

Phylogeny of Medusozoa and the evolution of cnidarian life cycles

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Hydrozoa;
Scyphozoa;
Stauromedusae.

Abstract

To investigate the evolution of cnidarian life cycles, data from the small subunit of the ribosome are used to derive a phylogenetic hypothesis for Medusozoa. These data indicate that Cnidaria is monophyletic and composed of Anthozoa and Medusozoa. While Cubozoa and Hydrozoa are well supported clades, Scyphozoa appears to be paraphyletic. Stauromedusae is possibly the sister group of either Cubozoa or all other medusozoans. The phylogenetic results suggest that: the polyp probably preceded the medusa in the evolution of Cnidaria; within Hydrozoa, medusa development involving the entocodon is ancestral; within Trachylina, the polyp was lost and subsequently regained in the parasitic narcomedusans; within Siphonophorae, the float originated prior to swimming bells; stauromedusans are not likely to be descended from ancestors that produced medusae by strobilation; and cubozoan polyps are simplified from those of their ancestors, which possessed polyps with gastric septa and four mesogleal muscle bands and peristomial pits.

Introduction

The considerable morphological disparity present within Cnidaria is evidenced by delicate siphonophores, massive medusae and corals, feathery hydroids, interstitial polyps and box jellies possessing complex eyes. Despite this diversity, these relatively simple metazoans are united in possessing nematocysts, most probably as a result of common ancestry. Corroborating evidence that cnidarians are monophyletic within Metazoa comes from molecular sequence data of the small subunit (SSU) of the ribosome (Collins, 1998; Kim *et al.*, 1999; Collins, 2000), as well as the large subunit (LSU) of the ribosome (Medina *et al.*, 2001). Most phylogenetic analyses of Cnidaria have focused on determining the relationships among the four main taxa that compose it – Anthozoa, Cubozoa, Hydrozoa, and Scyphozoa (Werner, 1973; Salvini-Plawen, 1978; Brusca & Brusca, 1990; Meglitsch & Schram, 1991; Bridge *et al.*, 1992, 1995; Schuchert, 1993). From this work, a consensus has emerged that

Anthozoa is the sister group of the remaining cnidarians, which are collectively referred to as Medusozoa (Petersen, 1979), or less often as Tesserazoa (Salvini-Plawen, 1978). Particularly convincing evidence for the monophyly of medusozoans is their shared possession of linear mitochondrial genomes (Bridge *et al.*, 1992) and medusae. However, relationships among the major medusozoan groups remain contentious (Collins, 2000).

There is a general correspondence between major taxonomic divisions and life-cycle differences within Cnidaria. Anthozoan ontogeny is most straight forward, involving a planula, settlement, and growth into a sessile polyp, which is the adult stage. Cubozoa, the most recently defined class of the phylum Cnidaria (Werner, 1975), is comprised of species that have planulae, which settle and develop into sessile polyps. The cubopolyp subsequently metamorphoses entirely into a single sexual medusa (Werner *et al.*, 1971; Arneson & Cutress, 1976). Many hydrozoans possess planulae, polyps and medusae. Usually, the hydrozoan medusa develops from a tissue mass termed the entocodon and is budded laterally from polyps. Of all the cnidarian groups, however, hydrozoans have the greatest variation in life cycles and the polyp or medusa stages are entirely lacking for some groups (as for example in the Trachymedusae

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and Hydridae, respectively). Scyphozoans generally have a life cycle that includes planulae that develop into sessile polyps. Scyphopolyps characteristically give rise to multiple juvenile medusae (ephyrae) by metamorphosis and transverse fission at their oral ends, a process termed strobilation. As in Cubozoa and Hydrozoa, the medusa is the typical sexual stage of the scyphozoan life cycle.

The goal of the work presented here is to move towards a better understanding of the phylogeny of Medusozoa to gain insight into some of the many evolutionary transitions in life cycle that have taken place during the history of Medusozoa. To this end, I have generated complete sequences of the SSU rRNA gene from 55 medusozoans (plus one anthozoan and two ctenophores). One advantage of phylogenetic analyses of speciose groups based on molecular sequences rather than morphological data is that individuals rather than supraspecific taxa are sampled. This allows, and in fact necessitates, that hypotheses of the monophyly of supraspecific taxa are tested when more than one individual of any given group are included in an analysis. When robust, results of these tests provide insight for understanding the evolution of the group under consideration. On the other hand, the advantage is nullified if the molecular sequences being sampled have not evolved at a rate that is appropriate for revealing the ages of the divergences being investigated. The SSU gene has been applied extensively to questions of metazoan phylogeny. The success achieved in these studies undoubtedly varies, and can best be judged in the light of independent lines of evidence. Analysis of complete SSU sequences for hydrozoans yielded results that are remarkably congruent with hydrozoan taxonomy (Collins, 2000) and cladistic analyses of morphological data (Marques, pers. comm.), suggesting that SSU may be suitable for revealing phylogenetic relationships among medusozoan cnidarians. Herein, I investigate the strength of phylogenetic hypotheses suggested by complete SSU sequences from 74 cnidarians, and discuss the implications of these hypotheses for testing and generating conjectures regarding the evolution of cnidarian life cycles.

Materials and methods

Genomic DNA was isolated from tissue samples of 55 species of medusozoans, one anthozoan, and two ctenophores (Table 1). Tissue samples were either fresh, preserved in 75–95% ethanol, or frozen (–80°). High molecular weight genomic DNA was extracted by pulverizing tissue in the reagent DNAzol (Chomczynski *et al.*, 1997), followed by centrifugation and ethanol precipitation. The complete sequences for SSU were amplified from genomic DNA preparations using eukaryotic-specific primers (Medlin *et al.*, 1988) via polymerase chain reaction (PCR) (30 cycles: 10 s at 94°, 60 s at 38–48°, and 180 s at 72°) after an initial 2-min 94° denaturation. PCR products were directly sequenced in

both directions with an ABI Prism 377 DNA Sequencer (Perkin-Elmer Instruments, Norwalk, CT, USA), with the exception of SSU for *Aequorea aequorea*, which was sequenced with a Li-Cor model 4000L infrared automated DNA sequencer (Li-Cor Inc., Lincoln, NB, USA).

The sequences were aligned with others obtained from GenBank by eye in BioEdit (Hall, 1999). Two data sets for analyses were culled from the complete sequences aligned in BioEdit. The first data set contains 1668 characters for a relatively broad sampling of 132 taxa: 74 cnidarians; 52 noncnidarian animals; and six choanoflagellates and mesomycetozoans as outgroups (Table 1). These data, referred to hereafter as the broad data set, were used to address hypotheses concerning the phylogenetic placement of Cnidaria within Metazoa, as well as the monophyly of Cnidaria and Medusozoa. A second Cnidaria-only data set, composed of 1768 nucleotide characters for the 74 cnidarian taxa, was used to investigate phylogenetic questions within Medusozoa. In both cases, characters were excluded if there was little confidence that the positions were homologous across the taxa being considered. A number of cnidarian groups of interest were not sampled as a result of a lack of access to tissues, including Laingiomedusae (Hydrozoa), Actinulidae (Hydrozoa), *Polypodium* (Cnidaria, *incertae sedis*), *Tetraplatia* (Cnidaria, *incertae sedis*), and Tesseranthinae (Stauromedusae).

Two hundred replicate searches for the most parsimonious trees, with taxa added randomly, were carried out on the broad data set using PAUP* (Swofford, 2000). Transversions were weighted three to two vs. transitions, in order to compensate for a slight bias in these types of substitutions (T ratio = 1.74) estimated using maximum likelihood (ML). Two bootstrap analyses (200 replicate searches) under maximum parsimony (MP) and minimum evolution (ME) criteria were carried out using the broad data set. The ME search assumed the HKY85 model of nucleotide evolution (γ set to 0.388, as estimated using ML and a neighbour-joining tree). Searches for optimal ML topologies were not possible with the broad data set because of computational limitations.

Searches for optimal trees using the Cnidaria-only data set were carried out under MP and ML criteria. One thousand replicate searches, with taxa added randomly, were carried out under MP. Transversions were weighted three to two vs. transitions. One thousand bootstrap replicates were carried out with the same weighting scheme. Bremer indices (Bremer, 1988) were calculated to evaluate the strength of support for nodes present in the MP trees. Just 10 replicate searches were carried out under ML. The model of nucleotide evolution was obtained by Modeltest (Posada & Crandall, 1998), which employs the likelihood ratio test to determine the model of evolution that best fits provided sequence data. In this case, a general-time-reversible model with rate heterogeneity was assumed (assumed nucleotide frequencies: $A = 0.2599$; $C = 0.1986$; $G = 0.2674$;

Table 1 Taxa used in this study, with GenBank accession numbers.

Cnidaria	
Anthozoa, Hexacorallia	
<i>Anthopleura kuogane</i>	Z21671
<i>Anthopleura midori</i>	Z86098
<i>Antipathes galapagensis</i>	AF100943
<i>Antipathes lata</i>	Z92907
<i>Parazoanthus axinellae</i>	U42453
<i>Rhizopsammia minuta</i>	Z92907
Anthozoa, Octocorallia	
<i>Bellonella rigida</i>	Z49195
<i>Calicogorgia granulosa</i>	Z92900
<i>Pachycerianthus fimbriatus</i>	AF358111*
<i>Virgularia gustaviana</i>	Z86106
Cubozoa, Carybdeidae	
Darwin carybdeid	AF358105*
<i>Carybdea sivickisi</i>	AF358110*
<i>Carybdea marsupialis</i>	AF358106*
<i>Tripedalia cystophora</i>	L10829
<i>Carybdea rastonii</i>	AF358108*
<i>Carybdea xaymacana</i>	AF358109*
<i>Carukia barnesi</i>	AF358107*
Cubozoa, Chirodropidae	
<i>Chironex fleckeri</i>	AF358104*
<i>Chiropsalmus</i> sp.	AF358103*
Hydrozoa, Capitata	
<i>Cladonema californicum</i>	AF358085*
<i>Millepora</i> sp.	AF358088*
<i>Moerisia</i> sp.	AF358083*
<i>Polyorchis haplus</i>	AF358089*
<i>Polyorchis penicillatus</i>	AF358090*
<i>Porpita</i> sp.	AF358086*
<i>Scrippisia pacifica</i>	AF358091*
<i>Solanderia secunda</i>	AJ133506
<i>Staurocladia wellingtoni</i>	AF358084*
<i>Velella</i> sp.	AF358087*
Hydrozoa, Filifera	
<i>Bougainvillia</i> sp.	AF358093*
<i>Eudendrium racemosum</i>	AF358094*
<i>Podocoryne carnea</i>	AF358092*
Hydrozoa, Hydridae	
<i>Chlorohydra viridissima</i>	AF358081*
<i>Hydra circumcincta</i>	AF358080*
<i>Hydra littoralis</i>	U32392
<i>Hydra littoralis</i> 2	AF358082*
Hydrozoa, Leptomedusae	
<i>Aequorea aequorea</i>	AF358076*
<i>Aequorea victoria</i>	AF358077*
<i>Blackfordia virginica</i>	AF358078*
<i>Clytia</i> sp.	AF358074*
<i>Gymnangium hians</i>	Z86122
<i>Melicertissa</i> sp.	AF358075*
<i>Obelia</i> sp.	Z86108
<i>Selaginopsis cornigera</i>	Z92899
<i>Tiaropsidium kelseyi</i>	AF358079*

Table 1 Continued.

Hydrozoa, Limnomedusae	
<i>Craspedacusta sowerbyi</i>	AF358057*
<i>Maeotias inexpectata</i>	AF358056*
Hydrozoa, Narcomedusae	
<i>Aegina citrea</i>	AF358058*
<i>Cunina frugifera</i>	AF358059*
<i>Solmissus marshalli</i>	AF358060*
Hydrozoa, Siphonophora	
<i>Hippopodius hippopus</i>	AF358069*
<i>Muggiaea</i> sp.	AF358073*
<i>Nanomia bijuga</i>	AF358071*
<i>Nectopyramus</i> sp.	AF358068*
<i>Physalia physalis</i>	AF358065*
<i>Physalia utriculus</i>	AF358066*
<i>Physophora hydrostatica</i>	AF358072*
<i>Praya</i> sp.	AF358067*
<i>Sphaeronectes gracilis</i>	AF358070*
Hydrozoa, Trachymedusae	
<i>Crossota rufobrunnea</i>	AF358063*
<i>Haliscera conica</i>	AF358064*
<i>Liriope tetraphylla</i>	AF358061*
<i>Pantachogon haeckeli</i>	AF358062*
Scyphozoa, Coronatae	
<i>Atolla vanhoeffeni</i>	AF100942
<i>Nausithoe rubra</i>	AF358095*
Scyphozoa, Rhizostomae	
<i>Catostylus</i> sp.	AF358100*
<i>Stomolophus meleagris</i>	AF358101*
Scyphozoa, Semaestomae	
<i>Chrysaora melanaster</i>	AF358099*
<i>Cyanea</i> sp.	AF358097*
<i>Chrysaora colorata</i>	AF358098*
<i>Phacellophora camtschatica</i>	AF358096*
Scyphozoa, Stauromedusae	
<i>Craterolophus convolvulus</i>	AF099104
<i>Halicystus sanjuanensis</i>	AF358102*
<i>Halicystus</i> sp.	AF099103
Bilateria	
<i>Ptychodera bahamensis</i>	AF236802
<i>Pteraster tessellatus</i>	AF088808
<i>Dorometra aegyptica</i>	AF088803
<i>Latimeria chalumnae</i>	L11288
<i>Halocynthia roretzi</i>	AB013016
<i>Oikopleura</i> sp.	AB013015
<i>Barentsia hildegardae</i>	AJ001734
<i>Philodina roseola</i>	AF154567
<i>Asplanchna sieboldi</i>	AF092434
<i>Onchidella celtica</i>	X70211
<i>Xenoturbella westbladi</i>	AF207993
<i>Modiolus modiolus</i>	AF124210
<i>Liolophura japonica</i>	X70210
<i>Prostoma eilhardi</i>	U29494
<i>Lineus</i> sp.	X79878
<i>Lanice conchilega</i>	X79873
<i>Eisenia fetida</i>	X79872

Table 1 Continued.

<i>Aspidosiphon misakiensis</i>	AF119090
<i>Phascolosoma granulatum</i>	X79874
<i>Phoronis hippocrepia</i>	AF202112
<i>Terebratulina retusa</i>	U08324
<i>Lingula adamsi</i>	U08329
<i>Ochetostoma erythrogrammon</i>	X79875
<i>Siboglinum fiordicum</i>	X79876
<i>Cristatella mucedo</i>	AF025947
<i>Pycnophyes kielensis</i>	U67997
<i>Priapulius caudatus</i>	AF025927
<i>Eusimonia wunderlichi</i>	U29492
<i>Odiellus troguloides</i>	X81441
<i>Macrobiotus</i> sp.	U49912
<i>Milnesium tardigradum</i>	U49909
Ctenophora	
<i>Hormiphora</i> sp.	AF100944
<i>Mnemiopsis leidyi</i>	L10826
<i>Beroe cucumis</i>	D15068
<i>Charistephane fugiens</i>	AF358113*
<i>Coeloplana agniae</i>	AF358112*
Placozoa	
<i>Trichoplax adhaerens</i>	L10828
<i>Trichoplax</i> sp.	Z22783
Porifera	
<i>Rhabdocalypus dawsoni</i>	AF100949
<i>Oopsacas minuta</i>	AF207844
<i>Ephydatia muelleri</i>	AF121110
<i>Eunapius fragilis</i>	AF121111
<i>Axinella polypoides</i>	U43190
<i>Tetilla japonica</i>	D15067
<i>Plakortis</i> sp.	AF100948
<i>Suberites ficus</i>	AF100947
<i>Microcionia prolifera</i>	L10825
<i>Mycale fibrexilis</i>	AF100946
<i>Leucosolenia</i> sp.	AF100945
<i>Scypha ciliata</i>	L10827
<i>Sycon calcaravis</i>	D15066
<i>Clathrina cerebrum</i>	U42452
Outgroups to Metazoa	
Choanoflagellata	
<i>Monosiga brevicollis</i>	AF100940
<i>Diaphanoeca grandis</i>	L10824
<i>Acanthocephopsis unguiculata</i>	L10823
Mesomycetozoa	
<i>Ichthyophonus hoferi</i>	U25637
Rosette agent	L29455
<i>Dermocystidium salmonis</i>	U21337

*Denotes sequences generated for this study.

$T = 0.2741$; substitution types = 6, proportion of invariable sites = 0.5007, γ for variable sites = 0.5646, substitution rates equal to 1.000 except between *A* and *G* = 2.481 and *C* and *T* = 4.069). One thousand bootstrap replicate searches under ME were carried out with distance set to the same model of evolution as used in the ML searches.

Additional analyses were carried out that included sequences for the enigmatic intracellular parasitic cnidarian *Polypodium hydriforme* (see Raikova, 1994) and myxozoans, which may have a common evolutionary history to the exclusion of other animals (Siddall *et al.*, 1995; Kent *et al.*, 2001). These sequences are highly diverged from those of the cnidarian sequences generated here and have been difficult to place phylogenetically (Kim *et al.*, 1999). The results are not presented here, but are in preparation for publication elsewhere.

Supplemental material including primer sequences, derived SSU sequences, the aligned BioEdit data set, and PAUP* data sets are publicly available at the archived data web pages of the University of California Museum of Palaeontology, www.ucmp.berkeley.edu/archdata/Collins-Medusozoa/, as well as upon request.

Results

Ninety-six most parsimonious trees resulted from the analysis of the data set with broad taxonomic sampling. The strict consensus of these trees (Fig. 1) shows Cnidaria to be a strongly supported clade. MP and ME bootstrap indices (bi) are equal to 100 and 98, respectively. Cnidaria is composed of two clades, Anthozoa (MP bi = 92, ME bi = 98) and Medusozoa (MP bi = 64, ME bi = 86). Figure 1 has Cnidaria as the sister group of Placozoa plus Bilateria. Bootstrap support indices for Placozoa plus Bilateria under the criteria of MP and ME are moderate, 85 and 70, respectively. Greater support (MP bi = 90, ME bi = 91) exists for the grouping of Cnidaria, Placozoa, and Bilateria to the exclusion of ctenophores and sponges.

Maximum parsimony searches with the Cnidaria-only data set found 22 most parsimonious trees (strict consensus shown as Fig. 2). Among the medusozoan taxa, both Cubozoa and Hydrozoa receive considerable support [bi = 100 and bremer support indices (bsi) = 82 and 71, respectively]. Monophyly of Scyphozoa is not supported. Nine additional steps are required to accommodate a monophyletic grouping of the scyphozoan taxa. One group traditionally included within Scyphozoa, Stauromedusae, groups with Cubozoa with low support (bi = 51, bsi = 4). The three other scyphozoan groups (Coronatae, Rhizostomae and Semaestomae) form a clade (bi = 78, bsi = 8), which is weakly suggested to be the sister group to Cubozoa plus Stauromedusae. Coronatae appears as the sister group to species of Rhizostomae and Semaestomae, which are united with high support (bi = 100, bsi = 50). Support indices indicating that Semaestomae is paraphyletic with respect to Rhizostomae are high (bi = 100 and bsi = 23 for the clade containing the semaeostome species *Phacellophora camtschatica* plus the sampled rhizostomes). Within Cubozoa, the two main families Chirodropidae and Carybdeidae are shown as monophyletic. Within Hydrozoa, there are two main clades, Trachylina and Hydroid-

olina (*sensu* Collins, 2000), which are reasonably well supported (bi = 95, bsi = 16 and bi = 89, bsi = 9, respectively). Within Trachylina, Limnomedusae is shown as the sister of Trachymedusae plus Narcomedusae. The sampled limnomedusans and narcomedusans fall into monophyletic groups, whereas the sampled trachymedusans do not. Instead, Trachymedusae is displayed as paraphyletic with respect to Narcomedusae. Little resolution exists among the major groups of the Hydroidolina clade. Strong support exists for the monophyly of Siphonophorae (bi = 91, bsi = 11) and Hydridae (bi = 100, bsi = 53). The sampled species of Capitata and Leptomedusae form monophyletic groups, although the former has low support (bi = 50, bsi = 1 and bi = 77, bsi = 8, respectively). The strict consensus does not have the sampled species of Filifera as monophyletic.

The ML topology of cnidarians (Fig. 3) is largely congruent with the strict consensus of most parsimonious trees (Fig. 2). In general, nodes that are well supported in one analysis are also well supported in the other. For example, the total number of nodes in both topologies that have bootstrap indices in excess of 85 is 47. All 47 of these nodes are present in both Figs 2 and 3. Thirty-three of the 47 nodes receive bootstraps greater than 85 under both parsimony and ME criteria, while six have just MP bootstrap indices greater than 85 and eight have only ME bootstraps greater than 85. In other words, contradictions between the two topologies only occur with nodes that have little support, as measured by bootstrap indices. Focusing on the higher level groups within Medusozoa, the following are monophyletic in both Figs 2 and 3: Cubozoa, Carybdeidae, Chirodropidae, Hydrozoa, Trachylina, Limnomedusae, Trachymedusae + Narcomedusae, Narcomedusae, Hydroidolina, Hydridae, Siphonophorae, Capitata (but with low support in both cases), Leptomedusae (but with moderate support in both cases), Stauromedusae, Coronatae + Semaestomae + Rhizostomae (but with moderate support in both cases), Semaestomae + Rhizostomae, *Phacellophora camtschatica* + Rhizostomae, and Rhizostomae. As in the MP topology, Scyphozoa is not monophyletic. However, in contrast with the MP topology, Stauromedusae is shown as the sister group to all other medusozoans, although support for the node joining the other medusozoans is weak (bi < 50).

Discussion

Cnidaria

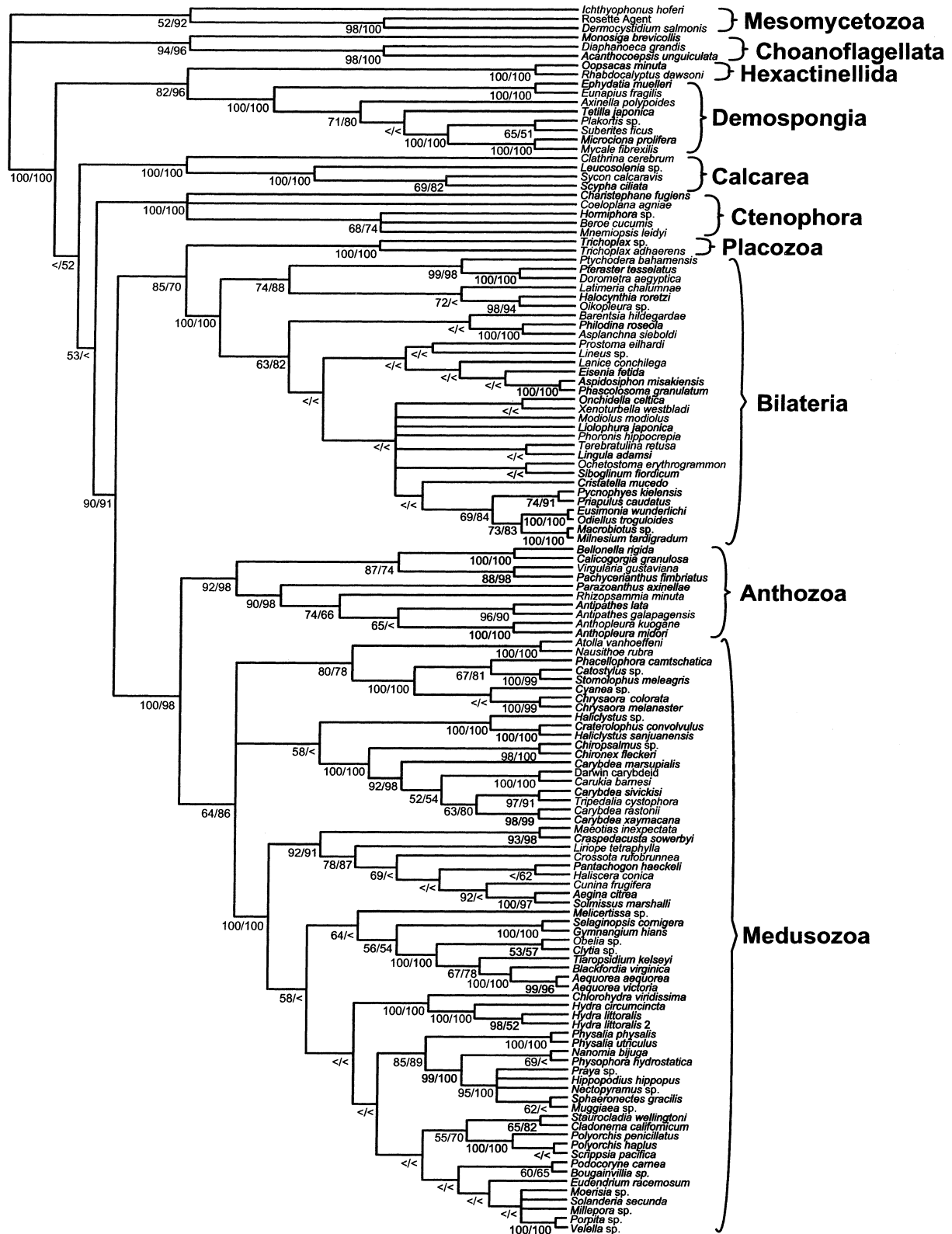
The phylogenetic position of Cnidaria within Metazoa is presently a controversial issue. This study, like others based on SSU rRNA data, suggests that Placozoa and Bilateria are the living clades most closely related to Cnidaria (Collins, 1998; Kim *et al.*, 1999; Peterson & Eernisse, 2001). In contrast, cladistic analyses based on morphology suggest that Cnidaria is the sister to Cteno-

phora plus Bilateria (Schram, 1991; Nielsen *et al.*, 1996; Peterson & Eernisse, 2001). Two recent analyses of combined data came to opposite conclusions on the question. Peterson & Eernisse (2001) combined morphological data with selected SSU sequences and derived a tree with Cnidaria as the sister group to Ctenophora plus Bilateria. Medina *et al.* (2001) took a different approach and combined SSU and LSU data for a small sample of metazoan taxa and tested competing hypotheses in a ML framework. They found that Ctenophora plus Bilateria is significantly less likely than Cnidaria plus Bilateria, given the combined SSU and LSU data, although this result was largely driven by the SSU, rather than the LSU, data (Medina *et al.*, 2001). Resolution of this important question in metazoan phylogeny awaits further data and analyses.

The molecular sequence data presented here, sampled from a diverse set of cnidarians, support the hypothesis that living cnidarians form a clade. This stands in contrast to a claim that cnidarians are triploblastic and gave rise to coelomate bilaterians (Boero *et al.*, 1998). More specifically, these workers observe that development in many hydrozoans involves a third tissue layer and the creation of a 'coelom-like' space that becomes the subumbrellar cavity in the free-living medusa. They infer that these characteristics were passed on to coelomate bilaterian descendants. However, even if one considers some hydrozoan cnidarians to be triploblastic during medusa development, SSU data strongly suggest that the third tissue layers of Hydrozoa and Bilateria are not homologous. Both Bilateria and Hydrozoa are strongly supported clades, and the latter is nested well within Cnidaria, a topology that conflicts with the idea that Bilateria is derived from hydrozoan ancestors. Moreover, the ancestral bilaterian probably did not possess a coelom (Collins & Valentine, 2001).

Within Cnidaria, three of the four groups of cnidarians recognized as Linnaean classes appear to be well supported as monophyletic. In agreement with other work, Anthozoa is a clade (Kim *et al.*, 1999) that is the sister group to Medusozoa (Werner, 1973; Salvini-Plawen, 1978, 1987; Brusca & Brusca, 1990; Meglitsch & Schram, 1991; Bridge *et al.*, 1992, 1995; Schuchert, 1993; Medina *et al.*, 2001). Within Medusozoa, Hydrozoa and Cubozoa are strongly supported as monophyletic. In contrast, taxa classified as Scyphozoa may not form a clade. Instead, scyphozoans appear to be paraphyletic with respect to Cubozoa (Fig. 2) or the remaining medusozoans (Fig. 3).

Fig. 1 Strict consensus of 96 most parsimonious trees of 126 animals plus six choanoflagellates and mesomycetozoans as outgroups, based on 1668 characters, of which 778 are parsimony informative. Length = 15113; CI = 0.286; RI = 0.733; RC = 0.210. Bootstrap indices under maximum parsimony and minimum evolution criteria are shown at each node (MP/ME); '<' denotes a bootstrap value of less than 50.



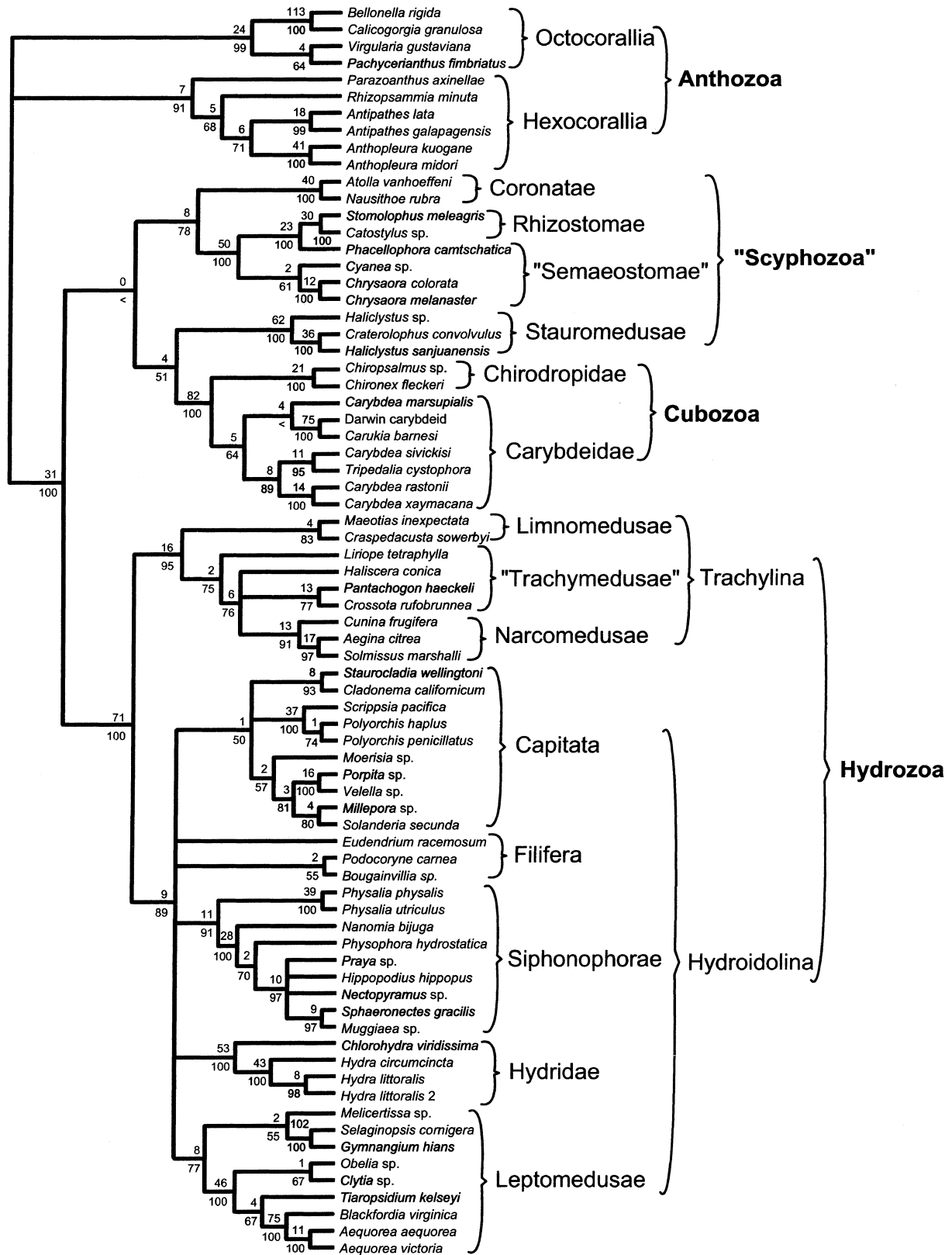


Fig. 2 Strict consensus of 22 most parsimonious trees of 64 medusozoans plus 10 anthozoans as outgroups, based on 1768 nucleotide characters, of which 495 are parsimony informative. Length = 6240; CI = 0.399; RI = 0.728; RC = 0.291. Bremer support and bootstrap indices are shown at each node, above and below, respectively; '<' denotes a bootstrap value of less than 50.

Most workers who have found Anthozoa to be the sister group of Medusozoa have concluded that the ancestral cnidarian likely did not possess a pelagic medusa stage (Werner, 1973; Salvini-Plawen, 1978; Salvini-Plawen, 1987; Bridge *et al.*, 1995; Nielsen, 1995, 2001). In contrast, Schuchert (1993) argued that a pelagic medusa is more likely to be plesiomorphic for Cnidaria based mainly on the hypothesis that Ctenophora, with an adult pelagic stage, was the most suitable outgroup for comparison to Cnidaria. However, Ctenophora is not likely to be the sister group of Cnidaria (Harbison, 1985; Collins, 1998). Therefore, an origin of the medusa in the lineage leading to Medusozoa is slightly more parsimonious because it requires a single origin of the medusa rather than an earlier origin and subsequent loss in the lineage leading to Anthozoa (Fig. 4). That said, as will be detailed below, the history of Cnidaria is replete with examples of life cycle modifications that involve the loss of either medusa or polyp. Therefore, if an ancestor of Anthozoa possessed a medusa stage that was evolutionarily lost in the last common ancestor of Anthozoa, one might expect homoplastic re-expression of a medusa-like stage to have subsequently occurred during anthozoan history. Indeed, active swimming has evolved within Anthozoa, at least three separate times in Actiniaria (true anemones), as well as in Ceriantharia (tube anemones), which have a long-lived pelagic larval stage (Robson, 1966). However, none of these cases represent the advent of a sexual pelagic stage, and are therefore not comparable with the medusa within Medusozoa. This is only weak evidence, however, that a medusa is not in the ancestry of Anthozoa. Other cnidarian groups that lack a medusa and for which we can be more certain that a medusa is in their ancestry (e.g. Hydridae) also have not re-evolved a medusa-like stage. Therefore, presently available information just slightly favours the idea that the medusa is a synapomorphy of Medusozoa rather than of Cnidaria. Perhaps comparative molecular developmental data from a diverse set of cnidarians may eventually resolve this primary issue in Cnidarian evolution, which has been debated for well over a century (Brooks, 1886).

A motile planula stage usually exists in the cnidarian life cycle between gastrulation and polyp. Planulae have anterior–posterior differentiation, can be either hollow or solid, and typically do not possess mouths or guts. However, feeding in planulae is known in a number of anthozoan groups, for example in Scleractinia (Fadlallah,

1983) and Actiniaria (Jennison, 1979), and is apparently absent or rare in medusozoan groups. It is unclear if a feeding planula arose one or more times in Cnidaria. If there was a single origin, it would be most parsimonious to assume that the origin occurred within Anthozoa rather than in the lineage leading to Cnidaria because the latter scenario would require an evolutionary loss as well as gain. However, as in the case of the medusa, this may not be the most compelling argument and certainly should be subjected to future tests. Nevertheless, in the absence of any indication of a feeding planula in the ancestry of Medusozoa, it seems most likely that feeding planulae arose one or more times within Anthozoa (Fig. 4). It may be that the existence of a dispersive stage, the medusa, has mitigated the likelihood for the evolution of feeding planulae in Medusozoa.

Hydrozoa

In many hydrozoans, medusae develop from masses of proliferating cells (the entocodon) located between the ectoderm and endoderm along the lateral portions of polyps. Bouillon & Boero (2000) hypothesized that the production of the medusa through the entocodon was a synapomorphy shared by Capitata, Filifera, Laingiomedusae (not sampled in this analysis), Leptomedusae, Limnomedusae, and Siphonophorae. They termed this assemblage Hydroidomedusa, a name that had earlier been applied to all nonsiphonophore hydrozoans (Bouillon *et al.*, 1992). The SSU data presented here contradict the monophyly of Hydroidomedusa in either sense (Figs 2 and 3). Rather, they suggest that Hydrozoa consists of two clades, Trachylina and Hydroidolina, both of which have subgroups characterized by the entocodon. Production of the medusa via the entocodon and lateral budding therefore is most likely a synapomorphy of Hydrozoa because it is characteristic of the basal group of Trachylina (Limnomedusae) and most of the groups that compose Hydroidolina (Fig. 4).

Trachylina consists of Limnomedusae, Narcomedusae, and Trachymedusae. Actinulidae (a group of solitary interstitial forms not sampled in this analysis) may also be a part of Trachylina. Actinulidae has been taxonomically grouped with Narcomedusae and Trachymedusae (as Automedusae) based on a number of developmental and morphological similarities (Bouillon & Boero, 2000). Another group, Laingiomedusae may also be part of Trachylina, as it shares a number of morphological characters with Narcomedusae, including a lobed umbrellar margin and solid tentacles that emerge above the bell margin (Bouillon & Boero, 2000). Unlike Narcomedusae, however, at least some laingiomedusans asexually produce medusae involving an entocodon. Knowing the phylogenetic positions of Actinulidae and Laingiomedusae among the other hydrozoan groups would enhance our understanding of the evolution of trachyline life cycles that is discussed below.

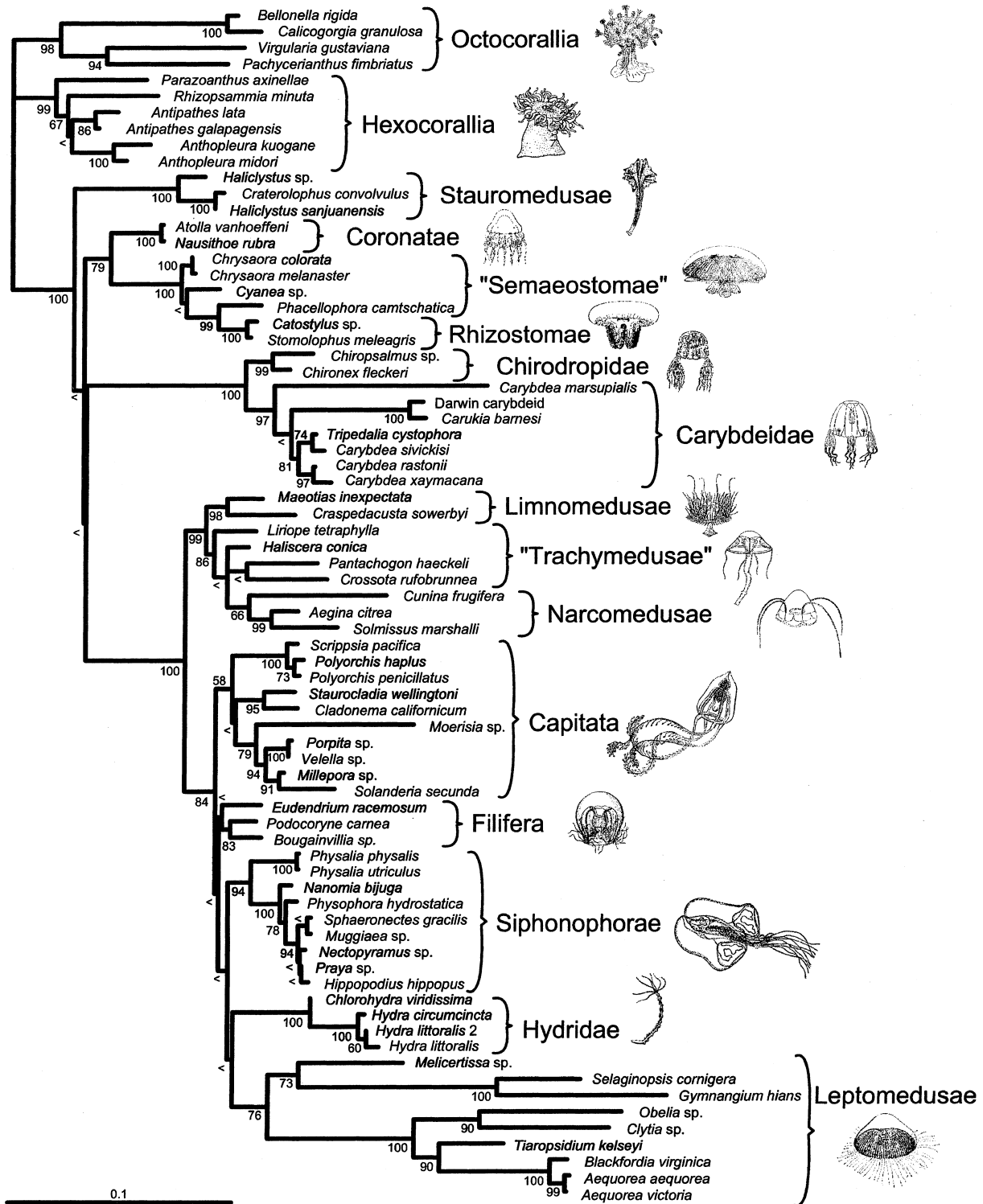


Fig. 3 Maximum likelihood (ML) tree of 64 medusozoans plus 10 anthozoans as outgroups, assuming a general-time-reversible model of nucleotide evolution with rate heterogeneity. Score = -15 595.976. Scale bar denotes 0.1 nucleotide substitutions per site. Minimum evolution bootstrap indices are shown at each node; '<' denotes a bootstrap value of less than 50.

Since the most basal group of the trachylines sampled here, Limnomedusae, has a life cycle most like that of other hydrozoans, consisting of a planula that develops into a benthic polyp and a medusa that develops via the entocodon, this life cycle is probably plesiomorphic for Trachylina (Fig. 4). Nearly all members of Trachymedusae and Narcomedusae are holopelagic, indicating that this habit evidently evolved in the lineage leading to them. Moreover, features that are characteristic of Trachymedusae (for instance, the lack of asexual reproduction and a polyp stage) may have evolved along the same lineage. This follows because Trachymedusae appears to be a paraphyletic group with respect to Narcomedusae. Therefore, characters uniquely shared by trachymedusans were most likely gained in an ancestor (i.e. shared because of common history) and lost in the lineage leading to Narcomedusans. That trachymedusans lack a polyp stage, which is typically benthic, makes sense for holopelagic organisms living without substrates. Trachymedusans also lack asexual reproduction, which may have more to do with the loss of a polyp stage than with the gain of the holopelagic habit. Mills (1987) noted that

open-ocean medusae with benthic stages in their life cycles do sometimes reproduce asexually by several types of fission or medusa-budding. Perhaps the loss of a polyp in trachymedusans has been accompanied by the loss of some portion of the developmental regulatory mechanisms necessary for regeneration of a whole individual from a part. After all, typically it is the polyp that asexually produces the medusa.

Trachymedusans either undergo direct development to the medusa form or have an ontogeny that includes a ciliated planula and tentacled form (actinuloid) between gastrula and medusa (Bouillon & Boero, 2000). Most narcomedusans also lack a polyp stage. However, the parasitic narcomedusans, which settle and develop mostly on the bells or in the gastric cavities of other medusae, do have a tentacled polyp-like stage that lives on a substrate, i.e. the host organism (Hyman, 1940). This polyp-like stage could be referred to as a polyp. However, in his detailed study of narcomedusan development, Bouillon (1987) observed that these polyp-like stages are extremely different from typical hydrozoan polyps, particularly in the way that they produce medu-

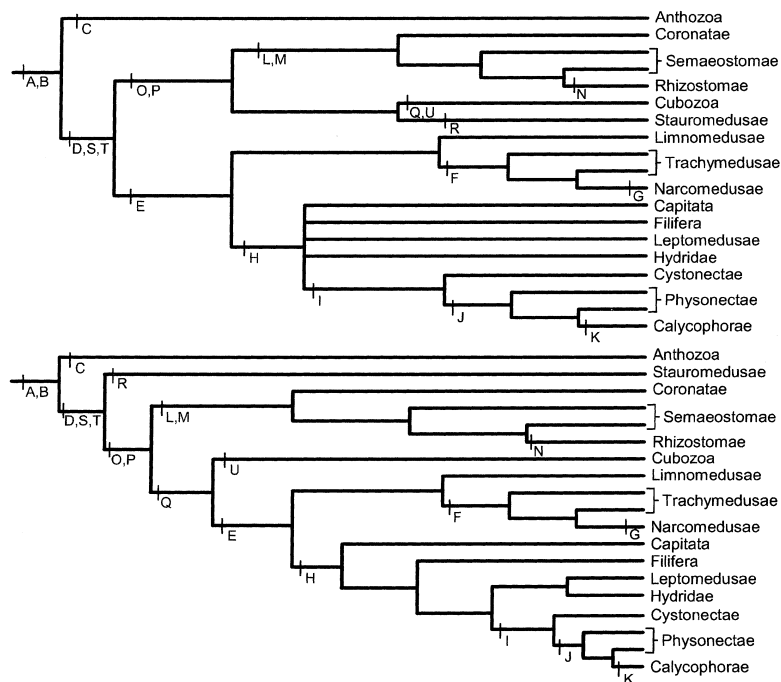


Fig. 4 Two hypotheses of the relationships among major medusozoan taxa, based on parsimony (above) and maximum likelihood (below) criteria, with selected life cycle characteristics placed at the various nodes. Uncertainties in the placement and potential reversals or losses of the life cycle characteristics are discussed in the text. A, ciliated/flagellated planula; B, benthic polyp; C, feeding planula (only in some members of Anthozoa); D, medusa; E, development of medusa through the entocodon and subsequent liberation by lateral budding from polyp; F, loss of polyp form; G, gain of benthic polyp form that gives rise to a single medusa by metamorphosis (only in some members of Narcomedusae); H, polymorphic zooids in benthic colonies of polyps (only in some members of Hydroidolina); I, float in pelagic colonies; J, swimming bell(s); K, loss of float; L, ephyrae; M, polydisk strobilation; N, monodisk strobilation; O, gastric septa in polyp; P, four intramesogleal longitudinal muscles associated with peristomial pits in polyp; Q, simplification of polyp/loss of gastric septa and four longitudinal muscles associated with peristomial pits; R, medusa not liberated/loss of transverse fission; S, metamorphosis at oral end of polyp produces adult medusa; T, medusa is liberated from polyp by transverse fission; and U, polyp ephemeral/does not persist after single medusa production.

sae. A narcopolyp, rather than laterally budding juvenile medusae, reproduces asexually and subsequently undergoes a complete metamorphosis into a single medusa. Because of this difference, he gave them a unique name, stolo-prolifers. One could however, use the word polyp for any polyp-like form that either is or gives rise to the adult stage in Cnidaria without implying homology. After all, the word is used to describe the – typically – benthic stage of all cnidarians, whether they produce medusae or not. The one-polyp to one-medusa metamorphosis of Narcomedusae is somewhat similar to what is observed in Cubozoa (see below) and for that reason a phylogenetic connection between the two groups has been postulated (Petersen, 1979; Bouillon, 1987). However, the SSU data presented here clearly contradict this hypothesis. Alternatively, they suggest that the narcopolyp and the process of metamorphosis from narcopolyp to medusa are uniquely evolved within Narcomedusae, presumably in concert with the assumption of a parasitic habit, a conclusion also derived from comparative anatomy (Bouillon & Boero, 2000). Apparently, the process of medusa production is different because the polyp form was essentially re-evolved (Fig. 4). Trachylina is not a particularly species rich group and a detailed species-level phylogeny of it (including Actinulidae and Laingiomedusae) would be able to address the phylogenetic distribution of direct development within Trachylina, parasitism within Narcomedusae, and which species are most closely related to Narcomedusae.

Hydroidolina is the other main group of hydrozoans, as indicated by SSU data, and consists of Capitata, Filifera, Hydridae, Leptomedusae, and Siphonophorae (Figs 2 and 3). Unfortunately, the SSU data presented here do not provide a clear resolution of the evolutionary relationships among these groups. Nevertheless, several observations can be made about this group. For instance, Hydroidolina contains a multitude of species that have colonies with polymorphic individuals. In fact, with one exception, all polymorphic species within Medusozoa fall within this group, specifically within Capitata, Filifera, Leptomedusae, and Siphonophorae (Fig. 4). Elsewhere within Cnidaria, species with polymorphic colonies are only found within the octocorallian anthozoans. The exception within Medusozoa is a little known limnomedusan genus *Monobrachium* (Bouillon & Boero, 2000). It would be interesting to include this group in future analyses to test whether or not its polymorphic individuals are independently derived. It should be noted, however, that many hydroidolinan species are not polymorphic suggesting that polymorphism might have arisen a number of times independently.

The only major group within Hydroidolina in which polymorphic species are not encountered is Hydridae. This fresh water group of hydrozoans consists mainly of the model organisms of the *Hydra* species complex. The group is characterized by the complete lack of a medusa in its life cycle. Identifying the sister group of Hydridae is

a necessary starting point for understanding its lack of medusa and its transition to fresh water. The latter is a particularly interesting question because a fresh-water habitat is relatively uncommon for cnidarians, known only in some Limnomedusae (*Craspedacusta*, *Limnocnida*) and *Polypodium*. As Limnomedusae is the basal group of Trachylina, and Hydridae is not ruled out as the basal group of Hydroidolina, it is possible that a fresh (or perhaps brackish) water condition could be traced back to the last common ancestor of Hydrozoa. However, there is no positive evidence for this hypothesis. Hydridae has been suggested to be closely related to Moerisidae, a family of Capitata that contains a number of species inhabiting brackish water (Naumov, 1960), based on similarities in early development including the presence of a resting stage, an embryo protected by periderm, and a planula without cilia (Petersen, 1990). However, while SSU data do not indicate the sister group of Hydridae, they do strongly contradict this hypothesis suggesting that the similarities may be convergent adaptations to life in environments with strong seasonal variation (Collins, 2000).

The vast majority of hydrozoan, and indeed medusozoan, diversity in terms of species numbers is present within Capitata, Filifera, and Leptomedusae (Schuchert, 1998). The former two groups are often classified together as Anthomedusae, but no known synapomorphies exist for the group (Schuchert, 1996) and its existence is neither supported nor contradicted by SSU data. The diversity of these groups is manifest both in terms of species richness, as well as in life cycle variation (Boero *et al.*, 1997). For some species, the polyp stage is relatively ephemeral. In one case, ephemeral polyps occur only during the dry season suggesting that de-emphasizing the polyp may be an evolutionary response to an ecological problem (Bouillon *et al.*, 1991). More usual among leptomedusan and anthomedusan species, however, is a de-emphasis of the medusa stage. In fact, the number of species with a reduced medusa is so large that it has led to a claim for a general trend towards medusa reduction within these groups (Boero *et al.*, 1992; Boero *et al.*, 1997). The hypothesized trend is presumably driven by the greater ecological difficulties faced by medusae, being top predators, than those faced by benthic colonies. Therefore, lineages that are able to suppress the medusa without jeopardizing their dispersal and reproductive abilities are able to diversify more than their evolutionary relatives with longer lived medusae (Boero *et al.*, 1992, 1997). Unfortunately, testing this interesting hypothesis is beyond the scope of the present study given its relatively thin sampling of anthomedusans and leptomedusans. A list of several extraordinary examples of life cycle evolution is provided by Boero *et al.* (1997) who also make a call for increased documentation of cnidarian life cycles, which are generally poorly known. Additional phylogenetic analyses of groups within Anthomedusae and Leptomedusae would also strengthen our ability to

recognize broad scale patterns in and test hypotheses relating to life cycle evolution.

Perhaps the most remarkable of all polymorphic animals are the pelagic siphonophores. Because of their distinctness, their phylogenetic position within Hydrozoa has been the subject of much debate. Nevertheless, larval similarities have led several siphonophore workers to conclude that Siphonophorae is closely allied to capitate anthomedusans (Haeckel, 1888; Garstang, 1946; Leloup, 1955; Totton, 1965). Other potential indications of this phylogenetic alliance are that both groups have gamete masses on the manubrium (Schuchert, 1996) and stenoteles nematocysts, although both characters are more broadly distributed. An alternative hypothesis, that siphonophores are the earliest diverging group of hydrozoans (Hyman, 1940; Bouillon *et al.*, 1992) is clearly contradicted by SSU (Collins, 2000) and morphological data (Bouillon & Boero, 2000), and should therefore be laid to rest (Marques, 2001).

Siphonophore colonies consist of such highly specialized zooids that they quite naturally beg the question of whether they should be considered colonies or individuals. The present analysis is consistent with the idea that siphonophores are derived from an ancestor with a typical hydrozoan life cycle. Nevertheless, siphonophore colonies have key differences from benthic hydrozoan colonies, including relatively determinant growth and composition by zooids that cannot replicate the colony form (Mackie *et al.*, 1987). Selection has clearly acted on the whole form of the colony in siphonophore species, but their descent is likely from a colony of less highly integrated zooids. Any debate about whether the siphonophore is an individual or a colony amounts more to a semantic discussion rather than a biological question.

Within Siphonophorae, species are classified in three groups based on the presence or absence of two characters that are associated with their pelagic life habit, swimming bells (nectophores) and floats (pneumatophores). Cystonect species possess a float, physonects have swimming bells, and calyphores are characterized by both nectophores and pneumatophores. The present analysis suggests that cystonect siphonophores (*Physalia*) hold a basal position within Siphonophorae and that the physonects (*Nanomia bijuga* and *Physophora hydrostatica*) gave rise to the calyphores (Figs 2 and 3). Therefore, within siphonophore evolution, the float appears to be an ancestral character (Fig. 4). The origin of the float was probably tied to the transition from benthic to pelagic life habit. Later in siphonophore evolution, swimming bells arose in the lineage leading to physonects and calyphores, presumably as an adaptation for locomotion in the pelagic realm (Fig. 4). In the lineage leading to calyphores, the float was lost (Fig. 4). These hypotheses are in line with Garstang's (1946) view that flotation preceded active swimming, but contradict Totton (1965), who argued that Cystonectae and Physonectae are more closely related to each other than either is to Calyphorae based on a

lengthy list of similarities. Apparently, these characters are plesiomorphic for Siphonophorae and lost in Calyphorae. Identifying a well-supported hypothesis for the phylogenetic position of Siphonophorae within Hydrozoa would likely provide additional insights into the origin of their pelagic habit and many unique features.

Scyphozoa and Cubozoa

It is intriguing that the scyphozoan taxa do not appear to form a clade, but rather may be paraphyletic. The optimal MP and ML trees (Figs 2 and 3) suggest two alternative hypotheses that have rather divergent evolutionary implications, as described below. In the MP topology, Stauromedusae is the sister to Cubozoa and the two taxa form the sister group of the remaining scyphozoans (Fig. 2). In the ML topology, Stauromedusae represents the earliest diverging medusozoan group (Fig. 3). In either case, the results are consistent for the remaining scyphozoan taxa. Coronatae is the sister group to the semaeostomes plus rhizostomes, a relationship also found by Thiel (1966) based on morphological characters. These three groups uniquely share lappets in adult medusae and distinctive juvenile medusae (ephyrae), which are produced by the process of strobilation, i.e. the serial production of ephyrae by transverse fission at the oral end of polyps (Fig. 4). Coronates and semaeostomes both undergo polydisk strobilation, meaning that multiple incipient ephyrae develop one on top of the next. In contrast, rhizostomes undergo monodisk strobilation; a single ephyra develops at the oral end of the polyp and is released by transverse fission. In either case, strobilating polyps regrow tentacles after juvenile medusae are liberated and persist, and the process may be repeated. As both SSU data and morphology (Mayer, 1910; Uchida, 1926; Thiel, 1966) suggest that semaeostomes are paraphyletic with respect to rhizostomes (Figs 2 and 3), monodisk strobilation is apparently derived from the polydisk state (Fig. 4).

Interestingly enough, the little-known stauromedusans do not produce free living medusae by strobilation or any other means. In fact, ultrastructural studies of stauromedusan ovaries and ocelli show that they are profoundly different from those observed in the other scyphozoans (Eckelbarger & Larson, 1993; Blumer *et al.*, 1995). Moreover, although the complete life cycle is not known for a single stauromedusan, the portions that are known are markedly peculiar. Rather than possessing free-swimming medusae, adult stauromedusans live attached to the substrate by a stalk. Unlike those of nearly all other cnidarians, the planulae of stauromedusans are nonciliated creepers, with an invariant number of stacked, nondividing endodermal cells (Otto, 1976, 1978). After creeping, the larva settles and develops into a juvenile polyp. Later, the oral end of the polyp metamorphoses (Uchida, 1929; Kikinger & von Salvini-Plawen, 1995) takes on a number of characters that resemble those in

adult medusae of other scyphozoans and cubozoans, e.g. hollow structures of tentacular origin (rhopalioids/rhopalia), circular coronal muscles, gonads, and ocelli. At the same time, the aboral portion of the adult retains polypoid characters such as gastric septa and four longitudinal muscles associated with four peristomial pits surrounding the mouth, precisely what is observed in other scyphozoan polyps. If the MP topology is correct, then the last common ancestor of scyphozoans and cubozoans probably possessed these characters (Fig. 4). If the ML (Fig. 3) topology is accurate, then the ancestral medusozoan is likely to have had a polyp with gastric septa and four longitudinal muscle fibres that ran through the mesoglea and connected the peristomial pits to the base of the polyp (Fig. 4). If either hypothesis is true, then these polyp characters were probably possessed by an ancestor of Cubozoa, whose polyps must then be secondarily simplified by their loss. Cubopolyps do possess intramesogleal muscles, but they are not grouped together as four tubular muscles (Chapman, 1978). If the ML topology is correct, then the polyps of both Cubozoa and Hydrozoa are likely to be simplified (Fig. 4).

The tube-shaped fossil conulariids with four-fold symmetry may also have been part of the phylogenetic alliance delineated by these characters. Conulariids possess septa, which have been specifically compared with those of Stauromedusae (Kiderlen, 1937; Jerre, 1994), and can be inferred to have had four longitudinal muscles associated with four opercular flaps (Moore & Harrington, 1956; Chapman, 1966). Arguments have also been made that conulariids were related to coronate scyphozoans based on similarities between the apatitic tests of conulariids and the chitinous thecae of coronate polyps (Werner, 1966; Van Iten, 1991; Van Iten *et al.*, 1996). A logical extension of the work presented here would be to include conulariids, and perhaps other fossil groups exhibiting four-fold symmetry, in a cladistic analysis of both molecular and morphological characters. Such an analysis might be able to distinguish among the alternative placements of these enigmatic fossils among the living scyphozoan and cubozoan taxa.

Before Cubomedusae was elevated to 'class' status as Cubozoa and taxonomically removed from Scyphozoa, several suggestions were made that cubozoans and stauromedusans were closely related (Haeckel, 1879; Uchida, 1929), a result also derived from the MP analysis (Fig. 2). Although many of the characters used to ally the groups are the shared lack of features distinctive of the other scyphozoan groups, both groups do possess a claustrum, a strip of tissue consisting of a double layer of endoderm, which connects the septa (Thiel, 1966). This fact may indicate that the MP topology is a better alternative than the ML topology. However, given the conflicting results using alternative optimality criteria and the lack of high support indices, it seems premature to strongly favour one topology over the other.

Cubozoans were originally classified within Scyphozoa because the medusae of both groups have distinctive hollow sensory structures, rhopalia, which are of tentacular origin. It was not until the full life cycle of a cubozoan was observed that they were removed from Scyphozoa and given 'class' status (Werner, 1975). At the time, the lack of strobilation and the complete metamorphosis from polyp to medusa in this group was somewhat of a surprise. Werner (1973) recognized the cubopolyp as secondarily simplified, although not by the reasoning outlined above, and concluded that Cubozoa is more closely related to Hydrozoa than it is to Scyphozoa. In contrast, Salvini-Plawen (1978, 1987) argued that Cubozoa and Scyphozoa were more closely related. What neither worker considered was that Scyphozoa may not be a monophyletic group. Unfortunately, the present analysis is not adequate to fully resolve the relationships among the major medusozoan groups, which now number four, Cubozoa, Hydrozoa, Stauromedusae and the strobilating scyphozoans.

On the other hand, the SSU data presented here do suggest that strobilation is a derived feature of Coronatae plus Semaestomae plus Rhizostomae (Fig. 4). In comparison, stauromedusan polyps undergo metamorphosis at their oral ends to produce their adult forms and cubozoan polyps metamorphose completely into single medusae. In all three cases, the orientation of the adult mouth is the same as in the polyp. The MP and ML topologies suggest that production of the medusa by metamorphosis at the oral end of the polyp is a character in the ancestry of Scyphozoa plus Cubozoa or Medusozoa, respectively (Fig. 4). Furthermore, the MP topology would suggest that stauromedusans are probably benthic as adults through the loss of transverse fission (Fig. 4). Even if the ML topology gains support through future analyses, it may be that stauromedusans are derived from ancestors with free-living medusae. One rare group of Stauromedusae not included in this analysis, Tesseranthinae, is comprised of pelagic members (Mayer, 1910; Goy, 1979). Knowing their life cycle and phylogenetic position could greatly further our understanding of the early history of Medusozoa.

The primary difference between strobilation and what occurs in Cubozoa may be the lack of persistence in the polyp. Furthermore, in a small fraction (2%) of at least one species, the cubopolyp produces a medusa while leaving behind a small rudiment that re-grows into a normal polyp capable of reproducing another medusa (Werner, 1973). One might expect the ephemeral nature of the cubopolyp to be associated with environmental conditions that change to such an extent that they not allow the polyp to persist. Few cubopolyps have been observed in nature. For one species, it has been shown that the polyps live in estuaries which are subject to annual rains and shifts in salinity that are beyond what the polyps can physiologically cope with (Hartwick, 1991a,b). Additional data on cubozoan life cycles and a species-level phylogeny of the group could be used to test

if the estuarine habitat with strong seasonal flooding could be traced back to the origin of the group.

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