

Medusozoan Phylogeny and Character Evolution Clarified by New Large and Small Subunit rDNA Data and an Assessment of the Utility of Phylogenetic Mixture Models

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Abstract.—A newly compiled data set of nearly complete sequences of the large subunit of the nuclear ribosome (LSU or 28S) sampled from 31 diverse medusozoans greatly clarifies the phylogenetic history of Cnidaria. These data have substantial power to discern among many of the competing hypotheses of relationship derived from prior work. Moreover, LSU data provide strong support at key nodes that were equivocal based on other molecular markers. Combining LSU sequences with those of the small subunit of the nuclear ribosome (SSU or 18S), we present a detailed working hypothesis of medusozoan relationships and discuss character evolution within this diverse clade. Stauromedusae, comprising the benthic, so-called stalked jellyfish, appears to be the sister group of all other medusozoans, implying that the free-swimming medusa stage, the motor nerve net, and statocysts of ecto-endodermal origin are features derived within Medusozoa. Cubozoans, which have had uncertain phylogenetic affinities since the elucidation of their life cycles, form a clade—named Acraspeda—with the scyphozoan groups Coronatae, Rhizostomeae, and Semaestomeae. The polyps of both cubozoans and hydrozoans appear to be secondarily simplified. Hydrozoa is comprised by two well-supported clades, Trachylina and Hydroidolina. The position of Limnomedusae within Trachylina indicates that the ancestral hydrozoan had a biphasic life cycle and that the medusa was formed via an entocodon. Recently hypothesized homologies between the entocodon and bilaterian mesoderm are therefore suspect. Laingiomedusae, which has often been viewed as a close ally of the trachylina group Narcomedusae, is instead shown to be unambiguously a member of Hydroidolina. The important model organisms of the *Hydra* species complex are part of a clade, Aplanulata, with other hydrozoans possessing direct development not involving a ciliated planula stage. Finally, applying phylogenetic mixture models to our data proved to be of little additional value over a more traditional phylogenetic approach involving explicit hypothesis testing and bootstrap analyses under multiple optimality criteria. [18S; 28S; Cubozoa; Hydrozoa; medusa; molecular systematics; polyp; Scyphozoa; Staurozoa.]

Cnidaria has been the subject of several recent molecular studies addressing the evolutionary relationships among its major component groups (Bridge et al., 1992, 1995; Odorico and Miller, 1997; Kim et al., 1999; Collins, 2000, 2002; Medina et al., 2001). This research has demonstrated nearly beyond doubt that Cnidaria consists of two large clades, Anthozoa and Medusozoa, the latter name being a reference to the typical, though far from universal, adult pelagic medusa stage of the group. More importantly, these studies have invigorated interest in medusozoan character evolution and are at least partly responsible for spurring a number of contemporary investigations of medusozoan relationships based on morphological and life history features (Schuchert, 1993; Bouillon and Boero, 2000; Marques and Collins, 2004). These phylogenetic endeavors, both molecular and morphological, continue the efforts going back well over a century (Haeckel, 1879; Brooks, 1886) to provide a historical context for understanding how the tremendous diversity of life-history strategies, morphology at the individual and colony levels, development, and species richness of Medusozoa originated.

Somewhat ironically, the bounty of recent and older analyses leaves us faced with an unprecedented number of alternative and somewhat contradictory ideas about the phylogeny of Medusozoa. A cursory glance

at Figure 1 might suggest that a great deal of uncertainty about medusozoan phylogeny exists. However, many specific hypotheses of cnidarian relationships are present in all or nearly all of the proposed relationships shown in Figure 1. Taken together, these studies provide strong evidence that their common elements are true because they are based upon different types of data assessed by diverse researchers. Moreover, these studies encompass methodologies ranging from scenario approaches, i.e., synthetic, narrative discussions of plausible histories, to more explicit cladistic and likelihood analyses. Each study also contains various caveats and focal points—e.g., Hydrozoa, Scyphozoa, etc.—and should be consulted directly for analytical details.

One of several examples of consensus (Fig. 1) involves the scyphozoan groups Semaestomeae and Rhizostomeae, which are universally viewed as close allies forming the clade Discomedusae. In fact, precladistic scenarios, being readily compatible with the concept of paraphyletic taxa, often viewed rhizostomes as direct descendants from semaestomes (Mayer, 1910; Hyman, 1940; Thiel, 1966), presaging results later obtained from small subunit (SSU) rDNA data (Collins, 2002). Within Hydrozoa, the holopelagic groups Trachymedusae and Narcomedusae form either a clade or a paraphyletic assemblage according to the vast majority of prior studies

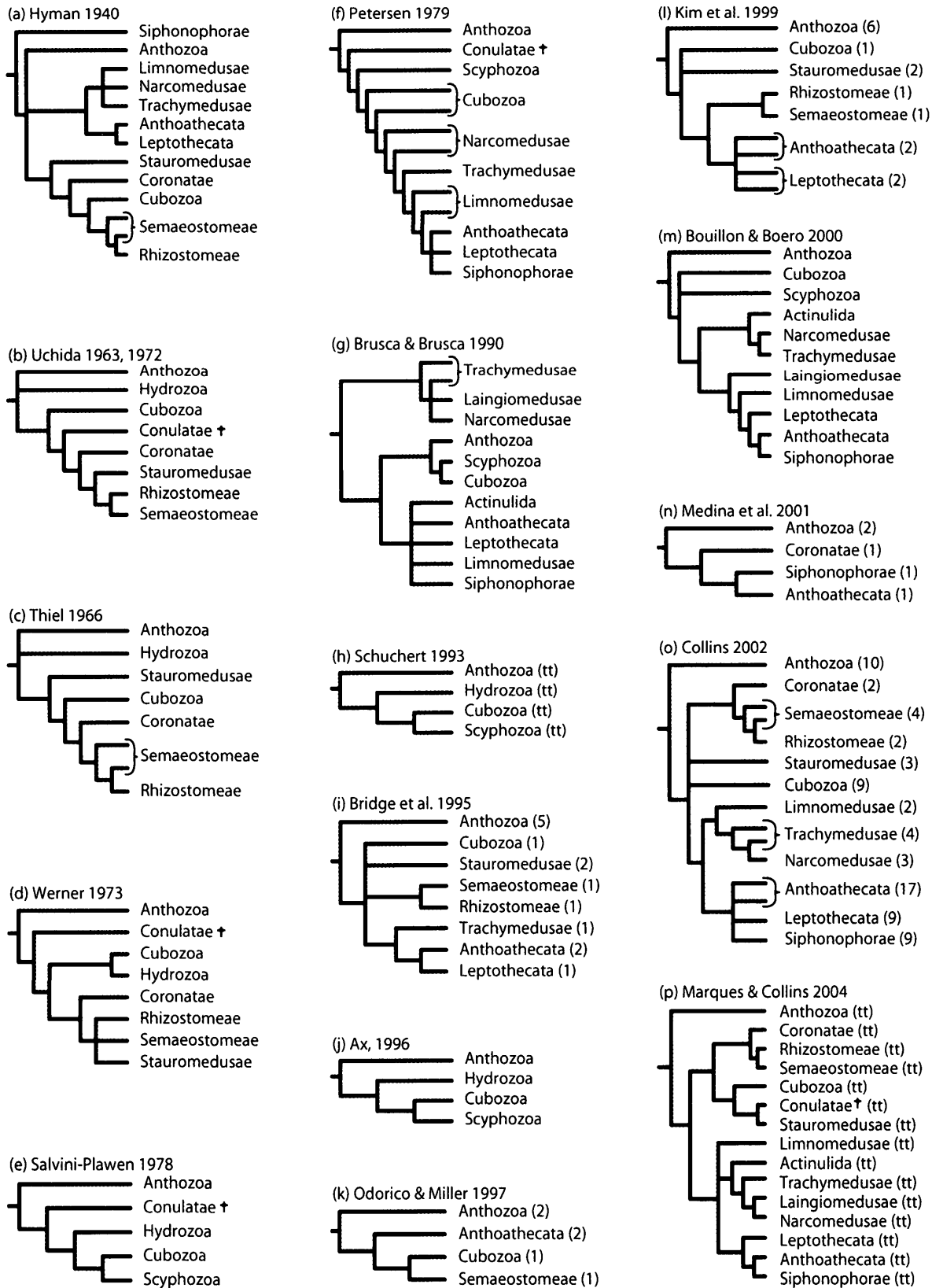


FIGURE 1. Comparison of hypotheses of phylogenetic relationships among medusozoan cnidarian groups published since 1940. Note that some precladistic, scenario-based hypotheses involve paraphyletic taxa, as indicated by parentheses. Taxa used in cladistic studies are marked (tt), for terminal taxon, in order to indicate that the monophyly of these groups was not explicitly tested in the analysis (though note that in some cases, as for example by Schuchert [1993], evidence supporting monophyly of the groups was presented.) For molecular studies, numbers in parentheses following taxon names indicate the number of species sampled. The cross next to the taxon Conulatae indicates that the group is extinct.

(Fig. 1). Similarly, nearly all analyses have suggested that the hydrozoan groups typified by highly polymorphic polyp colonies—Anthoathecata, Leptothezata, and Siphonophorae—are closely allied in a clade known as Hydroidolina (a name introduced by Collins, 2000). Finally, as noted above, all other considerations of cnidarian relationships since 1940, despite a couple of notable exceptions (i.e., Hyman, 1940; Brusca and Brusca, 1990), have concluded that Anthozoa is the sister group of Medusozoa.

If the aforementioned relationships are accepted as a reasonably certain backbone representing our present understanding of big-picture medusozoan phylogeny, then great progress can be made by clarifying the phylogenetic positions of a relatively small number of groups. For example, within Hydrozoa, Limnomedusae and two species-poor groups, Actinulida and Laingiomedusae, have uncertain positions, potentially being allied with either of the two hydrozoan clades about which there is little disagreement (Narcomedusae + Trachymedusae or Anthoathecata + Leptothezata + Siphonophorae; see Fig. 1). Similarly, among nonhydrozoan medusozoans, the class Cubozoa, and the groups Stauromedusae and Coronatae (traditionally classified as orders in the class Scyphozoa), have occupied a number of different hypothesized positions relative to each other and Discomedusae (Fig. 1). In order to reduce the uncertainty of the evolutionary relationships of these groups, additional evidence must be gathered and analyzed until a preponderance of results indicates relationships that can be accepted with some measure of confidence.

To further evaluate competing phylogenetic hypotheses involving medusozoan groups, we analyze sequences of the nearly complete (~3300 bp) large subunit of the nuclear ribosome (LSU or 28S) from 33 cnidarians, including 28 newly generated medusozoan sequences. In addition, we examine the most complete set (121 sequences, of which 25 medusozoan representatives are new) of cnidarian SSU (or 18S) data yet compiled. We complement more standard analyses of these data using phylogenetic mixture models in a Bayesian context (Pagel and Meade, 2004) in order to determine if explicitly accounting for heterogeneous patterns in our rDNA data helps us obtain the best possible inference of cnidarian phylogeny. These data, particularly the new LSU sequences, add further weight to some previously proposed hypotheses by strongly contradicting others and suggest a novel hypothesis for the phylogeny of Medusozoa as a whole. Our results establish a more robust working hypothesis of cnidarian phylogeny that can be used to examine character evolution within Cnidaria and should prove useful as a framework for comparative studies employing a growing number of different cnidarian species as model organisms.

MATERIALS

Genomic DNA was extracted from specimens using the DNAzol (Chomczynski et al., 1997) and Invisorb Spin Tissue (Invitek, Berlin) kits. Nearly complete genes cod-

ing for the large subunit of the nuclear ribosome (LSU or 28S) were amplified from 28 medusozoans using the primers F63mod and R3264 from Medina et al. (2001). In order to yield sufficient template quantities for sequencing, two internal primers were matched with terminal primers (F63sq+R2077sq and F1379+R3264 from Medina et al., 2001) in a second PCR to amplify approximately the first and last two-thirds of the entire fragment using diluted template obtained from the initial PCR. Cycle sequence reactions for LSU PCR products were carried out using primers from Medina et al. (2001), as well as the primers F2800 and R2800 (from Voigt et al., 2004). Sequences of the gene coding for SSU were obtained using standard PCR and sequencing primers (Medlin et al., 1988). We used DYEnamic™ E.T.-Terminator cycle sequencing kit (Amersham Biosciences) for all cycle sequencing reactions, which were subsequently visualized with a Megabace 500 Sequencer (Amersham Biosciences). Sequences and collection data for the source specimens are deposited in GenBank (see Appendix 1 for accession numbers).

An initial LSU alignment was obtained by employing Clustal W on a set of roughly 10 LSU sequences, including five obtained from GenBank (Appendix 1). Using Seaview (Galtier et al., 1996), this coarse alignment was adjusted with the aim of placing putatively homologous sites into columns. As additional LSU sequences were incorporated into the alignment, refinements guided by visual inspection were made by adjusting both single sequences and blocks of sequences. By a similar method, new SSU sequences were aligned to a preexisting data set (Collins, 2002). Positions from both the LSU and SSU alignments that were subjectively determined to be difficult to align were identified. In addition, the program Gblocks (Castresana, 2000)—using the default settings except that positions with gaps were allowed to be chosen—was used to determine nonconserved regions of the alignment. Those regions judged as nonconserved by Gblocks were excluded from phylogenetic analysis. Aligned data sets, including all exclusion sets, are available from TreeBase (S1383).

PHYLOGENETIC METHODS

Our general approach to phylogenetic inquiry is to first examine whether our new data provide substantial evidence against already existing hypotheses. Then we move on to explicitly assess those hypotheses favored by our data. In all phylogenetic analyses, anthozoans were used as outgroup taxa. Under the criterion of maximum likelihood (ML), searches (10 replicates with taxa added randomly to the starting tree) were conducted using PAUP*4.0 (Swofford, 2000) for optimal trees conforming to constraint topologies representing 13 different hypotheses of cnidarian relationships (Table 1, Fig. 1), given the LSU and combined data. The SSU data were not examined in this way because prior work already indicated the hypotheses likely to be favored by these data (Collins, 2002). In addition to the constrained analyses, searches in the absence of topological constraints were

TABLE 1. Results of approximately unbiased (AU) and Kishino-Hasegawa (KH) tests of different hypotheses identified a priori, given the LSU and combined data.

No.	Hypothesized clade	Publications (from Fig. 1)	P-values; AU/KH	
			LSU	Combined
1	Coronatae, Cubozoa, Rhizostomeae, Semaestomeae, Stauromedusae	a, b, c, e, g, h, j, p	0.072/0.061	0.074/0.051
2	Cubozoa, Hydrozoa	d, f	0.083/0.072	0.154/0.107
3	Coronatae, Rhizostomeae, Semaestomeae, Stauromedusae	b, d, e, f, g, h, j, m	0.006*/0.015*	0.003*/0.020*
4	Cubozoa, Rhizostomeae, Semaestomeae	a	0.021*/0.041*	0.001*/0.008*
5	Cubozoa, Stauromedusae	p	0.030*/0.028*	0.131/0.085
6	Coronatae, Cubozoa, Rhizostomeae, Semaestomeae	a, c	(best)	(best)
7	Coronatae, Rhizostomeae, Semaestomeae	c, o, p	(best)	(best)
8	Anthoathecata, Laingiomedusae, Leptothecata, Limnomedusae, Siphonophorae	m	0.027*/0.036*	0.006*/.016*
9	Laingiomedusae, Narcomedusae, Trachymedusae	g, p	0.000*/0.000*	0.000*/0.000*
10	Anthoathecata, Leptothecata, Limnomedusae, Siphonophorae	m	0.000*/0.000*	0.000*/0.000*
11	Limnomedusae, Narcomedusae, Trachymedusae	a, o	(best)	(best)
12	Anthoathecata, Leptothecata, Siphonophorae	m, o, p	0.152/0.117	0.180/0.155
13	Anthoathecata, Laingiomedusae, Leptothecata, Siphonophorae	Schuchert, 1996	(best)	(best)

*Significant *P*-value for rejection of specific hypothesis by the data.

carried out. For these analyses, an assumed model of nucleotide evolution was obtained by using the Akaike Information Criterion (AIC) as implemented in ModelTest (Posada and Crandall, 1998).

In order to gauge the extent to which the LSU and combined data conflict with prior hypotheses, we performed Approximately Unbiased (AU) and Kishino-Hasegawa (KH) tests (Hasegawa and Kishino, 1989; Shimodaira, 2002) using the software Consel (Shimodaira and Hasegawa, 2001), run with 10 sets of 100,000 bootstrap replicates. The KH test is somewhat controversial, having often been used inappropriately to discern among hypotheses chosen after preferences in the data have already been identified (Goldman et al., 2000), in contrast to how it is used here. Two alternative tests, the SH (Shimodaira and Hasegawa, 1999) and SOWH (Swofford et al., 1996) tests, were not employed because the latter may have a tendency to reject hypotheses that are true, whereas the former tends not to falsify unlikely alternatives (Buckley, 2002; Shimodaira, 2002).

Because the use of different optimality criteria may lead to incorrect results under some conditions, for instance, maximum parsimony (MP) when evolutionary rates vary strongly across taxa (Felsenstein, 1978) or ML when rates of evolution vary over time (Kolaczkowski and Thornton, 2004), we analyzed our data in a variety of ways. In addition to ML, we conducted MP (200 replicate searches with taxa added randomly to the starting tree) and minimum evolution (ME) searches for optimal topologies based on the LSU and combined data sets. For ME analyses, we used the same assumed model as was used for the ML searches. Bootstrap analyses under each criterion (500, 500, and 200 replicates for MP, ME, and ML, respectively) were conducted in order to assess node support deriving from the LSU and combined data. Using the SSU data set, tree searches were conducted for the optimal MP (1000 replicate searches keeping a maximum of 500 unique trees during each replicate) and ME topologies. Node support was assessed under MP and ME by bootstrap analysis (200 random replicate data sets, with 5 random replicate heuristic searches under MP).

ML analyses were not conducted with the SSU data sets due to computational limitations.

We also used phylogenetic mixture models (Pagel and Meade, 2004) in an attempt to obtain the best possible inference of cnidarian phylogeny using our rDNA data. Considerable variability in substitution rate is evident across both SSU and LSU (Van de Peer et al., 1997; Ben Ali et al., 1999), and typically a gamma parameter is used to take into account differences in the rate of evolution across sites. However, the evolution of different regions of ribosomal genes are likely to differ not just in rate but in kind, e.g., as a result of the complementary base-pairing involved in ribosomal structure. Even within categories of characters (such as stem or loop), there is likely to be significant variation in substitution patterns (Pagel and Meade, 2004). This observation led Pagel and Meade (2004) to develop the software BayesPhylogenies (Pagel and Meade, 2004), which can be used to fit two or more different models to a set of data without specifying which sites are best fit by which models. This approach, which is only implemented in BayesPhylogenies, appears to provide a powerful means for taking into account data heterogeneity.

Using BayesPhylogenies, we fit from one to six general time-reversible (GTR) models including a gamma parameter to our data sets—LSU, combined, and SSU—until we were reasonably sure of over-parameterization. We did this because there are uncertainties about how best to determine the most appropriate number of models for a given set of data (Pagel and Meade, 2004) and we wanted to observe how the number of models used impacts inferences of phylogeny. We analyzed the data by Markov chain Monte Carlo (MCMC) method, allowing the Markov chain to proceed for 500,000 iterations for the LSU and combined data sets and 1,000,000 times for the SSU data set. For each data set and model combination, we ran a minimum of four independent analyses to ensure that runs were converging to the same parameter space. Posterior probabilities (pp) were obtained by constructing a majority rule consensus of 100 trees sampled every 10,000 from 410,000 to 500,000 of the converged

LSU and combined runs and 910,000 to 1,000,000 from the converged SSU runs.

RESULTS

Of the 3619 characters in our LSU data set, 3247 were designated as conserved by Gblocks. Of these, 2130 are constant and 865 are parsimony-informative over the taxa we have examined. Our SSU data set contains 2139 positions, of which 1898 are conserved. Of these, 1142 are constant and 483 are parsimony-informative. In the combined data set, 1164 of the 5145 characters are parsimony-informative and 3620 are constant. For the LSU, SSU, and combined data sets, ModelTest indicated that the most appropriate model of nucleotide evolution is one that has six substitution rates, an assumed proportion of invariant sites, and a gamma-shape parameter (GTR+I+G), though estimates of the model parameters differ (see legends for Figs. 2 to 4).

Results of hypothesis testing are summarized in Table 1. In all cases, significance or non-significance of the AU and KH tests matched. Using the 95 percent level as the conventional arbitrary cutoff, five hypothesized clades (3, 4, 8, 9, and 10) are rejected by both the LSU and combined data sets. By these tests, one hypothesis (5) is significantly suboptimal based on the LSU data, but is not rejected by the combined data.

Four hypothesized clades listed in Table 1 (6, 7, 11, and 13) are present in the most optimal (ML) trees (Figs. 2 and 4). Gegenbaur's (1856) "Acraspeda" most closely approximates clade 6; this name refers to the absence of a velum typical of hydrozoan medusae. We call clade 7 Scyphozoa following Marques and Collins (2004), clade 11 Trachylina, and clade 13 Hydrozoa. Finally, three hypothetical clades (1, 2, and 12) are contradicted by the most optimal trees (Figs. 2, 4), but are not found to be so suboptimal that

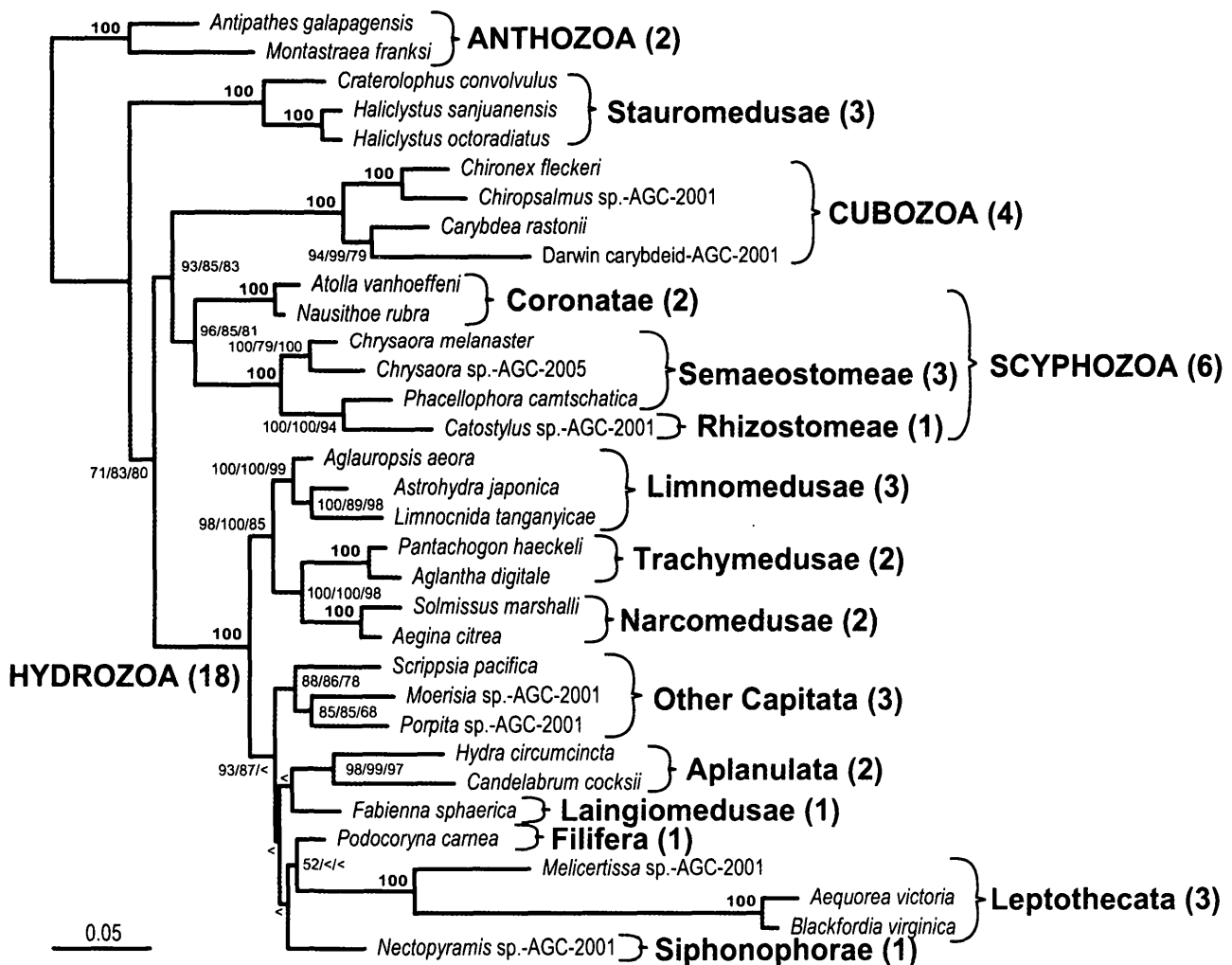


FIGURE 2. Unconstrained phylogenetic hypothesis of relationships among 31 medusozoan cnidarians, rooted with two anthozoans, based on ML analysis of LSU data. Numbers in parentheses indicate the number of species sampled for that taxon. Bootstrap values under ML, ME, and MP criteria are shown at the nodes. Nodes with bootstrap indices of 100 under all three criteria are indicated with a bold 100. Bootstrap values less than 50 are represented by <. The assumed model (GTR+I+G) of nucleotide evolution for ML and ME tree searches has six substitution rates (A-C, 0.8456; A-G, 2.6613; A-T, 1.0709; C-G, 0.8204; C-T, 4.9158; and G-T, 1.0000), an assumed proportion of invariant sites (0.4776), and a gamma-shape parameter (0.5251). The length of the bar indicates 0.05 substitutions per site.

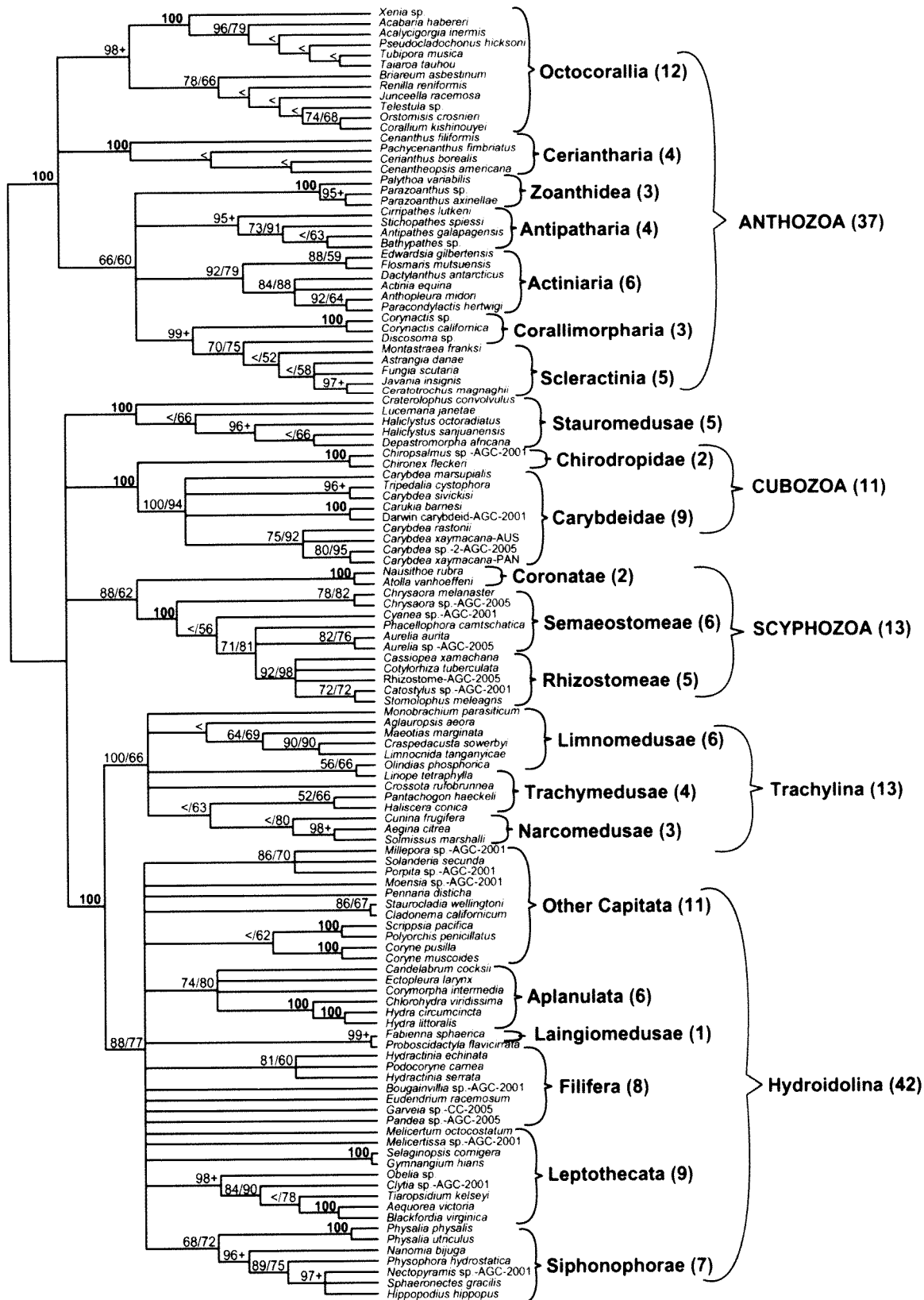


FIGURE 3. Phylogenetic hypothesis of relationships among 84 medusozoan cnidarians, rooted with 37 anthozoans, based on MP analysis of SSU data, which yielded this strict consensus of 68,528 MP trees of length 4062. Numbers in parentheses indicate the number of species sampled for that taxon. Bootstrap values under ME and MP criteria are shown at the nodes. Nodes with bootstrap indices of 100 under both criteria are indicated with a bold 100. Nodes with both bootstrap values above 95 are indicated with the lower of the two scores and +. Bootstrap values less than 50 are represented by <. The assumed model (GTR+I+G) of nucleotide evolution for ML and ME tree searches has six substitution rates (A-C, 1.2183; A-G, 3.1905; A-T, 1.3619; C-G, 1.1074; C-T, 5.5110; and G-T, 1.0000), an assumed proportion of invariant sites (0.4294), and a gamma-shape parameter (0.5314).

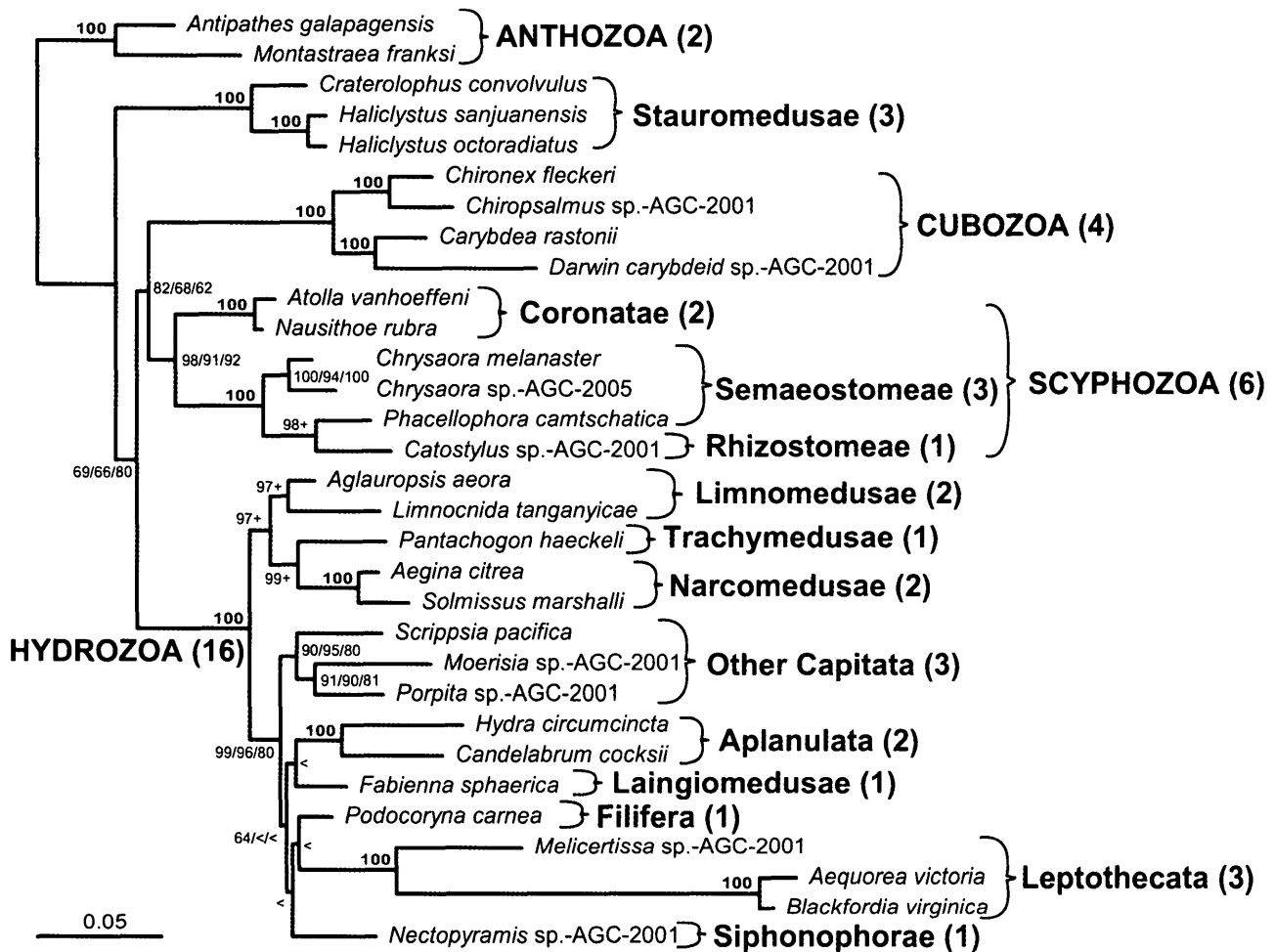


FIGURE 4. Phylogenetic hypothesis of relationships among 29 medusozoan cnidarians, rooted with two anthozoans, based on ML analysis of LSU and SSU data combined. Numbers in parentheses indicate the number of species sampled for that taxon. Bootstrap values under ML, ME, and MP criteria are shown at the nodes. Nodes with bootstrap indices of 100 under all three criteria are indicated with a bold 100. Bootstrap values less than 50 are represented by <. The assumed model (GTR+I+G) of nucleotide evolution for ML and ME tree searches has six substitution rates (A-C, 0.9431; A-G, 2.8946; A-T, 1.1569; C-G, 0.9034; C-T, 5.1750; and G-T, 1.0000), an assumed proportion of invariant sites (0.5333), and a gamma-shape parameter (0.5605). The length of the bar indicates 0.05 substitutions per site.

they are rejected by the AU and KH tests employed here.

Figure 2 shows the relationships for which our compiled LSU data are most probable. Tree searches using all three optimality criteria, ME, ML, and MP, are largely congruent (ME and MP not shown), differing only in the relationships among representatives of the hydroidolinan groups Aplanulata, Other Capitata, Filifera, Laingiomedusae, Leptothecata, and Siphonophorae. With the exception of nodes involving these taxa, bootstrap support indices under the three criteria are generally high and consistent. Figure 3 shows the topology that requires the fewest assumed changes in our SSU data. In terms of the larger groups, the strict consensus of the MP trees matches the LSU hypothesis (Fig. 2) closely. However, several key clades (e.g., Stauromedusae, Cubozoa, Scyphozoa, and Hydrozoa) form a polytomy based on the SSU data. A phylogenetic hypothesis for Cnidaria based on ML analysis of combined LSU and SSU data

is presented in Figure 4. The combined results mirror those of the LSU and SSU data sets examined separately (Figs. 2, 3).

In addition to those listed by Daly et al. (2002) and Collins and Daly (2005), i.e., Z92904, AF099103, and AF099104, two cnidarian SSU sequences in GenBank are potentially misidentified. An octocoral sequence labeled *Virgularia gustaviana* (GenBank accession no. Z86106) consistently branches with the ceriantharian tube anemones rather than within Octocorallia, which otherwise is a highly supported clade based on SSU data (Fig. 3; and see Song and Won, 1997; Berntson et al., 1999; Won et al., 2001). Another potentially erroneous sequence is that of *Cassiopea* sp. (GenBank accession no. AF099675), a rhizostome jellyfish. This sequence tends to branch ambiguously near the base of Hydrozoa or Medusozoa and far away from the two other rhizostomes that had been sampled for the SSU gene. Here, however, we present a new sequence for *Cassiopea*

xamachana, a culture of which is maintained in the Schierwater lab in Hannover, Germany. This sequence differs greatly from AF099675 and falls in a well supported clade with all other rhizostomes sampled (Fig. 3). Z92904, AF099103, AF099104, Z86106, and AF099675 are excluded from the analyses presented here.

Summarizing the results of the phylogenetic mixture models is somewhat difficult because of the large number of different analyses. We fit from one to six models with a gamma parameter to each data set resulting in 18 different estimates of cnidarian phylogeny. In each case, at least three out of the four independent runs of BayesPhylogenies converged to the same parameter space. Those runs that reached a suboptimal region of tree space, as judged by log-likelihoods, were discarded from calculations of posterior probabilities (pp). A comparison of all the average log-likelihoods of the resulting trees under the different number of models is shown Table 2. The use of an additional model always results in a better mean tree score. We always incorporated a gamma parameter because preliminary analyses indicated that use of a gamma parameter with n models (numbers of estimated parameters $6n + 1$) results in an average score that exceeded that of $n + 1$ models without a gamma parameter (number of estimated parameters $6n + 6$) for our data sets.

Picking the correct number of models that best fit the data is not a simple exercise. The most obvious potential indicator would be a positive log-Bayes factor (Gelman et al., 1995; Raftery, 1996), but these are dependent on the assumption that characters are independent, an assumption that is clearly violated by rDNA data. Nevertheless, approximate log-Bayes factors, calculated as twice the increase in log-likelihood score when going from n models to $n + 1$ less 32 (Pagel and Meade, 2005), are shown in Table 2. This approximation can be made because BayesPhylogenies MCMC runs assign uniform priors to trees and model parameters (Pagel and

Meade, 2004). Two other possible subjective indicators of overparameterization are a marked increase in the average standard deviation of the rate parameters and/or a precipitous decline in the improvements of the overall log-likelihood scores as models are added (Pagel and Meade, 2004, 2005). Both these measures are presented in Table 2.

By these three measures, there is no clear indication for what the most appropriate number of models is for any of our data sets (Table 2). The approximate Bayes factors are negative when going from Q4 to Q5, Q5 to Q6, and Q4 to Q5 for the LSU, combined, and SSU data sets, respectively. The average standard deviation of the rate parameters increases most markedly when going from Q3 to Q4, Q2 to Q3, and Q2 to Q3 for the three data sets, whereas there appear to be relatively steady declines in the improvements of the scores (Table 2).

In general, we found that the number of different models did not have a great impact on the resultant topologies for the LSU, combined, and SSU data sets. Figure 5 shows the preferred topology when three models (Q3) are applied to the LSU data. At each node, Bayesian pp's under all numbers of models fit to both the LSU or combined data illustrate that the majority of hypothesized relationships are equivalent when additional models are fit to both data sets. Most nodes have pp's equal to 100, and discrepancies arise only for those nodes that had low bootstrap support (Figs. 2, 4, and 5).

The placement of Stauromedusae and the monophyly of Acraspeda (Cubozoa plus Scyphozoa) are of particular importance to the ensuing discussion. As additional models are fit to the LSU and combined data, support for Stauromedusae being the sister group of all other medusozoans is eroded (Fig. 5). Instead, the pp for a clade including Stauromedusae and Hydrozoa exceeds 50 when four or more models are fit to the LSU data. When the combined data are analyzed, the probability of Stauromedusae having a basal position within Medusozoa

TABLE 2. Comparison of results under different numbers of phylogenetic mixture models.

Data set	No. of model	Number of parameters	Average log-likelihood	Increase in score	Percent increase	Average standard deviation of rate parameters	Approximate Bayes factor
LSU	Q1	6	-22,783.9	—	—	0.15	
	Q2	13	-22,602.9	180.9	0.79%	0.50	298
	Q3	20	-22,524.1	78.8	0.35%	0.88	94
	Q4	27	-22,474.7	49.4	0.22%	1.73	35
	Q5	34	-22,444.3	30.4	0.14%	1.79	-3
	Q6	41	-22,429.5	14.8	0.07%	1.81	-34
Combined	Q1	6	-30400.0	—	—	0.14	
	Q2	13	-30115.8	284.2	0.93%	0.66	504
	Q3	20	-30007.6	108.2	0.36%	1.60	152
	Q4	27	-29945.6	62.0	0.21%	1.46	60
	Q5	34	-29904.6	40.9	0.14%	1.69	18
	Q6	41	-29883.1	21.5	0.07%	1.51	-21
SSU	Q1	6	-19,852.8	—	—	0.20	
	Q2	13	-19,629.2	223.6	1.13%	0.83	383
	Q3	20	-19,517.8	111.3	0.57%	1.82	159
	Q4	27	-19,450.3	67.6	0.35%	2.65	71
	Q5	34	-19,426.1	24.2	0.12%	1.95	-16
	Q6	41	-19,395.5	30.5	0.16%	1.81	-3

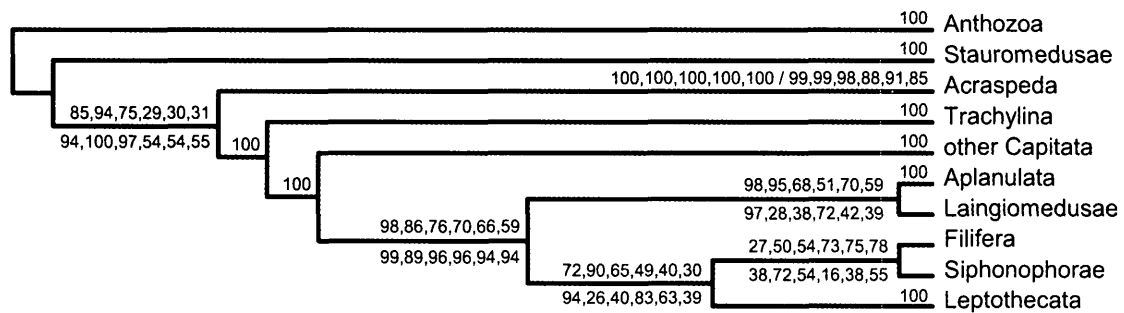


FIGURE 5. Preferred topology based on the Bayesian analysis with three GTR models and a gamma parameter (Q3 in Table 2) fit to the LSU data. Posterior probabilities under all six models for both the LSU (above) and combined data (below) are shown at all nodes. A single value of 100 is shown at those nodes receiving high support no matter how many models are fit to either the LSU or combined data sets. Note that several nodes have been collapsed, e.g., Acraspeda contains all scyphozoan and cubozoan representatives. Every hidden node received Bayesian posterior probabilities of 100 for every combination of models and data.

increases and the probability that Scyphozoa and Cubozoa form the clade Acraspeda decreases slightly (Fig. 5).

Similarly, fitting additional models to the SSU data has little impact on hypothesized relationships (Fig. 6). When three models and a gamma parameter are fit to the SSU data (Fig. 6), there are 68 resolved nodes within Medusozoa. Of these, 45 have pp's exceeding 90 (33 nodes with pp = 100) no matter how many models are fit to the data. Of the remaining 23 nodes, 15 are favored (pp > 50) irrespective of the number of models. Thus, just eight nodes are impacted by the number of models being fit to the data and the vast majority of these (seven) are present in most instances. In comparison to the strict consensus of most parsimonious trees (Fig. 3), 44 of the 51 nodes within Medusozoa have high support (pp > 90) no matter how many numbers of models are fit to the data. Of the remaining seven medusozoan nodes in the MP tree, five have pp's that exceed 70 under all models. Of the two remaining nodes, each one has a pp that is below 50 for just a single instance. In sum, applying phylogenetic mixture models to the SSU data results in topologies that are highly consistent with each other (Fig. 6). These topologies are also consistent with the strict consensus of MP trees (Fig. 3), but have greater resolution. The question then arises whether the increased resolution resulting from the Bayesian analyses represents true relationships. Because we cannot know the true phylogeny, these hypothetical nodes can only be judged in light of other information and future analyses.

DISCUSSION

Quality of the LSU Marker

The LSU marker appears to be of great use for phylogenetic analysis of this diverse group of metazoans. Hypotheses based on LSU data (Fig. 2) share many similarities with those based on the analysis of nonmolecular characters (Fig. 1), provide support for nodes that were equivocal based on SSU data alone, and are highly congruent with those derived from SSU data (Fig. 3; Collins, 2002). More importantly, there are no strong contradictions between conclusions drawn from the LSU and SSU data. One might suspect that both markers share similar

biases, given that LSU and SSU are part of the complex of ribosomal genes that evolve by concerted evolution. In fact, a simple visual comparison of the phylogram in figure 3 of Collins (2002), to Figure 2 here reveals that SSU and LSU exhibit similar lineage-specific variation in evolutionary rates, e.g., cubozoans and especially leptotheccates are relatively long-branched taxa.

On the other hand, SSU data are also congruent with the generally faster evolving data of partial mitochondrial 16S sequences in their indications of relationships among less inclusive groups of cnidarians, e.g., within Hydrozoa (Collins et al., 2005), Hexacorallia (Daly et al., 2003), and Stauromedusae (Collins and Daly, 2005). Although 16S is another ribosomal gene, there is no mechanism known that explains why this mitochondrial gene would be biased in the same way as nuclear SSU and LSU. The important conclusion that can be drawn is that combining data from all readily available rDNA markers—including complete mitochondrial 16S and 12S and complete nuclear SSU, LSU, and 5.8S—might be an effective and efficient strategy for pursuing large-scale studies aimed at elucidating cnidarian relationships at all taxonomic scales. Of course, this does not eliminate the need to develop and use alternative markers to test ribosomal-based hypotheses and seek resolution where it is presently lacking.

Utility of Phylogenetic Mixture Models

When employing phylogenetic mixture models, following a simple rule to determine the number of models to fit to the data is unwise, given the subjective nature of the decision. Instead, changes (if any) in topology that occur as models are added should be examined. Figures 5 and 6 show that this choice has relatively little influence on our phylogenetic inferences based on our data sets. Furthermore, applying phylogenetic mixture models to our data resulted in topologies that were overwhelmingly consistent with those derived from ML (Figs. 2, 4) and MP (Fig. 3) analyses. Not surprisingly, taxa that group with low bootstrap support are those most likely to have alternative relationships in the Bayesian results and whose inferred relationships are most influenced by the application of different numbers of models. In general,

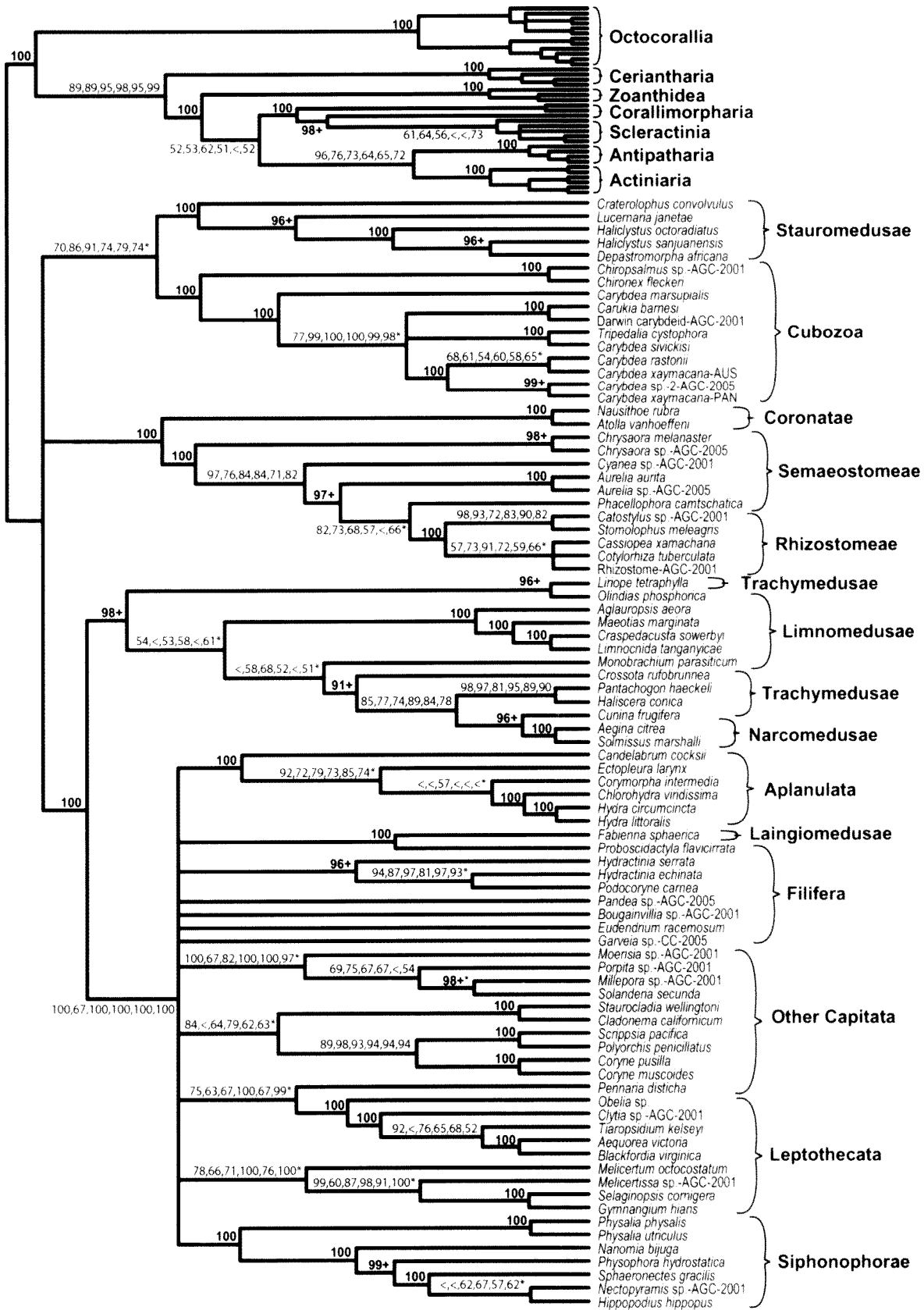


FIGURE 6. Preferred topology based on the Bayesian analysis with three GTR models and a gamma parameter (Q3 in Table 2) fit to the SSU data. Posterior probabilities when one to six different models are fit to the data are shown at each node, with the exception of internal nodes within the major anthozoan groups. A single value of 100 or a range of high values are shown at those nodes receiving high support (>90) no matter how many models are fit to the data. Posterior probabilities less than 50 are represented by <. Seventeen nodes not present in the MP analysis (Fig. 3) are indicated with*.

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we conclude that little insight was gained by applying phylogenetic mixture models to these data sets because there seems little justification to favor any one particular set of relationships over another.

Nevertheless, our SSU data set was too large to analyze using maximum likelihood and the Bayesian approach allowed us to examine our data using a method that takes advantage of the likelihood function. The Bayesian topology for the SSU data (Fig. 6) contains all of the medusozoan nodes revealed in the strict consensus of MP trees (Fig. 3), as well as 17 additional nodes (denoted with asterisks in Fig. 6). Whether these nodes accurately reflect phylogenetic history or not can only be judged in light of other evidence. For most of these hypothesized relationships, there is no relevant independent evidence, and so their veracity can only be judged through future phylogenetic analyses.

Evidence can, however, be brought to bear on a few of these hypothetical clades. First, Figure 6 suggests the possibility of a relationship between Stauromedusae and Cubozoa (Fig. 6). This hypothesis accords with the results of a cladistic analysis of morphological and life history characters (Marques and Collins, 2004), but a reanalysis of these same data, except that scoring of char-

acters dealing with the fossil group Conulatae was altered, found that Stauromedusae was the sister group to all other medusozoans (Van Iten et al., in press). Moreover, although LSU data do not have sufficient strength to statistically falsify a close relationship between Stauromedusae and Cubozoa (Table 1), this hypothesis has a pp of 0 no matter how many models are fit to the LSU data. Within the rhizostomes sampled here, a clade containing *Cassiopea* and *Cotylorhiza* agrees with the views of Stiasny (1921) and Thiel (1970). In addition, many of the relationships revealed among the capitate taxa sampled here were also found through an analysis of mitochondrial 16S data (Collins et al., 2005). In contrast, relationships among the members of Aplanulata contradict those derived from 16S data (Collins et al., 2005). On the whole, it seems premature to conclude that the application of phylogenetic mixture models has allowed us to efficiently extract more phylogenetic information from this relatively taxon-rich data set.

Relationships among the Cnidarian Classes

Our working hypothesis of relationships among the cnidarian classes (Fig. 7) has Anthozoa as the earliest diverging class, Staurozoa as the sister group of all other

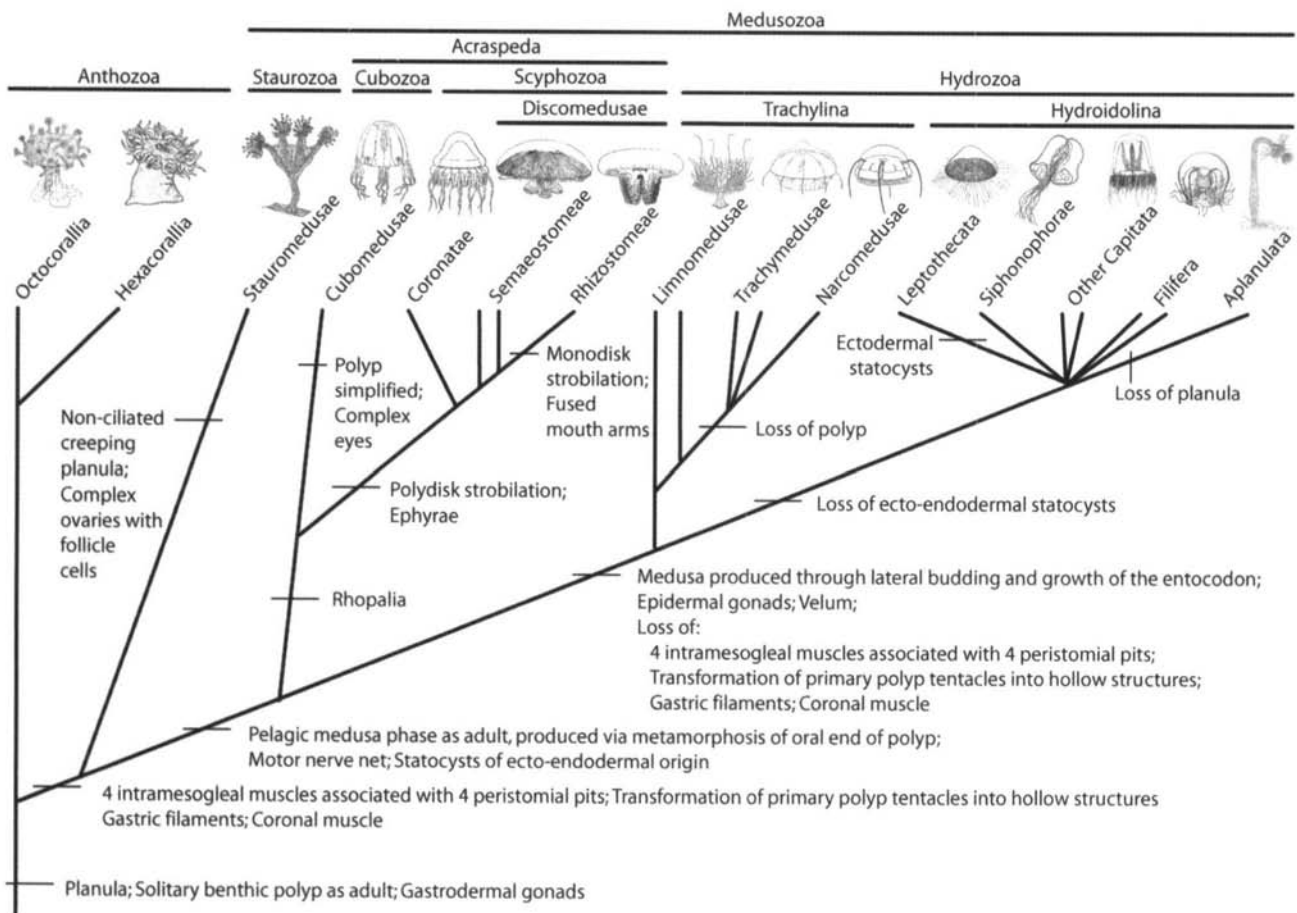


FIGURE 7. Working hypothesis for cnidarian relationships with selected hypothesized ancestral characters mapped at nodes. Diagrams representing taxa are after figures in Mayer (1910), with the exception of representatives of Octocorallia and Siphonophorae, which are modified from Hyman (1940), and Hexacorallia, which was drawn by Crissy Huffard.

medusozoans, and Cubozoa and Scyphozoa forming a clade that is the sister group of Hydrozoa. In order to communicate that Stauromedusae has a discrete history and that it possesses features that are correspondingly distinct from those of the other cnidarian classes, Marques and Collins (2004) recognized the new class Staurozoa. Our molecular data contradict the placement of Stauromedusae derived from their cladistic analysis of morphological and life-history characters. However, it reaffirms that Stauromedusae is a primary clade of Cnidaria and that it does not form a clade with the scyphozoan groups with which it has traditionally been classified. Instead, a growing body of both molecular (Collins and Daly, 2005) and morphological (Van Iten et al., in press) evidence suggests that Stauromedusae is the sister group to all other medusozoans (Fig. 7). For classification purposes, using Staurozoa as a separate class in Cnidaria conveys that the phylum is more diverse at a fundamental level than previously recognized.

Cubozoa was designated as a class (Werner, 1973) because its members have a life cycle and polyp morphology that are rather distinct from those of scyphozoans (Werner et al., 1971). Rather than producing ephyrae through strobilation, cubozoan medusae result from a total metamorphosis of the polyp (Werner et al., 1971; Studebaker, 1972; Arneson and Cutress, 1976; Yamaguchi and Hartwick, 1980). This difference is qualified by exceptions: in some species, part of the polyp sometimes remains after metamorphosis into a juvenile medusa and is capable of developing into a new polyp and producing a subsequent medusa (Stangl et al., 2002; Straehler-Prohl and Jarms, 2005). Some of the starkest differences between cubozoans and scyphozoans involve polyp morphology. Whereas scyphozoan polyps exhibit strong fourfold symmetry due to the presence of four gastric septa and four intramesogleal muscle strands, cubozoan polyp organization is essentially radial. These facts were stressed by Werner (1973), who championed the idea that Cubozoa is more closely related to Hydrozoa than it is to Scyphozoa.

Since Werner's 1973 work, the position of Cubozoa within Medusozoa has been rather contentious, with some favoring a hydrozoan affinity (Arneson and Cutress, 1976; Petersen, 1979; Leonard, 1980) and others a closer relationship to scyphozoans (Larson, 1976; Salvini-Plawen, 1978; Satterlie and Spencer, 1980; Schuchert, 1993; Ax, 1996; Marques and Collins, 2004). The two prior molecular analyses that contained samples of the relevant groups to test these competing alternatives (Bridge et al., 1995; Collins, 2002) provided no compelling evidence for a definitive placement of Cubozoa. Because LSU data provide strong evidence that Cubozoa forms a clade with Scyphozoa (Acraspeda), it seems likely that the absence of four gastric septae and interradial muscle fibers are due to evolutionary loss in the ancestry of Cubozoa (Fig. 7). This accords well with observations that cubopolyps contain intramesogleal muscles, though not concentrated in four fibers (Chapman, 1978) and that numerous similarities exist between cubozoan and scyphozoan medusae, including neuromuscular and

nerve net organization (Satterlie, 1979, 2002) and the presence of rhopalialia (Fig. 7).

Our working hypothesis suggests that hydrozoan polyps, which are radial and lack gastric pockets, were also derived from an ancestor that was more complex in these respects (Fig. 7). Furthermore, the ancestral hydrozoan likely had a biphasic life cycle with the medusa formed via lateral budding and development through a tissue mass known as the entocodon (Fig. 7). This complex trait involving an ontogenetic process as well as morphology is typical of Limnomedusae and the hydroidolinan groups (Anthoathecata, Leptothezata, and Siphonophorae), but it is absent in Actinulida, Narcomedusae, and Trachymedusae, and not yet determined for Laingiomedusae. Because Limnomedusae is at the base of Trachylina (Fig. 7; Collins, 2002) rather than among the hydroidolinan groups (Petersen, 1979; Bouillon and Boero, 2000), the last common ancestor of extant hydrozoans likely produced medusae by a developmental process involving an entocodon and lateral budding from polyps. The polyp stage and the attendant mode of medusa development were apparently lost in the lineage leading to Trachymedusae and Narcomedusae.

Origin of the Medusa Stage: A Link to Bilaterian Mesoderm?

Whether the adult form of the ancestral cnidarian was a polyp or a medusa has been a classic dispute. Brooks (1886) contended that a pelagic ancestor directly gave rise to the holopelagic trachylina hydrozoans, implying that even anthozoans were derived from hydrozoan ancestors. This opinion found its way into important textbooks (e.g., Hyman, 1940; Brusca and Brusca, 1990), but was probably a minority view. Instead, many argued that sessile polyps represent the ancestral cnidarian form and that the fundamental phylogenetic divergence within Cnidaria separated Anthozoa from the medusa-bearing groups (Haeckel, 1879; Hadzi, 1953; Werner, 1973; Salvini-Plawen, 1978). This latter phylogenetic hypothesis is widely accepted today because of new data, most notably that the mitochondrial genomes of medusozoan cnidarians are linear (Bridge et al., 1992). Despite the consensus phylogenetic view, however, the nature of the ancestral cnidarian remains somewhat controversial, with some authors supporting the idea that a pelagic medusa stage has probably been lost in the lineage leading to Anthozoa (Schuchert, 1993; Spring et al., 2002; Muller et al., 2003; Scholtz, 2004).

Most recent arguments championing the idea that the ancestral cnidarian possessed a medusa stage are built on the observation that several genes involved in specifying mesoderm and differentiating striated muscle in bilaterian animals are shared by the hydrozoan *Podocoryna carnea* during the ontogeny of its medusa as it develops from the entocodon (Spring et al., 2000, 2002; Muller et al., 2003). Because the medusae of cnidarians have striated muscles lining their subumbrellar surfaces and hydrozoan polyps do not, these authors conclude that a swimming medusa stage was present in the ancestral cnidarian. These conclusions are based on observations

of a hydrozoan and on the hypothesized homologies between this hydrozoan and bilaterians, implicitly suggesting that the ancestral process of medusa production for Cnidaria involved lateral budding of a medusa that grew from the entocodon.

Our working hypothesis, however, suggests that medusa production via lateral budding and growth of the entocodon is a synapomorphy of Hydrozoa, nested deeply within Medusozoa (Fig. 7). Therefore, hypothesized homologies between the entocodon and bilaterian mesoderm (Spring et al., 2002; Mueller et al., 2003; Seipel and Schmid, 2005) are suspect. Nevertheless, the intriguing similarities between the genes involved in myogenesis in the hydrozoan *Podocoryna carnea* and bilaterians still need to be explained. One potential key to understanding these similarities is that, in contrast with the lack of striated muscles in *Podocoryna* polyps, diverse cnidarian polyps do possess striated myofibers, including those of scyphozoans (Chia et al., 1984; Matsuno and Kawaguti, 1991), cubozoans (Chapman, 1978; Golz, 1993), and anthozoans (Amerongen and Peteya, 1980). Thus, the proposed homologies between the hydrozoan *Podocoryna* and bilaterians in myogenesis seem quite plausible. Study of additional model cnidarians may reveal that homologous genetic pathways involved in differentiation and specification of the striated muscles are broadly distributed within Cnidaria.

By providing reasonably strong evidence that Stauromedusae—comprising the sessile, so-called stalked jellyfishes—is the earliest diverging lineage within Medusozoa, our data further weaken the argument that there is a pelagic medusa stage in the ancestry of Anthozoa. Instead, the medusa appears to be derived within Medusozoa (Fig. 7). Furthermore, the motor nerve net (also known as giant fiber nerve net) and statocysts of ecto-endodermal origin likely developed in association with the pelagic, motile medusa stage. Despite these shared features, differences in the medusae of Hydrozoa and Acraspeda (e.g., lateral budding versus metamorphosis at the oral end of a polyp, presence versus absence of velum, absence versus presence of gastric filaments and coronal muscle, etc.; see Fig. 7) have led some to argue that the two are not homologous (Thiel, 1966; Salvini-Plawen, 1978).

A plausible, though speculative, scenario would involve a “reinvented” pelagic medusa in the ancestry of Hydrozoa. It is conceivable that the lateral budding of medusae in hydrozoans arose through the transformation of a laterally budding polyp into an entire medusa. The polyps of numerous species of cubozoans and scyphozoans laterally bud secondary polyps or vermiform stages known as frustules, which creep some distance before differentiating into polyps (Hofmann and Crow, 2002; Fischer and Hofmann, 2004). An interesting observation from the hydrozoan species *Podocoryna carnea* demonstrates that there may be some plasticity with respect to the morphologies that can be produced at budding sites. Polyps of *Podocoryna carnea*, if cultivated under stressful conditions (e.g., high temperature, sub-optimal sea water, etc.), will sometimes start to develop

little hydranths at the positions where medusae would otherwise arise (Peter Schuchert, personal observation).

The preceding discussion highlights the difficulties in assuming putative homology between entire life phases of the different cnidarian groups. For instance, the so-called “stalked jellyfishes” have frequently been viewed as possessing a mix of polyp and medusa characters that indicate a state of “degeneracy.” In other words, stauromedusans have been called stalked jellyfishes because they were thought to be descended from an ancestor with a pelagic medusa phase (Thiel, 1966; Uchida, 1972; Werner, 1973). Given the likely phylogenetic position of Stauromedusae (Fig. 7), it might actually be more accurate to think of medusae as free-swimming stauromedusans. However, attempting to homologize entire life stages across Cnidaria is almost certainly an oversimplification because some medusozoan characters may have arisen prior to the origin of a medusa and a benthic-pelagic life cycle. It follows that separating features into groups specific to polyp or medusa (as seen for instance in the cladistic scoring of Marques and Collins, 2004) is not entirely appropriate, though difficult to get around. Focusing on the potential homologies between characters rather than entire life stages may help us move beyond the old debate about which came first, polyp or medusa. As it stands, the possibility that anthozoan groups have or had characters that are presently thought of as features of the medusa is largely unexplored.

Relationships and Evolution within Scyphozoa

Ephyrae and polydisk strobilation are likely synapomorphies for Scyphozoa (Fig. 7). Within this clade, rhizostome jellyfishes have long been considered to be direct descendants from semaeostome ancestors because of similarities in their radial canal systems (Mayer, 1910; Hyman, 1940; Thiel, 1966). This hypothesis is confirmed by both SSU (Collins, 2002; Figs. 3, 6) and LSU (Figs. 2, 4, 5) data. Of the three semaeostome groups (Cyaneidae, Pelagiidae, and Ulmaridae), Pelagiidae (*Chrysaora* spp.) appears to be the earliest diverging. Ulmaridae (*Aurelia* and *Phacellophora*) most likely gave rise to Rhizostomeae (Figs. 3, 6). Within rhizostomes there are two main alliances of taxa, Cepheida and Rhizostomida (Stiasny, 1921; Thiel, 1970), which Thiel (1970) envisioned as independently derived from within Semaestomeae. However, members of Cepheida (*Cassiopea* and *Cotylorhiza*) and Rhizostomida (*Catostylus* and *Stomolophus*) form a well-supported monophyletic group (Figs. 3, 6). Thus, the monodisk strobilation and fused mouth arms of rhizostomes likely have a single origin (Fig. 7).

Relationships and Evolution within Hydrozoa

The new LSU and SSU data presented here greatly clarify the scope and likely phylogenetic position of Limnomedusae. Of the various groups that have been classified within Limnomedusae (Moerisiidae, Monobrachiidae, Olindiasidae, and Proboscidactylidae) only Monobrachiidae and Olindiasidae (= Olindiidae) are supported by molecular data as a paraphyletic

assemblage within the clade Trachylina (Figs. 3, 6). Our data reinforce hypotheses based on morphology that suggest that Moerisiidae and Proboscoidactylidae are members of Hydroidolina (Rees, 1958; Edwards, 1973; Petersen, 1990; Schuchert, 1996). Limnomedusae appears to be most easily characterized by two symplesiomorphies, i.e., ecto-endodermal statocysts and a life cycle that includes a polyp stage, shared with other members of Trachylina (Trachymedusae and Narcomedusae) and Hydroidolina, respectively.

The freshwater jellyfishes, here represented by the genera *Astrohydra* (LSU only), *Craspedacusta* (SSU only), and *Limnocrnida*, appear to have a single origin. Polyps of these genera are very similar (Bouillon, 1957; Hashimoto, 1981), but *Craspedacusta* and *Limnocrnida* have adult medusae that differ markedly. Little is known about the medusa stage of *Astrohydra*, which has only once been observed and then only in a juvenile state (Hashimoto, 1985). As with other members of Limnomedusae, *Craspedacusta* has medusae with four-parted mouths and gonads distributed along the radial canals. In contrast, the jellyfish of *Limnocrnida* has a large circular mouth and gonads distributed around the edge of its gut. Indeed, prior to knowledge of its polyp stage, *Limnocrnida* proved difficult to classify (Mayer, 1910). Nevertheless, both species possess the ecto-endodermal statocysts that are typical of other trachylines. The unusual medusa form of *Limnocrnida* appears to be relatively recently derived from a more typical limnomedusan form. The only brackish water species within Limnomedusae (*Maotias marginata*) is always well supported as the sister group to the freshwater species (Figs. 3, 6).

LSU and SSU data analyzed separately and together indicate that the single representative of Laingiomedusae sampled here is an unambiguous member of the clade Hydroidolina (Figs. 2 to 6). Laingiomedusae was erected by Bouillon (1978) as a new hydrozoan subclass (equivalent in his ranking system to Limnomedusae, Trachymedusae, etc.) and contains just four known species in three genera. The complete life cycle is unknown for any representative of Laingiomedusae, as only the medusa stages have been observed. They have tentacles that emerge on the exumbrellar side of a scalloped margin, characters reminiscent of Narcomedusae, and indeed some analyses of morphology have suggested that the two groups share a close alliance (Brusca and Brusca, 1990; Marques and Collins, 2004). In contrast, Schuchert (1996) argued that Laingiomedusae, in particular the genus sampled here, *Fabienna* (placed in Laingiomedusae by Bouillon and Barnett, 1999), may actually be closely related to anthoathecate hydrozoans of the family Proboscoidactylidae; both possess a solid radial canal and macrobasic euryteles. This hypothesis is strongly supported by SSU data (Figs. 3, 6). Furthermore, there is a strong similarity between Laingiomedusae and another filiferan, *Thecocodium quadratum*, which has a markedly lobed umbrella margin and tentacles that issue on the exumbrella (see Jarms, 1987). We predict that, upon discovery, the polyps of *Fabienna* will be filiferan hydroids. If future sampling shows that the name-giving species of

Laingiomedusae, *Laingia jaumotti*, forms a clade with *Fabienna* and the filiferan group Proboscoidactylidae, then the group should be demoted to family level and relegated to Anthoathecata.

Within Hydroidolina, relationships are uncertain (Figs. 2 to 6). We have no new data for Siphonophorae and there is little to add to the discussion of SSU data by Collins (2002). Similarly, the position of Leptothecata within Hydroidolina remains uncertain. We have added an SSU sequence for *Melicertum octocostatum*, a species that has a typical leptothecate medusa stage but has a hydroid stage lacking a theca. This species, though of uncertain position, appears to have diverged early in the history of Leptothecata (Figs. 3, 6), suggesting the possibility that the typical leptothecate medusa feature, gonads distributed along the radial canals, arose prior to the origin of thecae on polyps. Better sampling of Leptothecata and Hydroidolina is needed in order to pinpoint the origins of these characters.

Unlike Siphonophorae and Leptothecata, the phyletic status of Anthoathecata (= Capitata plus Filifera), a group for which we know of no synapomorphy (Schuchert, 1996), is unresolved by these data. Indeed, there is no molecular evidence for monophyly of either of the two anthoathecate subtaxa, Capitata or Filifera (Collins, 2000, 2002; Collins et al., 2005; and Figs. 2 to 6). It would not be surprising if both Leptothecata and Siphonophorae are found to be derived from within Anthoathecata. For Siphonophorae, this scenario has certainly been entertained (Haeckel, 1888; Garstang, 1946; Leloup, 1955; Totton, 1965). It is equally true that our data provide no strong evidence against monophyly of Anthoathecata, Capitata, or Filifera (with the exception of Laingiomedusae). These phylogenetic questions must be regarded as those that are most in doubt within Medusozoa, and their importance is underscored by the fact that Hydroidolina represents the majority of diversity within Medusozoa. It may be that an especially rapid evolutionary radiation separated the main groups within Hydroidolina. Regardless of the root cause for uncertainty about hydroidolinan phylogeny, the best strategy will be to continue sampling new taxa and markers and to compile a general phylogenetic hypothesis by identifying stable alliances among its component groups.

As an example, Collins et al. (2005) proposed, based on mitochondrial 16S data, that the important model organisms in the *Hydra* species group, Hydridae, form a clade with the anthoathecate families Candelabridae, Corymorphidae, and Tubulariidae. Species of Hydridae completely lack medusae, and different authors have come to conflicting conclusions concerning the group's phylogenetic position within Hydrozoa (e.g., Naumov, 1960; Petersen, 1990; Stepan'yants et al., 2000). Species of Hydridae have direct development that does not involve the ciliated planula typical of Cnidaria; this character is shared by the anthoathecate groups Acaulidae, Candelabridae, Corymorphidae, Margelopsidae, Paracorynidae, Tricyclusidae, and Tubulariidae. In reference to this putative synapomorphy, the name Aplanulata has been given to the putative clade uniting Hydridae

with Candelabridae, Corymorphidae, and Tubulariidae (Collins et al., 2005). The SSU data presented here (Figs. 3, 6), and to a lesser extent the LSU data (Fig. 2), affirm the validity of Aplanulata. Acaulidae, Margelopsidae, Paracorynidae, and Tricyclusidae have yet to be sampled for molecular data, but it seems likely that these groups are also part of Aplanulata.

Exploring Characters of Evolutionary Interest

Because Cnidaria is an early diverging lineage of Metazoa, it is likely that many characters of evolutionary interest have yet to be adequately examined. Such characters will need to be studied intensively in a small number of model organisms that are chosen with phylogenetic relationships in mind. Consider the case of gap junctions and the underlying proteins that form them. Prior work using antibodies to rat connexins suggested that connexins, the proteins underlying vertebrate gap junctions, are present in the hydrozoan *Hydra* (Fraser et al., 1987). However, sequencing of expressed sequence tags of *Hydra* have failed to reveal connexins, but instead have turned up innexins (Alexopoulos et al., 2004), proteins that form gap junctions in non-vertebrate bilaterians. This result prompted Alexopoulos et al. (2004) to conclude that innexin-based gap junctions arose in the common ancestor of cnidarians and bilaterians. However, Hydrozoa is nested relatively deeply in Cnidaria, gap junctions are not apparent in the other cnidarian groups (Mackie et al., 1984), and connexin-like proteins have been documented in an anthozoan (Germain and Anctil, 1996). Thus, further investigations of the genomes of distantly related cnidarians are necessary in order to evaluate the generality of the character states exhibited by *Hydra*.

Fortunately, recent efforts have established the anthozoan *Nematostella* as a new model organism (e.g., Darling et al., 2005). The synergistic impact of having two distantly related cnidarians developed as model organisms is likely to lead to many important insights into the evolution of form, development, and genomes. Nevertheless, when differences arise between results obtained from *Hydra* and those from *Nematostella*, there will be little justification, in the absence of relevant information obtained from outgroup taxa, for assuming one set of results is more likely to represent ancestral states in Cnidaria. Instead, other model organisms that are most likely to reveal ancestral character states for Anthozoa and Medusozoa will have to be developed in order to properly interpret the differences.

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APPENDIX 1. Sequences analyzed.

Class	Order/Family	Taxon ID	Accession numbers	
			SSU	LSU
Anthozoa	Actiniaria	<i>Actinia equina</i>	AJ133552	
Anthozoa	Actiniaria	<i>Anthopleura midori</i>	Z86098	
Anthozoa	Actiniaria	<i>Dactylanthus antarcticus</i>	AF052896	
Anthozoa	Actiniaria	<i>Edwardsia gilbertensis</i>	AF254377	
Anthozoa	Actiniaria	<i>Flosmaris nutsuensis</i>	Z92905	
Anthozoa	Actiniaria	<i>Paracondylactis hertwigi</i>	AJ133553	
Anthozoa	Antipatharia	<i>Antipathes galapagensis</i>	AF100943	AY026365
Anthozoa	Antipatharia	<i>Bathypathes</i> sp.	AF052901	
Anthozoa	Antipatharia	<i>Cirripathes lutkeni</i>	AF052902	
Anthozoa	Antipatharia	<i>Stichopathes spiessi</i>	AF052899	
Anthozoa	Ceriantharia	<i>Ceriantheopsis americana</i>	AF052898	
Anthozoa	Ceriantharia	<i>Cerianthus borealis</i>	AF052897	
Anthozoa	Ceriantharia	<i>Cerianthus filiformis</i>	AJ133557	
Anthozoa	Ceriantharia	<i>Pachycerianthus fimbriatus</i>	AF358111	
Anthozoa	Corallimorpharia	<i>Corynactis californica</i>	AF052895	
Anthozoa	Corallimorpharia	<i>Corynactis</i> sp.	AJ133559	
Anthozoa	Corallimorpharia	<i>Discosoma</i> sp.	AF052894	
Anthozoa	Octocorallia	<i>Acabaria habereri</i>	AJ133547	
Anthozoa	Octocorallia	<i>Acalycigorgia inermis</i>	AJ133545	
Anthozoa	Octocorallia	<i>Briareum asbestinum</i>	AF052912	
Anthozoa	Octocorallia	<i>Corallium kishinouyei</i>	AF052918	
Anthozoa	Octocorallia	<i>Junceella racemosa</i>	AF052937	
Anthozoa	Octocorallia	<i>Orstomisis crosnieri</i>	AF052916	
Anthozoa	Octocorallia	<i>Pseudocladochonus hicksoni</i>	AJ133544	
Anthozoa	Octocorallia	<i>Renilla reniformis</i>	AF052581	
Anthozoa	Octocorallia	<i>Taiaroa tauhou</i>	AF052908	
Anthozoa	Octocorallia	<i>Telestula</i> sp.	AF052914	
Anthozoa	Octocorallia	<i>Tubipora musica</i>	AF052909	
Anthozoa	Octocorallia	<i>Xenia</i> sp.	AF052931	
Anthozoa	Scleractinia	<i>Astrangia danne</i>	AY039209	
Anthozoa	Scleractinia	<i>Ceratotrochus magnaghii</i>	AF052886	

(Continued on the next page)

APPENDIX 1. (Continued)

Class	Order/Family	Taxon ID	Accession numbers	
			SSU	LSU
Anthozoa	Scleractinia	<i>Fungia scutaria</i>	AF052884	
Anthozoa	Scleractinia	<i>Javania insignis</i>	AJ133555	
Anthozoa	Scleractinia	<i>Montastraea franksi</i>	AY026382	AY026375
Anthozoa	Zoanthidea	<i>Palythoa variabilis</i>	AF052892	
Anthozoa	Zoanthidea	<i>Parazoanthus axinellae</i>	U42453	
Anthozoa	Zoanthidea	<i>Parazoanthus</i> sp.	AF052893	
Cubozoa	Carybdeidae	<i>Carukia barnesi</i>	AF358107	
Cubozoa	Carybdeidae	<i>Carybdea marsupialis</i>	AF358106	
Cubozoa	Carybdeidae	<i>Carybdea rastonii</i>	AF358108	AY920787
Cubozoa	Carybdeidae	<i>Carybdea sivickisi</i>	AF358110	
Cubozoa	Carybdeidae	<i>Carybdea</i> sp.—2-AGC-2005	AY920774	
Cubozoa	Carybdeidae	<i>Carybdea xaymacana</i> —Australia	AF358109	
Cubozoa	Carybdeidae	<i>Carybdea xaymacana</i> —Panama	AY920775	
Cubozoa	Carybdeidae	Darwin carybdeid—AGC-2001	AF358105	AY920788
Cubozoa	Carybdeidae	<i>Tripedalia cystophora</i>	L10829	
Cubozoa	Chirodropidae	<i>Chironex fleckeri</i>	AF358104	AY920785
Cubozoa	Chirodropidae	<i>Chiropsalmus</i> sp.—AGC-2001	AY920786	AY920786
Hydrozoa	Aplanulata	<i>Candelabrum cocksii</i>	AY920758	AY920796
Hydrozoa	Aplanulata	<i>Chlorohydra viridissima</i>	AF358081	
Hydrozoa	Aplanulata	<i>Corymorpha intermedia</i>	AY920759	
Hydrozoa	Aplanulata	<i>Ectopleura larynx</i>	AY920760	
Hydrozoa	Aplanulata	<i>Hydra circumcincta</i>	AF358080	AY026371
Hydrozoa	Aplanulata	<i>Hydra littoralis</i>	AF358082	
Hydrozoa	Filifera	<i>Bougainvillia</i> sp.—AGC-2001	AF358093	
Hydrozoa	Filifera	<i>Eudendrium racemosum</i>	AF358094	
Hydrozoa	Filifera	<i>Garveia</i> sp.—CC-2005	AY920766	
Hydrozoa	Filifera	<i>Hydractinia echinata</i>	AY920763	
Hydrozoa	Filifera	<i>Hydractinia serrata</i>	AY920764	
Hydrozoa	Filifera	<i>Pandea</i> sp.—AGC-2005	AY920765	
Hydrozoa	Filifera	<i>Podocoryna carnea</i>	AF358092	AY920802
Hydrozoa	Filifera	<i>Proboscoidactyla flavicirrata</i>	AY920768	
Hydrozoa	Laingiomedusae	<i>Fabienna sphaerica</i>	AY920767	AY920797
Hydrozoa	Leptothecata	<i>Aequorea victoria</i>	AF358077	AY920799
Hydrozoa	Leptothecata	<i>Blackfordia virginica</i>	AF358078	AY920800
Hydrozoa	Leptothecata	<i>Clytia</i> sp.—AGC-2001	AF358074	
Hydrozoa	Leptothecata	<i>Gymnangium hians</i>	Z86122	
Hydrozoa	Leptothecata	<i>Melicertissa</i> sp.—AGC-2001	AF358075	AY920798
Hydrozoa	Leptothecata	<i>Melicertum octocostatum</i>	AY920757	
Hydrozoa	Leptothecata	<i>Obelia</i> sp.	Z86108	
Hydrozoa	Leptothecata	<i>Selaginopsis cornigera</i>	Z92899	
Hydrozoa	Leptothecata	<i>Tiaropsidium kelseyi</i>	AF358079	
Hydrozoa	Limnomedusae	<i>Aglauroopsis aeora</i>	AY920754	AY920793
Hydrozoa	Limnomedusae	<i>Astrohydra japonica</i>		AY920794
Hydrozoa	Limnomedusae	<i>Craspedacusta sowerbyi</i>	AF358057	
Hydrozoa	Limnomedusae	<i>Limnocyclus tanganyicae</i>	AY920755	AY920795
Hydrozoa	Limnomedusae	<i>Maeotias marginata</i>	AF358056	
Hydrozoa	Limnomedusae	<i>Monobrachium parasiticum</i>	AY920752	
Hydrozoa	Limnomedusae	<i>Olindias phosphorica</i>	AY920753	
Hydrozoa	Narcomedusae	<i>Aegina citrea</i>	AF358058	AY920789
Hydrozoa	Narcomedusae	<i>Cunina frugifera</i>	AF358059	
Hydrozoa	Narcomedusae	<i>Solmissus marshalli</i>	AF358060	AY920790
Hydrozoa	Other Capitata	<i>Cladonema californicum</i>	AF358085	
Hydrozoa	Other Capitata	<i>Coryne muscoides</i>	AY920761	
Hydrozoa	Other Capitata	<i>Coryne pusilla</i>	Z86107	
Hydrozoa	Other Capitata	<i>Millepora</i> sp.—AGC-2001	AF358088	
Hydrozoa	Other Capitata	<i>Moerisia</i> sp.—AGC-2001	AF358083	AY920801
Hydrozoa	Other Capitata	<i>Pennaria disticha</i>	AY920762	
Hydrozoa	Other Capitata	<i>Polyorchis penicillatus</i>	AF358090	
Hydrozoa	Other Capitata	<i>Porpita</i> sp.—AGC-2001	AF358086	AY920803
Hydrozoa	Other Capitata	<i>Scrippisia pacifica</i>	AF358091	AY920804
Hydrozoa	Other Capitata	<i>Solanderia secunda</i>	AJ133506	
Hydrozoa	Other Capitata	<i>Staurocladia wellingtoni</i>	AF358084	
Hydrozoa	Siphonophorae	<i>Hippopodius hippopus</i>	AF358069	
Hydrozoa	Siphonophorae	<i>Nanomia bijuga</i>	AF358071	
Hydrozoa	Siphonophorae	<i>Nectopyramis</i> sp.—AGC-2001	AF358068	AY026377
Hydrozoa	Siphonophorae	<i>Physalia physalis</i>	AF358065	
Hydrozoa	Siphonophorae	<i>Physalia utriculus</i>	AF358066	

(Continued on the next page)

APPENDIX 1. (Continued)

Class	Order/Family	Taxon ID	Accession numbers	
			SSU	LSU
Hydrozoa	Siphonophorae	<i>Physophora hydrostatica</i>	AF358072	
Hydrozoa	Siphonophorae	<i>Sphaeronectes gracilis</i>	AF358070	
Hydrozoa	Trachymedusae	<i>Aglantha digitale</i>		AY920791
Hydrozoa	Trachymedusae	<i>Crossota rufobrunnea</i>	AF358063	
Hydrozoa	Trachymedusae	<i>Haliscera conica</i>	AF358064	
Hydrozoa	Trachymedusae	<i>Liriope tetraphylla</i>	AY920756	
Hydrozoa	Trachymedusae	<i>Pantachogon haeckeli</i>	AF358062	AY920792
Scyphozoa	Coronatae	<i>Atolla vanhoeffeni</i>	AF100942	AY026368
Scyphozoa	Coronatae	<i>Nausithoe rubra</i>	AF358095	AY920776
Scyphozoa	Rhizostomeae	<i>Cassiopea xamachana</i>	AY920771	
Scyphozoa	Rhizostomeae	<i>Catostylus</i> sp.—AGC-2001	AF358100	AY920777
Scyphozoa	Rhizostomeae	<i>Cotylorhiza tuberculata</i>	AY920773	
Scyphozoa	Rhizostomeae	Unidentified Rhizostome-AGC-2005	AY920772	
Scyphozoa	Rhizostomeae	<i>Stomolophus meleagris</i>	AF358101	
Scyphozoa	Semaeostomeae	<i>Aurelia aurita</i>	AY039208	
Scyphozoa	Semaeostomeae	<i>Aurelia</i> sp.—AGC-2005	AY920770	
Scyphozoa	Semaeostomeae	<i>Chrysaora melanaster</i>	AF358099	AY920780
Scyphozoa	Semaeostomeae	<i>Chrysaora</i> sp.—AGC-2005	AY920769	AY920779
Scyphozoa	Semaeostomeae	<i>Cyanea</i> sp.—AGC-2001	AF358097	
Scyphozoa	Semaeostomeae	<i>Phacellophora camtschatica</i>	AF358096	AY920778
Staurozoa	Stauromedusae	<i>Craterolophus convolvulus</i>	AY845344	AY920781
Staurozoa	Stauromedusae	<i>Depastromorpha africana</i>	AY845347	
Staurozoa	Stauromedusae	<i>Haliclystus octoradiatus</i>	AY845346	AH014894
Staurozoa	Stauromedusae	<i>Haliclystus sanjuanensis</i>	AF358102	AY920782
Staurozoa	Stauromedusae	<i>Lucernaria janetae</i>	AY845345	