TRENDS IN HYDROZOAN BIOLOGY - IV

Edited by

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FOREWORD

This volume represents the fourth in a series[1, 2, 3] published following workshops of the international Hydrozoan Society. Having previously met in Ischia, Italy (September 1985), Blanes, Spain (September 1991) and Roscoff, France (September 1994), this time the Society decided to venture into the New World, holding its Fourth Workshop at the Bodega Marine Laboratory in Bodega Bay, central California, from September 19 to October 3, 1998. Fifty participants, representing 16 countries and professional levels from advanced undergraduate students to professors emeritus, contributed to the two week workshop. This volume is composed of some of the presentations from that meeting. The Hydrozoan Society workshops provide a unique opportunity for those of us who study hydroids and hydromedusae, usually in comparative isolation, to really get to know each other at a personal level and to share ideas and promote future collaborations between people of similar interests, even if we come from different disciplines.

The Bodega Marine Laboratory, established in 1966, has a special place in the history of hydrozoan studies, as Cadet Hand, John Rees, Claudia Mills, and Nando Boero have all have worked there studying hydroids and medusae. When approached about hosting the Hydrozoan Society, both the Director James Clegg and Associate Director Paul Siri were enthusiastic, and thus the Bodega Marine Lab was selected as our venue. In addition to presenting original research papers and having daily topical round-table discussions, the Hydrozoan Society endeavors to do field-work during the course of the workshop. At the Bodega Marine Laboratory, we had a large teaching laboratory with running seawater tables and microscopes in addition to a conference room, projectors, library, dormitories and cafeteria. It was all very convenient and comfortable. We were surrounded by abundant wildlife, with large numbers of deer, songbirds and shorebirds, sea lions and even skunks. The lab residents were always smiling, willing to help and to do something for the "Hydrozoan people". This meant that our work was intense as usual, against a background of a happy environment. Being serious while smiling is the Bodega Bay formula. People work hard, but they are having fun; this is also the philosophy of the Hydrozoan Society. We gather not only to exchange our results and ideas, we get together to exchange our feelings. So Bodega Bay turned out to be a perfect place from every point of view. The success of the workshop resided in the number and diversity of attendees (this was the largest meeting in our short history) and in the quality of presentations and discussions. We saw unusual new live hydroid material, and are only sorry to report that a bloom of the freshwater jellyfish *Craspedacusta* occurred within a few miles of the meeting, but we did not learn about this unusual happening until after everyone had gone home; many of the attendees have never seen this species alive.

The Bodega Bay meeting occurred at a time of great change for international science, as the World Wide Web is coming into its own as a useful, authoritative venue. Within the last year, the essential and extensive hydrozoan bibliography compiled by Wim Vervoort[4] (who was bent over his computer working on this opus throughout our Third Workshop at Roscoff) has been made accessible over the Web (http://siba3.unile.it/ctle/mda/index.html) through the efforts of Cinzia Gravili and Ferdinando Boero and the expertise of the Library and Computer Services of the University of Lecce. The next step will be to scan these articles and put them up on the Web in their entirety, eventually leaving little excuse for nonfamiliarity with even the most obscure literature.

Some of the discussions at the Fourth Workshop of the Hydrozoan Society centered around the need to try to standardize data across a large number of species for future comparative work, requiring the collaborative efforts of a wide variety of scientists, including natural his-

torians, ecologists, developmental biologists, systematists, geneticists, molecular biologists and others. The concept of a giant matrix, available to all via the Web, including perhaps 100 species, was discussed – in which cells could be gradually filled in by any number of scientists, eventually yielding a much clearer picture of many kinds of patterns in the Hydrozoa. Such a matrix could guide future research towards filling in large gaps in our knowledge. In discussing our future needs as Hydrozoan scientists, the germ of a grand collaborative scheme was developed, which has now begun to blossom in the form of a Partnership for Enhancing Expertise in Taxonomy (PEET) grant from the American National Science Foundation. This effort to train new hydrozoan specialists stems directly from the Fourth Workshop and is continuing to link participants from all over the world, including senior taxonomists from the U.S. and Canada and students from Brazil and Italy, and has already resulted in a field workshop in Italy in the summer of 2000.

So we stand now looking forward to ever-more rapid advances in international science, as Web-accessible databases are beginning to be assembled on innumerable topics. No such database is yet in place for the Hydrozoa; we await the real work in building a useful tool. Scientists around the world are now connected electronically, so questions can be asked and answered overnight from even the most distant locations – the days of two to three week turnaround time for questions by mail are for the most part over. Still each scientist works in his or her own context, asking questions that arise from their own observations and interests. We present in this volume a wide variety of papers written by scientists living all over the world in highly different circumstances. The papers are all about Hydrozoa, but beyond that they represent a wide range of topics, and provide the reader with an overview of our knowledge and interests at the turn of the century and millenium.

THE EDITORS

References

1 Bouillon, J., F. Boero, F. Cicogna and P.F.S. Cornelius, eds. – 1987. *Modern Trends in the Systematics, Ecology and Evolution of Hydroids and Hydromedusae*. Clarendon Press, Oxford, 328 pages.

[2] Bouillon, J., F. Boero, F. Cicogna, J. M. Gili and R. G. Hughes, eds. – 1992. *Aspects of Hyrozoan Biology. Scientia Marina*, 56 (2-3): 296 pages.

[3] Piraino, S., F. Boero, J. Bouillon, P.F.S. Cornelius and J. M. Gili, eds. – 1996. *Advances in Hyrozoan Biology. Scientia Marina*, 60 (1): 243 pages.

[4] Vervoort, W. – 1995. Biography of Leptolida (non-Siphonophoran Hydrozoa, Cnidaria). Works published after 1910. *Zoologische Verhandelingen, Leiden*, 301, 29.xii.1995: 1-432.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

Towards understanding the phylogenetic history of Hydrozoa: Hypothesis testing with 18S gene sequence data*

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SUMMARY: Although systematic treatments of Hydrozoa have been notoriously difficult, a great deal of useful information on morphologies and life histories has steadily accumulated. From the assimilation of this information, numerous hypotheses of the phylogenetic relationships of the major groups of Hydrozoa have been offered. Here I evaluate these hypotheses using the complete sequence of the 18S gene for 35 hydrozoan species. New 18S sequences for 31 hydrozoans, 6 scyphozoans, one cubozoan, and one anthozoan are reported. Parsimony analyses of two datasets that include the new 18S sequences are used to assess the relative strengths and weaknesses of a list of phylogenetic hypotheses that deal with Hydrozoa. Alternative measures of tree optimality, minimum evolution and maximum likelihood, are used to evaluate the reliability of the parsimony analyses. Hydrozoa appears to be composed of two clades, herein called Trachylina and Hydroidolina. Trachylina consists of Limnomedusae, Narcomedusae, and Trachymedusae. Narcomedusae is not likely to be the basal group of Trachylina, but is instead derived directly from within Trachymedusae. This implies the secondary gain of a polyp stage. Hydroidolina consists of Capitata, Filifera, Hydridae, Leptomedusae, and Siphonophora. "Anthomedusae" may not form a monophyletic grouping. However, the relationships among the hydroidolinan groups are difficult to resolve with the present set of data. Finally, the monophyly of Hydrozoa is strongly supported.

Key words: Hydrozoa, Trachylina, Hydroidolina, Siphonophora, phylogeny, 18S, hypothesis testing.

INTRODUCTION

Hydrozoan classification and nomenclature have been infamous, posing difficulties for ecologists, taxonomists, biogeographers, as well as phylogeneticists who work with hydrozoans. This situation would appear to be an unfortunate backdrop as we move towards an understanding of the phylogenetic history of Hydrozoa because classification schemes, even those that were not explicitly aimed at grouping organisms based on common ancestry, often provide a first approximation of phylogeny. While by no means universal, many groups of organisms that were defined prior to the current trend toward phylogenetic classifications have held up as monophyletic clades. A pertinent example is presented by the present study, which strongly supports an assertion of monophyly for Hydrozoa, a finding in accordance with the conclusions of other students of cnidarian phylogeny (Schuchert, 1993; Bridge *et al.*, 1995). Unless or until contradictory information is brought into view, it will be accepted that this hypothesis accurately represents true evolutionary history.

The difficult nature of hydrozoan classification is a consequence of separate treatment having been

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given the polyp and medusa stages of hydrozoan life cycles. In the absence of adequate life history information connecting medusae to polyps, separate taxonomies arose. Luckily, substantial attempts have been made to integrate older taxonomic schemes in light of our growing knowledge of complete life cycles. Naumov (1960) was the first to take on this onerous task. However, far from being daunted by the undertaking, Naumov remarked that his classification of hydrozoans would need only modest alteration, as it was based on phylogenetic relationships. Since then, taxonomically broad-based contributions have been made by Bouillon (1985), who proposed a revised classification for non-siphonophoran hydrozoans, and Petersen (1990), who offered a phylogenetic classification for the capitate hydroids.

Herein, I evaluate hypotheses of phylogenetic relationships of the major groups of hydrozoans that have been offered in the past. Specifically, I ask whether complete sequences of the 18S gene, which codes for the small subunit of the ribosome, are consistent with each of the hypotheses. The value of molecular sequence data lies in their capacity to provide relatively large sets of heritable and variable characters that can be used to evaluate prior phylogenetic hypotheses and generate new ones. Of course, anatomic features and other characters are also variable and inherited, making them equally useful for phylogenetic inference. Today, a great value is placed on molecular characters in phylogenetic studies. Part of this emphasis is pragmatic. Technological advances make it possible to gather numerous molecular characters relatively inexpensively. Another reason that molecules are emphasized is possibly that they are fashionable. Fortunately, the current wave of molecular phylogenies is spurring on phylogenetic analyses based on non-molecular characters. All types of data that have the potential to reveal phylogenetic history should be investigated.

To simplify the discussion, I have compiled a list of phylogenetic hypotheses, derived mostly from a few major works, as outlined below. The principal focus of this analysis will be to evaluate the monophyly of and the relationships among the following taxa: Anthomedusae, Capitata, Filifera, Hydridae, Leptomedusae, Limnomedusae, Narcomedusae, Siphonophora, and Trachymedusae. Many of these names have roughly equivalent appellations (Anthomedusae equals Athecata, Gymnoblastea, and Anthoathecata etc.). Choosing to use the above names (which are mostly descended from the medusae-based classifications) is not based on priority, as there is no rule of precedence for taxonomic groups above the family level, nor for any considerations of what phase of the typical hydrozoan life cycle represents the adult stage. Instead, I argue that the choice is largely arbitrary and should be recognized as such. Reference will also be made to Actinulidae and Laingiomedusae, but hypotheses involving these groups cannot be explicitly tested with the present molecular dataset since these taxa have not been sampled for the 18S gene.

To an extent, this highlights the tentative nature of phylogenetic analyses. All phylogenetic trees, with the somewhat obscure exception of experimental phylogenies (Hillis *et al.*, 1992) are hypotheses of evolutionary relationships. Therefore, phylogenies are not final results. The analysis in this paper confirms that molecules and morphology often point to the same evolutionary relationships, but that there is not complete agreement. Therefore, the 18S data suggest some new phylogenetic hypotheses for hydrozoans. In turn, these hypotheses must be tested with other sets of data and additional analyses. The challenge of testing new possibilities forces us to look at old data in novel ways. 18S data will surely not reveal the complete truth about the evolutionary relationships among hydrozoans. However, through the process of testing, proposing, re-testing, and so forth, a coherent picture of hydrozoan phylogeny will emerge.

MATERIALS, METHODS, AND RESULTS

Compiling a list of phylogenetic hypotheses

Figure 1 shows three views of hydrozoan phylogeny that have been offered. The phylogeny of Hydrozoa that Hyman presented, stressing that it was "highly speculative", is redrawn as Figure 1A (Hyman, 1940). From this conception we can begin to enumerate hypotheses, shown in Table 1. 1) Hydrozoa is not a monophyletic group, having given rise to the other cnidarians. 2) Anthomedusae and Leptomedusae form a clade. 3) Limnomedusae, Narcomedusae, and Trachymedusae form a clade. Hyman did not explicitly mention Limnomedusae, but her discussion of Trachymedusae includes direct references to limnomedusan species. 4) Siphonophora is the earliest diverging branch of hydrozoans; Anthomedusae, Leptomedusae, Lim-

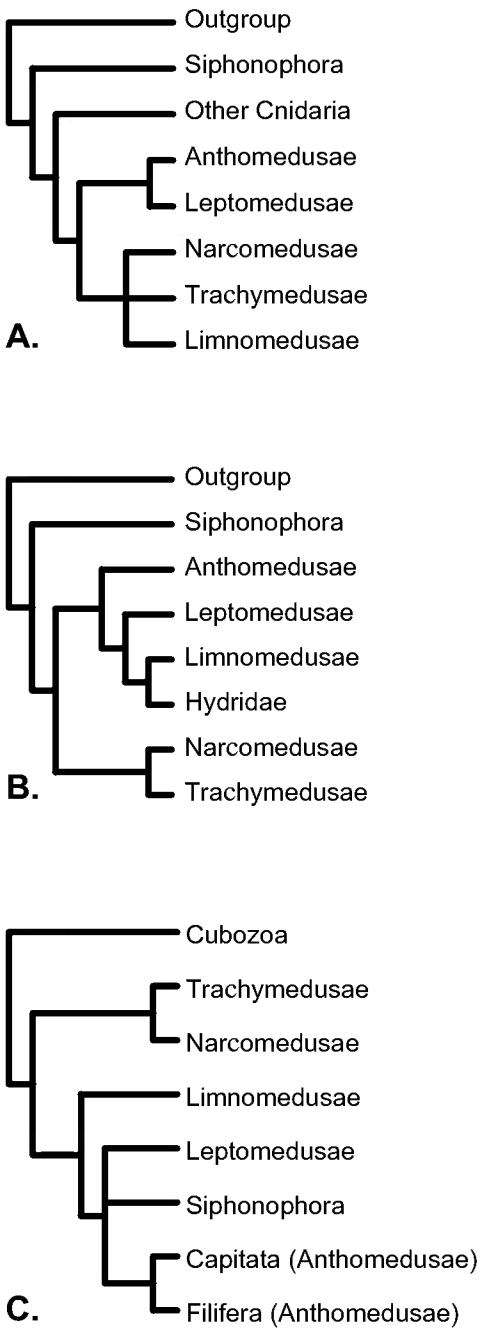

FIG. 1. – Three alternative views of the evolutionary relationships of Hydrozoa. A follows Hyman (1940), B follows Naumov (1960), and C follows Petersen (1979; 1990).

TABLE 1. – List of phylogenetic hypotheses for hydrozoan groups.

Hypothesis Number	Description of Hypothesis
(1)	Hydrozoa is not a monophyletic group, having given rise to the other cnidarians.
(2)	Anthomedusae and Leptomedusae form a clade.
(3)	Limnomedusae, Narcomedusae, and Trachymedusae form a clade.
(4)	Siphonophora is the earliest diverging branch of hydrozoans
(5)	Siphonophora is monophyletic.
(6)	Anthomedusae (containing Hydridae) is monophyletic.
(7)	Leptomedusae is monophyletic.
(8)	Narcomedusae is monophyletic.
(9)	Trachymedusae is monophyletic.
(10)	Limnomedusae is monophyletic.
(11)	Hydrozoa is monophyletic, the converse of hypothesis 1.
(12)	Hydridae is monophyletic.
(13)	Anthomedusae excluding Hydridae is monophyletic.
(14)	Hydridae and Limnomedusae form a clade.
(15)	Hydridae, Leptomedusae, and Limnomedusae form a clade.
(16)	Anthomedusae, Hydridae, Leptomedusae, and Limnomedusae form a clade.
(17)	Narcomedusae and Trachymedusae form a clade.
(18)	Capitata (containing Hydridae) is monophyletic.
(19)	Filifera is monophyletic.
(20)	Anthomedusae (with Hydridae), Leptomedusae, and Siphonophora form a clade.
(21)	Anthomedusae, Leptomedusae, Limnomedusae and Siphonophora form a clade.
(22)	Cubozoa is the sister group to Hydrozoa.
(23)	Capitata is a monophyletic group that does not contain Hydridae.
(24)	Anthomedusae is not monophyletic, the converse of hypothesis 6.
(25)	Hydridae and Leptomedusae form a clade.
(26)	Hydridae, Leptomedusae, and Siphonophora form a clade.
(27)	Trachymedusae are not monophyletic, having given rise to Narcomedusae.
(28)	Hydrozoa, Scyphozoa, and Cubozoa form a clade.

nomedusae, Narcomedusae, and Trachymedusae form a clade. In addition, Hyman's tree carries the implication that each of the major subgroups is monophyletic, augmenting the list of hypotheses. 5) Siphonophora is monophyletic. 6) Anthomedusae (containing Hydridae) is monophyletic. 7) Leptomedusae is monophyletic. 8) Narcomedusae is monophyletic. 9) Trachymedusae is monophyletic. 10) Limnomedusae is monophyletic (not argued by Hyman, but implied by Figure 1a). These hypotheses are mutually consistent, embodying a single view of hydrozoan evolutionary history. Entertaining alternative views of hydrozoan phylogeny expands the list greatly (Table 1).

The phylogeny of Hydrozoa according to Naumov is presented as Figure 1B (Naumov, 1960). Note that the position of Siphonophora is inferred. Naumov did not explicitly deal with siphonophores in his treatise on hydroids and hydromedusae of what is now the former Soviet Union. He considered them a separate subclass of Hydrozoa and thus they have been placed as the earliest branch of Hydrozoa. Some of the postulates of Naumov overlap with those already listed (4, 5, 7, 8, 9, and 10), but several are new. 11) Hydrozoa is monophyletic, the converse of hypothesis 1. 12) Hydridae is monophyletic. 13) Anthomedusae excluding Hydridae is monophyletic. 14) Hydridae and Limnomedusae form a clade. 15) Hydridae, Leptomedusae, and Limnome-

dusae form a clade. 16) Anthomedusae, Hydridae, Leptomedusae, and Limnomedusae form a clade. 17) Narcomedusae and Trachymedusae form a clade.

Petersen's account of the phylogeny of Hydrozoa is given in Figure 1C (Petersen, 1979). In addition to some conjectures already listed (5, 6, 7, 8, 9, 10, 11, and 17), several new hypotheses can be gleaned from Figure 1C. 18) Capitata (containing Hydridae) is monophyletic. 19) Filifera is monophyletic. 20) Anthomedusae (with Hydridae), Leptomedusae, and Siphonophora form a clade. 21) Anthomedusae, Leptomedusae, Limnomedusae and Siphonophora form a clade. 22) Cubozoa is the sister group to Hydrozoa, an assertion reiterated by Bouillon (Bouillon, 1985, 1987). Finally, hypotheses suggested by the 18S data, as detailed below, will complete this compilation of phylogenetic hypotheses of the major groups of Hydrozoa.

Accumulating molecular sequence data

All primers, sequences and molecular datasets used in this analysis are available upon request from the author. Genomic DNA was isolated from tissue samples of 23 hydrozoan species, seven scyphozoan species, and two anthozoan species. In addition, DNA samples from eight hydrozoan species and one cubozoan species were kindly provided by other researchers, as acknowledged below. Tissue samples were either fresh, preserved in 75 to 95 percent ethanol, or frozen (-80°). The extraction of high molecular weight genomic DNA was achieved by pulverization of tissue in the reagent DNAzol, followed by centrifugation and ethanol precipitation (Chomczynski *et al.*, 1997). The complete sequence for the 18S coding region was amplified from genomic DNA preparations using eukaryotic-specific primers (Medlin *et al.*, 1988) via PCR (30 cycles: 10s at 94°, 60s at 38° to 48°, and 180s at 72°, after an initial two minute 94°denaturation). The PCR products were directly sequenced with an ABI Prism 377 DNA Sequencer, with the exception of the 18S gene of *Aequorea aequorea*, which was sequenced with a Li-Cor model 4000L infrared automated DNA sequencer. The complete 18S sequences will be deposited in GenBank, as part of a publication that deals with the phylogeny of a broader taxonomic grouping, the medusa-bearing cnidarians, Medusozoa (Collins, in prep).

Sequences were entered into a data matrix that includes more than 150 other 18S gene sequences (derived from a wide array of metazoans and their allies). Sequences were aligned by eye using primary sequence similarity. Regions which were difficult to align were excluded from the analyses by using an alignment mask because putative homology of the sequence characters could not be asserted. Two subsets of the data matrix were used in the present analysis. The first dataset has 66 taxa, 56 cnidarians and a sample of 10 non-cnidarian metazoans to serve as outgroups (four poriferans, two ctenophores, two placozoans, and two bilaterians). Bilaterians are often excluded from phylogenetic analyses of lower metazoan groups (e.g. Bridge *et al.*, 1995). This may be unwise in light of evidence that bilaterians and cnidarians are relatively closely related (Collins, 1998; Kim *et al.*, 1999). Because of the inclusion of a wider diversity of outgroups, this 66-taxon dataset is more appropriate to address hypotheses that deal with Hydrozoa as a whole, e.g., whether Hydrozoa is or is not monophyletic and what group is the sister clade of Hydrozoa. The second dataset is limited to just the 56 cnidarian taxa (11 anthozoans, 8 scyphozoans, 2 cubozoans, and 35 hydrozoans). The 56-taxon dataset is used to address hypotheses concerning the various subgroups of Hydrozoa. In analyses carried out with this dataset, anthozoans are used as the outgroup, a hypothesis supported by prior phylogenetic investigations of morphological and molecular data (Bridge *et al.*, 1995; Schuchert, 1993).

Finding optimal trees and completing the list of hypotheses

The first step to explicitly testing prior phylogenetic hypotheses is to find an "optimal" or "best" tree implied by the 18S data. The optimal tree depends on how optimality is measured. There are a number of commonly-used measures of tree optimality (Swofford *et al.*, 1996). In this analysis, the primary optimality criterion is parsimony. The "best" tree obtained by a parsimony search is the one that minimizes the number of character changes or steps throughout a tree. PAUP* 4.0 (Swofford, 1998) was used for all phylogenetic analyses. A parsimony search (heuristic search option with 100 random replicates) with equally-weighted characters was performed. Ideally, the relative weight given a type of character change would reflect the relative likelihood of that type of change. That is, less likely character changes shared by two or more taxa should carry more weight than changes that occur more readily. Without any evidence that all

TABLE 2. – Maximum likelihood estimations of the ratio of transitions to transversions and the gamma shape parameter for most parsimonious trees with equally weighted characters and trees obtained by the neighbor-joining algorithm.

Description	T-Ratio	Gamma
66-Taxon Trees		
Most Pasimonious #2 of 10	1.61	0.273
Most Pasimonious #6 of 10	1.61	0.273
Neighbor-Joining	1.58	0.271
56-Taxon Trees		
Most Pasimonious #3 of 8	1.59	0.211
Most Pasimonious #7 of 8	1.60	0.213
Neighbor-Joining	1.58	0.212

changes in the 18S gene are equally likely, there is no reason to assume that all character changes are equally likely. In fact, there is a bias toward transitions in ribosomal genes, although the unequal rates of transitions and transversions is typically less than what is observed for other genes (Vawter and Brown, 1993). Fortunately, these rates can be estimated for a given set of taxa and molecular characters and appropriate weights can be implemented for subsequent analyses.

PAUP* 4.0 was used to make a maximum likelihood estimate of the relative difference in rates (T-Ratio) of transitions and transversions given the most parsimonious trees found in the search where character changes were weighted equally. The T-Ratio can then be used to weight transitions and transversions during subsequent parsimony analyses. The logic of such a method could be construed as circular. Is there a problem with taking parsimony trees, estimating the relative rates of transitions and transversions, and then building new parsimony trees with transitions and transversions weighted differently? In order to test this thought, an additional tree was obtained by the neighbor-joining algorithm and the T-Ratio was estimated with this tree. The results show that estimates using the unweighted parsimony trees are nearly identical to those made using the neighbor-joining tree. Table 2 reports the maximum likelihood estimates of the transition to transversion ratios for the 18S data given the 66-taxon and 56-taxon trees built by neighbor-joining and unweighted parsimony analyses. There is very little difference between the estimates; transitions are roughly 1.6 times as common as transversions. Thus, trees that serve as the "optimal" trees of this analysis are found by implementing a parsimony search where transitions were weighted 2/3 times (approximately 1/1.6) as heavily as transversions, according to their likelihood of occurrence.

A consensus of five most parsimonious trees (Fig. 2) was found using the 66-taxon dataset and weighted transitions and transversions (heuristic search option with 1000 random replicates). A single most parsimonious tree (Fig. 3) was detected using the 56-taxon dataset with weighted transitions and transversions (1000 random replicate searches). The relationships among the hydrozoans are similar in the two trees, but not exact. In fact, hydrozoan relationships revealed by the 18S data are not strongly influenced when different combinations of outgroups are used (results not shown). Several of the hypotheses enumerated in Table 1 are consistent with the most parsimonious trees (3, 5, 7, 8, 11, 12, 13, 17, 19, and 20). In addition, some novel hypotheses are suggested by these trees. 23) Capitata is a monophyletic group that does not contain Hydridae, in contrast with hypothesis 18. 24) Anthomedusae is not monophyletic, the converse of hypothesis 6. 25) Hydridae and Leptomedusae form a clade. 26) Hydridae, Leptomedusae, and Siphonophora form a clade. These last two hypotheses, drawn from the 56-taxon tree, are conflicted by the relationships shown in the 66-taxon tree. 27) Trachymedusae are not monophyletic, having given rise to Narcomedusae, in contrast to hypothesis 9. 28) Hydrozoa, Scyphozoa, and Cubozoa form a clade, sometimes referred to as Medusozoa.

Testing phylogenetic hypotheses

Each of the aforementioned hypotheses can be explicitly tested with the 18S data. However, it is difficult to devise a test of phylogenetic hypotheses that has a clear black-or-white result, e.g., pass versus fail. For instance, it is not sufficient to simply build trees with molecular data and to conclude that they are correct when different tree-building methodologies yield divergent results. Thus, concordance between a hypothesis and a given molecular analysis lends support to the hypothesis, but it is not conclusive. Similarly, discordance between a hypothesis and a molecular analysis casts some doubt on the hypothesis, but it does not completely falsify it. Knowing the extent to which a molecular analysis agrees or disagrees with a prior hypothesis would be useful. To this end, I follow a procedure that relies on imposing various topological constraints on tree-building analyses to determine the relative strengths of the hypotheses that are support-

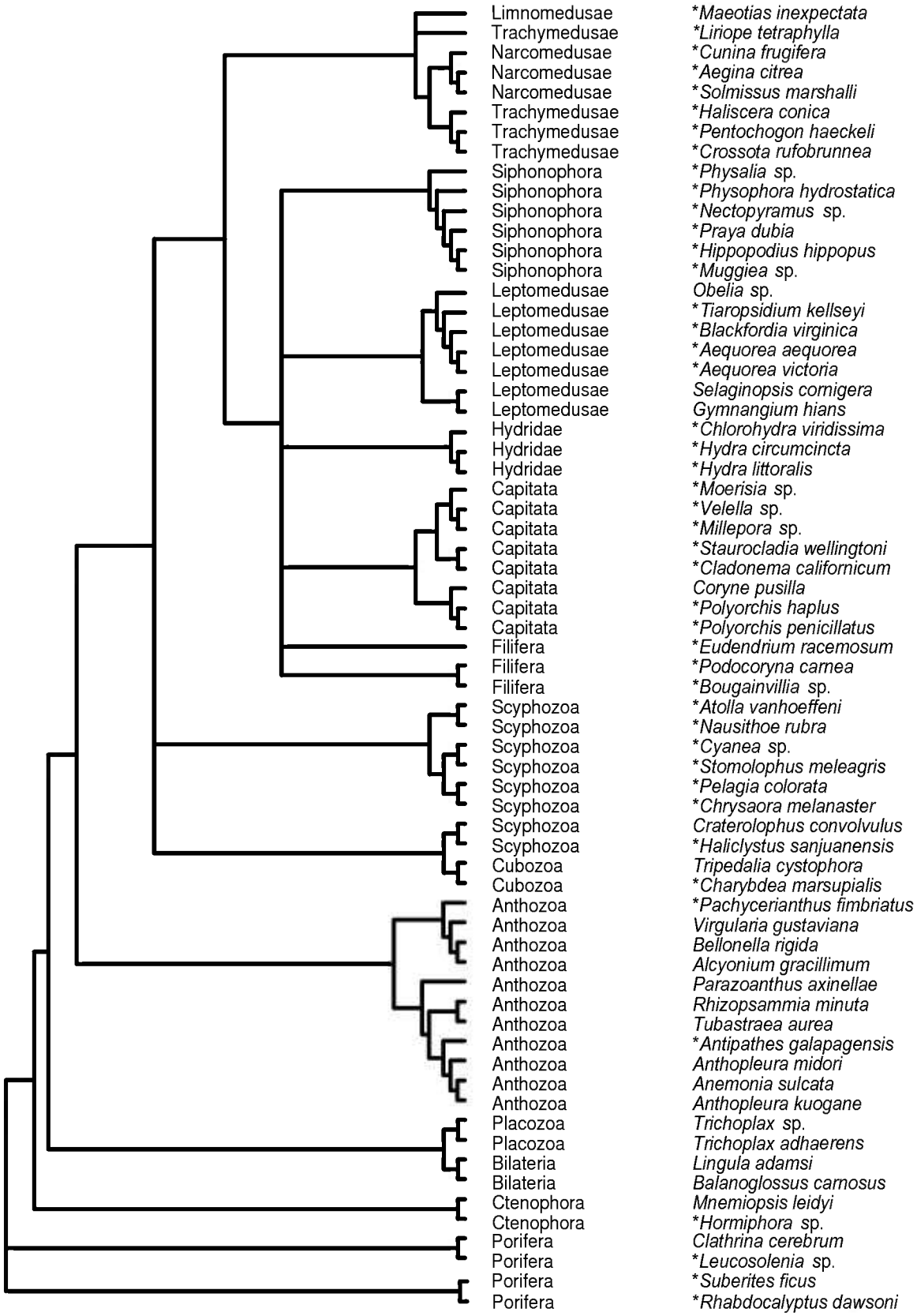

FIG. 2. – Consensus of five most parsimonious trees found by heuristic search (with 1000 random replicates) using the 66-taxon dataset, transitions are weighted 2/3 as heavily as transversions. The dataset consists of 1,807 characters, 635 of which are parsimony-informative. The five trees are 8,214 steps long, with consistency indices of .400, rescaled to .260, and retention indices of .650.

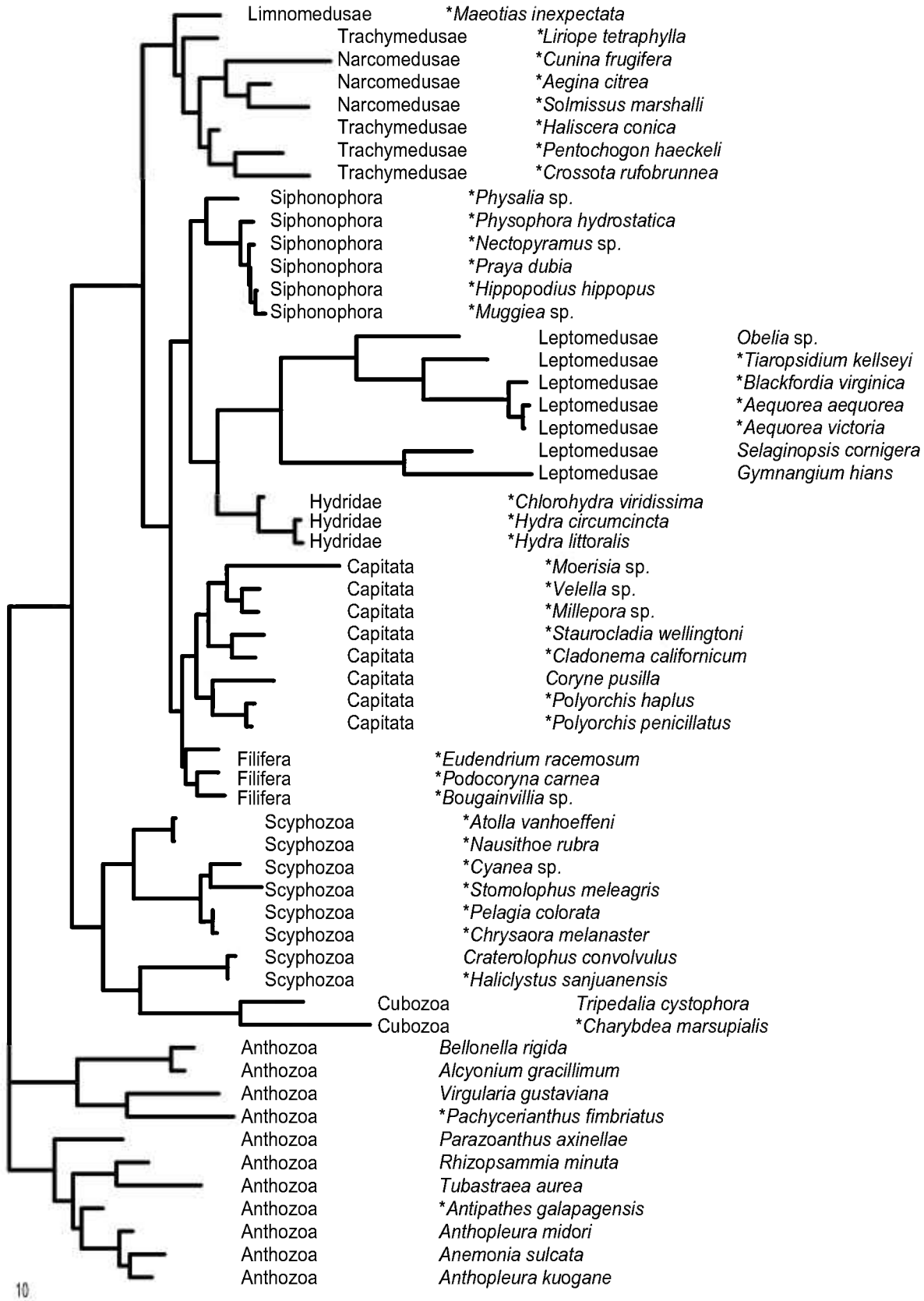

FIG. 3. – Most parsimonious tree found by heuristic search using the 56-taxon dataset, transitions are weighted 2/3 as heavily as transversions. The dataset consists of 1,807 characters, 531 of which are parsimony-informative. The tree is 5,472 steps long, with a consistency index of 0.446, rescaled to 0.306, and a retention index of 0.687.

TABLE 3. – List of hypotheses that are not consistent with the optimal parsimony trees. Column 1 reports the number of additional weighted-parsimony character changes it would take to accommodate the hypothesis. For instance, the most parsimonious tree that has Hydrozoa not monophyletic is 45 steps longer than the overall most parsimonious tree. The hypotheses are sorted by Column 2, which is Column 1 as a percent of the length of the most parsimonious tree (8,214 for 66 taxa and 5,472 for 56 taxa). Columns 3 and 4 show p-values for the Kishino-Hasegawa and Templeton tests. See text for interpretations of these values.

Hypothesis Number	Hypotheses not consistent with optimal trees	Number of steps to accommodate hypothesis (1)	As a percent of total number of steps (2)	Kishino Hasegawa Test P-value (3)	Templeton Test P-value (4)
(16)	Anthomedusae, Hydridae, Leptomedusae, and Limnomedusae form a clade	53	0.969%	0.001	0.001
(15)	Hydridae, Leptomedusae and Limnomedusae form a clade	46	0.841%	0.001	0.001
(14)	Hydridae and Limnomedusae form a clade	42	0.768%	0.001	0.001
(1)	*Hydrozoa is not monophyletic	45	0.548%	0.029	0.035
(21)	Anthomedusae, Leptomedusae, Limnomedusae and Siphonophora form a clade	24	0.439%	0.002	0.003
(4)	Siphonophora is the earliest diverging clade of Hydrozoa	23	0.420%	0.094	0.110
(18)	Capitata (including Hydridae) is monophyletic	12	0.219%	0.152	0.124
(9)	Trachymedusae is monophyletic	9	0.164%	0.170	0.119
(22)	*Cubozoa is the sister group of Hydrozoa	10	0.122%	0.551	0.593
(6)	Anthomedusae (containing Hydridae) is monophyletic	6	0.110%	0.396	0.314
(2)	Anthomedusae and Leptomedusae form a clade denotes . hypotheses addressed with the larger dataset	4	0.073%	0.157	0.157

ed by the 18S data, and the relative weaknesses of those that are contradicted by the 18S data.

With an optimal tree determined, it is now possible to divide the list of hypotheses listed in Table 1 into two groups, those that are consistent with the optimal parsimony trees and those that are not, shown in Tables 3 and 4 respectively. For each hypothesis that is inconsistent with the most parsimonious trees, an additional search (with 100 replicates and transitions and transversions weighted as before) was performed with the constraint that only trees that are consistent with the given hypothesis were considered. The length of the optimal tree that is consistent with the given hypothesis was then compared to the length of the optimal tree in the absence of constraints. Subtracting the two lengths yields a measure of the extent to which the hypothesis is controverted by the 18S data. A summary of hypotheses that are not consistent with the 18S data is presented as Table 3. For each hypothesis that is controverted by the 18S data, the number of extra weighted-character changes that it would take to accommodate the hypothesis is given in column 1. The hypotheses are sorted by column 2, which reports the number of steps (column 1) as a percent of the total number of steps in the most parsimonious trees.

In addition, PAUP* was used to implement two tests that aim to determine whether the optimal trees are significantly shorter in a statistical sense than the best trees that conform to each hypothesis. The first test (Kishino and Hasegawa, 1989) is a parametric test that compares the difference in length of the two trees to a distribution of differences whose mean is zero. The null hypothesis for this test is that there is no difference in the lengths of the phylogenetic arrangements derived from the molecular data, and so p-values can be interpreted as the probability of getting the observed difference in tree lengths if there is no true difference in tree lengths. The smaller the p-value, the lower the probability that the observed difference is due to chance alone, and consequently the higher the probability that the difference is due to phylogenetic signal. The second test (Templeton, 1983) is a non-parametric test that addresses the number of changes in each character implied by the two competing trees. In this test, randomness is expected to favor each of the competing trees equally. P-values from this test can be interpreted as the probability that the observed difference in character changes implied by the two trees is due to random error. Again, lower p-values should be associated with the most strongly controverted hypotheses. However, the validity of both the Kishino-Hasegawa and Templeton tests is somewhat suspect. First, an underlying assumption for these tests is that the data are randomly selected and independent. Phylogenetic history necessitates violation of independence of the data, while experimental design ensures that the choice of data, taxa and characters, is not random. Second, these two tests are two-tailed and should technically not be applied in a situation where one has an *a priori* expectation that one tree is shorter than the other, a situation which is true in

the present analysis. Nevertheless, results from these tests (p-values) are presented as columns 3 and 4 respectively on Table 3 in order to provide a sense of which hypotheses are most strongly contradicted by the 18S data. For instance, by any measure, the 18S data indicate that it is highly unlikely that Anthomedusae, Hydridae, Leptomedusae, and Limnomedusae form a clade.

Similarly, it is helpful to know the level of support for the hypotheses that are consistent with the optimal tree or trees. In order to achieve this, a search was performed (for each of the supported hypotheses) that was constrained to consider only those trees that are in violation of the given hypothesis. The difference in length between the unconstrained and constrained trees is equivalent to the number of extra character changes that are necessary to compromise the given hypothesis. Higher differences imply greater support for the hypotheses from the 18S data. The process of evaluating hypotheses consistent with the unconstrained trees is roughly equivalent to a Bremer analysis of clade support (Bremer, 1988; Bremer, 1994). Hypotheses that are concordant with the 18S data are presented in Table 4. For each hypothesis that is supported by the 18S data, the number of weighted-character changes necessary to compromise the hypothesis is shown in column 1. Column 2 shows the number of steps (column 1) as a percent of the total number of steps in the most parsimonious trees. Columns 3 and 4 contain p-values from Kishino-Hasegawa and Templeton tests that compare shortest constrained trees to the overall most parsimonious trees. These hypotheses are ordered from most to least support by sorting on column 2. The most strongly supported hypotheses are that Hydrozoa and Hydridae are each monophyletic. The hypothesis that Limnomedusae is monophyletic (10) cannot be tested in the current analysis because just a single representative limnomedusan taxon is included; monophyly of the group in these analyses is guaranteed.

Of course, this method begs the question of how to interpret the number of extra character changes needed to either compromise or accommodate a given hypothesis. The results of the Kishino-Hasegawa and Templeton tests are also difficult to understand given their limitations. It is largely arbitrary where the line is drawn. However, a comparison of results obtained using different tree-building methods may help. Phylogenetic relationships that are consistently inferred, regardless of the methodology used, should be considered the most robust results. Two methodologies that employ alternative measures of optimality were used to build trees in an attempt to determine the approximate number of steps (as a percent of the total number of steps) in the parsimony analyses that is indicative of support, or the lack thereof, irrespective of tree-building methodology.

TABLE 4. – List of hypotheses that are consistent with the optimal parsimony trees. Column 1 shows the number of additional weighted-parsimony character changes it would take to compromise the hypothesis. For instance, a tree that is just a single step longer than the most parsimonious tree contains an arrangement where Filifera is not monophyletic. The hypotheses are sorted by Column 2, which is Column 1 as a percent of the length of the most parsimonious tree (8,214 for 66 taxa and 5,472 for 56 taxa). Columns 3 and 4 show p-values for the Kishino-Hasegawa and Templeton tests. See text for interpretations of these values.

Hypothesis Number	Hypotheses consistent with optimal trees	Number of steps to compromise hypothesis (1)	As a percent of total number of steps (2)	Kishino Hasegawa Test P-value (3)	Templeton Test P-value (4)
(12)	Hydridae is monophyletic	45	0.822%	0.001	0.002
(11)	*Hydrozoa is monophyletic	45	0.548%	0.029	0.035
(3)	Limnomedusae, Narcomedusae, and Trachymedusae form a clade	22	0.402%	0.050	0.038
(7)	Leptomedusae is monophyletic	16	0.292%	0.312	0.295
(8)	Narcomedusae is monophyletic	14	0.256%	0.052	0.053
(20)	Anthomedusae (with Hydridae), Leptomedusae, and Siphonophora form a clade	13	0.238%	0.369	0.423
(5)	Siphonophora is monophyletic	10	0.183%	0.316	0.313
(27)	Trachymedusae is not monophyletic	9	0.164%	0.170	0.119
(24)	Anthomedusae is not monophyletic	6	0.110%	0.396	0.314
(17)	Narcomedusae and Trachymedusae form a clade	4	0.073%	0.520	0.491
(26)	Hydridae, Leptomedusae, and Siphonophora form a clade	4	0.073%	0.347	0.295
(25)	Hydridae and Leptomedusae form a clade form a clade	4	0.073%	0.556	0.449
(13)	Anthomedusae (excluding Hydridae) is monophyletic	2	0.037%	0.665	0.606
(23)	Capitata (excluding Hydridae) is monophyletic	2	0.037%	0.845	0.867
(19)	Filifera is monophyletic denotes hypotheses addressed with the larger dataset	1	0.018%	0.827	0.706

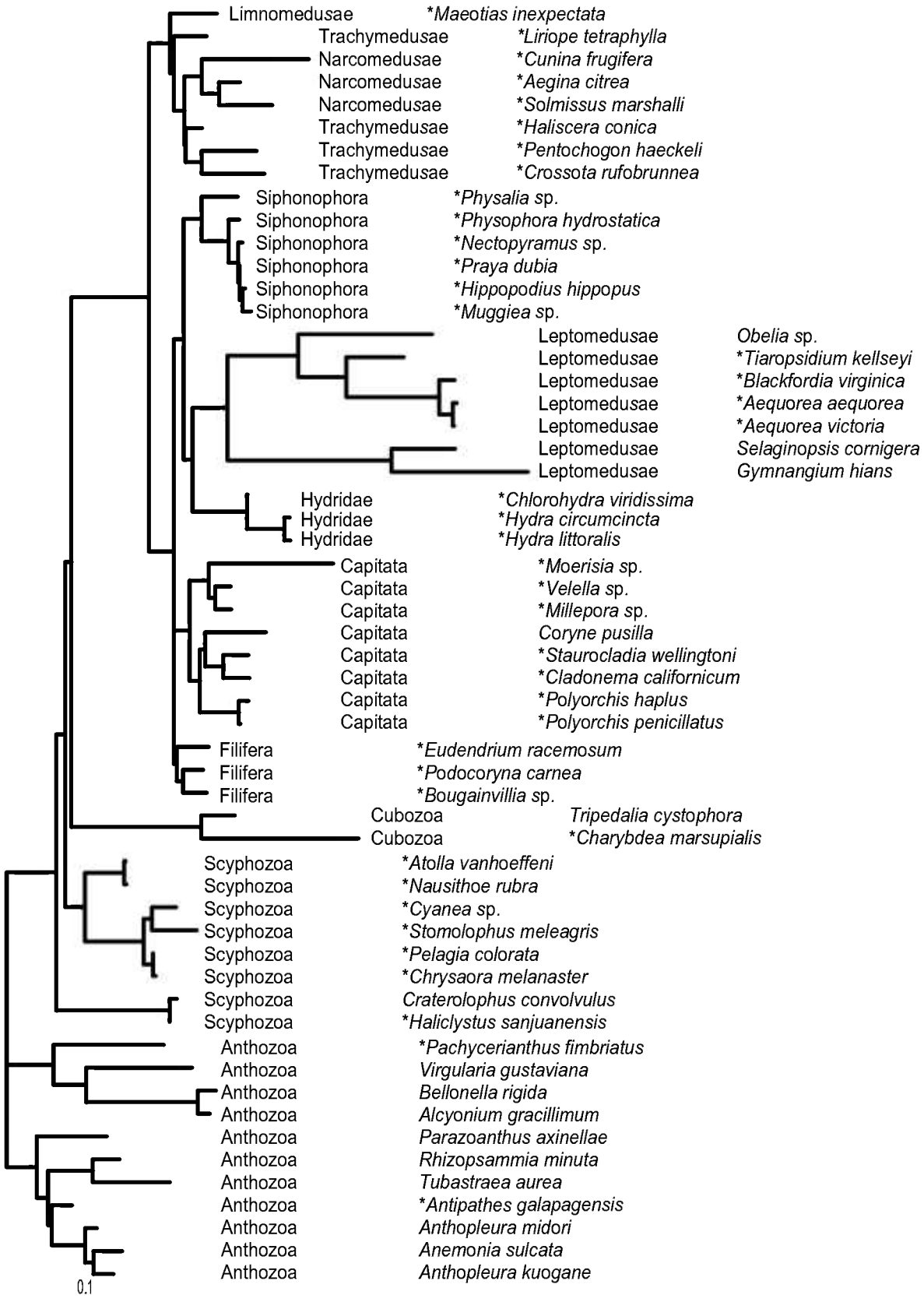

FIG. 4. – Maximum likelihood tree for the 56-taxon dataset. Model of nucleotide evolution is HKY85, with a T-Ratio assigned to be 1.59 and a gamma shape parameter of .212.

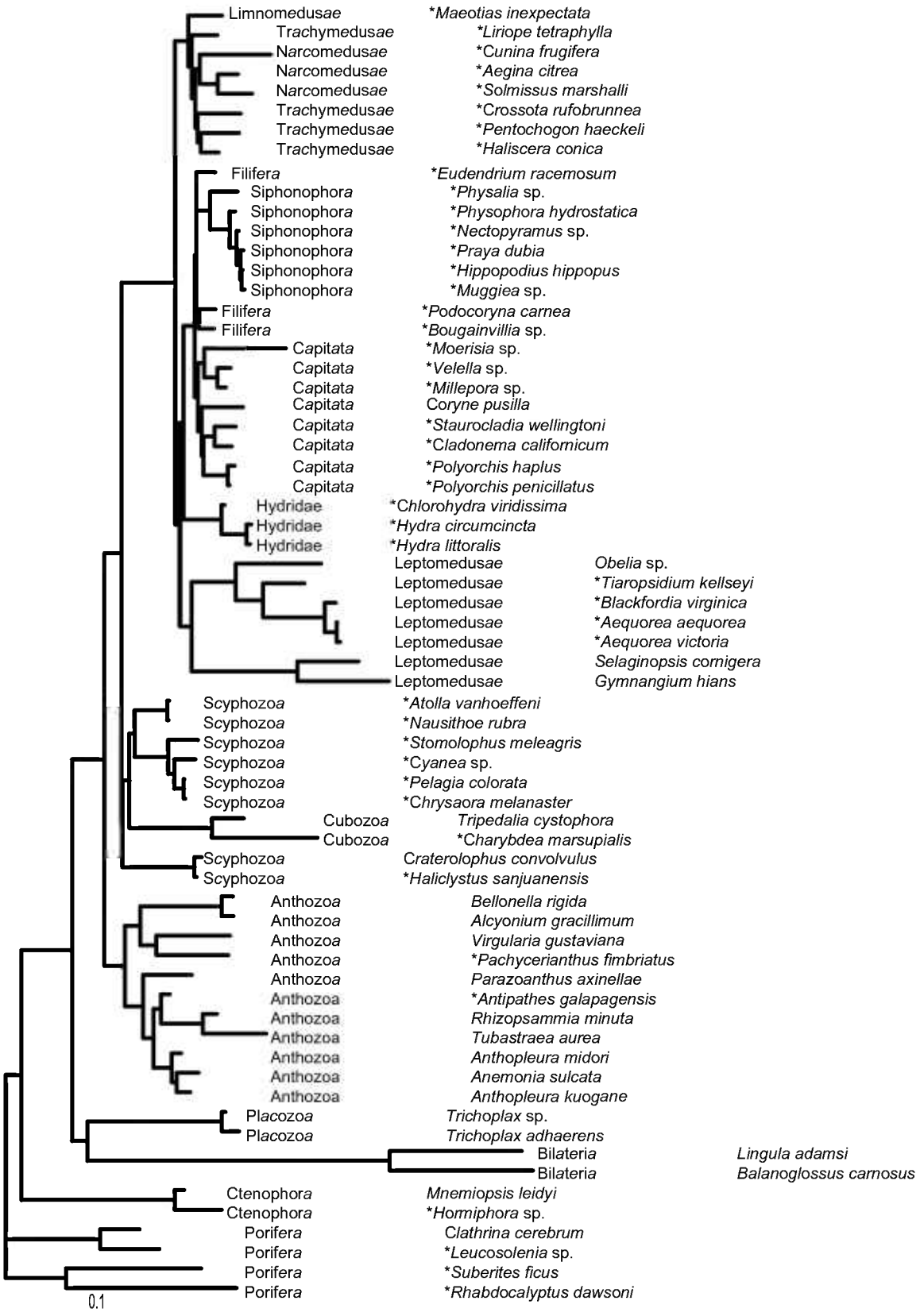

FIG. 5. – Minimum evolution tree for the 66-taxon dataset. The assumed model of nucleotide evolution is HKY85 with gamma shape parameter of .272.

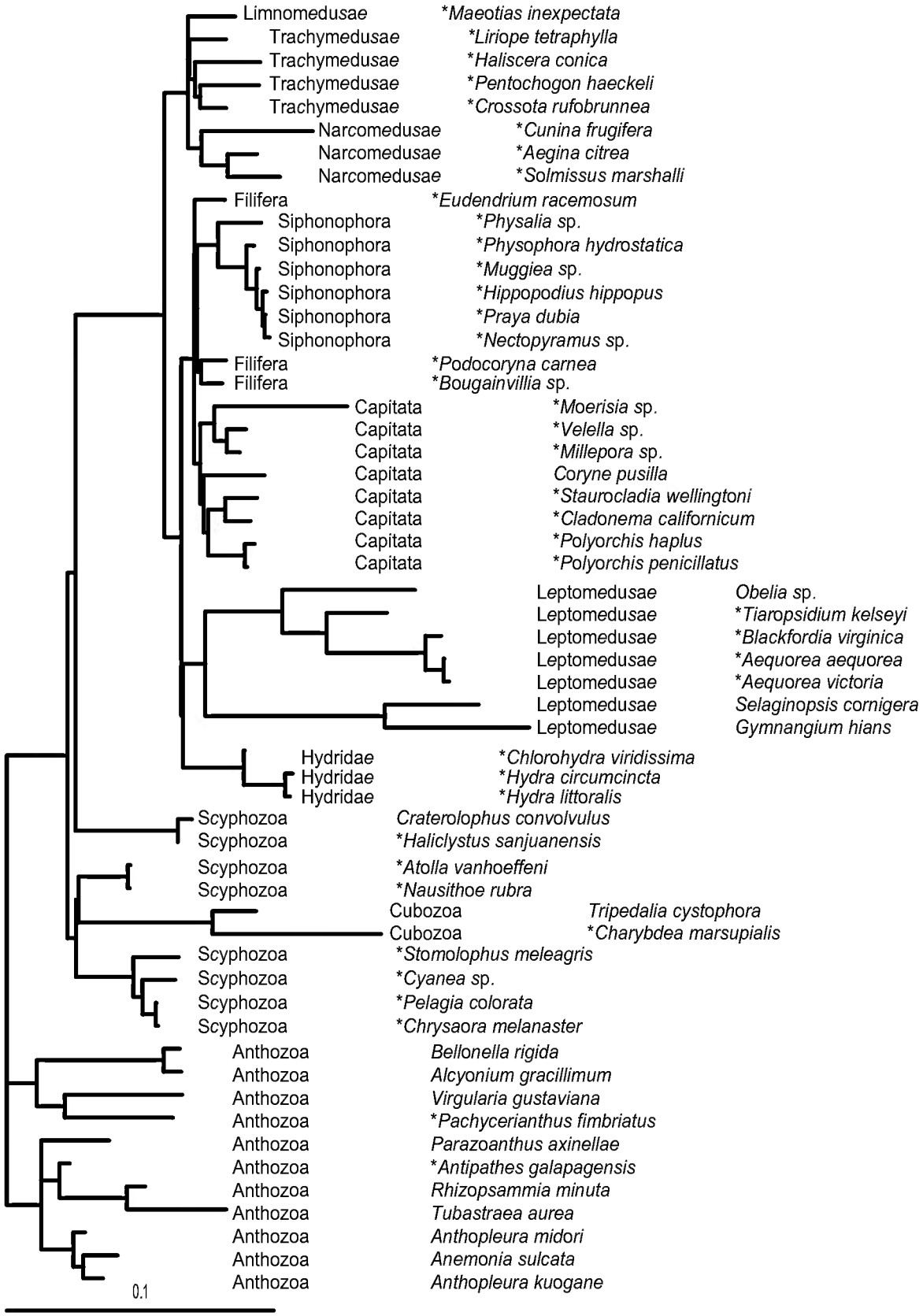

FIG. 6. – Minimum evolution tree for the 56-taxon dataset. The assumed model of nucleotide evolution is HKY85 with gamma shape para meter of .212.

The methods of maximum likelihood and minimum evolution take into account the possibility that a given nucleotide state may have evolved by character transformations from an identical state (e.g., from A to C to A again). The maximum likelihood method seeks the tree for which the data are most probable given an assumed model of nucleotide evolution. The model of nucleotide substitution used in this analysis (HKY85) allows for variation in the rate of evolution at different sites, unequal nucleotide frequencies, as well as different rates of transitions and transversions (Hasegawa et al., 1985). The two required parameters, the T-Ratio (as described above) and gamma (the shape of the distribution of substitution rates), were estimated using trees obtained by neighbor-joining and the unweighted parsimony analyses. These estimates are presented in Table 2. Due to computational difficulty, the maximum likelihood search was performed for just 10 replicates, and only on the smaller 56-taxon dataset. The maximum likelihood analysis performed on the 56-taxon dataset used a transition to transversion ratio of 1.59 and a gamma shape parameter of .212. The most probable tree given the 18S data and the specified model of nucleotide evolution is presented as Figure 4.

The minimum evolution method employs a distance-based optimality criterion (unweighted least-squares) and searches for the tree that minimizes the total sum of branch lengths given a model of nucleotide evolution. The HKY85 model was also assumed for minimum evolution searches, with gamma shape parameters of .272 used for the 66-taxon dataset and .212 for the 56-taxon dataset. 100 replicate searches were performed under the minimum evolution criterion. Negative branch lengths were disallowed. The trees with the smallest sum of unweighted least-squares distances between the taxa, given the specified models of evolution, are presented as Figures 5 and 6.

All of the hypotheses that are not consistent with the most parsimonious trees, listed in Table 3, are also not in harmony with trees built by the alternative methodologies. This increases the confidence one can have in the assertion that the 18S data contradict these hypotheses. Of the hypotheses consistent with the most parsimonious trees (Table 4), several (13, 17, 19, 25, and 26) are contradicted by trees that fit an alternative definition of optimal. However, contradictions occur only among those hypotheses for which four or fewer steps are required to be compromised. Thus, in this analysis 0.1 percent of the total number of weighted-character changes would appear to be an appropriate line of demarcation that is indicative of support, or the lack thereof, provided by the 18S data.

DISCUSSION

The optimal trees (Figs. 2, 3, 4, 5, and 6) inferred by 18S data reveal phylogenetic patterns that make sense in light of hydrozoan classifications and past phylogenetic hypotheses. Many well-defined groups are recognized as monophyletic, including Capitata (excluding Hydridae), Filifera (only Figs. 3 and 4), Hydridae, Leptomedusae, Narcomedusae, and Siphonophora. Some associations among these groups are expected, e.g., Trachymedusae with Narcomedusae, while others are more surprising, such as Leptomedusae or Siphonophora with Hydridae. However, just from looking at a tree, one cannot ascertain how much support there is for a given relationship. Nor does a tree indicate the extent to which an opposing view is contradicted by the tree. That is why this paper explicit tests competing hypotheses of hydrozoan relationships. Each of the groups, and the hypotheses associated with them, will be addressed in turn.

Anthomedusae

Anthomedusae are difficult to characterize; no synapomorphies for the group are known (Schuchert, 1996). The 18S data mirror this situation, indicating that Anthomedusae may not be a monophyletic group. The shortest 56-taxon trees that have a monophyletic Anthomedusae including Hydridae are six steps longer than the most parsimonious trees, which have Anthomedusae grouping with Siphonophora, Leptomedusae and Hydridae (Fig 3). A clade composed of these four groups is fairly strongly supported by the 18S data. Each of the "optimal" trees built by alternative methods reveal this grouping. Moreover, an additional 13 steps are required to find an alternative hypothesis in conflict with this arrangement (Table 4). Although, they appear to form a clade, the relationships among the following groups cannot be clearly distinguished with the present dataset: Capitata, Filifera, Hydridae, Leptomedusae, and Siphonophora.

Capitata and Filifera

The two recognized subgroups of the Anthomedusae, Capitata (excluding Hydridae) and Filifera may be monophyletic. In particular, a monophyletic Capitata is revealed in all "optimal" trees. Still, only two additional weighted-character changes are required to violate this relationship in the most parsimonious 56-taxon tree (Fig. 3). Two capitate families (Cladonematidae and Polyorchidae) are sampled more than once in this analysis. In all trees, the two families are revealed as monophyletic groupings. The relationships among the capitate families are unclear. The best supported result places the velellid (*Velella* sp.) and the milleporid (*Millepora* sp.) together, a situation anticipated by others (Bouillon, 1985; Petersen, 1979; Petersen, 1990). This relationship poses an interesting historical question, as both of these groups are derived morphologically and endowed with a fossil record. Fossil milleporids are known from the Cretaceous (Oliver and Coates, 1987). On the other hand, fossil velellids (or chondrophores as they are better known to the paleontological community) possibly date from the Neoproterozoic (Glaessner and Wade, 1966) and are probably known from the Cambrian (Waggoner and Collins, 1995). Of course, this phylogenetic relationship does not imply that one of these distinctive morphologies is necessarily derived from the other. If an excess of 500 million years has created the molecular divergence seen between these two species, then the 18S gene has evolved slowly in these lineages.

Even less support is provided for the hypothesis that Filifera is monophyletic. In all trees, the filiferan species branch somewhere near the base of Capitata. Interestingly, Naumov derived Capitata from Filifera (Naumov, 1960). This provides some support for the idea that filiform tentacles represent the ancestral condition for Capitata (Petersen, 1990). With just three filiferan species sampled and little resolution for the group as a whole, it is difficult to speculate on their relationships. However, the clavid species (*Podocoryna carnea*) and the bougainvilliid (*Bougainvillia* sp.) group together in these analyses. Filifera species appear to exhibit a relatively slow rate of 18S evolution. This may pose difficulties for using this gene to elucidate the more inclusive phylogenetic relationships among this group.

Hydridae

The finding that Hydridae is a monophyletic group has a great deal of support (Table 4). This is not too surprising in light of how different hydrids are from other hydrozoans. They completely lack the medusa stage and they are adapted to fresh water. The latter is presumably a difficult transition as it has only been made a few times in the history of cnidarians (e.g. Hydridae, Craspedacusta and Limnocnida). With just three species sampled (*Hydra littoralis*, *Hydra circumcincta*, and *Chlorohydra viridissima*), there is little one can say about the relationships among them. However, it has been unclear how distinctive the different species of the genera are. Some workers consider *Chlorohydra* to be a synonym of *Hydra* (Petersen, 1990). In all trees, the two *Hydra* species group together to the exclusion of *Chlorohydra viridissima* and so the two genera may delimit phylogenetically distinctive groups. Additional sampling is needed to investigate this issue further.

While the monophyly of the hydrids is likely, it is much less clear where the Hydridae fall within Hydrozoa. Petersen argued strongly that Hydridae and Moerisidae are closely related, 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). This analysis includes just a single representative moerisid (*Moerisia* sp.), but it does not cluster with the hydrid species. In fact, an additional 20 weighted-character changes are required in order to bring the two groups together, a relatively convincing indication that they do not constitute a clade. Moerisid species are often associated with brackish waters that have a strong fresh-water influence (Naumov, 1960). The similarities between the two groups may have arisen convergently as adaptations to the rigors imposed by strong seasonal changes in fresh and very low-saline brackish waters.

At a broader taxonomic level, Hydridae is often included as part of Capitata and/or Anthomedusae (Bouillon, 1985; Hyman, 1940; Petersen, 1979; Petersen, 1990). These hypotheses are contradicted by the 18S data. The Hydridae probably have an independent origin from that of Capitata. The shortest 56-taxon tree containing Hydridae within Capitata is 12 steps longer than the most parsimonious tree. Hypothesizing that Anthomedusae contains Hydridae requires six additional character changes. The most parsimonious 56-taxon tree has Hydridae grouped with Leptomedusae. Such a grouping has not been anticipated by any worker in the past, and specific morphological connections to support the grouping are not readily apparent. In fact, support for an assertion that Hydridae and Leptomedusae

form a clade is rather limited. Four extra character changes are enough to remove the relationship between the two groups in the 56-taxon analysis. Neither of the 66-taxon trees (Figs. 2 and 5) contain this grouping. In the absence of additional indications of a close phylogenetic affinity between these two groups, it may be best to consider this grouping a questionable result with little support.

Leptomedusae

18S data suggest that Leptomedusae is monophyletic. Not surprisingly, they are a reasonably well-characterized group. The polyps in this group usually have thecae, the medusae bear their gonads on the radial canals, and the statocysts are of ectodermal origin. Among the leptomedusae sampled, all tree-building methodologies reveal the same relationships. Although Bouillon (1985) stated that his tree of Leptomedusae families should not be read as a phylogeny, the 18S data agree remarkably well with it. In these analyses, the aglaopheniid (*Gymnangium hians*) and the sertulariid (*Selaginopsis cornigera*) form a clade. The one difference is the placement of the campanulariid (*Obelia* sp.), which would branch basal to the other Leptomedusae included in this analysis if it followed Bouillon's scheme. Proboscoida (all Leptomedusae other than campanulariids) may not be monophyletic. Instead, *Obelia* is at the base of a clade that includes exemplars of Mitrocomidae (*Tiaropsidium kellseyi*), Blackfordiidae (*Blackfordia virginica*), and Aequoridae (*Aequorea aequorea* and *A. victoria*). Among these groups, Aequoridae and Blackfordiidae appear to be the most closely related. The rate of evolution of the 18S gene appears to be relatively high in this group, as evidenced by their longer branch lengths. Additional sampling of the 18S gene from leptomedusan species should continue to help resolve their relationships.

Siphonophora

Forming fantastic colonies of highly polymorphic zooids, siphonophores are a distinctive group of hydrozoans and their monophyly is supported by 18S data. All three major subgroups of the siphonophores that are typically recognized are present in this analysis, Calycophorae, Cystonectae, and Physonectae. *Physalia*, the Portuguese man-of-war, is the lone representative of Cystonectae, and it always branches basal to the calycophorans and physonects in the analysis. This is in contrast to Totton's view that the cystonects and physonects are most closely related based on a fairly lengthy list of similarities (Totton, 1965). In light of the data presented here, these similarities, which include the possession of gas-filled floats, can be considered as ancestral to Siphonophora. In agreement with Totton, these data suggest that gas-filled floats were lost in calycophores. The calycophores, which hold together strongly as a monophyletic group based on 18S data, appear to have a great number of autapomorphies beyond those derived features that characterize the siphonophores as a whole.

Siphonophores are so distinct from other hydrozoans that they are often excluded from discussions that deal with the rest of Hydrozoa. In fact, Bouillon *et al.* (1992) were prompted to ask "Non-siphonophoran Hydrozoa: what are we talking about?" In this scholarly discourse on hydrozoan nomenclature, the authors wrestle with the question of what to call non-siphonophoran hydrozoans and conclude that "Hydroidomedusae" is the best name. However, the present analysis suggests that no formal taxonomic name should be used for the non-siphonophoran hydrozoans since they are very unlikely to be monophyletic. Siphonophores probably did not branch basally to the other hydrozoan groups. Such a scenario requires 23 additional weighted-character changes (Table 3).

Instead, Siphonophora may be allied with Anthomedusae. Haeckel was the first to assert this affiliation, suggesting that the ancestors of Siphonophora should be looked for among the capitate groups Corymorphidae and Tubularidae (Haeckel, 1888). Since Haeckel, there have been a number of workers who have also supported the idea of an ancestral connection between Siphonophora and Capitata primarily based upon larval similarities (Daniel, 1985; Garstang, 1946; Leloup, 1955; Totton, 1965). Such an explicit connection between Siphonophora and Capitata is not suggested by the present analysis, requiring eight extra steps to be accommodated. Schuchert (Schuchert, 1996) hinted that Siphonophora may share affinities with Anthomedusae because both share gonads on the manubrium and desmonemes, characters that Petersen listed as synapomorphies for Anthomedusae (Petersen, 1990). Still, the most parsimonious 56-taxon tree that places Siphonophora and Anthomedusae in a single clade is four steps longer than the overall most parsimonious 56-taxon tree (Fig. 3). It is possible that the Actinulidae and/or the Laingiomedusae also belong among these groups. Sampling these taxa would be a logical extension of this analysis.

Limnomedusae, Narcomedusae, and Trachymedusae

18S data provide a substantial buttress for the assertion that Narcomedusae species in this analysis comprise a clade. Still, the three Narcomedusae species in this analysis are members of just two of the four Narcomedusae subgroups, Cuninidae and Aeginidae, that are typically recognized (Bouillon, 1987). Surprisingly, the two members of Cuninidae, *Cunina frugifera* and *Solmissus marshalli*, do not group in the analyses. Additional taxa need to be sampled in order to address the internal relationships of Narcomedusae. The same can be said for the Trachymedusae. Just three subgroups of Trachymedusae are sampled in the present analysis, Geryonidae, Halicreatidae, and Rhopalonematidae. The two rhopalonematids (*Crossota rufobrunnea* and *Pentochogon haeckeli*) group together in most of the "optimal" trees (but see Fig. 6). *Haliscera conica*, the representative halicreatid, groups with the rhopalonematids, while the geryonid, *Liriope tetraphylla*, tends to branch basal to the other Trachymedusae as well as the Narcomedusae. The hypothesis of Trachymedusae monophyly is contradicted by the 18S data, for it would take an additional nine weighted character changes to accommodate (Table 3). With just a single limnomedusan species sampled, it is impossible to make any statements concerning the hypotheses that the group is or is not monophyletic.

In a phylogenetically broader view, Narcomedusae and Trachymedusae form a clade in all analyses. Bouillon asserted that the Trachymedusae were most likely derived from the Narcomedusae (Bouillon, 1987). In contrast, the 18S data actually imply that Narcomedusae are derived from Trachymedusae. If this hypothesis is true, then the polyp stage that some Narcomedusae species possess has been secondarily re-gained. This follows because all Trachymedusae for which the complete life cycle is known are direct developers. Moreover, this placement of Narcomedusae implies that the similarities that appear evident between Cubozoa and Narcomedusae, such as the complete metamorphosis of polyp into medusa and the sculpted medusa bell margin (Bouillon, 1987; Petersen, 1979) are convergent characters.

There can be little doubt that the limnomedusan species *Maeotias inexspectata* is part of a clade that includes Trachymedusae and Narcomedusae. The 18S data strongly indicate that Narcomedusae are related to Trachymedusae and Limnomedusae (Table 4). This is not too surprising since Limnomedusae were considered to be part of Trachymedusae prior to the discovery that they possess a polyp stage. In addition, Limnomedusae, like Narcomedusae and Trachymedusae, have statocysts that are ecto-endodermally derived. Further, the position of the gonads of Limnomedusae is typically on the radial canals, a character also seen in Trachymedusae (Hyman, 1940). An exception is the group Proboscidactylidae, which has been considered part of Limnomedusae in the past. However, the Proboscidactylidae are no longer considered to be Limnomedusae, but instead Filifera (Petersen, 1990). Sampling more limnomedusan species may be necessary to determine the phylogenetic limits of the group. Also, the hypothesis that Laingiomedusae are allied with Limnomedusae (Bouillon, 1987) can only be tested by obtaining additional samples of these taxa.

Hydrozoa

Among the hypotheses that are best supported by 18S data is the assertion that hydrozoans all share a more recent common ancestor with each other than any do with any other cnidarian. It would appear then that the velum, the medusa ring canal, and gonads of epidermal origin were present in the most recent common ancestor of hydrozoans (Schuchert, 1993). An additional character supporting the monophyly of Hydrozoa is a loss of nematocysts in the gastric cavity (Bridge *et al.*, 1995). It is unclear from this analysis which cnidarians constitute the sister group to the Hydrozoa and there is only limited support for a monophyletic grouping of the medusa-bearing cnidarians. Additional taxa and/or characters should be brought to bear on this question.

CONCLUSION

Phylogenetic classifications provide very tangible advantages over character-based classifications (De Queiroz and Gauthier, 1990; 1992). Phylogeny provides a natural and useful scheme for organizing life. By giving organisms names that correspond to evolutionary history, then learning names is equivalent to learning history. It is nice to know, therefore, that a phylogeny-based classification of Hydrozoa, which does not greatly contradict older classifications, can be offered in light of the present discus-

sion. Hydrozoa appears to be composed of two clades. One includes Limnomedusae, Narcomedusae, and Trachymedusae. A reasonable name for this clade is Trachylina, as it has been used in the past to encompass these groups (Haeckel, 1880; Bouillon *et al.*, 1992). The other main clade of Hydrozoa is comprised of Capitata, Filifera, Hydridae, Leptomedusae, and Siphonophora. This clade can be given a new name Hydroidolina (A. Marques, pers. comm.), because it is a novel grouping. In light of our present understanding, it is unclear where to place Actinulidae and Laingiomedusae.

Increasing the number of taxa in an analysis enhances phylogenetic accuracy (Graybeal, 1998; Hillis, 1996). Thus, future work should include sampling the 18S gene more widely. In addition, other sources of data need to be consulted. Alternative genes, life history information, nematocyst characters, other gene sequences etc. should all be used to test the results of this study. A combined data analysis would be particularly interesting and may hold the most hope for yielding a stable well-supported phylogeny of Hydrozoa.

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REFERENCES

Bouillon, J. – 1985. Essai de classification des Hydropolypes-Hydromeduses (Hydrozoa-Cnidaria). *Indo-Mal. Zool.,* 1: 29-243.

Bouillon, J. – 1987. Considerations sur le developpement des Narcomeduses et sur leur position phylogenetique. *Indo-Mal. Zool.,* 4: 189-278.

Bouillon, J., F. Boero, F. Cicogna, J.M. Gili, and R.G.Hughes. – 1992. Non-siphonophoran Hydrozoa: what are we talking about? *Scient. Mar.*, 56: 279-284.

Bremer, K. – 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution*, 42: 795-803.

Bremer, K. – 1994. Branch support and tree stability. *Cladistics*, 10: 295-304.

Bridge, D., C.W. Cunningham, R. DeSalle and L.W. Buss. – 1995. Class-level relationships in the phylum Cnidaria: Molecular and morphological evidence. *Mol. Biol. Evol.,* 12: 679-689.

Chomczynski, P., K. Mackey, R. Drews and W. Wilfinger. – 1997. DNAzol: A reagent for the rapid isolation of genomic DNA. *Biotechniques*, 22: 550-553.

Collins, A.G. – 1998. Evaluating multiple alternative hypotheses for the origin of Bilateria: An analysis of 18S molecular evidence. *Proc.Natl. Acad. Sci. USA*, 95: 15458-15463.

Daniel, R. – 1985. *The fauna of India and the adjacent countries. Coelenterata: Hydrozoa Siphonophora.* Zoological Survey of India, Calcutta.

De Queiroz, K. and J. Gauthier. – 1990. Phylogeny as a central principle in taxonomy: Phylogenetic definitions of taxon names. *Syst. Zool.,* 39: 307-322.

De Queiroz, K. and J. Gauthier. – 1992. Phylogenetic Taxonomy. In: D. G. Fautin (ed.), *Phylogenetic Taxonomy*, pp 449-480. Annual Reviews Inc., Palo Alto, California, USA.

Garstang, W. – 1946. The morphology and relations of the Siphonophora. *Quart. J. Micr. Sci.*, 87: 103-193.

Glaessner, M.F. and M. Wade. – 1966. The late Precambrian fossils from Ediacara, South Australia. *Paleontology*, 9: 599-628.

Graybeal, A. – 1998. Is it better to add taxa or characters to a difficult phylogenetic problems? *Sys. Biol.*, 47: 9-17.

Haeckel, E.H. – 1880. *Das System der Medusen: Erster Theil einer Monographie der Medusen.* G. Fischer, vormals F. Mauke, Jena, 672 .

Haeckel, E.H. – 1888. *Report on the Siphonophora collected by H.M.S. Challenger during the years 1873-76.* Her Majesty's Stationery Office, Edinburgh.

Hasegawa, M., H. Kishino, and T.A. Yano. – 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.*, 22: 160-174.

Hillis, D. M., J. J. Bull, M. E. White, M. R. Badgett and I. J. Molineux. – 1992. Experimental phylogenetics: Generation of a known phylogeny. *Science*, 255: 589-592.

Hillis, D.M. – 1996. Inferring complex phylogenies. *Nature,* 383: 130-131.

Hyman, L.H. – 1940. *The invertebrates. Vol. 1. Protozoa through Ctenophora.* McGraw-Hill, New York.

Kim, J.H., W. Kim and C.W. Cunningham. – 1999. A new perspective on lower metazoan relationships from 18S rDNA sequences. *Mol. Biol. Evol.*, 16: 423-427.

Kishino, H. and M. Hasegawa. – 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order of Hominoidea. *J. Mol. Evol.*, 29: 170-179.

Leloup, E. – 1955. Siphonophores. *Rep. Scient. Results Michael Sars N. Atlant. Deep Sea Exped.,* 5: 1-24.

Medlin, L., H.J. Elwood, S. Stickel and M.L. Sogin. – 1988. The characterization of enzymatically amplified eukaryotic 16S-like ribosomal RNA-coding regions. *Gene (Amsterdam).* 71: 491-500.

Naumov, D.V. – 1960. *Hydroids and Hydromedusae of the USSR.* Translated from Russian (1969). Israel Program for Scientific Translations, Jerusalem.

Oliver Jr., W.A. and A.G. Coates. – 1987. Phylum Cnidaria. *In*: R. S. Boardman, A. H. Cheetham and A. J. Rowell (eds.): *Fossil Invertebrates*, pp 140-193. Blackwell Scientific, Palo Alto, CA.

Petersen, K.W. – 1979. Development of coloniality in Hydrozoa. *In:* G. Larwood and B. R. Rosen (eds.): *Biology and Systematics of Colonial Organisms*, pp 105-139. Academic Press, New York.

Petersen, K.W. – 1990. Evolution and taxonomy in capitate hydroids and medusae (Cnidaria: Hydrozoa). *Zool. J. Linn. Soc.,* 100: 101-231.

Schuchert, P. – 1993. Phylogenetic analysis of the Cnidaria. *Zeit. Zool. Syst. Evol.*, 31: 161-173.

Schuchert, P. – 1996. *The marine fauna of New Zealand : athecate hydroids and their Medusae (Cnidaria: Hydrozoa).* New Zealand Oceanographic Institute, Wellington, 159 pp.
Swofford, D.L. – 1998. PAUP*. *Phylogenetic Analysis Using Parsimony* (*and Other Methods), Version 4b2a. Sinauer Associates, Sunderland, MA.
Swofford, D.L., G.J. Olsen, P.J. Waddell and D.M. Hillis – 1996. Phylogenetic inference. In: D.M. Hillis, C. Moritz and B.K. Mable (eds.), *Phylogenetic inference*, pp 407-514. Sinauer Associates, Sunderland, MA.
Templeton, A.R. – 1983. Convergent evolution and non-parametric inferences from restriction fragment and DNA sequence data. *In:* B. Weir (ed.): *Statistical Analysis of DNA Sequence Data*, pp. 151-179. Marcel Dekker, New York.
Totton, A.K. – 1965. *A Synopsis of the Siphonophora.* British Museum of Nat. Hist., London.
Vawter, L. and W.M. Brown. – 1993. Rates and patterns of base change in the small subunit ribosomal RNA gene. *Genetics*, 134**:** 597-608.
Waggoner, B.M. and A.G. Collins. – 1995. A new chondrophorine Cnidaria, Hydrozoa from the Cadiz Formation Middle Cambrian of California. *Palaeont. Zeit.,* 69**:** 7-17.