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journal homepage: www.elsevier.com/locate/ympevPhylogenetic placement of *Hydra* and relationships within Aplanulata (Cnidaria: Hydrozoa)Annalise M. Nawrocki^{a,e,*}, Allen G. Collins^b, Yayoi M. Hirano^c, Peter Schuchert^d, Paulyn Cartwright^e^a Pomona College, R.C. Seaver Biology Building, 175 W. Sixth Street, Claremont, CA 91711, USA^b NMFS, National Systematics Laboratory, National Museum of Natural History, MRC-153, Smithsonian Institution, P.O. Box 37012, Washington, DC 20013, USA^c Department of Biology, Graduate School of Science, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan^d Muséum d'histoire naturelle, CP 6434, CH-1211 Genève 6, Switzerland^e The University of Kansas, 1200 Sunnyside Avenue, Lawrence, KS 66045, USA

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ABSTRACT

The model organism *Hydra* belongs to the hydrozoan clade Aplanulata. Despite being a popular model system for development, little is known about the phylogenetic placement of this taxon or the relationships of its closest relatives. Previous studies have been conflicting regarding sister group relationships and have been unable to resolve deep nodes within the clade. In addition, there are several putative Aplanulata taxa that have never been sampled for molecular data or analyzed using multiple markers. Here, we combine the fast-evolving cytochrome oxidase 1 (CO1) mitochondrial marker with mitochondrial 16S, nuclear small ribosomal subunit (18S, SSU) and large ribosomal subunit (28S, LSU) sequences to examine relationships within the clade Aplanulata. We further discuss the relative contribution of four different molecular markers to resolving phylogenetic relationships within Aplanulata. Lastly, we report morphological synapomorphies for some of the major Aplanulata genera and families, and suggest new taxonomic classifications for two species of Aplanulata, *Fukaurahydra anthoformis* and *Corymorpha intermedia*, based on a preponderance of molecular and morphological data that justify the designation of these species to different genera.

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1. Introduction

The model organism *Hydra* belongs to the hydrozoan clade Aplanulata (Collins et al., 2005, 2006). Despite numerous studies on *Hydra* spanning as disparate fields as immunology (Bosch et al., 2009), stem cell biology (David, 2012; Hobmayer et al., 2012; Nishimiya-Fujisawa and Kobayashi, 2012) and evolutionary biology (Hemmrich et al., 2007; Kawaida et al., 2010; Martínez et al., 2010), as well as a complete sequenced *Hydra* genome (Chapman et al., 2010), and two sequenced mitochondrial genomes for *Hydra* (Kayal and Lavrov, 2008; Voigt et al., 2008), little is known about the precise phylogenetic placement of *Hydra* or the relationships among major lineages of Aplanulata. Recent studies have begun to shed light on these relationships, particularly within the families Hydridae (Martínez et al., 2010), Tubulariidae (Marques and Migotto, 2001) and Corymorphidae (Cartwright and Nawrocki, 2010; Nawrocki and Cartwright, 2012). However, there is little support for relationships between major lineages of

Aplanulata, and many putative Aplanulata taxa have not been sampled or studied in a phylogenetic context with multiple markers.

Aplanulata comprises eight families (Collins et al., 2006) and approximately 170 valid species (Daly et al., 2007). These species exhibit great morphological diversity (Fig. 1), inhabit several disparate ecological habitats, and display a wide variety of life cycles. Unlike members of all other major hydrozoan lineages, most species of Aplanulata display a solitary, as opposed to a colonial, polyp stage (but see Nawrocki and Cartwright, 2012). The solitary polyps range in size from a few millimeters in length (i.e. *Hydra*) to 70 cm tall (i.e. *Branchiocerianthus*, with some authors reporting that this species reaches two meters in length). This clade includes species that inhabit cold and deep waters, as well as intertidal species, and tropical species that may live symbiotically with sponges or corals. And while most species in Aplanulata are marine, this clade also includes *Hydra*, one of the few hydrozoan groups that inhabit fresh water environments (Jankowski et al., 2008) (Fig. 1). Aplanulata species also vary greatly in their possession of a pelagic medusa (jellyfish) stage, with some species producing fully independent, free-swimming medusae, whereas others exhibit structures demonstrating various earlier stages of medusa development. These structures, called gonophores, remain attached to the body of the polyp and often possess various elements of medusa morphology,

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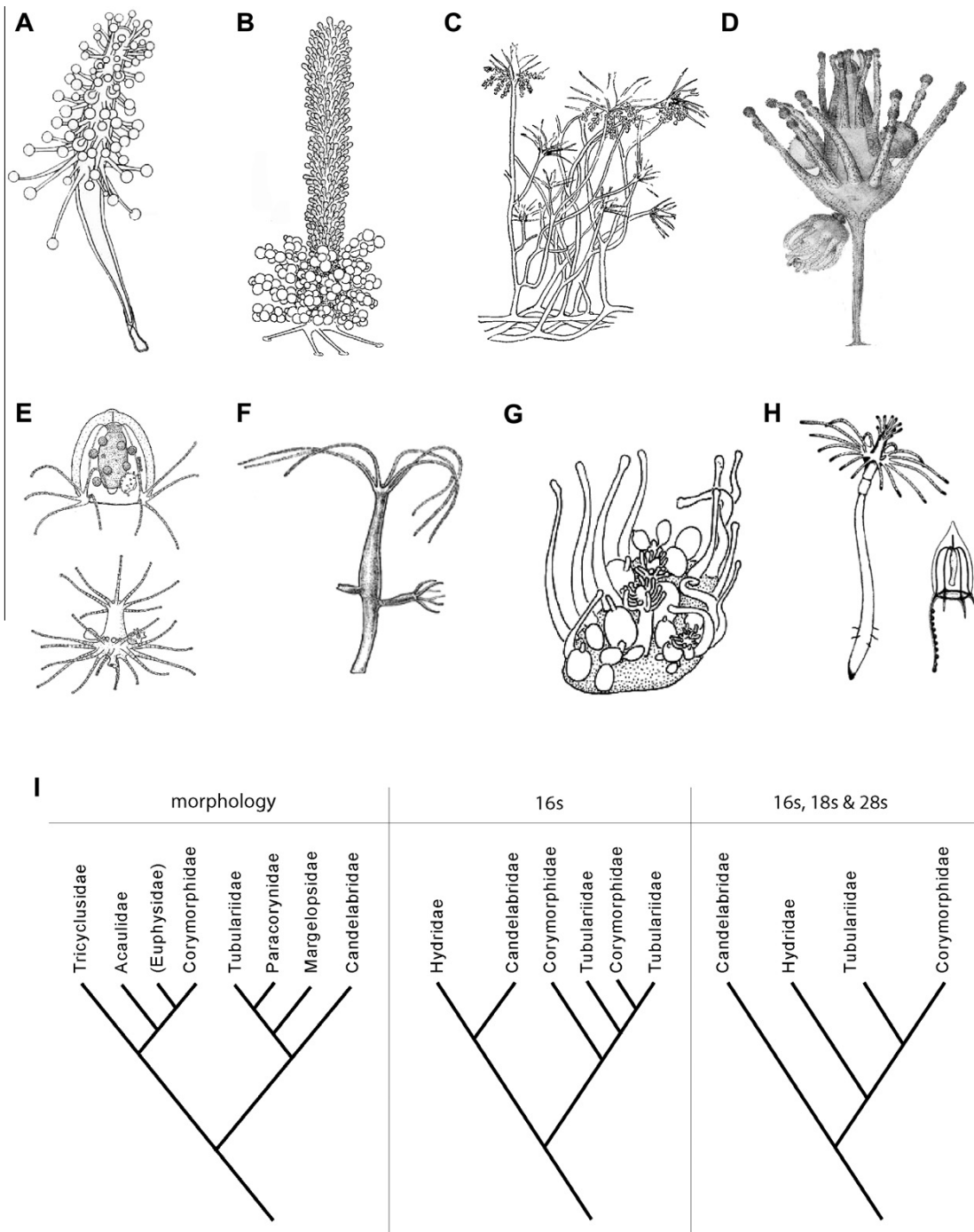


Fig. 1. Eight major families belonging to Aplanulata (Collins et al., 2006) (A–H) and current hypotheses of Aplanulata relationships (I). (A) *Acaulis ilonae*; Acaulidae. (B) *Candelabrum phrygium*; Candelabridae. (C) *Ectopleura crocea*; Tubulariidae. (D) *Tricyclusa singularis*; Tricyclusidae. (E) *Margelopsis haeckeli*; Margelopsidae. (F) *Hydra* sp.; Hydridae. (G) *Paracoryne huvei*; Paracorynidae. (H) *Corymorpha bigelowi*; Corymorphidae. (I) Recent morphological and molecular hypotheses of relationships of members of Aplanulata. Left: Hypothesis based on morphological data (Petersen, 1990). Center: Hypothesis of Aplanulata relationships based on mitochondrial 16S (Collins et al., 2005). Right: Hypothesis of Aplanulata relationships based on mitochondrial 16S, and nuclear 18S and 28S (Cartwright and Nawrocki, 2010; Nawrocki and Cartwright 2012). *Acaulis ilonae* modified from Brinckmann-Voss (1966); *Candelabrum phrygium* modified from Schuchert (2006); *Ectopleura crocea* modified from Hargitt (1901); *Tricyclusa singularis* modified from Schulze (1876). *Margelopsis haeckeli* modified from Schuchert (2006); *Paracoryne huvei* and *Hydra* images from Schuchert (2010). *Corymorpha bigelowi* modified from Sassaman and Rees (1978).

such as tentacles. Despite their diversity, the group is united by a striking developmental synapomorphy: individuals bypass a larval

stage typical of hydrozoans and instead develop directly into juvenile polyps inside a gonophore or within a cyst (for a description of

direct development in an Aplanulata species, see Berrill, 1952). This is in striking contrast to other hydrozoans, whose fertilized eggs develop into free-living, ciliated planula larvae that disperse in the water column or crawl before settling and metamorphosing into a primary polyp.

The phylogenetic relationships within families of Aplanulata have not been thoroughly investigated from a molecular perspective, with the exception of Hydridae (Martínez et al., 2010) and Corymorphidae (Nawrocki and Cartwright, 2012). Hydridae is reported to contain approximately 30 valid species (Daly et al., 2007), but a recent phylogenetic study of the family suggests that only eight species are likely valid (Martínez et al., 2010). However, no formal revision was done in this study (Martínez et al., 2010). Hydridae is comprised of the single genus *Hydra*. Members of *Hydra* are well documented throughout Europe (Schuchert, 2012) and the Americas, but have a global distribution and are found on all continents except Antarctica (Martínez et al., 2010). All members of this genus are solitary and inhabit freshwater environments. Hydridae is split into four large, well-supported and easily diagnosable clades, and the relationships between and within these major lineages are well resolved (Martínez et al., 2010).

Candelabridae comprises 20 valid species, most of which are solitary (but see Brinckmann-voss and Lindner, 2008) (Daly et al., 2007). Species in this family all have a large number of randomly scattered capitate (knobbed) tentacles along the body column, and reproductive structures are localized below the tentacles. No more than two species have been previously sampled for phylogenetic analyses.

Corymorphidae, with approximately 45 valid species (Daly et al., 2007), exclusively contains solitary species, and members possess a body column lacking both tentacles and a hard skeleton. However, most species do possess a thin or gelatinous covering over the body of the polyp. Two whorls of filiform (elongated with tapering ends) or moniliform (elongated but with batteries of nematocysts along them) tentacles are found towards the oral end of the polyp, and reproductive structures form between these two sets of tentacles. Occasionally, oral tentacles are capitate (with knobbed ends). Studies examining the relationships of this clade in a molecular phylogenetic context using 16S rDNA have failed to recover this family as monophyletic (Collins et al., 2005; Schuchert, 2010). More recently, an analysis using a combination of 18S, 28S and 16S data and including eleven corymorphid taxa did not recover a monophyletic Corymorphidae, and instead recovered *Corymorpha groenlandica* and *Hataia parva* separate from the rest of Corymorphidae (Nawrocki and Cartwright, 2012). This same study sampled the large, deep-water species *Branchiocerianthus imperator*, and recovered its placement at the base of the *Euphysa* clade within Corymorphidae; however, this placement was poorly supported. Its affiliation with *Euphysa* was surprising, given that members of this genus are some of the smallest polyps represented in Corymorphidae, being only 1–4 cm in length (Norenburg and Morse, 1983). This study also sampled *Corymorpha intermedia*, a taxon that recently was recovered as grouping with members of the genus *Euphysa* in molecular phylogenetic analyses (Cartwright and Nawrocki, 2010; Nawrocki and Cartwright, 2012). *Fukaurahydra anthoformis*, a species of Aplanulata with a unique squat polyp possessing a widened, flat platform bearing gonophores (Yamada et al., 1977), is classified as a corymorphid (Bouillon et al., 2006) but has never before been sampled for phylogenetic analyses.

Tubulariidae (approximately 60 valid species) (Daly et al., 2007) is comprised of both solitary and colonial species (see Nawrocki and Cartwright, 2012), with polyps sharing the general morphology of those of Corymorphidae, except that polyps are much smaller and have stalks covered with a hard exoskeleton (perisarc). Phylogenetic studies using 16S rDNA were unable to recover this family as monophyletic (Collins et al., 2005; Schuchert, 2010). A

more recent study with larger sampling of this family and two additional markers (18S and 28S) recovered a monophyletic Tubulariidae and suggested three well-supported lineages in the clade—an *Ectopleura* clade, a clade comprised of *Hybocodon* and *Tubularia*, and a clade comprised of *Ralpharia* and *Zyzyzus* (Nawrocki and Cartwright, 2012). Within the *Ectopleura* clade, there are three distinct lineages, which lack clear morphological synapomorphies. The results of this study disagree with a former phylogenetic analysis based on morphology alone, which suggested two major groupings of *Ectopleura* species based on the presence/absence of a medusa (Marques and Migotto, 2001).

Acaulidae is comprised of three genera and approximately five valid species (Daly et al., 2007). All members of this family are solitary and possess one set of tentacles surrounding the mouth, with scattered tentacles along the body column, and with or without an additional whorl of fleshy filiform tentacles at the base of the polyp (Bouillon et al., 2006; Petersen, 1990). Gonophores form between the tentacles on the body of the polyp (Bouillon et al., 2006; Schuchert, 2006). This family may (Cairns et al., 2003) or may not (Bouillon et al., 2006; Yamada and Kubota, 1991) include the solitary species *Hataia parva* Hirai and Yamada, 1965.

Margelopsidae has three genera and approximately five valid species (Daly et al., 2007) that are exclusively pelagic. Species belonging to this family resemble polyp hydranths, except that they lack a hydrocaulus underneath the hydranth. Instead, individuals float freely in the ocean. Members of this family are known to encyst (Kubota, 1993), but Petersen (1990) grouped this family with Paracorynidae and Tubulariidae based on the length of oral and aboral tentacles, as well as the shape of the hydranth.

Two putative Aplanulata families (Collins et al., 2006) are not sampled in this study. Tricyclusidae is a monotypic family containing the single species *Tricyclusa singularis* Schulze, 1876. This species is a solitary polyp with two whorls of tentacles with slightly capitate ends (Schulze, 1876; Schuchert, 2006). Gonophores form between the two sets of tentacles, and below the aboral tentacles (Fig. 1). Petersen (1990) affiliated this family with Acaulidae and Corymorphidae, based on the shared possession of gelatinous perisarc and encystment. Paracorynidae is another monotypic family containing the species *Paracoryne huvei* Picard, 1957 (Fig. 1). *Paracoryne huvei* is described as a polymorphic colony, containing gastrozooids, gonozooids and dactylozooids (Picard, 1957). However, it has been suggested that the colony is actually a flattened tubulariid hydranth (head) (Bouillon, 1974, 1975). Evidence for this lies in the morphology of the basal plate of the colony, which contains endodermal cavities and a large layer of parenchymatic tissue, similar to what is found in tubulariid heads (Bouillon, 1974, 1975). If this is the case, then dactylozooids are in actuality the tentacles of the hydranth, gonozooids are the blastostyles carrying gonophores, and gastrozooids are duplicated hypostomes (Petersen, 1990). Neither Tricyclusidae nor Paracorynidae has ever been sampled for molecular phylogenetic analyses.

Relationships among the component families of Aplanulata are also not well understood. Petersen (1990) provided a phylogenetic hypothesis based on morphological data for six of the major families (Fig. 1), with the exclusion of Hydridae, which he hypothesized to belong to a different hydrozoan lineage. Petersen split Aplanulata into two major clades based on developmental mode—one major lineage for families whose members encyst (Tricyclusidae, Acaulidae and Corymorphidae) and one for members that develop directly into actinulae, which are juvenile polyps (Tubulariidae, Paracorynidae, Margelopsidae and Candelabridae) (Petersen, 1990). However, subsequent phylogenetic analyses reveal that this division is likely not reflective of the clade's evolutionary history, and that Tubulariidae is more closely related to Corymorphidae than to Candelabridae (Fig. 1) (Cartwright and Nawrocki, 2010; Nawrocki and Cartwright, 2012; Collins et al., 2005, 2006). These

molecular-based results, suggesting a clade consisting of Corymorphidae and Tubulariidae, are more or less consistent with Bouillon's Tubularioidea (Bouillon, 1985), which is characterized by the presence of two whorls of tentacles (Cartwright and Nawrocki, 2010; Nawrocki and Cartwright, 2012; Collins et al., 2005, 2006). Bouillon's Tubularioidea also included some families not yet sampled (Margelopsidae and Paracorynidae), and one that is known to group outside of Aplanulata (Nawrocki et al., 2010). Furthermore, a phylogenetic analysis with mitochondrial 16S data places Candelabridae and Hydridae as sister taxa (Collins et al., 2005), which would suggest that their highly extensible bodies are shared due to common ancestry. In contrast, other studies incorporating nuclear 18S and/or 28S rDNA have suggested that Candelabridae might instead be a separate, early diverging lineage of Aplanulata, with Hydridae being sister to Corymorphidae + Tubulariidae (Cartwright and Nawrocki, 2010; Nawrocki and Cartwright, 2012; Collins et al., 2006). This latter hypothesis would suggest that oral tentacles organized in a whorl is a shared, derived character of Hydridae, Corymorphidae and Tubulariidae (Fig. 1).

Here, we provide the most complete sampling of 39 Aplanulata taxa, comprising members of six out of the eight described families, with four markers. We include new, previously unsampled taxa, including the monotypic *Fukaurahydra anthoformis*, in an effort to resolve relationships among the major lineages of Aplanulata and better understand the phylogenetic placement of *Hydra*. We compare our modern understanding of the evolution of characters in the clade to historical concepts of the evolution of the group, and demonstrate that reproductive characters that have been previously overlooked are likely important to understanding the evolution of this lineage. Based on these reproductive and morphological characters, we hypothesize phylogenetic affinities for four unsampled or under-sampled families of Aplanulata. Lastly, we offer new taxonomic classifications for two species of Aplanulata, *Corymorphia intermedia* and *Fukaurahydra anthoformis*, based on their recovered phylogenetic placement in our analyses, as well as a re-examination of their morphology.

2. Material and methods

2.1. DNA isolation and sequencing

Fresh, ethanol-preserved, or RNALater-preserved tissue was extracted using the Qiagen DNeasy tissue kit following manufacturer's instructions (Qiagen, Inc., Mississauga, ON, Canada), or using a standard phenol-chloroform protocol (available on request). A 640 base pair fragment of the mitochondrial 16S, 651 base pair fragment of the mitochondrial cytochrome oxidase 1 (CO1), 1800 base pair fragment of the nuclear 18S (small ribosomal subunit, SSU), and 3201 base pair fragment of the nuclear 28S (large ribosomal subunit, LSU) markers were amplified as previously described (Cartwright et al., 2008; Cunningham and Buss, 1993; Dawson, 2005; Evans et al., 2008; Folmer et al., 1994). PCR product was purified and sequenced directly by the University of Washington High Throughput Sequencing Unit (Seattle, WA, USA), or sequences were retrieved from GenBank. Contig assembly and sequence editing were conducted in Sequencher v4.9 (GeneCodes, 2005). Concatenation and matrix editing was conducted in Mesquite v2.74 (Maddison and Maddison, 2007). All new sequences generated for this study were deposited in GenBank (Table 1).

2.2. Sequence alignment and phylogenetic analyses

The DNA alignment for CO1 was generated in the program Translator X (Abascal et al., 2010), which uses the translated

protein code to guide the generation of a nucleotide alignment. Program settings were default, except that we used a 'coelenterate-specific' mitochondrial genetic code, MUSCLE for alignment (Edgar, 2004a, 2004b), and we inferred the most likely reading frame based on the aligned data. The final alignment was end-trimmed to remove characters missing from more than half of the sampled taxa.

DNA alignments for 16S, 18S and 28S were generated with MUSCLE (Edgar, 2004a,b), and were subsequently adjusted by hand based on developed secondary structure models for Hydridae (16S) following Nawrocki and Cartwright (2012), or models for Cnidaria (18S and 28S) (M.S. Barbeitos, personal communication). Alignments were run through Gblocks v0.91b (Castresana, 2000) to remove ambiguously aligned regions using the following settings: minimum block length = 5; gaps = with half. The final alignments were end-trimmed to remove characters missing in more than half of the aligned taxa. Analyses of partial datasets employing the doublet model, which incorporates information of secondary structure, did not show significant improvement in topology (not shown); thus, we applied a general time reversible model with rate-distributed variation across sites (GTRGamma) to all alignments used in this study. An additional proportion of invariant sites was not used, as the lowest rate category of the gamma distribution that accounts for rate heterogeneity in the GTRGamma model includes sites that are close to invariant (Ren et al., 2005). Analyses were run in the parallel version of RaxML v7.2.8 (Stamatakis, 2006) for all markers. For CO1, a GTRGamma model was applied to three different data partitions determined by codon position. For the concatenated analysis, PartitionFinder v1.0.1 (Lanfear et al., 2012) was used to explore the validity of various partitioning schemes, with the Akaike Information Criterion (AIC) used to evaluate different partitioning strategies (Akaike, 1987). One thousand bootstrap replicates were generated for each individual analysis, as well as the combined analysis. Trees were visualized in Mesquite v2.75 (Maddison and Maddison, 2007) and FigTree (Drummond and Rambaut, 2007).

3. Results

3.1. Taxon sampling, alignment and analyses

Sixty-five sequences were included in the 16S analysis, and a total of 508 bp, or 78% of the amplified 16S, were retained after removal of ambiguously aligned regions in Gblocks (Castresana, 2000). For 18S, 51 sequences were included in the analysis and a total of 1398 bp, or 78% of the amplified fragment, were retained after Gblocks. For 28S, 51 sequences were included in the analysis and a total of 3072 bp, or 96% of the amplified fragment, were retained after Gblocks. Forty-three CO1 sequences were included in the analysis and the entire amplified CO1 (645 bp with ends trimmed) was analyzed. For the combined analysis, all taxa with at least three sequenced markers were included in the final combined analysis, for a total of 51 taxa and 5623 alignment positions. This study contributed 36 new DNA sequences (including a new marker, CO1), and sampled 56 species, including 39 Aplanulata species from 14 genera representing six of the eight families in the clade. Species identifications, GenBank IDs, and voucher numbers are reported in Table 1.

PartitionFinder v1.0.1 suggested a 6-partition scheme over (A) partitioning the data by gene (4 partitions) (B) partitioning the data into "mitochondrial" and "nuclear ribosomal" sets (2 partitions), and (C) no partitioning (all data combined into a single partition) (Table 2). Thus, the data was split into data partitions by marker and by codon position (CO1 only), accounting for a total of six partitions in the combined analysis, and analyzed in RaxML

Table 1

Specimens and associated GenBank accession numbers. GenBank accession numbers for new sequences generated for this study are boldfaced.

Higher level	Family	Species	28s	18s	16s	CO1	Voucher or published reference sequence
Aplanulata	Candelabridae	<i>Candelabrum austrogeorgiae</i>	–	–	FN424120	–	Cantero et al. (2010)
Aplanulata	Candelabridae	<i>Candelabrum cocksii</i>	EU879928	EU876556	AY512520	JX121578	MHNGINVE29591
Aplanulata	Candelabridae	<i>Candelabrum</i> sp.	EU879929	EU876557	EU876530	JX121579	–
Aplanulata	Corymorphidae	<i>Branchiocerianthus imperator</i>	JN594035	JN594046	–	JX121580	–
Aplanulata	Corymorphidae	<i>Corymorpha bigelowi</i>	EU272563	EU876564	EU448099	JX121581	KUNHM2829
Aplanulata	Corymorphidae	<i>Corymorpha glacialis</i>	JN594036	JN594047	FN687549	JX121584	MHNGINVE67050
Aplanulata	Corymorphidae	<i>Corymorpha groenlandica</i>	–	–	FN687551	–	MHNGINVE67051
Aplanulata	Corymorphidae	<i>Corymorpha groenlandica</i>	–	–	FN687550	–	MHNGINVE63302
Aplanulata	Corymorphidae	<i>Corymorpha intermedia</i>	EU879930	AY920759	FN687910	JX121582	Collins et al. (2006) and Schuchert (2010)
Aplanulata	Corymorphidae	<i>Corymorpha nutans</i>	EU879931	EU876558	FN687546	JX121586	MHNGINVE48745
Aplanulata	Corymorphidae	<i>Corymorpha nutans</i>	–	–	FN687549	–	MHNGINVE67050
Aplanulata	Corymorphidae	<i>Corymorpha nutans</i>	–	–	FN687548	–	Schuchert (2010)
Aplanulata	Corymorphidae	<i>Corymorpha nutans</i>	–	–	FN687547	–	Schuchert (2010)
Aplanulata	Corymorphidae	<i>Corymorpha pendula</i>	EU879936	EU876565	EU876538	JX121583	KUNHMDIZ2962
Aplanulata	Corymorphidae	<i>Corymorpha sarsii</i>	JN594038	JN594049	–	JX121585	–
Aplanulata	Corymorphidae	<i>Corymorpha</i> sp.	–	–	FN424121	–	Cantero et al. (2010)
Aplanulata	Corymorphidae/Acaulidae	<i>Hataia parva</i>	JN594034	JN594045	JN594033	JX121608	UF5407
Aplanulata	Corymorphidae	<i>Euphysa aurata</i>	EU879934	EU876562	EU876536	JX121587	MHNGINVE48753
Aplanulata	Corymorphidae	<i>Euphysa aurata</i>	–	–	FN687552	–	Schuchert (2010)
Aplanulata	Corymorphidae	<i>Euphysa flammaea</i>	–	–	–	FJ602537	–
Aplanulata	Corymorphidae	<i>Euphysa japonica</i>	JX122505	EU301605	JX122503	JX121577	Lindsay et al. (2008)
Aplanulata	Corymorphidae	<i>Euphysa tentaculata</i>	EU879935	EU876563	EU876537	JX121588	Cartwright and Nawrocki (2010)
Aplanulata	Corymorphidae	<i>Fukaurahydra anthiformis</i>	JX122504	–	JX122502	–	–
Aplanulata	Corymorphidae	<i>Paragotea bathybia</i>	–	–	–	FJ602533	–
Aplanulata	Hydridae	<i>Hydra canadensis</i>	JN594039	JN594050	GU722797	GU722883	Martinez et al. (2010)
Aplanulata	Hydridae	<i>Hydra circumcincta</i>	EU879939	EU876568	GU722764	GU722857	Cartwright and Nawrocki (2010) and Martinez et al. (2010)
Aplanulata	Hydridae	<i>Hydra hymanae</i>	JN594040	JN594051	GU722760	GU722849	Martinez et al. (2010)
Aplanulata	Hydridae	<i>Hydra oligactis</i>	JN594041	JN594052	GU722781	GU722871	Martinez et al. (2010)
Aplanulata	Hydridae	<i>Hydra utahensis</i>	JN594042	JN594053	GU722774	GU722861	Martinez et al. (2010)
Aplanulata	Hydridae	<i>Hydra viridissima</i>	EU879940	EU876569	GU722756	GU722845	Martinez et al. (2010)
Aplanulata	Hydridae	<i>Hydra vulgaris</i>	JN594043	JN594054	GU722817	GU722914	Martinez et al. (2010)
Aplanulata	Margelopsidae	<i>Margelopsis hartlaubi</i>	–	–	–	GQ120059	–
Aplanulata	Tubulariidae	<i>Ectopleura crocea</i>	EU879932	EU876559	EU876533	JX121589	MHNGINVE34010
Aplanulata	Tubulariidae	<i>Ectopleura crocea</i>	EU883554	EU883548	EU883543	–	Cartwright and Nawrocki (2010)
Aplanulata	Tubulariidae	<i>Ectopleura dumortieri</i>	–	–	FN687542	–	Schuchert (2010)
Aplanulata	Tubulariidae	<i>Ectopleura dumortieri</i>	EU272561	EU876560	EU305474	–	Pers. Voucher: Alberto Lindner, AL525
Aplanulata	Tubulariidae	<i>Ectopleura dumortieri</i>	EU879933	EU876561	EU876534	JX121590	Cartwright and Nawrocki (2010)
Aplanulata	Tubulariidae	<i>Ectopleura dumortieri</i>	–	–	FN687543	–	Schuchert (2010)
Aplanulata	Tubulariidae	<i>Ectopleura larynx</i>	EU879943	EU876572	EU876545	–	KUNHMDIZ2963
Aplanulata	Tubulariidae	<i>Ectopleura larynx</i>	EU883549	AY920760	AY787877	JX121591	MHNGINVE29389
Aplanulata	Tubulariidae	<i>Ectopleura larynx</i>	–	–	FN687535	–	MHNGINVE54563
Aplanulata	Tubulariidae	<i>Ectopleura larynx</i>	–	–	FN687536	–	MHNGINVE62576
Aplanulata	Tubulariidae	<i>Ectopleura marina</i>	EU883553	EU883547	EU883542	JX121592	Cartwright and Nawrocki (2010)
Aplanulata	Tubulariidae	<i>Ectopleura wrighti</i>	JN594044	JN594055	FN687541	JX121593	MHNGINVE27331
Aplanulata	Tubulariidae	<i>Hybocodon chilensis</i>	EU879937	EU876566	EU876539	JX121594	MHNGINVE36023
Aplanulata	Tubulariidae	<i>Hybocodon prolifer</i>	EU879938	EU876567	EU876540	JX121595	Cartwright and Nawrocki (2010)
Aplanulata	Tubulariidae	<i>Ralpharia gorgoniae</i>	EU272590	EU272633	EU305482	GU812437	KUNHM2778
Aplanulata	Tubulariidae	<i>Ralpharia</i> sp.	–	JN594056	–	–	–
Aplanulata	Tubulariidae	<i>Tubularia indivisa</i>	EU879942	EU876571	EU876544	JX121596	Cartwright and Nawrocki (2010)
Aplanulata	Tubulariidae	<i>Tubularia indivisa</i>	–	–	FN687532	–	Schuchert (2010)
Aplanulata	Tubulariidae	<i>Tubularia indivisa</i>	–	–	FN687530	–	MHNGINVE60972
Aplanulata	Tubulariidae	<i>Tubularia</i> sp.	–	–	FN424153	–	Cantero et al. (2010)
Aplanulata	Tubulariidae	<i>Zyzyzus warreni</i>	EU272599	EU272640	EU305489	JX121597	KUNHM2777
Capitata	Corynidae	<i>Stauridiosarsia ophiogaster</i>	EU272560	EU272615	EU305473	JX121598	KUNHM2803
Capitata	Solanderiidae	<i>Solandaria secunda</i>	EU305533	EU305502	EU305484	JX121599	KUNHM2611
Filifera I	Proboscicactylidae	<i>Proboscicactyla flavicirrata</i>	EU305527	EU305500	EU305480	JX121600	USNM1074994
Filifera I	Ptilocodiidae	<i>Hydrichthella epigorgia</i>	EU272569	EU272622	EU305478	JX121601	KUNHM2665
Filifera II	Eudendriidae	<i>Eudendrium capillare</i>	–	–	–	JX121602	–
Filifera II	Eudendriidae	<i>Eudendrium californicum</i>	EU305513	EU305492	EU305475	–	KUNHM2850
Filifera II	Eudendriidae	<i>Eudendrium glomeratum</i>	FJ550440	FJ550583	AM991301	–	MHNGINVE49717

Table 1 (continued)

Higher level	Family	Species	28s	18s	16s	CO1	Voucher or published reference sequence
Filifera III	Hydractiniidae	<i>Clavactinia gallensis</i>	EU272553	EU272610	EU448101	–	MHNGINVE33470
Filifera III	Stylasteridae	<i>Lepidopora microstylus</i>	EU272572	EU272644	EU645329	JX121603	USNM1027724
Filifera IV	Bougainvillidae	<i>Garveia grisea</i>	EU272588	EU272632	AM183131	–	MHNGINVE34436
Filifera IV	Pandeidae	<i>Hydrichthys boycei</i>	EU272570	EU305496	EU448102	–	MHNGINVE37417
Leptothecata	Campanulariidae	<i>Obelia bidentata</i>	FJ550446	AY789754*	AY789815*	JX121604	MHNGINVE37294
Leptothecata	Sertulariidae	<i>Sertularella mediterranea</i>	FJ550403	FJ550546	FJ550479	–	MHNGINVE32948
Limnomedusae	Olindiasidae	<i>Olindias phosphorica</i>	EU247808	AY920753	AY512509	JX121605	MHNGINVE29811
Siphonophorae	Clausophyidae	<i>Clausophyes ovata</i>	EU305508	AY937336	AY935294	JX121606	YPM35349
Siphonophorae	Forskaliidae	<i>Forskalia edwardsi</i>	EU305516	AY937354	AY935312	JX121607	YPM35036
Trachymedusae	Rhopalonematidae	<i>Aglaura hemistoma</i>	EU247803	EU247818	EU293984	–	MHNGINVE31745

* Indicates that sequences are from different samples.

Table 2

AIC Values for different partitioning schemes for combined analysis.

6 Partitions ^a	4 Partitions ^b	2 Partitions ^c	1 Partition
87824.72062	88782.4634	89744.2679	93644.054

^a Data partitioned by gene for 16s, 18s and 28s and by codon position for CO1.

^b Dataset partitioned by gene.

^c Nuclear and mitochondrial genes combined into two different partitions.

using the command: raxmlHPC-PTHREADS-SSE3 -T 2 -m GTRGAM-MA -s All_final_regions.nxs2.phy -n Combined_1.3.2012 -q partitions.txt -f a -x 12345 -# 1000.

3.2. Relative contribution of markers to topology

A comparison of node support between markers (Fig. 2) demonstrates that 28S accounts for the most well-supported clades (66% of the nodes with $bs \geq 70$), with 16S supporting 45%, 18S supporting 34%, and CO1 supporting only 5% of nodes. In general, the mitochondrial markers (16S and CO1) recover some relationships at the tips of the trees and within Hydridae, but little support for deeper relationships, while 18S recovers some lower-level relationships and no deep nodes, and 28S provides the most resolution throughout the entire tree. The combined analysis recovers 88% of nodes with a $bs \geq 70$. Additionally, with the combined analysis, we recover strong support for the monophyly of Aplanulata ($bs = 100$),

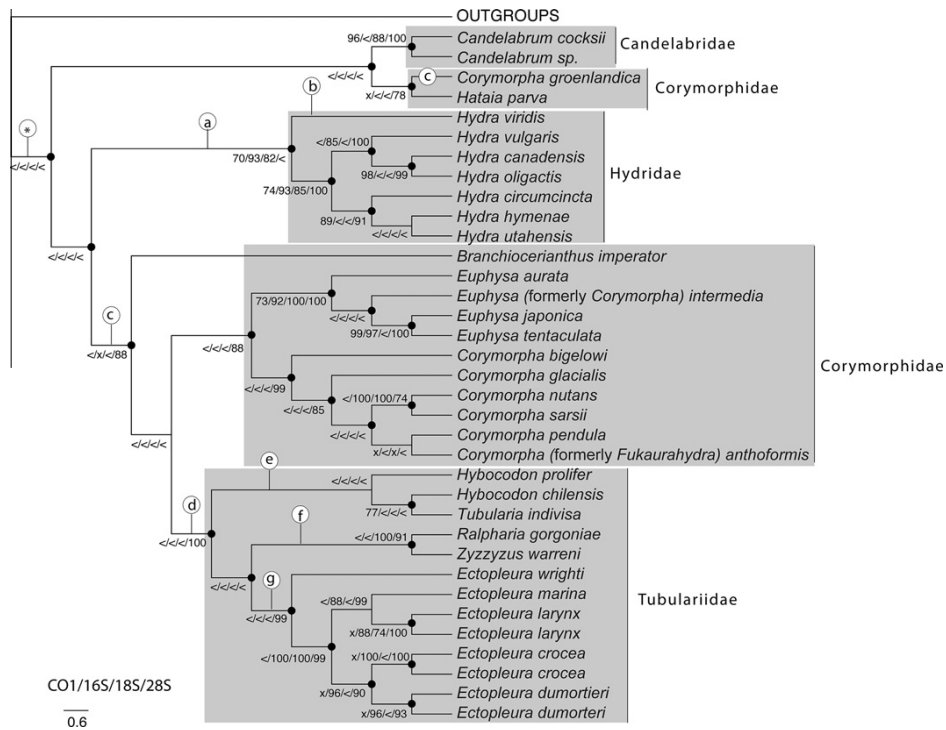


Fig. 2. Cladogram of relationships based on combined CO1, 16S, 18S and 28S, with nodes demonstrating bootstrap support for topology based on single-gene analyses. Node values are displayed if they are ≥ 70 . Darkened circles on nodes indicate bootstrap values ≥ 70 on combined, partitioned analysis in RaxML. X indicates that one of the sampled taxa was not in the analysis, thus the node did not exist. Mapped characters represent: (*) direct development; (a) freshwater; (b) intracellular algae; (c) two distinct sets of tentacles = Tubularioidae (d) divided hydrocaulus; (e) bilaterally symmetric gonophores; (f) symbiosis with other invertebrates; (g) single whorl of oral tentacles.

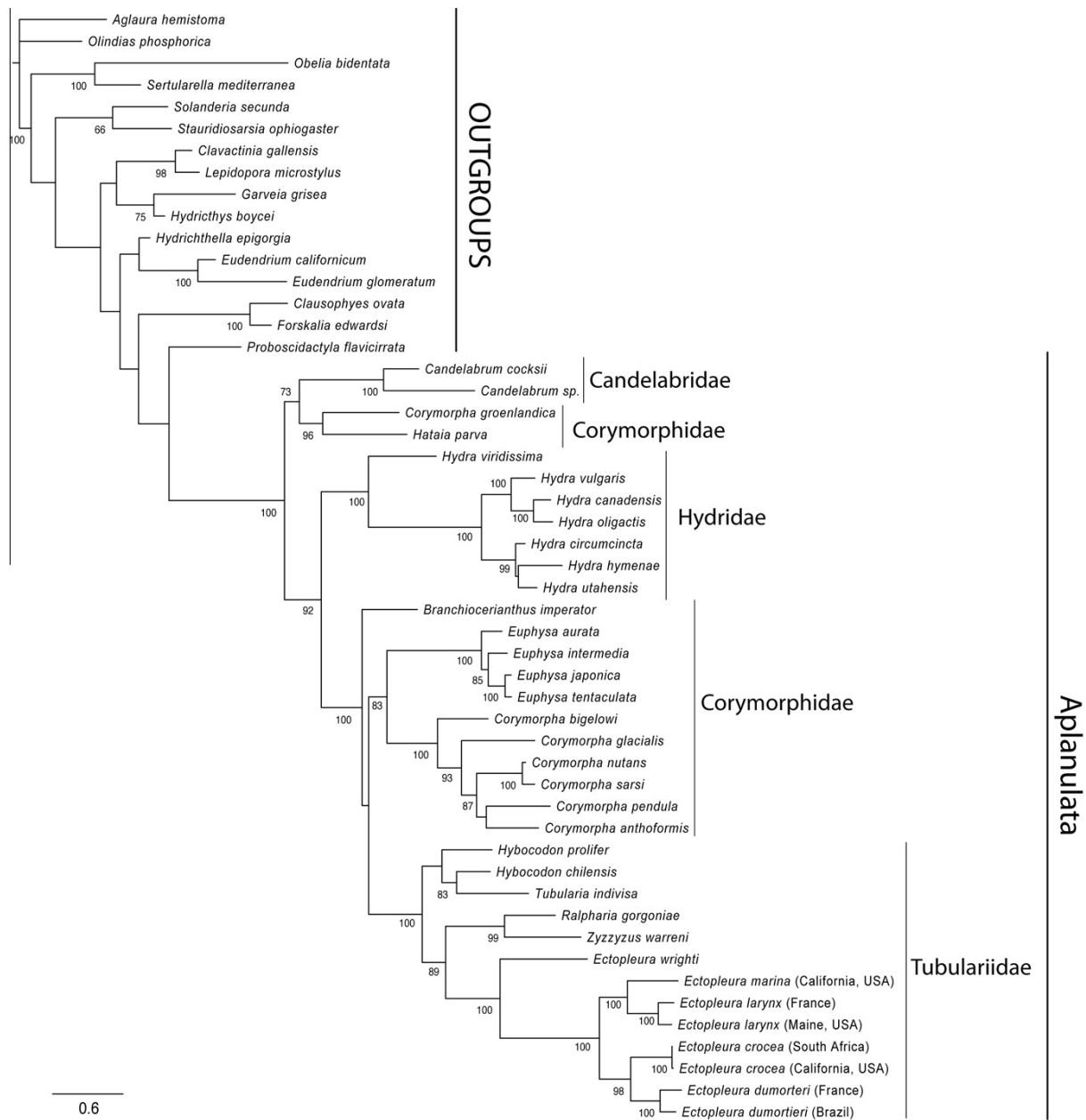


Fig. 3. Phylogenetic hypothesis of relationships of Aplanulata based on combined CO1, 16S, 18S and 28S analyzed under a partitioned GTR + Γ model in RaxML. Bootstrap values are reported if ≥ 70 . Node values indicate bootstrap support from 1000 replicates. See Appendix for single gene analyses.

strong support for a sister relationship between Hydridae and Tubularioidea (Tubulariidae + most Corymorphidae) and strong support for most of the deep nodes in the phylogeny. Thus, we consider the combined analysis our most robust hypothesis of relationships within Aplanulata.

3.3. Phylogenetic relationships of Aplanulata

CO1, 16S, 18S, 28S and combined analyses recover largely congruent topologies (see [Supplementary information](#) for single-gene analyses). Aplanulata is monophyletic in all analyses, although only the combined analysis shows strong support for the node

(Figs. 2 and 3; bs = 100). All analyses recover a monophyletic Hydridae, and our topology within *Hydra* is completely congruent with a recently published paper with much denser sampling of the family (Martínez et al., 2010).

Within Tubulariidae, the combined analysis recovers a monophyletic *Ectopleura* (also supported by 28S), a *Tubularia* + *Hybocodon* clade (but recovered with low support in all analyses), and a *Ralpharia* + *Zyzyzus* clade (also supported by 18S and 28S).

Corymorphidae is polyphyletic in all of the analyses that we conducted. *Corymorpha groenlandica*, an unidentified *Corymorpha* species from Cantero et al. (2010) (in 16S analysis only), and *Hataia parva* (supported by 28S and combined analyses) form a clade

sister to Candelabridae. Most of the sampled *Corymorpha* species, including the type species, *Corymorpha nutans*, and all of the sampled *Euphysa* species, including the type species *Euphysa aurata* fall into a well-supported clade that is sister to Tubulariidae (supported by 28S and combined analyses). Within this clade there is a split between members of the genus *Euphysa* + *Corymorpha* *intermedia* and a second group comprised of *Corymorpha* species (*C. bigelowi*, *C. glacialis*, *C. nutans*, *C. sarsii*, *C. pendula*) and *Fukaura-hydra anthoformis* (Figs. 2 and 3). We recover the corymorphid *Branchiocerianthus imperator* as the earliest diverging lineage of Tubularioidea (Tubulariidae + most other Corymorphidae) with good support in the 28S and combined analyses (Figs. 2 and 3).

4. Discussion

4.1. Relative contribution of CO1, 16S, 18S and 28S to topology

Our results suggest that faster evolving mitochondrial markers 16S and CO1 provide support at the tips of the tree, whereas the nuclear ribosomal marker 28S provides stronger overall support at deep nodes. These are congruent with previous phylogenetic analyses utilizing CO1 or 16S (Collins et al., 2005; Nawrocki et al., 2010; Ortman et al., 2010; Schuchert, 2010), and others utilizing nuclear ribosomal markers (Cartwright et al., 2008; Cartwright and Nawrocki, 2010; Collins et al., 2006; Nawrocki et al., 2010). In contrast to 28S, the ribosomal marker 18S does not provide strong support at deep nodes.

Our results are also for the most part congruent with a recent phylogenetic study that examined Aplanulata relationships, but did not remove ambiguously aligned regions with Gblocks (Nawrocki and Cartwright, 2012). One exception is that we recover a different placement for *Branchiocerianthus imperator*, but both Nawrocki and Cartwright, 2012 and our study here do not recover strong support for this taxon. This suggests that the inclusion of ambiguously aligned regions in rDNA data is not particularly informative for resolving relationships within Aplanulata. As the combined analysis recovers the most well-supported nodes (88% of the nodes), we consider the recovered topology from the combined analysis as the strongest hypothesis of relationships of component Aplanulata taxa.

4.2. Major Aplanulata lineages

Our combined topology supports an early-diverging lineage comprised of Candelabridae and the corymorphids *Corymorpha groenlandica* + *Hataia parva*. We recover the clade Tubularioidea *sensu lato*, which includes Corymorphidae (with the exclusion of *Corymorpha groenlandica* and *Hataia parva*) and a monophyletic Tubulariidae. The corymorphid *Branchiocerianthus imperator* is recovered as the earliest diverging branch of Tubularioidea, albeit with low support. Tubularioidea is recovered as sister to Hydridae, with strong support in the combined analysis.

4.3. Relationships within Hydridae

The monophyly of Hydridae is well supported by ours and previous studies (Martínez et al., 2010; Nawrocki and Cartwright, 2012; Cartwright and Nawrocki, 2010), and members of Hydridae are united by the strong synapomorphies of their freshwater habitat and the development of ovaries and testes directly in the epithelia of the polyp. Our combined analysis supports splitting Hydridae into four major clades (the Viridis group, the Braueri group, the Vulgaris group, and an Oligactis + Canadensis group). The Oligactis/Canadensis clade is sister to the Vulgaris clade. These data are congruent with a recent study with much denser sampling

of Hydridae (Martínez et al., 2010). Two of the major lineages of Hydridae that we recover have strong morphological synapomorphies. The *Hydra viridis* clade (represented in our analysis by only a single specimen) is united by both its distinctive green color (due to the presence of intracellular *Chlorella* algae) as well as the presence of an embryotheca with a cobbled surface, and the Braueri group has a flattened embryotheca and pyriform holotrichous isorhiza nematocysts (Martínez et al., 2010). The *oligactis-canadensis-vulgaris* clade is united by the presence of very long tentacles (longer than the length of the body of the animal) (Hyman, 1931; Schuchert, 2010).

4.4. Relationships within Tubulariidae

Tubulariidae is united by the combination of a presence of a thick skeletal covering (perisarc) over the polyp, direct development through a brooded actinula phase, and unbranched blastostyles (structures supporting developing gonophores). Members of this family also have a hydrocaulus (region below the polyp head) that is clearly divided into two distinct regions—the neck, which serves as a boundary between the polyp head and stalk (Nawrocki and Cartwright, 2012), and a stalk region. 28S and combined analyses recover a monophyletic Tubulariidae, congruent with previous analyses (Figs. 2 and 3) (Cartwright and Nawrocki, 2010; Nawrocki and Cartwright, 2012). As in a previous study (Nawrocki and Cartwright, 2012), we find support for two major groups within the family, with a third grouping only receiving low support. The earliest diverging group (recovered in the 16S, CO1 and combined analyses, but poorly supported) is comprised of the genera *Tubularia* and *Hybocodon*, which both contain solitary species with long, unbranched stalks covered in a hard, rigid perisarc. Members of this clade are often found in aggregates, with polyps often settling on one another, and have sometimes been mistaken for colonies (Nawrocki and Cartwright, 2012). Medusae of species in this clade have four tentacle bulbs along the margin of their bell, but a tentacle only develops from a single one of these bulbs, giving the medusae the appearance of bilateral symmetry. This is also the only group of tubulariids that produce bilaterally symmetric gonophores (either attached or detached).

The second clade of Tubulariidae is *Ralpharia gorgoniae* + *Zyzyzus warreni*. Both taxa live symbiotically imbedded in other invertebrate hosts—*Zyzyzus warreni* in a sponge, and *Ralpharia gorgoniae* in the body of a gorgonian coral. The evolution of symbiotic associations between hydroids and other invertebrates is of considerable interest and will be better understood when more species of sponge-associated *Zyzyzus* and octocoral-associated *Ralpharia* have been sampled.

The last well-supported clade of Tubulariidae (28S and combined analyses) is comprised of all sampled members of *Ectopleura*. This group is united by possession of a single whorl of oral tentacles (Petersen, 1990). *Ectopleura wrighti* is the earliest diverging sampled member of the group, followed by two sister clades comprised of *E. marina*–*E. larynx* and *E. crocea*–*E. dumortieri*. We find no support for grouping medusa-bearing species of this clade into one lineage, and non-medusa-bearing species into another, as suggested previously (Marques and Migotto, 2001).

4.5. Relationships within Corymorphidae

Our analyses find Corymorphidae as polyphyletic and recover three separate corymorphid lineages, two of which fall unexpectedly outside of Corymorphidae *sensu stricto*. In all of our analyses that included it, *Corymorpha groenlandica* grouped at the base of Aplanulata with *Hataia parva* and/or Candelabridae (Figs. 2 and 3), and in our 28S analysis, the grouping of *C. groenlandica* at the base of Aplanulata with *H. parva* has strong support (bs = 78). In

the 16S analysis, we were able to include another unidentified *Corymorpha* species from GenBank, which also grouped with the *Corymorpha groenlandica* samples, but was slightly divergent in sequence (see Supplemental file). This specimen was sampled only for 16S and was collected off of the Antarctic Peninsula, and could be one of any number of unsampled *Corymorpha* species, or alternatively, a more divergent sample of *Corymorpha groenlandica*, since this species has a broad range (Schuchert, 2010; Svoboda and Stepanjants, 2001). *Hataia parva* was originally classified in Clavidae based on the scattering of tentacles along the body (Hirai and Yamada, 1965). Later the development of *Hataia parva* was characterized, and authors noted its ability to encyst (Yamada and Kubota, 1991), clearly affiliating it with one of a number of Aplanulata families that have this capability. Later authors placed this species within Acaulidae (Cairns et al., 2003) or Corymorphidae (Bouillon et al., 2006), although there do not appear to be any strong synapomorphies that group it with either of these families, to the exclusion of the other. The placement of *Corymorpha groenlandica* + *Hataia parva* in our analyses with Candelabridae is not completely unexpected, given the gross similarity that *Hataia parva* polyps share with members of Candelabridae (scattered tentacles along the body column). However, its placement should be viewed as preliminary. While it is possible that the *Corymorpha groenlandica* + *Corymorpha* sp. + *Hataia parva* lineage is a valid grouping separate from other corymorphids given the strong support in the 16S analysis, the confident retrieval of its higher level placement will require much denser sampling of Corymorphidae.

Our results do not support the recent resurrection of the genus *Monocaulus* by Svoboda and Stepanjants (2001) for *C. groenlandica*, *C. glacialis* and *C. sarsii*. Svoboda and Stepanjants (2001) suggested that these corymorphid taxa, which lack branched gonophores and have some other unique morphological characteristics, should be classified in a separate genus, *Monocaulus*. This action is not universally recognized due to disagreement over the importance of branched blastostyles as a valid taxonomic character (Bouillon et al., 2006; Schuchert, 2010). Our study, though preliminary with regard to the placement of *C. groenlandica*, provides phylogenetic evidence that grouping these species into a separate genus, *Monocaulus*, is not warranted.

4.6. Phylogenetic placement of *Branchiocerianthus imperator*

Branchiocerianthus imperator is a morphologically distinct and large (usually 20–70 cm, but reported to reach sizes of up to 2 m) deep-sea hydrozoan classified within Corymorphidae (Schuchert, 2010). A previous analysis was unable to find strong support for the placement of this taxon, and instead placed it with weak



Fig. 4. Live specimen of *Corymorpha* (formerly *Fukaurahydra*) *anthoformis*. Photo credit: Y.M. Hirano.

support within Corymorphidae as sister to the genus *Euphysa* (Nawrocki and Cartwright, 2012). Our analysis, which added CO1 for this species, recovers *B. imperator* as the earliest diverging lineage of Tubularioidea (Fig. 3). We recover this relationship in the 28S analysis (bs = 88) and combined analysis (bs = 100), but not in the 18S or CO1 analyses (Figs. 2 and 3; species not sampled for 16S). The placement of *B. imperator* outside of Corymorphidae is surprising, given the morphological characters this species shares with species of Corymorphidae (such as rooting filaments and a reduced perisarc). However, this species also possesses a number of unique morphological apomorphies, such as its large size and the striking bilateral symmetry of the polyp not found in any other hydrozoan group. Furthermore, the precise relationship of *B. imperator*, as sister to everything else within Tubularioidea, is not well supported, and thus our data do not provide strong support for the assertion that this taxon is not part of Corymorphidae. In the interest of nomenclatural stability, we recommend keeping the current classification of *B. imperator* within Corymorphidae, pending further sampling of the genus, including its type species, *Branchiocerianthus urceolus* Mark, 1898.

4.7. Phylogenetic placement of *Fukaurahydra anthoformis*

Fukaurahydra anthoformis Fig. 4 is a morphologically distinct corymorphid in its own genus. This species' polyp stage has a short, squat body with a whorl of rooting filaments, in contrast to most corymorphid polyps, which have long bodies and a section at the base of the polyp with densely scattered rooting filaments. These morphological characteristics led to the erection of a new genus for the species (Yamada et al., 1977). Our analyses recover *F. anthoformis* as nested within the clade that includes most *Corymorpha* species (with the exception of *C. groenlandica*), and sister to the species *C. pendula* (combined analysis, bs = 69). All analyses that included *F. anthoformis* recover it as sister to *Corymorpha pendula*, but only the combined analysis provided some support for this relationship. Regardless, *F. anthoformis* is unequivocally nested within a clade of *Corymorpha* species, suggesting that a separate generic designation is unnecessary and that this species is a member of the genus *Corymorpha* (see Section 4.11).

4.8. Phylogenetic placement of *Corymorpha intermedia*

Within Corymorphidae *sensu stricto*, there is strong support for two major clades. One of these clades includes all sampled *Euphysa* species, as well as the species *Corymorpha intermedia*. The polyp stage of *C. intermedia* is currently unknown and the medusa possesses characteristics of both *Corymorpha* and *Euphysa* (see Section 4.11). The species was classified preliminarily in *Corymorpha* (Schuchert, 1996). Our analyses recover *Corymorpha intermedia* with *Euphysa*, and thus we recommend the new combination *Euphysa intermedia* (see Section 4.11). Members of *Euphysa* are morphologically distinct from other corymorphid species, in that they have markedly smaller polyps, the stalk (hydrocaulus) lacks endoderm canals characteristic of other corymorphids, they possess a single whorl of oral tentacles on the polyp, and the medusa lacks an apical canal or a peduncle (Bouillon et al., 2006; Petersen, 1990).

4.9. Phylogenetic placement of *Paragotea bathybia*

We sampled *Paragotea bathybia* Kramp, 1942 for our CO1 analysis using a sequence available on GenBank. This species grouped, albeit with low support, with *Euphysa*. This is an interesting result because although *Paragotea bathybia* has been traditionally classified within Corymorphidae (Bouillon et al., 2006; Pages and Bouillon, 1997), its exact phylogenetic placement is unknown. *Euphysa* and *Paragotea bathybia* have medusae with a single

tentacle, and their medusae also lack an apical canal. The affiliation of *Paragotea bathybia* with *Euphysa* should be viewed as preliminary and awaits sampling of additional markers.

4.10. Other Aplanulata taxa

4.10.1. Phylogenetic placement of *Margelopsis hartlaubi*

Margelopsis hartlaubi Browne, 1903 is a holopelagic species with a narrow distribution and is thus difficult to sample. This species closely resembles tubulariid polyps (two whorls of tentacles between which gonophores develop), except that it lacks a long stalk under the neck likely due to its pelagic existence (Fig. 1) (Bouillon et al., 2006; Mayer, 1910; Schuchert, 2006). We were only able to sample *M. hartlaubi* using a CO1 sequence available on GenBank (Ortman et al., 2010). Our CO1 analysis does not suggest affiliation with Tubulariidae or even Tubularioidea, as one would expect given the morphology of this species, and instead we recover it as sister to the rest of Aplanulata. However, we did not get strong support for this placement nor in the nodes separating *Margelopsis* from Tubularioidea, and this result should be viewed as preliminary. Thus, placement of this species and the scope of the family Margelopsidae await future sampling with more DNA markers and specimens, including the type species, *Margelopsis haeckelii* Hartlaub, 1897.

4.10.2. Phylogenetic placement of *Hataia parva*

Hataia parva Hirai and Yamada, 1965 was originally classified within Claviidae (in current usage = Oceaniidae), a clade of filiferan hydrozoans far removed from Aplanulata, based on its possession of scattered filiform tentacles (Bouillon, 1985; Hirai and Yamada, 1965). However, its solitary habit and direct development through encystment clearly affiliate it with Aplanulata, likely allied to one of the families whose members undergo encystment (Margelopsidae, Acaulidae, or Corymorphidae). Recently, authors have suggested that it is affiliated with Corymorphidae (Bouillon et al., 2006), although there are no strong morphological synapomorphies to group it with this family. Our examination of specimens of this species collected from Friday Harbor Laboratories (Gustav Paulay, pers. comm.) as well as photographs of specimens from Japan (Shin Kubota, pers. comm.) reveal that the most distal ends of scattered tentacles of *Hataia parva* are slightly rounded, lending them a capitate appearance. This characteristic, in combination with its possession of a pedal disc, reduced gonophores, encystment and reduction of perisarc, align this species morphologically with the family Acaulidae, whose members possess this combination of characteristics (Schuchert, 2006). Some authors have recently classified *Hataia parva* in Acaulidae, although this classification is not universally accepted (Cairns et al., 2003). Although we did not sample any other acaulid species, our analysis instead supports *Hataia parva* as grouping with the corymorphid *Corymorpha groenlandica* along with another family of Aplanulata with scattered tentacles along the body column – Candelabridae. At least one author has suggested that Acaulidae and Candelabridae are sister families (Bouillon, 1985), and morphological characteristics strongly align these two families. Based on both molecular and morphological evidence, we suspect that Acaulidae taxa would fall within our recovered *Corymorpha groenlandica* + *Hataia parva* clade. Clarification of the classification of *Corymorpha groenlandica* and *Hataia parva* and the phylogenetic affinity of these taxa to Acaulidae and Candelabridae awaits further sampling.

4.10.3. Phylogenetic placement of unsampled Aplanulata families

We were unable to sample other Aplanulata families for this study, including Paracorynidae and Tricyclusidae. Tricyclusidae has not been documented in the Mediterranean since it was first described in 1876, and has only rarely been reported in other

localities (Schuchert, 2006). Furthermore, we were unable to sample additional members of Acaulidae and Margelopsidae. All of these families include species that are rare and therefore difficult to sample for molecular analyses.

The strong affiliation between Corymorphidae and Tubulariidae into the superfamily Tubularioidea suggests that tentacle patterning may be an important evolutionary character for lineages in Aplanulata. Based on this character, we would hypothesize that members of Margelopsidae and Tricyclusidae are affiliated with this superfamily (both possess tentacles organized in groups, or whorls), while Acaulidae is associated with Candelabridae (both possess scattered, capitate tentacles). Reproductive characters also appear to be evolutionarily important in this lineage and may lend insight into relationships. We also did not sample Paracorynidae, but a number of features including reproduction through encystment and lack of a brooded actinula affiliate it with the Corymorphidae or Hydridae. Additional sampling and future phylogenetic studies that integrate morphological and molecular data will assist in determining a robust hypothesis for the phylogenetic placement of these divergent taxa, and will also likely reveal novel and interesting synapomorphies for evolutionary lineages within Aplanulata.

4.11. Taxonomic recommendations

Based on our results as well as a number of previous studies, we formally recommend the following changes to the taxonomy of Aplanulata and its component species, as compared to recent classifications by Schuchert (Schuchert, 2006, 2010) and Bouillon et al. (2006).

- (a) *Fukaurahydra anthoformis* falls within the genus *Corymorpha*, and is herein redesignated as *Corymorpha anthoformis*. We propose the following new diagnoses for the genus *Corymorpha* and for the species *Corymorpha anthoformis*.

Corymorpha M. Sars, 1835.

Type species: *Corymorpha nutans* M. Sars, 1835 by monotypy.

DIAGNOSIS: Solitary hydroids with more or less vasiform hydranth and long caulus, **or rarely with short, squat polyp with broad head.** Hydranth with one or several closelyset whorls of 16 or more moniliform or filiform tentacles and one **or more** aboral whorls of 16 or more long, non-contractile filiform tentacles. Gastrodermal diaphragm parenchymatic. Hydrocaulus stout, covered by a thin perisarc, filled with parenchymatic gastrodermis, with long peripheral canals; aboral end of caulus with papillae turning more aborally into rooting filaments, **rooting filaments scattered or gathered in a whorl**, rooting filaments composed of epidermis and solid gastrodermis, sometimes tips with non-ciliated statocysts. With or without asexual reproduction through constriction of tissue from aboral end of hydrocaulus.

Gonophores develop on blastostyles arranged in a whorl over aboral tentacles. Gonophores remain either as fixed sporosacs, **medusoids**, or are released as free medusae.

Medusa bell apex dome-shaped or pointed. Four marginal bulbs present, lacking long exumbrellar spurs. With a single tentacle or three short tentacles and one long tentacle that differ not merely in size but also in structure. Manubrium

(continued on next page)

thin-walled, sausage-shaped with flared mouth rim, reaching to umbrella margin. Cnidome comprises stenoteles, desmonemes, and haplonemes.

REMARKS: This diagnosis for the most part corresponds to Schuchert, 2010 (Schuchert, 2010) and Petersen, 1990 (Petersen, 1990), but with modifications (**indicated in bold**) to polyp body shape and arrangement of rooting filaments to include *Fukaurahydra* (*Corymorpha*) *anthoformis*. Medusoids are also added to diagnosis, as a number of species of *Corymorpha* produce these structures.

Corymorpha anthoformis (Yamada et al., 1977).
Fukaurahydra anthoformis Yamada et al., 1977, pp. 151–154, Fig. 1.

MATERIAL EXAMINED: Japan, exposed coast of Agheshima, near Senkaku Bay, Sado Island (Japan Sea); Collected by Dr. Yayoi M. Hirano on May 12, 2011. 6 mature polyps. Voucher Pending.

DIAGNOSIS: *Corymorpha* polyp with short, squat hydrocaulus, completely filled with parenchymatic endoderm. Base of polyp flat, with a ring of rooting filaments. Hydranth broad, plate-like. Live specimens with brightly-colored green, brown and red gonophores (see Fig. 4).

DESCRIPTION: See Yamada et al. (1977) and Yamada and Kubota (1991).

(b) The species *Corymorpha intermedia* groups with strong support within the genus *Euphysa*, and is herein redesignated as *Euphysa intermedia*.

Euphysa Forbes, 1848.

TYPE SPECIES: *Euphysa aurata* Forbes, 1848 by monotypy.

DIAGNOSIS: Corymorphid hydroid with hydrocaulus enveloped in gelatinous perisarc, covered by mud and detritus; hydrocaulus hollow, without peripheral longitudinal canals. Hydranth cylindrical to ovoid, with rounded hypostome, with 3–10 oral capitate tentacles and up to 20 aboral moniliform tentacles, no gastric diaphragm. Near base of hydranth papillae, each with an ecto-endodermal, statocyst-like structure. Gonophores singly or in clusters just above aboral tentacles, usually released as free medusae, rarely remaining as fixed sporosacs. Asexual reproduction through budding of polarity-reversed polyps from the hydranth above aboral tentacles and through asexual bodies constricted off from basal end of hydrocaulus. Medusa with an evenly rounded umbrella, **or rarely, a pointed umbrella with thickened apical mesoglea**. Umbrella without apical canal; with one to four tentacles, if more than one then usually unequally developed, but all of the same structure, usually moniliform; manubrium stout, cylindrical with small round mouth, shorter than bell cavity. **Phylogenetically, the least-inclusive clade containing *Euphysa intermedia*, *E. aurata*, *E. tentaculata*, *E. flammea* and *E. japonica*.**

REMARKS: The diagnosis of *Euphysa* follows Schuchert (2010), except for modifications made **in bold** to accommodate *Corymorpha* (*Euphysa*) *intermedia*.

Euphysa intermedia (Schuchert, 1996).

Corymorpha intermedia Schuchert, 1996: 104, fig. 62.

DIAGNOSIS: *Euphysa* medusa with apical process and a stout, cylindrical manubrium that narrows into a small, round mouth. Apical mesoglea thick. No apical canal or peduncle. Medusa with single moniliform tentacle and three non-tentacular bulbs.

DESCRIPTION: See Schuchert (1996).

NOTES: The medusa of *Euphysa intermedia* strongly resembles that of *Euphysa aurata*, with the exception of its possession of a thick, apical mesoglea and apical process.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympve.2012.12.016>.

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