

Phylogenetic analysis with multiple markers indicates repeated loss of the adult medusa stage in Campanulariidae (Hydrozoa, Cnidaria)

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Abstract

The Campanulariidae is a group of leptomedusan hydroids (Hydrozoa, Cnidaria) that exhibit a diverse array of life cycles ranging from species with a free medusa stage to those with a reduced or absent medusa stage. Perhaps the best-known member of the taxon is *Obelia* which is often used as a textbook model of hydrozoan life history. However, *Obelia* medusae have several unique features leading to a hypothesis that *Obelia* arose, in a saltational fashion, from an ancestor that lacked a medusa, possibly representing an example of a rare evolutionary reversal. To address the evolution of adult sexual stages in Campanulariidae, a molecular phylogenetic approach was employed using two nuclear (18S rDNA and calmodulin) and two mitochondrial (16S rDNA and cytochrome *c* oxidase subunit I) genes. Prior to the main analysis, we conducted a preliminary analysis of leptomedusan taxa which suggests that Campanulariidae as presently considered needs to be redefined. Campanulariid analyses are consistent with morphological understanding in that three major clades are recovered. However, several recognized genera are not monophyletic calling into question some “diagnostic” features. Furthermore, ancestral states were reconstructed using parsimony, and a sensitivity analysis was conducted to investigate possible evolutionary transitions in life-history stages. The results indicate that life-cycle transitions have occurred multiple times, and that *Obelia* might be derived from an ancestor with *Clytia*-like features.

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1. Introduction

Variation in life-cycle stages is a hallmark of Hydrozoa (Cnidaria), and even closely related species can have considerable differences in the contribution that the asexual polyp or sexual medusa makes to a single generation. In particular, Campanulariidae (Leptomedusae) exhibit considerable diversity in their reproductive strategies (Boero et al., 1996; Boero and Sarà, 1987; Cornelius, 1982) ranging from taxa with relatively long-lived medusa forms (e.g., *Clytia*, *Obelia*) to groups that lack medusae altogether (e.g., *Laomedea*). To many, *Obelia* is perhaps the most familiar

campanulariid as it is often used in textbooks as an example of a “typical” hydrozoan despite having many atypical features (see below).

As currently recognized (Cornelius, 1982, 1990), Campanulariidae consists of 11 genera with over 50 species. This taxon is placed within Leptomedusae in Proboscoidea, which also includes Bonneviellidae and Phialuciidae (Bouillon, 1985; Bouillon and Boero, 2000). Inclusion in Campanulariidae is based on hydranth morphology. In general, hydrozoan taxonomy has been plagued by researchers using features from only one part of the life cycle to develop classification schemes. For example, there are many cases where the hydroid stage is placed in one taxon (species/genus/family, etc.) and the medusa stage is placed in another (see Boero, 1980). Historically, separate classifications have been used for hydroids and medusae, and it

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was not until Naumov (1960) that the first unified classification was proposed. The apparent difference in rates of morphological evolution occurring in hydroids and medusae (Morton, 1957; Naumov, 1960; Rees, 1957) has further complicated matters. Researchers (e.g., Allman, 1864; Boero and Sarà, 1987; Cornelius, 1982; Millard, 1975; Rees, 1957; Vervoort and Watson, 2003) explicitly or implicitly assuming that losses or reductions of the medusa stage are rare have used life-cycle type to define genera. Others (e.g., Boero et al., 1996; Broch, 1916; Cunningham and Buss, 1993; Kramp, 1949; Petersen, 1979, 1990) have speculated that medusa loss happens relatively frequently, questioning whether current taxonomy accurately reflects phylogeny. The range of complex life cycles, the frequency of medusa loss (e.g., Petersen, 1990), and the possibility of “re-invented” medusae (e.g., Boero and Sarà, 1987) have fueled controversy on hydrozoan life-cycle evolution and systematics.

In campanulariids, the variation in the sexual adult portion of the life cycle is notable and has been used to delineate recognized genera (Table 1, Cornelius, 1982). The structure producing sexual adults is referred to as the gonophore, and in campanulariids there are four main types: fixed gonophores, meconidia, medusoids, and medusae. The three leading hypotheses of campanulariid phylogeny are depicted in Fig. 1. Differences in these hypotheses are based largely on concepts of gonophore evolution within Obeliinae (*Obelia*, *Laomedea*, *Gonothyrea*, and *Hartlaubella*). Cornelius (1982) hypothesized a progression from medusae to medusoids/meconidia to fixed gonophores (Fig. 1A). Östman (1987; Fig. 1B) suggested *Gonothyrea* was the basal obeliniid, based on the presence of a type of nematocyst also found in other campanulariids but not *Obelia*. This hypothesis built on the work of Boero and Sarà (1987) that the *Obelia* medusa was secondarily derived. Boero

et al. (1996; Fig. 1C), in contrast, placed fixed gonophore *Laomedea* as the basal obeliniid.

Much of the debate between these hypotheses centers on the nature of the *Obelia* medusa. *Obelia* medusae have morphological and developmental features that are unusual among the Hydrozoa (Boero et al., 1996; Chapman, 1968; Kühn, 1913). These features include anatomy of solid tentacles, shape of the manubrium (i.e., mouth), absence of a velum, statocyst position, absence of tentacular bulbs, position of developing gonads, and myofibril arrangement in the medusa bell. Some of these features (e.g., tentacles and manubrium) are more hydroid-like than medusa-like. *Obelia* medusa development also differs from all other hydrozoan medusae, in that the entocodon (a proliferation of ectoderm along the blastostyle that leads to the subumbrellar cavity) seems to disappear early in development (Boero et al., 1996; Kühn, 1913). Interestingly, another campanulariid, *Gonothyrea*, produces a sexual structure termed meconidia, that shares some “medusa” features with *Obelia* (e.g., solid tentacles; Boero et al., 1996). Because of their combination of original, medusa-like, and hydranth-like properties, Boero et al. (1996) suggest the possibility that *Obelia* medusae may have arisen via detaching hydranths that become sexually mature. Detachable hydranths are found in *Zeulonies estrambordi*, a species that appears to have campanulariid affinities (Boero et al., 1996; Gravier-Bonnet, 1992).

Campanulariid phylogenetic relationships and the evolution of their sexual stages have never been examined using an explicit phylogenetic reconstruction algorithm. Here, we have used data from nuclear (18S rDNA and calmodulin) and mitochondrial (16S rDNA and cytochrome *c* oxidase subunit I [COI]) markers in parsimony, maximum likelihood, and Bayesian analyses to reconstruct the group’s evolutionary history. To address hypotheses of the sexual medusa stage, we use parsimony to reconstruct ancestral character states on the

Table 1
Campanulariid subfamilies and genera and their associated sexual stage (gonophore type)

Subfamily	Genus	Gonophore type	Description
Campanulariinae	<i>Orthopyxis</i>	Medusoids	With some medusa features (i.e., radial canals) but not others (i.e., tentacles), some with extracapsular development (acrocysts); may or may not be released from the hydroid
	<i>Silicularia</i>	Medusoids	With some medusa features (i.e., radial canals) but not others; not known to be released from the hydroid
	<i>Campanularia</i>	Fixed gonophores	Gonophores with no medusa features; fixed on the hydroid
	<i>Rhizocaulus</i> ^a	Fixed gonophores	Gonophores with no medusa features; fixed on the hydroid
	<i>Tulpa</i>	Fixed gonophores	Gonophores with no medusa features; fixed on the hydroid
	<i>Billardia</i> ^c	Fixed gonophores	Gonophores with no medusa features; fixed on the hydroid
Clytiinae	<i>Clytia</i> ^b	Medusae	Typical hydrozoan medusae
Obeliinae	<i>Obelia</i>	Medusae	Atypical hydrozoan medusae
	<i>Gonothyrea</i> ^a	Meconidia	With some medusa features (i.e., tentacles), but not others; medusa features similar to <i>Obelia</i> , rather than typical, medusae; not released from the hydroid
	<i>Hartlaubella</i> ^a	Fixed gonophores	Gonophores with no medusa features; fixed on the hydroid
	<i>Laomedea</i>	Fixed gonophores	Gonophores with no medusa features; some with extracapsular development (acrocysts); fixed on the hydroid

^a Considered by Cornelius (1982) to be monotypic genus.

^b Including *Gastroblasta*.

^c *Billardia* is considered by some (e.g., Ralph, 1957; Vervoort and Watson, 2003) to be a campanulariid but not Cornelius (1982).

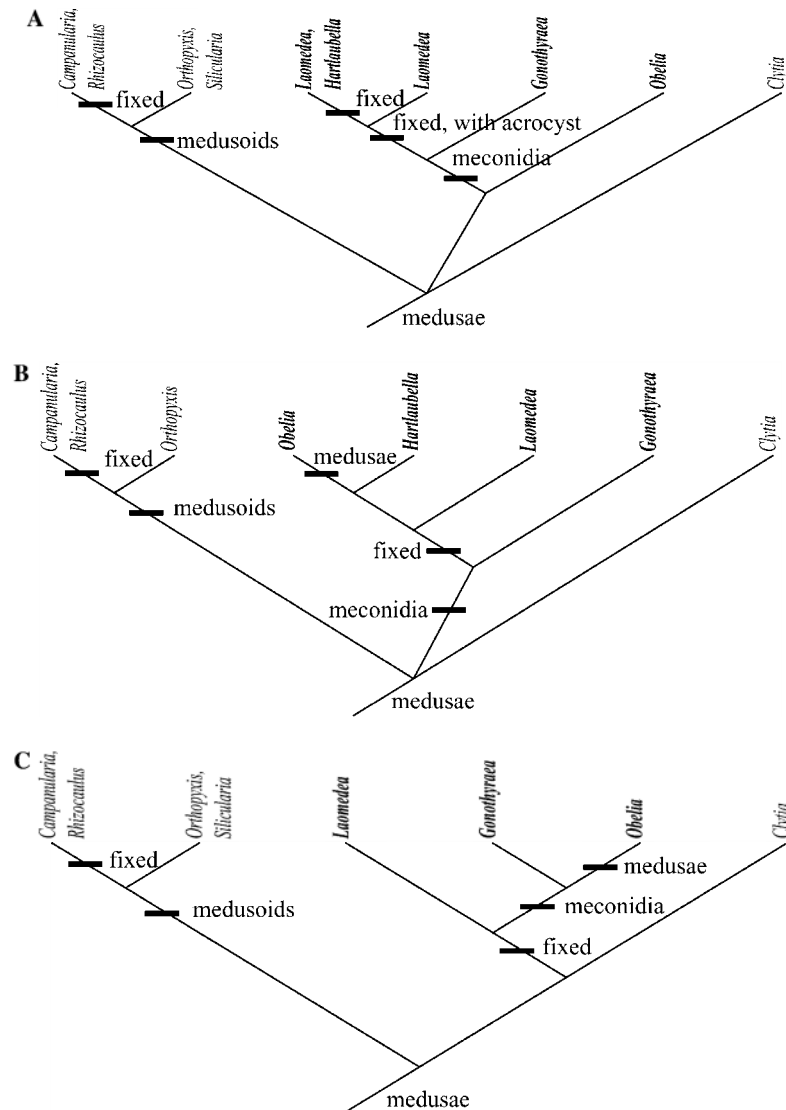


Fig. 1. Proposed relationships among the Campanulariidae, reflecting three possibilities for the evolution of *Obelia*. Note, the Obeliinae lineage (highlighted in bold) consists of *Obelia*, *Gonothyrax*, *Laomedea*, and *Hartlaubella*. (A) *Obelia* derived from typical medusae (Cornelius, 1982); (B) *Obelia* derived from fixed gonophores, which was derived from meconidia (Östman, 1987); (C) *Obelia* derived from fixed gonophores (Boero et al., 1996). Cornelius (1982) and Boero et al. (1996) considered *Silicularia* to have fixed gonophores. However, they have medusoids (Blanco, 1967; Ralph, 1956) and their figures are modified here. All hypotheses show a single loss of medusae in the Campanulariinae and Obeliinae lineages. Finally, Boero et al. consider *Clytia* to be part of the Obeliinae rather than a separate lineage as in Cornelius (1982) and Östman (1987).

resultant topology. Determining if the *Obelia* medusa was secondarily derived from ancestors without medusa and whether medusa loss was a rare event were of particular interest. Other studies (e.g., Emler, 1995; Hart et al., 1997; Wray, 1996) of life-cycle evolution usually consider benthic adults and planktonic larvae whereas, in the present study, we consider the adult, sexually competent medusa, and not the planula larva, as the main planktonic stage in the hydrozoan life cycle.

2. Materials and methods

2.1. Sample collection and outgroup selection

Field-collected hydroids were identified by taxonomic experts (F. Boero, D. Calder, A. Govindarajan, C. Gravili,

A. Lindner, S. Piraino, and P. Schuchert). Samples were split into morphological vouchers (10% formalin) and DNA samples (95% non-denatured ethanol). Taxa employed in this study, collection localities, and GenBank accession numbers are listed in Table 2. Morphological vouchers for most specimens were deposited in the Smithsonian Museum of Natural History invertebrate collections. Forty-seven of these operational taxonomic units (OTUs) are putative campanulariids with all recognized genera except *Hartlaubella* and *Tulpa* represented. Due to the potential existence of cryptic species, multiple representatives from different locations of some nominal species were included when available. Because relationships within Leptomedusae have not been well established, several thecate hydroids were used as outgroups (Table 2).

Table 2
Campanulariid taxa and outgroups used in this study, collection locality, genes sequenced, and GenBank accession numbers

Campanulariidae	Taxon	Locality	18S rDNA	Calmodulin	16S rDNA	COI
Campanulariinae	<i>Billardia subrifida</i> ^{a,b}	Antarctic peninsula	AY789779			
	<i>Campanularia hincksii</i> ^a	Otranto, Italy	AY789729	AY789837	AY789794	AY789882
	<i>Campanularia volubilis</i> ^a	Monterey, CA, USA	AY789739	AY789845	AY789804	AY789889
	<i>Orthopyxis everta</i> ^a	Torre del Serpe, Italy	AY789728		AY789793	AY789881
	<i>Orthopyxis integra</i> ^a	Italy	AY789734	AY789841	AY789799	AY789884
	<i>Orthopyxis integra</i> ^a	Friday Harbor, WA, USA	AY789733	AY789840	AY789798	
	<i>Orthopyxis integra</i> CA sp. 1 ^a	Monterey, CA, USA	AY789731	AY789839	AY789796	
	<i>Orthopyxis integra</i> CA sp. 2 ^a	Monterey, CA, USA	AY789732		AY789797	
	<i>Orthopyxis integra</i> ^a	Aleutians, USA	AY789735	AY789842	AY789800	AY789885
	<i>Orthopyxis integra</i> ^a	Sandgerdi, Iceland	AY789737		AY789802	AY789887
	<i>Orthopyxis integra</i> ^a	New Zealand	AY789736	AY789843	AY789801	AY789886
	<i>Orthopyxis sargassicola</i>	Brazil	AY789730	AY789838	AY789795	AY789883
	<i>Rhizocaulus verticillatus</i> ^a	Aleutians, USA	AY789738	AY789844	AY789803	AY789888
	<i>Silicularia rosea</i> ^a	Bay of Islands, New Zealand	AY789727	AY789836	AY789792	
Clytiinae	<i>Clytia elsaeoswaldae</i>	Brazil	DQ064796	DQ064799	DQ064793	DQ064800
	<i>Clytia gracilis</i> ^a	Georges Bank, North Atlantic	AY789750	AY789855	AY789811	AY789899
	<i>Clytia gracilis</i>	Italy	AY789749	AY789854	AY346364	AY789898
	<i>Clytia gracilis</i> ^a	Beaufort, NC, USA	AY789752	AY789857	AY789813	AY789901
	<i>Clytia gracilis</i>	Woods Hole, MA, USA	AY789751	AY789856	AY789812	AY789900
	<i>Clytia hemisphaerica</i> ^a	North Sea	AY789753	AY789858	AY789814	AY789902
	<i>Clytia hummelincki</i> ^a	S. Caterina, Italy	AY789745	AY789851	AY326363	AY789895
	<i>Clytia hummelincki</i>	South Africa	AY789744	AY789850	AY789809	AY789894
	<i>Clytia linearis</i>	Brazil	DQ064794	DQ064797	DQ064791	
	<i>Clytia linearis</i> ^a	Torre Inserraglio, Italy	AY789747	AY800196	AY346362	
	<i>Clytia linearis</i>	Beaufort, NC, USA	AY789748	AY789853	AY789810	AY789897
	<i>Clytia noliformis</i>	Brazil	DQ064795	DQ064798	DQ064792	
	<i>Clytia paulensis</i> ^a	Otranto, Italy	AY789746	AY789852	AY346361	AY789896
	<i>Clytia</i> sp.	CA, USA	AF358074	AY800197	AY800195	AY789903
Obeliinae	<i>Gonothyrea loveni</i> ^a	Dennis, MA, USA	AY789765	AY789869	AY789826	
	<i>Gonothyrea loveni</i>	Roscoff, France	AY789766	AY789870	AY789827	
	<i>Laomedea calceolifera</i> ^a	Woods Hole, MA, USA	AY789768	AY789872	AY789829	AY789914
	<i>Laomedea flexuosa</i>	Roscoff, France	AY789762	AY789866	AY789823	AY789910
	<i>Laomedea flexuosa</i> ^a	Iceland	AY789763	AY789867	AY789824	AY789911
	<i>Laomedea flexuosa</i> ^a	White Sea	AY789764	AY789868	AY789825	AY789912
	<i>Laomedea inornata</i> ^a	Friday Harbor, WA, USA	AY789761	AY789865	AY789822	
	<i>Obelia bidentata</i> ^a	Beaufort, NC, USA	AY789754	AY789859	AY789815	AY789904
	<i>Obelia bidentata</i> ^a	North Sea	AY789755	AY789860	AY789816	AY789905
	<i>Obelia dichotoma</i> ^a	Otranto, Italy	AY789767	AY789871	AY789828	AY789913
	<i>Obelia geniculata</i>	Roscoff, France	AY789769	AY789873	AY530359	AY530410
	<i>Obelia geniculata</i> ^a	New Brunswick, Canada	AY789770	AY789874	AY530344	AY530395
	<i>Obelia geniculata</i> ^a	Japan	AY789771	AY789875	AY530335	AY530386
	<i>Obelia geniculata</i> ^a	New Zealand	AY789772	AY789876	AY530378	AY530429
	<i>Obelia longissima</i> ^a	Rydgers Cove, MA, USA	AY789757	AY789860	AY789818	
	<i>Obelia longissima</i> ^a	Antarctic peninsula	AY789760	AY789864	AY789821	AY789909
	<i>Obelia longissima</i>	Dunedin, New Zealand	AY789756	AY789861	AY789817	AY789906
	<i>Obelia longissima</i> ^a	Sandgerdi, Iceland	AY789759	AY789863	AY789820	AY789908
	<i>Obelia longissima</i> ^a	White Sea	AY789758	AY789862	AY789819	AY789907
Outgroups						
Aequoreidae	<i>Aequorea aequorea</i>	GenBank	AF358076			
Aequoreidae	<i>Aequorea victoria</i>	GenBank	AF358077			
Aglaopheniidae	<i>Gymnangium hians</i>	GenBank	Z86122			
Blackfordiidae	<i>Blackfordia virginica</i>	GenBank	AF358078			
Bonneviellidae	<i>Bonneviella regia</i> ^a	Aleutians, USA	AY789740	AY789846	AY789805	AY789890
Bonneviellidae	<i>Bonneviella</i> sp. 2 ^a	Aleutians, USA	AY789741	AY789847	AY789806	AY789891
Bonneviellidae	<i>Bonneviella</i> sp. 3	Aleutians, USA	AY789742	AY789848	AY789807	AY789892
Bonneviellidae	<i>Bonneviella</i> sp. 4 ^a	Aleutians, USA	AY789743	AY789849	AY789808	AY789893
Campanulinidae	<i>Calycella syringa</i> ^a	Woods Hole, MA, USA	AY789776	AY789879	AY789833	AY789916
Campanulinidae	<i>Stegella lobata</i> ^a	Antarctic peninsula	AY789778			
Eirenidae	<i>Eugymnanthea inquilina</i>	Taranto, Italy	AY789775	AY789878	AY789832	AY789915
Eucheilotidae	<i>Eucheilota bakeri</i> ^a	CA, USA	AY789774	AY789877	AY789831	
Hydridae	<i>Chlorohydra viridissima</i>	GenBank	AF358081			

(continued on next page)

Table 2 (continued)

Campanulariidae	Taxon	Locality	18S rDNA	Calmodulin	16S rDNA	COI
Hydridae	<i>Hydra circumcincta</i>	GenBank	AF358080			
Laodiceidae	<i>Melicertissa</i> sp.	GenBank	AF358075			
Lovenellidae	<i>Lovenella gracilis</i> ^a	Wildwood Crest, New Jersey	AY789773		AY789830	
Phiallellidae	<i>Opercularella pumila</i> ^a	Woods Hole, MA, USA	AY789777	AY789880	AY789834	
Sertulariidae	<i>Selaginopsis cornigera</i>	GenBank	Z92899			
Tiaropsidae	<i>Tiaropsidium kelseyi</i>	GenBank	AF358079			

^a Voucher specimen deposited at the Smithsonian Museum.

^b *Billardia* is considered by some (e.g., Ralph, 1957; Vervoort and Watson, 2003) to be a campanulariid but not by Cornelius (1982).

We conducted a preliminary analysis of 18S leptomedusan data to determine the sister lineages and inclusiveness of Campanulariidae. Two species of Hydridae (Collins, 2000, 2002) were used to root the Leptomedusae analysis.

2.2. Data collection

DNA was extracted using DNEasy extraction kits (Qiagen) following the manufacturer's protocol. The entire 18S rDNA and portions of calmodulin, 16S rDNA, and COI were amplified using procedures and primers described in Halanych et al. (1998) for 18S rDNA, Lindner, 2005 for calmodulin, Cunningham and Buss (1993) for 16S rDNA, and Folmer et al. (1994) for COI. PCR products were visualized on a 1% agarose gel stained with ethidium bromide. In most cases, the target region was amplified in one reaction; however, in a few cases, the 18S rDNA gene was amplified in three overlapping segments. A few PCR products for calmodulin were re-amplified in order to obtain sufficient quantities for direct sequencing.

Overlapping 18S rDNA, calmodulin, 16S rDNA, and COI products were purified directly from PCR products using the Qiagen PCR purification kit according to manufacturer's protocol. In the case of full-length 18S rDNA, products were size selected on agarose gels (due to the occasional presence of multiple products) and purified using the Qiagen PCR gel purification kit. Some 18S fragments were cloned using the Promega T-GEM easy kit and colonies were screened by PCR amplification. The Promega Wizard miniprep kit was used following manufacturer's protocol.

After unincorporated dideoxynucleotides were removed with a Sephadex G-25 (Sigma) column, all templates were bi-directionally cycle-sequenced using either Big Dye 2 or 3 sequencing chemistry (ABI), as recommended, using an ABI 377 or 3700 automated DNA sequencer. For all gene fragments, amplification primers were used for sequencing. Additionally, the standard primers m13F and m13R, as well as six internal primers (Halanych et al., 1998) were used for the 18S fragment as appropriate. DNA sequences were checked using Autoassembler (ABI) and Sequencher 4.2.2 (Genecodes).

Sequences were aligned using ClustalX (Thompson et al., 1994) and confirmed by eye using MacClade (Maddison and Maddison, 2000). Regions that could not be unambiguously aligned from the rDNA were excluded from analyses. Alignments of calmodulin and COI were unam-

biguous. All alignments have been deposited in TREE-BASE (www.treebase.org).

2.3. Phylogenetic analysis

In addition to the leptomedusan 18S alignment, a master alignment of all four genes was produced for campanulariids. In this combined dataset, 18S and 16S were available for all OTUs, but fragments for calmodulin and COI were not available for some taxa and those data were coded as missing. Parsimony (PARS), maximum likelihood (ML), and Bayesian inference (BI) analyses were conducted for each gene fragment individually and with the all genes combined. We emphasize our results obtained with likelihood-based methods because of known problems of parsimony with rate variation.

PAUP* 4.0b10 (Swofford, 2002) was used for maximum likelihood and parsimony analysis. The best-fit models for maximum-likelihood analysis were determined with ModelTest using the hierarchical likelihood ratio tests Posada and Crandall, 1998). Heuristic searches were conducted using starting trees obtained by stepwise addition with 10 random addition sequence replicates and tree bisection–reconnection (TBR) branch swapping. Nodal support was obtained from non-parametric bootstrap analyses. For parsimony, 1000 bootstrap replicates were run with the maximum number of trees saved in each bootstrap replicate set to 1000 (100 per addition sequence replicate). Due to computational limitations, likelihood bootstrap analyses consisted of 100 replicates using one addition sequence replicate and limiting the number of rearrangements to 5000. Bayesian posterior probabilities were calculated with MRBAYES 3.0b4 (Ronquist and Huelsenbeck, 2003). Bayesian analyses were run for 1,000,000 generations with four chains and a sample frequency of 1000. Burn-in was set at 200, and a plot of log likelihoods versus generation was examined to confirm that the likelihood values had leveled off. MrBayes was used to calculate the model parameters after we assumed a general GTR+ Γ +I model. Bayesian posterior probabilities may sometimes overestimate support (Erixon et al., 2003; Simmons et al., 2004), and thus for the purpose of discussion, only nodes with high bootstrap values and post. prob. were considered well supported. Topological hypotheses were tested with the Shimodaira–Hasegawa (SH) test (Shimodaira and Hasegawa, 1999) implemented in PAUP* 4.0b10 using the RELL test distribution.

Table 3
Maximum-likelihood model parameters

Data sets	Maximum likelihood											Shape parameter α	Proportion of invariant sites		
	Model	Base frequencies				Substitution rates									
		A	C	G	T	A \leftrightarrow C	A \leftrightarrow G	A \leftrightarrow T	C \leftrightarrow G	C \leftrightarrow T	G \leftrightarrow T				
<i>Leptomedusae</i>															
18S (38 taxa)	TrN + I + G	0.2756	0.1885	0.2684	0.2675	1.0000		3.0829	1.0000	1.0000		7.0861	1.0000	0.7003	0.5234
<i>Campanulariidae</i>															
Combined (55 taxa)	GTR + I + G	0.3030	0.1646	0.2274	0.3051	3.4181		14.0885	10.4813	2.8637		43.5166	1.0000	0.4865	0.5886
18S (55 taxa)	TIM + I + G	0.2753	0.1897	0.2651	0.2699	1.0000		4.2396	1.8041	1.8041		9.3501	1.0000	0.6296	0.5823
Calmodulin (50 taxa)	TrN + I + G	0.4200	0.1567	0.1616	0.2617	1.0000		5.4449	1.0000	1.0000		14.8099	1.0000	0.7832	0.5910
16S (55 taxa)	GTR + I + G	0.4502	0.0942	0.1065	0.3490	130537.5078	722000.8750	92989.7578	0.0001	1258584.5000	1.0000	0.2875		0.5462	
COI (41 taxa)	GTR + I + G	0.3382	0.1081	0.1401	0.4135		7.1057	23.7533	12.4729	4.9198		180.0143	1.0000	0.7624	0.5391

For both ML and BI, substitution models were estimated for each dataset individually as well as the combined dataset (Table 3).

2.4. Ancestral character reconstruction

In order to understand the evolution of medusa form (i.e., gonophore evolution), OTUs were scored as having fixed gonophores, meconidia, medusoids, typical medusae, or *Obelia* medusae. Gonophore type was mapped on to the resultant phylogeny based on the combined dataset (see below), and the ancestral states were reconstructed using parsimony criteria in MacClade 4.0 (Maddison and Maddison, 2000). Based on Boero et al. (1996), the last common ancestral campanulariid was assumed to have a typical medusa stage. In particular, *Clytia* medusae bear striking morphological resemblance to other leptomedusa (e.g., *Phialella*) suggesting it is pleisomorphic for campanulariids. We implemented this assumption by using a single outgroup scored as possessing a typical medusa. The loss of a complex morphological character has been considered to be more common than a gain. Furthermore, frequent losses may bias inference of ancestral character states (Cunningham, 1999; Cunningham et al., 1998). Applying this logic to campanulariids, going from medusae to medusoids or fixed gonophores (i.e., a loss) may be easier than going from fixed gonophores and medusoids to medusae (i.e., a gain). In order to explore the robustness of conclusions on ancestral character states, a sensitivity analysis was conducted using differential weighting of gains as more costly than losses (e.g., Omland, 1997; Ree and Donoghue, 1998). Gain costs (weights) were tested at 0.1 increments between 1 and 2, and then periodically (3, 10, 25, 50, and 75) up to 99.

3. Results

3.1. Identifying outgroups and delineating Campanulariidae

The 18S rDNA alignment for the leptomedusan analysis included 38 OTUs and 2057 base pairs (bp). Of the 1595

nucleotide positions that could be unambiguously aligned, 27.2% (434 positions) were variable and 16.0% (255 positions) were parsimony informative. Fig. 2 shows the best ML tree ($-\ln$ likelihood = 7442.80525) reconstructed using the parameters indicated on Table 3.

The Campanulariidae (highlighted in Fig. 2) is not monophyletic as traditionally recognized because taxa not traditionally considered campanulariids fall within the clade. *Bonneviella* (Bonnieviellidae) were strongly supported (PARS = 95%; ML = 100%; post. prob. = 1.00) to be deeply nested within Campanulariinae forming a clade with *Campanularia volubilis* and *Rhizocaulus verticillatus* (PARS = 90%; ML = 85%; post. prob. = 1.00). *Lovenella gracilis* (Lovenellidae) and *Eucheilota bakeri* (Eucheilotiidae) are very similar genetically and constitute a weakly supported (PARS = <50%; ML = 67%; post. prob. = 1.00) clade within Campanulariidae. Additionally, *Billardia subrufa* fell outside the main “campanulariid” clade (bootstrap PARS and ML = 100%, posterior probabilities = 1.00, and SH test p = 0.000; Table 4). This result is contra Ralph (1957) and Vervoort and Watson (2003) and consistent with its placement in another family such as the Lafoeidae or Syntheciidae (Cornelius, 1982; Millard, 1975).

Based on results of this preliminary analysis, representatives of the sister clade to Campanulariidae and the next basal most lineage were chosen as outgroups subject to availability of tissue for data collection from additional markers. These taxa included *Calycella syringa* (Campanulinidae), *Opercularella pumila* (Phialellidae), and *Eugymnanthea inquilina* (Eirenidae). Note that Campanulinidae is not monophyletic as *Stegella lobata* falls in a basal leptomedusan clade with *B. subrufa* and *Melicertissa* sp. (Laodiceidae).

3.2. Relationships in Campanulariidae

Taxa incorporated into the phylogenetic analysis of Campanulariidae were adjusted to include *L. gracilis*, *E. bakeri*, and bonnieviellid taxa. *B. subrufa* was not included. The data were analyzed both together (Fig. 3) and

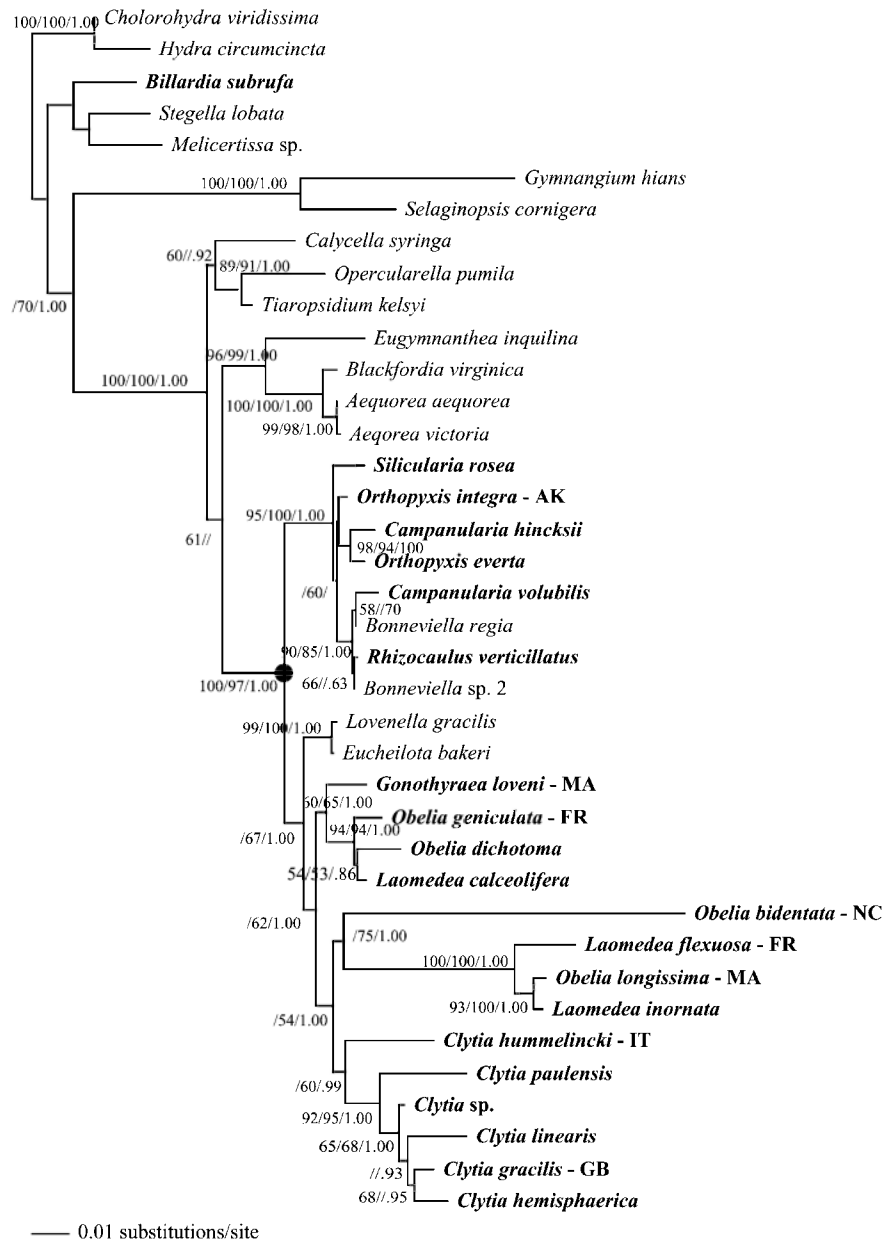


Fig. 2. Maximum-likelihood tree of the leptomedusan data set. Putative campanulariids are indicated in bold and a solid black circle is on the node defining the Campanulariidae as defined for subsequent analyses. The reconstructed topology is from a heuristic search based on 18S rDNA. The $-\ln$ likelihood for the topology = 7442.80525. First number along the branches refers to maximum-likelihood bootstrap values, second number refers to parsimony bootstrap values, and third number refers to Bayesian posterior probability values. Location codes for some OTUs (see Table 2): AN, Antarctic peninsula; AS, Aleutians; BR, Brazil; CA, California; FR, France; GB, Georges Bank; IC, Iceland; IT, Italy; JP, Japan; MA, Massachusetts; NB, New Brunswick; NJ, New Jersey; NZ, New Zealand; NC, North Carolina; NS, North Sea; SA, South Africa; WA, Washington.

separately (Figs. 4 and 5). Most phylogenetic signal came from 18S rDNA data, whereas other markers were generally very poorly resolved. Therefore, the combined and 18S rDNA (which allowed for increased taxon sampling) results are emphasized below due to space considerations. Combined and 18S analyses yielded similar results, with a few exceptions as noted. PARS, ML, and BI support usually agreed, although in some cases Bayesian posterior probabilities were considerably higher than likelihood and parsimony bootstrap values. Individually calmodulin, 16S nor COI placed all outgroups outside of campanulariids, but this result was never well supported (Fig. 5).

The combined alignment included 38 OTUs (three as outgroups) and 3546 bp. Of the 3108 bp (18S: 1612 bp; CAM: 401 bp; 16S: 434 bp; and COI: 661 bp) that could be unambiguously aligned, 28.6% (total: 890 positions; 18S: 359; CAM: 125; 16S: 131; and COI: 275) were variable and 21.7% (total: 673 positions; 18S: 216; CAM: 107; 16S: 105; and COI: 245) were parsimony informative when third positions were included. In the two protein-coding genes, most of the variation occurred in third codon positions (110/125 variable sites in CAM and 208/275 in COI). Fig. 3 shows the best ML tree ($-\ln$ likelihood = 20426.3370) reconstructed (model parameters in Table 3).

Table 4
Results of Shimodaira–Hasegawa tests for monophyly of recognized families and genera

Constraint	Monophyly tested	–ln <i>L</i> best	–ln <i>L</i> constraint	Diff –ln <i>L</i>	<i>p</i> value
Family 1	Campanulariidae, as defined with <i>Billardia</i> , the bonneviellids, and <i>Eucheilota</i> and <i>Lovenella</i>	7442.80525	7547.97789	105.17264	0.000
Family 2	Campanulariidae, as defined without <i>Billardia</i> , the bonneviellids, and <i>Eucheilota</i> and <i>Lovenella</i>	7442.80525	7514.35024	71.54499	0.001
Family 3	Campanulariidae, as defined without <i>Billardia</i> , and <i>Eucheilota</i> and <i>Lovenella</i> , but with the bonneviellids	7442.80525	7446.50419	3.69894	0.255
Family 4	Campanulariidae, as defined with <i>Eucheilota</i> and <i>Lovenella</i> , and without <i>Billardia</i> and the bonneviellids	7442.80525	7485.02453	42.21928	0.009
Obeliinae	<i>Obelia</i> + <i>Laomedea</i> + <i>Gonothyrax</i>	20426.69624	20426.85739	0.16110	0.483
<i>Obelia</i>	<i>Obelia</i>	20426.69624	20767.61421	340.91797	0.000
<i>Laomedea</i>	<i>Laomedea</i>	20426.69624	20746.78747	320.09123	0.000
<i>Campanularia</i>	<i>Campanularia</i> + <i>Rhizocaulus</i> + <i>Bonneviella</i> (Campanulariinae with fixed gonophores)	20426.69624	20431.36449	4.66825	0.294

The best tree (unconstrained) was compared with the best tree under the given constraints.

The combined analysis reveals considerable resolution in the campanulariid tree except near the base of the Obeliinae/Clytiinae clade (see below). In comparison to the preliminary 18S analysis, *E. bakeri* and *L. gracilis* appear basal to Campanulariidae, although this placement is not significantly supported by bootstrap or post. prob. The recognized “subfamilies” (Table 1) appear well resolved with some modification. The Campanulariinae is strongly supported (Fig. 3—PARS=100%; ML=100%; post. prob.=1.00) including a *Bonneviella* clade as sister to *R. verticillatus*. The Clytiinae are monophyletic, and even though the basal most node (including *Clytia hummelincki*) is not well supported, the remainder of the clade is moderately supported (PARS=96%; ML=80%; post. prob.=1.00). The Obeliinae as described in Table 1 forms three clades each with strong support (PARS=100%; ML=100%; post. prob.=1.00), but the *Obelia bidentata* clade clusters with Clytiinae rather than Obeliinae in the combined ML tree. Analyses of individual datasets are variable concerning placement of *O. bidentata*. However, both PARS and BI weakly support (values of 72 and 0.60, respectively) the placement of *O. bidentata* in Obeliinae, as the basal member of the clade containing *Laomedea flexuosa*, *Laomedea inornata*, and *Obelia longissima* (this arrangement not depicted in Fig. 3). The 18S rDNA only analysis is consistent with this position but only PARS finds strong support (bootstrap=95%). A heuristic ML analysis of the combined dataset with *O. bidentata* removed yields a topology otherwise identical to Fig. 3. Interestingly, the placement observed in the combined data set appears to be coming from CO1 data or possibly calmodulin (Fig. 5).

One other taxon that deserves mention is *Campanularia*, which is not monophyletic in either Fig. 3 or Fig. 4. In the combined analysis, *Orthopyxis everta* and *Orthopyxis integra* Italy (IT) group together strongly (Fig. 3, see also Fig. 5), while in the 18S rDNA analysis, *Or. everta* and *Campanularia hincksii* group together strongly. All three of the above taxa were collected in Italy, but were obtained and processed at different times so contamination appears unlikely.

In the combined analysis, three recognized campanulariid genera (*Campanularia*, *Obelia*, and *Laomedea*) and two species (*Or. integra* and *Clytia gracilis*) appear not to be monophyletic. In all of these cases, strong support is found for at least one node preventing monophyly. SH tests also significantly reject (both $p < 0.001$) monophyly of *Obelia* and *Laomedea*. Non-monophyly of these taxa is also supported by 18S only analyses.

3.3. Ancestral character reconstruction

The topology used for ancestral state reconstructions (Fig. 6) was derived from the analysis of the combined data. However, we choose to employ the PAR and BI topology because *O. bidentata* is placed with other obeliinids rather than clytiinids, an interpretation more consistent with morphology (*Obelia* medusae are morphologically indistinguishable). This topology is not significantly less likely than the most likely topology (Table 4, $p = 0.483$).

As expected based on the phylogenetic findings above, at least some campanulariid life cycles exhibit parallelism or convergence. Specifically, we must consider one of the following: fixed gonophores evolved multiple times, de novo *Obelia*-type medusa have evolved multiple times, or that the expression of the *Obelia* medusa has been turned on/off several times. Note that in the last case the medusa would be homologous across taxa but suppression could be due to a number of different non-homologous genetic changes (i.e., there may be numerous ways to turn off regulatory genes controlling medusa development). When gains and losses were equally weighted unequivocal character states could not be assigned from the campanulariid basal node up to several basal lineages within Obeliinae. However, as soon as gains were weighted slightly over losses, all equivocal nodes are lost and the Obeliinae ancestor is assumed to have an *Obelia*-type medusa with the next most basal ancestor possessing a typical medusa (Fig. 6, *sensu* Cornelius, 1982). Weighting gains two or more times the cost of losses switches the basal lineage of the Campanulariinae from being interpreted as possessing

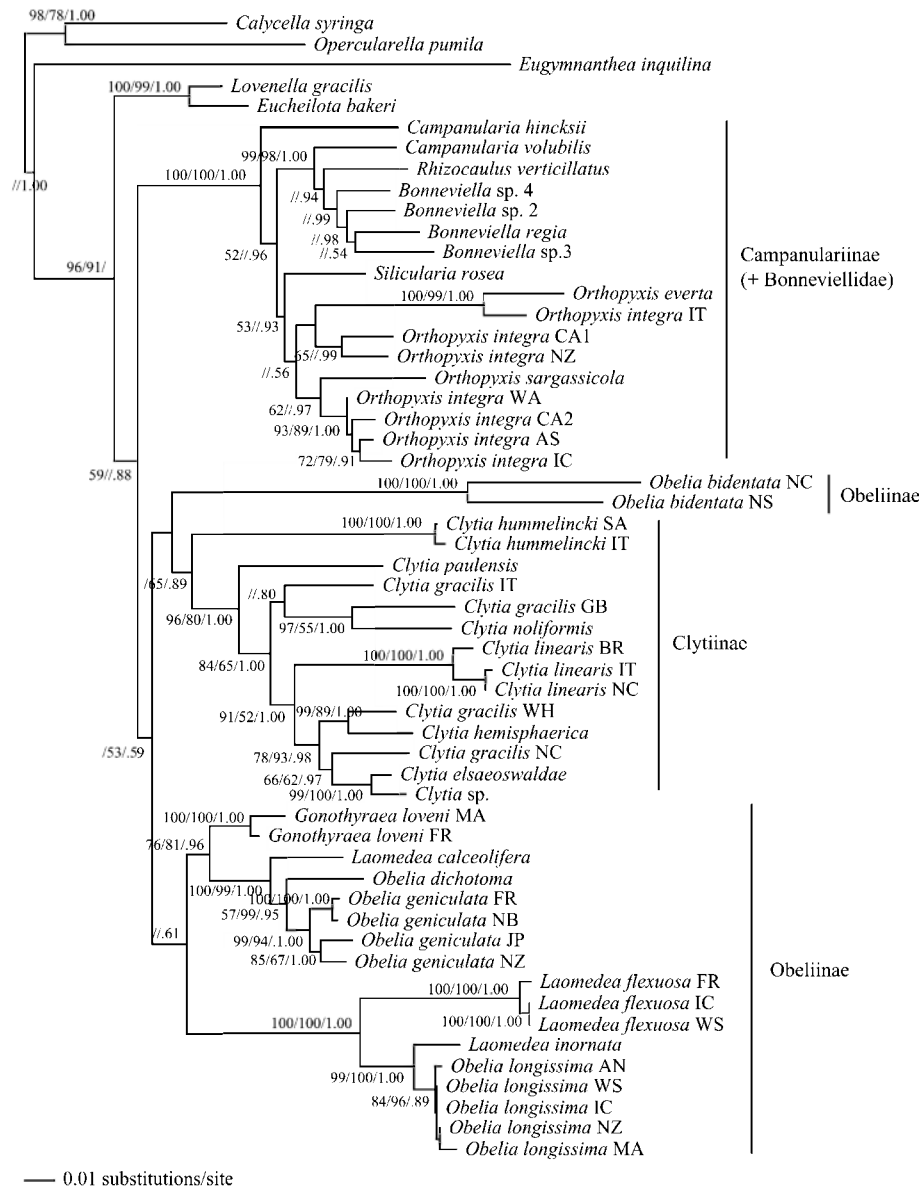


Fig. 3. Heuristic maximum-likelihood topology based on combined 18S rDNA, calmodulin, 16S rDNA, and COI. The $-\ln$ likelihood for the topology, 20426.3370. First number along the branches refers to maximum-likelihood bootstrap values, second number refers to parsimony bootstrap values, and third number refers to Bayesian posterior probability values. Subfamily lineages are indicated. Location codes are as in Fig. 2.

fixed gonophores to possessing either a typical medusa or medusoids. Increasing the gain cost up to 99 had no further effect. Given the limitation of only being able to sample extant taxa, conceivably extinct ancestors with intermediate character states may have existed. While this supposition is not parsimonious, the morphological difference between *Obelia* and *Clytia* medusae is pronounced.

4. Discussion

The combined analysis of four molecular markers indicates that evolution of the adult life-history stage, or medusa, in campanulariid hydrozoans is plastic. Although we could not definitively reconstruct ancestral character states for basal

nodes defining the three recognized subfamilies (Obeliinae, Campanulariinae, and Clytiinae), our results indicate that fixed gonophores have likely evolved independently in several lineages. Given that previous non-leptomedusan studies (e.g., Tubulariidae, Petersen, 1990; Hydractiniidae, Cunningham and Buss, 1993) also reported repeated medusa loss, this phenomenon is probably more widespread than traditionally recognized, suggesting that practices using life-history stages for taxonomic purposes are suspect (see Bouillon and Boero, 2000). Because formal taxonomic revisions based on this and additional morphological work will be published elsewhere, herein we focus on larger issues of phylogeny and evolutionary transitions.

The combined data set has considerably more resolution than any one individual marker. Not surprisingly, the 18S

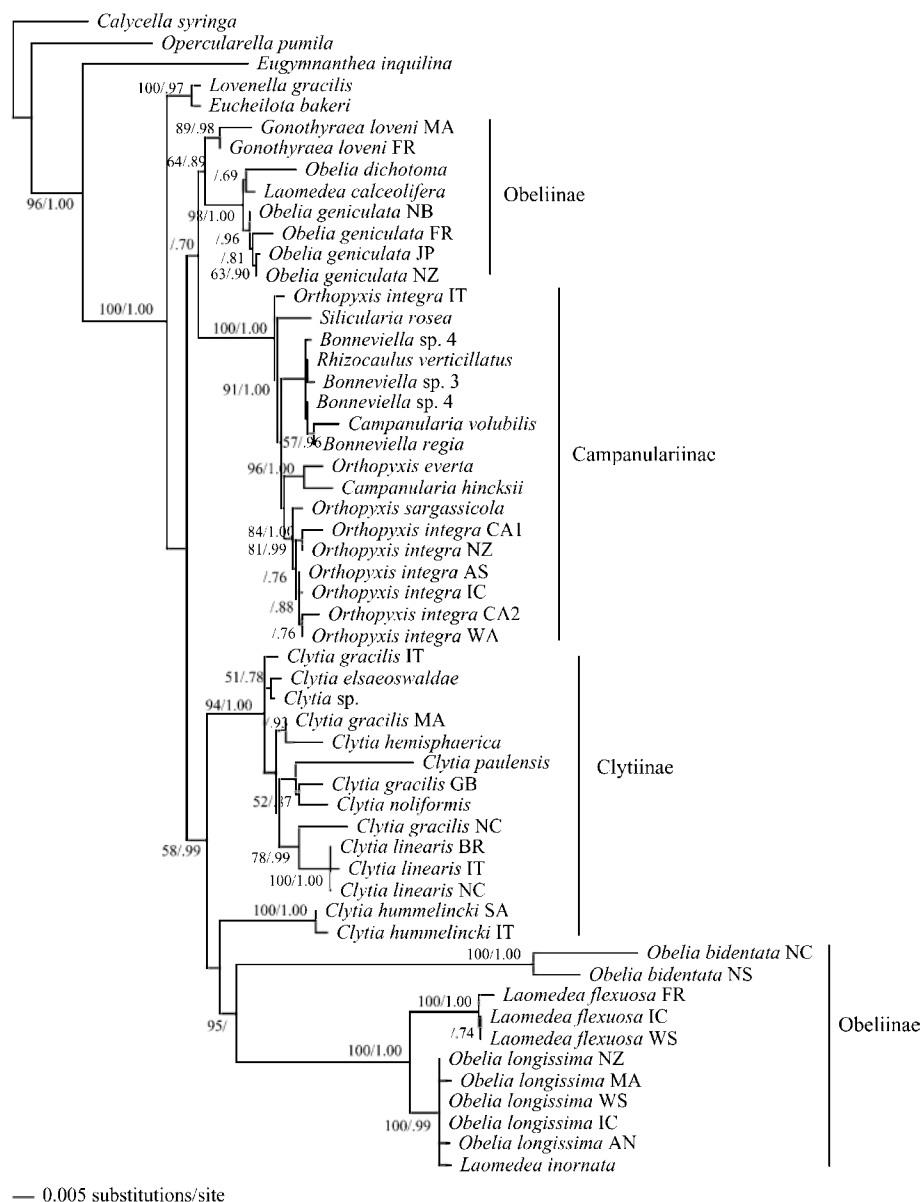


Fig. 4. Maximum-likelihood topology based only on 18S rDNA. The $-\ln$ likelihood of the topology = 6469.86106. Numbers before the slash refer to parsimony bootstrap values and numbers after the slash refer to Bayesian posterior probabilities. Subfamily lineages are indicated. Location codes are as in Fig. 2.

rDNA has the best resolution but also had the most data. Although there is considerable topological variation between markers, these differences were on poorly supported branches as judged by bootstrap values. Comparison of the combined tree to the topologies from individual genes reveals the several cases where the signal from a particular marker seemed to “win out.” For example, most signal for *Clytia* relationships is apparently from the 16 and CO1 genes and *O. bidentata*'s placement is due to calmodulin and COI.

4.1. Delineating Campanulariidae

In order to understand campanulariid phylogeny, we first had to determine inclusiveness of the lineage. Traditionally, diagnostic morphological features used to define Campanulariidae are a campanulate (wine-glass shaped) theca and a

peduncled hypostome (see Cornelius, 1982 for additional features). Soft tissue features, such as the peduncled hypostome, are most appropriately observed in live animals whereas the bulk of hydrozoan taxonomic work is conducted on preserved samples. Although the Leptomedusae is a large (>2000 nominal species) and ecologically important group, Fig. 2 represents the first molecular attempt to understand its phylogenetic history and highlights some of the known taxonomic problems (e.g., Campanulariidae).

Billardia, which our results indicate is clearly not a campanulariid (Fig. 2), is a poorly studied taxon with three recognized species occurring in the Southern Ocean (north to New Zealand). Although at least superficially possessing similar polyp morphology, Cornelius (1982), among others, has not included *Billardia* as part of the Campanulariidae, consistent with our findings. The other lineage in question, the



Fig. 6. Ancestral state reconstruction on the modified maximum-likelihood topology. *O. bidentata* is placed as the basal member of the *L. flexuosa*, *L. inornata*, and *O. longissima* clade. Location codes are as in Fig. 2. Gains ≥ 2.0 . Losses = 1.0. Gonophore type indicated in bold after the taxon name. T, typical medusae; D, medusoids; F, fixed gonophores; M, meconidia; O, *Obelia* medusae.

their large size relative to other leptomedusan polyps may account for some of their morphological uniqueness.

Representatives of Lovenellidae and Eucheilotidae form a well-supported clade that either falls within the Campanulariidae (Fig. 2) or as a sister lineage (Fig. 3). Unfortunately, support values were too weak to distinguish between these possibilities, but the node including Campanulariidae, *Lovenella*, and *Eucheilota* is well supported. Interestingly, when *E. bakeri* was first described, its hydroids were referred to as *Clytia bakeri* (Torrey, 1904). The morphological differences between *L. gracilis* and *E. bakeri* (Govindarajan, pers. obs.) are minimal, and further investigation is clearly needed to understand the relationship of these taxa to each other and to campanulariids. Herein, we favor the results based on the less-inclusive combined analysis and therefore consider the lovenellid/eucheilotid lineage as a sister taxon to Campanulariidae.

4.2. Campanulariid relationships

In general, the molecular data corroborate our understanding of campanulariid relationships based on morphological taxonomy. There are three major clades which

correspond well to Campanulariinae, Clytiinae, and Obeliinae, but only the Campanulariinae received strong support (Figs. 2 and 3). Our results differ with the traditional understanding in that at least three genera and two species are not monophyletic.

In the Campanulariinae, two main clades were found. All members of the *Campanularia/Rhizocaulus/Bonneviella* (CRB) clade were collected in the North Pacific and may represent a local, geographically restricted radiation. All known bonneviellids except *Bonneviella grandis* (which is also in the North Atlantic; Naumov, 1960) are found exclusively in this area which, incidentally, is known for many invertebrate species having unusually large size. Additional representation from non-North Pacific *Campanularia* will be useful in addressing the possibility of a local radiation. *Ca. hincksii*, which was collected in the Mediterranean and is a nearly cosmopolitan species (Cornelius, 1995), fell basal to the two main Campanulariinae clades.

The world-wide *Orthopyxis* and the Southern Ocean *Silicularia* form the other major Campanulariinae clade. *Or. integra* is not monophyletic, and this is consistent with the great number of nominal species that have been proposed in the past based on morphology

(see Cornelius, 1982; Vervoort and Watson, 2003). Many of these species are greatly variable in morphology, the champion being *Or. integra*, and this led to recognizing great overlaps in morphology and the merging of nominal species (Cornelius, 1982). Our data suggest the overlaps are due to convergence, probably in response to similar environmental pressures, and the genus is more speciose than previously recognized. *Silicularia* hydranths, in contrast with those of all other campanulariids, cannot withdraw completely into the hydrotheca, and have everted “caecae” when the hydranth is contracted. The rest of the colony morphology is similar to that of *Orthopyxis*, and it is possible that the ancestor of *Silicularia* was *Orthopyxis*-like.

The Clytiinae was somewhat paradoxical in that the base of the clade was perhaps the most problematic region in our analysis, but most of the clade was well resolved and supported. Assuming that *O. bidentata* is correctly placed in Obeliinae, *C. hummelincki*'s position is notable because, unlike other *Clytia*, it possesses a subhydrothecal spherule and a colony growth pattern similar to that of Campanulariinae (Boero et al., 1996). The possession of features in basal Campanulariinae and Clytiinae suggests these features are plesiomorphic of campanulariids in general. Additionally, the polyphyletic nature of *C. gracilis* is more thoroughly considered by (Lindner et al., 2003).

Obeliinae is split into three well-supported groups: *O. bidentata*, a *Laomedea/O. longissima* (LO) clade, and a *Gonothyræal/Laomedea/Obelia* (GLO) clade. Noteworthy, most of our analyses did not find Obeliinae as a monophyletic clade. For example, the 18S data alone placed the GLO clade with the Campanulariinae whereas the combined data place GLO and LO as sister taxa with *O. bidentata* outside. Even though Obeliinae relationships need further study, the current analyses vastly improve our understanding of this group. Both *Obelia*, diagnosed by having a unique medusa, and *Laomedea*, diagnosed by having fixed gonophores, are not monophyletic. *Obelia* has a particularly colorful taxonomic history in that Cornelius merged 120 nominal species into three (Cornelius, 1975), although he later increased that number to four (Cornelius, 1990). Other authors recognize additional species (e.g., Bouillon and Boero, 2000; Kubota, 1999; Stepenjants, 1998), and thus *Obelia* likely contains cryptic species. Although forming a monophyletic clade here, *Obelia geniculata* contains geographic subdivision and is not truly a cosmopolitan species (Govindarajan et al., 2004). In contrast, available data from *O. longissima* OTUs show little variation and lack phylogeographic structure suggesting that it may in fact be a true cosmopolitan species (Govindarajan, 2004). As for *Laomedea*, *L. calceolifera* females have a uniquely shaped gonotheca with a curl at the top and is thus distinct from other members of the genus. Furthermore, Miller (1973) found that gametes from *Gonothyræa loveni* and *L. calceolifera* were attracted to each other, but that gametes from *L. calceolifera* and *L. flexuosa* were not. Thus, its placement in the LO rather than the GLO clade may not be as unusual as the nomen suggests.

4.3. Gonophore evolution

The molecular phylogenetic results herein offer a novel perspective on gonophore evolution within Campanulariidae. As discussed earlier, debates about campanulariid evolution focus on gonophore characteristics in Obeliinae (Fig. 1). These hypotheses employ the assumption that the type of adult structure (i.e., gonophore) accurately delineates natural groups and thus one can reconstruct relationships using a stepwise evolutionary progression from fixed gonophore to medusa, or vice versa. Because *Obelia* and *Laomedea* are polyphyletic as currently recognized, evolution of the group is clearly more complicated than a stepwise progression. Our results support previous arguments (Petersen, 1990) that some gonophore types, specifically fixed gonophores (Boero et al., 1996), have evolved multiple times via medusa suppression within family-level lineages.

Under equally weighted parsimony, ancestral state reconstructions for Campanulariidae, Obeliinae, and *Clytia* could not be unequivocally inferred. However, when even a very small gain cost was applied, the ancestral campanulariid and obeliniid + *Clytia* ancestor appear to have had typical medusae (Fig. 6). This result raises the possibility that *Obelia* medusae derived directly from an ancestor with typical medusae, rather than one with fixed gonophores or meconidia. Thus, *Obelia* may not be an example of evolutionary reversal. However, because all *Obelia* medusae are nearly identical (Cornelius, 1995; Kramp, 1961) and multiple losses of life-history traits in marine invertebrates appear to be more general than gains (e.g., Hart, 2000), we argue that there was a single origin of *Obelia* medusae and that the fixed gonophore bearing *Laomedea* evolved multiple times within the *Obelia* clade. Whether the *Obelia* medusa was derived directly from a *Clytia*-like medusa is open to debate. Although our ancestral character reconstruction based on extant taxa suggests a direct relationship between these medusa types, morphology and developmental similarities are lacking. Future research on the genetic mechanisms of medusa development will be helpful in evaluating the evolutionary relationship between these morphologies.

One missing piece of information concerns the phylogenetic placement of *Hartlaubella*, a monotypic genus which could not be obtained for this study. Like *Laomedea*, *Hartlaubella* has fixed gonophores; however, hydroid colony morphology differs in some potentially important ways in the two genera. *Hartlaubella* hydroids are relatively large, polysiphonic, and have toothed hydrothecal margins. These characteristics are generally similar to those found in *Gonothyræa* and some *Obelia* and *Clytia* hydroids but not *Laomedea*. Unless gonophores are present, *Hartlaubella* is easily confused with *O. bidentata* (Cornelius, 1990). Additional studies are needed to determine if these features indicate a basal position in the Obeliinae. Interestingly, meconidia of *Gonothyræa*, which is basal in the GLO clade, has solid tentacles similar to those found in *Obelia* but no other Leptomedusae. This observation implies that some of

the unusual features of the *Obelia* medusa were present in the Obeliinae ancestor.

Our results suggest that if gains are even slightly more likely than losses, medusae may have been reduced multiple times. Heterochrony, or change in the timing of development, may be the underlying cause of medusa reduction. Weismann (1883; summarized in Berrill and Liu, 1948) related gonophore type to the location of germ cell differentiation. In free-living medusae, the germ site is on the ectoderm in the medusa. As medusa features are lost, the germ site moves to the entocodon (medusa bud) and gonophore endoderm and eventually to the hydroid coenosarc, or tissue. The germ cells then migrate to the gonophore ectoderm, where they mature, perhaps blocking medusa development (Boero and Bouillon, 1989; see also Boero et al., 1997). Given this developmental understanding in light of the phylogenetic results herein, the Campanulariidae offers an interesting opportunity to explore differences in the position and timing of germ cell proliferation across related taxa with different adult stages. Thus, it is a potential model system for examining the mechanism of gain or loss of life-history stages.

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References

- Allman, G.J., 1864. On the construction and limitation of genera among the Hydrozoa. *Ann. Mag. Nat. Hist.* 3, 345–380.
- Berrill, N.J., Liu, C.K., 1948. Germplasm, Weismann, and Hydrozoa. *Q. Rev. Biol.* 23, 124–132.
- Blanco, O.M., 1967. Estudio critico sobre las especies del genero *Silicularia* Meyen 1834. *Revta Mus. La Plata (n. ser.)* 9 Zool. 70, 217–241.
- Boero, F., 1980. Life cycles of hydroids and hydromedusae: some cases of difficult interpretation. *Mem. Biol. Marina Oceanogr.* X, 141–147.
- Boero, F., Bouillon, J., 1989. An evolutionary interpretation of anomalous medusoid stages in the life cycles of some Leptomedusae (Cnidaria). In: 23rd European Marine Biology Symposium: Reproduction, Genetics and Distributions of Marine Organisms, pp. 37–41.
- Boero, F., Bouillon, J., Piraino, S., 1996. Classification and phylogeny in the Hydroidomedusae (Hydrozoa, Cnidaria). *Sci. Mar.* 60, 17–33.
- Boero, F., Bouillon, J., Piraino, S., Schmid, V., 1997. Diversity of hydroidomedusan life cycles: ecological implications and evolutionary patterns. In: Proceedings of the 6th International Conference on Coelent, pp. 53–62.
- Boero, F., Sarà, M., 1987. Motile sexual stages and evolution of Leptomedusae (Cnidaria). *Boll. Zool.* 54, 131–139.
- Bouillon, J., 1985. Essai de classification des hydroides-hydromeduses (Hydrozoa-Cnidaria). *Indo-Malayan Zool.* 2, 29–243.
- Bouillon, J., Boero, F., 2000. Synopsis of the families and genera of the hydromedusae of the world, with a list of the worldwide species. *Thalassia Salentina* 24, 47–296.
- Broch, H., 1916. *Hydrozoa. Danish Ingolf. Exped.* 5, 1–66.
- Chapman, D.M., 1968. A new type of muscle cell from the subumbrella of *Obelia*. *J. Mar. Biol. Assoc. UK* 48, 667–688.
- Collins, A.G., 2000. Towards understanding the phylogenetic history of the Hydrozoa: hypothesis testing with 18S gene sequence data. *Sci. Mar.* 64 (Supl 1), 5–22.
- Collins, A.G., 2002. Phylogeny of the Medusozoa and the evolution of cnidarian life cycles. *J. Evol. Biol.* 15, 41–432.
- 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 the genera. *Bull. Br. Mus. Nat. Hist. (Zool.)* 42 (2), 37–148.
- Cornelius, P.F.S., 1990. European *Obelia* (Cnidaria, Hydrozoa): systematics and identification. *J. Nat. Hist.* 24, 535–578.
- Cornelius, P.F.S., 1995. North-west European thecate hydroids and their medusae. Part 2. Sertulariidae to Campanulariidae. *Synopsis of the British Fauna (New Series)* 50, 1–386.
- Cunningham, C.W., 1999. Some limitations of ancestral character-state reconstruction when testing evolutionary hypotheses. *Syst. Biol.* 48, 665–674.
- Cunningham, C.W., Buss, L.W., 1993. Molecular evidence for multiple episodes of paedomorphosis in the family Hydractiniidae. *Biochem. Syst. Ecol.* 21, 57–69.
- Cunningham, C.W., Omland, K.E., Oakley, T.H., 1998. Reconstructing ancestral character states: a critical reappraisal. *Trends Ecol. Evol.* 13, 361–366.
- Emlet, R.B., 1995. Developmental mode and species geographic range in regular sea urchins (Echinodermata: Echinoidea). *Evolution* 49, 476–489.
- Erixon, P., Svennblad, B., Britton, T., Oxelman, B., 2003. Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. *Syst. Biol.* 52, 665–673.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 3, 294–299.
- Govindarajan, A.F., 2004. Life cycle evolution and systematics in campanulariid hydrozoans. Ph.D. Thesis. Massachusetts Institute of Technology/Woods Hole Oceanographic Institution.
- Govindarajan, A.F., Halanych, K.M., Cunningham, C.W., 2004. Mitochondrial evolution and phylogeography in the hydrozoan *Obelia geniculata* (Cnidaria). *Mar. Biol.* 146, 213–222.
- Gravier-Bonnet, N., 1992. Cloning and dispersal by buoyant autotomised hydranths of a thecate hydroid (Cnidaria: Hydrozoa). *Sci. Mar.* 56 (2–3), 229–236.
- Halanych, K.M., Lutz, R.A., Vrijenhoek, R.C., 1998. Evolutionary origins and age of vestimentiferan tube-worms. *Cah. Biol. Mar.* 39, 355–358.
- Hart, M., 2000. Phylogenetic analyses of mode of larval development. *Cell Dev. Biol.* 11, 411–418.
- Hart, M.W., Byrne, M., Smith, M.J., 1997. Molecular phylogenetic analysis of life history evolution in asterinid starfish. *Evolution* 51, 1848–1861.
- Kramp, P.L., 1949. Origin of the hydroid family Corymorphidae. *Vidensk. Meddr. Dansk. Naturh. Foren.* 111, 183–215.
- Kramp, P.L., 1961. Synopsis of the medusae of the world. *J. Mar. Biol. Assoc.* 40, 7–469.

- Kubota, S., 1999. Fauna of *Obelia* (Cnidaria, Hydrozoa) in Japanese waters, with special reference to the life cycle of *Obelia dichotoma* (L., 1758). *Zoosystematica Rossica Suppl.* 1, 67–76.
- Kühn, A., 1913. Entwicklungsgeschichte und Verwandtschaftsbeziehungen der Hydrozoen. I. Teil: Die Hydroiden. *Erg. Fortschr. Zool.* 4 (1–2), 1–284.
- Lindner, A., 2005. Evolution and taxonomy of stylasterid corals (Cnidaria: Hydrozoa: Stylasteridae). Duke University. p. 165.
- Lindner, A., Migotto, A.E., Govindarajan, A.F., 2003. Diversity and species boundaries in cosmopolitan hydrozoans (Cnidaria): molecular and morphological evidence for cryptic species of *Clytia gracilis*. Fifth Workshop of the Hydrozoan Society, Program and Abstracts.
- Maddison, W.P., Maddison, D.R., 2000. *MacClade: Analysis of phylogeny and character evolution*. Version 4.0. Sinauer Associates, Sunderland, MA.
- Millard, N.A.H., 1975. Monograph of the Hydrozoa of southern Africa. *Ann. S. Afr. Mus.* 68, 1–513.
- Miller, R.L., 1973. The role of the gonomedusa and gonangium in the sexual reproduction (fertilization) of the Hydrozoa. In: Tokioka, T., Nishimura, S. (Eds.), *Recent Trends in Research in Coelenterate Biology*. The Proceedings of the Second International Symposium on Cnidaria. *Publ. Seto mar. biol. Lab.* 20, 367–400.
- Morton, J.E., 1957. Mosaic evolution in hydroids. *Nature* 180, 130–131.
- Naumov, D.V., 1960. *Hydroids and Hydromedusae of the USSR*. Israel Program for Scientific Translations, Jerusalem (translated from Russian (1969)).
- Nutting, C.C., 1915. American hydroids part III. The Campanulariidae and the Bonnevilliidae. *Spec. Bull. US Nat. Mus.* 5, 1–126.
- Omland, K.E., 1997. Examining two standard assumptions of ancestral reconstructions: repeated loss of dichromatism in dabbling ducks (Anatini). *Evolution* 51, 1636–1646.
- Östman, C., 1987. New techniques and old problems in hydrozoan systematics. 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. 67–82.
- Petersen, K.W., 1979. Development of coloniality in Hydrozoa. In: Larwood, G., Rosen, B.R. (Eds.), *Biology and Systematics of Colonial Organisms*. Academic Press, London.
- Petersen, K.W., 1990. Evolution and taxonomy in capitate hydroids and medusae (Cnidaria: Hydrozoa). *Zool. J. Linn. Soc.* 100, 101–231.
- Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14, 817–818.
- Ralph, P.M., 1956. Variation in *Obelia geniculata* (Linnaeus, 1758) and *Silicularia bilabiata* (Coughtrey, 1875) (Hydrozoa, f. Campanulariidae). *Trans. R. Soc. N. Z.* 84 (2), 279–296.
- Ralph, P.M., 1957. New Zealand thecate hydroids. Part I.—Campanulariidae and Campanulinidae. *Trans. R. Soc. N. Z.* 84 (4), 811–854.
- Ree, R.H., Donoghue, M.J., 1998. Step matrices and the interpretation of homoplasy. *Syst. Biol.* 47, 582–588.
- Rees, W.J., 1957. Evolutionary trends in the classification of capitate hydroids and medusae. *Bull. Br. Mus. Nat. Hist. Zool.* 4 (9), 1–534.
- Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574.
- Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Mol. Biol. Evol.* 16, 1114–1116.
- Simmons, M.P., Pickett, K.M., Miya, M., 2004. How meaningful are Bayesian support values? *Mol. Biol. Evol.* 21, 188–199.
- Stepenjants, S.D., 1998. *Obelia* (Cnidaria, Medusozoa, Hydrozoa): phenomenon, aspects of investigations, perspectives for utilization. *Oceanogr. Mar. Biol. Ann. Rev.* 36, 179–215.
- Swofford, D.L., 2002. *PAUP* (Phylogenetic Analysis Using Parsimony)*. Sinauer, Sunderland, MA.
- Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties, and weight matrix choice. *Nucleic Acids Res.* 22, 4673–4680.
- Torrey, H.B., 1904. *The hydroids of the San Diego region*. University of California Publications. *Zoology* 2 (1), 1–43.
- Vervoort, W., Watson, J., 2003. *The marine fauna of New Zealand: Leptothecata (Cnidaria: Hydrozoa) (thecate hydroids)*. NIWA Biodiversity Memoir 119, Wellington, NZ.
- Weismann, A., 1883. *Die Entstehung der Sexualzellen bei den Hydromedusen*. Gustav Fischer, Jena.
- Wray, G.A., 1996. Parallel evolution of nonfeeding larvae in echinoids. *Syst. Biol.* 45, 308–322.