

¹ **Shaped to kill: The evolution of siphonophore tentilla**
² **for specialized prey capture in the open ocean**

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¹⁰ **Abstract**

¹¹ As predators evolve to feed on different prey taxa, their apparatus for prey capture can adapt
¹² into a variety of forms. The study of this evolutionary process is facilitated by a predator
¹³ clade with structures used exclusively for prey capture and with significant morphological
¹⁴ variation in these structures. Siphonophores, a clade of colonial cnidarians, satisfy these
¹⁵ criteria particularly well. Their tentilla (tentacle side branches) are the exclusive means of
¹⁶ prey capture for the large majority of species and have no other known function. Earlier
¹⁷ work has shown that extant siphonophore diets correlate with the different morphologies and
¹⁸ sizes of their tentilla and nematocysts. We hypothesize that evolutionary specialization on
¹⁹ different prey types has driven the phenotypic evolution of these characters. To test this
²⁰ hypothesis, we: (1) measured multiple morphological characters from siphonophore tentacle
²¹ specimens from 45 species, (2) mapped these data to a phylogenetic tree, and (3) analyzed
²² the evolutionary associations between morphological characters and prey type data from the
²³ literature. Our results show that siphonophore tentillum morphology has strong evolutionary
²⁴ associations with prey type, and suggest that shifts between prey type are linked to shifts in
²⁵ tentillum and nematocyst size and shape. We found that predatory specialists can evolve

26 into generalists, and that specialists on one prey type have directly evolved into specialists
27 on other prey types. When there are changes in trophic niche, both trait optima and trait
28 correlation patterns showed significant shifts. The evolutionary history of tentilla shows that
29 siphonophores are an example of ecological niche diversification via morphological innovation
30 and evolution. The extreme modularity of tentilla may have released siphonophores from the
31 evolutionary constraints of adaptation to ecologically specialized niches. This contributes to
32 understanding how morphological evolution has shaped present-day oceanic food webs.

33 **Keywords**

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

35 **Introduction**

36 Most animal predators use specific structures to capture and subdue prey. Raptors have
37 claws and beaks, snakes have fangs, wasps have stingers, and cnidarians have nematocyst-
38 laden tentacles. The functional morphology of these structures is critical to their ability
39 to successfully capture prey (1). Long-term adaptive evolution in response to the defense
40 mechanisms of the prey (*e.g.*, avoidance, escape, protective barriers) leads to modifications
41 that can counter those defenses. The more specialized the diet of a predator is, the more
42 specialized its structures need to be to efficiently overcome the challenges posed by the
43 prey. Understanding the relationships between morphology and predatory specialization is
44 necessary to contextualize the phenotypic diversity of predators, quantify the importance
45 of ecological diversification in generating this diversity, and to understand the organismal
46 determinants of food web structure. However, for many clades of predators, there is scarce
47 knowledge on how these specializations evolved with each other. The primary questions
48 we set out to answer are: how do predator specialists and generalists evolve, and how does
49 predatory specialization shape morphological evolution?

50 Siphonophores (Cnidaria: Hydrozoa) are a clade of organisms bearing modular structures

51 that are exclusively used for prey capture: the tentilla (Fig. 1). The tentilla have great
52 morphological variation across species (2), which together with their unconfounded function,
53 makes them an ideal system to study the relationships between functional traits and prey
54 specialization. Like a head of coral, a siphonophore is a colony bearing many feeding polyps
55 (Fig. 1). Each feeding polyp has a single tentacle, which bears a series of side branches
56 known as tentilla. Like other cnidarians, siphonophores capture prey with nematocysts,
57 harpoon-like stinging capsules borne within specialized cells known as cnidocytes. Unlike the
58 prey capture apparatus of most other cnidarians, siphonophore tentacles carry their cnidocytes
59 in extremely complex and organized batteries (3) which are located in their tentilla. While
60 nematocyst batteries and clusters in other cnidarians are simple static scaffolds for cnidocytes,
61 siphonophore tentilla have their own reaction mechanism, triggered upon encounter with
62 prey. When it fires, a tentillum undergoes an extremely fast conformational change that
63 wraps it around the prey, maximizing the surface area of contact for nematocysts to fire on
64 the prey (4). In addition, some species have elaborate fluorescent and bioluminescent lures
65 on their tentilla to attract prey with aggressive mimicry (5–7).

66 Siphonophores bear four major nematocyst types in their tentacles and tentilla (Fig. 1F)..
67 The largest type, heteronemes, have open-tip tubules characterized by bearing a distinctly
68 wider spiny shaft at the proximal end of the everted tubule. These are typically found
69 flanking the proximal end of the cnidoband. The most abundant type, haplonemes, have no
70 distinct shaft, but similarly to heteronemes, their tubules have open tips and can be found
71 in the cnidoband. Both heteronemes and haplonemes bear short spines along the tubule.
72 Both can be toxic and penetrate the surface of some prey types. In the terminal filament,
73 siphonophores bear two other types of nematocysts, characterized by their adhesive function,
74 closed tip tubules, and lack of spines on the tubule. These are the desmonemes (a type of
75 adhesive coiled-tubule spironeme), and rhopalonemes (a siphonophore-exclusive nematocyst
76 type with wide tubules).

77 Many siphonophore species inhabit the deep pelagic ocean, which spans from ~200m

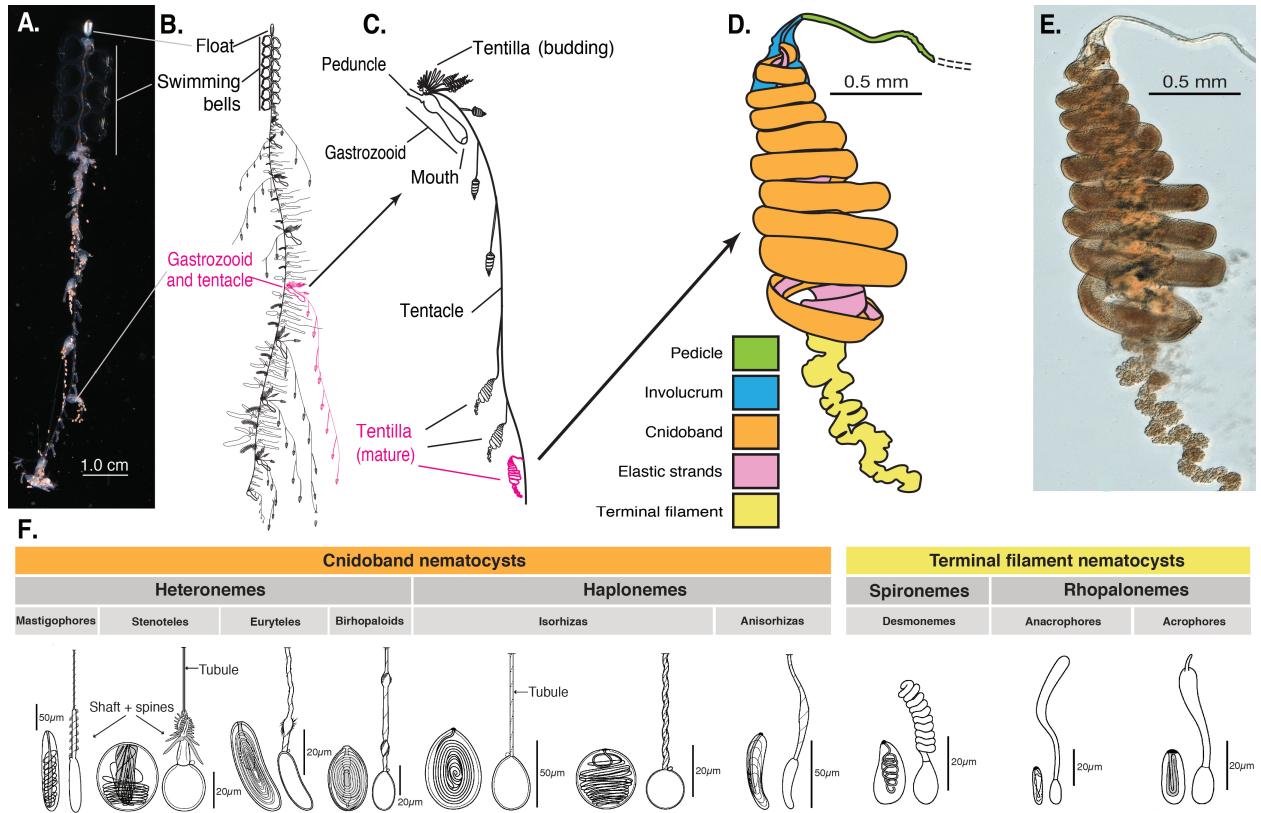


Figure 1: Siphonophore anatomy. A - *Nanomia* sp. siphonophore colony (photo by Catriona Munro). B, C - Illustration of a *Nanomia* colony, gastrozooid, and tentacle closeup (by Freya Goetz). D - *Nanomia* sp. Tentillum illustration and main parts. E - Differential interference contrast micrograph of 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.

78 to the abyssal seafloor (~4000m). This habitat has fairly homogeneous physical conditions
79 and stable zooplankton abundances and composition (8). With a relatively predictable
80 prey availability, ecological theory predicts that interspecific competition would inhibit the
81 coexistence of closely-related species unless evolution towards specialization reduces the
82 breadth of each species' niche (9–11). If this prediction holds true, we would expect the prey
83 capture apparatus morphologies of siphonophores to diversify with the evolution of increasing
84 specialization on a variety of prey types in different siphonophore lineages.

85 Specialization has been thought to be an evolutionary ‘dead-end’, meaning that specialized
86 lineages are unlikely to evolve into generalists or to shift the resource for which they are
87 specialized (12–16). However, recent studies have found that interspecific competition can
88 favor the evolution of generalists from specialists (17–19) and specialist resource switching
89 (20, 21). In addition to studying relationships with morphology, we seek to identify what
90 evolutionary transitions in trophic niche breadth are prevalent in open-ocean tactile predators.
91 To do so, we examine three alternative scenarios of siphonophore trophic specialization: (1)
92 predatory specialists evolved from generalist ancestors; (2) predatory specialists evolved from
93 specialist ancestors which targeted different resources, switching their primary prey type; and
94 (3) predatory generalists evolved from specialist ancestors. These scenarios are non-exclusive,
95 and each could apply to different transitions along the siphonophore phylogeny.

96 In the past, the study of siphonophore tentilla and diets have been limited due to the
97 inaccessibility of their oceanic habitat and the difficulties associated with the collection of
98 fragile siphonophores. Thus, the morphological diversity of tentilla has only been characterized
99 for a few taxa, and their evolutionary history remains largely unexplored. Contemporary
100 underwater sampling technology provides an unprecedented opportunity to explore the
101 trophic ecology (22) and functional morphology (23) of siphonophores. In addition, well-
102 supported phylogenies based on molecular data are now available for these organisms (24).
103 These advances allow for the examination of the evolutionary relationships between modern
104 siphonophore form, function, and ecology.

105 Our work builds upon previous pioneering studies that have explored the relationships
106 between tentilla and diet, and showed that siphonophores are a robust system for the study
107 of predatory specialization via morphological diversification. Purcell (25, 26) showed clear
108 relationships between diet, tentillum, and nematocyst characters in co-occurring epipelagic
109 siphonophores for a small subset of extant epipelagic siphonophore species.

110 In this study, we present the most extensive morphological characterization of tentilla
111 and their nematocysts across a broad variety of shallow and deep-sea siphonophore species
112 using modern imaging technologies, summarize decades of literature on siphonophore diets,
113 expand the phylogenetic tree of siphonophores by combining ribosomal gene sequences from a
114 broad range of taxa with a transcriptome-based backbone tree, and explore the evolutionary
115 histories and correlations between diet, tentillum, and nematocyst characters. Our results
116 suggest that siphonophores can evolve new specializations and generalism by modifying the
117 phenotypes and genetic correlations in their prey capture apparatus. These findings show
118 how studying elusive non-bilaterian predators can challenge traditional views on the evolution
119 of predatory specialization.

120 Results

121 *Novel phylogenetic relationships* – In order to analyze the relationships between morphology
122 and diet across the evolutionary history of siphonophores, we reconstructed a phylogeny with
123 extensive taxonomic representation. This phylogeny builds upon the published siphonophore
124 transcriptome phylogeny (24) to produce a tree with broader taxonomic representation using
125 ribosomal genes (18S & 16S). The topology of our tree recapitulates the resolved nodes in
126 (27) and (24). Only 5 nodes (blue dots in Figure 2) in the unconstrained tree inference
127 were incongruent with the (24) transcriptome tree, and these were constrained to the (24)
128 topology during estimation of the constrained 18S+16S tree inference (Fig. 2). Moreover,
129 with the inclusion of *Stephanomia amphytridis* sequences, our tree reveals a novel phylogenetic
130 relationship between the genus *Erenna* and *Stephanomia*.

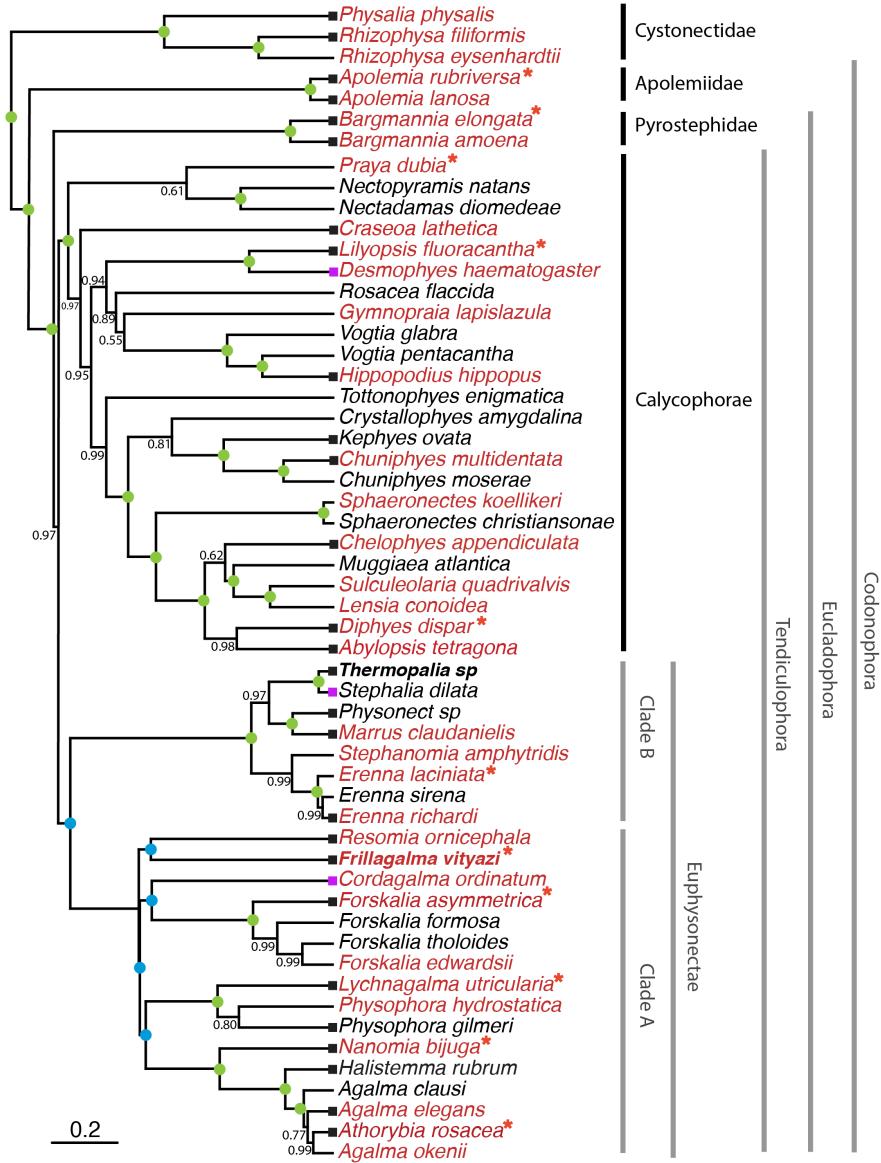


Figure 2: Bayesian time-tree built from 18S + 16S concatenated sequences. Branch lengths estimated using a relaxed molecular clock. Species names in red indicate replicated representation in the morphology data. Newly accessioned 16S data was used for species with names in bold. Nodes labeled with Bayesian 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.

¹³¹ We used the clade nomenclature defined in (27) and (24), including Codonophora to
¹³² indicate the sister group to Cystonectae, Euphysonectae to indicate the sister group to
¹³³ Calycophorae, Clade A and B to indicate the two main lineages within Euphysonectae. In
¹³⁴ addition, we define two new clades within Codonophora (Fig. 2): Eucladophora as the
¹³⁵ clade containing *Agalma elegans* and all taxa that are more closely related to it than to
¹³⁶ *Apolemia lanosa*, and Tendiculophora as the clade containing *Agalma elegans* and all taxa
¹³⁷ more closely related to it than to *Bargmannia elongata*. Eucladophora is characterized by
¹³⁸ bearing spatially differentiated tentilla with proximal heteronemes and a narrower terminal
¹³⁹ filament region. The etymology derives from the Greek *eu+kládos+phóros* for “true branch
¹⁴⁰ bearers”. Tendiculophora are characterized by bearing rhopalonemes and desmonemes in the
¹⁴¹ terminal filament, having a pair of elastic strands, and developing proximally detachable
¹⁴² cnidobands. The etymology of this clade is derived from the Latin *tendicula* for “snare or
¹⁴³ noose” and the Greek *phóros* for “carriers”.

¹⁴⁴ *Evolutionary associations between diet and tentillum morphology* – We reconstructed
¹⁴⁵ the evolutionary history of feeding guilds using stochastic mapping on the new phylogeny.
¹⁴⁶ Our reconstructions do not recover ancestral diet generalism. None of the transitions in
¹⁴⁷ diet are consistent with scenario 1 (specialists evolving from generalists). Feeding guild
¹⁴⁸ specializations have shifted from an alternative ancestral state at least five times, consistent
¹⁴⁹ with instances supporting scenario 2 (specialists evolving to feed on a different resource).
¹⁵⁰ Copepod specialization and fish specialization evolved twice, and ostracod specialization
¹⁵¹ evolved at least once. We also recover multiple independent origins of generalism from
¹⁵² specialist ancestors (Fig. 3). Large crustacean specialists evolve into generalists twice
¹⁵³ independently, consistent with instances of scenario 3 (generalists evolving from specialists).
¹⁵⁴ This finding is particularly compelling given in that it is the opposite of known biases in
¹⁵⁵ ancestral state reconstruction. (28) found that such methods tend to infer higher transition
¹⁵⁶ rates toward the more frequent state. In this case, that would lead to a bias for an increased
¹⁵⁷ rate of transition from generalists (the rarer state across the tips) to specialists (the more

¹⁵⁸ common state across the tips). We observe the opposite, indicating strong evidence that
¹⁵⁹ these generalists are indeed a derived state.

¹⁶⁰ To test whether measured morphological characters evolved in association with shifts in
¹⁶¹ feeding ecology, we analyzed the evolutionary history of each character on the phylogeny,
¹⁶² with the feeding guilds reconstructed on it as hypothetical selective regimes. We fitted
¹⁶³ and compared alternative evolutionary models for each continuous character. The models
¹⁶⁴ compared were the white noise (WN; non-phylogenetic model that assumes all values come
¹⁶⁵ from a single normal distribution with no covariance structure among species), the Brownian
¹⁶⁶ Motion (BM) model of neutral divergent evolution (29), the Early Burst (EB) model of
¹⁶⁷ decreasing rate of evolutionary change (30), and the Ornstein-Uhlenbeck (OU) model of
¹⁶⁸ stabilizing selection around a fitted optimum state (31, 32). The model comparison shows that
¹⁶⁹ out of 30 characters, 10 show significantly stronger support for the diet-driven multi-optima
¹⁷⁰ multi-rate OU model (SM15). These characters include terminal filament nematocyst size
¹⁷¹ and shape, involucrum length, elastic strand width, and heteroneme number. Most of these
¹⁷² characters are found exclusively in Tendiculophora, thus this may reflect processes that could
¹⁷³ be unique to this subtree. Five characters including cnidoband length, cnidoband shape, and
¹⁷⁴ haploneme length show maximal support for a diet-driven single-optimum OU model. The
¹⁷⁵ remaining 15 characters support BM (or OU with marginal AICc difference with BM).

¹⁷⁶ In order to investigate the associations between the evolutionary history of morphological
¹⁷⁷ characters and specific prey types found in the diet, we used phylogenetic logistic regressions.
¹⁷⁸ We found that several characters were significantly correlated with the gains and losses of
¹⁷⁹ these prey types (Fig. 3, right). Shifts toward ostracod presence in diet correlated with
¹⁸⁰ reductions in pedicle width and total haploneme volume. Shifts to copepod presence in
¹⁸¹ the diet were associated with reductions in haploneme width, cnidoband length and width,
¹⁸² total haploneme and heteroneme volumes, and tentacle and pedicle widths. Consistently,
¹⁸³ transitions to decapod presence in the diet correlated with more coiled cnidobands (SM21).
¹⁸⁴ Evolutionary shifts in these characters likely allowed the inclusion of these prey types in the

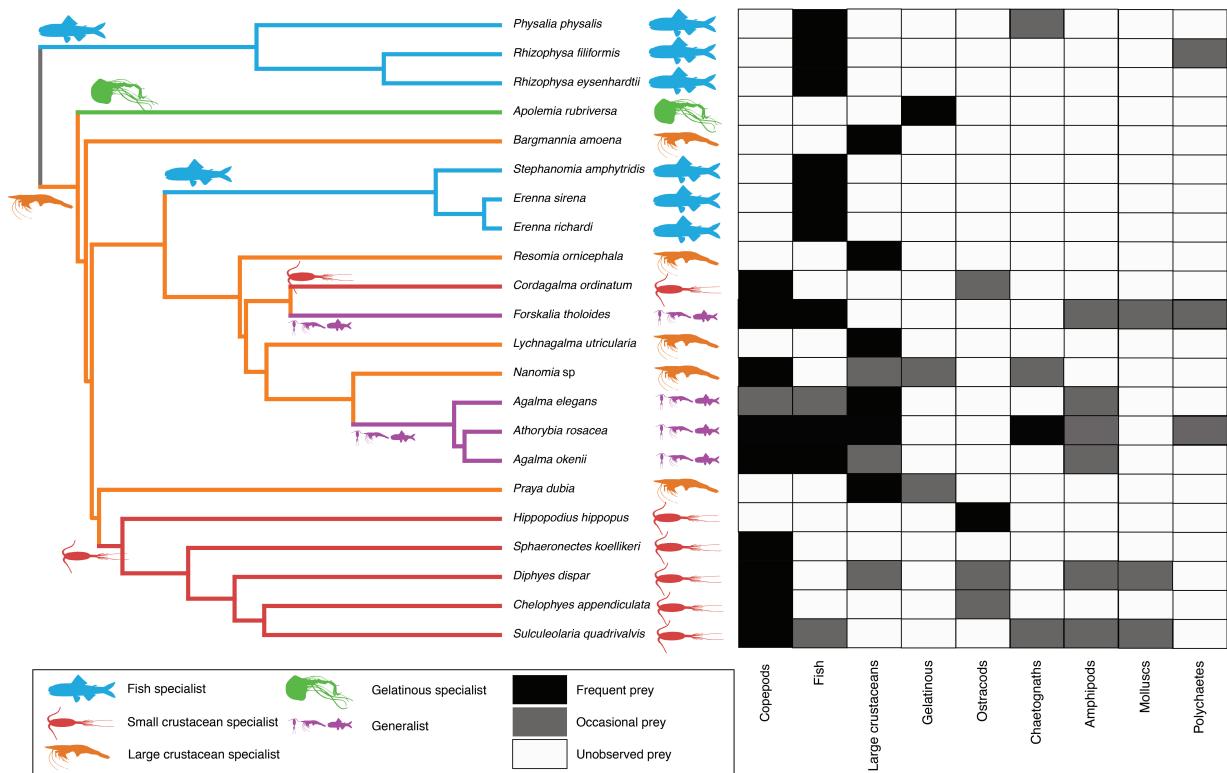


Figure 3: Left - Subset phylogeny showing the mapped feeding guild regimes that were used to inform the *OUwie* analyses. Right - Grid showing the prey items consumed from which the feeding guild categories were derived. Diet data were obtained from the literature review, available in the Dryad repository.

185 diet.

186 We also tested for correlations between shifts in prey selectivity and morphological
187 characters using phylogenetic linear models. We found that fish selectivity is associated
188 with increased number of heteronemes per tentillum, increased roundness of nematocysts
189 (desmonemes and haplonemes), larger heteronemes, reduced heteroneme/cnidoband length
190 ratios, smaller rhopalonemes, lower haploneme surface area to volume ratio (SA/V), and
191 larger the cnidoband, elastic strand, pedicle and tentacle widths. Decapod-selective diets
192 were associated with increasing cnidoband size and coiledness, haploneme row number,
193 elastic strand width, and heteroneme number. Copepod-selective diets evolved in associa-
194 tion with smaller heteroneme and total nematocyst volumes, smaller cnidobands, rounder
195 rhopalonemes, elongated heteronemes, narrower haplonemes with higher SA/V ratios, and
196 smaller heteronemes, tentacles, pedicles, and elastic strands. Selectivity for ostracods was
197 associated with reductions in size and number of heteroneme nematocysts, cnidoband size,
198 number of haploneme rows, heteroneme numbers, and cnidoband coiledness. Heteroneme
199 length and elongation also correlated negatively with chaetognath selectivity (SM21). These
200 results indicate that not only diet but also differential feeding selectivity has evolved in
201 correlation with changes in the prey capture apparatus of siphonophores.

202 We tested some of the diet-morphology associations previously proposed in the literature
203 (25, 26) for correlated evolution (Table 1). We found that most, such as heteroneme volume
204 and copepod prey size, do show evidence for correlated evolution. The sole exception was the
205 relationship between terminal filament nematocysts (rhopalonemes and desmonemes) and
206 crustaceans in the diet. Analyses that do not take phylogeny into account do recover this
207 correlation across the extant species studied, but it is not consistent with correlated evolution.
208 The latter is likely a product of the larger species richness of crustacean-eating species with
209 terminal filament nematocysts, rather than simultaneous evolutionary gains.

210 Table 1. Table 1. Tests of correlated evolution between siphonophore morphological
211 characters and aspects of the diet found correlated in the literature. We report the direction

212 and significance of the evolutionary association, the number of taxa used for the analysis,
 213 and the literature source where the morphology-diet association was first reported.

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	Non-Significant	0.200	19	Purcell & Mills, 1988
Number of nematocyst types	Soft-bodied prey	Phylogenetic logistic regression	-	0.040	22	Purcell & Mills, 1988

214

215

216 Table 2. Discriminant analysis of principal components for the presence of specific prey
 217 types using the morphological data. Top quartile variable (character) contributions to the
 218 linear discriminants are ordered from highest to lowest. Logistic regressions and GLMs
 219 were fitted to predict prey type presence and selectivity respectively. The sign of the slope
 220 of each predictor is reported, marked with an asterisk if significant ($p\text{-value} < 0.05$), and
 221 highlighted grey if it differs between prey presence in diet and prey selectivity. Pseudo- R^2
 222 (%) 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 (%)
Copepods	95.4	Total nematocyst volume	-	-*	
		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	-	+	
		Heteroneme volume	+	-	
		Total nematocyst volume	-	+	
		Total heteroneme volume	-	-	
		Cnidoband length	-	-	
		Cnidoband free length	+	+	
		Involucrum length	-	-	
		Pedicle width	+	+	
Large crustaceans	81.8	Involucrum length	+.*	+	
		Total heteroneme volume	-	-	
		Elastic strand width	-	+.*	
		Rhopaloneme length	+	+	
		Heteroneme volume	+	-	
		Haploneme elongation	-	+	
		Desmoneme length	-	-	
		Tentacle width	+	+	

223

224 *Evolution of the integrated tentillum morphology – Phenotypic integration results in*
 225 correlation patterns between morphological characters and their rates of evolution. To

study these patterns, we fit a set of evolutionary variance-covariance matrices (33). The quantitative characters we measured from tentilla and their nematocysts are highly correlated. The results indicate that the dimensionality (number of independent axes of variation) of tentillum morphology is low, that many traits are associated with size, but that nematocyst arrangement and shape are independent of it (SM4). The variance-covariance matrices (SM36-38) are congruent with the abundant positive correlations observed among simple measurement characters in SM3. This analysis more clearly reveals the diagonal blocks that constitute the evolutionary modules, such as the heteroneme block, the terminal filament nematocyst block, and the cnidoband-pedicle-tentacle block. These results were not sensitive to the transformation of inapplicable states and taxon sampling. These results indicate that siphonophore tentilla and nematocysts are phenotypically integrated and co-evolve within discrete evolutionary modules.

In order to test whether rate covariance matrices changed with evolutionary shifts in feeding guild regimes, we compared the rate covariance terms between characters across the subtrees occupied by the different feeding guild regimes (SM41). We found that half (48%) of the character pairs presented significantly distinct correlation coefficients across different regimes (SM39), indicating that the mode of phenotypic integration also shifts with trophic niche. When contrasting the regime-specific rate correlation matrices to the whole-tree matrix, we were able to identify the character dependencies that are unique to each predatory niche (SM42). These results indicate that the evolutionary dependencies in these integrated modules are changing across the phylogeny, and evolving together with changes in prey type specializations.

We were able to identify specific character correlations that shifted with the evolution of new diets. Under the majority of stochastic character mapping outcomes, large crustacean specialists are the ancestral feeding regime, and all other feeding regimes evolve from this ancestral specialization. Compared to the rate correlation matrix estimated over the whole tree, large crustacean specialists present strong negative correlations between haploneme

253 elongation and heteroneme size, and between rhopaloneme elongation and tentillum size,
254 as well as with involucrum length. Within generalist clades (*Forskalia* and the *Agalma-*
255 *Athorybia* clade), terminal filament nematocyst (desmonemes and rhopalonemes) sizes became
256 negatively correlated with the sizes of most characters, meaning that as some tentilla became
257 larger, their individual terminal nematocysts became smaller, observed to the extreme in
258 *Agalma*. In addition, heteroneme and rhopaloneme elongation became positively correlated
259 with cnidoband size. When large crustacean specialists switched to small crustacean prey in
260 *Cordagalma* and calycophorans, haploneme size became inversely correlated with heteroneme
261 elongation, which in turn developed a strong positive relationship with tentillum size. In
262 other words, as tentilla get smaller in this group, heteronemes get shorter and haplonemes
263 get larger. The extremes of this gradient can be seen in *Cordagalma* and *Hippopodius*. With
264 the evolution of fish prey specialization in cystonects and within Clade B (Fig. 1), haploneme
265 elongation became negatively correlated with heteroneme elongation (signal driven by Clade
266 B, since cystonects lack tentacular heteronemes), and the surface area to volume ratio of
267 haploneme nematocysts switched from a strong negative relationship with cnidoband size
268 (found in every other regime) to a positive correlation. Gelatinous specialization, albeit
269 appearing only once in our tree, also carries a unique signature in character rate correlation
270 shifts, with an increase in the strength of the correlation between heteroneme shape and shaft
271 width, consistent with the appearance of birrhopaloid nematocysts with swollen shafts that
272 are likely effective at anchoring gelatinous tissue (see reference to Narcomedusae nematocysts
273 in (26)).

274 *Evolution of nematocyst shape* – The greatest evolutionary change in haploneme nemato-
275 cyst shape occurred in a single shift towards elongation in the stem of Tendiculophora, which
276 contains the majority of described siphonophore species other than Cystonects, *Apolemia*,
277 and Pyrostephidae. There is one secondary return to more oval, less elongated haplonemes in
278 *Erenna*, but it does not reach the sphericity present in Cystonectae or Pyrostephidae (Fig.
279 4). Heteroneme evolution presents a less discrete evolutionary history, where Tendiculophora

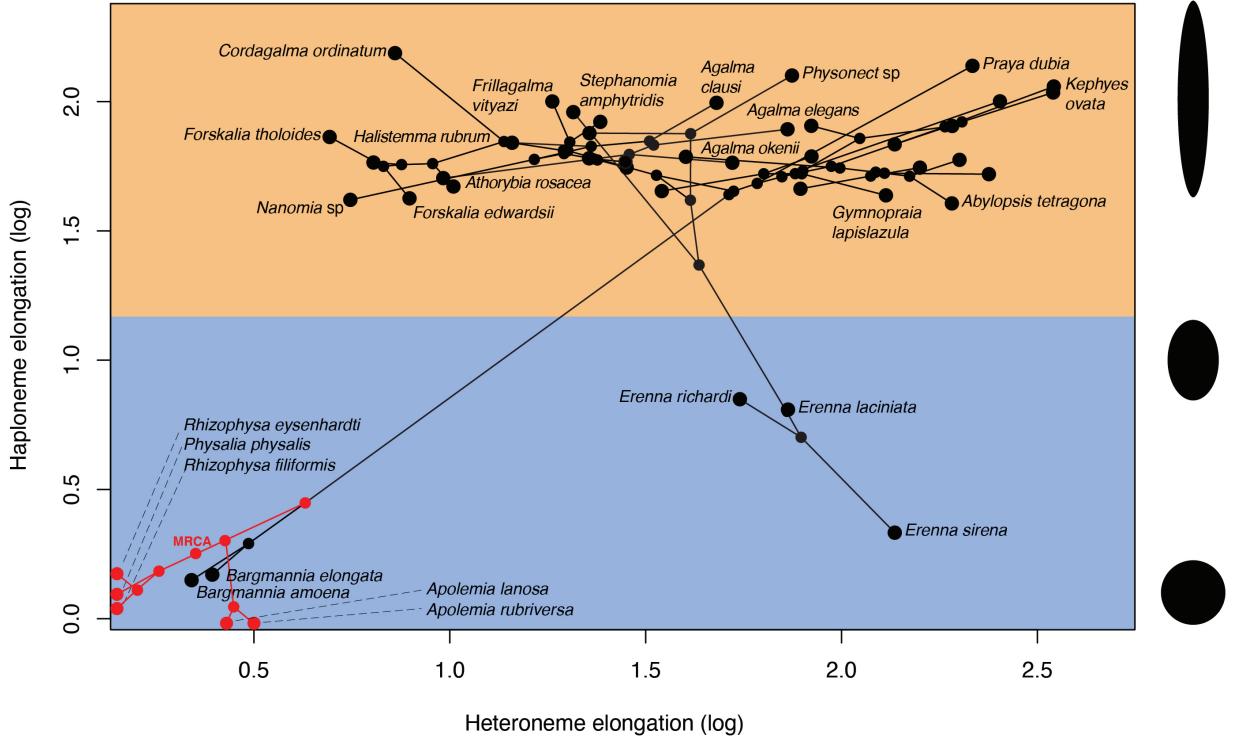


Figure 4: Phylomorphospace showing haploneme and heteroneme elongation (log scaled). Orange area delimits rod-shaped haplonemes, the blue area covers oval and round-shaped haplonemes. Smaller dots and lines represent phylogenetic relationships and ancestral states of internal nodes under BM. Species nodes in red lack either haplonemes or heteronemes, and their values projected onto the axis of the nematocyst type they bear. Cystonects have no tentacle heteronemes and are projected onto the haploneme axis. Apolemiids have no tentacle haplonemes and are projected onto the heteroneme axis.

280 evolved more elongate heteronemes, but the difference between theirs and other siphonophores
 281 is much smaller than the variation in shape within Tendiculophora, bearing no phylogenetic
 282 signal. In this clade, the evolution of heteroneme shape has diverged in both directions, and
 283 there is no correlation with haploneme shape (Fig. 4), which has remained fairly constant
 284 (elongation between 1.5 and 2.5).

285 Discussion

286 *Evolution of siphonophore trophic niche* – Several studies (12–16) have suggested that resource
 287 specialization is an irreversible dead-end due to the constraints posed by extreme phenotypic

specialization. Our results show that this is not the case for siphonophores, where the prey type on which they specialize has shifted at least 5 times. We find no support for any transitions from generalist to specialist (scenario 1, as described in the Introduction). We do find support for at least 3 instances of specialists switching from one prey type to another prey type, (scenario 2) and two switches from specialist to generalist (scenario 3).

This is consistent with the findings of recent studies on phytophagous insects (19), where the rate of evolution from generalists to specialists is comparable to the reverse, thus specialization does not limit further evolution. Our results are also consistent with analyses of lepidopterans (21), where specialized resource switching is the primary transition type while niche breadth remains fairly constant. The evolutionary history of tentilla shows that siphonophores are an example of trophic niche diversification via morphological innovation and evolution, which allowed transitions between specialized trophic niches. In more familiar predators, the prey capture apparatus is well integrated in the body (such as claws and jaws), leading to trade-offs and whole body adaptations to feeding specialization. The extreme modularity of the siphonophore prey capture apparatus could release them from the constraints typically imposed by adaptation to ecological specialization. This evolutionary mechanism is particularly important in a deep open ocean ecosystem, which is a relatively homogeneous physical environment, where the primary niche heterogeneity available is the potential interactions between organisms (8).

Siphonophores are an abundant group of zooplankton in oceanic ecosystems (34, 35). While little is known about siphonophore trophic ecology, what is known indicates that they occupy a central position in midwater food webs (22), serving as important trophic intermediaries between smaller zooplankton and higher trophic level predators. Our findings on the unique evolutionary history of siphonophore trophic specialization elucidate how they arrived to play this fundamental role in the oceanic food web.

Evolution of nematocyst shape – A remarkable feature of siphonophore haplonemes is that they are outliers to all other Medusozoa in their surface area to volume relationships,

315 deviating significantly from sphericity (36). This suggests a different mechanism for their
316 discharge that could be more reliant on capsule tension than on osmotic potentials (37),
317 and strong selection for efficient nematocyst packing in the cnidoband (3, 36). Our results
318 show that Codonophora underwent a shift towards elongation and Cystonectae towards
319 sphericity, assuming the common ancestor had an intermediate state. Since we know that the
320 haplonemes of other hydrozoan outgroups are generally spheroid, it is more parsimonious to
321 assume that cystonects retain this ancestral state. Later, we observe a return to more rounded
322 (ancestral) haplonemes in *Erenna*, concurrent with a secondary gain of a piscivorous trophic
323 niche, like that exhibited by cystonects. (25) showed that haplonemes have a penetrating
324 function as isorhizas in cystonects and an adhesive function as anisorhizas in Tendiculophora.
325 The two clades that have converged to feed primarily on fish (Cystonectae and Clade B, which
326 includes *Erenna*, *Stephanomia*, *Marrus*, and rhodaliids) have also converged morphologically
327 toward more compact haplonemes, significantly distinct from their closest relatives. Isorhizas
328 in cystonects are known to penetrate the skin of fish during prey capture, and to deliver
329 the toxins that aid in paralysis and digestion (38). *Erenna*'s anisorhizas are also able to
330 penetrate human skin and deliver a painful sting (39) (and pers. obs.), a common feature of
331 piscivorous cnidarians like the Portuguese man-o-war or box jellies.

332 The implications of these results for the evolution of nematocyst function are that an
333 innovation in the discharge mechanism of haplonemes may have occurred during the main shift
334 to elongation. Elongate nematocysts can be tightly packed into cnidobands. We hypothesize
335 this may be a Tendiculophora lineage-specific adaptation to packing more nematocysts into a
336 limited tentillum space, as suggested by (3). (36) hypothesized that smaller, more spherical
337 nematocysts, with a lower surface area to volume ratio, are more efficient in osmotic-driven
338 discharge and thus have more power for skin penetration. The elongated haplonemes of
339 crustacean-eating Tendiculophora have never been observed penetrating their crustacean
340 prey (25), and are hypothesized to entangle the prey through adhesion of the abundant
341 spines to the exoskeletal surfaces and appendages. Entangling requires less acceleration and

342 power during discharge than penetration, as it does not rely on point pressure. In fish-eating
343 cystonects and *Erenna* species, the haplonemes are much less elongated and very effective at
344 penetration, in congruence with the osmotic discharge hypothesis. Tendiculophora, comprised
345 of the clades Euphysonectae and Calycophorae, includes the majority of siphonophore species.
346 Within these clades are the most abundant siphonophore species, and a greater morphological
347 and ecological diversity is found. We hypothesize that this packing-efficient haploneme
348 morphology may have been a key innovation leading to the diversification of this clade.
349 However, other characters that shifted concurrently in the stem of this clade may have been
350 responsible for their extant diversity.

351 *Phenotypic integration of siphonophore tentilla* – While selection acting on character
352 states is a widely studied phenomenon, recent studies have shown that selection can also
353 act upon the patterns of character correlations and phenotypic dependencies (33, 40–45).
354 This evolution of character relationships can allow lineages to explore new regions of the
355 morphospace and facilitate the appearance of ecological novelties. Our results show that
356 the patterns of phenotypic integration in siphonophore tentilla vary among clades, and
357 appear to display different relationships across shifting feeding specializations. Similar to
358 what has been found in the feeding morphologies of fish (33, 46), siphonophore tentilla
359 may have accommodated new diets by altering the correlations between characters. For
360 example, changes in the size and shape relationships between nematocyst types gave rise to
361 the nematocyst complements specialized in ensnaring prey with different combinations of
362 defensive traits.

363 Our evolutionary rate covariance results (SM36-38) indicate that tentilla are not only
364 phenotypically integrated but also show patterns of evolutionary modularity, where different
365 sets of characters appear to evolve in stronger correlations among each other than with
366 other characters (47). This may be indicative of the underlying genetic and developmen-
367 tal dependencies among closely homologous nematocyst types (such as desmonemes and
368 rhopalonemes) and structures. In addition, these evolutionary modules point to hypothetical

369 functional modules. For example, the coiling degree of the cnidoband and the extent of the
370 involucrum have correlated rates of evolution, while high-speed videos (pers. obs.) show that
371 the involucrum helps direct the whiplash of the uncoiling cnidoband distally (towards the
372 prey).

373 *Limitations* - Our results unambiguously show that tentillum morphology evolved with
374 diet and strongly support deviations from the generalist-to-specialist evolution scenario.
375 However, the conclusions we can draw from these analyses are limited in several ways. The
376 biggest challenge at present is the sparse dietary data available in the literature. Additional
377 dietary data could reveal transitions from generalists to specialists we were unable to detect
378 for two reasons. First, some of the taxa in our dataset have a very limited number of
379 feeding observations, which could lead to apparent specialization. Second, some of the taxa
380 not included in our dataset could be undiscovered generalists. When interpreting these
381 results, it is also important to remember that diet is also dependent on environmental prey
382 availability. In addition, selectivity differences across siphonophore species could be also
383 driven by other phenotypes not accounted for in this study. Finally, further observations on
384 behavior, digestion biochemistry, and toxin composition are necessary to assess their relative
385 importance in determining diet.

386 **Conclusions**

387 Siphonophores occupy diverse predatory niches in the open ocean, ranging from mid-trophic
388 small-crustacean eaters to piscivorous super-carnivores. With the evolution of diversified
389 prey type specialization comes the evolution of morphologies adapted to the challenges posed
390 by different prey. The results presented here indicate that the associations found between
391 siphonophore tentilla and their prey are a product of correlated evolution in highly integrated
392 traits. While much of the feeding ecology literature focuses on how predatory generalists
393 evolve into predatory specialists, in siphonophores we find predatory specialists can evolve
394 into generalists, and that specialists on one prey type have directly evolved into specialists

395 on other prey types. We find that the character states, evolutionary optima, and genetic
396 correlations of many tentillum characters have evolved following these shifts in trophic niche.
397 Our extended morphological characterization shows that the relationships between form and
398 ecology hold across a large set of siphonophore taxa and characters, and can be used to
399 generate hypotheses on the feeding habits of uncharacterized species. We identify key aspects
400 of organismal trait evolution that are central to understanding the emergence of food web
401 complexity.

402 Materials and Methods

403 *Tentillum morphology* – The morphological work was carried out on siphonophore specimens
404 fixed in 4% formalin from the Yale Peabody Museum Invertebrate Zoology (YPM-IZ) collection
405 (accession numbers in Dryad repository). These specimens were collected intact across many
406 years of fieldwork expeditions, using blue-water diving (48), remotely operated vehicles
407 (ROVs), plankton net trawls, and human-operated submersibles. Tentacles were dissected
408 from non-larval gastrozooids, sequentially dehydrated into 100% ethanol, cleared in methyl
409 salicylate, and mounted onto slides with Canada Balsam or Permount mounting media.
410 The slides were imaged as tiled z-stacks using differential interference contrast (DIC) on an
411 automated stage at YPM-IZ (with the assistance of Daniel Drew and Eric Lazo-Wasem) and
412 with laser point confocal microscopy using a 488 nm Argon laser that excited autofluorescence
413 in the tissues. Thirty characters (defined in SM5) were measured using Fiji (49, 50). We did
414 not measure the lengths of contractile structures (terminal filaments, pedicles, gastrozooids,
415 and tentacles) since they are too variable to quantify. We measured at least one specimen for
416 96 different species (raw data available in Dryad). Of these, we selected 38 focal species across
417 clades based on specimen availability and phylogenetic representation. Three to five tentacle
418 specimens from each one of these selected species were measured to capture intraspecific
419 variation.

420 *Siphonophore phylogeny* – While the main goal of this work is not to elucidate a novel

421 phylogeny for Siphonophora, we did expand on the most recent transcriptome based phylogeny
422 (24) to accommodate a larger taxon sampling. In order to do this, we ran a constrained analysis
423 on an extensive 18S+16S dataset. The phylogenetic analysis included 55 siphonophore species
424 and 6 outgroup cnidarian species (*Clytia hemisphaerica*, *Hydra circumcincta*, *Ectopleura*
425 *dumortieri*, *Porpita porpita*, *Velella velella*, *Staurocladia wellingtoni*). The gene sequences we
426 used in this study are available online (accession numbers in Dryad repository). Some of the
427 sequences we used were accessioned in (27), and others we extracted from the transcriptomes
428 in (24). Two new 16S sequences for *Frillagalma vityazi* (MK958598) and *Thermopalia* sp.
429 (MK958599) sequenced by Lynne Christianson using the primers from (51) (read 3' to 5' F:
430 TCGACTGTTACCAAAACATAGC , R: ACGGAATGAACTCAAATCATGTAAAG) were
431 included and accessioned to NCBI. Additional details on the phylogenetic inference methods
432 are available in the Supplementary Methods.

433 Given the broader sequence sampling of the transcriptome phylogeny, we ran constrained
434 inferences (using both ML and Bayesian approaches, which produced fully congruent topologies
435 (SM8 and SM10)) after fixing the 5 nodes that were incongruent with the topology of the
436 consensus tree in (24). This topology was then used to inform a Bayesian relaxed molecular
437 clock time-tree in RevBayes, using a birth-death process (sampling probability calculated
438 from the known number of described siphonophore species) to generate ultrametric branch
439 lengths (SM11-12). Scripts available in the Dryad repository.

440 *Feeding ecology* – We extracted categorical diet data for different siphonophore species
441 from published sources, including seminal papers (4, 25, 52–56), and ROV observation data
442 (22, 57) with the assistance of Elizabeth Hetherington and C. Anela Choy (data available
443 in Dryad repository). In order to detect coarse-level patterns in feeding habits, the data
444 were merged into feeding guilds. For more details on how the diet data was curated and
445 summarized into guilds, please see Supplementary Methods.

446 We also extracted copepod prey length data from (25). To calculate specific prey
447 selectivities, we extracted quantitative diet and zooplankton composition data from (53),

⁴⁴⁸ matched each diet assessment to each prey field quantification by site, calculated Ivlev's
⁴⁴⁹ electivity indices (58), and averaged those by species (data available in the Dryad repository).

⁴⁵⁰ *Statistical analyses* – We used a series of phylogenetic comparative methods to test the
⁴⁵¹ evolutionary hypotheses presented in this study. We fitted different models generating the
⁴⁵² observed data distribution given the phylogeny for each continuous character using the
⁴⁵³ function fitContinuous in the R package *geiger* (59). We then ranked the models in order of
⁴⁵⁴ increasing parametric complexity (WN, BM, EB, OU), and compared the corrected Akaike
⁴⁵⁵ Information Criterion (AICc) support scores (60) to the lowest (best) score, using a cutoff
⁴⁵⁶ of 2 units to determine significantly better support. When the best fitting model was not
⁴⁵⁷ significantly better than a less complex alternative, we selected the least complex model
⁴⁵⁸ (SM13). We calculated model adequacy scores using the R package *arbutus* (61) (SM14).
⁴⁵⁹ We calculated phylogenetic signal in each of the measured characters using Blomberg's K
⁴⁶⁰ (62) (SM13). We reconstructed ancestral states using ML (R phytools::anc.ML (63)), and
⁴⁶¹ stochastic character mapping (R phytools::make.simmap) for categorical characters. For more
⁴⁶² details on the data wrangling prior to these analyses, please see the Supplementary Methods.
⁴⁶³ R scripts available in the Dryad repository.

⁴⁶⁴ In order to study the evolution of predatory specialization, we reconstructed components
⁴⁶⁵ of the diet and prey selectivity on the phylogeny using ML (R phytools::anc.ML). To identify
⁴⁶⁶ evolutionary associations of diet with tentillum and nematocyst characters, we compared the
⁴⁶⁷ performance of a neutral evolution model to that of a diet-driven directional selection model.
⁴⁶⁸ First, we collapsed the diet data into the five feeding guilds mentioned above (fish specialist,
⁴⁶⁹ small crustacean specialist, large crustacean specialist, gelatinous specialist, generalist), based
⁴⁷⁰ on which prey types they were observed consuming most frequently. Then, we reconstructed
⁴⁷¹ the feeding guild ancestral states using the ML function ace (package ape (64)), removing tips
⁴⁷² with no feeding data. The ML reconstruction was congruent with the consensus stochastic
⁴⁷³ character mapping (SM31). Then, using the package *OUwie* (65), we fitted an OU model with
⁴⁷⁴ multiple optima and rates of evolution matched to the reconstructed ancestral diet regimes,

475 a single optimum OU model, and a BM null model, inspired by the analyses in (66). Finally,
476 we compared their AICc support values to select the best fitting model (SM15). To model the
477 evolutionary associations between individual tentillum and nematocyst characters and the
478 ability to capture particular prey types in the diet, we ran a series of phylogenetic generalized
479 linear models (R `phylolm::phyloglm`) (SM21). In addition, we ran a series of comparative
480 analyses to address hypotheses of diet-tentillum relationships posed in the literature.

481 In order to study correlations between the rates of evolution between different characters,
482 we fitted a set of evolutionary variance-covariance matrices (33) (R `phytools::evol.vcv`). For
483 more details on the data wrangling preceding these analyses, please see Supplementary
484 Methods. To test whether phenotypic integration changes across selective regimes determined
485 by the reconstructed feeding guilds, we carried out character-pairwise variance-covariance
486 analysis comparing alternative models (R `phytools::evolvcv.lite`), including those where
487 correlations are the same across the whole tree and models where correlations differ between
488 selective regimes (SM42). Finally, we compared regime-specific variance-covariance matrices
489 to the general matrix and to their preceding regime matrix to identify the changes in character
490 dependences unique to each regime (SM43).

491 We carried out a linear discriminant analysis of principal components (DAPC) using
492 the `dapc` function (R `adegenet::dapc`) (67). This function allowed us to incorporate more
493 predictors than individuals. We generated discriminant functions for feeding guild, and
494 for the presence of copepods, fish, and shrimp (large crustaceans) in the diet (SM16-20).
495 From these DAPCs we obtained the highest contributing morphological characters to the
496 discrimination (characters in the top quartile of the weighted sum of the linear discriminant
497 loadings controlling for the eigenvalue of each discriminant). In order to identify the sign
498 of the relationship between the predictor characters prey type presence in the diet, we then
499 generated generalized logistic regression models (as a type of generalized linear model, or
500 GLM using R `stats::glm`) with the top contributing characters (from the corresponding DAPC)
501 as predictors. We also carried out these GLMs on the Ivlev's selectivity indices for each prey

502 type calculated from (53). Additional details on the DAPC optimization are available in the
503 Supplementary Materials.

504 **Supplementary Materials**

505 Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.NNNN>
506 Supplementary Materials are available in https://github.com/dunnlab/tentilla_morph/
507 Supplement_forShort.pdf

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