). *Sci. Rep.* 6, 18075. <http://dx.doi.org/10.1038/srep18075>.
- Marques, A.C., Peña Cantero, A., Migotto, A.E., 2006. An overview of the phylogeny of the families Lafoeidae and Hebellidae (Hydrozoa: Leptothecata): their composition and classification. *Invertebr. Syst.* 20, 43–58.
- Miglietta, M.P., Piraino, S., Kubota, S., Schuchert, P., 2007. Species in the genus *Turritopsis* (Cnidaria, Hydrozoa): a molecular evaluation. *J. Zool. Syst. Evol. Res.* 45 (1), 11–19.
- Miglietta, M.P., Schuchert, P., Cunningham, C.W., 2009. Reconciling genealogical and morphological species in a worldwide study of the family Hydractiniidae (Cnidaria, Hydrozoa). *Zool. Scr.* 38, 403–430.
- Migotto, A.E., Marques, A.C., Flynn, M.N., 2001. Seasonal recruitment of hydroids (Cnidaria) on experimental panels in the São Sebastião Channel, southeastern Brazil. *B. Mar. Sci.* 68 (2), 287–298.
- Millard, N.A.H., 1966. The Hydrozoa of the south and west coasts of South Africa. Part III. The Gymnoblastera and small families of Calyptoblastea. *Ann. S. Afr. Mus.* 48, 427–487.
- Millard, N.A.H., 1971. Hydrozoa. In: Bakker, E.M.Z., Winterbottom, J.M., Dyer, R.A. (Eds.), *Marion and Prince Edward Islands*. AA Balkema, Cape Town, pp. 396–408.
- Millard, N.A.H., 1975. Monograph on the Hydrozoa of Southern Africa. *Ann. S. Afr. Mus.* 68, 1–513.
- Miranda, L.S., Hirano, Y.M., Mills, C.E., Falconer, A., Fenwick, D., Marques, A.C., Collins, A.G., 2016. Systematics of stalked jellyfishes (Cnidaria: Staurozoa). *PeerJ* 4, e1951. <http://dx.doi.org/10.7717/peerj.1951>.

- Moura, C.J., Harris, D.J., Cunha, M.R., Rogers, A.D., 2008. DNA barcoding reveals cryptic diversity in marine hydroids (Cnidaria, Hydrozoa) from coastal and deep-sea environments. *Zool. Scr.* 37, 93–108.
- Moura, C.M., Cunha, M.R., Porteiro, F.M., Yesson, C., Rogers, A.D., 2011a. Evolution of *Nemertesia* hydroids (Cnidaria: Hydrozoa: Plumulariidae) from the shallow and deep Waters of the NE Atlantic and western Mediterranean. *Zool. Scr.* 41 (1), 79–96.
- Moura, C.J., Cunha, M.R., Porteiro, F.M., Rogers, A.D., 2011b. The use of the DNA barcode gene 16S mRNA for the clarification of taxonomic problems within the Family Sertulariidae (Cnidaria, Hydrozoa). *Zool. Scr.* 40 (5), 520–537.
- Moura, C.J., Cunha, M.R., Porteiro, F.M., Rogers, A.D., 2011c. Polyphyly and cryptic diversity in the hydrozoan families Lafoeidae and Hebellidae (Cnidaria: Hydrozoa). *Invertebr. Syst.* 25, 454–470. <http://dx.doi.org/10.1071/IS11045>.
- Nagata, R.M., Nogueira Júnior, M., Haddad, M.A., 2014. Faunistic survey of Hydromedusae (Cnidaria, Hydrozoa) from the coast of Paraná State, Southern Brazil. *Zootaxa* 3768 (3), 291–326. <http://dx.doi.org/10.11646/zootaxa.3768.3.3>.
- Naumov, D.V., 1969. Hydroids and Hydromedusae of the USSR. Israel Program for Scientific Translations, Jerusalem, p. 660.
- Nutting, C.C., 1915. American Hydroids. Part III. The Campanularidae and the Bonneviellidae. Government Printing Office, Washington, p. 126.
- Ortman, B.D., Bucklin, A., Pagès, F., Youngbluth, M., 2010. DNA barcoding the Medusozoa using mtCOI. *Deep-Sea Res. II* 57, 2148–2156. <http://dx.doi.org/10.1016/j.dsr2.2010.09.017>.
- Östman, C., 1979. Nematocysts in the *Phialidium* Medusae of *Clytia hemisphaerica* (Hydrozoa, Campanulariidae) studied by light and scanning electron microscopy. *Zoon* 7, 125–142.
- Östman, C., 1982a. Nematocysts and taxonomy in *Laomedea*, *Gonothyrea* and *Obelia* (Hydrozoa, Campanulariidae). *Zool. Scr.* 11 (4), 227–241.
- Östman, C., 1982b. Isoenzymes and taxonomy in Scandinavian hydroids (Cnidaria, Campanulariidae). *Zool. Scr.* 11 (3), 155–163.
- Palma, S., Córdova, P., Silva, N., Silva, C., 2014. Biodiversity and spatial distribution of medusae in the Magellan Region (Southern Patagonian Zone). *Lat. Am. J. Aquat. Res.* 42 (5), 1175–1188. <http://dx.doi.org/10.3856/vol42-issue5-fulltext-21>.
- Peña Cantero, A.L., García Carrascosa, A.M., 2002. The benthic hydroid fauna of the Chafarinas Islands (Albóran Sea, western Mediterranean). *Zool. Verh. Leiden* 337, 180.
- Peña Cantero, A.L., Sentandreu, V., Latorre, A., 2010. Phylogenetic relationships of the endemic Antarctic benthic hydroids (Cnidaria, Hydrozoa): what does the mitochondrial 16S rRNA tell us about it? *Polar Biol.* 33, 41–57.
- Petersen, K.W., 1990. Evolution and taxonomy in capitate hydroids and medusae (Cnidaria: Hydrozoa). *Zool. J. Linn. Soc. – Lond.* 100, 101–231.
- Ralph, P.M., 1956. Variation in *Obelia geniculata* (Linnaeus, 1758) and *Silicularia bilabiata* (Coughtrey, 1875) (Hydrozoa, F. Campanulariidae). *T. Roy. Soc. New Zeal.* 84 (2), 279–296.
- Ralph, P.M., 1957. New Zealand thecate hydroids. Part I. Campanulariidae and Campanulinidae. *T. Roy. Soc. New Zeal.* 84 (4), 811–854.
- Ralph, P.M., Thomson, H.G., 1968. Seasonal changes in growth of the erect stem of *Obelia geniculata* in Wellington Harbour, New Zealand. *Zool. Publ. Victoria Univ.* 44, 1–21.
- Rees, W.J., 1957. Evolutionary trends in the classification of capitate hydroids and medusae. *Bull. Br. Mus. Nat. Hist. Zool.* 4 (9), 453–534.
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61 (3), 539–542.
- Sars, M., 1850. Beretning om en i Sommeren 1849 foretagen zoologisk Reise i Lofoten og Finmarken. *Nyt Mag Naturvidensk* 6, 121–211.
- Schmidt-Roach, S., Lundgren, P., Miller, K.J., Gerlach, G., Noreen, A.M.E., Andreakis, N., 2013. Assessing hidden species diversity in the coral *Pocillopora damicornis* from Eastern Australia. *Coral Reefs* 32, 161–172.
- Schuchert, P., 2001. Hydroids of Greenland and Iceland (Cnidaria, Hydrozoa). *Medd. Grøn. Biosci.* 53, 1–184.
- Schuchert, P., 2014. High genetic diversity in the hydroid *Plumularia setacea*: a multitude of cryptic species or extensive population subdivision? *Mol. Phylogenet. Evol.* 76, 1–9.
- Schuchert, P., 2015. Campanulariidae Johnston, 1936. In: Schuchert, P. (Ed.), World Hydrozoa Database. Accessed through: World Register of Marine Species at <<http://www.marinespecies.org/aphia.php?p=taxdetails&id=1606>> (2015-08-22).
- Segura-Puertas, L., Damas-Romero, M., 1997. Variación estacional de la comunidad de medusas (Cnidaria) en la Laguna Bojórquez, Cancún, México. *Hidrobiológica* 7, 59–64.
- Silveira, F.L., Migotto, A.E., 1991. The variation of *Halocordyle disticha* (Cnidaria, Athecata) from the Brazilian coast: an environmental indicator species? *Hydrobiologia* 216 (217), 437–442.
- Stechow, E., 1921. Neue Genera und Species von Hydrozoen und anderen Evertabraten. *Arch. Naturgesch.* 87, 248–265.
- Swofford, D.L., 2002. PAUP* (Phylogenetic Analysis Using Parsimony and Other Methods). Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.
- Totton, A.K., 1930. Coelenterata. Part V. – Hydrozoa. *Nat. Hist. Rep. Br. Antarct. ("Terra Nova") Exped. 1910.* *Zool.* 5 (5), 131–252.
- Vervoort, W., 1972. Hydroids from the Theta, Vema and Yelcho cruises of the Lamont-Doherty Geological Observatory. *Zool. Verh.* 120, 1–247.
- Vervoort, W., Watson, J.E., 2003. The Marine Fauna of New Zealand: Leptothecata (Cnidaria: Hydrozoa) (Thecate Hydroids). NIWA Biodivers. Memoir, Wellington 119, 538.
- Watson, J.E., 1992. The hydroid community of *Amphibolis* seagrasses in south-eastern and south-western Australia. *Sci. Mar.* 56 (2-3), 217–227.
- Watson, J.E., 2005. Hydroids of the Archipelago of the Recherche and Esperance, western Australia: annotated list, redescription of species and description of new species. In: Wells, F.E., Walker, D.L., Kendrick, G.A. (Eds.), *The Marine Flora and Fauna of Esperance*. Western Australian Museum, Western Australia, Perth, pp. 495–612.
- Yamada, M., 1969. Notes on Japanese species of *Bonneviella* (Hydrozoa). *Bull. Mar. Biol. Stn. Asamushi.* 13 (3), 241–245.
- Zhou, K., Zheng, L., He, J., Lin, Y., Cao, W., Zhang, W., 2013. Detection of a new *Clytia* species (Cnidaria: Hydrozoa: Campanulariidae) with DNA barcoding and life cycle analyses. *J. Mar. Biol. Assoc. UK* 93 (8), 2075–2088.
- Zwickl, D.J., 2006. Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets Under the Maximum Likelihood Criterion Ph.D. Dissertation. The University of Texas at Austin, p. 115.