Loss of metagenesis and evolution of a parasitic life style in a group of open ocean jellyfish

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Abstract

Loss or stark reduction of the free-swimming medusa or jellyfish stage is common in the cnidarian class Hydrozoa. In the hydrozoan clade Trachylina, however, many species do not possess a sessile polyp or hydroid stage. Trachylines inhabiting freshwater and coastal ecosystems (i.e., Limnomedusae) possess a metagenetic life cycle involving benthic, sessile polyp and free-swimming medusa. In contrast, the paradigm is that open ocean inhabiting, oceanic trachylines (in the orders Narcomedusae and Trachymedusae) develop from zygote to medusa via a free-swimming larva, forgoing the polyp stage. In some open ocean trachylines, development includes a sessile stage that is an ecto- or endoparasite of other oceanic organisms. We expand the molecular-based phylogenetic hypothesis of trachylines significantly, increasing taxon and molecular marker sampling. Using this comprehensive phylogenetic hypothesis in conjunction with character state reconstructions we enhance understanding of the evolution of life cycles in trachyline hydrozoans. We find that the polyp stage was lost at least twice independently, concurrent with a transition to an oceanic life style. Further, a sessile, polypoid parasitic stage arose once, rather than twice as current classification would imply, in the open ocean inhabiting Narcomedusae. Our results also support the hypothesis that interstitial species of the order Actinulida are directly descended from direct developing, oceanic trachylines.

Keywords

Trachymedusae, Narcomedusae, Limnomedusae, *Tomopteris*, life cycle evolution

1. Introduction

The succession of sessile polyp and free-swimming medusa stage found in the moon jellyfish *Aurelia aurita* (Medusozoa: Scyphozoa) is the archetypal example of indirect, metagenetic development in medusozoan cnidarians (see Ceh et al., 2015; Morandini et al., 2016; Ceh et al., 2017 for discussions of cnidarian metagenesis). However, not all medusozoan cnidarians follow this mode of development and their life cycles are diverse, characterized by a suite of morphologically disparate and complex traits (e.g., Cartwright and Nawrocki, 2010). In the medusozoan class Hydrozoa in particular, the metagenetic life cycle has undergone many alterations, including multiple losses and modifications of the medusa or polyp stage. Medusozoan life cycles have historically been used as the basis for systematic classification, but molecular phylogenetic analyses demonstrate that homoplasy of complex life cycle characters is prevalent among hydrozoans (Collins, 2002; Cartwright and Nawrocki, 2010), making the group an interesting clade for studying the evolution of complex life cycle traits (Leclère et al., 2016).

Loss or stark reduction of the free-swimming medusa stage is common across Hydrozoa, for example, as a possible response to environmental constraints (e.g., Boero and Sarà, 1987). Regain of the medusa (or a reduced medusoid) appears less common, but Cartwright and Nawrocki (2010) suggested two possible cases, one each among capitates and leptothecates (Hydroidolina), hypotheses that have yet to be tested using a better-resolved and supported phylogeny of Hydroidolina. In contrast, for the majority of species comprising the hydrozoan subclass Trachylina, the sister clade to Hydroidolina, the polyp stage is thought to have been lost or modified while the medusa was retained (Collins, 2002; Collins et al., 2008), possibly as an adaptation or prerequisite for a transition to an open ocean or deep-sea life style. Trachylines inhabiting freshwater and coastal ecosystems (i.e., Limnomedusae) possess a metagenetic life cycle involving a sessile (benthic) polyp and free-swimming medusa that is formed via lateral budding from the polyp (cf., Collins et al., 2008).

For most open ocean inhabiting trachylines belonging to Trachymedusae and Narcomedusae,

the paradigm is that they develop in the water column (holoplanktonic) from zygote to medusa via a free-swimming actinuloid larva, forgoing the intermediate sessile polyp stage (Mayer, 1910). In some open ocean trachylines, however, development is indirect, including a sessile stage that lives on or parasitizes cnidarian medusae or polychaetes (Damas, 1936; Bouillon, 1987; Pagès et al., 2007).

While it is commonly thought that open ocean trachylines develop directly in the water column, this notion is based on a very limited number of observations (c.f., Bouillon et al., 2006). Furthermore, the current classification scheme of trachylines suggests that parasitism evolved twice in Narcomedusae (within the families Cuninidae and Solmarisidae). To elucidate the patterns of life cycle evolution in trachyline hydrozoans and test the current paradigm, we reconstructed a comprehensive phylogenetic hypothesis for Trachylina. We used character state reconstructions to assess our confidence in the hypothesis that the last common ancestor (and by extension its extant descendants) of open ocean trachylines was a direct developer. We also address the question of how many times parasitism evolved among trachylines.

2. Materials and Methods

2.1 Taxon Sampling and Molecular Phylogenetics

Specimens were collected during several scientific cruises using Hydrobios (Altenholz, Germany) multinets and/or remotely operated vehicles (ROVs) including the Monterey Bay Aquarium Research Institute's ROVs *Tiburon*, *Ventana*, and *Doc Ricketts*. When possible, a piece of tentacle tissue was preserved in 95% EtOH for later DNA extraction while the remainder of the specimen was preserved in 5% formalin as a morphological voucher (Supplementary Table SI). DNA was extracted using organic phenol-chloroform extraction protocols, as described in Collins et al. (2008) and Lindsay et al. (2017). We amplified and sequenced near complete nuclear large (28S) and small ribosomal subunits (18S), and mitochondrial ribosomal 16S. Polymerase chain reactions (PCRs) were carried out in 10 µl aliquots that comprised final concentrations of 0.5 units Biolase DNA polymerase (Bioline USA Inc., Taunton,

MA), 0.3 mM of each primer, 0.5 mM dNTPs (Bioline), 1.5 mM magnesium chloride, 2.5x Bovine serum albumin (BSA) (New England BioLabs Inc., Ipswich, MA), and 1x Buffer, 1 µl template DNA, and DNAase-free H_20. Nuclear 28S and 18S were amplified as follows: 5 minutes at 94^{o}C for initial denaturation followed by 35 cycles of 94^{o}C for 30 seconds, annealing at 57^{o}C for 30 seconds, extension at 72^{o}C for 2 minutes, and a final extension of 72^{o}C for 7 minutes. The thermocycler profile for mitochondrial 16S was 94^{o}C for 5 minutes denaturation followed by 35 cycles of 94^{o}C for 30 seconds, 50^{o}C for 30 seconds annealing, 72^{o}C for 1 minute extension, and a final extension of 72^{o}C for 5 minutes. PCR products were cycle sequenced (see Collins et al., 2008 for primers) on an Applied Biosystems 3130xl Genetic Analyzer or Applied Biosystems 3730xl DNA Analyzer following Sephadex G-50 Fine (GE Healthcare Life Sciences, Pittsburgh, PA) clean-up in 96-well MultiScreenHTS-HV Plates (Millipore, Billerica, MA). Sequences were assembled in Geneious (various versions; Biomatters Limited, NZ).

Individual genes were aligned using MAFFT (v7.205; Katoh and Standley, 2013) and unreliably aligned positions were identified and excluded using Gblocks (Talavera and Castresana, 2007), as implemented in the alignment viewer Seaview (version 4; Gouy et al., 2010), allowing for smaller blocks, gap positions, and less strict flanking positions. This resulted in three separate alignments that were concatenated in Mesquite (version 3.04; Maddison and Maddison, 2017). For the concatenated dataset, PartitionFinder (version 1.1.0; Lanfear et al., 2012) was used to identify the best partitioning scheme for phylogenetic analyses. The possible partition schemes were specified as follows: no separate partitions, each marker (16S, 18S, and 28S) treated separately, or two partitions, one for the mitochondrial 16S and one for the nuclear operon (i.e., 18S plus 28S). PartitionFinder was run setting the models to be evaluated to the ones implemented in MrBayes (Ronquist et al., 2012) or RaxML (Stamkatakis, 2006) respectively. Selection of the partitioning scheme was done using the Bayesian information criterion.

The most appropriate model of sequence evolution for each alignment was inferred using the

24 default models of sequence evolution encoded in MrModeltest (v. 2.3; Nylander, 2008); log likelihoods of models were calculated in PAUP* (v. 4.0a152; Swofford, 2002) and compared using the Akaike Information Criterion (AIC), as implemented in MrModeltest. Both maximum likelihood (ML) and Bayesian inference (BI) were used to reconstruct trachyline relationships. Concatenated phylogenetic analyses were conducted with a hydroidolinan outgroup to establish the root of Trachylina. RAxML (v. 8.2.7; Stamkatakis, 2006) was used to search for the ML topology for which the data are most probable using one hundred independent searches (`-N 100`), starting from a random tree each (`-d`) and using the slow hill-climbing algorithm (`-f o`) to thoroughly explore the likelihood surface. Robustness of the best ML tree was estimated with 1,000 non-parametric bootstrap replicate searches. BI was performed using MrBayes (v. 3.2.5; Ronquist et al., 2012). For the concatenated dataset, 4 separate runs were used with 8 Markov chain Monte Carlo simulations. Every 1,000 generations trees were sampled; the first third of the samples were discarded as burn-in. MrBayes' automatic stop-rule was used to terminate the analysis as soon as the average standard split frequencies deviation among runs fell below 0.01. Single-gene phylogenies were reconstructed from alignments with the same outgroup taxa as the concatenated analyses. Here, BI was performed using 2 separate runs with 4 chains each. ML inferences used RaxML's rapid bootstrapping algorithm with 1,000 non-parametric bootstrap replicate searches, followed by a search for the best-scoring ML tree (`-f a`).

2.2 Patterns of Life Cycle Evolution

To understand the patterns of life cycle evolution we used stochastic character mapping (Huelsenbeck et al., 2003), as implemented in the R (R Core Team, 2015) package phytools (version 0.6-00; Revell, 2012). In short, ancestral state probabilities are reconstructed for every interior node of the phylogenetic tree. Using these probabilities, character states for each node of the tree are assigned. These states serve as starting and end points among which a realization of the evolutionary process

from ancestral to descendant node is simulated (Huelsenbeck et al., 2003 provide a detailed description). This process is repeated several times to generate a posterior probability distribution of character histories.

Characters were coded following an extensive literature review, during which we searched for descriptions of life cycles for trachyline hydrozoans; we provide primary data on the life cycle of endoparasitic Narcomedusae (see 3.4). Considering that several trachyline families and genera are para- or polyphyletic (Fig. 1 herein; Collins et al., 2008; Lindsay et al., 2017; Grange et al., 2017), life cycles were only scored as known when it was possible to match the species name (in rare cases the genus name) in our phylogeny to the species name used in the publication describing the life cycle. One caveat is that, in some instances, taxonomic judgments had to be made to trace the history of names and evaluate whether specimens of the same nominal species from different geographic locations are indeed conspecific (see Lindsay et al., 2017 for a discussion on this issue). Hydroidolinan outgroups were scored following the character states outlined in Cartwright and Nawrocki 2010.

Characters were classified into 3 possible states: 1) direct medusoid development, medusa or free-living medusoid present but polyp absent; 2) indirect metagenetic development, polyp/hydroid plus free-living medusa or medusoid present; 3) direct polypoid development, polyp/hydroid present but medusa or free-living medusoid absent. For Narcomedusae, we considered early parasitic life cycle stages as polyps for the following reasons. Similar to other polyps or hydroids with an indirect, metagenetic life cycle that includes a medusa stage, the early, parasitic stages of some Narcomedusae do not produce gametes, but clone themselves via budding. Here, the primary polypoid "larva" gives rise to numerous secondary polypoid "larvae" that metamorphose into medusae later (Bouillon, 1987). In that sense, the early life stages of parasitic Narcomedusae function as the asexual polyp generation in the biphasic life cycle.

For numerous trachylines, the complete life cycle remains unknown (see 3.3). However, at least one life cycle stage is known for each species, allowing us to assign prior probabilities to each

character state for each species. For example, in the case of Trachymedusae for which only the medusa stage is known we can assign a probability of 0 to state 3 (direct polypoid development) while the absence of a polyp stage cannot be ruled out. Hence, states 1 and 2 receive equal probabilities of 0.5. For cases in which the full life cycle is known the respective state receives a probability of 1. By encoding characters in this fashion, we were not only able to reconstruct the posterior probabilities of character states at internal nodes on the phylogeny but also at the tips for those species that have incompletely known life cycles.

Stochasitic character mapping was performed with phytools' `make.simmap` function, using 1,000 Markov Chain monte Carlo simulations, sampling the posterior distribution every 100 generations after burn-in. Phytools implements three transition rate models by default: equal rates (ER), symmetric rates (SYM), and all rates differ (ARD). The best fit transition rate model was chosen using the AIC after calculating the fit of a Mk model of discrete character evolution using the `fitMk` function for each transition rate model. The posterior distribution of character state histories was summarized using the `describe.simmap` function of phytools.

3. Results

3.1 Phylogenetic Systematics

In agreement with Collins et al. (2008), we find that the root of Trachylina is located between a clade comprising Limnomedusae plus the trachymedusan family Geryoniidae and a clade comprising members of Trachymedusae, Actinulida, and a strongly supported Narcomedusae (Fig. 1; Supplementary Fig. 1). Performing both ML (Fig. 1) and Bayesian (Supplementary Fig. 1) phylogenetic reconstructions, we found highly resolved, supported and congruent phylogenetic hypotheses for Trachylina using the combined dataset. We further increase support for the sister-relationship between Halicreatidae (Trachymedusae) and a clade comprising the remainder of Trachymedusae, Actinulida, and Narcomedusae (Fig. 1) compared to Collins et al. (2008). Individual

gene datasets show that 28S and 16S are most consistent with the results of the concatenated analysis (Supplementary Figs. 2, 3, 6; note that the consensus of the Bayesian analysis of 16S lacks resolution, congruent with the low support for relationships found in the ML analysis of 16S). 18S by contrast provides less resolution overall and places Rhopalonematidae plus Actinulida as sister to Halicreatidae and Narcomedusae (Supplementary Figs. 4 and 5).

Our highly-resolved and supported phylogenetic hypothesis based on the concatenated dataset (Fig. 1) demonstrates the non-monophyly (paraphyly or polyphyly) of taxa at various levels. The majority of families are polyphyletic (i.e., Narcomedusae: Aeginidae, Cuninidae, Solmarisidae; Trachymedusae: Rhopalonematidae; Limnomedusae: Olindiidae), as are several genera of Trachymedusae (i.e., *Haliscera* and *Crossota*) and Narcomedusae (i.e., *Cunina*). Also note that Trachymedusae is polyphyletic (Fig. 1; Supplementary Fig. 1) with Geryoniidae being derived from within Limnomedusae, and Narcomedusae and Actinulida being derived from within Trachymedusae; these results support and extend the findings of Collins et al. (2006, 2008). To address some of the taxonomic challenges we emend the diagnosis of Limnomedusae and make suggestions for Trachymedusae (3.2).

3.2 Emendation of Limnomedusae

We formally suggest that the family Geryoniidae be transferred from the order Trachymedusae to the order Limnomedusae and provide an emended definition of Limnomedusae to reflect the change. Note that our phylogenetic hypothesis shows that the orders Actinulida and Narcomedusae are derived from within Trachymedusae. A clear taxonomic solution to this situation requires further study, in particular increased taxon sampling. A possible solution to trachyline classification would be to raise Halicreatidae to the order level (e.g., Halicreatida) and retain Trachymedusae as the clade that contains Trachymedusae plus Actinulida. Limnomedusae in the emended sense is monophyletic comprising the least inclusive clade containing *Liriope tetraphylla* and *Craspedacusta sowerbii*. Similarly,

Narcomedusae is monophyletic, being the least inclusive clade that contains *Aeginura grimaldii* and *Pegantha martagon*, but neither *Ptychogastria polaris* nor *Haliscera conica*.

Emended diagnosis for Limnomedusae (based on Schuchert, 2017): Hydroid, if present, small, simple, mostly solitary, some forming colonies; sessile; with or without tentacles; without theca but having a mucoprotein periderm, cysts and stolons can be covered by perisarc. Medusa mostly with 4 complete radial canals, 6 also possible, often with incomplete centripetal canals not reaching manubrium; with or without marginal nematocyst ring; gonads along radial canals or exceptionally on manubrium (genera *Armorhydra* and *Limnocnida*); marginal tentacles peripheral, hollow, or solid and hollow (Geryoniidae), without true basal bulb; marginal sense organs as internal enclosed statocysts of endo-ectodermal origin, embedded in the mesoglea near ring canal or in the velum; no ocelli; exceptionally reduced medusoids (genus *Monobrachium)*. Predominantly found in nearshore waters.

3.3 Character Reconstruction

We found descriptions of full life cycles, or reconstructions of life cycles from field-collected life cycle stages for most Limnomedusae included in our analyses (Fig. 2; Table 1). By contrast, we found limited information on trachymedusan (4 rhopalonematid species) and narcomedusan life cycles (Fig. 2; Table 1). Among Narcomedusae, parasitic, polypoid stages have been described for species falling into two genera, *Cunina* and *Pegantha* (Table 1). *Pegantha* and *Cunina* are considered to belong to two different families, Solmarisidae and Cuninidae. We show that both families are polyphyletic (Fig. 1) and that *Cunina* and *Pegantha* are each other's closest relatives (Fig. 1; Fig. 2). In addition to the description of parasitic life cycles obtained from the literature, we sampled the endo-parasitic stages of a narcomedusa field-identified as *Cunina* sp. (based on our phylogeny reassigned to *Pegantha* cf. *martagon*; Fig. 1) from the coelomic cavity of a holopelagic polychaete worm (*Tomopteris* sp.) (Fig. 3). Small polyp-like stages attached within the coelomic cavity were found budding off medusae complete with tentacles.

Stochastic character mapping was performed using a symmetric transition rate matrix (AICs: 138.92 [SYM]; 144.72 [ARD]; 146.69 [ER]). The most likely ancestral life cycle of Trachylina included both polyp and medusa stages. While not our focus here, the last common ancestor of Hydroidolina also possessed a polyp/hydroid and medusa stage. The ancestor of Limnomedusae most likely possessed the stereotypical metagenetic life cycle comprising polyp and medusa stage while the polyp stage was then most likely lost in the lineage leading to the members of Geryoniidae. The medusa stage, on the other hand, was lost at least in some species of *Monobrachium* (Fig. 2, 3). The polyp stage was also lost at the base of the clade uniting Trachymedusae, Narcomedusae, and Actinulida. We also found that a metagenetic life cycle, consisting of an asexually reproducing, parasitic and sexually reproducing medusa generation arose once in Narcomedusae and not twice, as the current classification scheme, which puts *Cunina* and *Pegantha* into separate families both containing multiple genera, might suggest. Surprisingly, we could not find any mention of the early life history of any species of Halicreatidae in the literature. Our character reconstruction assigned high posterior probabilities for halicreatids to possess a life cycle characterized by direct development, in which only a medusa stage is present. Likewise, the vast majority of Trachymedusae and Narcomedusae for which life cycles are unknown are inferred to most likely develop directly with only a medusa stage present; *Pseudaegina rhodina* is the exception. Being the closest relative of the clade of Narcomedusae that develop indirectly *via* a parasitic polypoid stage, *Pseudaegina rhodina* receives almost equal probabilities for developing directly (slightly higher posterior probability) or indirectly.

3.4 Observation and culture of parasitic Narcomedusae

KAR raised the parasitic polyps of *Cunina* sp. (field identified) in the laboratory from numerous different medusan hosts [*Haliscera bigelowi, Solmissus marshalli*, *Earleria* (*Foersteria) purpurea*, and *Ptychogena* sp.]. The various host medusae had these polyps on or in place of the gonads of the host. The parasitic polyps had many manubrial tubes that would attach to the gastrovascular cavity of the

host (as described in Bouillon, 1987). The polyps were kept alive and fed a variety of foods (*Artemia*, egg white, Aminoplex infused agar, homogenized plankton) for up to 45 days. The polyps budded off dozens of juvenile medusae which were raised for up to 98 days. The number of tentacles the medusae showed at release from a given polyp varied, ranging from 8 to 12 tentacles (11 being most common). Although the medusae did feed on many different types of food sources, none were successfully raised to adult size or maturity.

Twice, out of approximately 400 *Tomopteris* collected, KJO noticed medusae and polyp-like stages (stolon prolifer *sensu* Bouillon, 1987) of *Cunina* sp. (field identified and reassigned to *Pegantha* cf. *martagon* based on our phylogenetic analyses) within the coelomic cavity of two *Tomopteris* sp. (USNM 1423205 and USNM 1449088). Tomopterids have a large, undifferentiated (lacking any degree of separation between segments) coelomic cavity that extends from the head, along the body and into each parapodium. The circulatory system is completely open with circulation of coelomic fluid driven by regularly arranged bands of coelomic cilia (Meyer, 1929). Polypoid stages were attached within the head, parapodia and the main body cavity and were budding medusae laterally, similar to polyps in other hydrozoans. Medusae within the 57 mm long worm (USNM 1449088) ranged from 0.8 to 4.0 mm in diameter with tentacles reaching at least 5 mm in length. The 5 medusae within the 48 mm long worm (USNM 1423205) ranged from 2.5 to 6 mm in diameter. Eleven to 20 “polyps” and five to 20 medusae were observed within a single host (Fig. 3). Medusae did not appear to be pulsing within the coelomic cavity.

4. Discussion

4.1 Phylogeny of Trachylina

We present the most comprehensive, in terms of taxon and marker sampling, molecular phylogenetic hypothesis of Trachylina to date. While the goal of our phylogenetic analysis (Fig. 1) was to provide the backbone for a better understanding of life cycle evolution in the group, it is clear from this and

previous analyses (Collins et al., 2006; Lindsay et al., 2017; Collins et al., 2008; Grange et al., 2017) that current trachyline classification does not mirror the evolutionary history of the group. Numerous taxa at all levels are para- or polyphyletic. Trachymedusae in particular is not a natural group with Actinulida firmly nested within the trachymedusan Rhopalonematidae (Fig. 1; Collins et al., 2008; Grange et al., 2017), and Narcomedusae being derived from within Trachymedusae. One issue addressed herein (3.2) was the emendation of Limnomedusae to include the trachymedusan Geryoniidae.

Trachyline family- and genus-level classification remains problematic with numerous families and genera being para- or polyphyletic. To address some of these issues, we recently revised the classification of the narcomedusan genus *Aegina*, which was thought to constitute a single globally distributed species (Lindsay et al., 2017). Another interesting genus to point out for further study based on our phylogeny is the rhopalonematid genus *Crossota*. *Crossota* is paraphyletic with respect to both *Benthocodon* and *Pectis*. In addition, *Crossota millsae* is more closely related to *Tetrorchis erythrogaster* than to any other species of *Crossota*. We also find that the halicreatid genus *Haliscera* is poly- or paraphyletic, forming a clade with species of *Halicreas*, and *Botrynema*. While a comprehensive revision of trachyline classification is beyond the scope of this contribution, the phylogeny presented here suggests that revisions at the family and genus level, and reevaluation of species identities will be necessary in future studies with more complete taxon sampling.

4.2 Loss of Metagenesis and Gain of Parasitism

The general view (e.g., Bouillon et al., 2006) holds that Limnomedusae possess a metagenetic life cycle in which the polyp produces medusae via lateral budding while Trachymedusae develop directly in the water column via a pelagic actinuloid stage. Narcomedusae are generally considered to be direct developers as well, developing from zygote to medusa through an actinuloid larva. Some Narcomedusae deviate from this mode of development, as they are known to possess parasitic stages in

their life cycle (reviewed in Bouillon, 1987). This general model of trachyline development has not been scrutinized in a phylogenetic context thus far. Traditional classification places Geryoniidae within Trachymedusae due to the direct development of both *Geryonia proboscidalis* and *Liriope tetraphylla* (Geryoniidae), which was described in detail more than a century ago (Metschnikoff, 1886). Using a molecular phylogenetic approach, we have shown here and previously (Collins et al., 2008) that Geryoniidae is part of Limnomedusae and formally emended the definition of the latter to incorporate the former (3.2). Thus, direct development was likely acquired independently in Geryoniidae and the clade containing Trachymedusae, Narcomedusae, and Actinulida (Fig. 2).

Aside from Geryoniidae, we were able to find evidence for direct development in four species of Trachymedusae (out of 52 currently accepted species; WoRMS Editorial Board 2017) and six species of Narcomedusae (out of 38 currently accepted species; WoRMS Editorial Board 2017), all of which are included in our phylogenetic analysis (note that we were unable to match the exact species identification for *Solmaris* sp.; Table 1). Direct development has also been described in the genera comprising Actinulida (*Halammodhydra*, *Otohydra*, and *Marsipohydra*) (e.g., Swedmark and Teissier, 1966; Sanamyan and Sanamyana, 2012). While some life cycles were observed directly in the lab (e.g., *Aglantha digitale*, *Aglaura hemistoma*, *Rhopalonema velatum*: Metschnikoff, 1886; *Halammohydra schulzei*: Swedmark and Teissier, 1966), several were inferred using circumstantial evidence (e.g., the presence of brooded juveniles in *Crossota millsae*; Thuesen, 2003). Interestingly, Metschnikoff (1886) described geryoniid medusae as developing directly from the blastula stage, which he contrasted with Trachymedusae and Narcomedusae passing through a distinct actinuloid stage, suggesting two different modes of direct development consistent with their independent evolutionary origins. However, Thuesen (2003) describes the trachymedusan *Crossota millsae* to develop directly from egg to medusae without mentioning an actinuloid stage.

Considering that species of Limnomedusae live in shallow waters close to shore, in brackish environments, or freshwater, it seems unsurprising that we found descriptions of life cycles for the

majority of Limnomedusae in our analysis. Members of Limnomedusae (aside from species of Geryoniidae) develop indirectly (metagenetic) with a sessile polyp and free-swimming medusa stage, a life cycle that was likely present in the last common ancestor of Limnomedusae, and indeed *Trachylina* (Fig. 2). *Monobrachium parasiticum* represents a deviation from other Limnomedusae with a life cycle reminiscent of many hydroidolinan hydrozoans. Here, the medusa stage was lost and replaced by (or modified to) a sessile gonophore producing gametes (cf. Cartwright and Nawrocki, 2010); the gonophore develops attached to a polyp colony that is overgrowing the shell of its bivalve host (Fig. 3; Wagner, 1890). *Monobrachium parasiticum*'s putatively close relative *Monobrachium drachi* (not in our analysis), by contrast, possesses a rudimentary medusoid (cf. Cartwright and Nawrocki, 2010) that is the sexually reproductive stage and may disperse freely (Marche-Marchad, 1975). The medusoid of *Monobrachium drachi* lacks both a mouth and a velum (Marche-Marchad, 1975), suggesting that it neither feeds nor swims actively. Reduction or loss of the medusa stage is frequent in Hydrozoa (Cartwright and Nawrocki, 2010) and coincides with the acquisition of sexual reproduction in the polyp stage (but see Pyataeva et al., 2016 who describe an unusual case in which both polyp and medusa may reproduce sexually). While asexual reproduction is usually associated with the polyp stage, some medusae are capable of reproducing asexually via budding. For example, species of the metagenetic limnomedusans *Gonionemus* and *Scolionema* possess both polyp and medusa stages, and both polyp and medusa are capable of cloning themselves via budding, at least in some species of these genera (Uchida, 1970; Nagao, 1973). Likewise, several hydroidolinan medusae bud medusae (reviewed in Berrill, 1961) either from their manubrium (e.g., *Rathkea octopunctata*), the base of their tentacles (e.g., *Codonium proliferum*), or the radial canals of their gastrovascular system (e.g., *Proboscidactyla ornata*). Nonetheless, the medusa remains the life cycle stage producing gametes in these cases.

In contrast to limnomedusans, the lack of knowledge on narcomedusan and trachymedusan life cycles is likely attributable to the fact that many species inhabit midwater and deep-sea habitats. Interestingly, Actinulida is nested within Trachymedusae (Fig. 1; Collins et al. 2008) but its constituent

members are dwellers of the meiofaunal sand-interstitial. Actinulids have long been thought to be derived trachylines (Remane, 1927; Werner, 1965; Salvini-Plawen, 1987) and, in light of a molecular phylogenetic analysis, were hypothesized to have been derived from within Rhopalonematidae via paedomorphosis (Collins et al., 2008). Swedmark and Teissier (1966) dismissed the interpretation of actinulid morphology as a case of neoteny. However, considering the phylogenetic position of *Halammohydra* (Figs. 1 and 2; Collins et al., 2008) whose ancestor most likely possessed an actinuloid larva like all extant trachymedusans (Fig. 2), and development of gonads in an adult that resembles an actinuloid larva (e.g., Swedmark and Teissier, 1966; Sanamyan and Sanamyan, 2012), it seems likely that *Halammohydra*'s (and possibly all actinulids') development is indeed a case of paedomorphosis characterized by deceleration of growth rate during ontogeny (cf. Reilly et al., 1997; McNamara, 1986).

In general, culturing of trachymedusan and narcomedusan species is complex, explaining the apparent lack of life cycle information for these groups (see 3.3). It is noteworthy that we were unable to find any information on the development of any species of Halicreatidae, which hold a key position for understanding life cycle evolution in Trachylina (Figs. 1 and 2). While we do not know how the majority of trachymedusans and narcomedusans develop, the last common ancestor of the clade uniting Trachymedusae, Actinulida, and Narcomedusae likely developed directly (Fig. 2), suggesting that the general model of direct development in Trachymedusae and most Narcomedusae holds true. Using the stochastic mapping approach taken here, we were not only able to assign posterior probabilities to ancestral nodes on the phylogeny but also provide probabilities for unobserved life cycles of extant taxa. However, it could be possible that several Trachymedusae or Narcomedusae possess an as yet undiscovered polyp stage, and such discoveries would potentially alter the results of our character reconstructions.

Generally, a transition to direct development can be seen as a pre-requisite to an open ocean or midwater life style since habitat for the polyp to settle on is virtually absent. Some midwater

hydroidolinan hydrozoans solved the issue of a lack of substrate to settle on through free-floating polyp stages, as has been described in *Pelagohydra mirabilis* (e.g., Pilgrim, 1967) and *Eirene hexanemalis* (Bouillon, 1983). Others solved this problem by hitchhiking on other pelagic organisms. For example, the polyps of *Pandea rubra* are well-known to grow on the shells of pteropods (e.g., Bouillon et al., 2006) while some hydroids have been described to live on deep sea holothurians (Lindsay and Takeuchi, 2008). An interesting variation on this strategy is the epizoic, non-parasitic relationship between the hydroid of *Bythotiara dolioeques* and the doliolid (Tunicata: Thaliacea) *Doliolula equus* (Raskoff and Robison, 2005; Robison et al., 2005). The hydroid is found living exclusively on the pelagic tunicate, but the tentacles are able to expand a great distance away from the polyp, attach to another zooid of the doliolid, and then break their connection to the polyp, forming what has been termed autonomous tentacles. These autonomous tentacles then revert back to a polypoid form and grow a new mature polyp (Raskoff and Robison, 2005), in this way asexually populating large doliolid colonies made up of hundreds of zooids.

In this context, the evolution of parasitism in Narcomedusae is of particular interest. Among Narcomedusae, parasitic stages that infect other midwater organisms were described in some species of *Pegantha* and *Cunina* (Bouillon, 1987), which belong to the families Solmarisidae and Cunindae, respectively. Considering that neither family is monogeneric, this classification makes it appear as if parasitism evolved twice in Narcomedusae. Our phylogeny, however, shows, with high support, that both Cuninidae and Solmarisidae are polyphyletic, and that the parasitic members of both families are each other's closest relatives (Figs. 1 and 2). Hence, it seems most likely that parasitism evolved only once in Narcomedusae (Fig. 2). In addition to parasitizing other medusae, self-parasitism or brooding has been described as an alternative developmental route in species of *Cunina* whose larvae are known to parasitize other medusae (e.g., Lucas and Reed, 2009); brooding has been suggested as a gateway to parasitism in Narcomedusae (Boullion, 1987).

While some Narcomedusae infect hydroidoline or trachyline medusae (Bouillon, 1987; Pagès

et al., 2007), other hosts include holopelagic polychaete worms of the genus *Tomopteris* (Fig. 3; Damas, 1936). Here, the parasitic stage appears highly derived with a sessile, polypoid stage (stolon prolifer *sensu* Bouillon, 1987) attached to the coelomic wall budding medusae, not unlike the stereotypical hydrozoan polyps but lacking a mouth. Damas (1936) suggested that these parasites feed by absorbing nutrients from the coelomic fluid of their host. The presence of numerous, relatively large medusae clustered around the gut (Fig. 3) suggests that the highly structured, ciliary driven circulation, which is responsible for all circulation within tomopterid's open circulatory system, is greatly impacted by the medusae. It is unclear if the stolon prolifer clones itself like other hydrozoan polyps do but it seems likely that they do given the low occurrence of infection and number of stolon prolifer in each infected individual. The larvae of *Pegantha* likely enter the *Tomopteris* body through the gonoducts. It is unclear at this point how the numerous, large medusae (Fig. 3) exit their host, but rupture of the host body wall is likely.

4.3 Life Cycle Evolution in Hydrozoa

A shared genetic regulatory machinery (i.e., "deep homologies"; Shubin et al., 2009) can shape morphologically similar but phylogenetically disparate features, leading to parallelisms of complex traits due to shared constraints (Gould, 2002). Our contribution on life cycle evolution in Trachylina complements the examination of hydroidolinan patterns of life cycle evolution by Cartwright and Nawrocki (2010). Illuminating the processes underlying the evolution of indirect and direct development (c.f., Holstein and Laudet, 2014) in Hydrozoa, and Medusozoa in general, will greatly benefit from integrating targeted genome sequencing to identify the presence or absence of key regulators and associated pathways (see Fuchs et al., 2014 for a candidate pathway regulating the transition from polyp to medusa in Scyphozoa) in light of phylogenetic hypotheses and inferred patterns of life cycle evolution. Narcomedusae may be of particular interest in this regard, as at least some of the parasitic species have life cycles that contain stages resembling (or analogous to) polyps in

the sense that they bud medusae asexually and clone themselves.

5. Glossary

actinuloid larva – tentaculated embryonic stage (gastrula with tentacles) that develops directly into a medusa; not homologous with the hydroidolinan actinula larva that develops into a polyp

gonophore – incompletely developed medusa that remains attached to the polyp or hydroid (a sporosarc when completely reduced)

holopelagic – development occurs completely in the water column

medusa – free-swimming sexually reproductive life cycle stage (jellyfish); medusae can be more or less reduced and then be referred to as medusoids

Medusozoa – the cnidarian clade that contains Scyphozoa ("true" jellyfish), Staurozoa (stalked jellyfish), Cubozoa (box jellyfish), and Hydrozoa (hydroids and hydromedusae)

metagenesis – alternation of asexually and sexually reproducing generations

midwater – the part of a body of water that is neither close to the surface nor close to the bottom

polyp – usually sessile cnidarian life cycle stage; may be solitary or colonial (colonial forms in Hydrozoa commonly referred to as hydroids)

pelagic zone – the water column of a body of water beyond the coastal zones (e.g., the open ocean), not including the water near the seafloor

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TABLES

Table 1 Trachylines for which descriptions of developmental mode were found in the literature. Categorical character states for each developmental mode are shown.

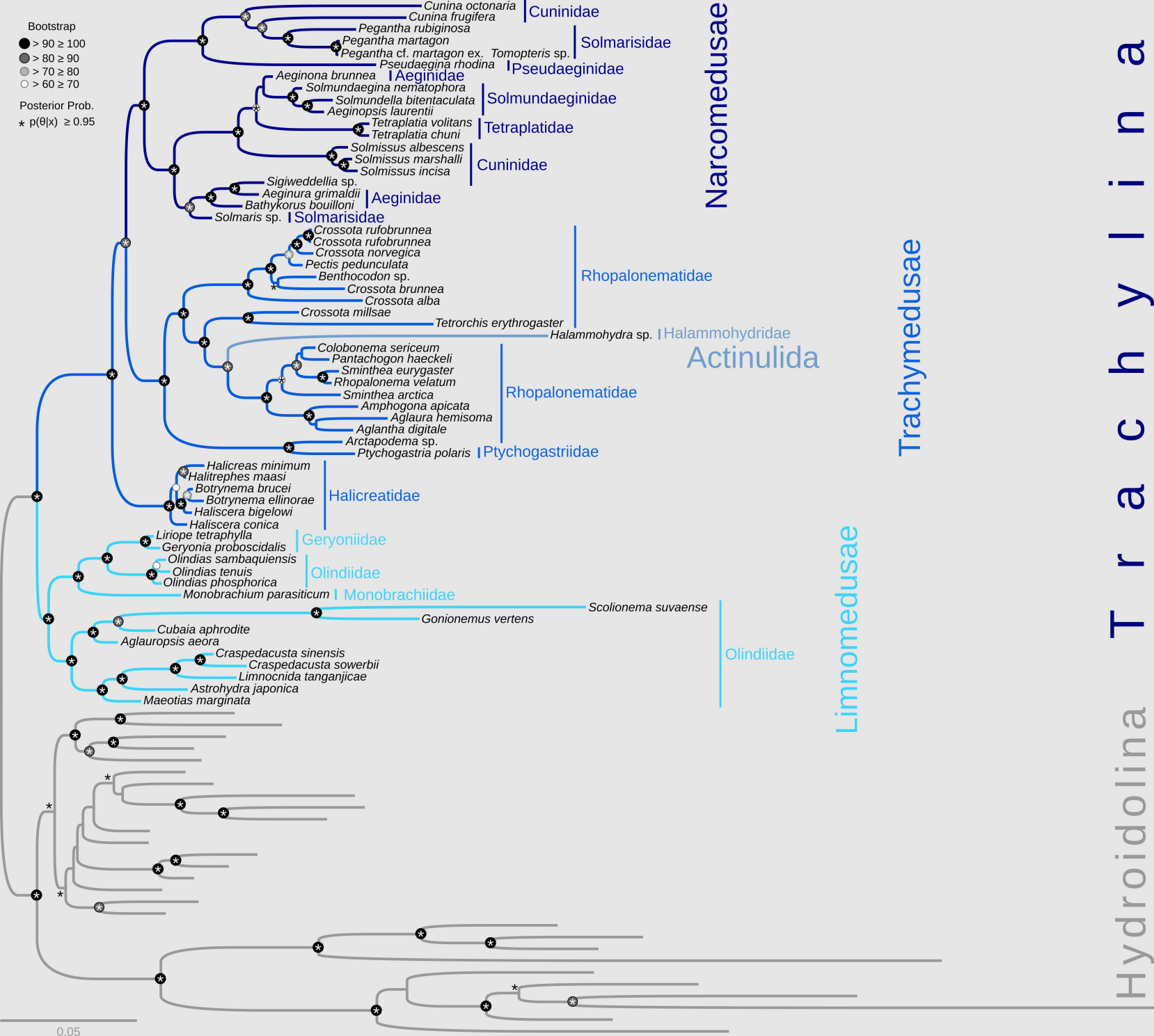
	Family	Genus	Species	Development	Character State	Reference
Limnomedusae	Geryoniidae	*Geryonia*	*proboscidalis*	direct medusoid	1	Fol (1873); Metschnikoff (1886); Maas (1908)
	Geryoniidae	*Liriope*	*tertraphylla*	direct medusoid	1	Metschnikoff (1886)
	Monobrachiidae	*Monobrachium*	*parasiticum*	direct polypoid	3	Fig. 3
	Olindiidae	*Aglauropsis*	*aeroa*	indirect metagenetic	2	Mills *et al.* (1976)
	Olindiidae	*Astrohydra*	*japonica*	indirect metagenetic	2	Hashimoto (1981; 1985)
	Olindiidae	*Craspedacusta*	*sowerbii*	indirect metagenetic	2	Payne (1924; 1926); Folino-Rorem *et al.* (2016)
	Olindiidae	*Gonionemus*	*vertens*	indirect metagenetic	2	Perkins (1903); Joseph (1925)
	Olindiidae	*Limnocnida*	*tanganjicae*	indirect metagenetic	2	Bouillon (1955)
	Olindiidae	*Maeotias*	*marginata*	indirect metagenetic	2	Rees and Gershwin (2000)
	Olindiidae	*Olindias*	*sambaquiensis*	indirect metagenetic	2	Zamponi et al. (1987)
	Olindiidae	*Olindias*	*tenuis*	indirect metagenetic	2	Weill (1936)
	Olindiidae	*Scolionema*	*suvaense*	indirect metagenetic	2	Goy (1970; 1973)
Trachymedsae	Rhopalonematidae	*Aglantha*	*digitale*	direct medusoid	1	Metschnikoff (1886)
	Rhopalonematidae	*Aglaura*	*hemistoma*	direct medusoid	1	Metschnikoff (1886)
	Rhopalonematidae	*Crossota*	*millsae*	direct medusoid	1	Thuessen (2003)
	Rhopalonematidae	*Rhopalonema*	*velatum*	direct medusoid	1	Metschnikoff (1886)
Actinulida	Halammohydridae	*Halammohydra*	sp.	direct medusoid	1	Swedmark and Teissier (1966)
Narcomedusae	Cuninidae	*Cunina*	*octonaria*	indirect metagenetic	2	Berrill (1950); Bouillon (1987)
	Cuninidae	*Solmissus*	*marshalli*	direct medusoid	1	Mackie and Mackie (1963/1964)
	Solmarisidae	*Pegantha*	sp.	indirect metagenetic	2	Damas (1936)
	Solmarisidae	*Pegantha*	*rubiginosa*	indirect metagenetic	2	Bouillion (1987)
	Solmarisidae	*Solmaris*	sp.	direct medusoid	1	Berrill (1950); Bouillon (1987)
	Solmundaeginidae	*Solmundella*	*bitentaculata*	direct medusoid	1	Metschnikoff (1886); Berrill (1950)

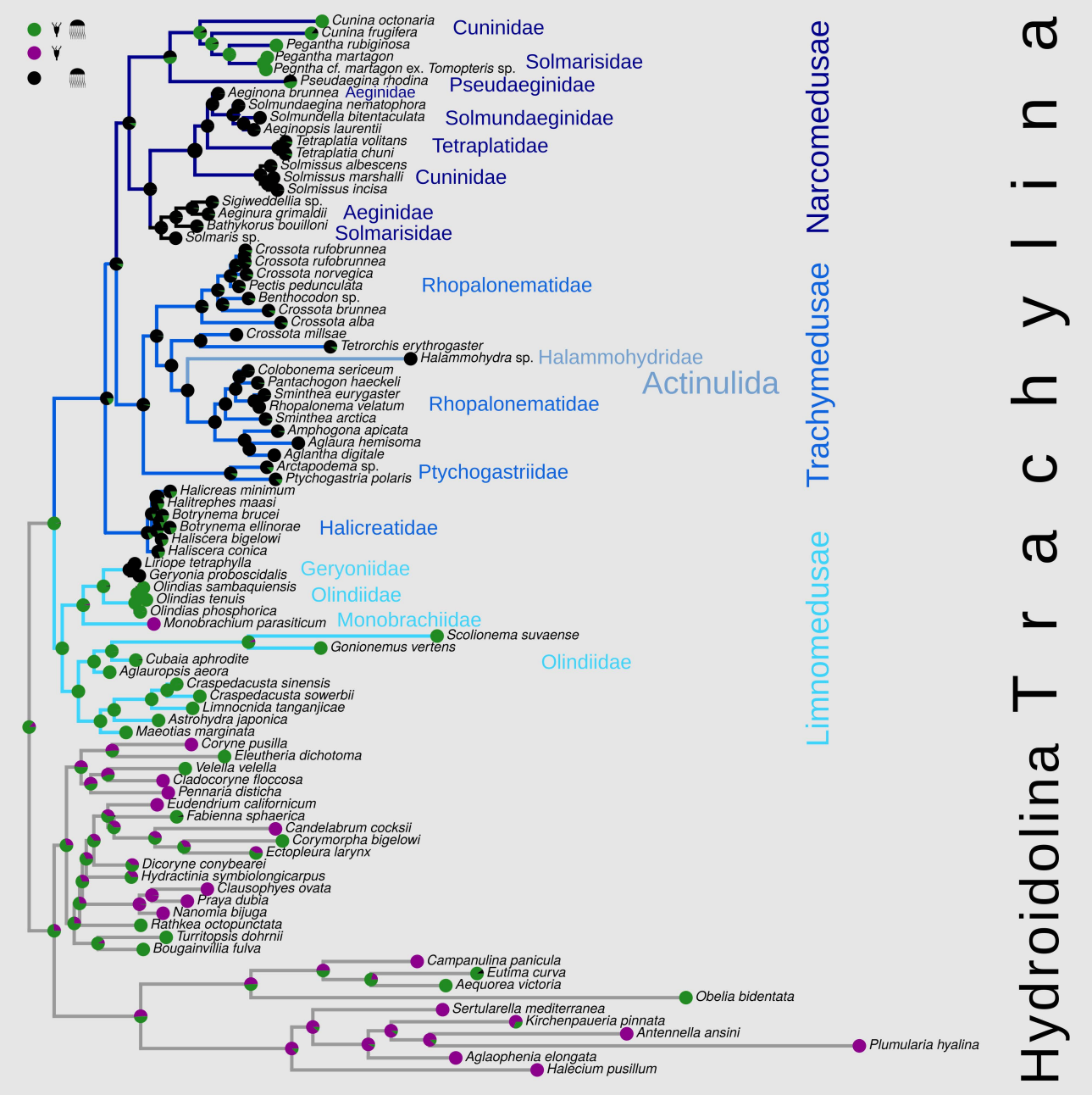
FIGURES

Fig. 1 Maximum likelihood phylogeny inferred from the concatenated dataset of nuclear ribosomal 28S and 18S, and mitochondrial ribosomal 16S using the GTR+I+G model of nucleotide evolution. Bootstrap support resulting from 1,000 non-parametric bootstrap replicates is shown in percent on each node in addition to posterior probabilities from the Bayesian inference greater than 0.95.

Fig. 2 Posterior probabilities of life cycle character states (pie charts) on nodes and tips of the maximum likelihood topology. All tips were assigned prior probabilities for each of 3 states (see 2.2). In cases where life cycles were unknown, posterior probabilities of tip states were inferred in the same fashion as ancestral nodes.

Fig. 3 Animals hosting trachylines. A. *Tomopteris* sp. (USNM 1423205) with medusae and polyps throughout body cavity including the head and parapodia. B. *Tomopteris* sp. (USNM 1449088) with medusae throughout the body cavity and polyps in the parapodia. C and D. Polyp from 1449088 and illustration of the same showing one late and one early budding medusa. E. Medusa from USNM 1423205 F. Detail illustration of medusae and polyps within USNM 1423205. G. *Monobrachium parasiticum* colony (USNM 1449088) growing on its bivalve host; note the prominent gonophore among the colonial polyps. Labels: ba, basal attachment, g, gut, lb, lateral bud, m, medusa, mbc, main body cavity, p, polypoid stage, pbc, parapodial body cavity, s, polypoid stalk, t, tentacle. Scale bars A and B 1 cm, C - E 1 mm.


Bootstrap
> 90 ≥ 100
> 80 ≥ 90
> 70 ≥ 80
> 60 ≥ 70
Posterior Prob.
* p(θ|x) ≥ 0.95
Cunina octonaria
Cunina frugifera
Cuninidae
Pegantha rubiginosa
Pegantha martagon
Pegantha cf. martagon ex. Tomopteris sp.
Solmarisidae
Pseudaegina rhodina
Pseudaeginidae
Aeginona brunnea
Aeginidae
Solmundaegina nematophora
Solmundella bitentaculata
Aeginopsis laurentii
Solmundaeginidae
Tetraplatia volitans
Tetraplatia chuni
Tetraplatidae
Solmissus albescens
Solmissus marshalli
Solmissus incisa
Cuninidae
Sigiweddellia sp.
Aeginura grimaldii
Bathykorus bouilloni
Aeginidae
Solmaris sp.
Solmarisidae
Narcomedusae
Crossota rufobrunnea
Crossota rufobrunnea
Crossota norvegica
Pectis pedunculata
Benthocodon sp.
Crossota brunnea
Crossota alba
Crossota millsae
Tetrorchis erythrogaster
Rhopalonematidae
Halammohydra sp.
Halammohydridae
Actinulida
Colobonema sericeum
Pantachogon haeckeli
Sminthea eurygaster
Rhopalonema velatum
Sminthea arctica
Amphogona apicata
Aglaura hemisoma
Aglantha digitale
Rhopalonematidae
Arctapodema sp.
Ptychogastria polaris
Ptychogastriidae
Trachymedusae
Halicreas minimum
Halitrephes maasi
Botrynema brucei
Botrynema ellinorae
Haliscera bigelowi
Haliscera conica
Halicreatidae
Liriope tetraphylla
Geryonia proboscidalis
Geryoniidae
Olindias sambaquiensis
Olindias tenuis
Olindias phosphorica
Olindiidae
Monobrachium parasiticum
Monobrachiidae
Scolionema suvaense
Gonionemus vertens
Cubaia aphrodite
Aglauropsis aeora
Craspedacusta sinensis
Craspedacusta sowerbii
Limnocnida tanganjicae
Astrohydra japonica
Maeotias marginata
Olindiidae
Limnomedusae
Trachylina
Hydroidolina
0.05


Hydroidolina
Trachylina
Narcomedusae
Trachymedusae
Limnomedusae
Cuninidae
Solmarisidae
Pseudaeginidae
Aeginidae
Solmundaeginidae
Tetraplatidae
Cuninidae
Aeginidae
Solmarisidae
Rhopalonematidae
Halammohydridae
Actinulida
Rhopalonematidae
Ptychogastriidae
Halicreatidae
Geryoniidae
Olindiidae
Monobrachiidae
Olindiidae
Cunina octonaria
Cunina frugifera
Pegantha rubiginosa
Pegantha martagon
Peqntha cf. martagon ex. Tomopteris sp.
Pseudaegina rhodina
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Tetraplatia chuni
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Solmissus marshalli
Solmissus incisa
Sigiweddellia sp.
Aeginura grimaldii
Bathykorus bouilloni
Solmaris sp.
Crossota rufobrunnea
Crossota rufobrunnea
Crossota norvegica
Pectis pedunculata
Benthocodon sp.
Crossota brunnea
Crossota alba
Crossota millsae
Tetrorchis erythrogaster
Halammohydra sp.
Colobonema sericeum
Pantachogon haeckeli
Sminthea eurygaster
Rhopalonema velatum
Sminthea arctica
Amphogona apicata
Aglaura hemisoma
Aglantha digitale
Arctapodema sp.
Ptychogastria polaris
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Haliscera conica
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Olindias tenuis
Olindias phosphorica
Monobrachium parasiticum
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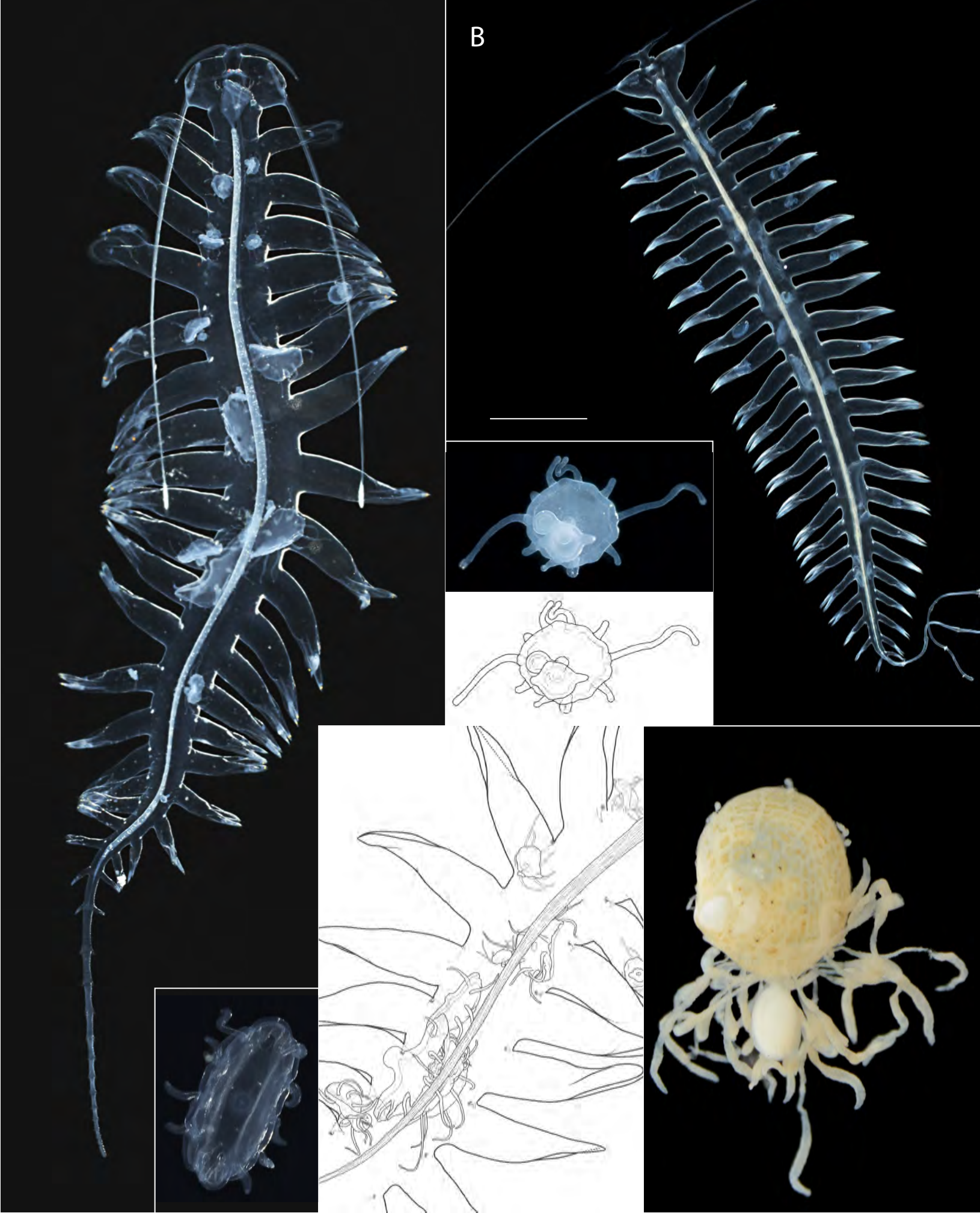
Gonionemus vertens
Cubaia aphrodite
Aglauropsis aeora
Craspedacusta sinensis
Craspedacusta sowerbii
Limnocnida tanganjicae
Astrohydra japonica
Maeotias marginata
Coryne pusilla
Eleutheria dichotoma
Velella velella
Cladocoryne floccosa
Pennaria disticha
Eudendrium californicum
Fabienna sphaerica
Candelabrum cocksii
Corymorpha bigelowi
Ectopleura larynx
Dicoryne conybearei
Hydractinia symbiolongicarpus
Clausophyes ovata
Praya dubia
Nanomia bijuga
Rathkea octopunctata
Turritopsis dohrnii
Bougainvillia fulva
Campanulina panicula
Eutima curva
Aequorea victoria
Obelia bidentata
Sertularella mediterranea
Kirchenpaueria pinnata
Antennella ansini
Plumularia hyalina
Aglaophenia elongata
Halecium pusillum


B

Hydrozoan (Cnidaria) life cycles