



SYMPOSIUM

Character Evolution in Hydrozoa (phylum Cnidaria)

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From the symposium “Assembling the Cnidarian Tree of Life” presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2010, at Seattle, Washington.

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Synopsis The diversity of hydrozoan life cycles, as manifested in the wide range of polyp, colony, and medusa morphologies, has been appreciated for centuries. Unraveling the complex history of characters involved in this diversity is critical for understanding the processes driving hydrozoan evolution. In this study, we use a phylogenetic approach to investigate the evolution of morphological characters in Hydrozoa. A molecular phylogeny is reconstructed using ribosomal DNA sequence data. Several characters involving polyp, colony, and medusa morphology are coded in the terminal taxa. These characters are mapped onto the phylogeny and then the ancestral character states are reconstructed. This study confirms the complex evolutionary history of hydrozoan morphological characters. Many of the characters involving polyp, colony, and medusa morphology appear as synapomorphies for major hydrozoan clades, yet homoplasy is commonplace.

Introduction

Hydrozoans are a group of cnidarians that are noted for their complexity and diversity in life cycles. In many hydrozoan species, the life cycle consists of a free-living planula larva that transforms into a primary polyp. The primary polyp buds other polyps to produce a benthic colonial stage. Upon reproductive maturity, the polyps bud pelagic medusae that ultimately form gametes and spawn in the water column. Within hydrozoans there exists an extraordinary variation in this life cycle that is reflected in a wide range of diversity of polyp, colony, and medusa morphologies, as well as complete loss or reduction of the polyp or medusa stage in some species. The diversity of hydrozoan characters that involve changes in the morphology of these life cycle stages has long been considered important for classification. However, hydrozoan classification schemes have historically been met with much confusion, in large part due to classification based exclusively, or almost exclusively, on either the medusa (Rees 1957; Brinkmann-Voss 1970; Bouillon 1985) or polyp (e.g., Petersen 1979), producing, in many instances, conflicting classifications for different life-cycle stages (discussed by Hyman 1940; Rees 1957; Boero and Bouillon 1987). In addition, polyps, colonies, and

medusae can differ dramatically between closely related species, making it difficult to disentangle the evolutionary history of these different characters. More recently, consideration of the entire life cycle has been used for classification and many of the previous inconsistencies have been reconciled (e.g., Bouillon 1985; Petersen 1990; Schuchert 1996; Bouillon and Boero 2000; Bouillon et al. 2004; Schuchert 2004; Bouillon et al. 2006; Schuchert 2006, 2007). The application of molecular phylogenetic methods has enabled further refinement of hydrozoan classification (Collins et al. 2006), including the placement of enigmatic taxa (Miranda et al. 2010; Evans et al. 2008), and sorting species into well supported clades (Collins et al. 2005; Dunn et al. 2005; Collins et al. 2006; Govindarajan et al. 2006; Leclère et al. 2007; Cartwright et al. 2008; Collins et al. 2008; Lindner et al. 2008; Leclère et al. 2009; Miglietta et al. 2009; Nawrocki et al. 2010). However, given that several key families have not yet been sampled for molecular data and many of the relationships between major hydrozoan clades are not yet resolved (Cartwright et al. 2008), more work is needed before results from molecular data can inform major revisions in hydrozoan classification.

Elucidating the evolutionary history of hydrozoan characters involving the various life-cycle stages would prove useful not only for classification, but also for our understanding of homology, evolutionary loss, and convergence of these characters. Robust reconstructions of ancestral character states require a comprehensive taxonomic sampling, careful coding of morphological character states in terminal taxa, and well-resolved phylogenies. Through the Cnidarian Tree of Life project, a relatively large taxonomic sampling of ribosomal DNA sequences has been made available for phylogenetic investigation. Here we use these data to reconstruct a phylogeny of hydrozoans, code terminal taxa for morphological character states of polyp, colony, and medusa life-cycle stages, and trace the evolutionary history of these characters within a phylogenetic framework.

Evolution Of Coloniality, Colonial Form, And Polymorphism

Hydrozoan colonies are composed of polyps that share a common gastrovascular cavity through the interconnection of tube-like stolons. Hydrozoans display a diversity of colonial forms ranging from stolonial and mat-like encrusting colonies; to upright, irregularly branched or regularly branched pinnate colonies; to pelagic colonies (Fig. 1A). The form of a colony is in large part dictated by the branching patterns of the stolons that connect the polyps (reviewed in Cartwright 2003; Cartwright 2004). Hydrozoans belonging to Porpitidae and Siphonophorae are pelagic colonies that display a highly organized arrangement of modified polyps and medusae within the colony that enables them to function efficiently in the water column (Fig. 1A, middle right). Other hydrozoans lack a colony and instead the benthic stage is represented by a solitary polyp (Fig 1A, right) or the polyp stage is lacking altogether and the planula larva develops directly into a medusa, such as found in Trachymedusae. Coloniality has generally been considered to be a derived state within Hydrozoa (Petersen 1979) and the presence of a polyp, presence of a colony, and colony form, appear important for defining hydrozoan clades. For example, species of the hydrozoan clade Trachylina either lack a polyp stage altogether or possess a very reduced one. Species of Leptothecata are almost all colonial, with many displaying an upright form. Within the polyphyletic Anthoathecata, the form of the colony is variable and some species have only a solitary polyp stage (e.g., Petersen 1979; Marques and Migotto 2001).

Some colonial hydrozoans display polymorphism of polyps, in which morphologically distinct and functionally specialized polyp types are found within the colony. In polymorphic colonies there is a division of labor between feeding and reproduction, and in some cases, defense. *Hydractinia*, a colonial hydrozoan that encrusts gastropod shells usually inhabited by hermit crabs, displays four different polyp types that specialize either in feeding (gastrozoid), reproduction (gonozoid), food gathering and defense (dactylozoid) (Fig. 1B), or defense only (tentaculozoid, not shown). Species of Siphonophorae are pelagic colonies comprising highly specialized polyp and medusoid forms (Fig. 1A, middle right).

Position of the gonophore bud and the evolution of medusae

Upon reproductive maturity, the polyp/colonial stage will bud gonophores. The position of the gonophore bud and the fate of the gonophore vary within Hydrozoa. Most species bud gonophores in proximity to the polyps' tentacles, usually towards the oral end of the polyp (Fig. 1C, left). Some species bud their gonophores at the base of the polyp or from the stalk or stolon (Fig. 1C, right). Rees (1957) noted that those that bud gonophores near the mouth of the polyp often go through "reproductive exhaustion," losing their mouth and thus ability to feed in the process. Given this observation, he viewed the placement of the gonophore proximal to the base of the polyp, and away from the mouth, as an important evolutionary innovation. Species that bear a gonozoid, a specialized polyp that buds gonophores but does not feed, also achieve a physical separation of feeding and reproduction.

In some hydrozoans the gonophore develops into a medusa that detaches from the colony (or solitary polyp), swims, and feeds in the water column, ultimately reaching sexual maturity and spawning gametes. Most hydrozoans however lack a free-swimming medusa and instead the gonophore reaches sexual maturity while remaining attached to the polyp. The developmental stage of the gonophore upon sexual maturity can range from a sporosac that lacks any trace of medusae-like characters (Fig. 1D, left), to gonophores called medusoids that possess some but not all medusae-like characters, such as a canal system and remnants of tentacles (Fig. 1D, middle left), which may or may not detach from the colony, to a fully formed, swimming, feeding medusa (Fig. 1D right). In the Hydridae and other species with solitary reduced polyps gonophores

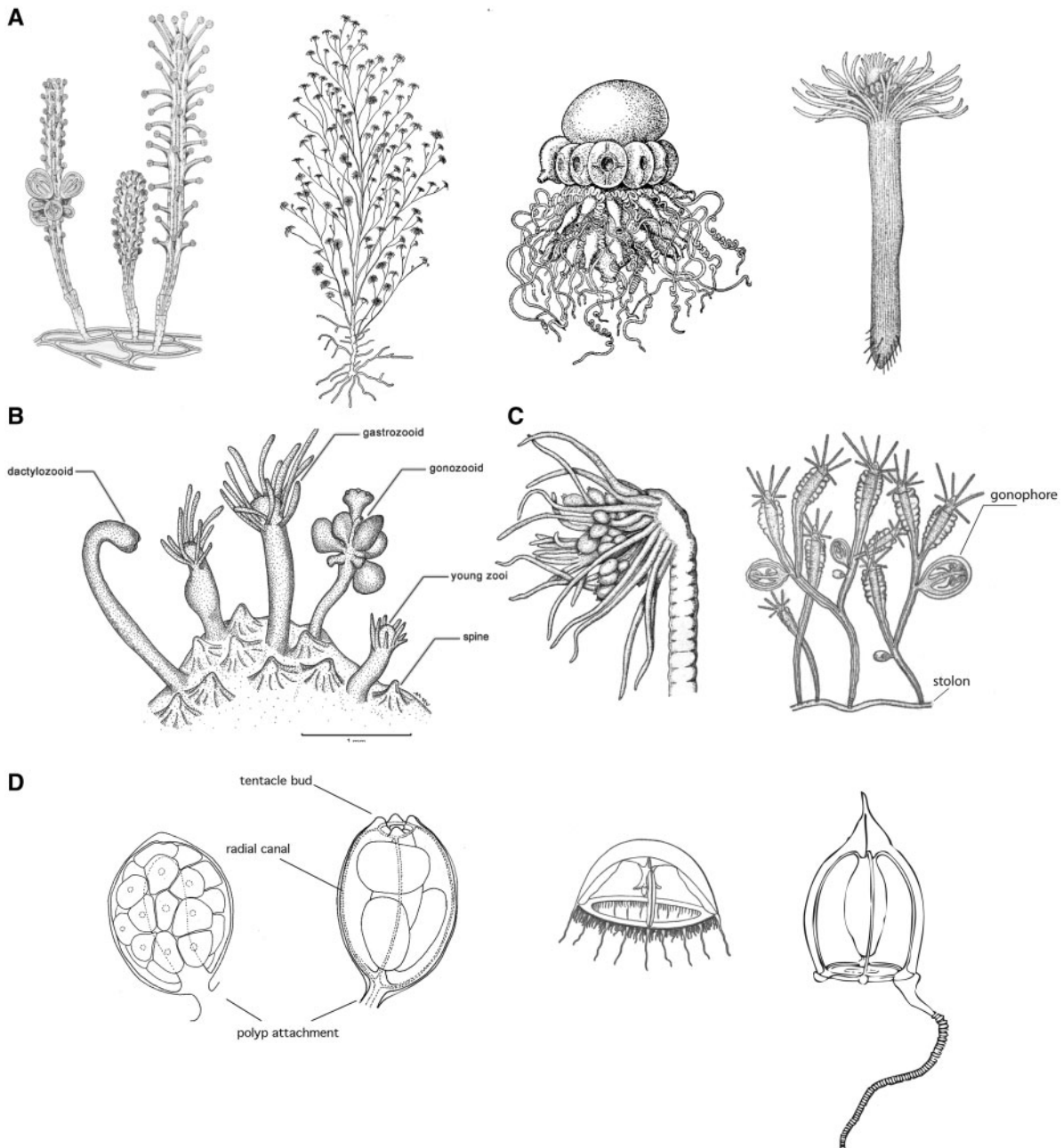


Fig. 1 Life cycle characters in Hydrozoa. (A) Colony Structure. Left to right: stolonial colony of *Zanclea implexa*, upright colony of *Eudendrium* sp., pelagic siphonophore colony of *Stephalia* sp., solitary *Corymorpha pendula*. (B) Polymorphism. A polymorphic colony of *Hydractinia echinata*. Note three distinct polyp types—feeding polyps (gastrozooids), reproductive polyps (gonozooids) and defensive polyps (dactylozooids). (C) Gonophore placement. Left: a distal placement of the gonophores in *Ectopleura* sp. Right: a proximal placement of the gonophores in *Leukartiara octona*. (D) Medusae and reduced reproductive structures. Left to right: reduced structures—sporosac of *Coryne pusilla*, medusoid of *Pennaria disticha*; Hydromedusae— *Mitrocoma* sp. and *Corymorpha bigelowi*. *Zanclea implexa* and *Mitrocoma* sp. from A.G. Mayer (1910), *Ectopleura* sp. from C. W. Hargitt (1901), *Stephalia* sp. and *Eudendrium* sp. modified from L. H. Hyman (1940) and E. Haeckel (1888), *Leukartiara octona* from F. S. Russell (1953), *Corymorpha pendula* from C. M. Fraser (1937), *Corymorpha bigelowi* from F. S. Russell (1939), *Pennaria disticha* medusoid adapted from P. Schuchert (2006) and *Coryne pusilla* sporosac adapted from P. Schuchert (1996).

have been completely lost and the gametes develop within the ectoderm (e.g., *Hydra*, *Sympagohydra*, *Boreohydra* and *Protohydra*) or endoderm (e.g., *Brinkmannia hexactinellidophila*) of the polyp. The

presence of a medusa has been asserted to be ancestral for Hydrozoa (Marques and Collins 2004) and reduced medusae are thought to have been derived during hydrozoan evolution (Boero and Bouillon

1987; Boero and Sarà 1987). Recent phylogenetic hypotheses have supported multiple instances of loss of the medusal stage (Cunningham and Buss 1993; Govindarajan et al. 2006; Leclère et al. 2007; Leclère et al. 2009; Nawrocki et al. in review), partial regain as re-evolved medusoids (Leclère et al. 2009), and instances of re-evolved, fully formed medusae (Marques and Migotto 2001; Nawrocki et al. in review).

Given the apparent complex evolutionary history of hydrozoan life cycles with concomitant changes in polyp, colony, and medusa morphologies, we chose to investigate the evolution of some of these hydrozoan characters within a phylogenetic context. Specifically we coded character states involving overall colony form, polymorphism, position of the gonophore bud and type of gonophore upon sexual maturity. The characters were mapped onto the phylogeny and ancestral states were reconstructed in an effort to better understand the evolutionary history of these prominent components of hydrozoan diversity.

Materials and methods

Molecular data set

The 216 hydrozoan taxa and 14 other medusozoan outgroup taxa sampled in this study are arranged taxonomically in Table 1. DNA sequences used in the phylogenetic analysis are from the small and large nuclear ribosomal subunits (18S and 28S, respectively), and the large mitochondrial ribosomal subunit (16S). All sequences were retrieved from GenBank and accession numbers are shown in Table 1. The 28S and 18S DNA sequences were aligned according to secondary structure models generated for Cnidaria (M.S. Barbeitos, unpublished). Secondary structure models were constructed first by starting with DCSE (De Rijk and Wachter 1993) annotated templates from the scleractinian coral *Montastraea franksi*. For 18S, the template was downloaded from the European Ribosomal RNA Database (<http://bioinformatics.psb.ugent.be/webtools/rRNA/index.html>). For 28S, a starting template from Schnare et al. (1996) was used to hand code into DCSE format the 28S sequence from *Montastraea franksi* (GenBank# AY026375). The secondary structure models were further refined using representatives of all orders within Cnidaria. Sequences in this analysis were hand aligned in Genedoc v.2.6.002 (Nicholas et al. 1997) according to the models (M.S. Barbeitos, pers. comm.). Those regions that could not be confidently aligned according to the model were refined using MFold

(Zuker 2003). Base pairing in stems were evaluated using the PERL script *ReNATon* v0.88 (M.S. Barbeitos, University of Kansas) and alignment was corrected to maximize base pairing (M.S. Barbeitos, personal communication). Loops of variable length were realigned individually in Muscle (v. 3.7) (Edgar 2004) as implemented in SeaView (v.4.2.4) (Gouy et al. 2010).

Given the higher rate of evolution for 16S in Hydrozoa, generating a reliable secondary structure model proved problematic. Instead, 16S rDNA sequences were aligned in the program MAFFT (v.6) (Katoh and Kuma 2002) by employing the E-INS-i strategy (Katoh and Toh 2008). Ambiguously aligned regions in the 18S and 28S loops and in the 16S MAFFT alignment were removed using Gblocks v0.91b (Castresana 2000) under default parameters, except with minimum block length set to five and allowing up to half the taxa to have gaps. Sequences were concatenated in Mesquite (v. 2.72) (Maddison and Maddison 2007) to produce a combined DNA sequence matrix comprising 4513 characters (2664 from 28S, 1472 from 18S and 377 from 16S).

Phylogenetic analysis

Phylogenetic analysis was performed on the combined partitioned data set using maximum likelihood (ML) criteria as implemented in RAxML 7.0.1 (Stamatakis 2006). Two-hundred independent ML searches were conducted under the GTRMIX model using default settings for both number of categories of the gamma distribution and number of re-arrangements of the starting maximum parsimony tree. Node support was also assessed in RAxML using 1000 bootstrap replicates. Support was mapped onto the ML tree using the program SumTrees (Sukumaran and Holder 2009).

Morphological character coding

Morphological characters were coded by consultation of relevant literature. We relied heavily on a recent book by Bouillon et al. (2006) as well as other relevant monographs (Hyman 1940; Petersen 1990; Svoboda and Cornelius 1991; Marques and Migotto 2001; Schuchert 2001, 2006, 2007) and when prudent, original species descriptions. Four morphological characters were coded: the degree of development of the gonophore upon sexual maturity (medusa/medusoid/sporosac/none), the organization of the polyp stage (encrusting colony/upright colony/pelagic colony/solitary/not applicable), types of polymorphic polyps (gastrozoid only/gastrozoid and gonozoid/gastrozoid and dactylozoid/all three polyp types),

Table 1 Samples with GenBank accession numbers, voucher numbers when available, and coding of morphological characters

Taxonomic hierarchy	Species	GenBank accession numbers			Voucher	Characters			
		28s	18s	16s		1	2	3	4
Hydrozoa									
Anthoathecata									
Aplanulata									
Candelabridae	<i>Candelabrum cocksii</i>	AY920796	AY920758	AY512520	MHNGINVE29591	2	3	0	1
	<i>Candelabrum</i> sp.	EU879929		EU876530		?	3	0	?
Corymorphidae	<i>Corymorpha bigelowi</i>	EU272563	EU272618	EU448099	KUNHM2829	0	3	0	1
	<i>Corymorpha intermedia</i>	EU879930	AY920759	AY512526		0	3	0	1
	<i>Corymorpha nutans</i>	EU879931	EU876558	EU876532	MHNGINVE48745	0	3	0	1
	<i>Corymorpha pendula</i>	EU879936	EU876565	EU876538	KUNHM2962	1	3	0	1
	<i>Euphysa aurata</i>	EU879934	EU876562	EU876536	MHNGINVE48753	0	3	0	1
	<i>Euphysa tentacula</i>	EU879935	EU876563	EU876537		0	3	0	1
Hydridae	<i>Hydra circumcincta</i>	EU879939	EU876568	EU876541		3	3	0	2
	<i>Hydra viridissima</i>	EU879940	EU876569	EU876542		3	3	0	2
	<i>Hydra vulgaris</i>	EU879941	EU876570	EU876543		3	3	0	2
Tubulariidae	<i>Ectopleura crocea</i>	EU879932	EU876559	EU876533	MHNGINVE34010	2	1	0	1
	<i>Ectopleura dumortieri</i>	EU879933	EU876561	EU876534		0	3	0	1
	<i>Ectopleura larynx</i>	EU879943	EU876572	EU876545	KUNHMDIZ2963	2	1	0	1
	<i>Ectopleura marina</i>	EU883553	EU883547	EU883542		2	1	0	1
	<i>Hybocodon chilensis</i>	EU879937	EU876566	EU876539	MHNGINVE36023	0	3	0	1
	<i>Hybocodon prolifer</i>	EU879938	EU876567	EU876540		0	3	0	1
	<i>Ralpharia gorgoniae</i>	EU272590	EU272633	EU305482	KUNHM2778	1	3	0	1
	<i>Tubularia indivisa</i>	EU879942	EU876571	EU876544		2	3	0	1
	<i>Zyzyzus warreni</i>	EU272599	EU272640	EU305489	KUNHM2777	2	3	0	1
Capitata									
Asyncorynidae	<i>Asyncoryne ryniensis</i>	GQ424289	EU876578	EU876552	KUNHMDIZ2639	0	0	0	1
Cladocorynidae	<i>Cladocoryne floccosa</i>	EU272551	EU272608	EU876554		2	0	0	1
Cladonematidae	<i>Eleutheria claparedei</i>	GQ424292	GQ424320	AM088486	MHNGINVE34228	0	0	0	1
	<i>Eleutheria dichotoma</i>	GQ424291	GQ424321	AY512538		0	0	0	1
	<i>Staurocladia radiatum</i>	GQ424290	EU448096	AM088482		0	0	0	1
	<i>Staurocladia wellingtoni</i>	EU879948	GQ424323	EU876550	MHNGINVE25379	0	1	0	1
Corynidae	<i>Codium prolifera</i>	GQ424302		GQ395318	MHNGINVE49490	0	?	?	?
	<i>Coryne epizoica</i>	GQ424295		GQ395314	MHNGINVE37171	?	0	0	?
	<i>Coryne eximia</i>	GQ424296	GQ424325	AJ878713	MHNGINVE34009	0	1	0	1
	<i>Coryne muscoides</i>	GQ424298	GQ424328	GQ395315		2	1	0	1
	<i>Coryne pintneri</i>	GQ424303	GQ424330	AJ878717	MHNGINVE31976	2	0	0	1
	<i>Coryne pusilla</i>	GQ424304	AJ133558	AY512552	MHNGINVE35756	2	1	0	1
	<i>Coryne uchidai</i>	GQ424306	GQ424332	GQ395320	MHNGINVE49102	2	0	0	1
	<i>Dipurena halterata</i>	EU883550	EU883544	AM084261	MHNGINVE31741	0	0	0	1
	<i>Polyorchis haplus</i>	GQ424317	GQ424344	AY512549		0	?	?	?
	<i>Sarsia lovenii</i>	GQ424310	GQ424337	GQ395329	MHNGINVE48736	1	0	0	1
	<i>Sarsia princeps</i>	EU879947	EU876575	EU876549		0	0	0	1
	<i>Sarsia striata</i>	GQ424311	GQ424338	GQ395328	MHNGINVE35765	0	0	0	1
	<i>Sarsia tubulosa</i>	EU879946	EU876574	EU876548	MHNGINVE35763	0	0	0	1
	<i>Scrippisia pacifica</i>	AY920804	AF358091	AY512551		0	?	?	?
	<i>Stauridiosarsia cliffordi</i>	GQ424294	GQ424324	GQ395313	MHNGINVE36025	0	1	0	1
	<i>Stauridiosarsia gemmifera</i>	EU879945	EU876573	EU876547		0	0	0	1
	<i>Stauridiosarsia nipponica</i>	GQ424299	GQ424329	GQ395333	KUNHM2627	0	0	0	1
	<i>Stauridiosarsia ophiogaster</i>	EU272560	EU272615	EU305473	KUNHM2803	0	0	0	1
<i>Stauridiosarsia producta</i>	GQ424301	GQ424331	GQ395317	MHNGINVE48751	0	0	0	1	
<i>Stauridiosarsia reesi</i>	GQ424308	GQ424334	GQ395321		0	0	0	1	
Hydrocorynidae	<i>Hydrocoryne miurensis</i>	GQ424313			KUNHM2814	0	3	0	1
Milleporidae	<i>Millepora</i> sp.	EU879950	EU876577	EU876551		1	1	2	0
Moerisiidae	<i>Moerisia</i> sp.	AY920801	AF358083	AY512534		0	3	0	1
	<i>Odessia maeotica</i>	GQ424314	GQ424341	GQ395324	MHNGINVE53642	0	3	0	1

(continued)

Table 1 Continued

Taxonomic hierarchy	Species	GenBank accession numbers			Voucher	Characters			
		28s	18s	16s		1	2	3	4
Pennariidae	<i>Pennaria disticha</i>	GQ424316	GQ424342	AM088481	MHNGINVE29809	1	1	0	1
Porpitidae	<i>Porpita porpita</i>	EU883551	GQ424319	AY935322		0	2	3	1
	<i>Porpita</i> sp.	AY920803	AF358086			0	2	3	1
	<i>Velella velella</i>	EU879949	EU876576	EU305487		0	2	3	1
Solanderiidae	<i>Solandaria ericopsis</i>	EU272593	EU272636	AY512530	MHNGINVE29593	1	1	0	0
	<i>Solandaria secunda</i>	EU305533	EU305502	EU305484	KUNHM2611	1	1	0	0
Sphaerocorynidae	<i>Sphaerocoryne agassizii</i>	GQ424318		GQ395323		0	0	0	1
Zanclidae	<i>Zanclia costata</i>	EU879951	EU876579	EU876553	MHNGINV26507	0	0	0	1
	<i>Zanclia prolifera</i>	EU272598	EU272639		KUNHM2793	0	?	?	?
Filifera I									
Eudendriidae	<i>Eudendrium californicum</i>	EU305513	EU305492	EU305475	KUNHM2858	2	1	1	1
	<i>Eudendrium capillare</i>	EU305514		EU305476	KUNHM2625	2	1	1	1
	<i>Eudendrium glomeratum</i>	FJ550440	FJ550583	AM991301	MHNGINVE49717	2	1	1	1
	<i>Eudendrium racemosum</i>	EU272562		AY787896	MHNGINVE32164	2	1	1	1
Filifera II									
<i>incerta sedis</i>	<i>Brinckmannia hexactinellidophila</i>	EU272550	EU272607	AM183123	MHNGINVE38148	3	3	0	1
Laingiidae	<i>Fabienna sphaerica</i>	AY920797	AY920767	AM183133	MHNGINVE33453	0	?	?	?
Ptilocodiidae	<i>Hydrichthella epigorgia</i>	EU272569	EU272622	EU305478	KUNHM2665	1	0	3	1
Proboscoidactylidae	<i>Proboscoidactyla flavicirrata</i>	EU305527	EU305500	EU305480		0	0	3	1
	<i>Proboscoidactyla ornata</i>	EU272587	EU272631	EU305481	KUNHM2767	0	0	3	1
Filifera III									
Bougainvilliidae	<i>Dicoryne conybeari</i>	EU272559	EU272614	AM183141	MHNGINVE32949	2	0	1	1
Hydractiniidae	<i>Clava multicornis</i>	EU272552	EU272609	EU305471		2	0	0	1
	<i>Clavactinia gallensis</i>	EU272553	EU272610	EU448101	MHNGINVE33470	2	0	1	1
	<i>Hydractinia symbiolongicarpus</i>	EU272568	EU272621	FJ214380		2	0	3	1
	<i>Podocoryna exigua</i>	AY920802	AF358092	AY512513		0	0	1	1
	<i>Podocorynoides minima</i>	EU883552		AM183125		0	?	?	?
Stylasteridae	<i>Adelopora crassilabrum</i>	EU272541	EU272642	EU645356	USNM1027760	2	1	3	0
	<i>Conopora anthohelia</i>	EU305509	EU645429	EU645268	USNM1027755	2	1	3	0
	<i>Crypthelia cryptotrema</i>	EU272558	EU272641	EU645281	USNM1027758	2	1	3	0
	<i>Lepidopora microstylus</i>	EU272572	EU272644	EU645329	USNM1027724	2	1	3	0
	<i>Pseudocrypthelia pachypoma</i>	EU272589	EU272643	EU645280	USNM1027728	2	1	3	0
Filifera IV									
Bougainvilliidae	<i>Bimeria vestita</i>	EU272548	EU272605	AM183130		2	1	0	0
	<i>Bougainvillia carolinensis</i>	EU272549	EU272606			0	1	0	0
	<i>Bougainvillia fulva</i>	EU305507	EU305490	EU305470	KUNHM2816	0	1	0	0
	<i>Bougainvillia muscus</i>	FJ550439	FJ550582	AM411410	MHNGINVE48761	0	1	0	0
	<i>Garveia annulata</i>	EU272564	AY920766		KUNHM2860	2	1	0	0
	<i>Garveia grisea</i>	EU272588	EU272632	AM183131	MHNGINVE34436	2	1	0	0
	<i>Koellikerina fasciculata</i>	EU272571	EU272623	AM183129		0	0	0	0
	<i>Pachycordyle pusilla</i>	EU272579	EU272627	AM183132	MHNGINVE32953	2	0	0	0
Cytaeidae	<i>Perarella schneideri</i>	HM357628	HM357626			1	0	2	0
Oceaniidae	<i>Cordylophora caspia</i>	EU272556	EU272612	EU305472		2	1	0	0
	<i>Rhizogeton nudus</i>	EU272592	EU272635	AY787883	MHNGINVE35757	2	0	0	0
	<i>Turritopsis dohrnii</i>	EU272596	EU272638	AY787889	MHNGINVE29753	0	1	0	0
	<i>Turritopsis</i> sp.	EU305538	EU305504	EU305486	KUNHM2817	0	1	0	0
Pandeidae	<i>Hydrichthys boycei</i>	EU272570	EU305496	EU448102	MHNGINVE37417	0	0	1	0
	<i>Leuckartiara octona</i>	EU272573	EU272624	AM411421		0	0	0	0
	<i>Neoturris brevicornis</i>	EU305524	EU448097	EU448103		0	0	0	0
	<i>Pandea</i> sp.	EU272580				0	0	2	0
Rathkeidae	<i>Lizzia blondina</i>	EU272574	EU272625	AM411417		0	?	?	0
	<i>Rathkea octopunctata</i>	EU272591	EU272634	EU305483	KUMIP314321	0	0	?	?

(continued)

Table 1 Continued

Taxonomic hierarchy	Species	GenBank accession numbers			Voucher	Characters			
		28s	18s	16s		1	2	3	4
Leptothecata									
Macrocolonia									
Aglaopheniidae	<i>Aglaophenia elongata</i>	FJ550450	FJ550593	FJ550508	MHNGINVE37539	2	1	3	1
	<i>Aglaophenia harpago</i>	FJ550449	FJ550592	FJ550506	MHNGINVE37531	2	1	3	1
	<i>Aglaophenia octodonta</i>	FJ550397	FJ550541	AM887989	MHNGINVE32875	2	1	3	1
	<i>Aglaophenia pluma</i>	FJ550398	FJ550542	DQ855916	MHNGINVE38220	2	1	3	1
	<i>Aglaophenia tubiformis</i>	EU272543	EU272601	AM887991	MHNGINVE31751	2	1	3	1
	<i>Cladocarpus integer</i>	FJ550453	FJ550597	FJ550512	MHNGINVE48754	2	1	3	1
	<i>Gymnangium gracilicaule</i>	FJ550442	FJ550585	DQ855934	MHNGINVE36839	2	1	3	1
	<i>Lytocarpia</i> sp.	FJ550448	FJ550591	AY787921	MHNGINVE36828	2	1	3	1
	<i>Macrorhynchia phoenicea</i>	FJ550441	FJ550584	DQ855935	MHNGINVE36813	2	1	3	1
	Haleciidae	<i>Halecium labrosum</i>	FJ550407	FJ550550	AY787916	MHNGINVE29030	2	1	1
<i>Halecium lenticulare</i>		FJ550387		FJ550469	MHNGINVE33461	2	1	1	1
<i>Halecium muricatum</i>		FJ550408	FJ550551	AY787915	MHNGINVE29028	2	1	1	1
<i>Halecium pusillum</i>		FJ550437	FJ550580	FJ550499	MHNGINVE36295	2	1	1	1
<i>Hydranthea margarica</i>		FJ550424	FJ550567	DQ855932		1	0	1	1
<i>Hydrodendron mirabile</i>		FJ550425	FJ550568	DQ855933	MHNGINVE34779	2	1	3	1
Halopterididae	<i>Antennella ansini</i>	FJ550388	FJ550533	AY787890	MHNGINVE32157	2	1	3	1
	<i>Antennella kiwiana</i>	FJ550389	FJ550534	DQ855918	MHNGINVE33623	2	1	3	1
	<i>Antennella secundaria</i>	FJ550432	FJ550575	DQ883445	MHNGINVE32969	2	1	3	1
	<i>Halopteris carinata</i>	FJ550433	FJ550576	DQ855919	MHNGINVE35473	2	1	3	1
	<i>Halopteris diaphana</i>	FJ550378	FJ550525	DQ855921	MHNGINVE30116	2	1	3	1
	<i>Halopteris liechtensternii</i>	FJ550379	FJ550526	AY787888	MHNGINVE30116	2	1	3	1
	<i>Halopteris minuta</i>	EU272567	EU272620	AY787912	MHNGINVE25073	2	1	3	1
	<i>Halopteris schucherti</i>	FJ550434	FJ550577		MHNGINVE35930	2	1	3	1
	<i>Monostaechas quadridens</i>	EU305521	EU305497	DQ855941		2	1	3	1
Kirchenpaueriidae	<i>Kirchenpaueria pinnata</i>	FJ550435	FJ550578	AY787911	MHNGINVE36294	2	1	3	1
	<i>Kirchenpaueria similis</i>	FJ550438	FJ550581	DQ855923	MHNGINVE36296	2	1	3	1
Plumulariidae	<i>Plumularia obliqua</i>	FJ550401	FJ550544	DQ855929		1	1	3	1
	<i>Nemertesia antennina</i>	EU305523	EU305498	AM888329	MHNGINVE29954	2	1	3	1
	<i>Plumularia filicaulis</i>	FJ550422	FJ550565	DQ855926	MHNGINVE34020	2	1	3	1
	<i>Plumularia habereri</i>	FJ550428	FJ550571			2	1	3	1
	<i>Plumularia hyalina</i>	EU305525	EU305499	AY787913	MHNGINVE25333	2	1	3	1
	<i>Plumularia</i> sp.	FJ550421	FJ550564	FJ550491	MHNGINVE34019	2	1	3	1
	<i>Plumularia margaretta</i>	FJ550410	FJ550553	AY787892	MHNGINVE29760	2	1	3	1
	<i>Plumularia pulchella</i>	FJ550419	FJ550562		MHNGINVE34016	2	1	3	1
	<i>Plumularia setacea</i>	EU272583	EU272628	AY787886	MHNGINVE36298	2	1	3	1
	<i>Plumularia setaceoides</i>	FJ550394	FJ550538	DQ855931	MHNGINVE33460	2	1	3	1
	<i>Plumularia spiralis</i>	FJ550426	FJ550569	AY787920	MHNGINVE32600	2	1	3	1
	<i>Plumularia strictocarpa</i>	HM357629	HM357627	DQ855940		2	1	3	1
Sertulariidae	<i>Abietinaria abietina</i>	FJ550411	FJ550554	AY787898		2	1	1	1
	<i>Abietinaria filicula</i>	EU272540	EU272600	AY787899	MHNGINVE29947	2	1	1	1
	<i>Amphisbetia minima</i>	EU272544	EU272602	AY787903	MHNGINVE25071	2	1	1	1
	<i>Amphisbetia operculata</i>	FJ550418	FJ550561	FJ550489	MHNGINVE34014	1	1	1	1
	<i>Diphasia fallax</i>	FJ550414	FJ550557	AY787901	MHNGINVE29950	2	1	1	1
	<i>Dynamena moluccana</i>	FJ550429	FJ550572	FJ550494		2	1	1	1
	<i>Hydrallmania falcata</i>	FJ550416	FJ550559	AY787900	MHNGINVE29948	2	1	1	1
	<i>Sertularella africana</i>	FJ550420	FJ550563	FJ550490	MHNGINVE34017	2	1	1	1
	<i>Sertularia cupressina</i>	FJ550395	FJ550539	AY787905	MHNGINVE29949	2	1	1	1
	<i>Sertularella mediterranea</i>	FJ550403	FJ550546	FJ550479	MHNGINVE32948	2	1	1	1
	<i>Sertularia perpusilla</i>	EU305532		AY787894	MHNGINVE29765	2	1	1	1
	<i>Symplectoscyphus turgidus</i>	FJ550377	FJ550524	FJ550462	MHNGINVE29467	2	1	1	1
	<i>Thyrososcyphus marginatus</i>	FJ550430	FJ550573	FJ550495	MHNGINVE35477	2	1	1	1
	<i>Thuaria thuja</i>	EU305536	EU305503	AY787908	MHNGINVE29951	2	1	1	1
	Statocysta								
Aequoreidae	<i>Aequorea aequorea</i>	EU305505	AF358076	AY512518		0	0	1	1
	<i>Aequorea floridana</i>	EU305506				0	0	1	1
	<i>Aequorea victoria</i>	AY920799	AF358077	EU305469	KUNHM2867	0	0	1	1
	<i>Rhacostoma atlantica</i>	EU305528	EU305501			0	?	?	?

(continued)

Table 1 Continued

Taxonomic hierarchy	Species	GenBank accession numbers			Voucher	Characters			
		28s	18s	16s		1	2	3	4
Blackfordiidae	<i>Blackfordia virginica</i>	AY920800	AF358078	AY512516		0	0	1	1
Campanulariidae	<i>Clytia hemisphaerica</i>	FJ550457	AY789753	EU999221		0	0	1	1
	<i>Clytia noliformis</i>	EU272554	DQ064795	DQ064792		0	0	1	1
	<i>Eucheilota maculata</i>	FJ550444	FJ550587	FJ550501		0	0	1	1
	<i>Eucheilota menoni</i>	FJ550427	FJ550570	FJ550493	MHNGINVE33457	0	0	1	1
	<i>Gonothyrax loveni</i>	FJ550404	AY789766	AY789827	MHNGINVE29034	2	1	1	1
	<i>Laomedea calceolifera</i>	FJ550447	AY789768	AY789829	MHNGINVE37296	2	1	1	1
	<i>Obelia bidentata</i>	FJ550446	AY789754	AY789815	MHNGINVE37294	0	1	1	1
	<i>Obelia geniculata</i>	FJ550405	AY789769	AY530362		0	1	1	1
	<i>Silicularia rosea</i>	FJ550406	AY789727	AY789792	MHNGINVE25072	1	0	1	1
Campanulinidae	<i>Calycella syringa</i>	FJ550372	AY789776	AY789833		0	0	1	1
	<i>Campanulina panicula</i>	FJ550452	FJ550596	FJ550511	MHNGINVE48748	2	1	1	1
Eirenidae	<i>Eirene viridula</i>	FJ550445	FJ550588	FJ550502		0	0	1	1
	<i>Eutima curva</i>	FJ550455	FJ550599	FJ550514	MHNGINVE33468	0	?	1	1
	<i>Eutima gegenbauri</i>	FJ550456	FJ550600	FJ550515	MHNGINVE31748	0	0	1	1
	<i>Eutima sapinhua</i>	EU305515	EU305493			0	?	1	1
Malagazziidae	<i>Octophialucium indicum</i>	EU272577	EU272626	AY787897	MHNGINVE29970	0	0	1	1
Mitrocomidae	<i>Mitrocomella niwai</i>	FJ550392	FJ550536	FJ550473		0	0	1	1
	<i>Tiaropsidium kelseyi</i>	EU305537	AF358079	EU305485		0	0	1	1
	<i>Tiaropsis multicirrata</i>	FJ550386	FJ550531	FJ550468		0	0	1	1
Phialellidae	<i>Phialella quadrata</i>	FJ550393	FJ550537	FJ550474	MHNGINVE33466	0	1	1	1
	<i>incerta sedis</i>								
Hebellidae	<i>Anthohebella parasitica</i>	EU272545	EU272603	AY787918	MHNGINVE29762	1	0	1	1
Lafoeidae	<i>Hebella venusta</i>	FJ550431	FJ550574	FJ550496	MHNGINVE35476	0	0	1	1
	<i>Lafoea dumosa</i>	EU305520		AY787917	MHNGINVE29952	2	1	1	1
Melicertidae	<i>Melicertum octocostatum</i>	EU272575	AY920757	EU305479	G.Mackie:AGC209	0	0	0	1
Tiarannidae	<i>Stegopoma plicatile</i>	FJ550454	FJ550598	FJ550513	MHNGINVE48755	2	1	1	1
Laodiceidae	<i>Staurodiscus gotoi</i>	FJ550391	FJ550535	FJ550472	MHNGINVE33467	0	0	1	1
Siphonophorae									
Calycophorae									
Clausophyidae	<i>Clausophyes ovata</i>	EU305508	AY937336	AY935294	YPM35349	1	2	0	0
Diphyidae	<i>Sulculeolaria quadrivalvis</i>	EU272594	AY937329	AY935288	YPM35357	1	2	0	0
Hippopodiidae	<i>Hippopodius hippopus</i>	EU305517	AY937356	AY935314	YPM35045	1	2	0	0
Prayidae	<i>Nectopyramis</i> sp.	AY026377	AY937349	AY935307		1	2	0	0
	<i>Praya dubia</i>	EU305526	AY937326	AY935285	YPM35346	1	2	0	0
	<i>Rosacea flaccida</i>	EU305529	AY937328	AY935287	YPM35041	1	2	0	0
Cystonectae									
Physaliidae	<i>Physalia physalis</i>	EU448095	AF358065		YPM35345	1	2	3	1
Physonectae									
Agalmatidae	<i>Cordagalma cordiforme</i>	EU272555	AY937317	AY935275	YPM35032	1	2	1	1
	<i>Halistemma rubrum</i>	EU272566	AY937323	AY935281	YPM35359	1	2	1	1
	<i>Nanomi bijuga</i>	EU272576	AY937338	AY935296	YPM35043	1	2	1	1
	<i>Apolemia</i> sp.	EU272546	AY937315	AY935273	YPM35090	1	2	3	1
Erennidae	<i>Erenna</i> sp.	EU305512	AY937361		YPM35362	1	2	1	1
Forskaliidae	<i>Forskalia edwardsi</i>	EU305516	AY937354	AY935312	YPM35036	1	2	1	1
Physophoridae	<i>Physophora hydrostatica</i>	EU272582	AY937342	AY935300	YPM35046	1	2	1	1
Rhodaliidae	<i>Stephalia dilata</i>	EU305534	AY937357	AY935315	YPM35358	1	2	1	1

(continued)

Table 1 Continued

Taxonomic hierarchy	Species	GenBank accession numbers			Voucher	Characters			
		28s	18s	16s		1	2	3	4
Trachylina									
Limnomedusae									
Olindiasidae	<i>Aglauropsis aeora</i>	AY920793	AY920754	EU293973		0	3	0	1
	<i>Astrohydra japonica</i>	AY920794		EU293975		0	3	0	1
	<i>Limnocnida tanganyicae</i>	AY920795	AY920755	EU293972		0	0	0	1
	<i>Maeotias marginata</i>	EU247810	AF358056	AY512508		0	3	0	1
	<i>Olindias phosphorica</i>	EU247808	AY920753	AY512509	MHNGINVE29811	0	3	0	1
	<i>Olindias sambaquiensis</i>	EU247809	EU247814	EU293977		0	3	0	1
Narcomedusae									
Aeginidae	<i>Aegina citrea</i>	AY920789	EU247813	EU293997		0	4	4	2
	<i>Solmundella bitentaculata</i>	EU247795	EU247812	EU293998	MHNGINVE31746	0	4	4	2
Cuninidae	<i>Sigiweddella</i> sp.	EU247796		EU293996	JAMSTECI060320CN1	0	4	4	2
	<i>Solmissus marshalli</i>	AY920790	AF358060	EU294001		0	4	4	2
Tetraplatiidae	<i>Tetraplatia volitans</i>	DQ002502	DQ002501	EU293999	KUMIP314322	0	4	4	2
Trachymedusae									
Geryoniidae	<i>Geryonia proboscidalis</i>	EU247807	EU247816	EU293979	JAMSTEC RB-BWD-4	0	4	4	2
Haliscratidae	<i>Botrynema brucei</i>	EU247798	EU247822	EU293982	JAMSTECI060319bN4	0	4	4	2
	<i>Haliscera conica</i>	EU247797	EU247825	EU293981		0	4	4	2
Rhopalonematidae	<i>Aglantha digitale</i>	AY920791	EU247821	EU293985		0	4	4	2
	<i>Aglaura hemistoma</i>	EU247803	EU247818	EU293984	MHNGINVE31745	0	4	4	2
	<i>Amphogona apicata</i>	EU247801		EU293994	JAMSTEC I060314A2	0	4	4	2
	<i>Crossota rufobrunnea</i>	EU247800	EU247824	EU293986	JAMSTECI060319bN4	0	4	4	2
	<i>Pantachogon haeckeli</i>	AY920792	AF358062	EU293988		0	4	4	2
<i>Rhopalonema velatum</i>	EU247804	EU247819	EU293992		0	4	4	2	
Scyphozoa									
Coronatae									
Atollidae	<i>Atolla vanhoeffeni</i>	AY026368	AF100942			0	3	0	2
Nausithoidae	<i>Nausithoe rubra</i>	AY920776	AF358095			0	3	0	2
Rhizostomeae									
Catostylidae	<i>Catostylus</i> sp.	AY920777	AF358100			0	3	0	2
Semaestomeae									
Pelagiidae	<i>Chrysaora melanaster</i>	AY920780	AF358099			0	3	0	2
Ulmaridae	<i>Phacellophora camtschatica</i>	AY920778	AF358096			0	3	0	2
Staurozoa									
Cleistocarpida									
	<i>Craterolophus convolvulus</i>	AY920781	AY845344	AY845343		0	3	0	1
Cubozoa									
Carybdeida									
Alatinidae	<i>Alatina mordens</i>	GQ849058	GQ849082		USNM1124421	0	3	0	2
Carukiidae	<i>Carukia barnesi</i>	GQ849059	AF358107	GQ849097	USNM1124558	0	3	0	2
Carybdeidae	<i>Carybdea rastonii</i>	AY920787	AF358108	GQ849112	AMSG17493	0	3	0	2
	<i>Carybdea xaymacana</i>	GQ849067	GQ849090	GQ849114	USNM1073334	0	3	0	2
Tamoyidae	<i>Tamoya haplonema</i>	GQ849062	GQ849085	GQ849122		0	3	0	2
Tripedaliidae	<i>Tripedalia cystophora</i>	GQ849065	GQ849088	GQ849124	USNM1124454	0	3	0	2
Chiropodidae	<i>Chiropsella bronzie</i>	AY920786	AF358103	GQ849099	QMG327938	0	3	0	2
	<i>Chiropsalmus quadrumanus</i>	GQ849056	GQ849079	GQ849111	DZUFRJ1104	0	3	0	2

Characters are described in detail in the Appendix 1.

KUMIP=University of Kansas Museum of Invertebrate Paleontology, KUNHM=University of Kansas Natural History Museum; MHNG=Muséum d'histoire naturelle de Genève; YPM=Yale Peabody Museum; USNM=US National Museum of Natural History; JAMSTEC=Japan agency for marine-earth science and technology.

and the position of the gonophore (proximal to the base of the polyp/towards the oral end of the polyp/not applicable). Morphological characters and character states are described in detail in the Appendix 1 and character coding is summarized in Table 1.

Reconstruction of ancestral character states

Reconstructions of characters using a global likelihood criterion were performed in Mesquite (Maddison and Maddison 2007) on the ML tree generated from the combined analysis (Supplementary Fig. S1). ML reconstructions were performed using the MK1 (Markov k-state 1 parameter model), which corresponds to the Mk model from Lewis (2001). This model estimates a single parameter (the rate of change in character state) from the data. All transition rates of character states are thus set as equal. For each node in the tree, reconstruction techniques determined the character state that maximized the global distribution of characters at the tips of the tree (while allowing all other node values to vary). Such reconstructions must be carried out on a fully resolved tree with branch lengths, and thus are dependent both on the tree's topology and its branch lengths. A likelihood decision threshold (LDT) of 2.0 log units was used as a cutoff to determine the best estimate for a character state at each particular node (Pagel 1999). The assignment of the best estimate of character state was determined by taking the difference in log likelihood scores between character states (Pagel 1999). If the difference between states differed by 2.0 log units or more, the state with the lower likelihood score was rejected, and the state with the higher likelihood score was reported as the best estimate for the character state at that node. If the difference in log likelihood scores did not exceed 2.0 log units, the node was reported as uncertain.

Results and discussion

Phylogenetic patterns

The phylogeny constructed from the combined data matrix with the bootstrap values is shown in Supplementary Figure S1. This topology is largely congruent with previous studies using the same markers (e.g., Cartwright et al. 2008; Collins et al. 2008; Leclère et al. 2009). The monophyly of Hydrozoa is well supported, with a bootstrap value (bt) of 99. In addition, the hydrozoan clade Trachylina is well supported (bt = 91) but the more diverse Hydroidolina is not (bts = 56). Within Hydroidolina, this phylogeny contains several

well-supported clades (bts >95), including Capitata, Aplanulata, Siphonophorae and Leptothecata. Anthoathecata (Capitata, Aplanulata, Siphonophorae and Filifera) is polyphyletic, consistent with previous studies (Collins et al. 2006; Cartwright et al. 2008). Filifera is polyphyletic and the filiferan clades are labeled I–IV (Figs. 2 and 3, Supplementary Figure) according to Cartwright et al. (2008). These clades are recognized on the basis of their monophyly in the molecular analysis (although some are weakly supported) and on their morphological synapomorphies (discussed in Cartwright et al. 2008). This analysis failed to recover strong support for the deeper nodes that reflect relationships among major clades of Hydroidolina. Thus, robust reconstructions of ancestral states for these deep nodes are unreliable. Instead, we focus our discussion on the well-supported clades within Hydroidolina. Figures 2 and 3 depict topologies identical to Supplementary Fig. S1. Although the names of the terminal taxa are not shown in Figs. 2 and 3, the order of the taxa from top to bottom is exactly the same as Supplementary Fig. S1.

Evolution of hydrozoan medusae

Figure 2A depicts reconstructions of ancestral character states for medusae and reduced forms of medusae (sporosacs and medusoids). The presence of a medusa was recovered as ancestral for hydrozoans. Within Capitata, medusae are reduced to medusoids three separate times and to sporosacs two times. The phylogenetic patterns within the filiferan clades indicate that there are likely several independent reductions to medusoids and sporosacs, but because of poorly-supported sister-group relationships most of the reconstructions are uncertain. One instance of medusal re-evolution is recovered in *Podocoryna exigua* (Filifera III). Medusae are absent in the leptothecate clade Macrocolonia (Leclère et al. 2009), with one independent regain of a medusoid in *Amphisbetia operculata*. Species with medusae, medusoids and sporosacs are found in the leptothecate clade Statocysta, with multiple instances of medusal reduction, consistent with the findings of Leclère et al. (2009). Within Aplanulata, one instance of medusal re-evolution in *Ectopleura dumortieri* and several independent reductions to medusoids and sporosacs were recovered, consistent with the findings of Nawrocki et al. (in review). Gonophores were lost completely in *Brinkmannia hexactinellidophila* (Filifera II) and in the Hydra lineage (Aplanulata).

The complex history of hydrozoan medusae has been discussed extensively amongst researchers of

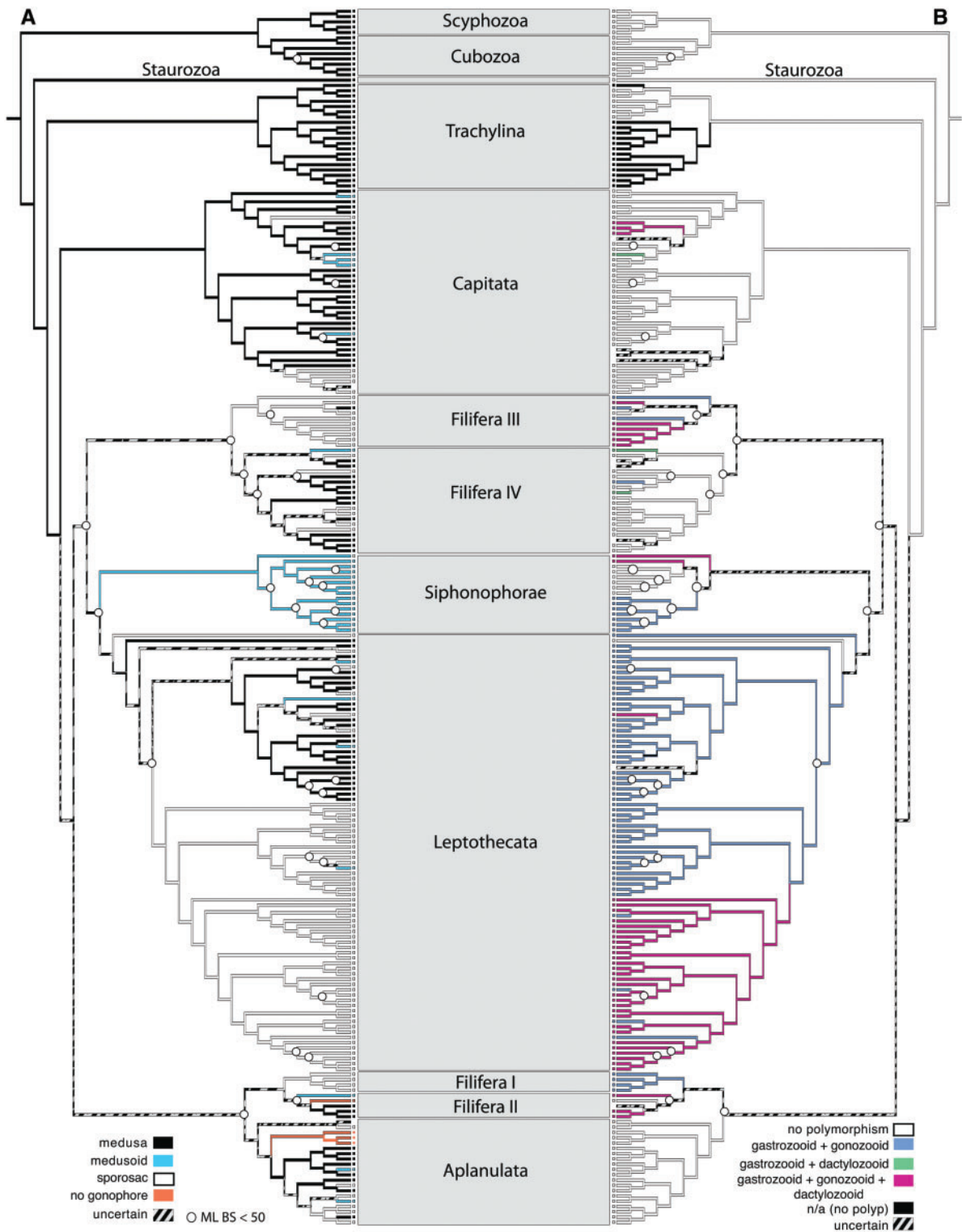


Fig. 2 (A) Reconstructions of ancestral character states of taxa with medusa (black), medusoid (blue), sporosac (white), or of uncertain status (hatched). (B) Reconstructions of ancestral character states of taxa displaying polymorphism: no polymorphism (white), gonozoid (blue), dactylozoid (green), gonozoid plus dactylozoid (pink), no polyp (black), or of uncertain status (hatched). Topology is the ML tree recovered from the combined data set. Open circles at nodes represent poorly supported nodes (bootstrap support <50). A LDT was set at 2.0 log units. The identical topology with the exact order of terminal taxa, but including the species names and bootstrap values, can be found in the Supplementary Figure.

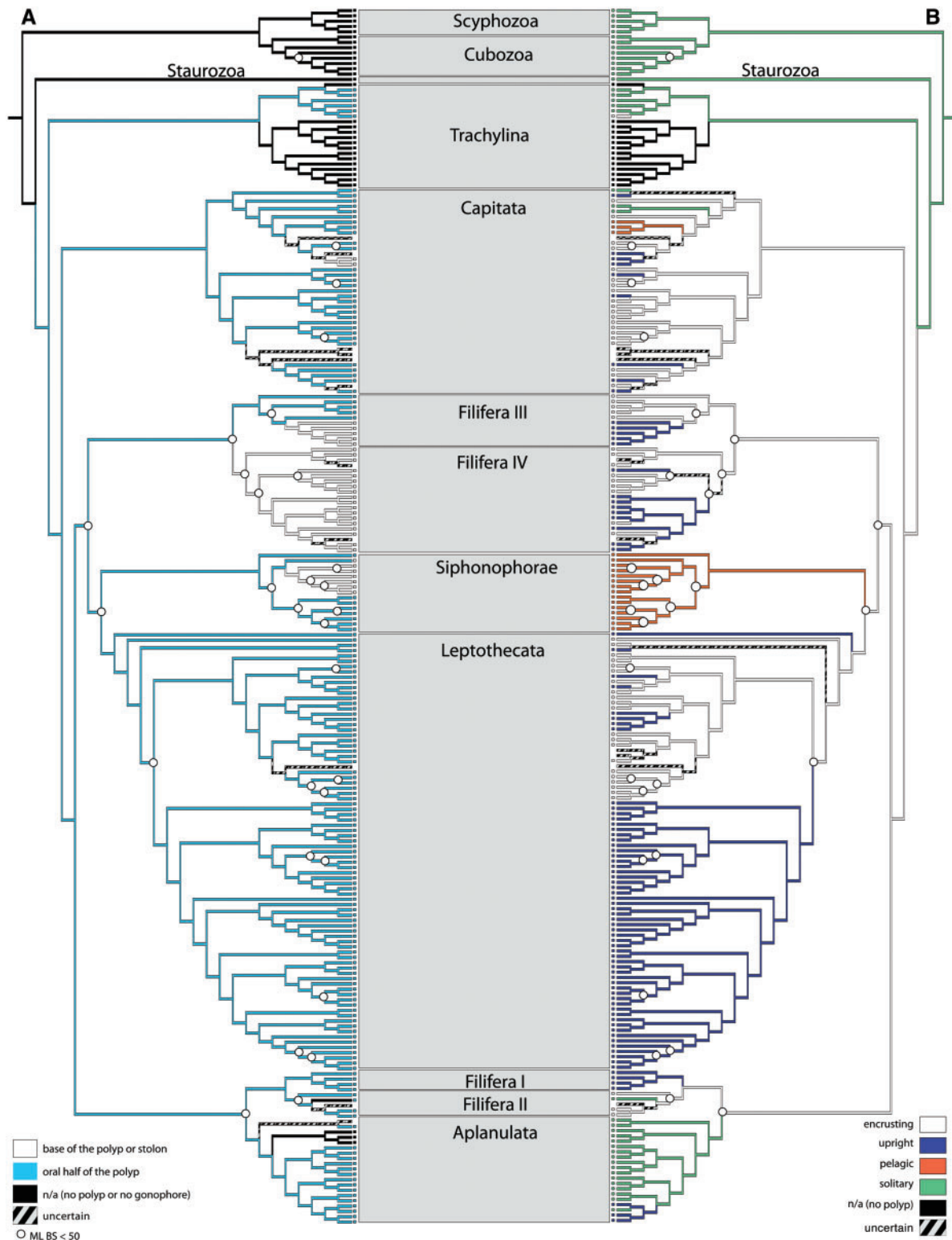


Fig. 3 (A) Reconstructions of ancestral character states of position of gonophore bud. Position at the base of the polyp or stolon (white), position towards the oral end of the polyp (blue), position not applicable (no gonophore or no polyp) (black), or status uncertain (hatched). (B) Reconstructions of ancestral character states of taxa displaying the organization of the polyp stage. Encrusting colony (white), upright colony (blue), pelagic colony (orange), solitary polyp (green), polyp absent (black), of status uncertain (hatched). Topology is the ML tree recovered from the combined dataset. Open circles at nodes represent poorly supported nodes (bootstrap support <50). A LDT was set at 2.0 log units. The identical topology with the exact order of terminal taxa, but including the species names and bootstrap values, can be found in the Supplementary Figure.

hydrozoans. Allman (1864) and many to follow, argued that the degree of gonophore development from a fixed sporosac to a free-living medusa should be used to distinguish different hydrozoan genera. This classification scheme was questioned by many including Broch (1916), who recognized species with very similar or identical polyps possessed divergent gonophores. Later, molecular phylogenetic analyses were able to show from independent data, that indeed closely related species can have highly divergent types of gonophores (Cunningham and Buss 1993; Leclère et al. 2009, Nawrocki et al. in review) and thus the presence/absence of a gonophore type would not be appropriate for classifying genera. In this analysis, even after disregarding the poorly supported nodes, we can conservatively conclude that hydrozoan medusae have been lost several times, and re-gained at least twice, and that these patterns appear in closely related taxa.

Evolution of polymorphism

Figure 2B depicts reconstructions of ancestral character states for polymorphic polyps (gastrozooids, gonozooids and dactylozooids). Polymorphism is found in all major hydroidolinan clades except Aplanulata, where it is inferred to be lost in the Aplanulata ancestor. Within Capitata, polymorphism evolved independently in the pelagic colonies of Porpitidae and in *Millepora* sp. Although all members of Siphonophorae are undoubtedly polymorphic, members of Calyophorae do not possess the types of polyps that were coded in this study (gonozooids and dactylozooids). All species of Leptothecata possess gonozooids, except *Melicertum octocostatum*, which buds gonophores from the gastrozooid body column. In Leptothecata, dactylozooids are inferred to have evolved in the ancestor of leptothecate clade that includes *Hydrodendron mirabile* and Plumularioidea. All species in Filifera III, with the exception of *Clava multicornis*, possess gonozooids, and dactylozooids are found in *Hydractinia symbiolongicarpus* and Stylasteridae. In Filifera IV, gonozooids evolved independently in *Hydrichthys boycei* and dactylozooids evolved independently in *Perarella schneideri* and *Pandea* sp. Given the unique morphology of *P. schneideri* dactylozooids (Appendix 1) and phylogenetic placement, these polyps are clearly not homologous with other hydrozoan dactylozooids. Also, dactylozooids in *Pandea* species are rarely observed (Appendix 1) so it is unclear if the *Pandea* sp. included in this study actually possesses dactylozooids.

The prevalence of polymorphism in hydroidolinan clades (excluding Aplanulata) provides clear evidence that the division of labor is a key evolutionary innovation in colonial hydrozoans. The evolution of the gonozooid, separating reproduction from feeding, is the most common strategy for division of labor. The evolution of other polyp types, including dactylozooids, appears to have evolved independently multiple times and most often from ancestors with gonozooids, as in the pattern recovered in Leptothecata.

The position of the gonophore

With reference to Fig. 3A, the most common position of the gonophore in hydrozoans is towards the oral end of the polyp. Proximal placement of the gonophore occurs in *Solanderia* (Capitata), Stylasteridae (Filifera III) and Filifera IV (Fig. 3A). Cartwright et al. (2008) named the Filifera IV clade Gonoproxima in reference to the significance of the position of the gonophore in this clade. It is interesting to note that species with a proximal position of the gonophore are closely related to species bearing polymorphic polyps. The sister taxon to *Solanderia*, which has a proximal position of the gonophore, is *Millepora*, which possesses dactylozooids. Filifera IV possesses two taxa with dactylozooids and the clade is the putative sister lineage to Filifera III which includes polymorphic species. Rees (1957) speculated that the proximally placed gonophore is actually a reduced gonozooid. The patterns reported here support the idea that the physical separation of feeding and reproduction, either as in the proximal placement of the gonophore or as in the evolution of the gonozooid, may be linked in evolution.

Organization of the polyp stage

In Fig. 3B, the presence of a solitary polyp was recovered as ancestral for hydrozoans, with coloniality evolving after the divergence of Trachylina and Hydroidolina. The small colonies found in some Limnomedusae are likely of independent origin. Coloniality was lost or greatly reduced in the capitate family Moerisiidae (*Moerisia* and *Odessia*) and in Filifera II, represented by the species *Brinckmannia hexactinellidophila*. In both lineages, the polyps are either solitary or may bud one or a few polyps that remain attached, forming pseudo-colonies. Coloniality was also lost in the ancestor of Aplanulata and upright colonies regained from a solitary ancestor in Tubulariidae. Pelagic colonies evolved twice, represented in the capitate family

Porpitidae and in the Siphonophorae. Transitions between upright and stolonial colonies occurred multiple times in hydrozoan evolution. Upright colonies appear to be a synapomorphy for the Leptothecata clade Macrocolonia as previously reported by Leclère et al. (2009).

Loss of coloniality is likely underestimated in our analysis as many solitary hydrozoans were not sampled, including Tricyclusidae, Margolopsidae, *Protohydra*, Boreohydridae, Acaulidae and *Nemopsis*. Aplanulata, which contains mostly solitary species, likely includes the families Tricyclusidae, Margolopsidae, and Acaulidae. The earliest diverging lineage of Aplanulata is Candalabridae, which comprise species with solitary polyps that can bud other polyps through root-like processes at their base, forming pseudo-colonies. *Hydra*, also part of Aplanulata, is a solitary polyp that adheres to the substrate with a specialized pedal disk. Also included in this clade are large, solitary deep-sea corymorphids such as *Branchiocerianthus imperator*, and the solitary meiofaunal corymorphids of the genus *Euphysa*. Sister to Corymorphidae is Tubulariidae (Collins et al. 2006; Nawrocki et al. in review), which includes colonial and solitary species.

Conclusion

Although hydrozoan diversity, as revealed in the complexity of life cycles, has been appreciated for centuries, only recently have the data and tools become available to investigate the evolution of hydrozoan characters in a phylogenetic context. Previous phylogenetic studies on clades within Hydrozoa revealed multiple instances of loss, and sometimes regain, in prominent aspects of the hydrozoan life cycle (Cunningham and Buss 1993; Collins 2002; Collins et al. 2006; Dunn et al. 2005; Govindarajan et al. 2006; Leclère et al. 2007; Cartwright et al. 2008; Collins et al. 2008; Leclère et al. 2009; Nawrocki et al. in review). Through reconstructions of ancestral states of characters involving the morphologies of different life-cycle stages, we confirm the complexity of the evolution of hydrozoan characters and the importance of evaluating these characters in a phylogenetic context. Although these reconstructions of characters should be viewed as preliminary estimates, given that many key taxa are missing and there is low support at many of the deep nodes, it is clear that many of the characters used in this study serve as synapomorphies for major hydrozoan clades (i.e., upright colonies for the leptothecate clade Macrocolonia, solitary polyps for Aplanulata, medusae for Capitata and polymorphism for

Filifera III), but that homoplasy (independent loss and regain of these characters) is common in hydrozoan evolution. In fact, our evaluation of loss and regain of characters is likely an underestimate and a clearer picture awaits more complete sampling. In addition, it is anticipated that more data will resolve some of the deeper nodes so that evolutionary transitions between major hydrozoan clades can be evaluated.

Hydrozoans are unique amongst metazoans in that their component parts are relatively simple, constructed of two epithelial cell layers, a handful of cell types, and very little in the way of internal anatomy. Given this simplicity, hydrozoans have achieved remarkable diversity in their life-history stages. This study illustrates that hydrozoan evolution is replete with repeated patterns of convergence in characters involving the forms of polyps, colonies and medusae. These complex evolutionary patterns can, in part, be explained by the simple construction of hydrozoans; there is likely a limited number of ways to construct a complex character with two epithelial layers and a few cell types. Hence, given the constraints imposed by the simplicity of their component parts, combined with the diverse ecological and evolutionary strategies inherent in the different stages of the life cycle, it is not surprising that the evolution of hydrozoan characters is marked with multiple instances of convergence. Insight into the genetic controls responsible for these convergent characters will illuminate our understanding of the mechanisms underlying these intricate evolutionary patterns.

Supplementary material

Supplementary material is available at *ICB* online.

Acknowledgments

We would like to thank the Society of Integrative and Comparative Biology and the divisions of Invertebrate Zoology, and Systematics and Evolutionary Biology, for support of this symposium. We also thank M.S. Barbeitos for providing secondary structure alignments and for help with the analyses, the Ohio Supercomputer Center for use of their cluster and C.W. Dunn for discussions on siphonophores.

Funding

The National Science Foundation AToL EF-0531779 and DEB-0953571 (to P.C.); The KU Madison and Lila Self Foundation and the National Science Foundation DEB-0910211 (to A.M.N.).

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Appendix 1

Description of characters and character state assignments. Characters and character states are numbered in the order in which they appear in Table 1.

Character 1

Degree of development of the gonophore upon sexual maturity. 0 = medusa, 1 = medusoid, 2 = sporosac, 3 = no gonophore.

Free-living medusae are defined as swimming, feeding, autonomous individuals that spawn after separation from the polyp. Staurozoa, although they do not swim, were coded as having medusae because they have a mouth and feed. Medusoids (also referred to as eumedusoids), as defined here, do not feed (lack a functional mouth) but have radial canals, which in free-living medusae are part of the digestive system. Sporosacs (also referred to as cryptomedusoids and heteromedusoids), as defined here are gonophores that upon reproductive maturity lack a mouth and canal system. Most medusoids and sporosacs remain attached to the polyp upon spawning, but swimming medusoids (e.g., *C. pendula*) and abortive medusae (e.g., *Millepora* spp.) were coded as medusoids and swimming sporosacs (e.g., *D. conybeari*) were coded as sporosacs because of how they are defined above. Hydrozoan species that lack gonophores, and instead develop gametes in the epithelial tissues of the polyps (e.g., *Hydra* sp., *B. hexactinellidophila*), were coded as a separate character state.

Character 2

Polyp organization. 0 = encrusting colony, 1 = upright colony, 2 = pelagic colony, 3 = solitary polyp, 4 = polyp stage absent (n/a).

Hydrozoan colonies either encrust a substrate, grow upright from a substrate, or swim in the water column. Colonies were coded as encrusting if they grow horizontally on a substrate in a stolonal or hydrohizal form. Upright colonies were defined as those that attach to the substrate but grow erect in

the water column. Upright colonies include branching colonies such as *Pennaria* sp., bush-like colonies such as *E. larynx*, and erect chitinous forms such as *S. ericopsis*. Pelagic colonies are those whose polyps are interconnected in a colony but function as an individual unit to swim in the water column. These include siphonophores and the pelagic members of Capitata (*Velella* sp., *Porpita* sp.). Species were coded as solitary if polyps do not form permanent stolonal connections with each other, even if they transiently share a gastrovascular cavity (e.g., *Candelabrum* sp.). Species that lack polyps as part of their life cycle were coded as non-applicable for this character.

Character 3

Polymorphism. 0 = no polymorphism (gastrozooids only) 1 = gastrozooids + gonozooids, 2 = gastrozooids + dactylozooids, 3 = gastrozooids + gonozooids + dactylozooids, 4 = non-applicable (polyp stage absent).

Polyps of a colony can be identical or polymorphic. All colonies possess feeding polyps (gastrozooids) and in non-polymorphic colonies these polyps also serve other functions, such as gonophore budding, food gathering and defense. Polymorphic polyps are functionally specialized and morphologically distinct. Gonozooids are polyps specialized for reproduction (gonozooid) that bear gonophores on the polyp body column, and are morphologically distinct from gastrozooids, usually lacking a mouth and tentacles or with reduced tentacles. In siphonophores, gonozooids are interpreted as any palpon that is associated with reproductive structures. Dactylozooids are polyps specialized for food gathering and/or defense. They are morphologically distinct from gastrozooids, usually highly extensile with a dense population of nematocysts, and lacking a mouth and tentacles or with reduced tentacles.

In siphonophores, dactylozooids are interpreted as any palpon associated with defense. *Perarella schneideri* possesses two types of polyps, a short gastrozooid, as well as a long 'gastrozooid' with four small tentacles. We coded the long 'gastrozooid' as a dactylozooid based on its role in food acquisition (Bavestrello 2000). We coded *Pandea* sp. as having dactylozooids because it has been observed (rarely) that some species belonging to the genus possess tentaculozooids and/or dactylozooids. Schuchert (2007) reported tentaculozooids on *P. conica*, which lives on the shell of a pelagic gastropod, but noted that this was not found on all colonies of this species.

Character 4

Placement of the gonophore bud. 0 = proximal to the polyp base, 1 = distal, towards the oral end of the polyp, 2 = non-applicable (no polyp or no gonophore). Most hydrozoan species bud gonophores on their mid-body region or toward the mouth of the polyp (distal end). Species that were found to bud gonophores at the polyp base (proximal end) or on the stolons or stalks of the colony were coded as proximal. In siphonophores, proximal gonophores were coded in those taxa that lack palpons associated with reproduction and have gonophores that are borne directly on the stem. Some filiferan species such as *Neoturris brevicornis*, *Koellikerina fasciculata*, *Turritopsis* sp., *B. vestia*, *C. caspia*, *Bougainvillia* sp., and *G. grisea* possess gonophores on the stalk below the polyp body column. In some cases these structures may be close to the polyp, but in all cases they are on the stalk and not the polyp body column. Thus, they are also coded as proximal. In many of these species, the stalk can be distinguished from the polyp body column by the presence of a perisarc (a chitinous exoskeleton).