Phylogeny of Capitata and Corynidae (Cnidaria, Hydrozoa) in light of mitochondrial 16S rDNA data

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New sequences of the partial rDNA gene coding for the mitochondrial large ribosomal subunit, 16S, are derived from 47 diverse hydrozoan species and used to investigate phylogenetic relationships among families of the group Capitata and among species of the capitate family Corynidae. Our analyses identify a well-supported clade, herein named Aplanulata, of capitate hydrozoans that are united by the synapomorphy of undergoing direct development without the ciliated planula stage that is typical of cnidarians. Aplanulata includes the important model organisms of the group Hydridae, as well as species of Candelabridae, Corymorphidae, and Tubulariidae. The hypothesis that Hydridae is closely related to brackish water species of Moerisiidae is strongly controverted by 16S rDNA data, as has been shown for nuclear 18S rDNA data. The consistent phylogenetic signal derived from 16S and 18S data suggest that both markers would be useful for broad-scale multimarker analyses of hydrozoan relationships. Corynidae is revealed as paraphyletic with respect to Polyorchidae, a group for which information about the hydroid stage is lacking. *Bicorona*, which has been classified both within and outside of Corynidae, is shown to have a close relationship with all but one sampled species of *Coryne*. The corynid genera *Coryne*, *Dipurena*, and *Sarsia* are not revealed as monophyletic, further calling into question the morphological criteria used to classify them. The attached gonophores of the corynid species *Sarsia lovenii* are confirmed as being derived from an ancestral state of liberated medusae. Our results indicate that the 16S rDNA marker could be useful for a DNA-based identification system for Cnidaria, for which it has been shown that the commonly used cytochrome c oxidase subunit 1 gene does not work.

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Introduction

In order to understand the evolutionary causes and consequences of the great diversity of cnidarian life cycles, robust phylogenetic hypotheses at all hierarchical levels within Cnidaria are needed. At the broadest scale, progress is being made as analyses of both morphology (Bouillon & Boero 2000; Marques & Collins 2004) and 18S rDNA (Collins 2000, 2002) converge in indicating that the two cnidarian groups having undergone the most frequent and dramatic life history evolution, Anthoathecata and Leptothecata, form a clade (along with Siphonophorae) within Hydrozoa known as Hydroidolina. Hydroidolinan hydrozoans provide excellent opportunities for generating hypotheses explaining evolutionary shifts in life cycle (Bouillon *et al.* 1991; Boero *et al.* 1992, 1997). However, these ideas have not yet been tested because they require sound phylogenetic hypotheses for multiple groups within Hydroidolina in order to establish whether or not broad-scale associations exist among hydrozoan life-history characteristics, such as geographical range, speciation rates, ecological circumstances, population genetic structures, etc.

Two groups that could eventually be key to understanding life-cycle evolution in Hydroidolina are Capitata and the capitate family Corynidae. The former comprises roughly 25 families, many of which exhibit marked life-cycle variation, and is classified along with its putative sister group, Filifera, in the hydrozoan order Anthoathecata. Capitata has been the subject of just a single character-based phylogenetic analysis

(Petersen 1990). Corynidae is a capitate family of approximately 90 species (following Schuchert 2001), whose colonial hydroids are common, though often not appreciated, components of shallow water marine communities around the world. Subsequent to the hydroid stage, corynid life cycles vary considerably. Whereas some species liberate free-swimming medusae, others have fixed gonophores, and still others lack any semblance of medusae. Classical taxonomy of Corynidae separated *Coryne* spp. from other corynid species based on absence and presence, respectively, of the medusa stage (Rees 1957; Brinckmann-Voss 1970; Bouillon 1985). However, this criterion for classification, while practical, appears to be artificial in the sense that it is unlikely to be reflective of evolutionary history (Petersen 1990; Schuchert 2001), a conclusion already confirmed for another hydroidolinan group, the filiferan family Hydractiniidae, with a similar taxonomic history (Cunningham & Buss 1993).

Despite recognizing that past taxonomy most likely fails to reflect the phylogeny of the group, Schuchert (2001), in his comprehensive review of Corynidae, concluded that evolutionary relationships, and even species identifications, are difficult if not impossible to determine based on morphological characters because they are limited in number and likely to be highly labile. Therefore, molecular data are necessary in order to accomplish the basic goals of systematics for Corynidae. In order to address this need, we have derived sequences (420–520 bp) from the mitochondrial 16S rRNA gene (16S) for 11 corynid species, 24 species representing 12 capitate families that may potentially be closely allied to Corynidae, 9 species of noncapitate hydroidolinans, and 4 trachyline hydrozoans as definitive outgroups. We evaluate whether the mitochondrial 16S gene fragment contains suitable information to assess the phylogenetic status of Corynidae, identify species groups that are closely related to corynids, and generate reliable hypotheses of phylogenetic relationships among capitate families and corynid species.

Materials and methods

DNA was obtained from a wide diversity of hydrozoan species, arranged taxonomically in Table 1. For higher-level hydrozoan taxonomy, we followed Marques & Collins (2004), whereas for Capitata we followed Petersen (1990) with changes effected by Schuchert (1996). DNA was extracted by using either the DNA extraction kits, DNAzol (Molecular Research Center Inc., Cincinnati) or Invisorb (Invitek GmbH, Berlin), or through a basic protocol involving tissue homogenization, proteinase K digestion, extraction with phenol/chloroform/isoamylalcohol (25 : 24 : 1), centrifugation at 8000 **g** for 15 min, and precipitation with 0.1 vol. 5 M NH_4Ac and 2.5 vol. ice-cold 98% EtOH. Two pairs of primer sets were used to amplify nearly identical regions of mitochondrial 16S rDNA. For some samples, hydrozoan specific primers (fwd: GGTGWHRCC TGCCCAVTG; rev: TAAAGGTCGAACAGACCTAC; Werner Schroth, Hannover) were used to amplify approximately 420–480 bp of the mitochondrial 16S rRNA gene. Alternatively, the forward primer from Cunningham & Buss (1993) was combined with the reverse primer from Schroth *et al.* (2002) to amplify roughly 450–520 bp. PCR products were subsequently purified and sequenced in both directions using an automated sequencer (either an ABI 310 or a Megabace 500).

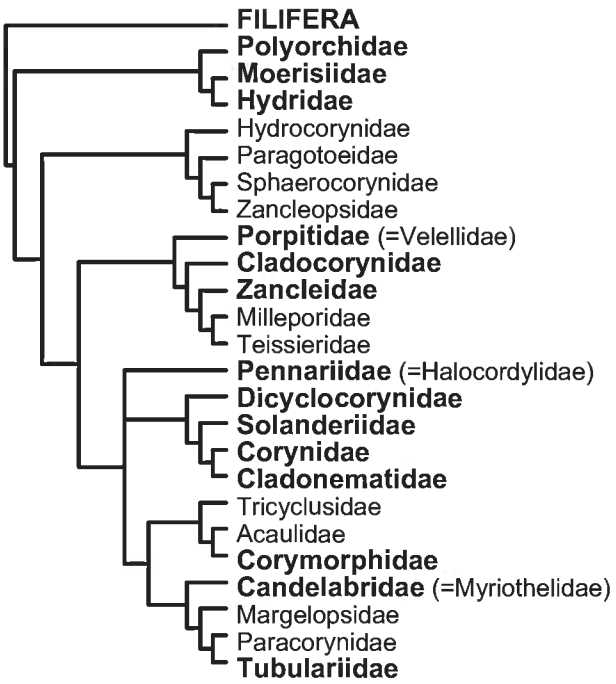

Fig. 1 Phylogenetic hypothesis of relationships among capitate hydrozoans from Petersen (1990). Taxon names in bold are sampled in the present analysis of partial mitochondrial 16S rDNA sequences.

Edited 16S sequences were aligned by eye, along with sequences from 5 additional hydrozoans obtained from GenBank (Table 1), using the software SEAVIEW. Our aligned data set (available upon request) covers 14 of 24 families treated in Petersen's (1990) phylogenetic analysis of Capitata (Fig. 1). One of Petersen's (1990) families, Halocorynidae, is assumed to fall within Zancleidae (following Schuchert 1996). We have not been able to sample Protohydridae, which may be allied to Corymorphidae (Stepanjants *et al.* 2000), the recently erected Boeromedusidae (Bouillon 1995), or Halimedusidae, which was recently transferred from Filifera (Mills 2000). Both of the latter two groups may be closely related to Polyorchidae (Mills 2000).

Sites of ambiguous homology within the alignment were excluded from phylogenetic analyses, which were carried out using PAUP*4.0 (Swofford 1998). Maximum parsimony (MP), minimum evolution (ME), and maximum likelihood (ML)

Table 1 Hydrozoan samples used in this analysis, with life cycle notes for capitate species, GenBank accession numbers, and locality of source material. Life cycle notes: cp — colonial polyp stage; sp — solitary polyp stage; ap — absence of polyp stage; fm — free swimming medusa stage; eu — liberated eumedusoids; fg — fixed gonophores; am — complete absence of medusa stage and/or gonophores; nk — not known.

Higher Taxonomy	Species; Life cycle notes	Acc. No.	Locality of material
Trachylina			
Limnomedusae	*Craspedacusta sinensis*	AY512507	China
Limnomedusae	*Maeotias marginata*	AY512508	Northern California
Limnomedusae	*Olindias phosphorica*	AY512509	Mallorca
Narcomedusae	*Aegina citrea*	AY512510	Coast of California
Trachymedusae	*Liriope tetraphylla*	U19377	not known
Hydroidolina			
Leptothecata	*Aequorea aquorea*	AY512518	Woods Hole, MA
Leptothecata	*Blackfordia virginica*	AY512516	Northern California
Leptothecata	*Clytia* sp.	AY512519	Coast of California
Leptothecata	*Melicertissa* sp.	AY512515	Guam
Leptothecata	*Tiaropsidium kelseyi*	AY512517	Coast of California
Siphonophorae	*Nectopyramis* sp.	AY512512	Coast of California
Siphonophorae	*Physalia utriculus*	AY512511	Tasmania
Anthoathecata			
Filifera	*Podocoryna carnea*	AY512513	not known
Filifera	*Thecocodium quadratum*	AY512514	not known
Capitata			
Candelabridae	*Candelabrum cocksii*; sp., fg	AY512520	France, Atlantic
Cladocorynidae	*Cladocoryne floccosa*; cp, fg	AY512535	Mallorca
Cladonematidae	*Cladonema radiatum*; cp, fm	AY512539	France, Atlantic
Cladonematidae	*Eleutheria dichotoma*; cp, fm	AY512538	France
Mediterranean			
Cladonematidae	*Staurocladia bilateralis*; nk, fm	AY512537	Japan
Cladonematidae	*S. oahuensis*; nk, fm	AY512536	Japan
Cladonematidae	*S. wellingtoni*; cp, fm	AJ580934	New Zealand
Corymorphidae	*Corymorpha intermedia*; nk, fm	AY512526	New Zealand
Corymorphidae	*C. nutans*; sp., fm	AY512527	France, Atlantic
Corynidae	*Bicorona tricycla*; cp, fg	AJ608640	New Zealand
Corynidae	*Coryne eximia*; cp, fm	AY512541	France, Atlantic
Corynidae	*C. japonica*; cp, fm	AY512540	New Zealand
Corynidae	*C. muscoides*; cp, fg	AY512553	France, Atlantic
Corynidae	*C. pintneri*; cp, fg	AY512542	France, Mediterannean Sea
Corynidae	*C. producta*; cp, fm	AY512543	Sandgerdi, Iceland
Corynidae	*C. pusilla*; cp, fg	AY512552	France, Atlantic
Corynidae	*Dipurena reesi*; cp, fm	AY512546	Brazil
Corynidae	*D. simulans*; cp, fm	AY512547	France, Atlantic
Corynidae	*Sarsia lovenii*; cp, fg	AJ608796	Sandgerdi, Iceland
Corynidae	*S. marii*; cp, fm	AY512544	France, Mediterannean Sea
Corynidae	*S. mirabilis*; cp, fm	AY512548	Woods Hole, MA
Corynidae	*S. tubulosa*; cp, fm	AY512545	not known
Hydridae	*Hydra circumcinta*; sp., am	AY512521	Switzerland
Hydridae	*H. vulgaris*; sp., am	AY512522	Switzerland
Moerisiidae	*Moerisia* sp.; sp., fm	AY512534	Northern California
Pennariidae	*Pennaria disticha*; cp, eu	AY512533	Mallorca
Polyorchidae	*Polyorchis haplus*; nk, fm	AY512549	Northern California
Polyorchidae	*P. penicillatus*; nk, fm	AY512550	Friday Harbor, WA
Polyorchidae	*Scrippsia pacifica*; nk, fm	AY512551	San Diego, CA
Porpitidae	*Porpita* sp.; cp, fm	AY512529	Guam
Porpitidae	*Velella velella*; cp, fm	AY512528	Coast of California
Solanderiidae	*Solanderia ericopsis*; cp, fg	AY512530	New Zealand
Tubulariidae	*Ectopleura larynx*; cp, fg	AY512523	France, Atlantic
Tubulariidae	*E. wrighti*; cp, fm	AY512524	Mallorca
Tubulariidae	*Hybocodon prolifer*; sp., fm	AY512525	France, Atlantic
Tubulariidae	*Tubularia indivisa*; sp., fg	U19379	not known
Zancleidae	*Zanclea costata*; cp, fm	AY512531	France, Mediterannean Sea
Zancleidae	*Z. sessilis*; cp, fm	AY512532	Mallorca

criteria were used to perform replicate searches (500, 500, and 10, respectively) for optimal trees. Likelihood ratio tests employed by ModelTest (Posada & Crandall 1998) were used to determine an appropriate model of nucleotide evolution assumed for the ME and ML searches. Bootstrap analyses with 500 replicates under MP and ME were conducted in order to assess node support. Finally, parsimonious searches were carried out to find optimal trees that are constrained to conform to prior hypotheses of relationships among capitate hydrozoan groups.

Results

After excluding sites of ambiguous alignment, the data set used for phylogenetic analysis contains 415 nucleotide characters, of which 161 do not vary across our samples. Of the 254 variable characters, 226 are parsimony informative. This partial mitochondrial 16S fragment is AT-rich for the sampled hydrozoans, with mean nucleotide frequencies of 40.5, 11.6, 15.6, and 32.3% for A, C, G, and T, respectively. Hierarchical likelihood ratio tests implemented using ModelTest (Posada & Crandall 1998) indicate that the model that best fits our data (TVM + I + G) has different rates for each type of transversion, one rate for transitions, an assumed proportion of invariable sites (0.337), and a gamma shape parameter (0.656).

The ML topology (Fig. 2) is broadly similar to the consensus MP (nine MP trees of length 1756) and ME topologies (not shown). Numerous nodes throughout the topologies do not receive strong support from the partial mitochondrial 16S sequences. Not surprisingly, these are the nodes that most often differ when the various criteria are used for evaluation. Many taxa are revealed as nonmonophyletic, e.g. Capitata, Cladonematidae, Corymorphidae, Corynidae, Tubulariidae, and Zancleidae. Although there is a well-supported break between Trachylina and Hydroidolina, relationships among the hydroidolinan taxa are resolved, but with little support. Anthoathecata (Capitata + Filifera) is revealed as paraphyletic, as is Capitata with respect to Filifera, Leptothecata and Siphonophorae.

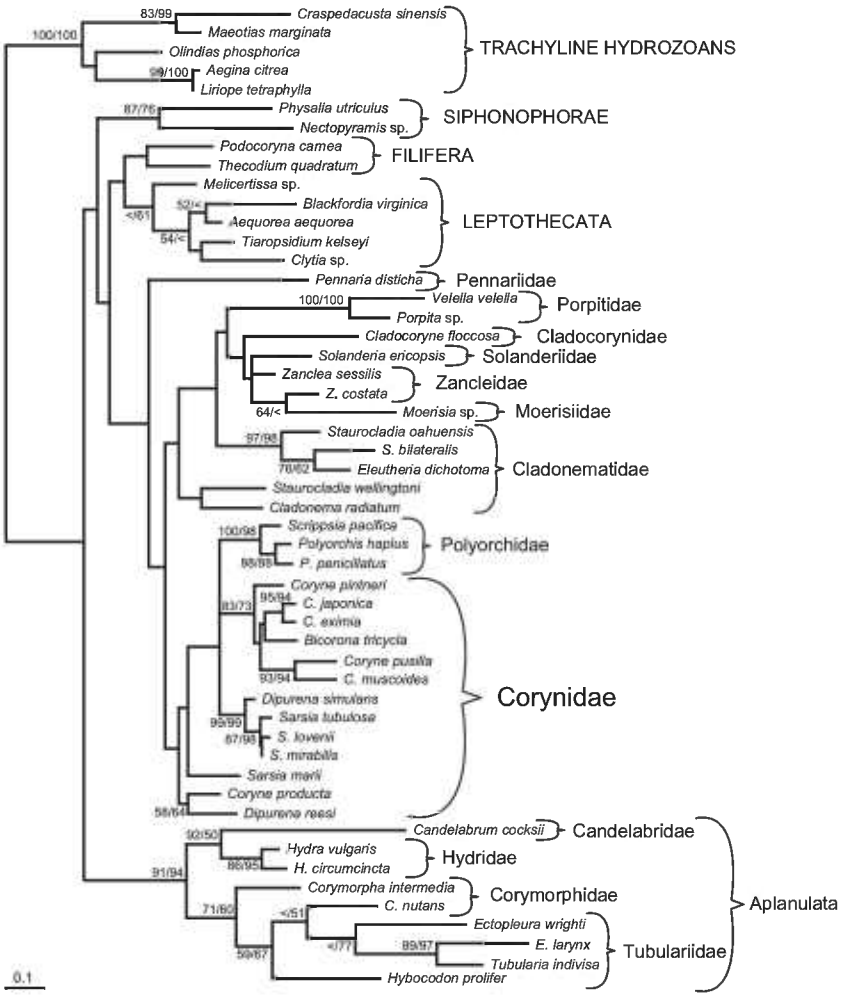

Fig. 2 ML topology. Hydrozoans of the group Trachylina are used as definitive outgroups to the analysis of Hydroidolina. Within Hydroidolina, noncapitate groups are indicated in all capitals. Bootstrap indices under ME and MP are shown, separated by a slash, at nodes when one or both of these values exceed 50. '<' indicates a bootstrap index less than 50. Scale bar denotes 0.1 nucleotide substitutions per site.

Within Capitata, a strongly supported association of Candelabridae, Corymorphidae, Hydridae, and Tubulariidae is shown. Within this clade, herein referred to as Aplanulata (see below for explanation of the name), Candelabridae appears to be the taxon most closely related to the important model organisms of Hydridae. Although the node support is relatively weak, Corymorphidae plus Tubulariidae appears as a clade in optimal topologies under each of the criteria. The non-Aplanulata capitates also form a clade in optimal ML, ME and MP topologies. With the exception of the well-supported associations among *Staurocladia oahuensis*, *S. bilateralis* and *Eleutheria dichotoma*, relationships among representatives of the capitate families Cladocorynidae, Cladonematidae, Moerisiidae, Pennariidae, Porpitidae, Solanderiidae, and Zancleidae shown in the ML topology receive no substantive bootstrap support.

On the other hand, several well-supported clades are revealed within Corynidae. *Dipurena simulans*, *Sarsia tubulosa*, *S. lovenii* and *S. mirabilis* appear to be closely allied, as do *Coryne japonica* and *C. eximia* as well as *C. pusilla* and *C. muscoides*. These latter two species pairs fall within a well-supported clade that also includes *C. pintneri* and *Bicorona tricyda*. Finally, in all optimal topologies, species of Polyorchidae (revealed as a strongly supported clade) group within Corynidae, specifically in a clade containing all examined corynid species other than *Coryne producta*, *Dipurena reesi* and *Sarsia marii*.

Discussion

This work addresses phylogenetic questions dealing with hydrozoans at various hierarchical levels using partial sequences of the mitochondrial 16S rDNA gene. At the highest level, this marker is consistent with the phylogenetic signal contained in, and the inferences based upon, 18S data (Collins 2000, 2002), which separate hydrozoans into two major clades, Trachylina (Limnomedusae, Narcomedusae, and Trachymedusae) and Hydroidolina (Anthoathecata, Leptothecata, and Siphonophorae). These two groupings are also present in recent phylogenetic analyses of Bouillon & Boero (2000) and Marques & Collins (2004) based on morphology and life history characteristics, though the two analyses differ on the inferred placement of the root among these taxa, and therefore contradict one another on whether the two groupings are monophyletic or not. The analysis presented here does not test these two alternatives because it does not include any nonhydrozoans to establish a root within Hydrozoa. Other higher-level hydrozoan groups such as Laingiomedusae and the interstitial species of Actinulida have yet to be sampled for any molecular markers.

Within Hydroidolina, neither partial 16S nor complete 18S (Collins 2000, 2002) data provide a strong signal resolving the major taxa, indicating that historical divergences among hydroidolinan taxa may have occurred within a narrow time frame. If so, a great deal of additional molecular data from markers that have evolved at appropriate rates will be necessary to clarify hydroidolinan relationships. Our analyses of partial 16S data further mirror results based upon 18S by suggesting that both Anthoathecata and Capitata may be paraphyletic with respect to the other hydroidolinan groups. Both sets of data indicate that the important model organisms classified in the family Hydridae are somewhat removed phylogenetically from other capitates. Given that partial mitochondrial 16S data are largely consistent with nuclear 18S data, both markers would be suitable for use in future multimarker phylogenetic analyses of Capitata, as well as Hydroidolina, Hydrozoa, and perhaps even Cnidaria as a whole.

While our data set suffers somewhat from limited sampling within Capitata, it is the most complete molecular data set yet compiled, and it furthers the 18S results by revealing a well-supported alliance between Hydridae and species classified in Tubulariidae, Corymorphidae, and Candelabridae. Identifying the closest living relatives of Hydridae is of obvious interest because more is known about the biology of *Hydra* than all other cnidarians. That Hydridae is closely related to Tubulariidae, Corymorphidae, and Candelabridae is a result unanticipated by all but one prior study. Naumov (1960) hypothesized that Hydridae was not part of a clade of capitate hydrozoans, but he viewed the group as closely allied to Limnomedusae, a result strongly contradicted by 18S data (Collins 2000) as well as the present analysis. Similarly, our results, as well as those based on 18S, contradict the hypothesis that Hydridae is closely related to Moerisiidae (Petersen 1990). In contrast, Stepanjants *et al.* (2000) envisioned that Hydridae might have originated from aberrant corymorphids, a view that is reasonably consistent with our data. However, rather than Corymorphidae, our 16S data suggest that Hydridae may share a particularly close relationship to Candelabridae.

Our 16S data are also consistent with morphology-based hypotheses that Tubulariidae, Corymorphidae and Candelabridae are closely related to each other (Naumov 1960; Petersen 1990). Other taxa not sampled here have also been hypothesized to be part of this group (Fig. 1) and continued sampling may reveal that the clade includes additional taxa. The synapomorphy identified by Petersen (1990) as uniting these groups, namely development from egg to polyp via a nonciliated stereogastrula stage, as opposed to the ciliated planula more typical of hydrozoans, also describes what occurs in species of Hydridae. Therefore, we suggest that this character is likely derived from the most recent common ancestor of the clade containing Candelabridae, Corymorphidae, Hydridae and Tubulariidae, for which we accordingly provide the name Aplanulata. Finally, subsequent work confirms that 18S data also strongly support the monophyly of Aplanulata (Collins *et al.* unpublished observation).

Although support for monophyly of the group is weak, under all optimality criteria the non-Aplanulata capitates included in this analysis form a clade. As judged by bootstrap indices, relationships among these nine sampled families are uncertain. While the ML topology (Fig. 2) contradicts in various ways past views about capitate relationships (Rees 1957; Naumov 1960; Petersen 1990), the lack of support suggests that our 16S data may not be used to argue strongly against most of these past hypotheses. However, this is not the case. By constraining tree searches to find only the best topologies conforming to a particular hypothesis, we can begin to understand to what extent our partial mitochondrial 16S data contradict prior hypotheses.

For instance, Petersen (1990) proposed that Polyorchidae is the sister group to a clade consisting of Moerisiidae and Hydridae. Such a grouping does not appear in optimal trees under any criteria, and the most parsimonious trees that conform to Petersen's hypothesis would require 41 additional nucleotide substitutions than do those without any constraints. However, given that the putative synapomorphy uniting these three groups — gonads arranged interradially on stomach and enlarged gastric pouches of medusa — is only shared by species of Moerisiidae and Polyorchidae, it is reasonable to investigate the optimality of a grouping of Polyorchidae and Moerisiidae.

Even without involving Hydridae, which has a well-supported position elsewhere within Capitata (Fig. 2), a grouping of Moerisiidae and Polyorchidae also seems to be rather unlikely since the most parsimonious tree that contains a grouping of *Moerisia* sp. and the three polyorchid species sampled here is 18 steps longer than the unconstrained MP trees. Instead, under all optimality criteria, Polyorchidae groups within Corynidae. Polyp stages are not known for any species of Polyorchidae and would likely provide key observations bearing on the hypothesis represented here (Fig. 2) that *Polyorchis* and *Scrippsia*, along with potentially closely related species of *Boeromedusa*, *Halimedusa*, *Tiaricodon*, and *Urashimea* (Mills 2000), are descended from within Corynidae. Interestingly enough, the supposed polyp stage of *Polyorchis penicillatus* was once erroneously thought to be that of a corynid species that produces juvenile medusae closely resembling those of *P. penicillatus* (Brinckmann-Voss 2000).

Petersen (1990) grouped Cladocorynidae, Porpitidae, and Zancleidae (along with Milleporidae and Teissieridae not sampled here; Fig. 1) because of their shared lack of desmonemes in both polyp and medusa. This grouping is controverted by our optimal topologies (Fig. 2), which place Moerisiidae and Solanderiidae among these groups. However, the most parsimonious trees that conform to Petersen's hypothesis are only a single step longer than those without constraints. Petersen (1990) proposed that Moerisiidae is the most closely related capitate group to Hydridae, but this hypothesis is strongly controverted by our data, requiring 31 additional nucleotide substitutions. Solanderiidae has been thought to be a close ally of Cladonematidae and Corynidae (Petersen 1990; Schuchert 2001), but this grouping is also relatively suboptimal in light of our 16S data, as it would require 21 extra character changes.

However, given the results presented above, it seems reasonable to investigate grouping Solanderiidae with Cladonematidae, Corynidae, as well as Polyorchidae. Most parsimonious trees consistent with such a grouping are 13 steps longer. Without knowing the true tree, it is of course impossible to know how much weight to give 13 additional steps, as opposed to three or 20, when comparing competing hypotheses. At present we must conclude that our results favour the hypothesis that both Moerisiidae and Solanderiidae are more closely related to Cladocorynidae, Porpitidae, and Zancleidae than has previously been supposed. However, our data provide little basis for determining the specific relationships among these five taxa. Future studies with additional characters and taxon sampling are required in order to resolve them.

The group Pennariidae has been proposed as either closely related to Candelabridae, Cladonematidae, and Corynidae (Rees 1957; Naumov 1960) or as a member of an even larger clade including Candelabridae, Cladonematidae, Corynidae, Corymorphidae, Solanderiidae, and Tubulariidae (as well as Acaulidae, Margelopsidae, and Tricyclusidae, not sampled here, Petersen 1990). Both of these groups are suboptimal, given our data, requiring 34 and 42 additional character changes, respectively. Allowing for the inclusion of Polyorchidae within these hypothetical clades reduces the number of additional steps to 26 and 35. Alternatively, species of Pennariidae have been thought of as closely related to Tubulariidae (Petersen 1979), a hypothesis that is less parsimonious by 20 additional character changes.

Our results show strong support for a clade of *Eleutheria dichotoma*, *Staurocladia bilateralis*, and *S. oahuensis*, a result of interest because *E. dichotoma* is used as a model organism in studies of Hox gene evolution (Kuhn *et al.* 1996). These two genera are sometimes classified within Cladonematidae (as in Petersen 1990) but are often placed separately in Eleutheriidae (Kramp 1961; Bouillon & Boero 2000). However, it is widely appreciated that the morphology of these species, particularly the branched medusa tentacles bearing adhesive pads, indicates that they share a close affinity with species of *Cladonema* (Naumov 1960; Petersen 1990; Schuchert 1996; Schierwater & Ender 2000). The ML topology (Fig. 2) contains a nonmonophyletic grouping of these taxa, but our data do not provide strong evidence against monophyly of this group because it is present in the optimal ME topology and requires just three additional character changes under parsimony.

Turning to Corynidae, our optimal trees are not consistent with a hypothesis that the corynid species we have sampled

are monophyletic, nor do they contain a well-supported monophyletic grouping of Corynidae plus Polyorchidae. In some ways this is not very surprising since no morphological synapomorphies have been identified for Corynidae (Schuchert 2001). However, these two hypotheses of monophyly would only necessitate five and one additional character changes to accommodate and therefore our data do not warrant strong conclusions that either is false. One group that has been thought of as closely related to Corynidae is Zancleidae (Naumov 1960), but most parsimonious trees containing a grouping of these species are 26 steps longer (15 including polyorchid species). Alternatively, Petersen (1990) proposed, and Schuchert (2001) concurred, that the most likely sister group to Corynidae is Cladonematidae based on their shared possession (in most species) of thin filiform polyp tentacles bereft of nematocysts. While this hypothesis is not present in any optimal topologies, just four more substitutions are required under parsimony tree searches constrained to fit the hypothesis that Cladonematidae is the sister group of Corynidae plus Polyorchidae (10 steps if Polyorchidae is excluded).

Only two analyses have been conducted on phylogenetic relationships within Corynidae, those of Petersen (1990) and Schuchert (2001). The results presented here (Fig. 2), while limited by having sampled just 13 of *c.* 90 species, are informative. For example, of the 10 species of *Sarsia* (excluding problematic and indeterminable species, Schuchert (2001)), one, *S. lovenii*, is unlike the others in that it has medusoids that remain attached to the sessile polyp stage and a cnidome including oval-shaped microbasic mastigophores and isorhizas. Nevertheless, other characters indicate a close relationship with *Sarsia* and *Dipurena* species and Schuchert (2001) provisionally assigned the species to *Sarsia* awaiting molecular investigations. Partial mitochondrial 16S sequences confirm a close relationship of this species with *S. tubulosa*, and *S. mirabilis* (Fig. 2), suggesting that evolution from free-swimming medusae to attached gonophores has occurred independently in the lineage leading to *S. lovenii*.

Not all *Sarsia* species included in our analysis group together, however. Schuchert (2001) considers *S. marii* insufficiently described and suggests that the species might actually be more closely related to species of *Dipurena* based on its possession of capitate medusa tentacles. Indeed, in all of our analyses, *S. marii* falls near the base of Corynidae, often in association with *Dipurena reesi* and *Coryne producta*. This result might warrant a taxonomic shift in this species from *Sarsia* to *Dipurena*, but given that our two representatives (out of nine) of the genus *Dipurena* do not group together (Fig. 2), it would be premature to do so at this time. Given that it requires 16 and 19 additional character changes to find topologies that contain monophyletic *Dipurena* and *Sarsia*, respectively, the utility of the morphological characters, which indeed vary within the groups, used to unite species into these genera are called into question. Moreover, *Dipurena* and *Sarsia* have been postulated as being closely related to each other based upon their shared possession of manubria that are proximally thin, distally thick, contain stomachs, and extend lower than the medusa bell (Petersen 1990; Schuchert 2001). Thirteen additional character changes are needed to accommodate a monophyletic grouping of all six sampled *Dipurena* and *Sarsia* species, although the number of extra changes drops to five if *C. producta* is also included in the putative clade.

We have been able to sample one of two species in the genus *Bicorona*. Petersen (1990) considered *Bicorona* to be a synonym of *Dicyclocoryne* and excluded the group from Corynidae, decisions that were criticized by Schuchert (2001). We have not been able to sample the sole representative of *Dicyclocoryne* (*sensu* Schuchert 2001), but we can conclude that *Bicorona tricycla* clearly groups with species of *Coryne* and its inclusion within Corynidae appears to be warranted. Moreover, this species, which lacks a free swimming medusa stage but instead has gonophores that remain fixed to the hydranth, falls within a clade that contains three other species (*Coryne pintneri*, *C. pusilla* and *C. muscoides*) that also have fixed gonophores. However, two species (*C. japonica* and *C. eximia*) that liberate medusae also appear in the clade. Their nested position potentially indicates that having released medusae (in these two species) is derived from an ancestral character state of fixed gonophores. On the other hand, just two additional character changes are necessary to accommodate the hypothesis that these two taxa are basal within this clade and that their liberated medusae potentially represent the plesiomorphic state for the group.

Coryne pusilla and *C. muscoides* form a strongly supported clade (Fig. 2), confirming the idea that the two species are closely related (Petersen 1990; Schuchert 2001). Our samples derive from the north-eastern Atlantic, where these two species, though similar, appear to be readily distinguishable by taxonomic experts. Within the Mediterranean, on the other hand, the individuals are highly variable and determining their species status is difficult (Schuchert 2001). Mitochondrial 16S data could prove useful in illuminating the precise relationship between Mediterranean and Atlantic populations.

The result that *C. producta* groups with *Dipurena reesi* is rather confusing because the former, in both polyp and medusa stages, very closely resembles *C. japonica*, which is also included in our analysis. On the other hand, our own sampling raises some issues for consideration. First, our analysed individuals of *C. producta* were sampled without knowledge of the complete life cycle and a misidentification of some species is possible. Second, tissue from *C. japonica* was collected from New Zealand populations, which differ in terms of size of nematocysts, arrangement of tentacles, development of gonophores,

and size of medusa (Nagao 1962; Schuchert 1996; Schuchert 2001). It is possible that our sample from New Zealand represents an undescribed species. Further replicate sampling is clearly advisable in order to correctly and fully interpret our analyses.

Conclusion

Mitochondrial 16S structure has been shown to be phylogenetically informative at the class level within at least two phyla (Lydeard *et al.* 2000; Ender & Schierwater 2003). We have found that at the sequence level, this marker is not a perfect indicator of Corynidae and Capitata phylogeny because it does not provide a basis for making strong conclusions about the phyletic status of Corynidae or about the identity of those capitate hydrozoans most closely related to corynids. Nevertheless, we have shown that this gene fragment contains historical signal that helps evaluate various hypotheses at hierarchical levels ranging from those that deal with taxa traditionally given order status to those at the species level. These sequences are helpful for addressing phylogenetic questions at different scales probably because different portions of this gene are evolving at different rates.

Partial 16S sequences are easy to amplify and sequence and should prove to be efficient and useful in more exhaustive studies of populations or closely related species. Given that this marker has also been successful in sorting out the scyphozoan *Aurelia* species complex (Schroth *et al.* 2002), it may fill a void for a DNA-based identification system for Cnidaria, for which it has been shown that the commonly used cytochrome c oxidase subunit 1 gene does not work (Hebert & Ratnasingham 2003). Finally, partial 16S sequences appear to contain information that is congruent with that present in nuclear 18S data, suggesting that both sets of data reflect phylogenetic history and are therefore appropriate for use in large-scale multigene analyses aimed at providing stable phylogenetic hypotheses of speciose hydrozoan groups.

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