

The Evolution of Siphonophore Tentilla as Specialized Tools for Prey Capture

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Abstract

Predators have evolved dedicated body parts to capture and subdue prey. As different predators specialize on distinct prey taxa, their tools for prey capture diverge into a variety of adaptive forms. Studying the evolution of predation is greatly facilitated by a predator clade with structures used exclusively for prey capture that present significant morphological variation. Siphonophores, a clade of colonial cnidarians, satisfy these criteria particularly well, capturing prey with their tentilla (tentacle side branches). Earlier work has shown that extant siphonophore diets correlate with the different morphologies and sizes of their tentilla and nematocysts. We hypothesize that evolutionary specialization on different prey types has driven the phenotypic evolution of these characters. To test this hypothesis, we: (1) measured multiple morphological traits from fixed siphonophore specimens using microscopy and high speed video techniques, (2) built a phylogenetic tree of 45 species, and (3) characterized the evolutionary associations between siphonophore nematocyst characters and prey type data from the literature. Our results show that siphonophore tentillum structure has strong evolutionary associations with prey type and size specialization, and suggest that shifts between prey-type specializations are linked to shifts in tentillum and nematocyst size and shape. In addition, we generated hypotheses about the diets of understudied siphonophore species based on these characters. Thus, the evolutionary history of tentilla shows that siphonophores are an example of ecological niche diversification via morphological innovation and evolution. This study contributes to understanding how morphological evolution has shaped present-day oceanic food-webs.

Keywords

Siphonophores, tentilla, nematocysts, predation, specialization, character evolution

Most animal predators have characteristic biological tools that they use to capture and subdue prey. Raptors have claws and beaks, snakes have fangs, wasps have stingers, and cnidarians have nematocyst-laden tentacles. The functional morphology of these structures tend to be finely attuned to their ability to successfully capture specific prey (Schmitz 2017). Long-term adaptive evolution in response to the defense mechanisms of the prey (*e.g.* avoidance, escape, protective barriers) leads to modifications that can counter those defenses. The more specialized the diet of a predator is, the more specialized its tools need to be to meet the specific challenges posed by the prey. Understanding the relationships between predatory specializations and morphological specializations is necessary to contextualize the phenotypic diversity of predators, and to quantify the importance of ecological diversification in generating this diversity.

Siphonophores (Cnidaria : Hydrozoa) are a clade of organisms bearing modular structures that are exclusively used for prey capture: the tentilla (Fig. 1). These present a significant morphological variation across species (Mapstone 2014) (Fig. 2), which makes it ideal to study the relationships between functional traits and prey specialization. A siphonophore is a colony bearing many feeding polyps (Fig. 1), each with a single tentacle, which branches into several tentilla carrying the functional cnidocytes (specialized neural cells carrying nematocysts, the stinging capsules). Unlike most other cnidarians, siphonophores carry their tentacle nematocysts in extremely complex and organized batteries (Skaer 1988), built into their tentilla. While nematocyst batteries and clusters in other cnidarians are simple static scaffolds for cnidocytes, siphonophore tentilla have their own reaction mechanism, triggered upon encounter with prey. When it fires, a tentillum undergoes an extremely fast conformational change that wraps it around the prey, maximizing the surface area

48 of contact for nematocysts to fire on the prey (Mackie et al. 1987). In addition, some species have elaborate
49 fluorescent and bioluminescent lures on their tentilla to attract prey with aggressive mimicry (Purcell 1980;
50 Haddock et al. 2005; Haddock and Dunn 2015).

51 Many siphonophore species inhabit the deep pelagic ocean, which spans from ~200m to the oceanic
52 seafloor. This habitat has fairly homogeneous physical conditions and stable abundances of zooplanktonic
53 animals (Robison 2004). With a relatively predictable prey availability, ecological theory would predict
54 evolution to drive coexisting siphonophore lineages towards specialization, increasing their feeding efficiencies
55 and reducing interspecific competition (Hardin 1960; Hutchinson 1961). If this prediction holds true, we
56 expect the prey capture apparatus morphologies of siphonophores to diversify with the evolution of increased
57 specialization on a variety of prey types in different siphonophore lineages.

58 Coexisting siphonophores feeding on the same planktonic community may have substantial niche overlap
59 and compete for prey resources. Traditional ecological coexistence theory (Simpson 1944) predicts that
60 competition between species would select for increasing ecological specialization. This specialization is often
61 thought to be an evolutionary ‘dead end’, meaning that specialized lineages are unlikely to evolve into
62 generalists or to shift the resource for which they are specialized (Futuyma and Moreno 1988). However,
63 recent studies have found that interspecific competition can favor the evolution of resource generalism
64 (Stireman-III 2005; Johnson et al. 2009) and resource switching (Hoberg and Brooks 2008). Here we examine
65 three alternative hypotheses on siphonophore trophic specialization: (1) predatory specialists evolved from
66 generalist ancestors; (2) predatory specialists evolved from ancestral predatory specialists which specialized on
67 a different resource, switching their primary prey type; and (3) predatory generalists evolved from specialist
68 ancestors.

69 The study of siphonophore tentilla and diets has been limited in the past due to the inaccessibility of
70 their oceanic habitat and the difficulties associated with the collection of fragile siphonophores. Thus, the
71 morphological diversity of tentilla has only been characterized for a few taxa, and their evolutionary history
72 remains largely unexplored. Contemporary underwater sampling technology provides an unprecedented
73 opportunity to explore the trophic ecology (Choy et al. 2017) and functional morphology (Costello et al.
74 2015) of siphonophores. In addition, well-supported phylogenies based on molecular data are now available
75 for these organisms (Munro et al. 2018). These advances allow for the examination of relationships between
76 modern siphonophore form, function, and ecology, as well as reconstructing their evolutionary history.

77 The few pioneering studies that have addressed the relationships between tentilla and diet suggest that
78 siphonophores are a robust system for the study of predatory specialization via morphological diversification.
79 (Purcell 1984) and (Purcell and Mills 1988) showed clear relationships between diet, tentillum, and nematocyst
80 characters in co-occurring epipelagic siphonophores. These correlations, while studied for a small subset of
81 extant epipelagic siphonophore species, might be generalizable to all siphonophores. We hypothesize that
82 these relationships reflect correlated evolution between prey selection and tentillum (and nematocyst) traits.
83 Furthermore, we hypothesize that with an extensive characterization of tentilla morphology, we can generate
84 hypotheses about the diets of understudied siphonophore species. In addition, our study design allows us
85 to address other interesting questions about the morphology and evolution of these unique structures. In
86 particular, we aim to address the evolutionary origins of giant tentilla, the phenotypic integration of tentilla,
87 the evolution of the extreme shapes of siphonophore haploneme nematocysts (Thomason 1988), and the
88 mechanical implications of tentillum morphologies on cnidoband discharge.

89 In this study, we characterize the morphological diversity of tentilla and their nematocysts across a broad
90 variety of shallow and deep sea siphonophore species using modern imaging technologies, we expand the
91 phylogenetic tree of siphonophores by combining a broad taxon sampling of ribosomal gene sequences with a
92 transcriptome-based backbone tree, and we explore the evolutionary histories and correlations among diet,
93 tentillum, and nematocyst characters.

94 **Methods**

95 *Tentillum morphology* – The morphological work was carried out on siphonophore specimens fixed in 4%
96 formalin from the Yale Peabody Museum Invertebrate Zoology (YPM-IZ) collection (accession numbers
97 in Appendix 1). These specimens were collected intact across many years of fieldwork expeditions, using
98 of blue-water diving (Haddock and Heine 2005), remotely operated vehicles (ROVs), and human-operated
99 submersibles. Tentacles were dissected from non-larval gastrozooids, sequentially dehydrated into 100%

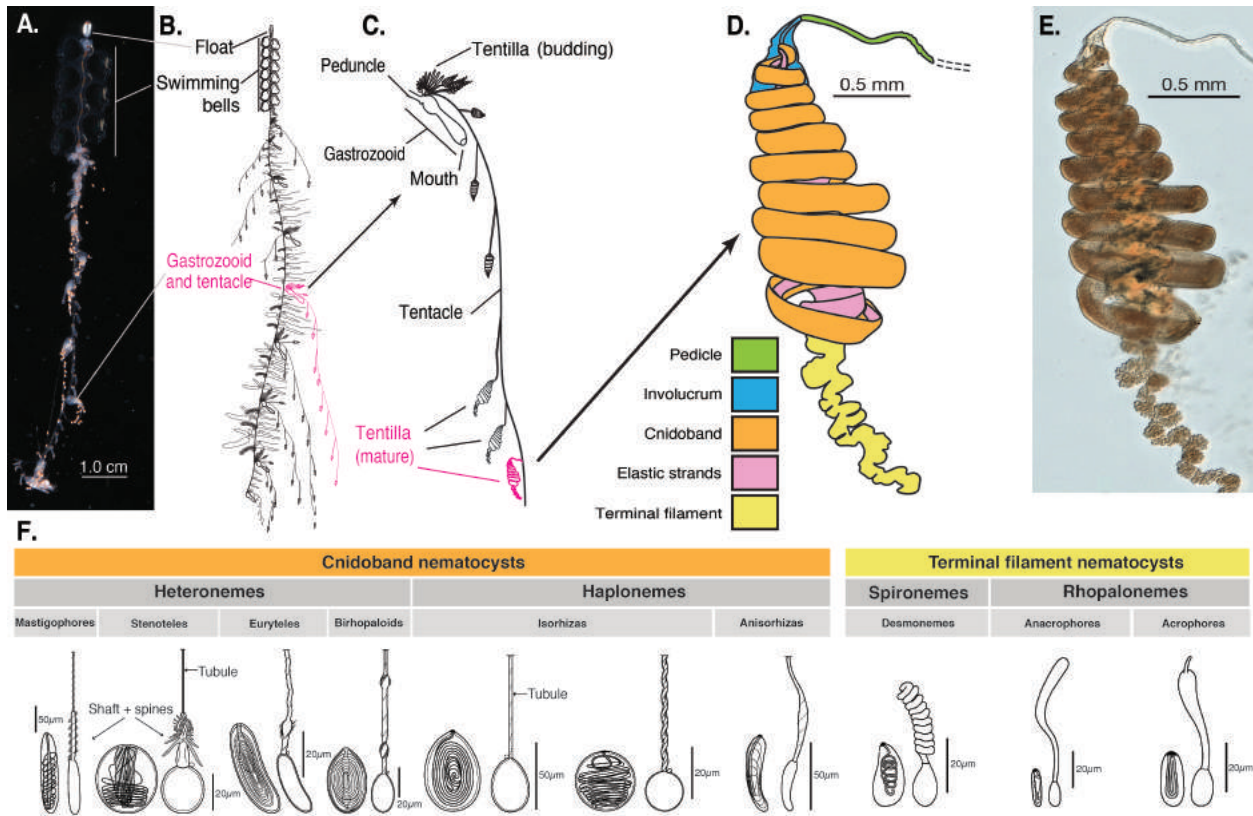


Figure 1: Siphonophore anatomy. A - *Nanomia* sp. siphonophore colony (photo by Catriona Munro). B,C - Illustration of a *Nanomia* colony, gastrozooid, and tentacle (by Freya Goetz). D - *Nanomia* sp. Tentillum illustration and main parts. E - Transmission micrograph of the tentillum illustrated in D. F - Nematocyst types (illustration reproduced with permission from Mapstone 2014), hypothesized homologies, and locations in the tentillum. Undischarged to the left, discharged to the right.

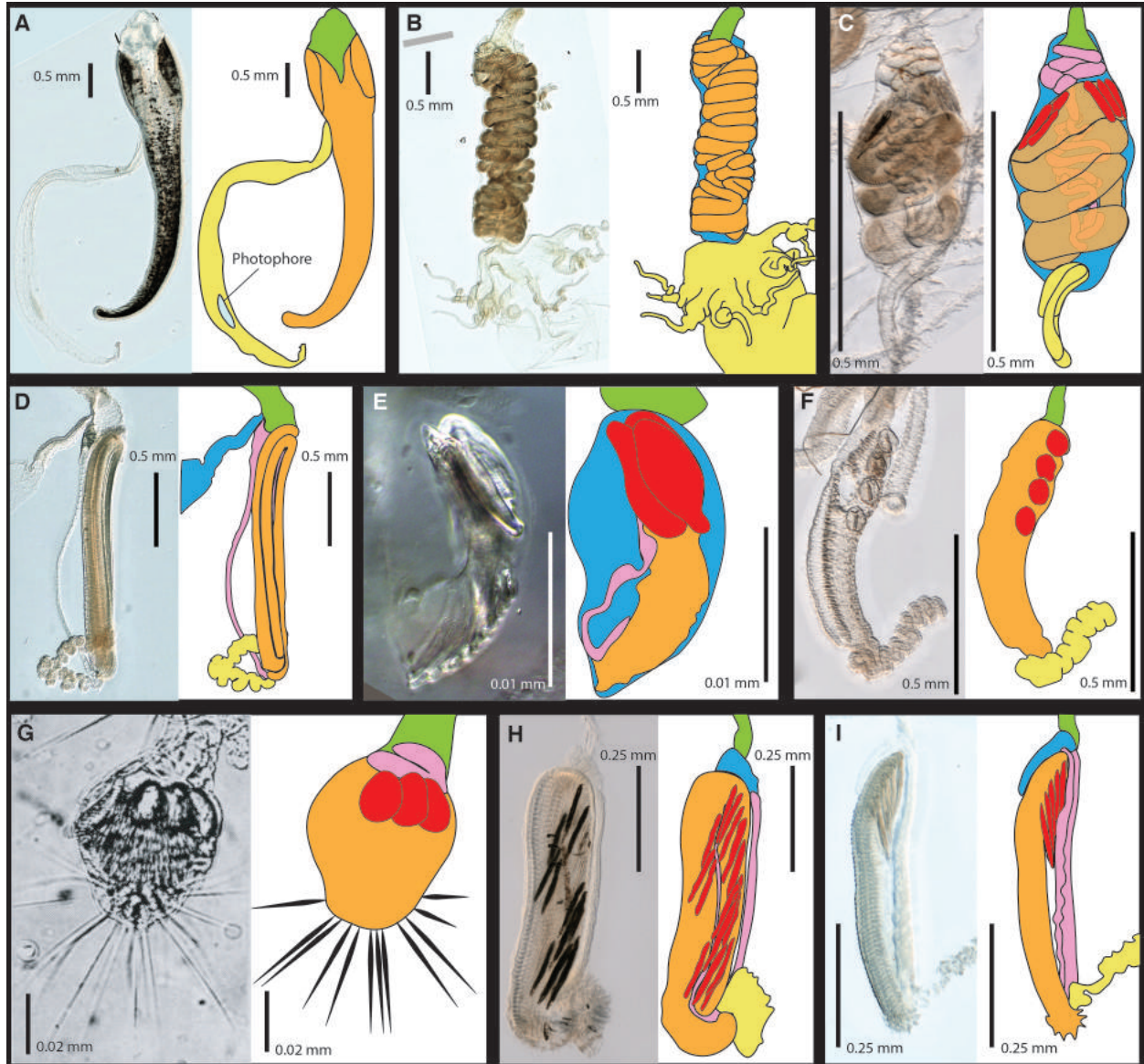


Figure 2: Tentillum diversity plate. The illustrations delineate the pedicle (green), involucrum (blue), cnidoband (orange), elastic strands (pink), terminal structures (yellow). Heteroneme nematocysts (stenoteles in C,E,F,G and mastigophores in H,I) are depicted in red for some species. A - *Erenna laciniata*, 10x. B - *Lychnagalma utricularia*, 10x. C - *Agalma elegans*, 10x. D - *Resomia ornicephala*, 10x. E - *Frillagalma vityazi*, 20x. F - *Bargmannia amoena*, 10x. G - *Cordagalma* sp., reproduced from Carré 1968. H - *Lilyopsis fluoracantha*, 20x. I - *Abylopsis tetragona*, 20x.

100 ethanol, cleared in methyl salicylate, and mounted into slides with Canada Balsam or Permount mounting
101 media. The slides were imaged as tiled z-stacks using differential interference contrast (DIC) on an automated
102 stage at YPM-IZ (with the assistance of Daniel Drew and Eric Lazo-Wasem) and with laser point confocal
103 microscopy using a 488 nm Argon laser that excited autofluorescence in the tissues. Thirty characters (defined
104 in Appendix 2) were measured using Fiji (Collins 2007; Schindelin et al. 2012). We did not measure the
105 lengths of contractile structures (terminal filaments, pedicles, gastrozooids, and tentacles), since they are too
106 variable to quantify. We measured at least one specimen for 96 different species (Appendix 3, Fig. 3). Of
107 these, we selected 38 focal species across clades based on specimen availability and phylogenetic representation.
108 Three to five tentacle specimens from each one of these selected species were measured to capture intraspecific
109 variation.

110 In order to observe the discharge behavior of different tentilla, we recorded high speed footage (1000-3000
111 fps) of tentillum and nematocyst discharge by live siphonophore specimens (26 species) using a Phantom
112 Miro 320S camera mounted on a stereoscopic microscope. We mechanically elicited tentillum and nematocyst
113 discharge using a fine metallic pin. We used the Phantom PCC software to analyze the footage. For the
114 10 species recorded, we measured total cnidoband discharge time (ms), heteroneme filament length (μm),
115 and discharge speeds (mm/s) for cnidoband, heteronemes, haplonemes, and heteroneme shafts when possible
116 (data in Appendix 4).

117 *Siphonophore phylogeny* – The phylogenetic analysis included 55 siphonophore species and 6 outgroup
118 cnidarian species (*Clytia hemisphaerica*, *Hydra circumcincta*, *Ectopleura dumortieri*, *Porpita porpita*, *Velella*
119 *velella*, *Staurocladia wellingtoni*). The gene sequences we used in this study are available online (accession
120 numbers in Appendix 5). Some of the sequences we used were accessioned in (Dunn et al. 2005), and
121 others we extracted from the transcriptomes in (Munro et al. 2018). Two new 16S sequences for *Frillagalma*
122 *vityazi* (MK958598) and *Thermopalma* sp. (MK958599) sequenced by Lynne Christianson were included and
123 accessioned to NCBI. We aligned these sequences using MAFFT (Katoh et al. 2002) (alignments available
124 in Dryad). We inferred a Maximum Likelihood (ML) phylogeny (Appendix 6) from 16S and 18S ribosomal
125 rRNA genes using IQTree (Nguyen et al. 2014) with 1000 bootstrap replicates (iqtree -s alignment.fa -nt
126 AUTO -bb 1000). We used ModelFinder (Kalyaanamoorthy et al. 2017) implemented in IQTree v1.5.5. to
127 assess relative model fit. ModelFinder selected GTR+R4 for having the lowest Bayesian Information Criterion
128 score. Additionally, we inferred a Bayesian tree with each gene as an independent partition in RevBayes
129 (Höhna et al. 2016) (Appendix 7 and 9), which was topologically congruent with the unconstrained ML tree.
130 The *alpha* priors were selected to minimize prior load in site variation.

131 Given the broader sequence sampling of the transcriptome phylogeny, we ran constrained inferences (using
132 both ML and Bayesian timetree approaches, which produced fully congruent topologies (Appendix 6 and 7))
133 after fixing the 5 nodes that were incongruent with the topology of the consensus tree in (Munro et al. 2018).
134 This topology was then used to inform a Bayesian relaxed molecular clock time-tree in RevBayes, using
135 a birth-death process (sampling probability calculated from the known number of described siphonophore
136 species) to generate ultrametric branch lengths (Appendix 8). Scripts available in Appendix 9.

137 *Feeding ecology* – We extracted categorical diet data for different siphonophore species from published
138 sources, including seminal papers (Biggs 1977; Purcell 1981, 1984; Andersen 1981; Mackie et al. 1987; Pugh
139 and Youngbluth 1988; Bardi and Marques 2007), and ROV observation data (Hissmann 2005; Choy et
140 al. 2017) with the assistance of Elizabeth Hetherington and Anela Choy (Appendix 10). We removed the
141 gelatinous prey observations for *Praya dubia* eating a ctenophore and a hydromedusa, and for *Nanomia* sp.
142 eating *Aegina*, since we believe these are rare events that have a much larger probability of being detected by
143 ROV methods than their usual prey, and it is not clear whether the medusae were attempting to prey upon
144 the siphonophores. Personal observations on feeding (from SHDH, CAC, and Philip Pugh) were also included
145 for *Resomia ornicephala*, *Lychnagalma utricularia*, *Bargmannia amoena*, *Erenna richardi*, *Erenna laciniata*,
146 *Erenna sirena*, and *Apolemia rubriversa*. In order to detect coarse-level patterns in the feeding habits, the
147 data were merged into feeding guilds. The feeding guilds described here are: small-crustacean specialist
148 (feeding mainly on copepods and ostracods), large crustacean specialist (feeding on large decapods, mysids,
149 or krill), fish specialist (feeding mainly on actinopterygian larvae, juveniles, or adults), gelatinous specialist
150 (feeding mainly on other siphonophores, medusae, ctenophores, salps, and/or doliolids), and generalist
151 (feeding on a combination of the aforementioned taxa, without favoring any one prey group). These were
152 selected to minimize the number of categories while keeping the most different types of prey separate. We
153 extracted copepod prey length data from (Purcell 1984). To calculate specific prey selectivities, we extracted

154 quantitative diet and zooplankton composition data from (Purcell 1981), matched each diet assessment to
155 each prey field quantification by site, calculated Ivlev's electivity indices (Jacobs 1974), and averaged those
156 by species (Appendix 11).

157 *Statistical analyses* – For subsequent comparative analyses, we removed species present in the tree but not
158 represented in the morphology data, and *vice versa*. Although we measured specimens labeled as *Nanomia*
159 *bijuga* and *Nanomia cara*, we are not confident in some of the species-level identifications, and some specimens
160 were missing diagnostic zooids. Thus, we decided to collapse these into a single taxonomic concept (*Nanomia*
161 sp.). All *Nanomia* sp. observations were matched to the phylogenetic position of *Nanomia bijuga* in the
162 tree. We carried out all phylogenetic comparative statistical analyses in the programming environment R
163 (Team 2017), using the bayesian ultrametric species tree (Fig. 4), and incorporating intraspecific variation
164 estimated from the specimen data as standard error (Appendix 3). R scripts available in Dryad. For each
165 character (or character pair) analyzed, we removed species with missing data and reported the number of
166 taxa included. We tested each character for normality using the Shapiro-Wilk test (Shapiro and Wilk 1965),
167 and log-transformed those that were non-normal.

168 We fitted different models generating the observed data distribution given the phylogeny for each continuous
169 character using the function `fitContinuous` in the R package *geiger* (Harmon et al. 2007). The models
170 compared were the white noise (WN; non-phylogenetic model that assumes all values come from a single
171 normal distribution with no covariance structure among species), the Brownian Motion (BM) model of
172 neutral divergent evolution (Martins 1996), the Early Burst (EB) model of decreasing rate of evolutionary
173 change (Harmon et al. 2010), and the Ornstein-Uhlenbeck (OU) model of stabilizing selection around a fitted
174 optimum state (Uhlenbeck and Ornstein 1930; Butler and King 2004). We then ranked the models in order
175 of increasing parametric complexity (WN,BM,EB,OU), and compared the corrected Akaike Information
176 Criterion (AICc) support scores (Sugiura 1978) to the lowest (best) score, using a cutoff of 2 units to determine
177 significantly better support. When the best fitting model was not significantly better than a less complex
178 alternative, we selected the least complex model (Appendix 12). We calculated model adequacy scores using
179 the R package *arbutus* (Pennell et al. 2015) (Appendix 13). We calculated phylogenetic signal in each of the
180 measured characters using Blomberg's K (Blomberg et al. 2003) (Appendix 12), and for the morphological
181 dataset as a whole using the R package *geomorph* (Adams et al. 2016). We reconstructed ancestral states
182 using Maximum Likelihood (anc.ML (Revell 2012)), and stochastic character mapping (`make.simmap`) for
183 categorical characters. R scripts available in Dryad.

184 In order to study the evolution of predatory specialization, we reconstructed components of the diet and
185 prey selectivity on the phylogeny using ML (R `phytools::anc.ML`). To identify evolutionary associations of
186 diet with tentillum and nematocyst characters, we compared the performance of a neutral evolution model to
187 that of a diet-driven directional selection model. First, we collapsed the diet data into the five feeding guilds
188 mentioned above (fish specialist, small crustacean specialist, large crustacean specialist, gelatinous specialist,
189 generalist), based on which prey types they were observed consuming most frequently. We reconstructed
190 the feeding guild ancestral states using the ML function `ace` (package *ape* (Paradis et al. 2019)), removing
191 tips with no feeding data. The ML reconstruction was congruent with the consensus stochastic character
192 mapping (Appendix 18). Then, using the package *OUwie* (Beaulieu and O'Meara 2012), we fitted an OU
193 model with multiple optima and rates of evolution matched to the reconstructed ancestral diet regimes, a
194 single optimum OU model, and a BM null model, inspired by the analyses in (Cressler et al. 2015). Finally,
195 we compared their AICc support values to select the best fitting model (Appendix 14).

196 To model the evolutionary associations between individual tentillum and nematocyst characters and
197 the ability to capture particular prey types in the diet, we ran a series of phylogenetic generalized linear
198 models (R `phytools::phyloglm`) (Appendix 17). In addition, we ran a series of comparative analyses to address
199 hypotheses of diet-tentillum relationships posed in the literature. To test for correlated evolution among
200 binary characters, we used Pagel's test (Pagel 1994). To characterize and evaluate the relationship between
201 continuous characters, we used phylogenetic generalized least squares regressions (PGLS) (Grafen 1989).
202 To compare the evolution of continuous characters with categorical aspects of the diet, we carried out a
203 phylogenetic logistic regression (R `nlme::gls`).

204 To generate hypotheses about the diets of understudied siphonophores for which no feeding observations
205 have yet been reported (but for which we have tentacle morphology data), we carried out linear discriminant
206 analysis of principal components (DAPC) using the `dapc` function (R `adegenet::dapc`) (Jombart et al. 2010).
207 This function allowed us to incorporate more predictors than individuals. We generated discriminant functions

208 for feeding guild, soft/hard bodied prey, presence of copepods, fish, and shrimp (large crustaceans) in the
209 diet (Appendix 15). Some taxa have inapplicable states for certain absent characters (such as the length
210 of a nematocyst subtype that is not present in a species), which are problematic for DAPC analyses. We
211 tackled this by transforming the absent states to zeroes. This approach allows us to incorporate all the
212 data, but creates an attraction bias between small character states (*e.g.* small tentilla) and absent states
213 (*e.g.* no tentilla). Absent characters are likely to be very biologically relevant to prey capture and we
214 believe they should be accounted for. We limited the number of linear discriminant functions retained to
215 the number of groupings in each case. We selected the number of principal components retained using
216 the a-score optimization function (R `adegenet::optim.a.score`) (Jombart et al. 2010) with 100 iterations,
217 which yielded more stable results than the cross validation function (R `adegenet::xval`). This optimization
218 aims to find the compromise value with highest discrimination power with the least overfitting. From these
219 DAPCs we obtained the highest contributing morphological characters to the discriminaton (characters in
220 the top quartile of the weighted sum of the linear discriminant loadings controlling for the eigenvalue of each
221 discriminant). For each DAPC we generated hypotheses about the diets of siphonophores outside the training
222 set (R `adegenet::predict.dapc`), incorporating prediction uncertainty as posterior probabilities (Appendix 15).
223 In order to identify the sign of the relationship between the predictor characters prey type presence in the
224 diet, we then generated generalized logistic regression models (as a type of generalized linear model, or GLM
225 using R `stats::glm`) with the top contributing characters (from the corresponding DAPC) as predictors. We
226 also carried out these GLMs on the Ivlev's selectivity indices for each prey type calculated from (Purcell
227 1981) (in Appendix 11).

228 In order to explore the correlational structure among continuous characters and among their evolutionary
229 histories, we used principal component analysis (PCA) and phylogenetic PCA (Revell 2012). Since the
230 character data contains many gaps due to missing characters and inapplicable states, we carried out these
231 analyses on a subset of species and characters that allowed for the most complete dataset. This was done by
232 removing the terminal filament characters (which are only shared by a small subset of species), and then
233 removing species which had inapplicable states for the remaining characters. In addition, we obtained the
234 correlations between the phylogenetic independent contrasts (Felsenstein 1985) using the package `rphylip`
235 (Revell and Chamberlain 2014).

236 To test how many times extreme nematocyst morphologies evolved, we reconstructed the ancestral states
237 of $\log(\text{length}/\text{width})$ of the different nematocyst types, and identified the branches with the greatest shifts. In
238 addition to characterizing the shifts in the state values of haploneme and heteroneme elongation, we identified
239 and located regime shifts for the rate of evolution using a Bayesian Analysis of Macroevolutionary Mixtures
240 (BAMM) (Rabosky et al. 2014) (Appendix 16).

241 Results

242 *Phylogeny* – Only 5 nodes in the unconstrained inference were incongruent with the (Munro et al. 2018)
243 transcriptome tree. The topology of the constrained tree presented here (Fig. 4) is congruent with the
244 resolved nodes in (Dunn et al. 2005) and (Munro et al. 2018).

245 We retained the clade nomenclature defined in (Dunn et al. 2005) and (Munro et al. 2018), such
246 as Codonophora to indicate the sister group to Cystonectae, Euphysonectae to indicate the sister group
247 to Calycophorae, Clade A and B to indicate the two main lineages within Euphysonectae. In addition,
248 we define two new clades within Codonophora (Fig. 4): Eucladophora as the clade containing *Agalma*
249 *elegans* and all taxa that are more closely related to it than to *Apolemia lanosa*, and Tenticulophora as
250 the clade containing *Agalma elegans* and all taxa more closely related to it than to *Bargmannia elongata*.
251 Eucladophora is characterized by bearing spatially differentiated tentilla with proximal heteronemes and
252 a narrower terminal filament region. The etymology derives from the Greek *eu+kládos+phóros* for “true
253 branch bearers”. Tenticulophora are characterized by bearing rhopalonemes and desmonemes in the terminal
254 filament, having a pair of elastic strands, and developing proximally detachable cnidobands. The etymology
255 of this clade is derived from the Latin *tenticula* for “snare or noose” and the Greek *phóros* for “carriers”.

256 *Evolutionary dynamics between diet and tentillum morphology* – The reconstructions of feeding guilds show
257 that generalism is not likely to be ancestral, and it appears to have evolved at least two times independently
258 (Fig. 5). Generalism evolves twice independently from large crustacean specialist lineages, supporting
259 hypothesis 3. Feeding guild specializations have shifted from an alternative ancestral state at least five times,

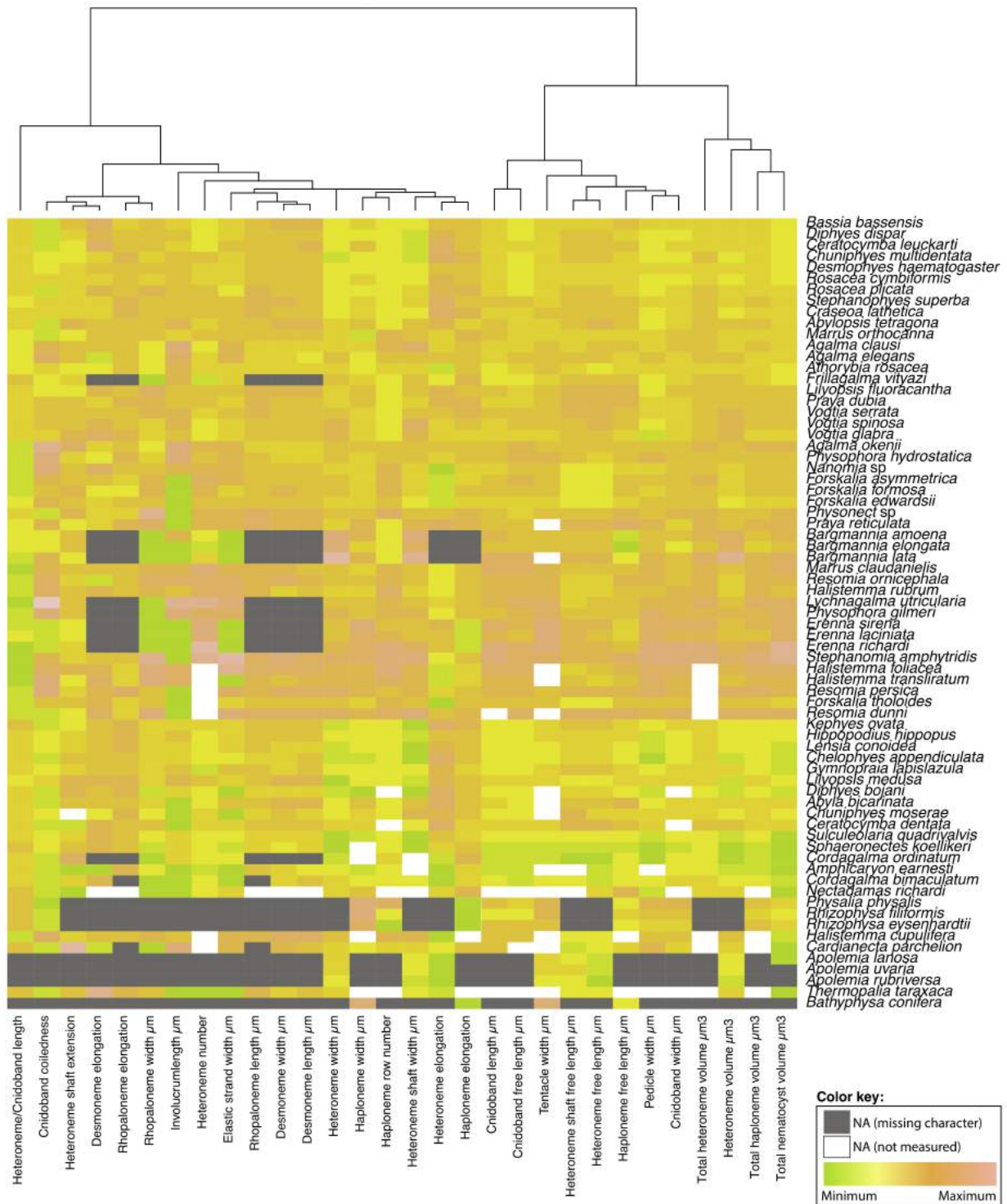


Figure 3: Heatmap summarizing the morphological diversity measured for 96 species of siphonophores clustered by similarity (raw data in Appendix 3). Missing values from absent characters presented as dark grey cells, missing values produced from technical difficulties presented as white cells. Values scaled by character.

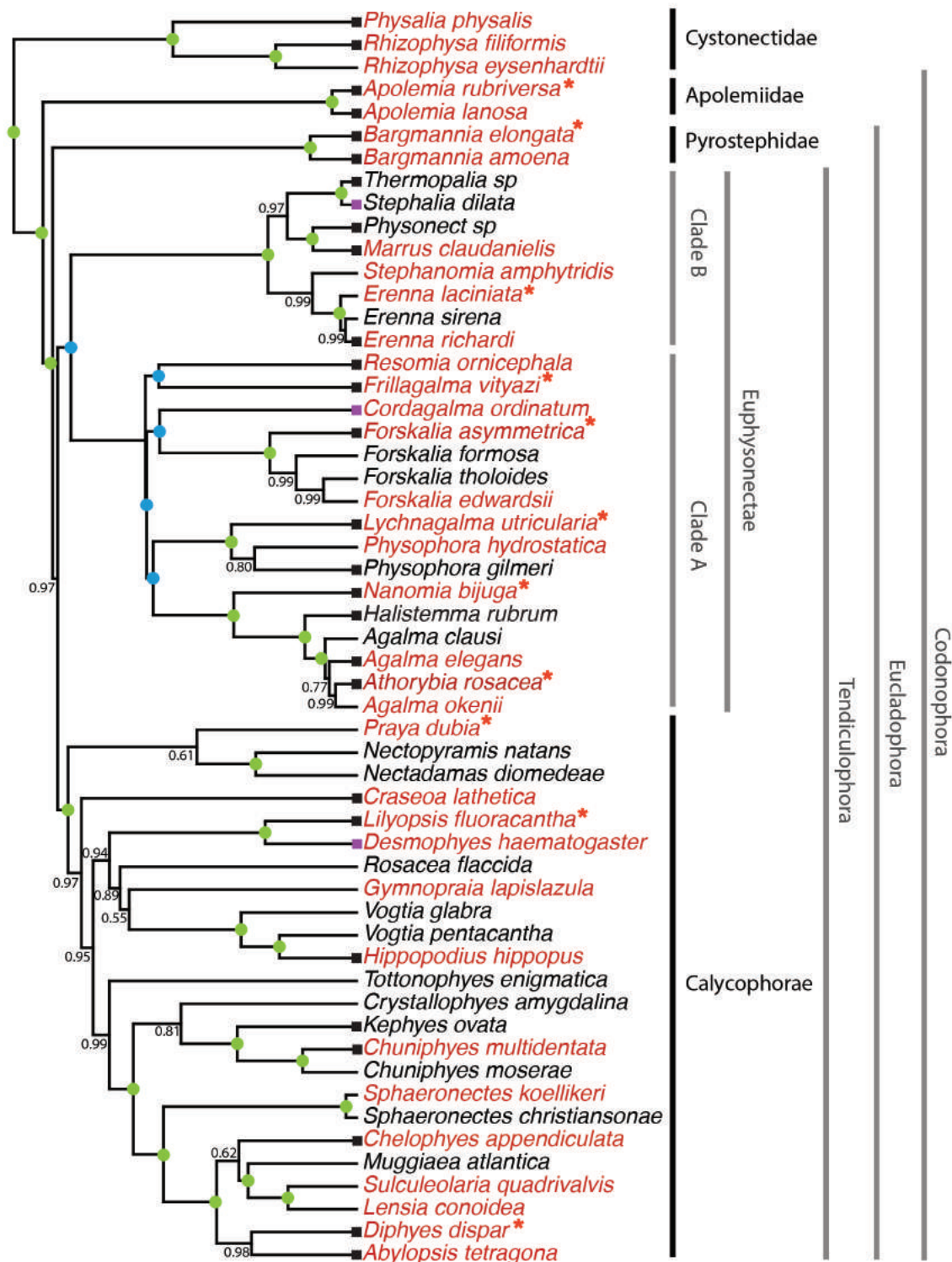


Figure 4: Bayesian time-tree built from 18S + 16S concatenated sequences. Branch lengths estimated using relaxed molecular clock. Species names in red indicate replicated representation in the morphology data. Species marked with an asterisk were recorded using high speed video. Nodes labeled with bayesian posteriors (BP). Green circles indicate BP = 1. Blue circles indicate nodes constrained to be congruent with (Munro *et al.* 2018). Tips with black squares indicate the species with transcriptomes used in (Munro *et al.* 2018). Tips with grey squares indicate genus-level correspondence to taxa included in (Munro *et al.* 2018). The main clades are labeled: in black for described taxonomic units, and in grey for operational phylogenetic designations.

260 supporting hypothesis 2. Individual prey type presence reconstructions show that copepod specialization and
 261 fish specialization evolved twice, ostracod specialization evolved at least once. The OUwie model comparison
 262 shows that out of 30 characters, 10 show significantly stronger support for the diet-driven multi-optima
 263 multi-rate OU model (Appendix 14). These characters include terminal filament nematocyst size and shape,
 264 involucre length, elastic strand width, and heteroneme number. Most of these characters are found
 265 exclusively in Tenticulophora, thus this reflects processes that could be unique to this subtree. Five characters
 266 including cnidoband length, cnidoband shape, and haploneme length show maximal support for a diet-driven
 267 single-optimum OU model. The remaining 15 characters support BM (or OU with marginal AICc difference
 268 with BM).

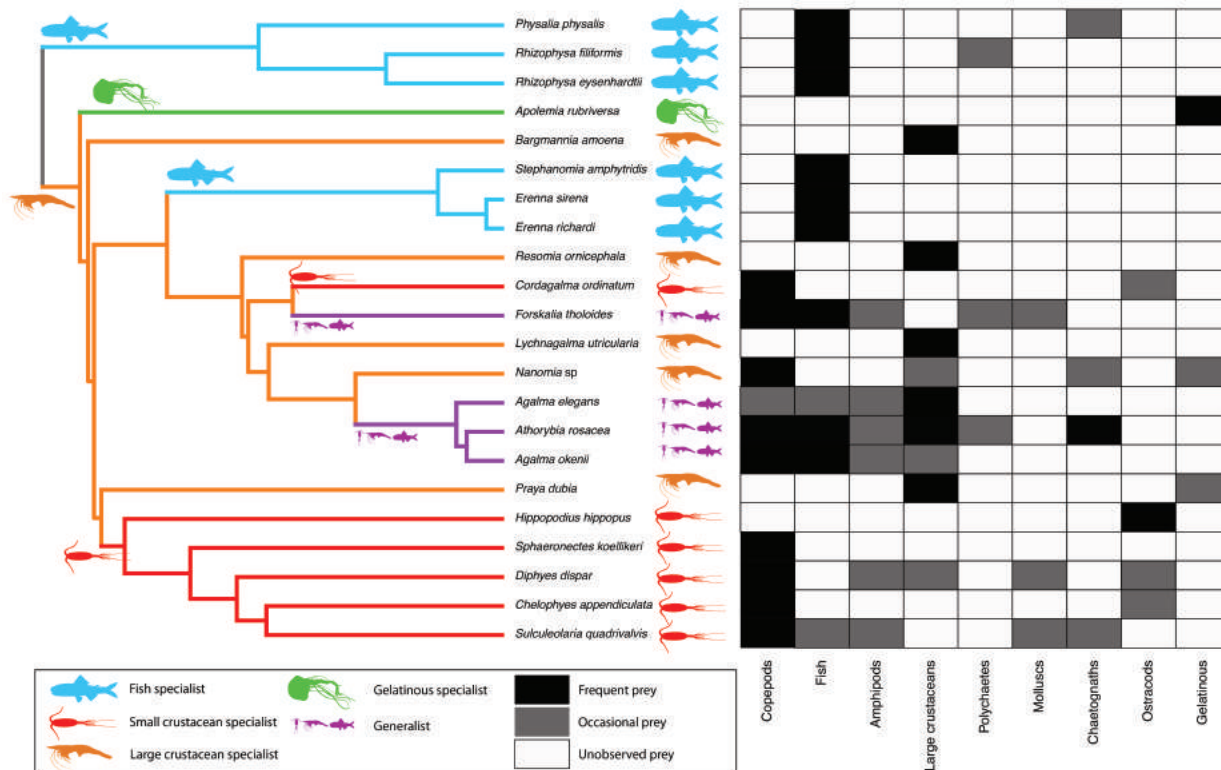


Figure 5: Left - Subset phylogeny showing the mapped feeding guild regimes that were used to inform the OUwie analyses. Right - Grid showing the prey items consumed from which the feeding guild categories were derived. Diet data were obtained from the literature review in Appendix 10.

269 Phylogenetic logistic regressions identified evolutionary associations between individual characters and
 270 the presence of particular prey types in the diet (Fig. 5, right). Shifts toward ostracod presence in diet
 271 correlated with reductions in pedicle width and total haploneme volume. Shifts to copepod presence in the
 272 diet were associated with reductions in haploneme width, cnidoband length and width, total haploneme and
 273 heteroneme volumes, and tentacle and pedicle widths. Consistently, transitions to decapod presence in the
 274 diet correlated with more coiled cnidobands (Appendix 17).

275 Phylogenetic regressions of continuous characters against prey selectivity data produced additional insights.
 276 Fish selectivity is associated with increased number of heteronemes per tentillum, increased roundness of
 277 nematocysts (desmonemes and haplonemes), larger heteronemes, reduced heteroneme/cnidoband length
 278 ratios, smaller rhopalonemes, lower haploneme SA/V ratios, and increased size of the cnidoband, elastic
 279 strand, pedicle and tentacle widths. Decapod-selective diets were associated with increasing cnidoband size
 280 and coiledness, haploneme row number, elastic strand width, and heteroneme number. Copepod-selective
 281 diets evolved in association with smaller heteroneme and total nematocyst volumes, smaller cnidobands,
 282 rounder rhopalonemes, elongated heteronemes, narrower haplonemes with higher SA/V ratios, and smaller
 283 heteronemes, tentacles, pedicles and elastic strands. Selectivity for ostracods was associated with reductions

284 in size and number of heteroneme nematocysts, reductions in cnidoband size, number of haploneme rows,
 285 heteroneme number, and cnidoband coiledness. Heteroneme length and shape also correlated negatively with
 286 chaetognath selectivity.

287 When some of the diet-morphology associations reported in the literature (Purcell 1984; Purcell and Mills
 288 1988) were tested for correlated evolution (Table 1), we found that most were consistent with an evolutionary
 289 explanation except the relationship between terminal filament nematocysts (rhopalonemes and desmonemes)
 290 and crustaceans in the diet. The latter is likely a product of the larger species richness of crustacean-eating
 291 species with terminal filament nematocysts, rather than simultaneous evolutionary gains.

292 Table 1. Tests of correlated evolution between morphological characters and aspects of the diet found
 293 correlated in the literature.

| Character | Aspect of diet | Test of evolutionary association | Relationship sign | P-value | Number of taxa | Association first report |
|-------------------------------|-------------------|----------------------------------|-------------------|---------|----------------|--------------------------|
| Differentiated cnidobands | Hard bodied prey | Pagel's test | + | 0.017 | 19 | Purcell, 1984 |
| Heteroneme volume | Copepod prey size | pGLS | + | 0.002 | 8 | Purcell, 1984 |
| Terminal filament nematocysts | Crustacean diet | Pagel's test | + | 0.200 | 19 | Purcell & Mills, 1988 |
| Number of nematocyst types | Soft-bodied prey | Phylogenetic logistic regression | - | 0.040 | 22 | Purcell & Mills, 1988 |

294 *Generating dietary hypotheses using tentillum morphology* – The discriminant analysis of principal components
 295 for feeding guild (7 principal components, 4 discriminants) produced 100% discrimination, and the highest
 296 loading contributions were found for the characters (ordered from highest to lowest): Involucrum length,
 297 heteroneme volume, heteroneme number, total heteroneme volume, tentacle width, heteroneme length, total
 298 nematocyst volume, and heteroneme width (Appendix 15.1). We used the predictions from this discriminant
 299 function to generate hypotheses about the feeding guild of 45 species in our morphological data (Fig.
 300 @figure6)). This projection predicts that two other *Apolemia* species may also be gelatinous prey specialists
 301 like *Apolemia rubriversa*, and that *Erenna laciniata* may be a fish specialist like *Erenna richardi*.

302 Table 2. Discriminant analysis of principal components for the presence of specific prey types using the
 303 morphological data. Top quartile variable (character) contributions to the linear discriminants are ordered
 304 from highest to lowest. Logistic regressions and GLMs were fitted to predict prey type presence and selectivity
 305 respectively. The sign of the slope of each predictor is reported, and highlighted green if significant (p value
 306 < 0.05). Pseudo-R² (%) approximates the percent variance explained by the model.

| Prey type | DAPC | | GLM for prey type presence (22 taxa) | | Best fitting GLM for prey type selectivity (Purcell, 1981) (7 taxa) | |
|-------------------|--------------------|-------------------------------------|--------------------------------------|-----------------------|---|-----------------------|
| | Discrimination (%) | Top quartile variable contributions | Sign | Pseudo-R ² | Sign | Pseudo-R ² |
| Copepods | 95.4 | Total nematocyst volume | - | 67.8 | - | 97.9 |
| | | Tentacle width | - | | + | |
| | | Haploneme elongation | - | | + | |
| | | Haploneme surface area/volume ratio | + | | - | |
| | | Haploneme row number | + | | + | |
| | | Cnidoband length | - | | + | |
| | | Cnidoband width | - | | - | |
| | | Cnidoband free length | + | | + | |
| Fish | 68.1 | Total haploneme volume | - | 45.8 | + | 96.0 |
| | | Heteroneme volume | + | | - | |
| | | Total nematocyst volume | - | | + | |
| | | Total heteroneme volume | - | | - | |
| | | Cnidoband length | - | | - | |
| | | Cnidoband free length | + | | + | |
| | | Involucrum length | - | | - | |
| | | Pedicle width | + | | + | |
| Large crustaceans | 81.8 | Involucrum length | + | 73.2 | + | 98.7 |
| | | Total heteroneme volume | - | | - | |
| | | Elastic strand width | - | | + | |
| | | Rhopaloneme length | + | | + | |
| | | Heteroneme volume | + | | - | |
| | | Haploneme elongation | - | | + | |
| | | Desmoneme length | - | | - | |
| | | Tentacle width | + | | + | |

308 When predicting soft and hard bodied prey specialization, the DAPC achieved 90.9% discrimination success,
 309 only marginally confounding hard-bodied specialists with generalists (Appendix 15.4). The main characters
 310 driving the discrimination are involucrum length, heteroneme number, heteroneme volume, tentacle width,
 311 total nematocyst volume, total haploneme volume, elastic strand width, and heteroneme length. Discriminant
 312 analyses and GLM logistic regressions were also applied to specific prey type presence and selectivity (Table
 313

2), revealing the sign of their predictive relationship to each prey type. We only selected prey types with sufficient variation in the data to carry out these analyses (copepods, fish, and large crustaceans). While the presence of fish or large crustaceans in the diet cannot be unambiguously discriminated using tentillum morphology (Appendix 15), specialization on fish or large crustacean prey can be fully disentangled (Appendix 15.1). For each prey type studied, tentilla morphology is a much better predictor of prey selectivity than of prey presence, despite prey selectivity data being available for a smaller subset of species. Interestingly, many of the morphological predictors had opposite slope signs when predicting prey selectivity *versus* predicting prey presence in the diet (Table 2).

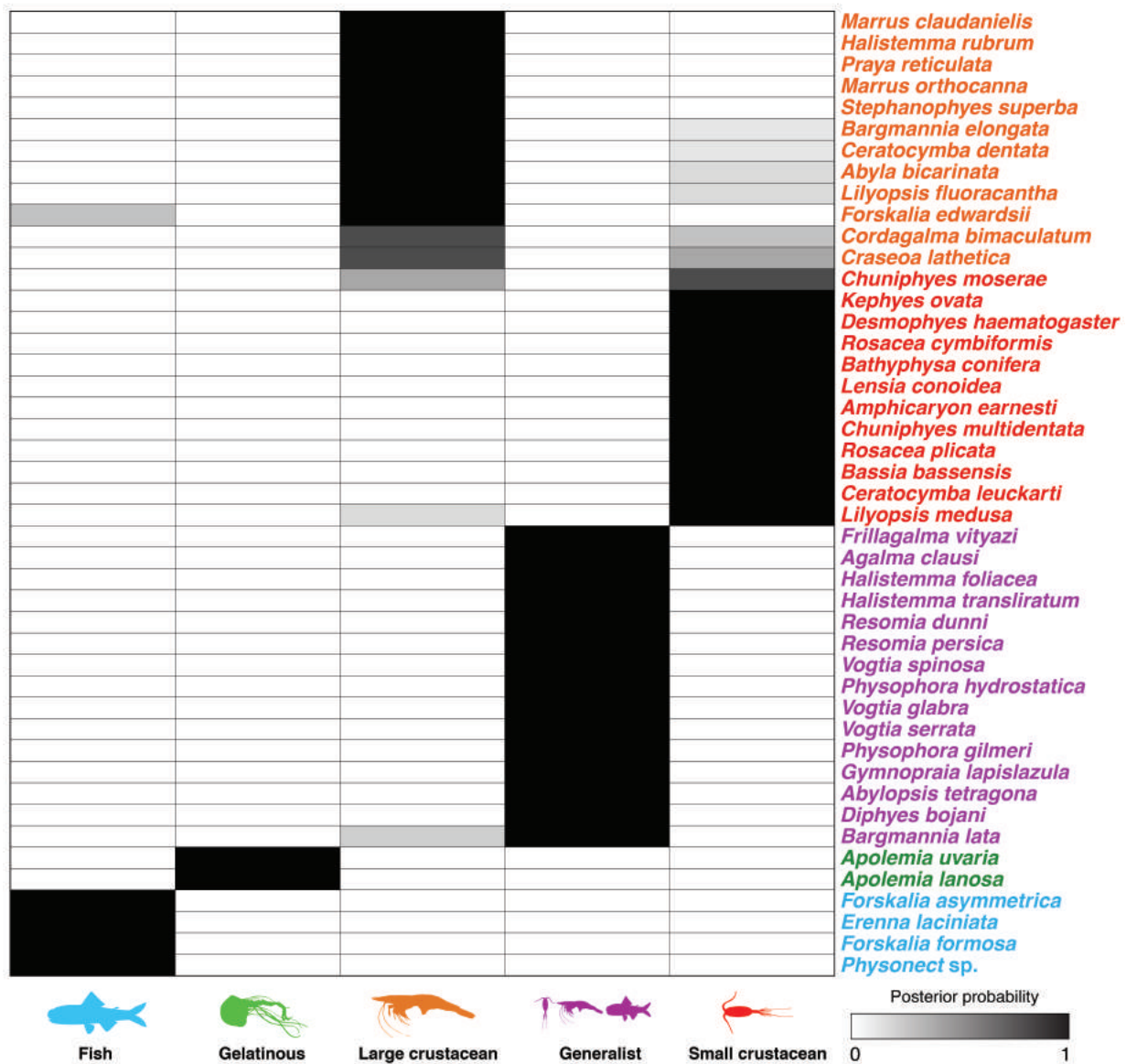


Figure 6: Hypothetical feeding guilds for siphonophore species predicted by a 6 PCA DAPC (in Appendix 15.1). Cell darkness indicates posterior probability of belonging to each guild. Training data set transformed so inapplicable states are computed as zeroes. Species ordered and colored according to their predicted feeding guild.

322 *Evolution of tentillum and nematocyst characters* – One third of the characters measured support a non-
 323 phylogenetic generative model, indicating they are not likely to be phylogenetically distributed (Appendix 12).

324 Total nematocyst volume and cnidoband-to-heteroneme length ratio showed strongly conserved phylogenetic
325 signals. 74% of characters present a significant phylogenetic signal, yet only total nematocyst volume,
326 haploneme length, and heteroneme-to-cnidoband length ratio had a phylogenetic signal $K > 1$. 67% of
327 characters support BM models, indicating a history of neutral constant divergence. No relationship between
328 phylogenetic signal and BM model support was found. Haploneme nematocyst length is the only character
329 with support for an EB model of decreasing rate of evolution with time. No character had support for a
330 single-optimum OU model (when uninformed by feeding guild regime priors).

331 The phylogenetic positions of the main categorical character shifts were reconstructed using stochastic
332 character mappings (Appendix 18), and summarized in Figure 7. Haploneme nematocysts are likely ancestrally
333 present in the tentacles, since they are present in the tentacles of many other hydrozoans. Haplonemes
334 diverged into spherical isorhizas of 2 size classes in Cystonectae, and elongated anisorhizas of one size class in
335 Codonophora. Haplonemes were likely lost in the tentacles of *Apolemia*, but spherical isorhizas are retained in
336 other *Apolemia* tissues (Siebert et al. 2013). Similarly, while heteronemes exist in other tissues of cystonects,
337 they only appear in the tentacles of codonophorans as birhopaloids in *Apolemia*, ancestral stenoteles in
338 eucladophoran physonects, and microbasic mastigophores in calycophorans.

339 Eucladophora (the clade containing Pyrostephidae, Euphysonectae, and Calycophorae, see Fig. 4)
340 encompasses most of the extant Siphonophore species (178 of 186). Innovations evolved in the stem of this
341 group include spatially segregated heteroneme and haploneme nematocysts, terminal filaments, and elastic
342 strands (Fig. 7). Pyrostephids evolved a unique bifurcation of the axial gastrovascular canal of the tentillum
343 known as the “saccus” (Totton and Bargmann 1965). The stem to the clade Tenticulophora (clade containing
344 Euphysonectae and Calycophorae, see Fig. 4) subsequently acquired further novelties such as the desmoneme
345 and rhopaloneme (acrophore subtype ancestral) nematocysts on the terminal filament (Fig. 7), which bear
346 no other nematocyst type (Fig. 1). These are arranged in sets of 2 parallel rhopalonemes for each single
347 desmoneme (Skaer 1988, 1991). The involucrem is an expansion of the epidermal layer that can cover part or
348 all of the cnidoband (Fig. 2). This structure, together with differentiated larval tentilla, appeared in the stem
349 branch to Clade A physonects. Calycophorans evolved unique novelties such as larger desmonemes at the
350 distal end of the cnidoband, pleated pedicles with a “hood” (here considered homologous to the involucrem) at
351 the proximal end of the tentillum, anacrophore rhopalonemes, and microbasic mastigophore-type heteronemes.
352 While calycophorans have diversified into most of the extant described siphonophore species (108 of 186), their
353 tentilla have not undergone any major categorical gains or losses since their most recent common ancestor.
354 Nonetheless, they have spreaded over a broad span of variation in nematocyst and cnidoband sizes.

355 *Phenotypic integration of the tentillum* – The quantitative characters we measured from tentilla and their
356 nematocysts are highly correlated. The results indicate that the dimensionality of tentillum morphology is
357 low, that many traits are associated with size, but that nematocyst arrangement and shape are independent
358 of it. Of the phylogenetic correlations (Fig. 8a, lower triangle), 81.3% were positive and 18.7% were negative,
359 while of the ordinary correlations (Fig. 8a, upper triangle) 74.6% were positive and 25.4% were negative. Half
360 (49.9%) of phylogenetic correlations were >0.5 , while only 3.6% are < -0.5 . Similarly, of the across-species
361 correlations, 49.1% were >0.5 and only 1.5% were < -0.5 . 13.9% of character pairs had opposing phylogenetic
362 and ordinary correlation coefficients. Just 4% have negative phylogenetic and positive ordinary correlations
363 (such as rhopaloneme elongation ~ heteroneme-to-cnidoband length ratio and haploneme elongation, or
364 haploneme elongation ~ heteroneme number), and vice versa for 9.9% of character pairs (such as heteroneme
365 elongation ~ cnidoband convolution and involucrem length, or rhopaloneme elongation with cnidoband
366 length). These disparities can be caused by Simpson’s paradox (Blyth 1972), the reversal of the sign of a
367 relationship when a third variable (or a phylogenetic topology (Uyeda et al. 2018)) is considered. However,
368 no character pair had correlation coefficient differences larger than 0.64 between ordinary and phylogenetic
369 correlations (heteroneme shaft extension ~ rhopaloneme elongation has a Pearson’s correlation of 0.10 and a
370 phylogenetic correlation of -0.54). Rhopaloneme shape shows the most incongruences between phylogenetic
371 and ordinary correlations with other characters.

372 In the non-phylogenetic PCA morphospace using only simple characters (Fig. 9), PC1 (aligned with
373 tentillum and tentacle size) explained 69.3% of the variation in the tentillum morphospace, whereas PC2
374 (aligned with heteroneme length, heteroneme number, and haploneme arrangement) explained 13.5%. In
375 a phylogenetic PCA, 63% of the evolutionary variation in the morphospace is explained by PC1 (aligned
376 with shifts in tentillum size), while 18% is explained by PC2 (aligned with shifts in heteroneme number and
377 involucrem length).

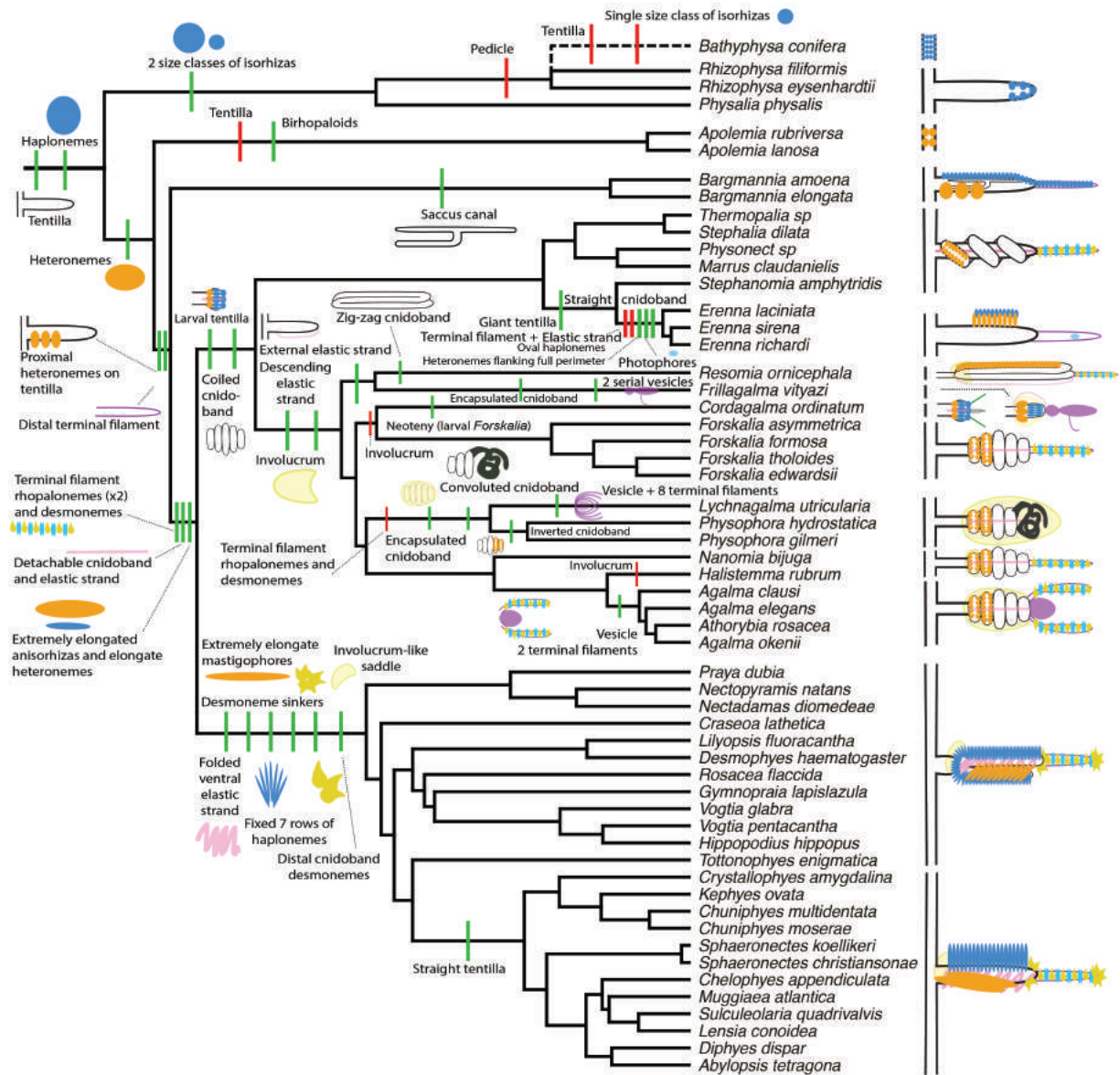


Figure 7: Siphonophore cladogram with the main categorical character gains (green) and losses (red) mapped. Some branch lengths were modified from the Bayesian chronogram to improve readability. The main visually distinguishable tentillum types are sketched next to the species that bear them, showing the location and arrangement of the main characters. In large complex-shaped tentilla, haplonemes were omitted for simplification. The rhizophysid *Bathypphysa conifera* branch was appended manually as a polytomy (dashed line).

378 *Evolution of nematocyst shape* – Haploneme nematocyst evolution has been mainly driven by a single
 379 large shift towards elongation in Tenticulophora, which contains the majority of described siphonophore
 380 species. There is one secondary return to more oval, less elongated haplonemes in *Erenna*, but it doesn't
 381 reach the sphericity present in Cystonectae or Pyrostephidae (Fig. 10). Heteroneme evolution presents a less
 382 radical evolutionary history, where Tenticulophora evolved more elongate heteronemes, but the difference
 383 between theirs and other siphonophores is much smaller than the variation in shape within Tenticulophora,
 384 bearing no phylogenetic signal. In this group, evolution of heteroneme shape has diverged in both directions,
 385 and there is no correlation with haploneme shape, which has remained fairly constant (elongation between
 386 1.5 and 2.5).

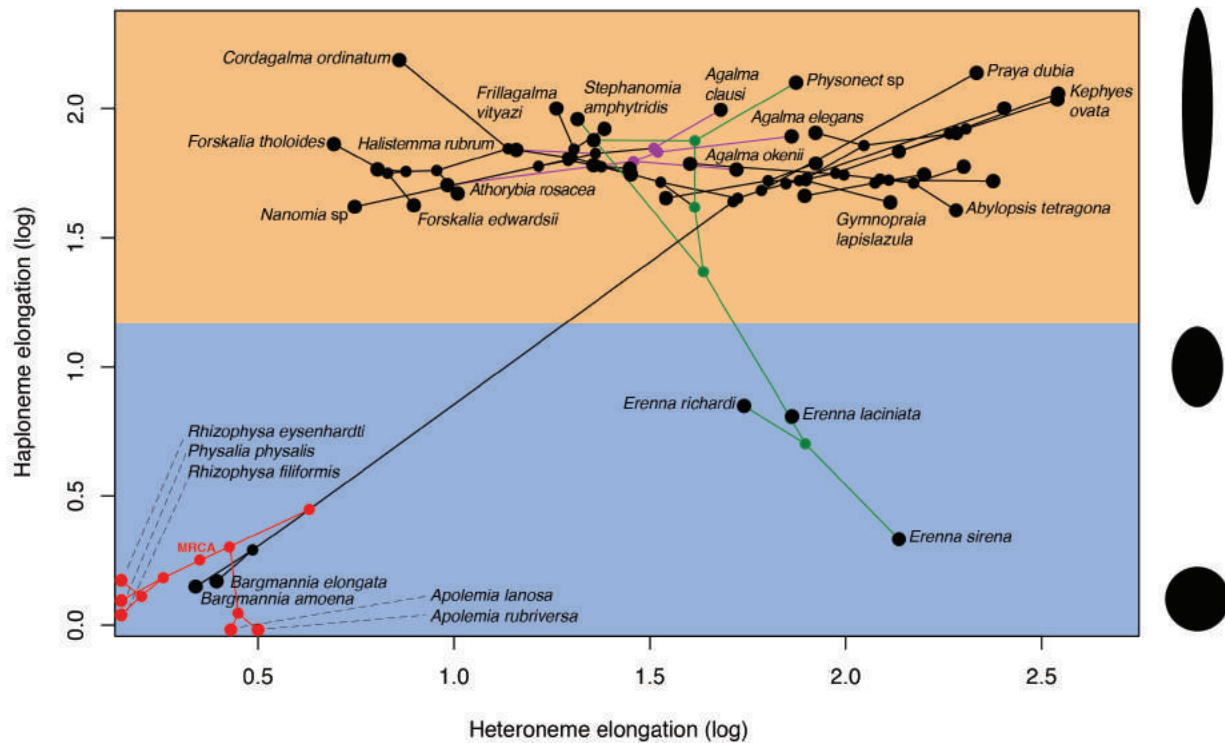


Figure 10: Phylomorphospace showing haploneme and heteroneme elongation (log scaled). Orange area delimits rod-shaped haplonemes, blue area covers oval and round shaped haplonemes. Smaller dots and lines represent phylogenetic relationships and ancestral states of internal nodes under BM. Species nodes in red were manually added to the plot. Cystonects have no tentacle heteronemes and are projected onto the haploneme axis. Apolemids have no tentacle haplonemes and are projected onto the heteroneme axis. Colored branches and nodes correspond to BMM regimes of accelerated haploneme shape (green) and heteroneme shape (violet) evolution.

387 Haploneme and heteroneme shape share 21% of their variance across extant values, and 53% of variance
 388 in their shifts along the branches of the phylogeny. However, much of this correlation is due to the contrast
 389 between Pyrostephidae and their sister group Tenticulophora (Fig. 4). BMM identified a regime shift in
 390 heteroneme shape evolution on the branches leading to *Agalma* and *Athorybia*. For the rates of haploneme
 391 shape evolution, BMM identified two main independent regime shifts (Fig. 10): one in the branch leading to
 392 Clade B physonects. Clade B includes *Erenna*, *Stephanomia*, *Marrus*, and rhodaliids. Most of these taxa
 393 have rod-shaped anisorhizas, but *Erenna* has oval ones). No clear regime shift patterns were identified in the
 394 evolution of desmoneme and rhopaloneme shape.
 395

396 *Functional morphology of tentillum and nematocyst discharge* – Tentillum and nematocyst discharge
 397 high speed measurements are available in Appendix 4. While the sample sizes of these measurements were

398 insufficient to draw reliable statistical results at a phylogenetic level, we did observe patterns that may be
399 relevant to their functional morphology. For example, cnidoband length is strongly correlated with discharge
400 speed (p value = 0.0002). This is probably the sole driver of the considerable difference between euphysonect
401 and calyphoran tentilla discharge speeds (average discharge speeds: 225.0mm/s and 41.8mm/s respectively;
402 t-test p value = 0.011), since the euphysonects have larger tentilla than the calyphorans among the species
403 recorded.

404 We also observed that calyphoran haploneme tubules fire faster than those of euphysonects (T-test p
405 value = 0.001). Haploneme nematocysts discharge 2.8x faster than heteroneme nematocysts (T-test p value
406 = 0.0012). Finally, we observed that the stenoteles of the Euphysonectae discharge a helical filament that
407 “drills” itself through the medium it penetrates as it everts.

408 Discussion

409 The core aims of this study are to examine the evolutionary history of siphonophore tentilla and diet,
410 characterize the evolutionary shifts in their trophic niches, and identify the morphological characters that
411 evolve with changes in prey type. We inquire whether the relationships between form and function observed
412 in extant taxa are due to correlated evolution or non-evolutionary causes, whether the evolution of their
413 trophic specializations supports or challenges traditional ecological theory (such as the idea specialists evolve
414 from generalists), and whether the diets of siphonophores can be hypothesized by observing their tentacles. In
415 addition, we produced novel findings on tentillum morphology, siphonophore phylogeny, nematocyst character
416 evolution, and tentillum discharge dynamics.

417 *Evolution of tentillum morphology with diet* – Siphonophores are an abundant group of zooplankton
418 in oceanic ecosystems (Longhurst 1985; O’Brien 2007). While little is known about siphonophore trophic
419 ecology, what is known indicates that they occupy a central position in midwater food webs (Choy et al.
420 2017), serving as trophic intermediaries between smaller zooplankton and higher trophic level predators.
421 Siphonophore species have been observed to feed on a variety of prey with very different sizes, traits, and
422 behaviors. Because there is a total absence of siphonophores in the fossil record, how they became established
423 as the ubiquitous and diversified predators in today’s oceans remains an open question. Predators that use
424 similar tools for prey capture tend to capture similar prey, so their abundance and coexisting species diversity
425 are inversely related due to competitive exclusion by resource limitation (Schluter 2000). However, this is not
426 consistent with what we observe in siphonophores, which have been found to be both very abundant and
427 locally diverse (Longhurst 1985, Mapstone (2014)). We hypothesize that siphonophores have escaped this by
428 specializing on different prey resources.

429 According to our reconstructions, the evolutionary history of siphonophore diets indicates that being a
430 specialist was an ancestral aspect of their trophic niche, while trophic generalism is likely a derived condition.
431 Several studies (reviewed in (Futuyma and Moreno 1988)) have suggested that resource specialization is
432 an irreversible dead end due to the constraints posed by phenotypic specialization. Our reconstructions
433 show that this is not the case for siphonophores, where the prey type on which they specialize has shifted at
434 least 5 times, and generalism has evolved independently at least twice. Among the evolutionary hypotheses
435 considered, we find support for both hypotheses 2 (specialist resource switching) and 3 (specialist to generalist),
436 but no support for hypothesis 1 (generalist to specialist). The evolutionary history of tentilla shows that
437 siphonophores are an example of trophic niche diversification via morphological innovation and evolution,
438 which allowed transitions between specialized trophic niches. This strategy is particularly important in a
439 deep open ocean ecosystem, which is a relatively homogeneous physical environment, where the primary
440 niche heterogeneity available is the potential interactions between organisms (Robison 2004).

441 One of the most common prey items found in siphonophore diets is copepods (Fig. 5). Copepod-specialized
442 diets have evolved convergently in *Cordagalma* and some Calyphorans. These evolutionary transitions
443 happened together with transitions to smaller tentilla with fewer cnidoband nematocysts. Tentilla are
444 expensive single-use structures, therefore we would expect that specialization in small prey would beget
445 reductions in the size of the prey capture apparatus to the minimum required for the ecological function.
446 *Cordagalma*’s tentilla strongly resemble the larval tentilla (only found in the first-budded feeding body of the
447 colony) of their sister genus *Forskalia* spp. This indicates that the evolution of *Cordagalma* tentilla could be
448 a case of paedomorphosis associated with predatory specialization.

449 (Purcell 1984) showed that haplonemes have a penetrating function as isorhizas in cystonects and an

450 adhesive function as anisorhizas in Tenticulophora. The two clades that have been observed primarily feeding
451 on fish (Cystonectae and Clade B, which includes *Erenna*, *Stephanomia*, *Marrus*, and Rhodaliids) present an
452 accelerated rate of haploneme shape evolution towards more compact haplonemes, significantly distinct from
453 their closest relatives. Isorhizas in cystonects are known to penetrate the skin of fish during prey capture,
454 and to deliver the toxins that aid in paralysis and digestion (Hessinger 1988). *Erenna* anisorhizas are also
455 able to penetrate human skin and deliver a painful sting (Pugh 2001) (and pers. obs.), a common feature of
456 piscivorous cnidarians like cystonects or cubozoans.

457 (Thomason 1988) hypothesized that smaller, more spherical nematocysts, with a lower surface area to
458 volume ratio, are more efficient in osmotic-driven discharge and thus have more power for skin penetration.
459 The elongated haplonemes of crustacean-eating Tenticulophora have never been observed penetrating their
460 crustacean prey ((Purcell 1984) and our unpublished observations), and are hypothesized to entangle the prey
461 through adhesion of the abundant spines to the exoskeletal surfaces and appendages. Entangling requires less
462 acceleration and power during discharge than penetration, as it does not rely on point pressure. In fish-eating
463 cystonects and *Erenna* species, the haplonemes are much less elongated and very effective at penetration, in
464 congruence with the osmotic discharge hypothesis. The accelerated rate of heteroneme shape diversification
465 in the smallest clade containing *Agalma* and *Nanomia* may indicate a rapid dietary differentiation. However,
466 our limited ecological data do not show any significant dietary differentiation in this group.

467 When we tested the diet-morphology correlation hypotheses supported in the literature from a macroevo-
468 lutionary perspective, we found that most of them were consistent with correlated evolution (Table 2). The
469 ecomorphological association between rhopalonemes, desmonemes, and crustacean eaters was not congruent
470 with a scenario of correlated evolution. This could be due to the broader set of taxa in our analyses, including
471 multiple species without desmonemes or rhopalonemes but which effectively capture crustaceans (such as
472 *Cordagalma ordinatum*, *Lychnagalma utricularia*, and *Bargmannia amoena*).

473 While our results unambiguously show that tentillum morphology evolved with diet, the conclusions
474 we can draw from these analyses are limited by the sparse dietary data available. Moreover, our analyses
475 are not sufficient to adequately test hypotheses of adaptation, since that would require evidence of changes
476 within a population exposed to different selective pressures. When interpreting these results, it is important
477 to remember that diet is a product of environmental prey availability and predator selectivity. Selectivity
478 differences across siphonophore species could be driven by other phenotypes not accounted for this study.
479 For example, tentacle-deploying behavior, positioning in the water column, or thresholds for discharging on
480 or ingesting an encountered animal. Further observations on these behaviors in the field are necessary to
481 assess their relative importance in determining dietary composition. In addition to behavior, there is much
482 biochemistry in the prey capture and digestion processes that remains unexplored. Part of the success in
483 siphonophore prey capture is likely determined by the effectivity of the toxins delivered by the nematocysts on
484 different taxa. Comparative toxin assays and venom protein evolution studies could shed light on this question.
485 Moreover, siphonophore trophic specialization may have brought changes in the digestive biochemistry of
486 gastrozooids and palpons. A comparison of the gene expression levels for different enzymes in the gastrozooids
487 of different species, together with digestive enzyme sequence evolution studies, and a toxicological assay of
488 the different venoms in siphonophore nematocysts on different prey taxa, would provide a great complement
489 to our results.

490 *Generating hypotheses on siphonophore feeding ecology* – One motivation for our research was to understand
491 the links between predator capture tools and their diets so we can generate hypotheses about the diets
492 of siphonophores based on morphological characteristics. Indeed, our discriminant analyses were able to
493 distinguish between different siphonophore diets based on morphological characters alone. The models
494 produced by these analyses generated testable predictions about the diets of many species for which we only
495 have morphological data of their tentacles. While the limited dataset used here is informative for generating
496 tentative hypotheses, the empirical data are still scarce and insufficient to cast robust predictions. This
497 reveals the need to extensively characterize siphonophore diets and feeding habits. In future work, we can
498 test these ecological hypotheses and validate these models by directly characterizing the diets of some of
499 those siphonophore species. Predicting diet using morphology is a powerful tool to reconstruct food web
500 topologies from community composition alone. In many of the ecological models found in the literature,
501 interactions among the oceanic zooplankton have been treated as a black box (Mitra 2009). The ability
502 to predict such interactions, including those of siphonophores and their prey, will enhance the taxonomic
503 resolution of nutrient flow models constructed from plankton community composition data.

504 *Phenotypic integration of siphonophore tentilla* – Tentillum characters, such as nematocysts, arose from
505 the subfunctionalization of serial homologs (David et al. 2008). Serial homologs have shared genetic elements
506 underlying their development, and are expected to have phylogenetic correlations (Wagner and Schwenk
507 2000). In addition, these sub-structures must fit and work together in synchrony to ensnare prey successfully
508 (functional integration). Character complexes that satisfy these conditions tend to be phenotypically integrated.
509 Phenotypic integration is the set of functional and genetic correlations among the traits of an organism
510 (Pigliucci 2003). These correlations have been hypothesized to direct and constrain adaptive evolution
511 (Wagner and Schwenk 2000). The siphonophore tentillum morphospace has a fairly low extant dimensionality
512 due to an evolutionary history with many synchronous, correlated changes. This is consistent with strong
513 phenotypic integration where genetic and developmental correlations are maintained by natural selection to
514 preserve function.

515 Part of the tentillum structural correlations are to be expected from shared regulatory networks for elements
516 that develop together from common positional bud (budding tentilla in the tentacle). Similarly, correlations
517 between nematocyst subtypes are also expected given their common evolutionary and developmental origin.
518 None of these explanations for correlated evolution are surprising, nor require natural selection. However, we
519 also found correlations between nematocyst and tentillum characters. Siphonophore tentacle nematocysts (in
520 their cnidocytes) are not produced nor matured in the developing tentillum. These cnidocytes are produced
521 by dividing cnidoblasts in the basigaster (basal swelling of the gastrozoid). Once the cnidocytes have
522 assembled the nematocyst, they migrate outward along the tentacle (Carré 1972) and position themselves
523 in the tentillum according to their type and size (Skaer 1988). Thus, the developmental programs that
524 produce the observed nematocyst morphologies are spatially separated from those producing the tentillum
525 morphologies. Therefore, we hypothesize the genetic correlations and phenotypic integration between tentillum
526 and nematocyst characters are maintained through natural selection on separate regulatory networks, out
527 of the necessity to work together and meet the spatial, mechanical, and functional constraints of their prey
528 capture behavior.

529 *Evolutionary history of tentillum morphology* – This study produced the most speciose siphonophore
530 molecular phylogeny to date, while incorporating the most recent findings in siphonophore deep node
531 relationships. This revealed for the first time that *Erenna* is the sister to *Stephanomia amphytridis*. *Erenna*
532 and *Stephanomia* bear the largest tentilla among all siphonophores, thus their monophyly indicates that
533 there was a single evolutionary transition to giant tentilla. Siphonophore tentilla range in size from ~30 μm in
534 some *Cordagalma* specimens to 2-4 cm in *Erenna* species, and up to 8 cm in *Stephanomia amphytridis* (Pugh
535 and Baxter 2014). Most siphonophore tentilla measure between 175 and 1007 μm (1st and 3rd quartiles),
536 with a median of 373 μm . The extreme gain of tentillum size in this newly found clade may have important
537 implications for access to large prey size classes.

538 Tentillum size, as well as the majority of the characters studied, supported BM evolutionary models.
539 There are two alternative hypotheses about the generative process of BM. One hypothesis would suggest
540 that these characters are not under selection, and therefore diverging neutrally (Lande 1976). The second
541 hypothesis suggests that they are under selection, but the adaptive landscape was rapidly shifting (Hansen
542 and Martins 1996), without leaving clear patterns across the phylogeny. Some of the BM supported characters
543 are likely to have evolved under the second hypothesis, since when a diet-driven regime tree was provided,
544 these characters preferentially supported an OU model (Appendix 14).

545 Siphonophore tentilla are defined as lateral, monostichous evaginations of the tentacle gastrovascular
546 lumen with epidermal nematocysts (Totton and Bargmann 1965). The buttons on *Physalia* tentacles were not
547 traditionally regarded as tentilla, but (Bardi and Marques 2007) and our observations (Munro et al. 2018),
548 confirm that the buttons contain evaginations of the gastrovascular lumen, thus satisfying all the criteria
549 for the definition. In this light, and given that most Cystonectae bear conspicuous tentilla, we conclude (in
550 agreement with (Munro et al. 2018)) that tentilla are likely ancestral to all siphonophores, and secondarily
551 lost in *Apolemia* and *Bathyphysa conifera*.

552 The clade Tenticulophora contains far more species than its relatives Cystonectae, Apolemiidae, and
553 Pyrostephidae. An increase in clade richness and ecological diversification can be triggered by a 'key innovation'
554 (Simpson 1955). The evolutionary innovation of the Tenticulophora tentilla with shooting cnidobands and
555 modular regions may have facilitated further dietary diversification to unfold. In addition, our work identifies
556 an interesting example of convergent evolution. The calycophoran tentillum morphospace (Fig. 9) was
557 independently occupied by the physonect *Frillagalma vityazi*. Like calycophorans, *Frillagalma* tentilla have

558 small C-shaped cnidobands with a few rows of anisorhizas. Unlike calyphorans, they lack paired elongate
559 microbasic mastigophores. Instead, they bear three elongated stenoteles, and their cnidobands are followed
560 by a branched vesicle, unique to this genus. Their tentillum morphology is very different from that of other
561 related physonects, which tend to have long, coiled, cnidobands with many paired oval stenoteles. Most
562 calyphoran diet studies have reported their prey to be small crustaceans such as copepods or ostracods.
563 The diet of *Frillagalma vityazi* is unknown, but this morphological convergence presents the hypothesis that
564 they evolved to capture similar kinds of prey. Our DAPCs predict that *Frillagalma* has a generalist niche
565 with both soft and hard bodied prey, including copepods.

566 *Evolution of nematocyst shape* – The phylogenetic placement of siphonophores among the Hydrozoa
567 remains an unresolved question (Munro et al. 2018). The most recent work on this front sets them as
568 sister group to all other Hydroidolina (Kayal et al. 2015). All reconstructions of hydrozoan relationships
569 recover siphonophores as an early diverging lineage within Hydroidolina, with many unique apomorphic
570 characters. Therefore, there is a great uncertainty around the ancestral plesiomorphies of the common
571 ancestor of all siphonophores. This is especially true for those characters that present extreme differences
572 between Cystonectae and Codonophora (the earliest split in the siphonophore phylogeny). One such character
573 is the shape of haploneme nematocysts. A remarkable feature of siphonophore haplonemes is that they are
574 outliers to all other Medusozoa in their surface area to volume relationships, deviating significantly from
575 sphericity (Thomason 1988). This suggests a different mechanism for their discharge that could be more
576 reliant on capsule tension than on osmotic potentials (Carré and Carré 1980), and strong selection for efficient
577 nematocyst packing in the cnidoband (Thomason 1988; Skaer 1988). Our results show that Codonophora
578 underwent a shift towards elongation and Cystonectae towards sphericity, assuming the common ancestor
579 had an intermediate state. Since we know that the haplonemes of other hydrozoan outgroups are generally
580 spheroid, it is more parsimonious to assume that cystonects retain this ancestral state. Later, we observe a
581 return to more rounded (ancestral) haplonemes in *Erenna*, associated with a secondary gain of a piscivorous
582 trophic niche, like that exhibited by cystonects.

583 Simultaneous with this shift in haploneme shape, heteroneme shape evolution also presents a single
584 transition to elongation. In addition, the clade defined by the most recent common ancestor of *Agalma* and
585 *Nanomia* shows an increased rate of divergence for heteroneme shape, spanning extremes (from oval *Nanomia*
586 stenoteles to the elongate *Agalma okenii* stenoteles) in relatively short evolutionary time. While cystonects
587 do not bear heteronemes in their tentacles, *Physalia physalis* bears stenoteles in other zooids, hypothetically
588 used for defense rather than for prey capture. These stenotele heteronemes are rounded like those found in
589 pyrostephids and apolemiids, which is consistent with the story of a single transition leading to the elongated
590 heteronemes in the stem of Tenticulophora.

591 The implications of these results to the evolution of nematocyst function suggests that an innovation in
592 the discharge mechanism of haplonemes may have occurred during the main shift to elongation. Elongate
593 nematocysts can be tightly packed into cnidobands. We hypothesize this may be a Tenticulophora lineage-
594 specific adaptation to packing more nematocysts into a limited tentillum space, as suggested by (Skaer
595 1988). Tenticulophora is the most abundant, speciose, and diverse (ecologically and morphologically) clade
596 of siphonophores, containing the clades Euphysonectae and Calyphorae. We hypothesize that this packing-
597 efficient haploeme morphology may have been a key innovation leading to the diversification of this clade.
598 However, other characters that shifted concurrently in the stem of this clade may have been responsible for
599 their extant diversity.

600 Some siphonophore clades have more nematocyst types than others in the tentacles (Tenticulophora
601 has 4 types, Cystonectae and Apolemiidae have 1), or different subtypes (e.g. stenoteles, mastigophores,
602 birhopaloids). Siphonophores bear nematocysts in different parts of the colony (tentacles, gastrozooids,
603 papons, palpacles, bracts, nectophores, and gonozooids) (Totton and Bargmann 1965). In this paper we only
604 look at the presence of nematocyst types in the tentacles, therefore the gains and losses reported are not
605 necessarily morphological innovations, but developmental allocations. For instance, stenoteles (a type of
606 heteroneme) are absent from the tentacles of *Physalia* and seem to reappear in Euphysonectae, but we know
607 that *Physalia* has stenoteles in other body parts (Totton and Bargmann 1965). Nonetheless, siphonophores
608 have evolved unique nematocyst types and subtypes, not present in any other cnidarian, such as the two
609 types of rhopalonemes (acrophores and anacrophores), and the haploneme homotrichous anisorhizas (Werner
610 1965). Both these nematocyst types evolved in the stem to Tenticulophora, and are likely morphological
611 innovations, since they have not been yet found in any other tissue of any other organism. The gain of

612 extreme elongation in the haplonemes of Tenticulophora can be interpreted as part of the character shift to a
613 novel anisorhiza subtype.

614 *Diversity of discharge dynamics* – A fundamental corollary in functional morphology is that structural
615 morphology determines functional performance (Wainwright and Reilly 1994). We expected the discharge
616 dynamics exhibited by siphonophore tentilla should vary with their morphological diversity. Our results are
617 consistent with this expectation, and we observe, for example, that cnidoband size largely correlates with
618 cnidoband discharge speed. This suggests that prey escape response speed may determine the minimum
619 cnidoband length for successful capture.

620 *Insights from tentillum morphology* – The measurements taken illustrate that the morphological diversity
621 of siphonophore tentilla and nematocysts spans clades, from the overall shape and size, to the dimensions of
622 the nematocysts. Siphonophores bear the largest nematocysts among Hydrozoans, and present a wide variety
623 of nematocyst sizes within the clade. The largest nematocysts in our dataset (*Bargmannia lata* by volume
624 and *Resomia dunni* by length), are the largest of all nematocysts reported for cnidarians, and therefore
625 possibly the largest intracellular organelles among all living things.

626 In addition to the insights produced in this study, the newly collected morphological data provide a
627 unique resource for future studies, and a reference dataset for siphonophore identification. Many conspicuous
628 categorical characters in siphonophore tentilla are very diagnostic, such as: the fluorescent lures of *Resomia*
629 *ornicephala*, the bioluminescent lures of *Erenna* species, the unique branched vesicle of *Frillagalma vityazi*,
630 the buoyant medusa-resembling vesicle of *Lychnagalma* with 8 pseudo-tentacles, the zig-zag morphology of
631 *Resomia* species, the inverted orientation of *Physophora* cnidobands, the button-like tentilla of *Physalia*, or the
632 acorn-shaped minute tentilla of *Cordagalma* species (Fig. 7). Some categorical characters are synapomorphic
633 diagnostic characters for large clades, such as the proximal tentillum heteronemes of Eucladophora, the
634 elastic strand, rhopalonemes, and desmonemes of Tenticulophora, the larval tentilla of Euphysonectae, the
635 two-sized isorhizas of Cystonectae, the saccus canal of Pyrostephidae, or the seven rows of anisorhizas in
636 Calycophorae. These characters should be used together with the classical nectophore and bract characters
637 to identify species or at least impute phylogenetic affiliation from incomplete material.

638 Conclusions

639 Siphonophores have diverse predatory niches in the open ocean, ranging from mid-trophic small crustacean
640 eaters to piscivorous super-carnivores. With the evolution of diversified prey type specializations comes the
641 evolution of morphologies adapted to the challenges posed by different prey. The results presented here
642 indicate that the associations found between siphonophore tentilla and their prey are a product of correlated
643 evolution in highly integrated traits. While much of the literature focuses on how predatory generalists evolve
644 into predatory specialists, in siphonophores we find predatory specialists can evolve into generalists, and
645 that specialists on one prey type have directly evolved into specialists on other prey types. Our extended
646 morphological characterization shows that the relationships between form and ecology hold across a large set of
647 taxa and characters, and can be used to generate hypotheses on the feeding habits of uncharacterized species.
648 We conclude that the siphonophores were able to establish as abundant oceanic predators by occupying a
649 variety of trophic niches facilitated by the evolution and diversification of extraordinary prey capture tools on
650 their tentacles.

651 Supplementary Materials

652 Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.NNNN> (Temporarily,
653 for pre-print purposes, the Supplementary Materials and Aonline Appendices are all available in https://github.com/dunnlab/tentilla_morph/Supplementary_materials)
654

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670 Author contributions

671 Alejandro Damian-Serrano (Yale University) collected specimens and morphological data, executed phylo-
672 genetic analyses (tree inference and character evolution), elaborated the figures, wrote and reviewed the
673 manuscript.

674 Steven H.D. Haddock (Monterey Bay Aquarium Research Institute) contributed by facilitating the access
675 to the field and collection tools, directing the ROV and blue water diving operations in the field. He also
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