Class-Level Relationships in the Phylum Cnidaria: Molecular and Morphological Evidence

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The evolutionary history of cnidarian life cycles has been debated since the 1880s, with different hypotheses favored even by current textbooks. Contributing to the disagreement is the fact that the systematic relationships of the four cnidarian classes have received relatively little examination using modern systematic methods. Here we present analyses of class-level relationships based on 18S ribosomal DNA (rDNA) sequence, mitochondrial 16S rDNA sequence, mitochondrial genome structure, and morphological characters. DNA sequences were aligned using a repeatable parsimony-based approach incorporating a range of alignment parameters. Analyses of individual data sets and of all data combined are unanimous in grouping the classes possessing a medusa stage, leaving the holobenthic Anthozoa basal within the phylum.

Introduction

The Cnidaria are noted for both the unusual alternation of generations and the life cycle diversity within the phylum. The metagenetic life cycle, in which a sessile polyp stage is followed by a morphologically distinct, free-swimming medusa stage, occurs in three of the four cnidarian classes, the Hydrozoa, Cubozoa, and Scyphozoa. However, within the phylum, each life cycle stage can be found without the other (fig. 1). In the fourth class, the Anthozoa, the free-swimming medusa stage is always absent. A corresponding life cycle with the sessile polyp stage absent characterizes the hydrozoan order Trachymedusae.

Two competing hypotheses regarding the diversification of cnidarian life cycles have been predominant (fig. 1). One view is that the polyp-medusa life cycle is derived from the polyp-only life cycle. This view has been presented together with arguments for monophyly of the three classes with medusae, the Hydrozoa, Cubozoa, and Scyphozoa (fig. 1A) (Jägersten 1955; Pantin 1960; Petersen 1979; Werner 1984), which are sometimes termed the Medusozoa. An alternative view is that the polyp-only life cycle is secondarily reduced from the polyp-medusa life cycle, which in turn is derived from an original medusa-only life cycle (Brooks 1886; Hyman

1940, p. 635; Hand 1959; Barnes 1987, p. 113; Ax 1989). This proposal has been accompanied by arguments that the classes Anthozoa, Scyphozoa, and Cubozoa form a monophyletic group and often by arguments that the Hydrozoa is paraphyletic, with the order Trachymedusae basal within the Cnidaria (fig. 1B).

Despite the existence of these alternative phylogenetic hypotheses, the relationships of the cnidarian classes have received relatively little examination using modern systematic methods. The single parsimony-based study focusing on this question (Schuchert 1993a) used 11 morphological characters and a molecular character, presence of mitochondrial DNA as circular or linear molecules (Bridge et al. 1992). This phylogenetic analysis supported monophyly of the three classes with medusae, the "Medusozoa" hypothesis (fig. 1A). The earlier analysis of Brusca and Brusca (1990, p. 256) agreed with the alternative "basal Trachymedusae" hypothesis (fig. 1B) but used character polarizations based on undescribed criteria and included fewer informative characters than Schuchert's study.

Nucleotide sequence data are an obvious source of additional evidence about cnidarian class-level relationships. However, only one published data set includes information for members of more than two cnidarian classes. A distance analysis of these data, 120 base pairs of 5S ribosomal RNA from seven cnidarian species, tentatively supported grouping the classes with medusae (fig. 1A) (Hori and Satow 1991). To bring more evidence to bear on the long-standing problem of cnidarian class-level systematics, we obtained new ribosomal DNA (rDNA) sequence data and analyzed it together with

- polyp
┌─ Trachymedusae   M ⎤
│   ⎥ Hydrozoa
+ medusa └── other Hydrozoa   P M ⎦

+ polyp ── Cubozoa   P M

── Scyphozoa   P M

── Anthozoa   P

A

- medusa
┌─ Anthozoa   P
│
+ polyp ── Cubozoa   P M

── Scyphozoa   P M

+ medusa ── other Hydrozoa   P M ⎤
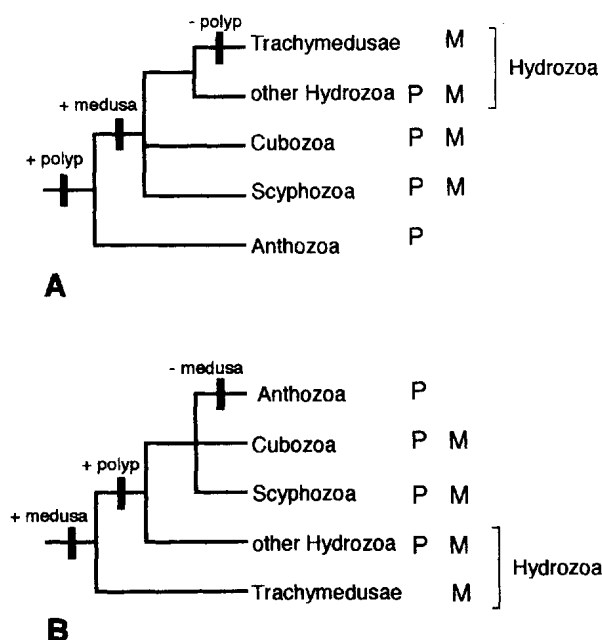   ⎥ Hydrozoa
── Trachymedusae   M ⎦

B

Fig. 1.—Alternative views of cnidarian life-cycle evolution and systematic relationships. *A*, "Medusozoa" hypothesis: the polyp-only life cycle is pleisiomorphic for the Cnidaria and the classes with the medusa stage form a monophyletic group, based on Werner (1984) and Petersen (1979). *B*, "Basal Trachymedusae" hypothesis: the medusa-only life cycle is pleisiomorphic and the hydrozoan order Trachymedusae is the sister group of the rest of the Cnidaria, based on Hyman (1940, pp. 635–640) and Brusca and Brusca (1990, p. 256); *P* indicates the presence of a polyp stage, *M* the presence of a medusa stage. Pleisiomorphy of the metagenic life cycle is not illustrated, since no outgroup to the Cnidaria shows metagenesis.

morphological characters and data on mitochondrial genome structure.

Methods and Material

Choice of Taxa

Nuclear 18S rDNA sequence was obtained from representatives of multiple subclasses within the three more speciose cnidarian classes. Species represented are shown in table 1, and the 12 for which new sequence data were collected are indicated. Members of all three anthozoan subclasses (Cairns et al. 1991) were sampled, as were members of three of the four scyphozoan orders (Cairns et al. 1991) and three of the eight hydrozoan orders (Bouillon 1985; Cairns et al. 1991), including the holopelagic Trachymedusae. Because the class Cubozoa comprises only 17 described species and one order (Barnes and Harrison 1991), a single cubozoan representative was used.

Representatives of three phyla were used as outgroups in the analyses, because there is little consensus as to the sister group of Cnidaria. Traditionally, the Ctenophora has been placed as the sister group of the

phylum (Harbison 1985; Ax 1988), so a member of each of the two ctenophore classes (Harbison 1985) was included. However, a recent maximum likelihood analysis of 18S rDNA sequence data supported a sister group relationship between the Cnidaria and the simple multicellular Placozoa (Wainright et al. 1993), so the placozoan *Trichoplax* was also included. Two sponge classes were sampled to provide a third outgroup.

Mitochondrial 16S rDNA sequences were obtained for nine of the cnidarian taxa sampled. Unpublished sequence data for two additional taxa were kindly provided by G. A. Pont-Kingdon, C. T. Beagley, C. G. Vassort, R. Okimoto, R. Warrior, and D. R. Wolstenholme (table 1). For two cnidarian representatives and the outgroup taxa, 18S rDNA data were available from the literature but 16S rDNA data were not. Attempts to amplify 16S rDNA from the two ctenophore outgroup representatives using standard metazoan 16S rDNA primers (DeSalle et al. 1993) and cnidarian-specific primers (Cunningham and Buss 1993) were not successful. A separate analysis involving fewer taxa was therefore performed with the 16S data included.

Molecular Data

Animals were starved for at least 3 d before DNA extraction. DNA was isolated using a urea buffer, following the method of Shure et al. (1983). For representatives of 12 cnidarian and one ctenophore species (table 1), approximately 665 aligned base pairs of 18S rDNA were amplified and sequenced as four contiguous fragments designated "A," "D1," "D2," and "B." Primers used were as follows (positions of primers in the human 18S rDNA sequence follow each sequence): 5'-CCTGA-GAAACGGCTACCACATC-3' 441–462 and 5'-TA-ACCGCAACAACTTTAAT-3' 668–650 for the A fragment; 5'-AATTAAAGTTGTTGCGGTTA-3' 650–668 and 5'-TGCTTTRAGCACTCTAA-3' 829–813 for the D1 fragment; 5'-TTAGAGTGCTYAAAGC-3' 814–829 and 5'-GACGGTCCACACATTTCACC-3' 976–957 for the D2 fragment; and 5'-GGTGAAATTCTTGGACCGTC-3' 958–977 and 5'-GTTTCAGCTTTGCAA CCAT-3' 1191–1173 for the B fragment. Primers for regions A and B were designed by Wheeler et al. (1993) and primers for regions D1 and D2 by W. C. Wheeler (pers. comm.). To amplify DNA from the ctenophore *Beroe,* the B fragment primers were modified to 5'-CAGAGGTGA-AATTCTTGGATT-3' 954–977 and 5'-GTTTCATTC-TTGCGAACATA C-3' 1191–1171.

Double-stranded amplification products of the 18S rDNA gene were sequenced directly. Primers were removed from amplified DNA using the Prep-a-Gene (Bio-Rad) procedure. DNA sequencing was carried out using the Sequenase 2.0 (U.S. Biochemical) dideoxy protocol

Table 1
Taxa Used in the Study

Cnidaria	
class Anthozoa	
subclass Hexacorallia	*Anemonia sulcata*
	Metridium senile[a,b]
subclass Ceriantipatharia	*Ceriantheopsis americanus*[a]
subclass Octocorallia	*Leptogorgia virgulata*[a,b]
	Renilla muelleri[a,b]
class Cubozoa	*Tripedalia cystophora*
class Scyphozoa	
order Semaeostomeae	*Aurelia aurita*[a,b]
order Rhizostomeae	*Cassiopea sp.*[a,b]
order Stauromedusae	*Haliclystus sp.*[a,b]
	Craterolophus convolvulus[a,b]
class Hydrozoa	
order Trachymedusae	*Liriope tetraphylla*[a,b]
order Thecata	*Obelia dichotoma*[a,b]
order Athecata	*Hydra vulgaris*[a,b]
	Tubularia indivisa[a,b]
Ctenophora	
class Nuda	*Beroe ovata*[a]
class Tentaculata	*Mnemiopsis leidyi*
Placozoa	*Trichoplax adhaerens*
Porifera	
class Calcarea	*Scypha lingua*
class Demospongiae	*Microciona prolifera*

[a] 18S rDNA sequence obtained in this study; for other species 18S data are from the literature.

[b] 16S rDNA sequence used in this study; unpublished sequences for *Hydra vulgaris* and *Metridium senile* were provided by G. A. Pont-Kingdon, C. T. Beagley, C. G. Vassort, R. Okimoto, R. Warrior, and D. R. Wolstenholme.

(Sanger et al. 1977). The manufacturer's protocol was followed except in the initial steps of denaturation and annealing. The template DNA and primer were boiled in 1X Sequenase reaction buffer for 4 min; then the mixture was placed on ice for 3 min. DNA was sequenced in both directions to avoid compression artifacts. Sequences were deposited in Genbank under accession numbers U19541 through U19553.

For representatives of nine species (table 1), approximately 583 aligned base pairs of mitochondrial 16S rDNA were amplified using the primers and conditions given in Cunningham and Buss (1993). Primers were modified from "universal" 16S rDNA primers (DeSalle et al. 1993) based on unpublished sequence data from *Hydra vulgaris* and *Metridium senile* provided by G. A. Pont-Kingdon, C. T. Beagley, C. G. Vassort, R. Okimoto, R. Warrior, and D. R. Wolstenholme. The 16S rDNA sequences were determined either by direct sequencing of single-stranded PCR products or by sequencing of cloned PCR products. Single-stranded products were prepared and sequenced following the protocol in Hillis et al. (1990). The PCR fragments were cloned and sequenced as described in Cunningham and Buss (1993). To detect PCR error, sequence for each species was determined from at least two clones or from one clone and single-stranded product. Approximately one substitution attributable to PCR error was found per 1,000 bases sequenced. GenBank accession numbers for these sequences are U19371 through U19379.

Morphological Data

Twenty-nine morphological characters were compiled from the literature (App. A). All but three of the 11 morphological characters used in Schuchert's study (1993a) were included. Of these three, presence of solid tentacles in the polyp was not used because of arguments that two types of hollow tentacles should be distinguished (Petersen 1990). Presence of pedalia and presence of podocysts were not used because they were uninformative given the taxa sampled. Holotrichous, basitrichous, and atrichous isorhizae were combined into a single character because of claims that the terms for these nematocysts are used with some overlap within the literature (Mariscal and Bigger 1976).

Characters present only in the polyp or medusa stage were coded as inapplicable for taxa lacking the relevant stage and were treated as missing in analyses. In some cases, the presence of missing data for inapplicable characters can have undesirable effects on cladogram reconstruction (Maddison 1993). In particular, the length of cladograms may be evaluated inappropriately if groups of taxa for which the character is applicable are separated by multiple taxa for which the character is inapplicable. In the present study, problems would not be expected to occur because of characters present only in the polyp stage, since they are inapplicable only for a single ingroup taxon, *Liriope*. However, coding characters present only in the medusa stage as inapplicable could lead to overestimation of the length of a particular set of cladograms: those in which the Anthozoa are paraphyletic and are not basal within the Cnidaria. While these cladograms do not agree with any existing view of cnidarian relationships, they could potentially have been specified by the data in this study. An alternative to coding characters as inapplicable would be to add the absence of the medusa stage as a third state for these characters (Maddison 1993). This was not done, because it would bias the results toward a single origin of the medusa (Coddington 1988; Brooks and McLennan 1991). The potential problem was instead addressed by performing those analyses that included the morphological data with the medusa-specific characters removed as well as with them present.

Data Analysis

Sequences were aligned using the parsimony-based multiple alignment program MALIGN 1.85 (Wheeler

and Gladstein 1993) on a Sun Sparcstation2. MALIGN can be used to search for alignments that minimize cladogram length, so that parsimony provides a criterion for alignment quality (Gatesy et al. 1993; Wheeler and Gladstein 1993). The MALIGN options "build," "alignswap," "alignaddswap," "keepaligns 20," "treeswap," and "keeptrees 100" were used to specify relatively intensive searches for alignments.

Alignments of rDNA sequences can vary when different weights are assigned to gaps relative to nucleotide substitutions. It is thus of interest to identify regions in sequences where sequence alignment is not sensitive to changes in the gap cost used. In this study, such alignment-stable regions were identified in a repeatable way by aligning sequences using a range of gap weights. Nucleotide positions whose alignment was the same when different gap costs were used could then be identified, as proposed by Gatesy et al. (1993). Both the 18S rDNA and the 16S rDNA sequences were aligned using a range of arbitrarily chosen gap costs (1–8, 16, 32, 64, and 128), with nucleotide substitution cost was set at 1. Each single-base deletion was assigned equal cost.

Although there is a lower limit on the gap cost to be used in aligning sequences (Wheeler 1993), the upper limit is not obvious. It would be desirable to compare alignments produced with different gap costs using a property of the cladograms they specify, such as a measure of character homoplasy. But it is not clear that measures of homoplasy will increase as alignments become more extreme, if the gaps in the data are given greater weight. In order to set an upper limit on the range of gap costs used, we examined the percentage of invariant (constant) characters in each aligned data set. For the 18S sequence, the percentage of invariant characters showed no trend as gap cost increased for gap costs of eight and lower. Above a gap cost of eight, the percentage of invariant characters decreased with increasing gap weight. Alignments produced using gap weights of one through eight were therefore used to generate a data set of alignment-stable positions. For the 16S sequence, the percentage of invariant positions decreased above a gap cost of four, and alignments produced using gap costs of one through four were used to generate an alignment-stable data set.

Data sets of alignment-stable positions were produced by excluding those positions that differed between alignments (Gatesy et al. 1993). To examine the phylogenetic signal in 18S rDNA alignment-dependent regions, an additional data set was produced by combining the alignments generated using gap costs of one through eight, without removing positions that differed between alignments. Alignment-stable and combined data sets were produced from the original sequence alignments

using the program CULL (Wheeler 1994). The sequence alignments used in phylogenetic analyses are available from D. Bridge or from EMBL; EMBL accession numbers are DS20353 for alignments of 18S sequence and DS20404 for alignments of 16S sequence.

Phylogenetic analyses were performed using PAUP version 3.1.1 (Swofford 1993). When the number of taxa examined precluded use of the branch-and-bound algorithm, the heuristic algorithm of PAUP was used, with tree bisection and reconnection branch swapping of 200 randomly constructed initial trees. The "constraints" option was used to determine how many additional steps were required for phylogenetic hypotheses other than the one best supported.

The 18S rDNA and morphological data were analyzed both separately and together, with the single character of mtDNA genome structure (Bridge et al. 1992) also included in the combined analysis. Because of difficulty amplifying 16S rDNA in the outgroup taxa, the 16S data were only used in a combined analysis. The 16S characters were coded as missing for the outgroup taxa. Only two outgroup taxa, a ctenophore representative (*Mnemiopsis*) and the placozoan representative *Trichoplax* were used in order to minimize the amount of missing data. In this way evidence from the 16S sequence data provided information on relationships within the Cnidaria, with the other data sets serving to root the resulting cladograms.

Results
18S rDNA Data

Phylogenetic analyses of 18S rDNA data grouped together the "Medusozoa," the three classes with medusa stages (Hydrozoa, Scyphozoa, and Cubozoa), regardless of treatment of alignment-dependent positions. The data with alignment-dependent positions removed consisted of 534 positions, 90 of which were informative. Aligned sequences with alignment-dependent positions retained consisted of between 663 and 670 positions, with between 180 and 186 positions informative. The alignment-stable sequence yielded 10 most parsimonious cladograms, whose strict consensus is shown in figure 2. The data set produced by combining alignments with gap costs of one through eight, retaining alignment-dependent positions, specified a single cladogram, also with the Hydrozoa, Scyphozoa, and Cubozoa grouped together (not shown). Finally, individual sequence alignments produced using gap costs of 1–6 and 8 also supported grouping these three classes, with the Anthozoa basal (not shown).

Two features of the tree based on alignment-stable data were unexpected. First, the placozoan *Trichoplax* was placed within the Cnidaria, not as an outgroup to the phylum. Second, the Anthozoa were paraphyletic,
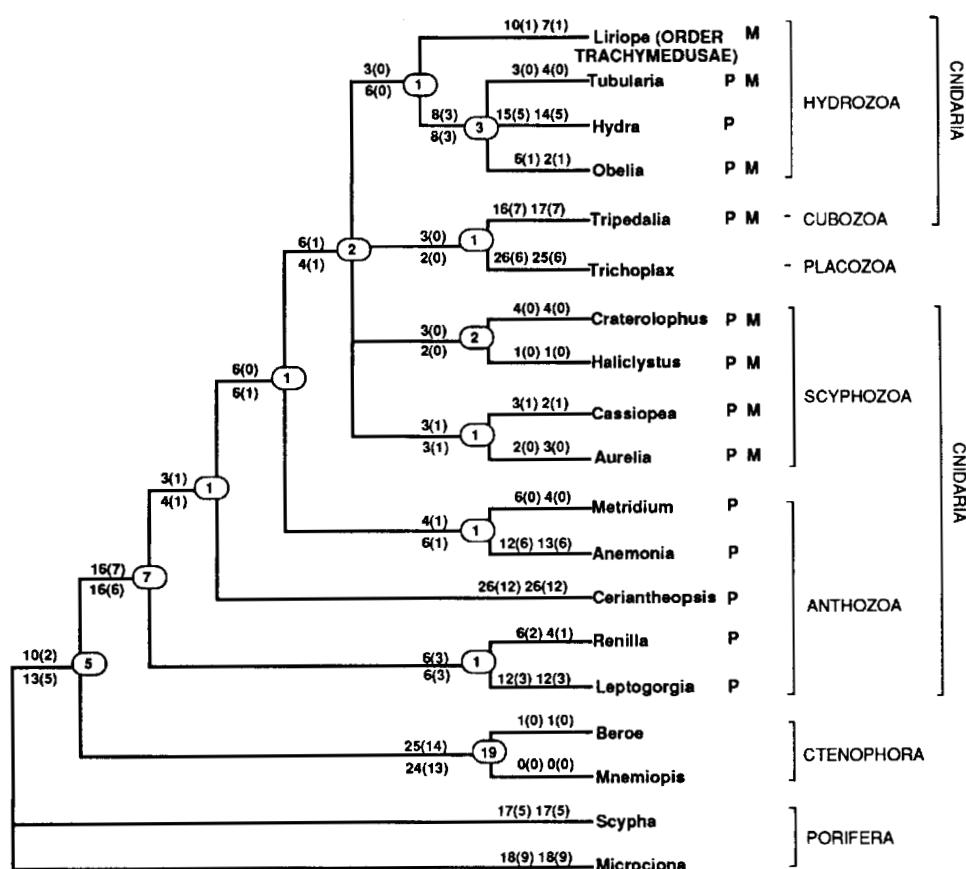
FIG. 2.—Consensus of cladograms from alignment-stable 18S rDNA sequence data. The strict consensus of 10 minimum-length trees is shown. Trees are 283 steps long, with CI (informative sites only) 0.540 and RI 0.620. The Bremer support (decay index), the number of additional steps in the shortest cladogram lacking a particular node (Bremer 1988; Donoghue et al. 1992) is shown at that node. Branch lengths for one of the cladograms, with DELTRAN character optimization, are shown above the branch. The number of nonhomoplasious changes are in parenthesis. Branch lengths with ACCTRAN optimization are also shown to give an idea of the range of branch lengths seen under different optimization methods; they appear below each branch, or to the right of the DELTRAN length for terminal branches; *P* indicates the presence of a polyp stage; *M* indicates the presence of a medusa stage (in some cases an identifiable reduced medusa stage which remains attached to the polyp). Note that the absence of the medusa in the genus *Hydra* is considered secondary (Hyman 1940, p. 635).

which has not been proposed based on morphology. The cladograms specified by the individual sequence alignments bear on these results, however. The alignments generated with gap weights in the middle of the range of gap weights used (three and four) yielded a single cladogram with *Trichoplax* outside the Cnidaria and Anthozoa monophyletic (not shown).

To assess the strength of competing hypotheses, the most parsimonious cladograms consistent with the "basal Hydrozoa" and the "basal Trachymedusae" (fig. 1*B*) views were determined. For the alignment-stable data the shortest trees that united the Anthozoa, Scyphozoa, and Cubozoa, leaving the Hydrozoa basal, were 287 steps long, four steps longer than the overall shortest trees. These cladograms included some with the representative of the hydrozoan order Trachymedusae basal, so this hypothesis also required four extra steps (table 2).

Morphological Data

The morphological data (App. A) yielded 110 most parsimonious arrangements, the strict consensus of which is shown in figure 3. In all the shortest trees the three classes possessing medusae formed a monophyletic group, in agreement with the Medusozoa hypothesis. The data provided less information about relationships within the Scyphozoa and the Anthozoa. Monophyly of the Scyphozoa was not supported because the Cubozoa share features with both the Scyphozoa and the Hydrozoa. Medusa nerve rings and the absence of septae in the polyp are shared with the Hydrozoa, while septae, gastric filaments, and rhopalia in the medusa are shared with some Scyphozoa. Monophyly of the Anthozoa was not supported because existing outgroups did not provide information about the polarity of potential synapomorphies such as siphoglyphs in the polyp.

Table 2
Length and Consistency Indices of the Most Parsimonious Trees Consistent with Different Phylogenetic Hypotheses

	18S rDNA		MORPHOLOGY		18S rDNA, 16S rDNA, MORPHOLOGY, MtDNA STRUCTURE		18S rDNA, MORPHOLOGY, MtDNA STRUCTURE	
	Steps	CI	Steps	CI	Steps	CI	Steps	CI
"Medusozoa"	283	0.540	38	0.842	500	0.634	329	0.570
Hydrozoa basal	287	0.530	40	0.800	502	0.630	332	0.564
Trachymedusae basal	287	0.530	40	0.800	503	0.629	333	0.562

To investigate the effects of treating characters present only in the medusa stage as missing (inapplicable) in the anthozoan representatives, an analysis without these seven characters (characters 17–19, 21, 25–27 in App. A) was also performed. The strict consensus of the most parsimonious cladograms was as shown in figure 3 except that the monophyly of *Haliclystus* and *Craterolophus* was not retained.

The most parsimonious trees with the Hydrozoa basal and the Anthozoa, Scyphozoa, and Cubozoa forming a monophyletic group were two steps longer than the shortest trees (table 2). A basal position for the hydrozoan order Trachymedusae also required two steps more than the shortest tree. The two alternative hypotheses still required two steps more than the most parsimonious cladogram when the medusa-specific characters were not included in the analysis.

Combined Data

When alignment-stable 18S rDNA sequence, morphological characters, and existing data on mtDNA genome structure (Bridge et al. 1992) were analyzed together, the combined data also supported monophyly of the three classes with medusae (fig. 4). The combined data provided better resolution than the individual data sets, yielding six most parsimonious cladograms. Although the separate data sets did not specify monophyly of the Anthozoa, the combined data did. The same six most parsimonious cladograms were obtained when the seven characters specific to the medusa stage and inapplicable for the Anthozoa were excluded.

The most parsimonious arrangements uniting the Anthozoa, Scyphozoa, and Cubozoa required three extra steps, while those with the Trachymedusae basal required four extra steps. The difference between the most parsimonious cladogram and the two alternative hypotheses was the same when the medusa-specific characters were not included in the analysis.

Data from the mitochondrial 16S rDNA gene were available for 11 of the 19 taxa studied (table 1). The 16S rDNA data with alignment-dependent positions removed consisted of 286 positions, 95 of which were informative. In order to root the cladograms obtained, alignment-stable 16S sequence was combined with 18S rDNA sequence, morphological characters, and data on mtDNA genome structure. Like the results of the previous phylogenetic analyses, the single minimum-length cladogram produced (not shown) agreed with the Medusozoa view. The classes with medusae represented in the analysis (Hydrozoa and Scyphozoa) formed a monophyletic group. The Hydrozoa were monophyletic, while the Scyphozoa and Anthozoa were paraphyletic.

Anthozoan paraphyly may be an artifact of the poorer taxonomic sampling in this analysis. To examine the effect of including only 11 taxa, the analysis of combined 18S rDNA, morphology, and mtDNA genome structure data was performed with the 11 taxa for which 16S data was available, as well as with 19 taxa. The analysis with 11 taxa included agreed with the 16S rDNA results in specifying Anthozoan paraphyly, with *Metridium* basal. Analysis of the same data for 19 taxa specified Anthozoan monophyly, as reported above.

Monophyly of the classes with medusae was still supported when the seven characters specific to the medusa stage and inapplicable for the Anthozoa were excluded. The shortest trees with the Hydrozoa basal were two steps longer than the minimum-length trees (table 2). A basal position for the Trachymedusae required three steps more than the shortest cladogram.

Discussion

The molecular and morphological data agreed in grouping the classes with medusae, the Hydrozoa, Scyphozoa, and Cubozoa. Combined analyses of rDNA sequences, morphological characters, and data
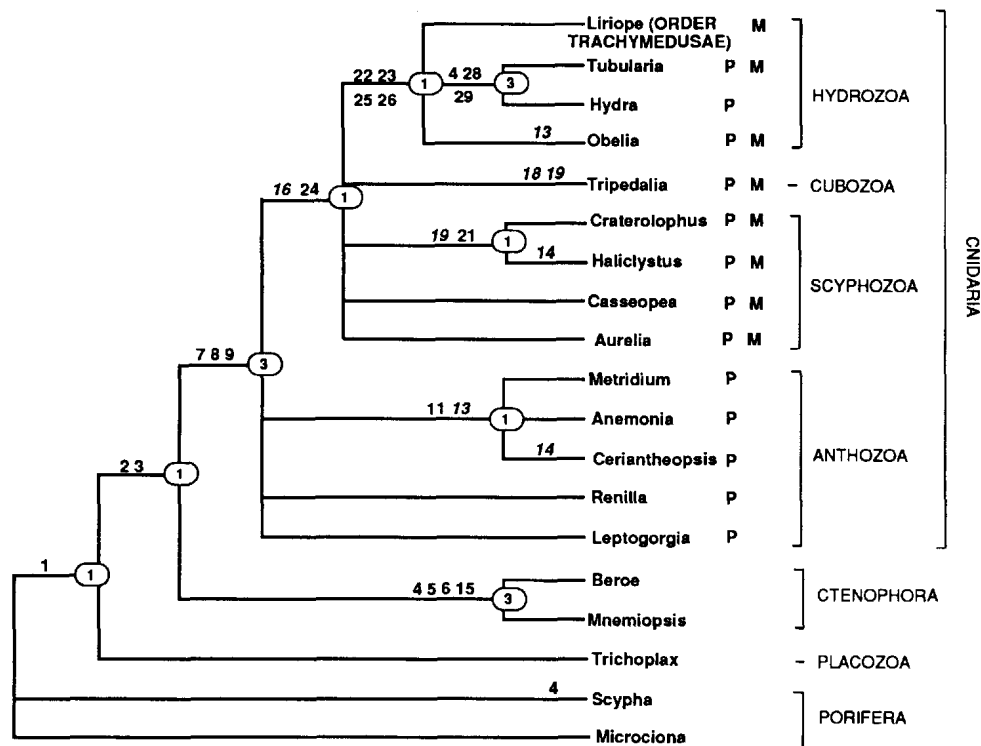
FIG. 3.—Consensus of cladograms from morphological data. The strict consensus of 110 minimum-length trees is shown. Trees are 38 steps long (CI, 0.842; RI, 0.918). The Bremer support of each node is shown at that node. The characters changing on each branch for one of the trees are listed above each branch. DELTRAN character optimization is shown, to favor appearance of character changes as autapomorphies. Homoplasious characters are italicized; *P* indicates the presence of a polyp stage, *M* the presence of a medusa stage.

on mtDNA genome structure also supported the Medusozoa hypothesis (fig. 1*A*). In addition, all analyses specified monophyly of the class Hydrozoa, in accordance with the Medusozoa hypothesis but not the alternative basal-Trachymedusae hypothesis (fig. 1*B*). Since cladograms consistent with the basal-Hydrozoa and basal-Trachymedusae views require only two to four steps more than the most parsimonious cladograms (table 2), additional data on this question will be of interest. However, the agreement of the different data sets is reassuring.

While this study focuses on resolution of relationships between the cnidarian classes, it is relevant to note that the cladogram nodes resolved by the combined data (fig. 4) are largely in agreement with previous, morphology-based ideas about relationships within the classes. Within the Hydrozoa, *Hydra vulgaris* and *Tubularia indivisa* (order Athecata) form the sister group of *Obelia dichotoma* (order Thecata), with *Liriope tetraphylla* (order Trachymedusae) basal, in accordance with Petersen (1979, 1990). Within the Scyphozoa, *Aurelia aurita* (order Semaeostomeae) and *Cassiopea* sp. (order Rhizoatomeae) group together, as do *Haliclystus* sp. and *Craterolophus convolvulus* (order Stauromedusae), in agreement with Thiel

(1966). The inability to group the cubozoan with either the Hydrozoa or the Scyphozoa reflects incongruence among relevant morphological characters and resulting disagreement between investigators (Werner 1975; Schuchert 1993*a*), as well as a need for additional data. Within the Anthozoa, *Anemonia sulcata* and *Metridium senile* (subclass Hexacorallia) are the sister group of *Ceriantheopsis americanus* (subclass Ceriantipatharia), and *Leptogorgia virgulata* and *Renilla muelleri* form a monophyletic group, in agreement with Hyman (1940, pp. 371–372) and Salvini-Plawen (1987).

The phylogenetic results support the hypothesis that cnidarian alternation of generations is derived from a polyp-only life cycle (fig. 1*A*). Transition from an anthozoan polyp-only life cycle to a polyp-medusa life cycle to a medusa-only life cycle requires two steps on the cladogram in figure 4. The alternative hypothesis of transitions from a plesiomorphic medusa-only life cycle requires at least one additional evolutionary event. A cladogram with the Anthozoa paraphyletic, like the ones generated by the alignment-stable 18S rDNA data set, would have provided even stronger support for the idea that the anthozoan life cycle is pleisomorphic. However, the more conventional tree produced using the combined 18S, morphological, and
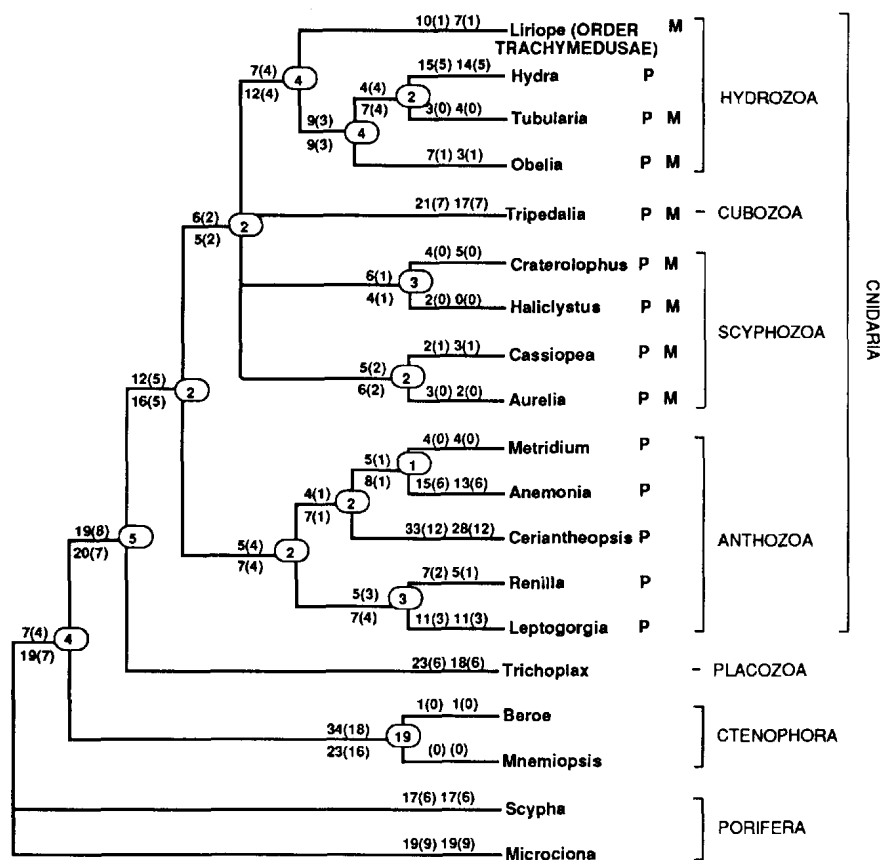
FIG. 4.—Consensus of cladograms from 18S rDNA, mtDNA structure, and morphological data. The strict consensus of six minimum-length trees is shown. Trees are 329 steps long (CI, 0.570; RI, 0.668). The Bremer support of each node is shown at that node. Branch lengths for one of the cladograms are as marked in fig. 2; *P* indicates the presence of a polyp stage, *M* the presence of a medusa stage.

mtDNA structure data also favors this long-debated hypothesis.

All cladograms are also consistent with a single origin for the medusa stage. It has been proposed that this life cycle stage, although unique to the Cnidaria, arose independently in the Hydrozoa and the Scyphozoa (Thiel 1966). Independent origins were proposed because the development, as well as the morphology, of the medusa is different in each class. The medusa is usually produced by budding from the side of the polyp in Hydrozoa, by metamorphosis of the whole polyp in Cubozoa, and by repeated transverse fission of the top of the polyp in Scyphozoa. Phylogenetic hypotheses grouping the Anthozoa, Scyphozoa, and Cubozoa (fig. 1B) imply either two gains of the medusa or a secondary loss of the stage, whereas the present analysis requires no homoplasy with respect to the medusa.

Life cycles and the related developmental phenomenon of asexual reproduction are remarkably diverse in the Cnidaria. However, it is difficult to investigate either the causes of this variation or its effects on morphological

evolution without evidence about cnidarian systematic relationships. Phylogenetic studies are necessary steps toward addressing the causes and effects of the "almost unbelievable protean plasticity" (Berrill 1949) seen in this phylum.

Acknowledgments

APPENDIX A
Table A1 Morphological Character Matrix

	5	10	15	20	25	....
Scypha	10000	0000N	0N01N	0NNNN	NNN0N	NN00
Microciona	10010	0000N	0N01N	0NNNN	NNN0N	NN00
Beroe	01131	1000N	0N012	0NNNN	NN00N	NN00
Mnemiopsis	01131	1000N	0N012	0NNNN	NN00N	NN00
Trichoplax	00??0	0000N	0N01N	0NNNN	NNN0N	NN00
Cassiopea	01110	01110	00010	11101	010?1	0000
Aurelia	01110	01110	00010	11101	01011	0000
Craterolophus	01110	01110	000?0	10011	110?1	0000
Haliclystus	01110	01110	00000	10011	110?1	0000
Tripedalia	01110	01110	00000	10110	01011	0100
Liriope	01110	0111N	0N0?0	1000N	001?0	1100
Tubularia	01120	01110	000?0	00000	001?0	1111
Hydra	01120	01110	00000	0NNN0	N011N	NN11
Obelia	01110	01110	001?0	00000	001?0	1100
Anemonia	01110	01111	111?1	0NNN0	N10?N	NN00
Metridium	01110	01111	11111	0NNN0	N100N	NN00
Cerantheopsis	01110	01111	11101	0NNN0	N10?N	NN00
Leptogorgia	01110	01111	010?1	0NNN0	N100N	NN00
Renilla	01110	01111	01011	0NNN0	N10?N	NN00

NOTE.—The matrix includes both binary and unordered multiple state characters. Character states assigned were 0, 1, 2, 3, ? (unknown), and N (inapplicable). The inapplicable state was assigned principally in cases where the life cycle stage showing the character was absent. Inapplicable states were treated as unknown in analysis. Presence or absence of specific nematocyst types and of cells in the mesoglea were coded as reported for the genus in question. The 29 columns correspond to the character numbers in the list below: (1) Multiple pores and canals with unidirectional water flow conducted with choanocytes (absent/present) (Brusca and Brusca 1990, p. 182), (2) Axial patterning and gut in adult (absent/present) (Brusca and Brusca 1990, pp. 171–279); (3) Nerve net with synapses (absent/present) (Brusca and Brusca 1990, pp. 171–279; Schuchert 1993*b*); (4) Amphiblastula: 0, planula (free-swimming gastrula): 1, juvenile released as polyp: 2, cydippid: 3 (Hyman 1940, pp. 672, 679; Campbell 1974; Bergquist 1978, pp. 105–112); (5) Multiciliate cells (absent/present) (Brusca and Brusca 1990, pp. 172, 882); (6) Colloblasts (absent/present) (Brusca and Brusca 1990, pp. 265–268); (7) Cnidocytes (absent/present) (Brusca and Brusca 1990, p. 214); (8) Tentacle ring surrounding mouth (absent/present) (Brusca and Brusca 1990, pp. 214–269); (9) Atrichous, basitrichous, or holotrichous isorhizae (absent/present) (Weill 1934; Westfall 1965; Werner 1975; Conklin and Mariscal 1976; Mariscal and Bigger 1976; Chapman 1978; Calder 1983; Bouillon 1985; Rifkin 1991); (10) Siphonoglyphs in polyp (absent/present) (Hyman 1940, pp. 538–632); (11) Spirocysts (absent/present) (references as for character 9); (12) Septae in polyp with gastrodermal musculature (absent/present) (Hyman 1940, pp. 369–372, Schuchert, 1993*a*); (13) Microbasic mastigophores (absent/present) (references as for character 9); (14) Cells in the mesoglea (Hyman 1940, p. 630; Grimstone et al. 1958; Chapman 1966; Dunkelberger and Watabe 1974; Bergquist 1978, p. 9; Chapman 1978; Grell and Ruthmann 1991; Hernandex-Nicaise 1991); (15) Gut lined with endoderm only: 0, actinopharynx: 1, true pharnyx: 2 (Hyman 1940, pp. 369–372; Brusca and Brusca 1990, pp. 171–279); (16) Microbasic euryteles (absent/present) (references as for character 9); (17) Strobilation producing ephyrae (absent/present) (Thiel 1966; Hyman 1940, pp. 366–372); (18) Rhopalia in medusa (absent/present) (Thiel 1966; Brusca and Brusca 1990, pp. 214–217); (19) Septae in medusa (absent/ present) (Thiel 1966; Hyman 1940, pp. 369–372); (20) Septae in polyp with ectodermally-derived musculature (absent/ present) (Hyman 1940, pp. 369–372; Werner 1975; Schuchert 1993); (21) Aboral, stalked adhesive disk in medusa (absent/ present) (Hyman 1940, pp. 369–372); (22) Nematocysts located in gut (absent/present) (references for character 9) (Hyman 1940, pp. 369–371); (23) Gonads derived from endoderm: 0, derived from ectoderm: 1 (Hyman 1940, pp. 369–372); (24) Cnidocil (absent/present) (Holstein and Hausmann 1988; Brusca and Brusca 1990; Schuchert 1993*a*); (25) Gastric filaments in medusa (absent/present) (Hyman 1940, pp. 369–372; Thiel 1966); (26) Velum in medusa (absent/present) (Hyman 1940, pp. 369–372, 413); (27) Nerve rings in medusa (absent/present) (Schuchert 1993*a*); (28) Stenoteles (absent/present) (references as for character 9); (29) Desmonemes (absent/present) (references as for character 9).

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