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Molecular Phylogenetics of Thecata (Hydrozoa, Cnidaria) Reveals Long-Term Maintenance of Life History Traits despite High Frequency of Recent Character Changes

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Abstract.—Two fundamental life cycle types are recognized among hydrozoan cnidarians, the benthic (generally colonial) polyp stage either producing pelagic sexual medusae or directly releasing gametes elaborated from an attached gonophore. The existence of intermediate forms, with polyps producing simple medusoids, has been classically considered compelling evidence in favor of phyletic gradualism. In order to gain insights about the evolution of hydrozoan life history traits, we inferred phylogenetic relationships of 142 species of Thecata (=Leptothecata, Leptomedusae), the most species-rich hydrozoan group, using 3 different ribosomal RNA markers (16S, 18S, and 28S). In conflict with morphology-derived classifications, most thecate species fell in 2 well-supported clades named here Statocysta and Macrocolonia. We inferred many independent medusa losses among Statocysta. Several instances of secondary regain of medusoids (but not of full medusa) from medusa-less ancestors were supported among Macrocolonia. Furthermore, life cycle character changes were significantly correlated with changes affecting colony shape. For both traits, changes did not reflect graded and progressive loss or gain of complexity. They were concentrated in recent branches, with intermediate character states being relatively short lived at a large evolutionary scale. This punctuational pattern supports the existence of 2 alternative stable evolutionary strategies: simple stolonal colonies with medusae (the ancestral strategy, seen in most Statocysta species) versus large complex colonies with fixed gonophores (the derived strategy, seen in most Macrocolonia species). Hypotheses of species selection are proposed to explain the apparent long-term stability of these life history traits despite a high frequency of character change. Notably, maintenance of the medusa across geological time in Statocysta might be due to higher extinction rates for species that have lost this dispersive stage. [Cnidaria; colony; Dollo's law; gradualism; Hydrozoa; Leptomedusae; Leptothecata; life cycle; phylogeny; punctuated evolution; reverse evolution; Thecata.]

Alternation of morphologically and ecologically divergent life stages, each produced by the previous one through sexual or asexual reproduction, is a common life history strategy in multicellular algae, plants, and fungi, but among animals, it is a rare phenomenon occurring only in Cnidaria and a few derived bilaterian lineages (e.g., cycliophorans, some parasitic flatworms, and some planktonic tunicates; Brusca R.C. and Brusca G.J. 2003). The "typical" life cycle of nonanthozoan cnidarians is indeed unique in comprising a planktonic medusa stage derived from a benthic polyp stage (metagenetic life cycle). In hydrozoans, medusae are issued from asexual buds (gonophores) produced on the body surface of a polyp. The medusa is capable of active swimming due to its bell shape and striated subumbrellar musculature. It grows by feeding on other planktonic animals and releases gametes, leading to the development of planula larvae. These will fix on the bottom and transform into a polyp, generally the founder individual of a polyp colony. According to recent molecular phylogenies (Marques and Collins 2004; Collins et al. 2006), the medusa stage was acquired in a common ancestor of Hydrozoa, Cubozoa, and Scyphozoa (i.e., the medusozoans). However, a large number (about 70%) of hydrozoan species lack the typical metagenetic life cycle, with the pelagic stage either absent (gametes being produced directly by polyps, in gonophores that do not develop into medusae and are thus called "fixed gonophores") or consisting in somewhat reduced and short-lived medusae called medusoids. Recent cladistic analyses (Petersen 1990) and molecular phylogenies (e.g., Cunningham and Buss 1993; Govindarajan et al. 2006) have indicated that medusa loss has occurred repeatedly in Hydrozoa, but several important aspects of hydrozoan life cycle evolution remain unexplored.

For example, medusoids have been traditionally considered as intermediate stages of medusa reduction and thereby hydrozoan life cycle evolution has been conceived as a typical instance of phyletic gradualism (see Boero and Sarà 1987), although these assumptions have never been tested explicitly. Medusoids are generally capable of swimming, but they are devoid of a functional mouth opening and often lack other anatomical structures usually found in medusae (e.g., gastrovascular system and tentacles) (Millard 1975; Boero and Sarà 1987; Cornelius 1990; Boero et al. 1997). The various medusoid morphotypes among living hydrozoans range from relatively complex anatomies (approaching full medusae) to very simple ones (more similar to fixed gonophores). Because each of them evokes a particular ontogenetic stage of medusa budding in species with full metagenetic life cycles, they have been interpreted as reflecting successive steps of medusa reduction through paedomorphosis (Boero and Sarà 1987; Boero et al. 1997). However, the existence of phenotypic intermediates does not necessarily imply that shifts between the extreme states follow a gradualistic pattern (see Blackburn 1995, 1998). Under the phyletic gradualism model, a continuum of character states should exist among living species, and clades should contain

species representing primitive (e.g., medusa), intermediate (e.g., medusoid), and advanced (e.g., no pelagic stage) evolutionary stages in paraphyletic arrangement (Blackburn 1995). Therefore, a primary objective of the present study was to test the gradualist hypothesis of hydrozoan life cycle evolution under a phylogenetic framework.

Furthermore, the high number of convergent medusa losses/reductions observed among hydrozoans strongly suggests that getting rid of the pelagic stage is positively selected under some circumstances, leading Cornelius (1990) to underscore a challenging paradox: "if medusa loss is advantageous, and if it can evolve easily, then why have not all recent forms dispensed with the medusa long ago?" To revisit the Cornelius paradox, we should first consider the directionality of character change. A heterodox explanation to the paradox could be that, in addition to being repeatedly lost, medusae can also been frequently regained. That such re-acquisitions from medusa-less ancestors might have taken place at least occasionally has been suggested for the somewhat unusual medusae of *Obelia* (Boero and Bouillon 1987; Boero and Sarà 1987; but this hypothesis was refuted by phylogenetic analyses: Govindarajan et al. 2006) and more recently for the simple medusoids of Plumularioidea (Boero and Bouillon 1989), in this latter case with support from molecular phylogeny (Leclère et al. 2007). However, the frequency and potential impact at the macroevolutionary level of such regains remain to be evaluated. Another pivotal issue regarding the Cornelius paradox is whether or not macroevolutionary forces favoring retention of the medusa are operating above the species level. For example, medusa-less species might undergo higher extinction rates than metagenetic species as a result of lower dispersal capacity. Finally, previous studies (Naumov 1960; Cornelius 1990) have indicated that small annual colonies with a stolonal organization (polyps being directly connected to a creeping stolon) tend to release medusae, whereas larger perennial colonies with relatively complex branching patterns generally lack a pelagic stage. This observation suggests that the 2 extreme types of life cycles might indeed represent aspects of 2 more generally different ecological strategies, but this correlation between life cycle and colony architecture characters remains to be tested phylogenetically.

We chose Thecata (=Leptothecata, Leptomedusae), a subclade of Hydrozoa, as a model taxon to investigate the evolution of hydrozoan life history traits. Thecata includes more than half of all known extant hydrozoan species (with almost 2000 species and 32 families), is present in all marine environments worldwide, and comprises the greatest diversity of life cycles (including species with medusoids of various types) and of colony architecture found among hydrozoans. Unlike the polyps of other hydrozoans, Thecata polyps are surrounded and protected by a chitinous exoskeleton called a theca, and Thecata medusae have their mature gametes located under the radial canals. Thecata colonies usually present a zooid polymorphism with specialization of polyps according to their function, that is, gastrozooid (nutrition), gonozooid (reproduction), and dactylozooid (defense of the colony). The 2 first polyp types are present in almost all species of thecates, but dactylozooids are present in only some thecate groups (Plumularioidea, *Hydrodendron*, and some species within Campanulinidae and Lafoeidae).

As is the case for other hydrozoan groups, the thecates have a confused taxonomic history with current morphology-based classifications resulting from a compromise between earlier separate polyp- and medusa-based systems (Naumov 1960; Bouillon 1985; Petersen 1990; Bouillon et al. 2006). Indeed, until the second part of the 20th century, 2 classification systems coexisted, with 2 names for the 2 parts of the life cycle, 1 for the polyp (classification based on the thecae and the colony shape) and 1 for the medusae (classification based on the medusae sense organs). A high level of incongruence between these 2 systems rendered the synthesis particularly difficult owing to different patterns and rates of character evolution between both semaphoronts (a phenomenon previously called "mosaic evolution" or "inconsistent evolution;" Morton 1957; Boero and Bouillon 1987). A united classification can now be elaborated with molecular characters as a data source independent from polyp and medusa morphology, and this is one of the desired outcomes of this study.

We analyzed evolutionary patterns of life cycle and colony shape characters in Thecata on a phylogeny reconstructed from 3 different molecular markers (16S ribosomal RNA [rRNA], 18S rRNA, and 28S rRNA) for 119 species. The well-resolved phylogeny enabled us to reconstruct the evolution of life cycle and colony architecture and to examine correlations between these characters. We demonstrate that the evolution of these life history traits does not follow a phyletic gradualism model. We show that life cycle and colony shape character changes are correlated and that they determine 2 alternative evolutionary stable strategies. Based on these findings, we propose an explanation for the Cornelius paradox invoking species selection.

Material and Methods

Taxonomic Sampling

The total number of sampled species of Thecata is 142, supplemented by 16 nonthecate outgroup species. We generated new sequences from 92 thecate samples; 9 new sequences from the CnidToL project were provided by P. Cartwright. Additional sequences were retrieved from the following previous studies: Leclère et al. (2007) for 16S rRNA, Collins et al. (2006) for 28S and 18S rRNA, and Govindarajan et al. (2006) for 16S and 18S rRNA (see Appendix 1). The newly sequenced specimens were collected and identified to the species level by PS, usually with voucher samples deposited in the Museum of Natural History of Geneva (Switzerland).

We estimated the representativeness of the ingroup taxonomic sampling with respect to the known taxonomic and morphological diversity of Thecata (Bouillon et al. 2006). The sampled species represent 22 of 32 currently recognized families. All thecate families that comprise more than 10 species are sampled in the present study, with the exception of Syntheciidae. Species with a pelagic stage in their life cycle are slightly overrepresented, with 28.2% (33 sp.), 9.4% (11 sp.), and 62.3% (73 sp.) of our species sampling having, respectively, a cycle with medusa, with medusoid, or without a pelagic phase, compared with the estimated proportions of 17.8% (346 sp.), 2.2% (44 sp.), and 79.9% (1550 sp.) for all described species of Thecata (Bouillon et al. 2006). The main weakness of our taxonomic sampling is the under-representation of Lafoeidae (only 1 species sampled, *Lafoea dumosa*) and Syntheciidae (no species sampled), 2 families that comprise only species with fixed gonophores.

The 16 outgroup species comprise 11 nonthecate species of Hydroidolina and 5 species belonging to Trachylina, the latter being included only in 18S and 28S rRNA analyses but not in the 16S rRNA analyses to avoid topological artefacts due to extreme sequence divergence of the trachylines for this marker (Leclère et al. 2007).

Sequencing

Total genomic DNA was extracted using the cetyl trimethylammonium bromide method (Coffroth et al. 1992). The primers used for 16S amplification are described in Cunningham and Buss (1993). 18S primers are the same as used in Govindarajan et al. (2006) (18SFb 5′GCTGTATGTACTGTGAAACTGCG3′; 18SRb 5′CACCTACGGAAACCTTGTTACGAC3′). Two partial 28S rRNA fragments of about 2000 bp each were amplified using, respectively (F10, R2077) and (F1379, R3264) as primer sets (F10: 5′TCCCCTAGTAACGGCGAGTGAAGCG3′; other primers are from Medina et al. 2001).

Polymerase chain reactions (PCRs) were performed in a total volume of 50 μl with 5 μl 10 × PCR buffer (Bioline, London, UK), 0.3 μl BioTaq polymerase (Bioline, London, UK), 1 μl 10 mM deoxyribonucleotide, 1.5 μl 50 mM MgCl2, 1 μl 10 μM forward primer, and 1 μl 10 μM reverse primer. PCR cycles comprised an initial denaturation step at 94˚C for 2 min followed by 30 cycles of 1 min at 94˚C, 1 min at the annealing temperature, and 3 min at 72˚C. There was then a final extension for 10 min at 72˚C. Annealing temperatures were 47˚C for 16S rRNA amplification, 55 ˚C for 18S rRNA amplification, and 63˚C for 28S rRNA amplifications.

Sequencing was done at the Genoscope (the French National Sequencing Centre, Evry, France) with the primers used for PCR amplification. To complete the sequencing of the 18S PCR fragment, 2 internal primers were designed (18SR1028:5′CTGCGAAAGCATTTGCCAAG3′ and 18SF970:5′CTAGGACGGTATCTGATCGTCTTCG3′). Likewise, 28S sequences were obtained by using the F780, F1379, and R1379 internal primers for the first fragment and the F2077, F2800, and R2800 internal primers for the second fragment (F780: 5′ACCCGTCTTGAAACACGGACCAAGG3′; R1379: 5′CCATGGCCACCGTCCTGCTGTC3′; F2077: 5′AACTTCGGGAAAAGGATTGGCTC3′; other primers are from Medina et al. 2001 and Voigt et al. 2004). 18S and 28S rRNA sequences from *Clytia hemisphaerica* were retrieved from expressed sequence tag sequence data available for this species. Forward and reverse sequences were assembled in BioEdit (Hall 1999).

Alignment and Combination of Data Sets

The different data sets (16S, 18S, and 28S) were aligned independently with the software MUSCLE (Edgar 2004) under default parameters. Final positional homology was derived by visual adjustment in BioEdit with reference to secondary structure models available for *Hydra circumcincta* 18S rRNA (Medina et al. 2001, AF358080), *Hydra vulgaris* 16S rRNA (Pont-Kingdon et al. 2000, AF100773), and *Saccharomyces cerevisiae* 28S rRNA (comparative RNA Web site http://www.rna.icmb.utexas.edu, Cannone et al. 2002). Optimal secondary structures for the most highly variable positions were calculated using MFold v3.0 under default parameters (Zuker 2003; http://frontend.bioinfo.rpi.edu/applications/mfold/cgi-bin/rna-form1-2.3.cgi). Nonalignable loops were removed from the alignments.

Positions containing more than 33% of gaps and/or missing data were deleted. Based on preliminary (not shown) analyses of the data set, we defined empirically this value as a reasonable compromise for eliminating parts of the alignment where primary homology is doubtful, but at the same time minimizing data loss. Excluding all sites with a single gap (or missing data) results in loosing a large amount of data because very few positions (less than 32%) have no gap or missing data. Conversely, keeping all positions would have the inconvenient to include a number of highly ambiguous alignment regions (especially in the 28S data set).

To avoid the existence of pairs of taxa with no data in common, species represented by only 1 of the 3 markers were not included in the combined data set (these are indicated by an asterisk in the separate analyses, Figs. 1 and 2, Supplementary Fig. 1 [available from http://www.oxfordjournals.org/our journals/sysbio/]). Indeed, our preliminary analyses indicated that pairs of taxa with no data in common tend to be attracted in the combined maximum likelihood (ML) tree. When a single species was represented by several sequenced samples in separate analyses forming a monophyletic group, only 1 (randomly chosen) sequence was retained for combined analyses. All sequence data sets have been deposited in TreeBASE (Study accession number S2436; Matrix accession number M4625-M4628). The number of sampled species in the combined (16S + 18S + 28S rRNA) alignment is 135 (including 119 species of Thecata and 16 outgroup species).

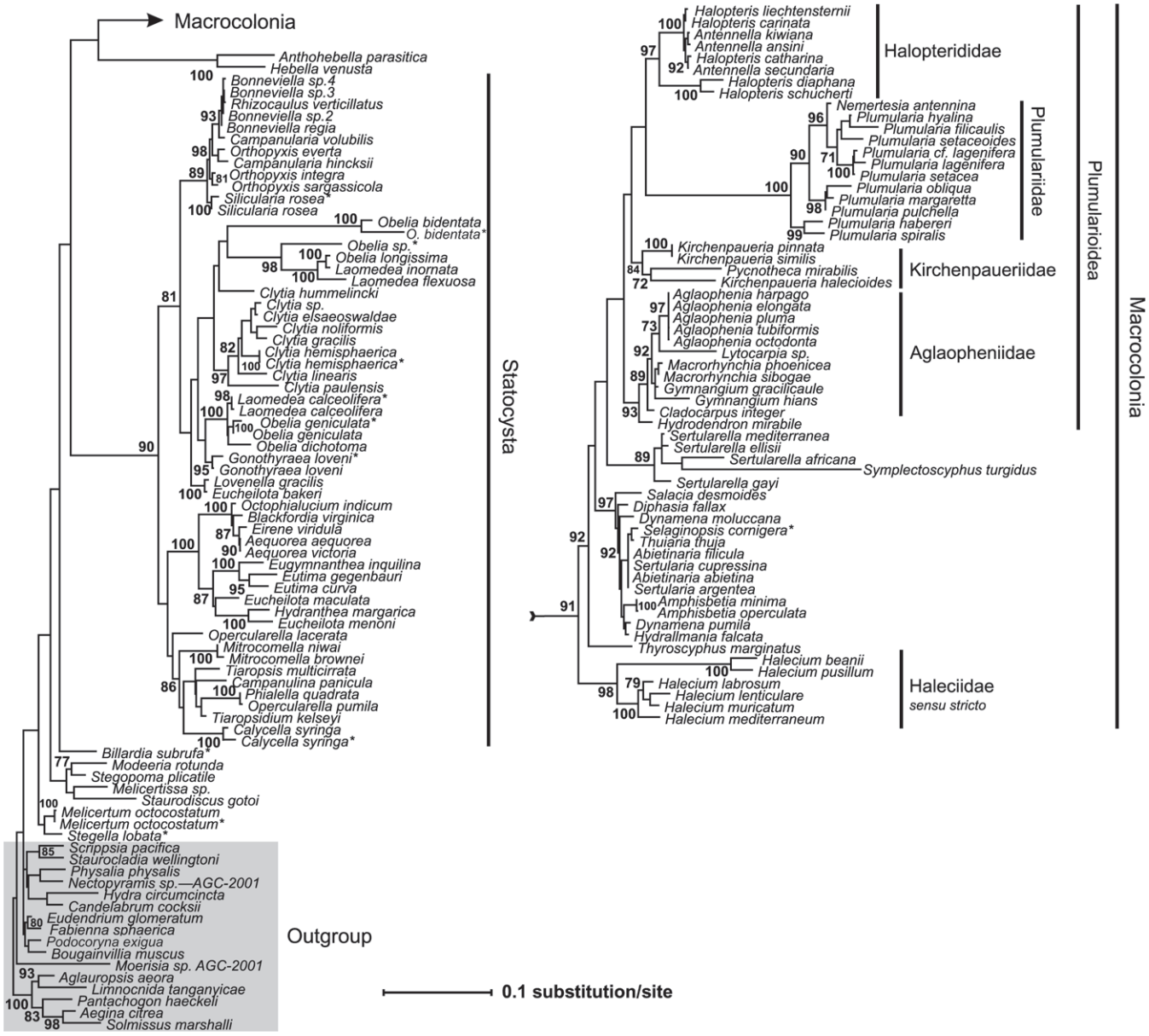

FIGURE 1. Phylogram of the ML analysis of 18S rRNA sequences under the GTR + G + I model. ML bootstrap values higher than 70% (500 bootstrap replicates) are indicated above or below branches (according to space available). Asterisks indicate sequences that were not included in the combined data set. Names of monophyletic higher level taxa comprising more than 3 samples are indicated.

Phylogenetic Analyses

Each data set was analyzed by maximum parsimony (MP), ML, and Bayesian inference. Details on analyzed data sets and estimated parameters and models are summarized in Table 1. The PhyML (Guidon and Gascuel 2003) program was used for ML analyses. Models of nucleotide evolution for 16S, 18S, and 28S rRNA and combined data sets were selected using the Akaike information criterion in the MrModelTest v.2 program (Nylander 2004). Among-site rate variation was estimated using a discrete approximation to the gamma distribution with 8 rate categories. The starting trees were BioNJ trees. MP analyses were performed using PAUP 4.1 (Swofford 1998). All characters were treated as equally weighted and unordered and gaps were treated as missing data. Heuristic analyses were performed with 500 random addition sequences of taxa and the TBR algorithm for branch swapping. Branch robustness in the MP and ML trees was estimated by bootstrapping (Felsenstein 1985) with 500 replicates (10 random addition sequences for each MP bootstrap replicate).

Bayesian analyses of the combined data set were performed using MrBayes 3.0b4 (Ronquist and Huelsenbeck 2003) using either a simple GTR + G + I model or partitioned (GTR + G + I) models for each marker (16S, 18S, and 28S rRNA). For each data set, 3 searches were run

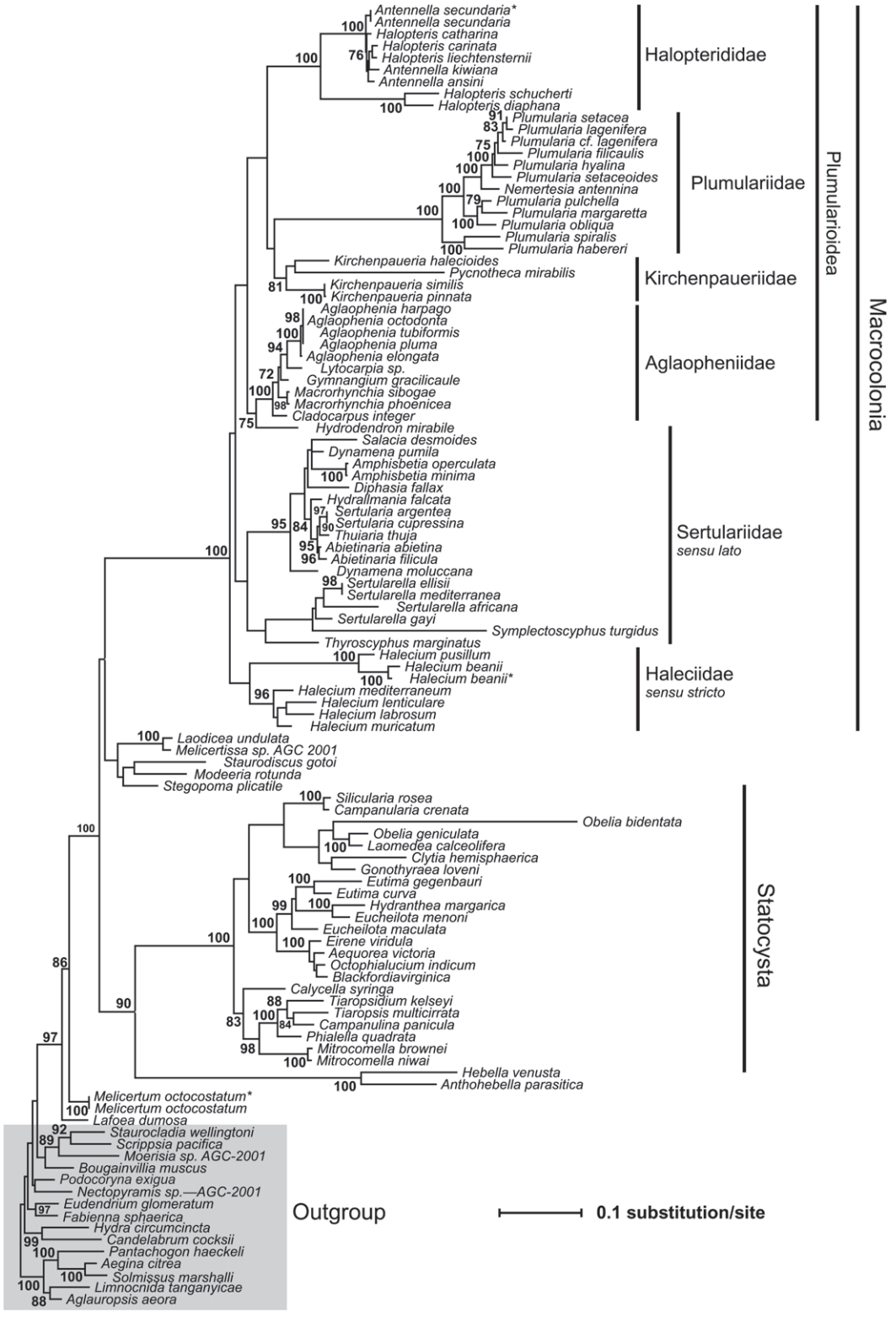

FIGURE 2. Phylogram of the ML analysis of 28S rRNA sequences under the GTR + G + I model. ML bootstrap values higher than 70% (500 bootstrap replicates) are indicated above or below branches (according to space available). Asterisks indicate sequences that were not included in the combined data set. Names of monophyletic higher level taxa comprising more than 3 samples are indicated.

TABLE 1. Information about data sets and analysis parameters, with proportion of invariant positions and α parameter of the Gamma distribution from ML analyses

	16S	18S	28S	Combined	Combined without *Symplectoscyphus tugidus*
Number of terminal taxa	149	144	109	135	134
Number of characters	488	1645	3074	5207	5207
Variable characters	319	711	1273	2257	2227
Parsimony informative characters	284	509	1027	1802	1791
% Guanine + Cytosine	32	47	48	47	47
Tree length (MP)	3155	4010	9831	16 549	16137
Number of minimal trees (MP)	14149	40526	2215	24	8
MrModelTest model	GTR + G + I	GTR + G + I	GTR + G + I	GTR + G + I	GTR + G + I
Proportion invariant	0.114	0.328	0.414	0.173	0.175
α parameter	0.340	0.352	0.369	0.250	0.247
-Ln likelihood	−14311.6	−22703.1	−47818.2	−83988.8	−82391.0

for 2 million generations and trees were sampled every 100 generations. We estimated convergence for each search by checking stasis of the "average standard deviation in partition frequency values across independent analyses" (with a threshold value of 0.03) and by checking stasis of the likelihood values (using the "sump" command). For all searches, convergence was already reached at 1,000,000 generations. Posterior probabilities were estimated by constructing a majority rule consensus of 1000 trees, sampled every 1000 generations from 1,000,000 to 2,000,000 generations. Finally, the consensus trees of the 3 searches were compared with check that they converged on the same topology.

Some alternative topological hypotheses were tested. Their likelihoods, obtained by constraining the ML analysis for each alternative hypothesis of interest (using PAUP, see Table 2), were compared with the likelihood of the best ML tree by the approximately unbiased (AU) test (Shimodaira 2002), using CONSEL (Shimodaira and Hasegawa 2001). Constrained analyses were also done using MP in PAUP to estimate differences in parsimony score between the best MP trees and the best trees obtained under particular topological constraints (Table 2).

TABLE 2. Results of topological constraints using MP and hypothesis testing using the AU test

	Steps	*P* AU test
(a) Groups that share the same life cycle		
Campanularia (FG)	11	0.025
Lafoea + Macrocolonia (FG)	54	<0.01
Medusoids of the Macrocolonia (MD)	603	<0.01
Eugymanthea + *Hydranthea* (MD)	38	<0.01
Laomedea (FG)	129	<0.01
Opercularella + *Calycella* + *Campanulina* (FG)	102	<0.01
(b) Taxonomic groups		
Plumularioidea without *Hydrodendron*	10	0.49
Campanulariidae	44	<0.01
Bonneviellidae	2	0.37
Lovenellidae	38	<0.01
Eirenidae	72	<0.01
Campanulinidae	102	<0.01
Mitricomidae	44	<0.01
Hebellidae	13	0.27
Conica	258	<0.01
Proboscoida	5	0.18

Note: "Steps" is the difference in number of steps between the best unconstrained MP tree and the best MP tree under constraint. $P < 0.05$ indicates rejection of the monophyly of the hypothetic clade under the AU test. In (a), we tested for the monophyly of a number of groupings that share the same life cycle type and are para- or polyphyletic in the ML analyses (Figs. 1–3) but without being very distantly related. In all cases, monophyly was rejected, thus supporting a maximal number of FG and MD acquisitions. In (b), we tested for the monophyly of some recognized taxonomic groups that were not monophyletic in the ML analyses (Figs. 1–3). FG = fixed gonophore; MD = medusoid.

Optimization of Morphological Characters on Molecular Trees

For character reconstruction, life cycle and colony shape characters were coded as multistate with the following character states: colony shape: "stolonal," "erect unbranched," "erect and branched;" type of life cycle: "with medusa," "with medusoid," "with fixed gonophore." The influence of character coding was checked by recoding these characters as binary ("presence/absence of fixed gonophore," "presence/absence of medusoid," etc.). This gave the same pattern of character changes as with multistate coding (not shown). For character correlation tests (see below), these unordered multistate characters were transformed into binary characters by combining states "erect unbranched" and "erect and branched" into "erect (branched or not)" and states "medusa" and "medusoid" into "presence of a pelagic sexual stage". Other morphological features of the polyp and medusa were coded as binary characters: dactylozooid (defensive polyp type)—present or absent, statocyst (gravitation sense organ of the medusae)—present or absent; thecae (chitinous cups surrounding the polyps)—present or absent; position of mature gametes in the medusa—on the radial canals or on the manubrium.

Parsimony and ML reconstructions of morphological character evolution and reweighting of character transformations were done with Mesquite (Maddison WP and Maddison DR 2009) on the tree resulting from the ML analysis of the combined data set (16S + 28S + 18S rRNA) recomputed after removal of *Symplectoscyphus turgidus*. Preliminary analyses indicated that excluding this long-branched species significantly increases support values of Plumulariida (Sertulariidae +Plumularioidea), Sertulariidae and (*Sertularella* +*Thyroscyphus*)

clades, without affecting topology and support in the rest of the tree. Prior to ML reconstruction, the molecular tree was ultrametrized (after exclusion of Trachylina species), using the command "ultrametrize" in Mesquite. Both the MK1 ("Markov *k* state 1 parameter model") and the AssymmMK ("Asymmetrical Markov *k* state 2 parameter model") models were used for ML reconstruction.

Bias in character changes in favor of recent events were estimated by comparing the number of events in terminal versus internal branches. Correlation between life cycle and colony form character changes (using binary recoding of the characters—see above) was estimated using 2 methods: (i) Pagel's (1994) ML test for association among discrete variables, as implemented in Mesquite. This method estimates transition rates from the data and uses a probabilistic model for inferring the likelihood of joint changes between 2 characters versus a model of independent evolution. The test was done on the ultrametrized combined ML tree (without *S. turgidus*). The *P* value was estimated from 1000 simulations. (ii) Bayesian approach to correlation of characters as implemented in BayesDiscrete (Pagel and Meade 2006) available through Pagel's BayesTraits software (http://www.evolution.rdg.ac.uk/). This method takes into account phylogenetic uncertainty in reconstructing ancestral characters by examining characters over a posterior distribution of trees rather than just on a single consensus tree. The test was done using trees from the Bayesian analysis of the combined data set without *S. turgidus* and the partitioned model.

Results

Levels of Resolution in the Separate and Combined Analyses

Levels of phylogenetic resolution in the ML analyses differed greatly between 16S and 28S/18S rRNA trees. In the 16S rRNA tree (Supplementary Fig. 1), Thecata is not monophyletic probably due to a long-branch attraction between Plumularioidea and the outgroup. As found in previous studies (Leclère et al. 2007; Moura et al. 2008), substitution rates in the 16S rRNA sequences are much higher within Plumularioidea than in other hydrozoans. Although internal nodes of Plumularioidea are well resolved in this 16S rRNA analysis (as in Leclère et al. 2007), for the rest of Thecata only a few external nodes are significantly supported. In contrast, the 18S and 28S rRNA analyses retrieved a well-supported Thecata clade and resolved many internal nodes with high statistical support (Figs. 1 and 2). The topologies resulting from 18S (Fig. 1) and 28S (Fig. 2) rRNA analyses are mostly congruent. There are nevertheless some significant differences including the paraphyly of Sertulariidae and the phylogenetic position of Hebellidae, Tiarannidae, and Laodiceidae species. Topologies resulting from the combined analyses of the 3 markers are very similar to those obtained through separate analyses of the 28S rRNA data set, with a high degree of resolution of internal relationships within Thecata.

Relationships among Thecate Hydrozoans

Monophyly of Thecata is strongly supported in all analyses, except when using 16S rRNA data alone. Most thecate species fall within 2 main clades (Figs. 1–3) which we call, respectively, Macrocolonia (from the large size of their colonies) and Statocysta (from their main synapomorphy, acquisition of gravitation sense organ, or statocysts, in the medusa). Macrocolonia comprises notably Plumularioidea and Haleciidae, and Statocysta includes Campanulariidae and Mitricomidae among other families (Fig. 3). A minority of species, belonging to the families Melicertidae, Tiaranidae, Laodiceidae, Hebellidae, and Lafoeidae, are positioned outside these 2 major clades. The classical subdivision of Thecata into Conica (Campanulariidae and Bonneviellidae) and Proboscoida (the rest of Thecata, see Bouillon 1984, 1985), based on the morphology of the oral region of the polyp, is clearly contradicted by our molecular analyses, as indicated by bootstrap values and phylogenetic tests (Table 2). In order to update the classification of Thecata, we propose here to phylogenetically define Macrocolonia as the least inclusive clade containing *Plumularia setacea*, *Dynamena pumila*, and *Halecium muricatum* and Statocysta as the least inclusive clade containing *C. hemisphaerica*, *Aequorea aequorea*, and *Phialella quadrata* (node-based definitions).

Within Macrocolonia, most of the recognized families (Bouillon et al. 2006) are monophyletic. The only unconventional results are *Hydrodendron mirabile* as the sister group of Aglaopheniidae, whereas it has previously been classified in Haleciidae, and the nesting of *Thyroscyphus marginatus* (Thyroscyphidae) within Sertulariidae in all analyses. The taxonomic position of Thyroscyphidae has been unclear—some authors (e.g., Bouillon et al. 2006) treat it as a separate family, whereas others include it in Sertulariidae (e.g., Millard 1975), consistent with our results.

We are confronted with a radically different situation within Statocysta because all families with at least 2 species sampled are para- or polyphyletic (with significant support from AU test—see Table 2), apart from Aequoreidae and Bonneviellidae. That the incongruence between the traditional taxonomy and our phylogenetic results is higher for Statocysta than Macrocolonia probably has to do with the prevalence of metagenetic life cycles in Statocysta, and with the long history of separate medusa and polyp-based classifications. Indeed, in Macrocolonia, the existence of the polyp stage but not of the medusa stage has made the classification easier to construct, and the relatively complex colonial organization has provided more taxonomic characters than with the medusae and simple colonies of Statocysta.

Evolution of Medusa Characters

A medusa must have been present in the ancestral life cycle of Thecata (Fig. 4). We infer 4 unambiguous simplifications (medusa transformed into a medusoid) and 13 complete losses of the pelagic stage (gametes produced

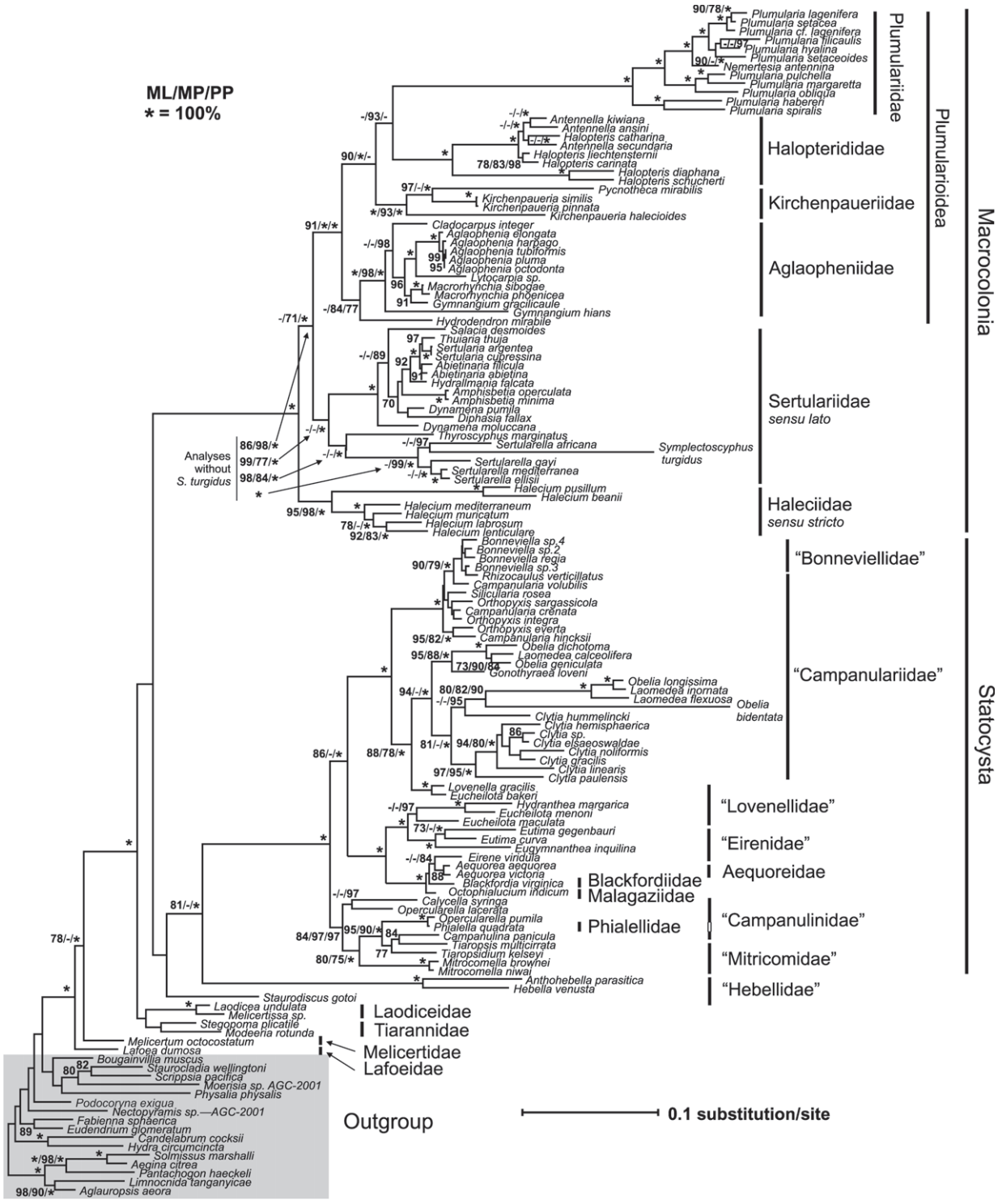

FIGURE 3. Phylogram of the ML analysis of the combined (16S + 18S + 28S rRNA) data set under the GTR + G + I model. ML bootstraps (left value), MP bootstraps (middle value), and Bayesian posterior probabilities (PP, right value) higher than 70% are indicated above or below branches (according to space available). These values are replaced by an asterisk when maximal (100%) and an asterisk alone means that all 3 values are maximal. In a few cases, only ML bootstrap values are shown because of lack of space. Supraspecific taxa are indicated by vertical lines. Note that Haleciidae sensu *stricto* correspond to Haleciidae without the genus *Hydrodendron;* Sertulariidae sensu *lato* include *Thyroscyphus marginatus*, sometimes classified in a separate family Thyroscyphidae (e.g., Bouillon et al. 2006) but classified by others as members of the Sertulariidae (e.g., Millard 1975).

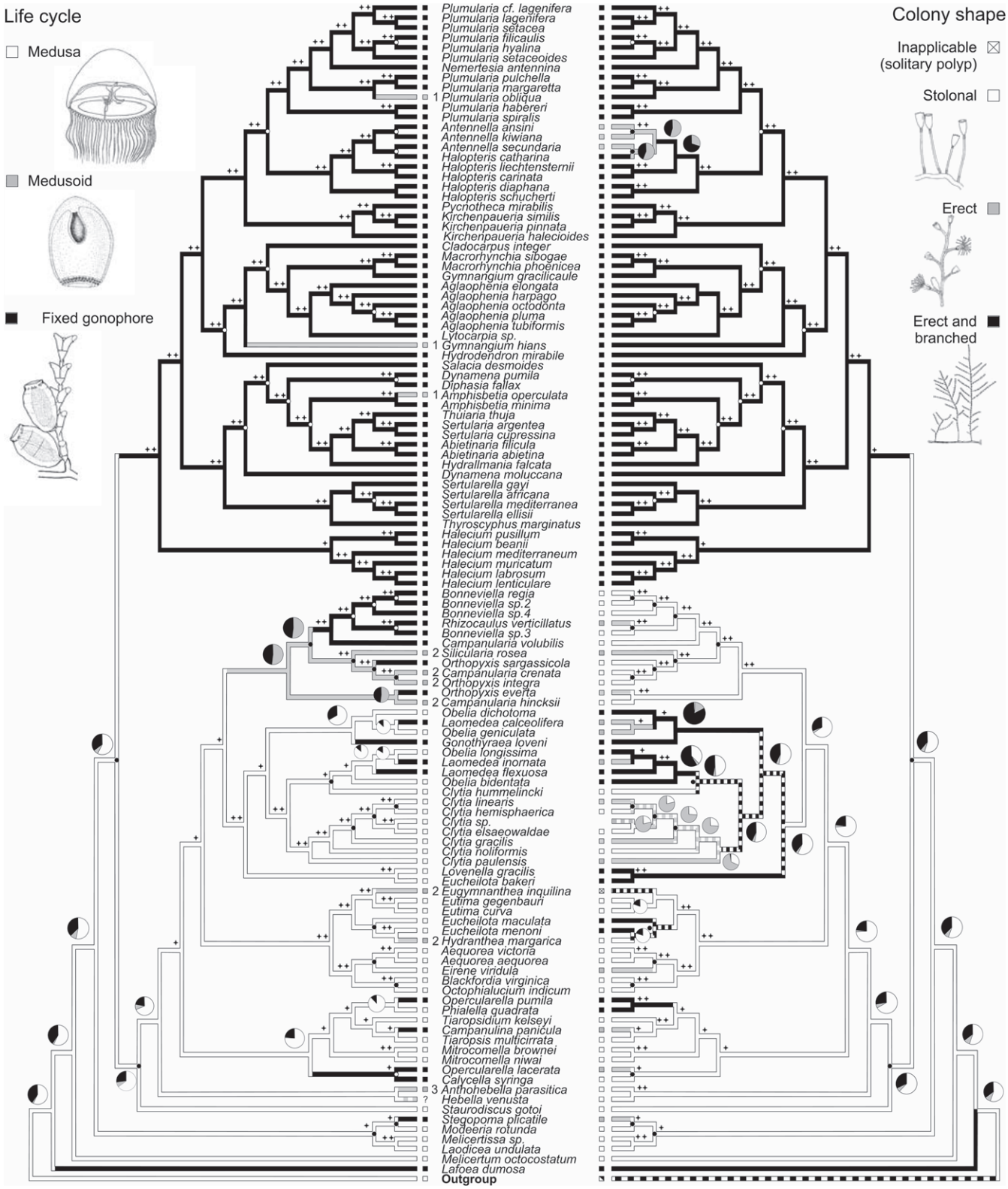

FIGURE 4. MP and ML ancestral character state reconstructions on the topology resulting from the ML analysis of the combined data set (tree in Fig. 3) for characters of the life cycle (left side) and of colony shape (right side). Nodes with less than 70% of ML bootstrap are indicated by a dot on the node. The MP reconstruction was unweighted and unordered, with ACCTRAN optimization. Branches labeled with vertical lines correspond to unknown character states. Plus indicate support from ML character reconstruction (Mk1 model) to the character state obtained by the MP analysis on the considered branch (a single plus: probability >90% and 2 plus: probability >95%).

(*Continued next page*)

by fixed gonophores). These scenarios are supported by unweighted parsimony and by the statistically supported polyphyly of groups with fixed gonophore or with medusoid (Table 2). The medusoid morphology is supported as an evolutionary intermediate between full medusa and absence of a pelagic stage in only 1 case (in the Bonneviellidae + Campanulariidae clade), but in all other instances among Statocysta, events of medusa reduction or loss are independent from each other.

Macrocolonia is characterized by an ancient loss of the pelagic stage in its ancestor. Within this clade, we infer 3 gains of medusoids from a plesiomorphic medusa less condition (Fig. 4), in 3 families: Aglaopheniidae (*Gymnagium hians*), Plumulariidae (*Plumularia obliqua*), and Sertulariidae (*Amphisbetia operculata)* (see Motz-Kossowska 1907; Tessier 1922; Bourmaud and Gravier-Bonnet 2004). According to the AssymMK model, gains have to be weighted at least 86 times more than losses to lose statistical support in favor of medusoid regains. In the MP reconstruction, gains are suppressed with a weight of gains at least 6 times higher than the weight of losses, and this implies at least 10 additional losses of the medusa within Macrocolonia with the 70% bootstrap ML tree (soft polytomy) and 13 with the unmodified ML tree. MP and ML estimates of the number and rate of loss and regains for medusae are summarized in Table 3.

The vast majority of the character transformations affecting life cycle evolution are concentrated in the terminal branches of the tree (Fig. 4). This holds true for medusa losses (10/13) as well as for medusa simplifications (3/4) and regains of medusoids (3/3).

Two types of medusae, anthomedusae, and leptomedusae are traditionally recognized in Hydrozoa, the latter type being considered characteristic for Thecata. Although anthomedusae have their mature gametes located on the manubrium (the stomach) and lack equilibration organs (statocysts), leptomedusae typically bear mature gametes along the radial canals and posses statocysts (Bouillon et al. 2006). The few instances of leptomedusae lacking statocysts have been considered derived (Bouillon 1984, 1985). Our optimizations place the transition of mature gametes localization (from the manubrium to the radial canals) in the common branch of Thecata (see Supplementary Fig. 2a), but this reconstruction is obtained only under ACCTRAN optimization because of the placement of *L. dumosa*, a species without medusa, as sister to the rest of Thecata. In addition, the localization of mature gametes reverted to a position on the manubrium in the medusoids of *Anthohebella parasitica* and *Hydranthea margarica*.

TABLE 3. MP and ML estimates of the number and rate of loss and regains for medusae

Rate	Estimated parameter	ML tree		70% bp ML tree
		MP	ML	MP
Loss = gain	Number of losses[a]	13	5	12
	Number of medusa simplifications[a]	4	4	4
	Number of regains[a]	3	3	3
	Rate of gain or loss	—	3.79	—
Loss ≠ gain	Rate of loss (α)	—	4.22	—
	Rate of gain (β)	—	3.38	—
	Number of loss if no regain	27	—	22
	Minimum ratio α/β to allow for medusa regain	1.7	26[b]	1.7
	Minimum ratio β/α to avoid medusoid regains	6	86[c]	6

[a]In the ML estimate, only changes involving 2 states with each more than 95% of likelihood are counted.
[b]Minimum ratio α/β, so that at least 1 branch with a 95% probability for "fixed gonophore" occurs in the ancestry of extant species with medusa.
[c]Minimum ratio β/α, so that all ancestor of extant species with medusoids of the Macrocolonia have less than 95% probability for the fixed gonophore state. All reconstructions were done on the ultrametrized ML tree using Mesquite.

Unexpectedly, our analyses obtained monophyly for species of thecates having medusae with statocysts (See Supplementary Fig. 2b) (clade Statocysta). Consequently, the absence of statocysts in some leptomedusae (e.g., *Staurodiscus gotoi, Modeeria secunda, Melicertum octocostatum*) is ancestral and not derived as traditionally thought (Bouillon 1984, 1985). It is interesting to note that, leaving apart those species that have completely lost their pelagic stage, there is no instance of statocyst loss among Statocysta because even species with highly simplified medusae (medusoids) have retained them. This contrasts with the medusoids of Macrocolonia, which lack statocysts.

Evolution of Colony Morphology and its Connection to Life Cycle Evolution

Adult colonies of Thecata have distinct shapes that can be classified into 3 major types: stolonal colonies (polyps are directly connected to the creeping common part of the colony), erect colonies with an unbranched stem (polyps on pedicels are borne on a vertical unbranched common axis) and erect and branched colonies (see drawings on Fig. 4, a branched stem bears the hydranths on pedicels). Here, colonies are inferred as ancestrally stolonal, and the erect unbranched and erect branched morphologies are derived. This is coherent with the reported ontogenetic sequence in which a

FIGURE 4. (*continued*.) When this value was lower than 90%, the ML reconstruction is shown in a pie chart. Pie charts indicate the relative degree of support for alternative character states. Illustrations of the characters are adapted from Bouillon et al. (2005). For terminal taxa with medusoids in their life cycle, numbers associated with the grey square indicate the type of medusoid (Millard 1975; Bouillon et al. 2006), from the most simple to most complex type: 1 = cryptomedusoid (possessing only the subumbrellar cavity, striated muscle used for swimming, and velum, but lacking gastro-vascular apparatus and tentacles); 2 = eumedusoid without tentacles (having a reduced gastrovascular system in addition to the structures present in cryptomedusoids); and 3 = eumedusoid with small tentacles (in addition to structures present in type 2). Note that the gonophores of *Gonothyraea loveni* have been described as cryptomedusoids (Bouillon et al. 2006), but as they are not liberated, they are simply coded here as fixed gonophores. More generally, there is also a described gradation in the complexity of fixed gonophore anatomies, but as data are available for only a limited number of species, we did not take this into account.

stolonal stage precedes the erect stage in all species with a stem (Bryant 1991).

Colony shape is highly stable within Macrocolonia, a clade characterized by a synapomorphic transition from the stolonal to the erect and branched morphology (Fig. 4), with reversion(s) to an unbranched stem occurring only in the genus *Antennella* and perhaps in species of *Halecium* (not sampled). There are very rare instances of reversion to the stolonal condition in Sertulariidae, but these species are not represented in our analysis. The situation is radically different within Statocysta, with at least 10 inferred transitions from the stolonal to the erect form. In addition, the stolonal morphology of some species of *Clytia* (notably *C. hemisphaerica*) seems to result from a reversion from the erect branched state. However, this conclusion is not statistically supported by ML optimization (<95%, Fig. 4). As for life cycle types, most changes affecting colony shape are located in the terminal branches of the tree (15/20, Fig. 4), and notably, the acquisitions of the intermediate state (erect unbranched) were significantly concentrated in terminal branches (8/10).

Some degree of correlation between life cycle and colony shape evolution can be suspected by comparing the patterns of evolutionary changes for these 2 characters (Fig. 4). We checked for this correlation using the Pagel's (1994) ML test and BayesTraits bayesian test for correlated (discrete) character evolution. The correlation was clearly supported by both methods (Pagel's ML test: $P < 0.001$ from 1000 simulations; BayesTraits test: $P < 0.001$ from 5,000,000 generations with 50,000 discarded as burn in). Medusae tend to be associated with stolonal colonies and fixed gonophores with erect colonies.

Evolution of Other Important Polyp Characters

A classical synapomorphy of Thecata is the acquisition of the theca, a usually rigid, skeletal chitinous envelope surrounding the polyps. In our taxonomic sampling, thecae are missing in all non-Thecata hydrozoans as well as in 2 species of Thecata, *M. octocostatum* and *Eugymanthea inquilina*. In the latter species, absence of thecae is unambiguously the result of a loss (Supplementary Fig. 2c), easily explainable by its endobiotic life style within mussels. The case of *M. octocostatum* is more problematic because of its basal position among Thecata. The absence of thecae in *M. octocostatum* and its presence in *L. dumosa* (sister group of all other Thecata in our combined analyses) result in an uncertainty for this character state at the base of Thecata (Supplementary Fig. 2c). In addition, the branching order between *Lafoea, Melicertum* and the other thecates remains uncertain (low support values in this study: Figs. 1–3; incongruent topologies between MP and ML analyses in Cartwright et al. 2008). However, in 18S rRNA analyses (Fig. 1), *M. octocostatum* is the sister group of *Stegella lobata* (excluded from the combined analysis because of missing data for 16S and 28S rRNAs), a species with thecae. This observation, together with the presence of small thecae in another species of *Melicertum* (*Melicertum campanula*, Gemmill 1921) favors the assumption that thecae were present in the last common ancestor of Thecata.

According to our character optimization (Supplementary Fig. 2d), defensive polyps (dactylozoids) were acquired only once within Macrocolonia in the ancestor of Plumularioidea (which includes *Hydrodendron*) in conflict with a previous phylogenetic study suggesting their convergence between Plumularioidea and *Hydrodendron* (Leclère et al. 2007). In our tree (Fig. 3), however, there were probably 2 additional acquisitions of defensive polyps outside Macrocolonia, in some species of *Lovenella* and in some members of Lafoeidae (Bouillon et al. 2006), but these events cannot be inferred from our trees because dactylozooid-bearing species of these taxa were not included in our analyses.

Discussion

Our combined analyses of 16S, 18S, and 28S rRNAs provide a well-resolved phylogeny of Thecata, strongly supporting the hypothesis that a majority of Thecata species belong to 2 highly diversified monophyletic groups, Macrocolonia (large erect and branched colonies) and Statocysta (medusae with statocysts). These conclusions are in agreement with the topology retrieved in a recently published phylogeny of hydrozoans (Cartwright et al. 2008), based on the same molecular markers but with a much more reduced sampling of Thecata (25 species) than ours.

Patterns of Life Cycle Character Transformations in Thecate Hydrozoans

The traditional view that the ancestral life cycle of Thecata comprised an alternation between polyp and medusa stages, with medusa less life cycles (as in most species of Macrocolonia) representing a derived situation, is clearly confirmed by our character optimization onto the molecular tree, although with low ML support (Fig. 4). Absence of medusae in the ancestor of Thecata would imply convergence between thecate and nonthecate hydromedusae, a highly unlikely scenario as they share many common anatomical features absent in scyphomedusae and cubomedusae (velum, 2 nerve rings, 4 radial canals, tentacle bulbs, etc.). Complete loss of the pelagic stage is inferred to have occurred at least 13 times within Thecata, the convergent nature of these losses being strongly supported by AU tests rejecting the monophyly of medusa less taxa (Table 2).

Simple medusae devoid of a mouth opening (medusoids) have been acquired through 2 different evolutionary pathways, according to our analyses (Fig. 4), that is, either by medusa simplification or by *de novo* re-acquisition of a pelagic stage from medusa-less ancestors. Thus, outside Macrocolonia, medusoids clearly represent secondarily simplified medusae (e.g., in species of *Orthopyxis* or in *H. margarica,* see Cornelius 1992). These simplifications are believed to have occurred through paedomorphosis (more specifically, progenesis,

Gould 1977), that is, truncation of medusa development (Boero and Bouillon 1987; Boero et al. 1997). By contrast, the medusoids occurring in some species of Macrocolonia (*A. operculata*, *G. hians*, *and P. obliqua*) are probably not secondary simplified medusae but simple pelagic forms re-acquired independently and repeatedly from polyp-only ancestors, as already demonstrated in a recent phylogenetic study of Plumularioidea (Leclère et al. 2007). These medusoids can thus be considered swimming gonophores.

In fact, these analyses probably underestimate the number of medusoid acquisitions among Macrocolonia because there are additional species with medusoids that were not included in the combined molecular phylogeny. Notably, the 2 plumularioid species, *Macrorhynchia philippina* and *Dentitheca bidentata* for which we have only 16S rRNA data, produce medusoids (Gravier 1970; Migotto and Marques 1999; Bourmaud and Gravier-Bonnet 2004) but do not group with other species with medusoids of Macrocolonia in the 16S rRNA analyses (Supplementary Fig. 1). Furthermore, there are several described species of Macrocolonia with medusoids that were not sequenced here (*Sertularia loculosa*, *Sertularia turbinata*, *Sertularia marginata*, Migotto 1998; *Sertularella diaphana* and *Sertularella* sp., Gravier-Bonnet and Lebon 2002; *Gymangium ferlusi* and *Hydrodendron* sp., Gravier-Bonnet personal communication; and *Nemalecium lighti*, Gravier-Bonnet and Migotto 2000), these species being taxonomically distant from each other and from species with medusoids sampled in this study, at least according to their colony and polyp morphology.

Medusoid evolution from polyp-only ancestors in Macrocolonia offers an interesting example of "reverse evolution" (Teotónio and Rose 2001), with re-acquisition of a set of characters (most notably, the striated musculature of the medusa bell and the velum, a muscular membrane characteristic for hydrozoan medusae) that had been previously lost (Leclère et al. 2007). That such re-evolution of complex features is possible (against the famous "Dollo's law," Dollo 1893, see Gould 1970; Collin and Miglietta 2008) is gaining more and more credibility from recent phylogenetic studies, as illustrated by the proposed re-acquisition of pelagic larvae in echinoderms (Hart et al. 1997, but see Cunningham 1999), of a coiled shell in *Crepidula* (Collin and Cipriani 2003), of posterior limbs in some cetaceans (Bejder and Hall 2002), or of the wings in stick insects (Whiting et al. 2003; but see Trueman et al. 2004).

However, in the case of life cycle in Macrocolonia, this reverse evolution is incomplete because there is no documented case of reversal to a fully developed medusa capable of feeding and growing autonomously. Notably, a previous suggestion that the morphologically aberrant medusa of *Obelia* evolved through a reversal from a medusa less ancestor (Boero and Sarà 1987) is contradicted by our character optimization (Fig. 4), supporting instead the homology between the medusae of *Obelia* and of other Hydrozoa, in agreement with a published molecular phylogeny of the Campanulariidae (Govindarajan et al. 2006).

A major weakness of all proposals of "complex" character re-acquisition cited above (including ours) is the incapacity to evaluate the relative probabilities of loss and gain for these structures, independently from the phylogeny. Insights into the genetic factors conditioning complex structure loss and re-acquisition are expected to come from comparative studies of developmental genes. For example, the investigation in various thecate species with medusae and with medusoids of genes such as *Mef2* and *Snail*, previously identified as striated muscle markers in the *Podocoryna* medusa (Spring et al. 2002), could help test the hypothesis that re-acquisition of a pelagic stage involved the re-activation of a genetic program still persisting in species with fixed gonophores but functioning in other developmental contexts (Leclère et al. 2007).

Life Cycle Evolution does not Follow the "Phyletic Gradualism" Model

Our projection of character evolution onto the molecular phylogeny (Fig. 4) clearly indicates that life cycle evolution in Thecata does not conform to a "phyletic gradualism" model. Under phyletic gradualism, we would expect to observe clades containing successively branching species in ancestral, intermediate, and advanced evolutionary stages (Blackburn 1995). This is not the emerging pattern (Fig. 4) because most medusa suppressions (8/11) fail to be preceded by a medusoid evolutionary stage. The same conclusion emerges from the phylogenetic distribution of medusoid morphotypes, which differ from each other in terms of anatomical complexity (Fig. 4, see Materials and Methods section for details). These medusoid types (labeled 1, 2, and 3 from more simple to more complex on Fig. 4) are clearly dispersed in the tree rather than being arranged in evolutionary sequences. They correspond to independent simplifications of the medusa (in non-Macrocolonia) and independent re-acquisitions from a polyp-only ancestor (in Macrocolonia), not to transitional forms on the way toward medusa suppression. That species sampling was not exhaustive cannot explain this pattern because species with medusoid are largely overrepresented in this study and because all genera with medusoids described among non-Macrocolonia have been included in the data set with the exception of the 2 monospecific genera *Tripoma* and *Clathrozoon* (Bouillon et al. 2006). Phyletic gradualism for life cycle evolution is, however, supported in a single case, in the "*Campanularia/Bonneviella/Orthopyxis*" clade, among Statocysta (Fig. 4), with a succession of full medusa (in the outgroup), medusoid (acquired in the common branch of the clade), and fixed gonophore (acquired 3 times independently). Branch lengths in this clade are very short (see Fig. 3), suggesting that these transitions occurred within a very short time frame relatively to the antiquity of Thecata.

An additional striking characteristic of life cycle evolution among Thecata is the concentration of character changes in terminal branches (Fig. 4). This is true of most

(8/10) suppressions of the medusa stage among Statocysta (Fig. 4). A particularly persuasive case of rapid suppression of the medusa stage concerns *Opercularella pumila* for which complete medusa loss happens in a very short terminal branch (cf. Figs. 3 and 4). Acquisitions of medusoids are also clearly concentrated in recent lineages among Statocysta (medusa reductions) as well as among Macrocolonia (transitions from fixed to swimming gonophore) (cf. Figs. 3 and 4). Because there is no reason to invoke an acceleration of the rate of character changes in recent times, this pattern reveals that life cycle type tends to be conserved in the long term within each of the main clades (Macrocolonia and Statocysta), through recurrent elimination of deviating lineages, and in particular that "intermediate" life cycles (with medusoids) are short-lived at a large evolutionary scale. The recent (not yet eliminated) losses inferred in our analyses would thus be the "tip of the iceberg" of all losses that have occurred in Thecata during their history.

Two Alternative Evolutionarily Stable Strategies in Thecata

The evolution of colony shape follows similar trends. All changes from (ancestral) stolonal to (derived) erect + branched colonies fail to be associated with the intermediate erect unbranched form (Fig. 4). The only potential instance of gradual evolution for colonial shape is observed among Statocysta in the *Obelia/Laomedea/Clytia* clade (Fig. 4), with parsimony reconstruction suggesting reverse gradual evolution from erect branched toward "stolonal," but this scenario is not statistically supported in the ML analysis (Fig. 4). Furthermore, transitions to the intermediate phenotype (erect unbranched) are concentrated in terminal/recent branches (Fig. 4), and thereby lineages harboring this intermediate type of colonies are short-lived.

Using Pagel's (1994) and BayesTraits Bayesian character correlation tests, we further demonstrated a correlation between character changes concerning the life cycle and colony structure. The correlation is not perfect but it is statistically significant. Consistent with our observed pattern, previous statistical analyses among extant species have demonstrated that stolonal colonies are often associated with the presence of a medusa, whereas complex colonies usually have fixed gonophores (Naumov 1960; Ryland and Warner 1986; Cornelius 1990).

These phylogenetic inferences thus support the existence among Thecata of 2 evolutionarily stable and mutually exclusive life history strategies, with intermediate situations possible but short-lived at the geological scale, and a low frequency of full transitions from one strategy to the other. The ancestral life history strategy (hereafter called "Strategy 1") involves stolonal colonies that are generally annual (Cornelius 1990) and produce numerous medusae, permitting long-range dispersal. The alternative (derived) strategy ("Strategy 2") is represented by large, erect, and branched colonies, usually perennial (Cornelius 1990) and having poor dispersal capacities (Cornelius 1990; Sommer 1992). Not only do they fail to liberate medusa and thus do not disperse their gametes across wide distances but they also tend to brood their embryos and to produce planula larvae that move very little and settle near the parent colony, sometimes with a special mucus thread connecting the released planulae with the parent colony (Hughes 1977; Sommer 1992), thus maximizing philopatry and local maintenance of the population. Strategy 1 is an investment in reproductive/dispersive tissue (medusa) to the reduction of colony and Strategy 2 is the investment of somatic tissue to the reduction of reproductive/dispersive function.

These distinctive dispersal characteristics are likely correlated with distinctive colony capacities with respect to competition for the substrate. The tree-shaped perennial colonies of Macrocolonia (Strategy 2) are optimized for long-term settlement on the substrate. The stolonal colonies of most Statocysta (Strategy 1) feature the typical morphology of runners exploring spatial refuges (Buss 1979; Jackson 1979), some species being furthermore specialized on living substrates (e.g., algae or other animals) where they encounter few competitors. Thus, using the terminology introduced by Grime (1977), we suggest as a stimulating hypothesis to be tested in future ecological studies that erect and branched colonies are of the competitive type, whereas stolonal colonies are either of the ruderal type (for species behaving as pioneers on free substrates or as runners) or of the stress-tolerant type (for specialist species, i.e., species adapted to overcome the arsenal deployed by their living substrate against epibiont settlement). A common feature of pioneers, runners, and specialist epibionts is that they reside in spatial refuges. They must constantly colonize new refuges to maintain viable populations, and this probably represents a selection pressure for the maintenance of high dispersal capacity.

A Punctuational Analogue at the Clade Level?

The phylogenetic pattern described here for life history traits of thecate hydrozoans closely matches the operational criteria presented by Blackburn (1995, 1998) as predictions derived from the "punctuated equilibrium model": character states are not arranged into evolutionary series between extreme states, intermediate states are evolutionarily short-lived, and character states present a bimodal distribution among living species (see Supplementary Fig. 3). The original theory of punctuated equilibrium (Eldredge and Gould 1972) describes the appearance of species in geological time in terms of rapid episodes of speciation associated with phenotypic changes, alternating with long periods of morphological stasis. Gould (2002) argued in favor of a generalization of the theory at larger scales and used the terms "punctuation above the species level" or "punctuational analogs in lineages" for macroevolutionary patterns "conceptually homologous" to punctuated equilibra, even though the proximal causes responsible for these patterns at the

species level and above the species level are clearly not the same. According to him, clade "nontrending" (i.e., conservativeness) is similar to stasis in the history of species. Likewise, concentration of phenotypic change in "very short episodes relative to periods of stability in basic design during the normal waxing and waning of clades" is similar to punctuation in the punctuated equilibrium model.

Empirical cases presented as instances of such punctuational patterns in the literature include transitions from oviparity to viviparity in squamates (Blackburn 1995, 1998), the evolution of larval types in metazoans (Wray 1995), and mitochondrial gene losses in angiosperms (Adams et al. 2002).

According to this conceptual framework, the 2 evolutionary stable strategies defined above for Thecata can be viewed as stasis analogs and the concentration of changes affecting life cycle and colony shape in recent branches as "punctuational" (as for multiple recent acquisitions of viviparity in squamates, Blackburn 1995, 1998). A particularly central claim in Gould's (2002) considerations about punctuated evolution above the species level is that character stability across evolutionary time, such as long-term maintenance of the medusa in Statocysta despite frequent recent losses, must be regarded as an active phenomenon calling for causal explanations.

The Causal Explanation of Macroevolutionary Patterns: Evidence for Species Selection in Thecata

As pointed out by Cornelius (1990, 1992), the high frequency of medusa loss and the absence of full reversals should have led long ago to the total disappearance of the medusa, which is clearly not the case for non-Macrocolonia thecates. The observed concentration of medusa losses in a relatively recent period suggests higher extinction rates for species having lost the medusa phase (see above). Therefore, the Cornelius paradox can be easily resolved by invoking species selection. This macroevolutionary process occurs when the extinction or diversification of a species is affected by fitness differences of heritable species-level traits (Grantham 1995; Duda and Palumbi 1999; Gould 2002). Medusa loss might be advantageous under some circumstances at the individual level (e.g., because more metabolic resources becomes available for colony growth) but it implies a drastic reduction in long-range dispersive capacity, with the probable consequence of limiting the geographic distribution of the species (an emergent species-level trait, Grantham 1995) as well as increasing its susceptibility to local environmental changes (Duda and Palumbi 1999) (a trait reducible to the organismic level, Grantham 1995). These lineages might rarely persist long enough for a shift to the other stable strategy to occur, that is, for the shape and biology of the colony to become adjusted to a life cycle without medusa.

Macrocolonia has successfully lost the medusa and shifted to Strategy 2. This group has undergone strong diversification (around 1350 known species belong to Macrocolonia, Bouillon et al. 2006), possibly because of a higher speciation rate than in species with medusa (Statocysta includes probably only about 380 described species, Bouillon et al. 2006). As pointed out by Duda and Palumbi (1999) in their phylogenetic study of dispersion modes (with or without planktonic phase) in the gastropod genus *Conus*, strong clade diversification following reduction of dispersal capacity is good evidence for species selection. Limited dispersal in Macrocolonia probably implies low levels of gene flow, and thereby frequent local speciation, as opposed to larger, panmictic populations in thecate species with medusa (as in most Statocysta). In this explanation, an emergent species-level trait (geographical range) affect an emergent component of species-level fitness (speciation rate) (a case of "class A" species sorting according to the classification of Grantham 1995). The hypotheses of species selection proposed here are closely akin to Jablonski's (1987) interpretation of macroevolutionary patterns in fossil gastropods, where planktotroph species have significantly larger geographic ranges, longer temporal duration, and lower speciation rates than nonplanktotroph species.

Finally, gains of medusoids inferred among Macrocolonia are relatively recent and concern only a few isolated species, suggesting that species of Macrocolonia with medusoid are eliminated in the long term. In addition, acquisition of a medusoid stage in a Macrocolonia species is not likely to increase dispersal significantly, as medusoids are very short-lived (a few hours) and liberate gametes just after their release (Bourmaud and Gravier-Bonnet 2004). Thus, rather than lower speciation rate, higher extinction rate probably explains the low prevalence of species with medusoids among Macrocolonia. Although transition to a life cycle with medusoids may be advantageous in the short term (e.g., because more eggs are produced, Tessier 1922), the concomitant loss of embryo brooding on the colony (a trait reducible to the organismic level, Grantham 1995) perhaps increases extinction rate in these species.

Prospects for future studies include field experiments designed to test our predictions about the ecological aspects of both evolutionary stable strategies identified among Thecata, as well as phylogenetic studies on other hydrozoan groups comprising a diversity of life cycle and colony architecture types (e.g., Capitata, Filifera, Aplanulata), to assess whether or not the macroevolutionary trends described here for the evolution of life history traits among thecates are generalizable to the whole class Hydrozoa.

Supplementary Material

Supplementary material can be found at: http://www.sysbio.oxfordjournals.org/.

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APPENDIX 1. List of taxa examined in this study, GenBank accession numbers (16S, 18S, 28S rRNA), voucher numbers and origin data

Order/family	Species	16S	18S	28S	Voucher MHNG	Geographic origin/reference
Aplanulata	*Candelabrum cocksii*	AY512520	AY920758	AY920796		Collins et al. (2006)
Aplanulata	*Hydra circumcincta*	AY512521	AF358080	AY026371		Collins et al. (2006)
Capitata	*Moerisia* sp.	AY512534	AF358083	AY920801		Collins et al. (2006)
Capitata	*Scrippsia pacifica*	AY512551	AF358091	AY920804		Collins et al. (2006)
Capitata	*Staurocladia wellingtoni*		FJ550523	FJ550376	INVE25379	Wellington, New Zealand
Filifera	*Bougainvillia muscus*		FJ550582	FJ550439		Roscoff, France
Filifera	*Eudendrium glomeratum*		FJ550583	FJ550440	INVE49717	Marseille, France
Filifera	*Podocoryna exigua*	AY512513	AF358092	AY920802		Collins et al. (2006)
Laingiomedusae	*Fabienna sphaerica*	AM183133	AY920767	AY920797		Collins et al. (2006)
Limnomedusae	*Aglauropsis aeora*		AY920754	AY920793		Collins et al. (2006)
Limnomedusae	*Limnocnida tanganyicae*		AY920755	AY920795		Collins et al. (2006)
Narcomedusae	*Aegina citrea*		AF358058	AY920789		Collins et al. (2006)
Narcomedusae	*Solmissus marshalli*		AF358060	AY920790		Collins et al. (2006)
Siphonophorae	*Nectopyramis* sp.	AY512512	AF358068	AY026377.		Collins et al. (2006)
Siphonophorae	*Physalia physalis*	AY935284	AF358066			Collins et al. (2006)

Continued.

Order/family	Species	**16S**	18S	28S	Voucher MHNG	Geographic origin/reference
Trachymedusae	*Pantachogon haeckeli*		AF358062	AY920792		Collins et al. (2006)
Aequoreidae	*Aequorea aequorea*	AY512518	AF358076			Collins et al. (2006)
Aequoreidae	*Aequorea victoria*	EU305469	AF358077	AY920799		Collins et al. (2006)
Aglaopheniidae	*Aglaophenia acacia*[a]	FJ550507			INVE37535	Ria de Ferrol, Galicia, Spain
Aglaopheniidae	*Aglaophenia elongata*	FJ550508	FJ550593	FJ550450	INVE37539	Isola del Giglio, Italy
Aglaopheniidae	*Aglaophenia harpago*	FJ550506	FJ550592	FJ550449	INVE37531	Giglio Island, Italy
Aglaopheniidae	*Aglaophenia latecarinata*[a]	DQ855936				Leclère et al. (2007)
Aglaopheniidae	*Aglaophenia octodonta*	DQ855915	FJ550541	FJ550397	INVE32875	Villefranche sur mer, France
Aglaopheniidae	*Aglaophenia parvula*[a]	DQ855914			INVE34013	Leclère et al. (2007)
Aglaopheniidae	*Aglaophenia picardi*[a]	AY787891				Leclère et al. (2007)
Aglaopheniidae	*Aglaophenia pluma*	DQ855916	FJ550542	FJ550398	INVE38220	Villefranche-sur-Mer, France
Aglaopheniidae	*Aglaophenia tubiformis*	DQ855917	FJ550543	FJ550399	INVE32960	Banyuls-sur-Mer, France
Aglaopheniidae	*Cladocarpus integer*	FJ550512	FJ550597	FJ550453	INVE48754	Raunefjord, Norway
Aglaopheniidae	*Gymnangium gracilicaule*	DQ855934	FJ550585	FJ550442	INVE36839	Nosy Ranj, Madagascar
Aglaopheniidae	*Gymnangium hians*	AY787922	Z86122		INVE32586	Pee Pee Island, Thailand
Aglaopheniidae	*Lytocarpia* sp.	FJ550505	FJ550591	FJ550448	INVE36828	Sakatia, Madagascar
Aglaopheniidae	*Macrorhynchia philippina*[a]	DQ855937				Leclère et al. (2007)
Aglaopheniidae	*Macrorhynchia phoenicea*	DQ855935	FJ550584	FJ550441	INVE36813	Sakatia, Madagascar
Aglaopheniidae	*Macrorhynchia sibogae*	FJ550500	FJ550586	FJ550443	INVE36832	Nosy Ranj, Madagascar
Blackfordiidae	*Blackfordia virginica*	AY512516	AF358078	AY920800		Collins et al. (2006)
Bonneviellidae	*Bonneviella regia*	AY789805	AY789740			Govindarajan et al. (2006)
Bonneviellidae	*Bonneviella* sp.2	AY789806	AY789741			Govindarajan et al. (2006)
Bonneviellidae	*Bonneviella* sp.3	AY789807	AY789742			Govindarajan et al. (2006))
Bonneviellidae	*Bonneviella* sp.4	AY789808	AY789743			Govindarajan et al. (2006)
Campanulariidae	*Campanularia crenata*	FJ550466		FJ550383		Wellington, New Zealand
Campanulariidae	*Campanularia hincksii*	AY789794	AY789729			Govindarajan et al. (2006)
Campanulariidae	*Campanularia volubilis*	AY789804	AY789739			Govindarajan et al. (2006)
Campanulariidae	*Clytia elsaeoswaldae*	DQ064793	DQ064796			Govindarajan et al. (2006)
Campanulariidae	*Clytia gracilis*	AY789811	AY789750			Govindarajan et al. (2006)
Campanulariidae	*Clytia hemisphaerica*		FJ550601	FJ550457		Villefranche-sur-mer, France
Campanulariidae	*C. hemisphaerica*[a]	AY789814	AY789753			Govindarajan et al. (2006)
Campanulariidae	*Clytia hummelincki*	AY789809	AY789744			Govindarajan et al. (2006)
Campanulariidae	*Clytia linearis*	AY789810	AY789748			Govindarajan et al. (2006)
Campanulariidae	*Clytia noliformis*	DQ064792	DQ064795			Govindarajan et al. (2006)
Campanulariidae	*Clytia paulensis*	AY346361	AY789746			Govindarajan et al. (2006)
Campanulariidae	*Clytia* sp.	AY800195	AF358074			Govindarajan et al. (2006)
Campanulariidae	*Gonothyraea loveni*	FJ550480	FJ550547	FJ550404	INVE29034	Sandgerdi, Iceland
Campanulariidae	*G. loveni*[a]	AY789826	AY789765			Govindarajan et al. (2006)
Campanulariidae	*Laomedea calceolifera*	FJ550504	FJ550590	FJ550447	INVE37296	Herquemoulin, France
Campanulariidae	*L. calceolifera*[a]	AY789829	AY789768			Govindarajan et al. (2006)
Campanulariidae	*Laomedea flexuosa*	AY789823	AY789762			Govindarajan et al. (2006)
Campanulariidae	*Laomedea inornata*	AY789822	AY789761			Govindarajan et al. (2006)
Campanulariidae	*Obelia bidentata*	FJ550503	FJ550589	FJ550446	INVE37294	Utah Beach, France
Campanulariidae	*O. bidentata*[a]	AY789815	AY789754			Govindarajan et al. (2006)
Campanulariidae	*Obelia dichotoma*	AY789828	AY789767			Govindarajan et al. (2006)
Campanulariidae	*Obelia geniculata*	FJ550481	FJ550548	FJ550405		Sandgerdi, Iceland
Campanulariidae	*O. geniculata*[a]	AY530359	AY789769			Govindarajan et al. (2006)
Campanulariidae	*Obelia longissima*	AY789821	AY789760			Govindarajan et al. (2006)
Campanulariidae	*Obelia* sp.[a]		Z86108			Collins et al. (2006)
Campanulariidae	*Orthopyxis everta*	AY789793	AY789728			Govindarajan et al. (2006)
Campanulariidae	*Orthopyxis integra*	AY789802	AY789737			Govindarajan et al. (2006)
Campanulariidae	*Orthopyxis sargassicola*	AY789795	AY789730			Govindarajan et al. (2006)
Campanulariidae	*Rhizocaulus verticillatus*	AY789803	AY789738			Govindarajan et al. (2006)
Campanulariidae	*Silicularia rosea*	FJ550482	FJ550549	FJ550406	INVE25072	Wellington, New Zealand
Campanulariidae	*S. rosea*[a]	AY789792	AY789727			Govindarajan et al. (2006)
Campanulinidae	*Billardia subrufa*[a]		AY789779			Govindarajan et al. (2006)
Campanulinidae	*Calycella syringa*	FJ550460	FJ550519	FJ550372		Roscoff, France
Campanulinidae	*C. syringa*[a]	AY789833	AY789776			Govindarajan et al. (2006)
Campanulinidae	*Campanulina panicula*	FJ550511	FJ550596	FJ550452	INVE48748	Korsfjord, Norway
Campanulinidae	*Opercularella lacerata*	FJ550509	FJ550594		INVE48734	Raunefjord, Norway
Campanulinidae	*Opercularella pumila*	AY789834	AY789777			Govindarajan et al. (2006)
Campanulinidae	*Stegella lobata*[a]		AY789778			Govindarajan et al. (2006)
Eirenidae	*Eirene viridula*	FJ550502	FJ550588	FJ550445		Luc-sur-mer, France
Eirenidae	*Eugymnanthea inquilina*	AY789832	AY789775			Govindarajan et al. (2006)
Eirenidae	*Eutima curva*	FJ550514	FJ550599	FJ550455	INVE33468	Devonport, New Zealand
Eirenidae	*Eutima gegenbauri*	FJ550515	FJ550600	FJ550456	INVE31748	Villefranche sur mer, France
Haleciidae	*Halecium beanii*[a]	FJ550477		FJ550400	INVE32968	Banyuls-sur-Mer, France
Haleciidae	*H. beanii*	FJ550488	FJ550560	FJ550417	INVE34009	Simons Town, South Africa
Haleciidae	*Halecium halecinum*[a]	FJ550463			INVE26671	Roscoff, France
Haleciidae	*Halecium labrosum*	AY787916	FJ550550	FJ550407	INVE29030	Gardur, Iceland
Haleciidae	*Halecium lenticulare*	FJ550469	FJ550532	FJ550387	INVE33461	Wellington, New Zealand
Haleciidae	*Halecium mediterraneum*	FJ550492	FJ550566	FJ550423	INVE34437	Calanque Port d'Alon, France
Haleciidae	*Halecium muricatum*	AY787915	FJ550551	FJ550408	INVE29028	Gardur, Iceland
Haleciidae	*Halecium pusillum*	FJ550499	FJ550580	FJ550437	INVE36295	Roscoff, France
Haleciidae	*Hydrodendron gardineri*[a]	AY787923				Leclère et al. (2007)
Haleciidae	*Hydrodendron mirabile*	DQ855933	FJ550568	FJ550425	INVE34779	Cantabria, Noja, Spain
Halopteridiae	*Antennella ansini*	FJ550470	FJ550533	FJ550388	INVE32157	Mallorca, Spain
Halopteridiae	*Antennella kiwiana*	DQ855918	FJ550534	FJ550389	INVE33623	Devonport, New Zealand

Continued.

Order/family	Species	16S	18S	28S	Voucher MHNG	Geographic origin/reference
Halopteridiae	*Antennella secundaria*	DQ883445	FJ550575	FJ550432	INVE32969	Banyuls-sur-Mer, France
Halopteridiae	*A. secundaria*[a]	FJ550467		FJ550384		Roscoff, France
Halopteridiae	*Halopteris alternata*[a]	DQ855939				Leclère et al. (2007)
Halopteridiae	*Halopteris carinata*	DQ855919	FJ550576	FJ550433	INVE35473	Honduras
Halopteridiae	*Halopteris catharina*	DQ855920	FJ550517	FJ550370		Roscoff, France
Halopteridiae	*Halopteris diaphana*	DQ855921	FJ550525	FJ550378	INVE30116	Mallorca, Spain
Halopteridiae	*Halopteris liechtensternii*		FJ550526	FJ550379	INVE30116	Mallorca, Spain
Halopteridiae	*Halopteris minuta*[a]	AY787912				Leclère et al. (2007)
Halopteridiae	*Halopteris polymorpha*[a]	DQ855922			INVE30117	Banyuls-sur-Mer, France
Halopteridiae	*Halopteris schucherti*		FJ550577	FJ550434	INVE35930	Punta Huinay, Chile
Halopteridiae	*Halopteris tenella*[a]	DQ855938				Leclère et al. (2007)
Halopteridiae	*Monostaechas quadridens*[a]	DQ855941				Leclère et al. (2007)
Hebellidae	*Anthohebella parasitica*	AY787918	EU272603	EU272545	INVE29762	Spain, Mallorca
Hebellidae	*Hebella venusta*	FJ550496	FJ550574	FJ550431	INVE35476	Honduras
Hebellidae	*Scandia gigas*[a]	AY787919				Leclère et al. (2007)
Hebellidae	*Staurodiscus gotoi*	FJ550472	FJ550535	FJ550391	INVE33467	Devonport, New Zealand
Kirchenpaueriidae	*Kirchenpaueria halecioides*	AY787895	FJ550530	FJ550385	INVE29766	Banyuls-sur-Mer, France
Kirchenpaueriidae	*Kirchenpaueria pinnata*	FJ550497	FJ550578	FJ550435	INVE36294	Roscoff, France
Kirchenpaueriidae	*Kirchenpaueria similis*	DQ855923	FJ550581	FJ550438	INVE36296	Roscoff, France
Kirchenpaueriidae	*Pycnotheca mirabilis*	FJ550465	FJ550529	FJ550382	INVE25847	Wellington, New Zealand
Lafoeidae	*Lafoea dumosa*	AY787917		EU305520		Govindarajan et al. (2006)
Laodiceidae	*Laodicea undulata*	FJ550471		FJ550390	INVE31753	Villefranche sur mer, France
Laodiceidae	*Melicertissa* sp.	AY512515	AF358075	AY920798		Collins et al. (2006)
Lovenellidae	*Eucheilota bakeri*	AY789831	AY789774			Govindarajan et al. (2006)
Lovenellidae	*Eucheilota maculata*	FJ550501	FJ550587	FJ550444		Luc-sur-mer, France
Lovenellidae	*Eucheilota menoni*	FJ550493	FJ550570	FJ550427	INVE33457	Motutapu Island, New Zealand
Lovenellidae	*Hydranthea margarica*	DQ855932	FJ550567	FJ550424		Las Negras, Andalucia, Spain
Lovenellidae	*Lovenella gracilis*	AY789830	AY789773			Govindarajan et al. (2006)
Malagazziidae	*Octophialucium indicum*		FJ550522	FJ550375	INVE29970	Wellington, New Zealand
Melicertidae	*Melicertum octocostatum*	FJ550510	FJ550595	FJ550451	INVE48744	Raunefjord, Norway
Melicertidae	*M. octocostatum*[a]	EU305479	AY920757	EU272575		Collins et al. (2006)
Mitrocomidae	*Mitrocomella brownei*		FJ550521	FJ550374		Roscoff, France
Mitrocomidae	*Mitrocomella niwai*	FJ550473	FJ550536	FJ550392		Devonport, New Zealand
Mitrocomidae	*Tiaropsidium kelseyi*	AY512517	AF358079	EU305537		Collins et al. (2006)
Mitrocomidae	*Tiaropsis multicirrata*	FJ550468	FJ550531	FJ550386		Sandgerdi, Iceland
Phialellidae	*Phialella quadrata*	FJ550474	FJ550537	FJ550393	INVE33466	Whangaparoa, New Zealand
Plumulariidae	*Dentitheca bidentata*[a]	DQ855942				Leclère et al. (2007)
Plumulariidae	*Nemertesia antennina*	FJ550458	FJ550516	FJ550369		Roscoff, France
Plumulariidae	*Nemertesia perrieri*[a]	DQ855925			INVE32971	Banyuls-sur-Mer, France
Plumulariidae	*Plumularia cf lagenifera*	FJ550491	FJ550564	FJ550421	INVE34019	Simons Town, South Africa
Plumulariidae	*Plumularia filicaulis*	DQ855926	FJ550565	FJ550422	INVE34020	Simons Town, South Africa
Plumulariidae	*Plumularia habereri*	DQ855927	FJ550571	FJ550428		Bunaken Island, Indonesia
Plumulariidae	*Plumularia hyalina*	AY787913	FJ550552	FJ550409	INVE25333	Goat Island, New Zealand
Plumulariidae	*Plumularia lagenifera*	DQ855928	FJ550527	FJ550380	INVE25120	Friday Harbour, USA
Plumulariidae	*Plumularia margaretta*	FJ550483	FJ550553	FJ550410	INVE29760	Mallorca, Spain
Plumulariidae	*Plumularia obliqua*	DQ855929	FJ550544	FJ550401		Banyuls-sur-Mer, France
Plumulariidae	*Plumularia pulchella*	DQ855930	FJ550562	FJ550419	INVE34016	Simons Town, South Africa
Plumulariidae	*Plumularia setacea*	FJ550459	FJ550518	FJ550371		Roscoff, France
Plumulariidae	*Plumularia setaceoides*	DQ855931	FJ550538	FJ550394	INVE33460	Wellington, New Zealand
Plumulariidae	*Plumularia spiralis*	AY787920	FJ550569	FJ550426	INVE32600	Koh Bida Nok, Thailand
Plumulariidae	*Plumularia strictocarpa*[a]	DQ855940				Leclère et al. (2007)
Sertulariidae	*Abietinaria abietina*	FJ550484	FJ550554	FJ550411	INVE29946	Gardur, Iceland
Sertulariidae	*Abietinaria filicula*	FJ550485	FJ550555	FJ550412	INVE29947	Gardur, Iceland
Sertulariidae	*Amphisbetia minima*	FJ550486	FJ550556	FJ550413	INVE25071	Devonport, New Zealand
Sertulariidae	*Amphisbetia operculata*	FJ550489	FJ550561	FJ550418	INVE34014	Simons Town, South Africa
Sertulariidae	*Diphasia fallax*	AY787901	FJ550557	FJ550414	INVE29950	Gardur, Iceland
Sertulariidae	*Dynamena moluccana*	FJ550494	FJ550572	FJ550429		Bunaken Island, Indonesia
Sertulariidae	*Dynamena pumila*	AY787902	FJ550558	FJ550415	INVE29026	Sandgerdi, Iceland
Sertulariidae	*Hydrallmania falcata*	FJ550487	FJ550559	FJ550416	INVE29948	Gardur, Iceland
Sertulariidae	*Salacia desmoides*	FJ550464	FJ550528	FJ550381		Banyuls-sur-Mer, France
Sertulariidae	*Selaginopsis cornigera*[a]		Z92899			Collins et al. (2006)
Sertulariidae	*Sertularella africana*	FJ550490	FJ550563	FJ550420	INVE34017	Simons Town, South Africa
Sertulariidae	*Sertularella ellisii*	FJ550478	FJ550545	FJ550402	INVE32156	Mallorca, Spain
Sertulariidae	*Sertularella gayi*		FJ550579	FJ550436	INVE36302	Roscoff, France
Sertulariidae	*Sertularella mediterranea*	FJ550479	FJ550546	FJ550403	INVE32948	Banyuls-sur-Mer, France
Sertulariidae	*Sertularella rugosa*[a]	AY787906				Leclère et al. (2007)
Sertulariidae	*Sertularia argentea*	FJ550461	FJ550520	FJ550373		Roscoff, France
Sertulariidae	*Sertularia cupressina*	FJ550475	FJ550539	FJ550395	INVE29949	Gardur, Iceland
Sertulariidae	*Symplectoscyphus tricuspidatus*[a]	AY787907				Leclère et al. (2007)
Sertulariidae	*Symplectoscyphus turgidus*	FJ550462	FJ550524	FJ550377	INVE29467	California, USA
Sertulariidae	*Thuiaria thuja*	AY787908	EU305503	EU305536		Collins et al. (2006)
Thyroscyphidae	*Thyroscyphus marginatus*	FJ550495	FJ550573	FJ550430	INVE35477	Honduras
Tiarannidae	*Modeeria rotunda*	FJ550476	FJ550540	FJ550396	INVE32967	Banyuls-sur-Mer, France
Tiarannidae	*Stegopoma plicatile*	FJ550513	FJ550598	FJ550454	INVE48755	Raunefjord, Norway

Note: MHNG = Muséum d'Histoire Naturelle de Genève. Taxa with geographic origin indicated are new samples obtained for this study.
[a]Sample not included in the combined data set.