

¹ **Classification: Biological Sciences**

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

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¹³ **Keywords**

¹⁴ Siphonophores, nematocysts, predation, specialization, character evolution

¹⁵ **Abstract**

¹⁶ Predator specialization has often been considered an evolutionary ‘dead-end’ due to the
¹⁷ constraints associated with the evolution of morphological and functional optimizations
¹⁸ throughout the organism. However, in some predators, these changes are localized in separate
¹⁹ structures dedicated to prey capture. One of the most extreme cases of this modularity can
²⁰ be observed in siphonophores, a clade of pelagic colonial cnidarians that use tentilla (tentacle
²¹ side branches armed with nematocysts) exclusively for prey capture. Here we study how
²² siphonophore specialists and generalists evolve, and what morphological changes are associated
²³ with these transitions. To answer these questions, we: (1) measured 29 morphological
²⁴ characters of tentacles from 45 siphonophore species, (2) mapped these data to a phylogenetic
²⁵ tree, and (3) analyzed the evolutionary associations between morphological characters and prey
²⁶ type data from the literature. Instead of a dead-end, we found that siphonophore specialists
²⁷ can evolve into generalists, and that specialists on one prey type have directly evolved into
²⁸ specialists on other prey types. Our results show that siphonophore tentillum morphology has
²⁹ strong evolutionary associations with prey type, and suggest that shifts between prey types
³⁰ are linked to shifts in the morphology, mode of evolution, and genetic correlations of tentilla
³¹ and their nematocysts. The evolutionary history of siphonophore specialization helps build
³² a broader perspective on predatory niche diversification via morphological innovation and
³³ evolution. These findings contribute to understanding how specialization and morphological
³⁴ evolution have shaped present-day food webs.

³⁵ **Significance Statement**

³⁶ Predatory specialization is often associated with the evolution of modifications in the mor-
³⁷ phology of the prey capture apparatus. Specialization has been considered an evolutionary
³⁸ ‘dead-end’ due to the constraints associated with these morphological changes. However,
³⁹ in predators like siphonophores, armed with modular structures used exclusively for prey
⁴⁰ capture, this assumption is challenged. Our results show that siphonophores can evolve

41 generalism and new prey-type specializations by modifying the morphological states, modes of
42 evolution, and genetic correlations between the parts of their prey capture apparatus. These
43 findings demonstrate how studying open-ocean non-bilaterian predators can reveal novel
44 patterns and mechanisms in the evolution of specialization. Understanding these evolutionary
45 processes is fundamental to the study of food-web structure and complexity.

46 Introduction

47 Most animal predators use specific structures to capture and subdue prey. Raptors have
48 claws and beaks, snakes have fangs, wasps have stingers, and cnidarians have nematocyst-
49 laden tentacles. The functional morphology of these structures is critical to their ability
50 to successfully capture prey (1). Long-term adaptive evolution in response to the defense
51 mechanisms of the prey (*e.g.*, avoidance, escape, protective barriers) leads to modifications
52 that can counter those defenses. The more specialized the diet of a predator is, the more
53 specialized its structures need to be to efficiently overcome the challenges posed by the
54 prey. Characterizing the relationships between morphology and predatory specialization
55 is necessary to understand how the phenotypic diversity of predators determines food-web
56 structure. However, for many clades of predators, there is scarce knowledge on how these
57 specializations evolved. The primary questions we set out to answer are: how do predator
58 specialists and generalists evolve, and how does predatory specialization shape morphological
59 evolution?

60 Siphonophores (Cnidaria: Hydrozoa) are a clade of gelatinous, colonial organisms that
61 swim in the open ocean, feeding on a wide diversity of prey (often fish, crustaceans, and
62 jellyfish). Siphonophores carry modular structures that are exclusively used for prey capture:
63 the tentilla (Fig. 1). The tentilla have great morphological variation across species (2).
64 Together with their well understood function, this makes them an ideal system to study
65 the relationships between functional traits and prey specialization. Like a head of coral, a
66 siphonophore is a colony bearing many feeding polyps (Fig. 1). Each feeding polyp has a

67 single tentacle, which branches into a series of tentilla (side branches). Like other cnidarians,
68 siphonophores capture prey with nematocysts, harpoon-like stinging capsules borne within
69 specialized cells known as cnidocytes. Unlike the prey capture apparatus of most other
70 cnidarians, siphonophore tentacles carry their cnidocytes in extremely complex and organized
71 batteries (3) which are located in their tentilla. While nematocyst batteries and clusters in
72 other cnidarians are simple static scaffolds for cnidocytes, siphonophore tentilla have their
73 own reaction mechanism, triggered upon encounter with prey. When it fires, a tentillum
74 undergoes an extremely fast conformational change that wraps it around the prey, maximizing
75 the surface area of contact for nematocysts to fire on the prey (4). In addition, some species
76 have elaborate fluorescent and bioluminescent lures on their tentilla to attract prey with
77 aggressive mimicry (5–7).

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

89 Many siphonophore species inhabit the deep pelagic ocean, which spans from ~200m to the
90 abyssal seafloor (~4000m). This habitat has fairly homogeneous physical conditions and stable
91 zooplankton abundances and composition (8). With relatively predictable prey availability,
92 ecological theory predicts that interspecific competition would inhibit the coexistence of
93 closely-related species unless evolution towards specialization reduces the breadth of each

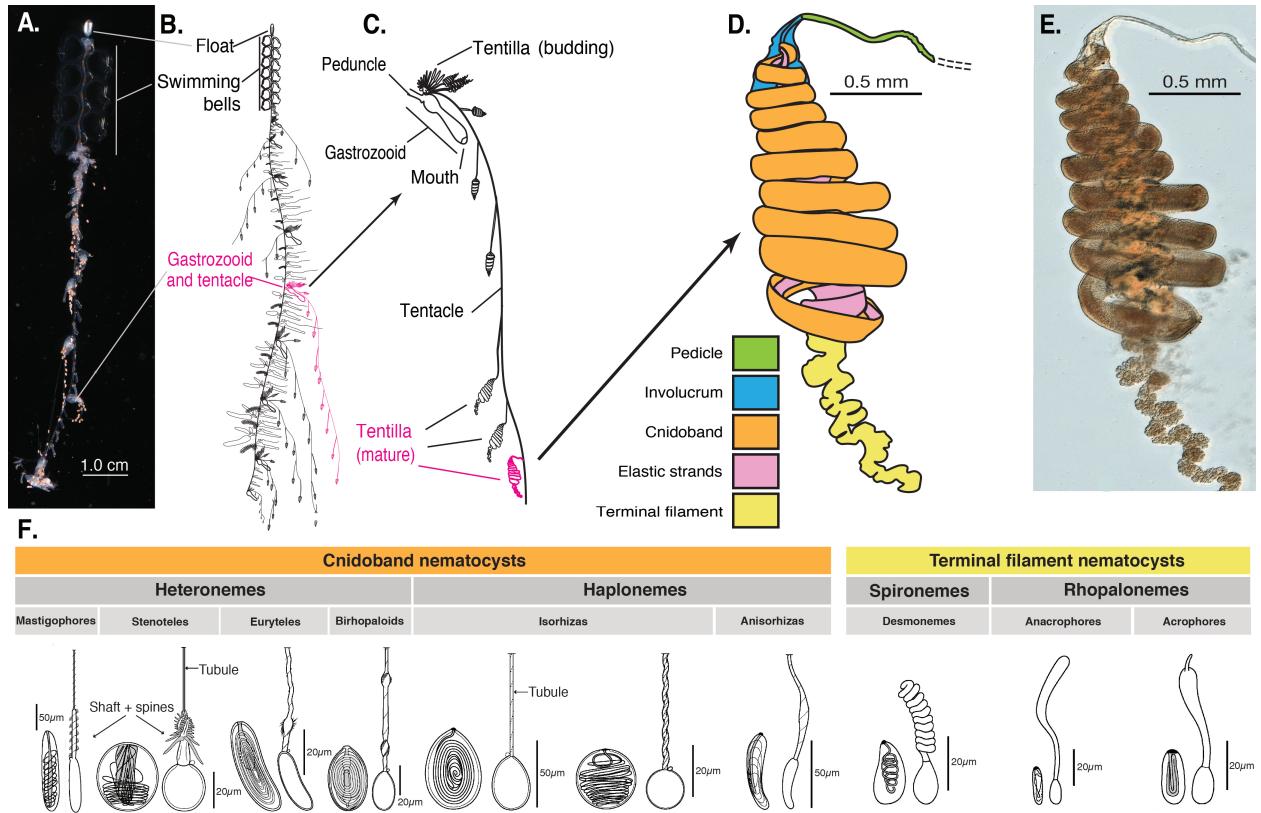


Figure 1: Siphonophore anatomy. A - *Nanomia* sp. siphonophore colony (photo by Catriona Munro). B, C - Illustration of a *Nanomia* colony, gastrozooid, and tentacle closeup (by Freya Goetz). D - *Nanomia* sp. Tentillum illustration and main parts. E - Differential interference contrast micrograph of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

94 species' niche (9–11). If this prediction holds true, we would expect the prey-capture apparatus
95 morphologies of siphonophores to diversify with the evolution of specializations on a variety
96 of prey types in different siphonophore lineages.

97 Specialization has been thought to be an evolutionary 'dead-end', meaning that specialized
98 lineages are unlikely to evolve into generalists or to shift the resource for which they are spe-
99 cialized (12–16). However, recent studies have found that interspecific competition can favor
100 the evolution of generalists from specialists (17–19) and specialist resource switching (20, 21).
101 In addition to studying relationships with morphology, we seek to identify what evolutionary
102 transitions in trophic niche breadth are prevalent in these open-ocean tactile predators. To
103 do so, we examine three alternative scenarios of siphonophore trophic specialization: (1)
104 predatory specialists evolved from generalist ancestors; (2) predatory specialists evolved from
105 specialist ancestors which targeted different resources, switching their primary prey type; and
106 (3) predatory generalists evolved from specialist ancestors. These scenarios are non-exclusive,
107 and each could apply to different transitions along the siphonophore phylogeny.

108 In the past, the study of siphonophore tentilla and diets has been limited due to the inac-
109 cessibility of their oceanic habitat and the difficulties associated with the collection of fragile
110 siphonophores. Thus, the morphological diversity of tentilla has only been characterized for a
111 few taxa, and their evolutionary history remains largely unexplored. Contemporary underwa-
112 ter sampling technology provides an unprecedented opportunity to explore the trophic ecology
113 (22) and functional morphology (23) of siphonophores. In addition, well-supported phylogenies
114 based on molecular data are now available for these organisms (24). These advances allow
115 for the examination of the evolutionary relationships between modern siphonophore form,
116 function, and ecology. Our work builds upon previous pioneering studies that have explored
117 the relationships between tentilla and diet, and have shown that siphonophores are a robust
118 system for the study of predatory specialization via morphological diversification. Purcell
119 (25, 26) showed clear relationships between diet, tentillum, and nematocyst characters in
120 co-occurring epipelagic siphonophores for a small subset of extant epipelagic siphonophore

¹²¹ species.

¹²² In this study, we present an extensive morphological characterization of tentilla and
¹²³ their nematocysts across a broad variety of shallow and deep-sea siphonophore species using
¹²⁴ modern imaging technologies, summarize the literature on siphonophore diets, expand the
¹²⁵ phylogenetic tree of siphonophores by combining ribosomal gene sequences from a broad range
¹²⁶ of taxa with a transcriptome-based backbone tree, and explore the evolutionary histories and
¹²⁷ correlations between diet, tentillum, and nematocyst characters. Our results suggest that
¹²⁸ siphonophores can evolve new specializations and generalism by modifying the phenotypes
¹²⁹ and genetic correlations in their prey capture apparatus. These findings show how studying
¹³⁰ elusive non-bilaterian predators can challenge traditional views on the evolution of predatory
¹³¹ specialization.

¹³² Results

¹³³ *Novel phylogenetic relationships* – In order to analyze the relationships between morphology
¹³⁴ and diet across the evolutionary history of siphonophores, we generated a siphonophore
¹³⁵ phylogeny that had broader taxonomic sampling than was available in previously published
¹³⁶ analyses. We first inferred a new tree with the needed taxon sampling with publicly available
¹³⁷ ribosomal RNA genes (18S & 16S) and new data from one species. This tree is essentially an
¹³⁸ extended version of that published in (27), and the two are congruent. We then compared
¹³⁹ the new extended ribosomal RNA tree to a recently published siphonophore transcriptome
¹⁴⁰ phylogeny (24). The topology of the extended ribosomal RNA tree recapitulates the resolved
¹⁴¹ nodes in (27) and most of the nodes in (24). Only five nodes in the unconstrained tree
¹⁴² inference were incongruent with the transcriptome tree in (24), with four of them poorly
¹⁴³ supported (bootstrap values <84), and only one of them strongly supported (*Frillagalma*
¹⁴⁴ *vityazi-Nanomia bijuga*, 100 bootstrap support). We constrained the incongruent nodes to
¹⁴⁵ the (24) topology during estimation of the constrained 18S+16S tree inference (Fig. 2).
¹⁴⁶ Since the transcriptome-based placement of *Nanomia bijuga* is more consistent with the

¹⁴⁷ morphological data, that relationship was also constrained. Moreover, with the inclusion of
¹⁴⁸ sequences from *Stephanomia amphytridis* and multiple *Erenna* species, our tree reveals a
¹⁴⁹ novel sister relationship between the genus *Erenna* and *Stephanomia*.

¹⁵⁰ We used the clade nomenclature defined in (27) and (24), including Codonophora to
¹⁵¹ indicate the sister group to Cystonectae, Euphysonectae to indicate the sister group to
¹⁵² Calycophorae, Clade A and B to indicate the two main lineages within Euphysonectae. In
¹⁵³ addition, we define two new clades within Codonophora (Fig. 2): Eucladophora as the
¹⁵⁴ clade containing *Agalma elegans* and all taxa that are more closely related to it than to
¹⁵⁵ *Apolemia lanosa*, and Tendiculophora as the clade containing *Agalma elegans* and all taxa
¹⁵⁶ more closely related to it than to *Bargmannia elongata*. Eucladophora is characterized by
¹⁵⁷ bearing spatially differentiated tentilla with proximal heteronemes and a narrower terminal
¹⁵⁸ filament region. The etymology derives from the Greek *eu+kládos+phóros* for “true branch
¹⁵⁹ bearers”. Tendiculophora are characterized by bearing rhopalonemes and desmonemes in the
¹⁶⁰ terminal filament, having a pair of elastic strands, and developing proximally detachable
¹⁶¹ cnidobands. The etymology of this clade is derived from the Latin *tendicula* for “snare or
¹⁶² noose” and the Greek *phóros* for “carriers”.

¹⁶³ *Evolutionary associations between diet and tentillum morphology* – We reconstructed the
¹⁶⁴ evolutionary history of feeding guilds using stochastic mapping on the new phylogeny (Fig.
¹⁶⁵ 3). Our reconstructions do not recover generalism as the ancestral siphonophore diet. None
¹⁶⁶ of the transitions in diet are consistent with scenario 1 (specialists evolving from generalists).
¹⁶⁷ Feeding guild specializations have shifted from an alternative ancestral state at least five
¹⁶⁸ times, consistent with instances supporting scenario 2 (specialists evolving to feed on a
¹⁶⁹ different resource). We also recover multiple independent origins of generalism from specialist
¹⁷⁰ ancestors (Fig. 3). Large crustacean specialists evolve into generalists twice independently,
¹⁷¹ consistent with instances of scenario 3 (generalists evolving from specialists). This finding
¹⁷² is particularly compelling given in that it is the opposite of known biases in ancestral state
¹⁷³ reconstruction. Nosil and Mooers (28) found that such methods tend to infer higher transition

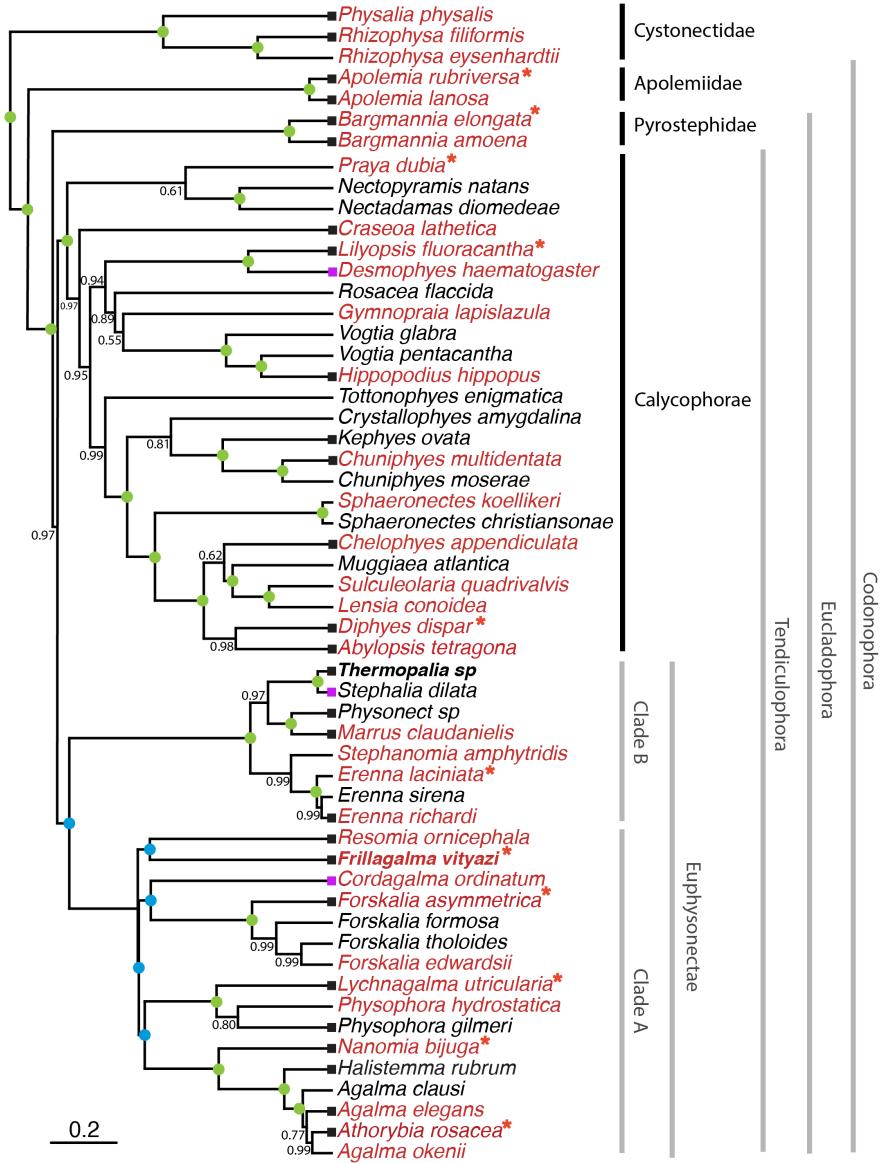


Figure 2: Bayesian time-tree inferred from 18S + 16S concatenated sequences and constrained to be congruent with a published transcriptome phylogeny. Branch lengths estimated using a relaxed molecular clock. Species names in red indicate replicated representation in the morphology data. All data were publicly available, apart from new sequences produced for *Thermopalria taraxaca* and *Frillaglma vityazi* (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 purple squares indicate genus-level correspondence to taxa included in Munro *et al.* (2018). The main clades are labeled: with black bars for described taxonomic units, and grey bars for operational phylogenetic designations.

¹⁷⁴ rates toward the more frequent state. In this case, that would lead to a bias for an increased
¹⁷⁵ rate of transition from generalists (the rarer state across the tips) to specialists (the more
¹⁷⁶ common state across the tips). We observe the opposite, indicating strong evidence that
¹⁷⁷ these generalists are indeed a derived state.

¹⁷⁸ To test whether measured morphological characters evolved in association with shifts in
¹⁷⁹ feeding ecology, we analyzed the evolutionary history of each character on the phylogeny, with
¹⁸⁰ the feeding guilds reconstructed on it as hypothetical selective regimes. We fit and compared
¹⁸¹ alternative evolutionary models for each continuous character. The models compared were the
¹⁸² Brownian Motion (BM) model of neutral divergent evolution (29), the Ornstein-Uhlenbeck
¹⁸³ (OU) model of stabilizing selection around a single fitted optimum state (30, 31), and an OU
¹⁸⁴ model with multiple optima (OUM) corresponding to each reconstructed selective regime
¹⁸⁵ (feeding guild). The model comparison shows that out of 30 characters, 10 show significantly
¹⁸⁶ stronger support for the diet-driven OUM (S15). These characters include terminal filament
¹⁸⁷ nematocyst size and shape, involucrum length, elastic strand width, and heteroneme number.
¹⁸⁸ Most of these characters are found exclusively in Tendiculophora, thus this may reflect
¹⁸⁹ processes that could be unique to this clade. Five characters including cnidoband length,
¹⁹⁰ cnidoband shape, and haploneme length show maximal support for a diet-driven single-
¹⁹¹ optimum OU model. The remaining 15 characters support BM (or OU with marginal AICc
¹⁹² difference with BM).

¹⁹³ In order to investigate the associations between the evolutionary history of morphological
¹⁹⁴ characters and specific prey types found in the diet, we used phylogenetic logistic regressions.
¹⁹⁵ We found that several characters were significantly correlated with the gains and losses of
¹⁹⁶ specific prey types (Fig. 3, right). Shifts toward ostracod presence in diet correlated with
¹⁹⁷ reductions in pedicle width and total haploneme volume. Shifts to copepod presence in
¹⁹⁸ the diet were associated with reductions in haploneme width, cnidoband length and width,
¹⁹⁹ total haploneme and heteroneme volumes, and tentacle and pedicle widths. Consistently,
²⁰⁰ transitions to decapod presence in the diet correlated with more coiled cnidobands (S21).

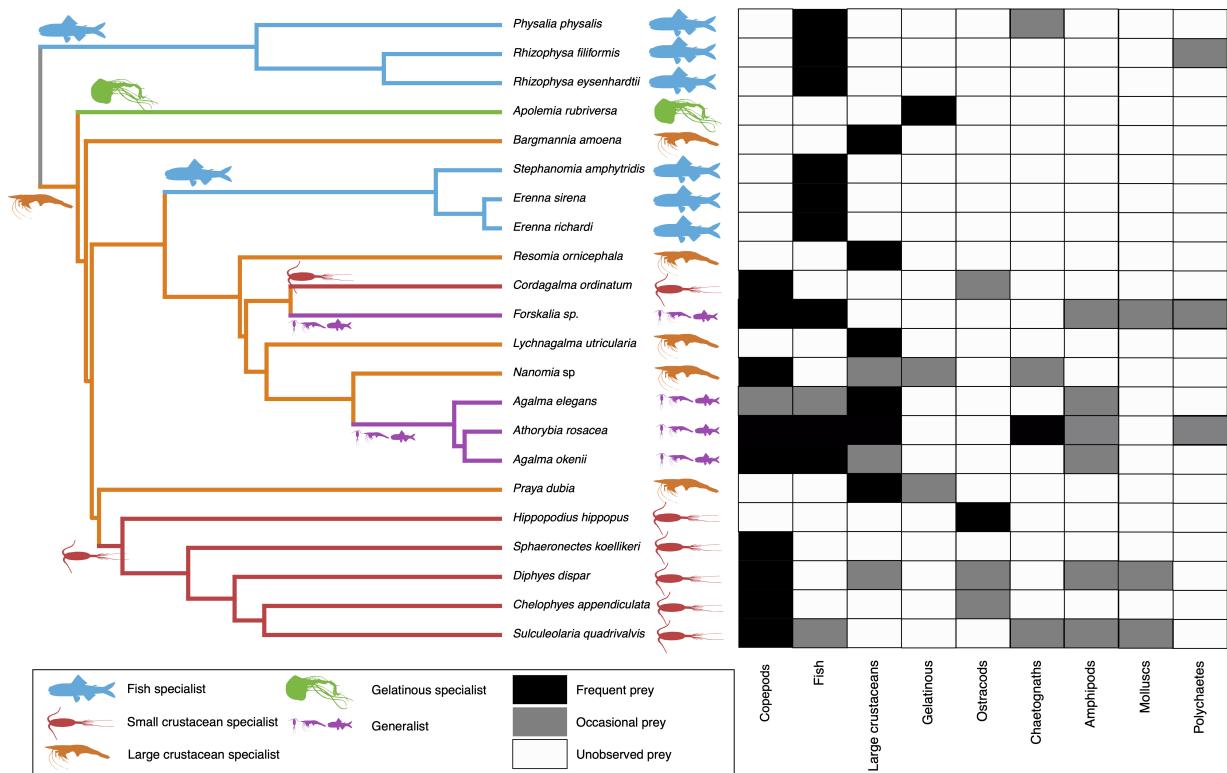


Figure 3: Left - Subset phylogeny showing the mapped feeding guild regimes that were used to inform the *OUwie* analyses. Right - Grid showing the prey items consumed from which the feeding guild categories were derived. Diet data were obtained from the literature review, available in the Dryad repository.

201 Evolutionary shifts in these characters may have allowed the inclusion of these prey types in
202 the diet.

203 In addition to studying correlations with prey type presence/absence in the diet, we also
204 tested for correlations between morphological characters and shifts in prey selectivity using
205 phylogenetic linear models. Prey selectivity values were calculated from (32) by contrasting
206 the gut content frequencies to the corresponding environmental abundances of prey. We
207 found that fish selectivity is associated with increased number of heteronemes per tentillum,
208 increased roundness of nematocysts (desmonemes and haplonemes), larger heteronemes,
209 reduced heteroneme/cnidoband length ratios, smaller rhopalonemes, lower haploneme surface
210 area to volume ratio (SA/V), and larger the cnidoband, elastic strand, pedicle and tentacle
211 widths. Decapod-selective diets were associated with increasing cnidoband size and coiledness,
212 haploneme row number, elastic strand width, and heteroneme number. Copepod-selective
213 diets evolved in association with smaller heteroneme and total nematocyst volumes, smaller
214 cnidobands, rounder rhopalonemes, elongated heteronemes, narrower haplonemes with higher
215 SA/V ratios, and smaller heteronemes, tentacles, pedicles, and elastic strands. Selectivity
216 for ostracods was associated with reductions in size and number of heteroneme nematocysts,
217 cnidoband size, number of haploneme rows, heteroneme number, and cnidoband coiledness.
218 Heteroneme length and elongation also correlated negatively with chaetognath selectivity
219 (S21). These results indicate that not only diet but also differential feeding selectivity has
220 evolved in correlation with changes in the prey capture apparatus of siphonophores. For each
221 prey type studied, tentillum morphology is a much better predictor of prey selectivity than
222 of prey presence in the diet, despite prey selectivity data being available for a smaller subset
223 of species. Interestingly, many of the morphological predictors had opposite slope signs when
224 predicting prey selectivity *versus* predicting prey presence in the diet (Table 2).

225 We tested some of the diet-morphology associations previously proposed in the literature
226 (25, 26) for correlated evolution (Table 1). We found that most, such as heteroneme volume
227 and copepod prey size, do show evidence for correlated evolution. The sole exception was the

228 relationship between terminal filament nematocysts (rhopalonemes and desmonemes) and
229 crustaceans in the diet. Analyses that do not take phylogeny into account do recover this
230 correlation across the extant species studied, but it is not consistent with correlated evolution.
231 The latter is likely a product of the larger species richness of crustacean-eating species with
232 terminal filament nematocysts, rather than simultaneous evolutionary gains.

233 Table 1. Tests of correlated evolution between siphonophore morphological characters and
234 aspects of the diet found correlated in the literature. We report the direction and significance
235 of the evolutionary association, the number of taxa used for the analysis, and the literature
236 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	Page's test	+	0.017	19	Purcell, 1984
Heteroneme volume	Copepod prey size	pGLS	+	0.002	8	Purcell, 1984
Terminal filament nematocysts	Crustacean diet	Page'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

238

239 Table 2. Discriminant analysis of principal components for the presence of specific prey
240 types using the morphological data. Top quartile variable (character) contributions to the
241 linear discriminants are ordered from highest to lowest. Logistic regressions and GLMs
242 were fitted to predict prey type presence and selectivity respectively. The sign of the slope
243 of each predictor is reported, marked with an asterisk if significant ($p\text{-value} < 0.05$), and
244 highlighted grey if it differs between prey presence in diet and prey selectivity. Pseudo- R^2
245 (%) 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	+	+	67.8
		Cnidoband length	-	+	
		Cnidoband width	-	-	
		Cnidoband free length	+	+	
		Total haploneme volume	-	+	
		Heteroneme volume	+	-	
Fish	68.1	Total nematocyst volume	-	+	
		Total heteroneme volume	-	-	45.8
		Cnidoband length	-	-	
		Cnidoband free length	+	+	
		Involucrum length	-	-	
		Pedicle width	+	+	
		Involucrum length	+.*	+	
		Total heteroneme volume	-	-	
		Elastic strand width	-	+.*	
		Rhopaloneme length	+	+	
Large crustaceans	81.8	Heteroneme volume	+	-	73.2
		Haploneme elongation	-	+	
		Desmoneme length	-	-	
		Tentacle width	+	+	
					98.7

246

247 *Evolution of relationships between characters with diet – Phenotypic integration results*
248 in correlation patterns between morphological characters and their rates of evolution. To
249 study these patterns, we fit a set of evolutionary variance-covariance matrices (33). The
250 quantitative characters we measured from tentilla and their nematocysts are highly correlated.
251 The results indicate that the dimensionality (number of independent axes of variation) of
252 tentillum morphology is low, that many traits are associated with size, but that nematocyst
253 arrangement and shape are independent of it (S4). The variance-covariance matrices (S21-23)
254 are congruent with the abundant positive correlations observed among simple measurement
255 characters in S3. This analysis more clearly reveals the diagonal blocks that constitute
256 the evolutionary modules, such as the heteroneme block, the terminal filament nematocyst
257 block, and the cnidoband-pedicle-tentacle block. These results were not sensitive to the
258 transformation of inapplicable states and taxon sampling. These results indicate that
259 siphonophore tentilla and nematocysts are phenotypically integrated and co-evolve within
260 discrete evolutionary modules.

261 In order to test whether rate covariance matrices changed with evolutionary shifts in

262 feeding guild regimes, we compared the rate covariance terms between characters across the
263 subtrees occupied by the different feeding guild regimes (S21). We found that half (48%)
264 of the character pairs presented significantly distinct correlation coefficients across different
265 regimes (S19), indicating that the mode of phenotypic integration also shifts with trophic
266 niche. When contrasting the regime-specific rate correlation matrices to the whole-tree
267 matrix and to the preceding ancestral regime matrix, we were able to identify the character
268 dependencies that are unique to each predatory niche (S22-23).

269 We were able to identify specific character correlations that shifted with the evolution of
270 new diets. Under the majority of stochastic character mapping outcomes, large crustacean
271 specialists are the ancestral feeding regime, and all other feeding regimes evolve from this
272 ancestral specialization. Compared to the rate correlation matrix estimated over the whole
273 tree, large crustacean specialists present strong negative correlations between haploneme
274 elongation and heteroneme size, and between rhopaloneme elongation and tentillum size, as
275 well as with involucrum length. Within generalist clades (*Forskalia* and the *Agalma-Athorybia*
276 clade), terminal filament nematocyst (desmoneme and rhopaloneme) sizes became negatively
277 correlated with the sizes of most characters, meaning that as some tentilla became larger,
278 their individual terminal nematocysts became smaller, observed to the extreme in *Agalma*.
279 In addition, heteroneme and rhopaloneme elongation became positively correlated with
280 cnidoband size. When large crustacean specialists switched to small crustacean prey in
281 *Cordagalma* and calycophorans, haploneme size became inversely correlated with heteroneme
282 elongation, which in turn developed a strong positive relationship with tentillum size. The
283 extremes of this gradient can be seen in *Cordagalma* and *Hippopodius*, genera subspecialized
284 in copepods and ostracods respectively. With the evolution of fish prey specialization in
285 cystonects and within Clade B (Fig. 1), haploneme elongation became negatively correlated
286 with heteroneme elongation (signal driven by Clade B, since cystonects lack tentacular
287 heteronemes), and the surface area to volume ratio of haploneme nematocysts switched from
288 a strong negative relationship with cnidoband size (found in every other regime) to a positive

²⁸⁹ correlation. This is consistent with Clade B haplonemes becoming rounder, more similar
²⁹⁰ to cystonect haplonemes specialized in fish prey penetration and envenomation. Gelatinous
²⁹¹ specialization, albeit appearing only once in our tree, also carries a unique signature in
²⁹² character rate correlation shifts, with an increase in the strength of the correlation between
²⁹³ heteroneme shape and shaft width, consistent with the appearance of birrhopaloid nematocysts
²⁹⁴ with swollen shafts. These are likely effective at anchoring gelatinous tissue in a similar way
²⁹⁵ to the nematocysts of the Narcomedusae (26).

²⁹⁶ Discussion

²⁹⁷ Several studies (12–16) have suggested that resource specialization can be an irreversible
²⁹⁸ dead-end due to the constraints posed by extreme phenotypic specialization. Our results show
²⁹⁹ that this is not the case for siphonophores, where the prey type on which they specialize has
³⁰⁰ shifted at least 5 times. We find no support for any transitions from generalist to specialist
³⁰¹ (scenario 1, as described in the Introduction). We do find support for at least 3 instances of
³⁰² specialists switching from one prey type to another prey type, (scenario 2) and two switches
³⁰³ from specialist to generalist (scenario 3). This is consistent with the findings of recent studies
³⁰⁴ on phytophagous insects (19), where the rate of evolution from generalists to specialists is
³⁰⁵ comparable to the reverse, thus specialization does not limit further evolution. Our results
³⁰⁶ are also consistent with analyses of lepidopterans (21), where specialized resource switching
³⁰⁷ is the primary transition type while niche breadth remains fairly constant. The evolutionary
³⁰⁸ history of tentilla shows that siphonophores are an example of trophic niche diversification via
³⁰⁹ morphological innovation and evolution, which allowed transitions between specialized trophic
³¹⁰ niches. In more familiar predators, the prey capture apparatus (such as claws and jaws) is
³¹¹ well integrated in the body, leading to trade-offs and whole-body adaptations for feeding
³¹² specialization. The extreme modularity of the siphonophore prey capture apparatus could
³¹³ release them from constraints typically imposed by adaptation to ecological specialization.
³¹⁴ This evolutionary mechanism is particularly important in a deep open-ocean ecosystem, which

³¹⁵ is a relatively homogeneous physical environment, where the primary niche heterogeneity
³¹⁶ available is the potential interactions between organisms (8).

³¹⁷ While selection acting on character states is a widely studied phenomenon, recent studies
³¹⁸ have shown that selection can also act upon the patterns of character correlations and
³¹⁹ phenotypic dependencies (33–39). This evolution of character relationships can allow lineages
³²⁰ to explore new regions of the morphospace and facilitate the appearance of ecological
³²¹ novelties. Our results show that the patterns of phenotypic integration in siphonophore
³²² tentilla vary among clades, and appear to display different relationships across shifting feeding
³²³ specializations. Similar to what has been found in the feeding morphologies of fish (33, 40),
³²⁴ siphonophore tentilla may have accommodated new diets by altering the correlations between
³²⁵ characters. For example, changes in the size and shape relationships between nematocyst
³²⁶ types gave rise to the nematocyst complements specialized in ensnaring prey with different
³²⁷ combinations of defensive traits.

³²⁸ Our results unambiguously show that tentillum morphology evolved with diet and strongly
³²⁹ support deviations from the generalist-to-specialist evolution scenario. However, the conclu-
³³⁰ sions we can draw from these analyses are limited in several ways. The biggest challenge at
³³¹ present is the sparse dietary data available in the literature. Additional dietary data could
³³² reveal transitions from generalists to specialists we were unable to detect for two reasons:
³³³ some of the taxa in our dataset have a very limited number of feeding observations, which
³³⁴ could lead to apparent specialization; and some of the taxa not included in our dataset could
³³⁵ be undiscovered generalists. When interpreting these results, it is also important to remember
³³⁶ that diet is also dependent on environmental prey availability. In addition, selectivity differ-
³³⁷ ences across siphonophore species could be also driven by other phenotypes not accounted
³³⁸ for in this study. Finally, further observations on behavior, digestion biochemistry, and toxin
³³⁹ composition are necessary to assess their relative importance in determining diet.

³⁴⁰ **Conclusions**

³⁴¹ Most studies on the evolution of predation have focused on vertebrate systems with an inte-
³⁴² grated feeding apparatus serving multiple functions. This has led to a narrow understanding of
³⁴³ the evolutionary outcomes of specialization, where extreme morphological evolution constrains
³⁴⁴ further shifts in their ecology. Siphonophores differ in many ways from commonly-known
³⁴⁵ predators, using modular weapons for prey capture (the tentilla) that are fully decoupled from
³⁴⁶ other structures and body functions. Our analysis of the evolutionary history of dietary spe-
³⁴⁷ cialization and morphological change in these elusive animals has revealed notable deviations
³⁴⁸ from traditional expectations. While much of the feeding ecology literature focuses on how
³⁴⁹ predatory generalists evolve into predatory specialists, in siphonophores we find predatory
³⁵⁰ specialists can evolve into generalists, and that specialists on one prey type have directly
³⁵¹ evolved into specialists on other prey types. We find that the character states, evolutionary
³⁵² optima, and genetic correlations of many morphological characters have evolved following
³⁵³ these ecological shifts. We find that the relationships between form and ecology hold across a
³⁵⁴ large set of siphonophore taxa and characters. These findings are central to understanding
³⁵⁵ the evolutionary mechanisms driving the emergence of food web complexity.

³⁵⁶ **Materials and Methods**

³⁵⁷ *Tentillum morphology* – The morphological work was carried out on siphonophore specimens
³⁵⁸ fixed in 4% formalin from the Yale Peabody Museum Invertebrate Zoology (YPM-IZ) collection
³⁵⁹ (accession numbers in Dryad repository). These specimens were collected intact across many
³⁶⁰ years of fieldwork expeditions, using blue-water diving (41), remotely operated vehicles
³⁶¹ (ROVs), plankton net trawls, and human-operated submersibles. Tentacles were dissected
³⁶² from non-larval gastrozooids, sequentially dehydrated into 100% ethanol, cleared in methyl
³⁶³ salicylate, and mounted onto slides with Canada Balsam or Permount mounting media.
³⁶⁴ The slides were imaged as tiled z-stacks using differential interference contrast (DIC) on an
³⁶⁵ automated stage at YPM-IZ (with the assistance of Daniel Drew and Eric Lazo-Wasem) and

366 with laser point confocal microscopy using a 488 nm Argon laser that excited autofluorescence
367 in the tissues. Thirty characters (defined in S1) were measured using Fiji (42, 43). We did not
368 measure the lengths of contractile structures (terminal filaments, pedicles, gastrozooids, and
369 tentacles) since they are too variable to quantify. We measured at least one specimen for 96
370 different species (raw data available in Dryad). Of these, we selected 38 focal species across
371 clades based on specimen availability and phylogenetic representation. Three to five tentacle
372 specimens from each one of these selected species were measured to capture intraspecific
373 variation.

374 *Siphonophore phylogeny* – While the main goal of this work is not to elucidate a novel
375 phylogeny for Siphonophora, we did expand on the most recent transcriptome based phylogeny
376 (24) to accommodate a larger taxon sampling. In order to do this, we ran a constrained analysis
377 on an extensive 18S+16S dataset. The phylogenetic analysis included 55 siphonophore species
378 and 6 outgroup cnidarian species (*Clytia hemisphaerica*, *Hydra circumcincta*, *Ectopleura*
379 *dumortieri*, *Porpita porpita*, *Velella velella*, *Staurocladia wellingtoni*). The gene sequences we
380 used in this study are available online (accession numbers in Dryad repository). Some of the
381 sequences we used were accessioned in (27), and others we extracted from the transcriptomes
382 in (24). Two new 16S sequences for *Frillagalma vityazi* (MK958598) and *Thermopalia* sp.
383 (MK958599) sequenced by Lynne Christianson using the primers from (44) (read 3' to 5' F:
384 TCGACTGTTACCAAAAAACATAGC , R: ACGGAATGAACCAAATCATGTAAG) were
385 included and accessioned to NCBI. Additional details on the phylogenetic inference methods
386 can be found in the Supplementary Methods.

387 Unconstrained ML and Bayesian phylogenies were congruent (S2,S5). Given the broader
388 sequence sampling of the transcriptome phylogeny, we ran constrained inferences (using
389 both ML and Bayesian approaches, which produced fully congruent topologies (S4,S6)) after
390 clamping the 5 nodes (S3, blue circles in Fig. 2) that were incongruent with the topology
391 of the consensus tree in (24). This topology was then used to inform a Bayesian relaxed
392 molecular clock time-tree in RevBayes, using a birth-death process (sampling probability

393 calculated from the known number of described siphonophore species) to generate ultrametric
394 branch lengths (S7-8). Scripts and tree files available in the Dryad repository.

395 *Feeding ecology* – We extracted categorical diet data for different siphonophore species
396 from published sources, including seminal papers (4, 25, 32, 45–48), and ROV observation
397 data (22, 49) with the assistance of Elizabeth Hetherington and C. Anela Choy (data available
398 in Dryad repository). In order to detect coarse-level patterns in feeding habits, the data
399 were merged into feeding guilds. For more details on how the diet data was curated and
400 summarized into guilds, please see Supplementary Methods.

401 We also extracted copepod prey length data from (25). To calculate specific prey
402 selectivities, we extracted quantitative diet and zooplankton composition data from (32),
403 matched each diet assessment to each prey field quantification by site, calculated Ivlev's
404 electivity indices (50), and averaged those by species (data available in Dryad repository).

405 *Statistical analyses* – We used a series of phylogenetic comparative methods to test the
406 evolutionary hypotheses presented in this study. We reconstructed ancestral states using ML
407 (R phytools::anc.ML (51)), and stochastic character mapping (R phytools::make.simmap) for
408 categorical characters. For more details on the data wrangling prior to these analyses, please
409 see the Supplementary Methods. R scripts available in the Dryad repository.

410 In order to study the evolution of predatory specialization, we reconstructed components
411 of the diet and prey selectivity on the phylogeny using ML (R phytools::anc.ML). To identify
412 evolutionary associations of diet with tentillum and nematocyst characters, we compared the
413 performance of a neutral evolution model to that of a diet-driven directional selection model.
414 First, we collapsed the diet data into the five feeding guilds mentioned above (fish specialist,
415 small crustacean specialist, large crustacean specialist, gelatinous specialist, generalist), based
416 on which prey types they were observed consuming most frequently. Then, we reconstructed
417 the feeding guild ancestral states using the ML function ace (package ape (52)), removing tips
418 with no feeding data. The ML reconstruction was congruent with the consensus stochastic
419 character mapping (S15). Then, using the package *OUwie* (53), we fitted an OU model with

420 multiple optima and rates of evolution (OUm) matched to the reconstructed ancestral diet
421 regimes, a single optimum OU model, and a BM null model, inspired by the analyses in (54).
422 We then ranked the models in order of increasing parametric complexity (BM, OU, OUm),
423 and compared the corrected Akaike Information Criterion (AICc) support scores (55) to the
424 lowest (best) score, using a cutoff of 2 units to determine significantly better support. When
425 the best fitting model was not significantly better than a less complex alternative, we selected
426 the least complex model (S9). In addition, we calculated and reported the model adequacy
427 scores using the R package *arbutus* (56).

428 In order to study correlations between the rates of evolution between different characters,
429 we fitted a set of evolutionary variance-covariance matrices (33) (R phytools::evol.vcv). For
430 more details on the data wrangling preceding these analyses, please see Supplementary
431 Methods. To test whether phenotypic integration changed across selective regimes determined
432 by the reconstructed feeding guilds, we carried out character-pairwise variance-covariance
433 analysis comparing alternative models (R phytools::evolvcv.lite), including those where
434 correlations are the same across the whole tree and models where correlations differ between
435 selective regimes (S19). Number of taxa used in each pairwise comparison is reported in S20.
436 Finally, we compared regime-specific variance-covariance matrices to the general matrix and
437 to their preceding regime matrix to identify the changes in character dependences unique to
438 each regime (S21-22).

439 We carried out a linear discriminant analysis of principal components (DAPC) using
440 the dapc function (R adegenet::dapc) (57). This function allowed us to incorporate more
441 predictors than individuals. We generated discriminant functions for feeding guild, and
442 for the presence of copepods, fish, and shrimp (large crustaceans) in the diet (S10-13).
443 From these DAPCs we obtained the highest contributing morphological characters to the
444 discrimination (characters in the top quartile of the weighted sum of the linear discriminant
445 loadings controlling for the eigenvalue of each discriminant). In order to identify the sign of
446 the relationship between the predictor characters and prey type presence in the diet, we then

⁴⁴⁷ generated generalized logistic regression models (as a type of generalized linear model, or
⁴⁴⁸ GLM using R stats::glm) and phylogenetic generalized linear models (R phylolm::phylolm)
⁴⁴⁹ with the top contributing characters (from the corresponding DAPC) as predictors (S14). We
⁴⁵⁰ also carried out these GLMs on the Ivlev's selectivity indices for each prey type calculated
⁴⁵¹ from (32). In addition, we ran a series of comparative analyses to address hypotheses of diet-
⁴⁵² tentillum relationships posed in the literature. Additional details on the DAPC optimization
⁴⁵³ are available in the Supplementary Methods.

⁴⁵⁴ **Supplementary Materials**

⁴⁵⁵ Data will be available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.NNNN>
⁴⁵⁶ Supplementary Materials (Supplementary Methods and figures) are appended following this
⁴⁵⁷ manuscript text, data available in https://github.com/dunnlab/tentilla_morph/tree/master/
⁴⁵⁸ Supplementary_materials

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