

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

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## **Abstract**

For predators to specialize on different prey taxa, their apparatus for prey capture has to evolve into a variety of forms. Specialization has been considered an evolutionary ‘dead-end’ due to the constraints associated with these morphological changes. However, in predators with modular structures used exclusively for prey capture, this may not be the case. Siphonophores, a clade of colonial cnidarians, use tentilla (tentacle side branches armed with nematocysts) exclusively for prey capture. Here we study how siphonophore specialists and generalists evolve, and how their predatory specialization shapes morphological evolution. To answer these questions, we: (1) measured 29 morphological characters of tentacles from 45 siphonophore species, (2) mapped these data to a phylogenetic tree, and (3) analyzed the evolutionary associations between morphological characters and prey type data from the literature. Instead of a dead-end, we found that predatory specialists can evolve into generalists, and that specialists on one prey type have directly evolved into specialists on other prey types. Our results show that siphonophore tentillum morphology has strong evolutionary associations with prey type, and suggest that shifts between prey types are linked to shifts in the morphology, mode of evolution, and genetic correlations of tentilla

<sup>26</sup> and their nematocysts. The evolutionary history of tentilla shows that siphonophores are a  
<sup>27</sup> unique example of ecological niche diversification via morphological innovation and evolution.  
<sup>28</sup> These findings contribute to understanding how specialization and morphological evolution  
<sup>29</sup> have shaped present-day food webs.

## <sup>30</sup> **Keywords**

<sup>31</sup> Siphonophores, tentilla, nematocysts, predation, specialization, character evolution

## <sup>32</sup> **Significance Statement**

<sup>33</sup> Predatory specialization is often associated with the evolution of modifications in the mor-  
<sup>34</sup> phology of the prey capture apparatus. Specialization has been considered an evolutionary  
<sup>35</sup> ‘dead-end’ due to the constraints associated with these morphological changes. However,  
<sup>36</sup> in predators like siphonophores, armed with modular structures used exclusively for prey  
<sup>37</sup> capture, this assumption becomes challenged. Our results show that siphonophores can evolve  
<sup>38</sup> new prey type specializations and generalism by modifying the morphological states, modes  
<sup>39</sup> of evolution, and genetic correlations among the parts of their prey capture apparatus. These  
<sup>40</sup> findings demonstrate how studying open-ocean non-bilaterian predators can reveal novel  
<sup>41</sup> patterns and mechanisms in the evolution of specialization. Understanding these evolutionary  
<sup>42</sup> processes is fundamental to the study of food web structure and complexity.

## <sup>43</sup> **Introduction**

<sup>44</sup> Most animal predators use specific structures to capture and subdue prey. Raptors have  
<sup>45</sup> claws and beaks, snakes have fangs, wasps have stingers, and cnidarians have nematocyst-  
<sup>46</sup> laden tentacles. The functional morphology of these structures is critical to their ability  
<sup>47</sup> to successfully capture prey (1). Long-term adaptive evolution in response to the defense  
<sup>48</sup> mechanisms of the prey (*e.g.*, avoidance, escape, protective barriers) leads to modifications

49 that can counter those defenses. The more specialized the diet of a predator is, the more  
50 specialized its structures need to be to efficiently overcome the challenges posed by the  
51 prey. Characterizing the relationships between morphology and predatory specialization  
52 is necessary to understand how the phenotypic diversity of predators determines food web  
53 structure. However, for many clades of predators, there is scarce knowledge on how these  
54 specializations evolved. The primary questions we set out to answer are: how do predator  
55 specialists and generalists evolve, and how does predatory specialization shape morphological  
56 evolution?

57 Siphonophores (Cnidaria: Hydrozoa) are a clade of predatory, gelatinous, colonial organ-  
58 isms that swim in the open ocean, feeding on a wide diversity of prey (often fish, crustaceans,  
59 and jellyfish). Siphonophores carry modular structures that are exclusively used for prey  
60 capture: the tentilla (Fig. 1). The tentilla have great morphological variation across species  
61 (2). Together with their well understood function, this makes them an ideal system to study  
62 the relationships between functional traits and prey specialization. Like a head of coral, a  
63 siphonophore is a colony bearing many feeding polyps (Fig. 1). Each feeding polyp has a  
64 single tentacle, which branches into a series of tentilla (side branches). Like other cnidarians,  
65 siphonophores capture prey with nematocysts, harpoon-like stinging capsules borne within  
66 specialized cells known as cnidocytes. Unlike the prey capture apparatus of most other  
67 cnidarians, siphonophore tentacles carry their cnidocytes in extremely complex and organized  
68 batteries (3) which are located in their tentilla. While nematocyst batteries and clusters in  
69 other cnidarians are simple static scaffolds for cnidocytes, siphonophore tentilla have their  
70 own reaction mechanism, triggered upon encounter with prey. When it fires, a tentillum  
71 undergoes an extremely fast conformational change that wraps it around the prey, maximizing  
72 the surface area of contact for nematocysts to fire on the prey (4). In addition, some species  
73 have elaborate fluorescent and bioluminescent lures on their tentilla to attract prey with  
74 aggressive mimicry (5–7).

75 Siphonophores bear four major nematocyst types in their tentacles and tentilla (Fig. 1F)..

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

86 Many siphonophore species inhabit the deep pelagic ocean, which spans from ~200m to the  
87 abyssal seafloor (~4000m). This habitat has fairly homogeneous physical conditions and stable  
88 zooplankton abundances and composition (8). With relatively predictable prey availability,  
89 ecological theory predicts that interspecific competition would inhibit the coexistence of  
90 closely-related species unless evolution towards specialization reduces the breadth of each  
91 species' niche (9–11). If this prediction holds true, we would expect the prey-capture apparatus  
92 morphologies of siphonophores to diversify with the evolution of specializations on a variety  
93 of prey types in different siphonophore lineages.

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

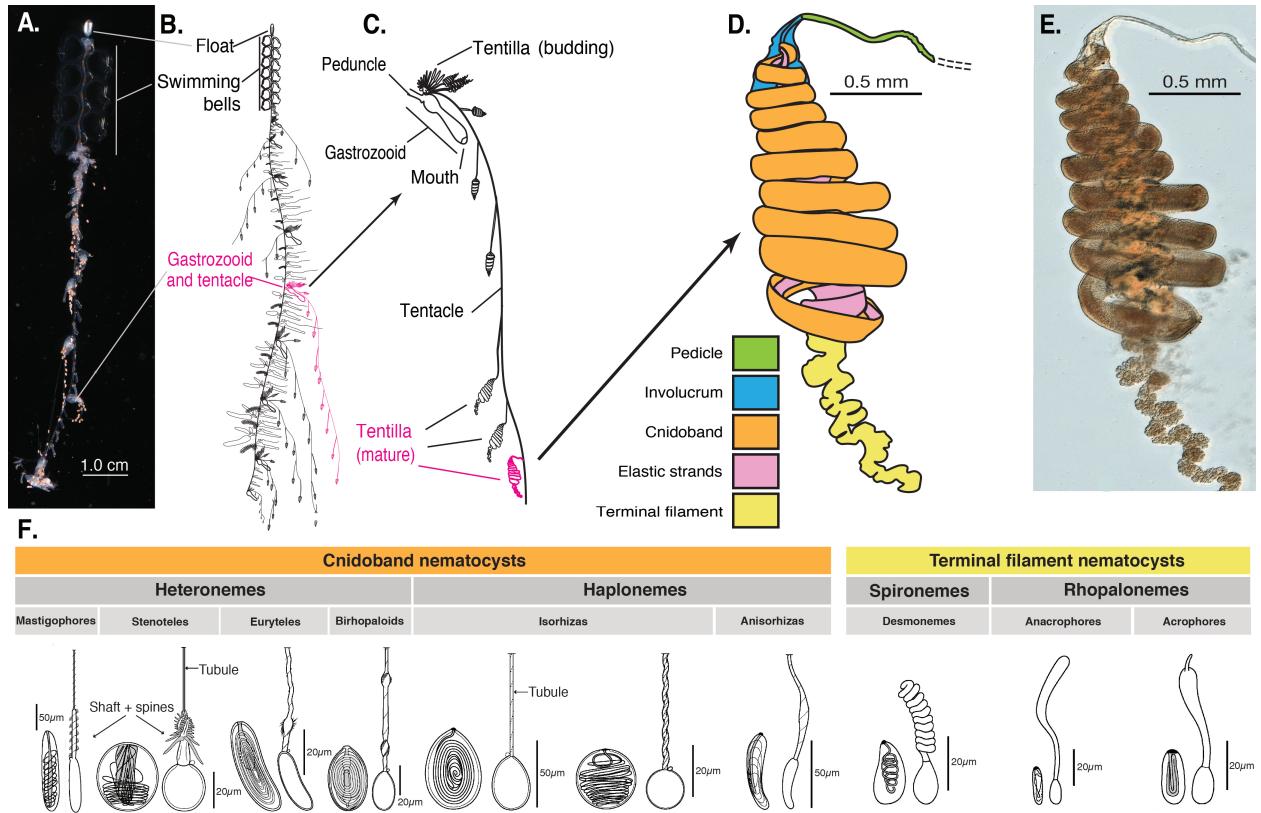


Figure 1: Siphonophore anatomy. A - *Nanomia* sp. siphonophore colony (photo by Catriona Munro). B, C - Illustration of a *Nanomia* colony, gastrozooid, and tentacle closeup (by Freya Goetz). D - *Nanomia* sp. Tentillum illustration and main parts. E - Differential interference contrast micrograph of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

<sup>103</sup> (3) predatory generalists evolved from specialist ancestors. These scenarios are non-exclusive,  
<sup>104</sup> and each could apply to different transitions along the siphonophore phylogeny.

<sup>105</sup> In the past, the study of siphonophore tentilla and diets has been limited due to the  
<sup>106</sup> inaccessibility of their oceanic habitat and the difficulties associated with the collection of  
<sup>107</sup> fragile siphonophores. Thus, the morphological diversity of tentilla has only been characterized  
<sup>108</sup> for a few taxa, and their evolutionary history remains largely unexplored. Contemporary  
<sup>109</sup> underwater sampling technology provides an unprecedented opportunity to explore the  
<sup>110</sup> trophic ecology (22) and functional morphology (23) of siphonophores. In addition, well-  
<sup>111</sup> supported phylogenies based on molecular data are now available for these organisms (24).  
<sup>112</sup> These advances allow for the examination of the evolutionary relationships between modern  
<sup>113</sup> siphonophore form, function, and ecology. Our work builds upon previous pioneering studies  
<sup>114</sup> that have explored the relationships between tentilla and diet, and showed that siphonophores  
<sup>115</sup> are a robust system for the study of predatory specialization via morphological diversification.  
<sup>116</sup> Purcell (25, 26) showed clear relationships between diet, tentillum, and nematocyst characters  
<sup>117</sup> in co-occurring epipelagic siphonophores for a small subset of extant epipelagic siphonophore  
<sup>118</sup> species.

<sup>119</sup> In this study, we present an extensive morphological characterization of tentilla and  
<sup>120</sup> their nematocysts across a broad variety of shallow and deep-sea siphonophore species using  
<sup>121</sup> modern imaging technologies, summarize the literature on siphonophore diets, expand the  
<sup>122</sup> phylogenetic tree of siphonophores by combining ribosomal gene sequences from a broad range  
<sup>123</sup> of taxa with a transcriptome-based backbone tree, and explore the evolutionary histories and  
<sup>124</sup> correlations between diet, tentillum, and nematocyst characters. Our results suggest that  
<sup>125</sup> siphonophores can evolve new specializations and generalism by modifying the phenotypes  
<sup>126</sup> and genetic correlations in their prey capture apparatus. These findings show how studying  
<sup>127</sup> elusive non-bilaterian predators can challenge traditional views on the evolution of predatory  
<sup>128</sup> specialization.

<sup>129</sup> **Results**

<sup>130</sup> *Novel phylogenetic relationships* – In order to analyze the relationships between morphology  
<sup>131</sup> and diet across the evolutionary history of siphonophores, we needed a siphonophore phylogeny  
<sup>132</sup> that had broader taxonomic sampling than was available in previously published analyses.  
<sup>133</sup> We first inferred a new tree with the needed taxon sampling with publicly available ribosomal  
<sup>134</sup> RNA genes (18S & 16S) and new data from one species. This tree is essentially an extended  
<sup>135</sup> version of that published in (27), and the two are congruent. We then compared the new  
<sup>136</sup> extended ribosomal RNA tree to a recently published siphonophore transcriptome phylogeny  
<sup>137</sup> (24). The topology of the extended ribosomal RNA tree recapitulates the resolved nodes in  
<sup>138</sup> (27) and most of the nodes in (24). Only 5 nodes in the unconstrained tree inference were  
<sup>139</sup> incongruent with the (24) transcriptome tree, 4 of them were poorly supported (bootstrap  
<sup>140</sup> values <84), and only one of them was strongly supported (*Frillagalma vityazi-Nanomia*  
<sup>141</sup> *bijuga*, 100 bootstrap support). We constrained the incongruent nodes to the (24) topology  
<sup>142</sup> during estimation of the constrained 18S+16S tree inference (Fig. 2). Since the transcriptome-  
<sup>143</sup> based placement of *Nanomia bijuga* is more consistent with the morphological data, that  
<sup>144</sup> relationship was also constrained. Moreover, with the inclusion of sequences from *Stephanomia*  
<sup>145</sup> *amphytridis* and multiple *Erenna* species, our tree reveals a novel sister relationship between  
<sup>146</sup> the genus *Erenna* and *Stephanomia*.

<sup>147</sup> We used the clade nomenclature defined in (27) and (24), including Codonophora to  
<sup>148</sup> indicate the sister group to Cystonectae, Euphysonectae to indicate the sister group to  
<sup>149</sup> Calycophorae, Clade A and B to indicate the two main lineages within Euphysonectae. In  
<sup>150</sup> addition, we define two new clades within Codonophora (Fig. 2): Eucladophora as the  
<sup>151</sup> clade containing *Agalma elegans* and all taxa that are more closely related to it than to  
<sup>152</sup> *Apolemia lanosa*, and Tendiculophora as the clade containing *Agalma elegans* and all taxa  
<sup>153</sup> more closely related to it than to *Bargmannia elongata*. Eucladophora is characterized by  
<sup>154</sup> bearing spatially differentiated tentilla with proximal heteronemes and a narrower terminal  
<sup>155</sup> filament region. The etymology derives from the Greek *eu+kládos+phóros* for “true branch

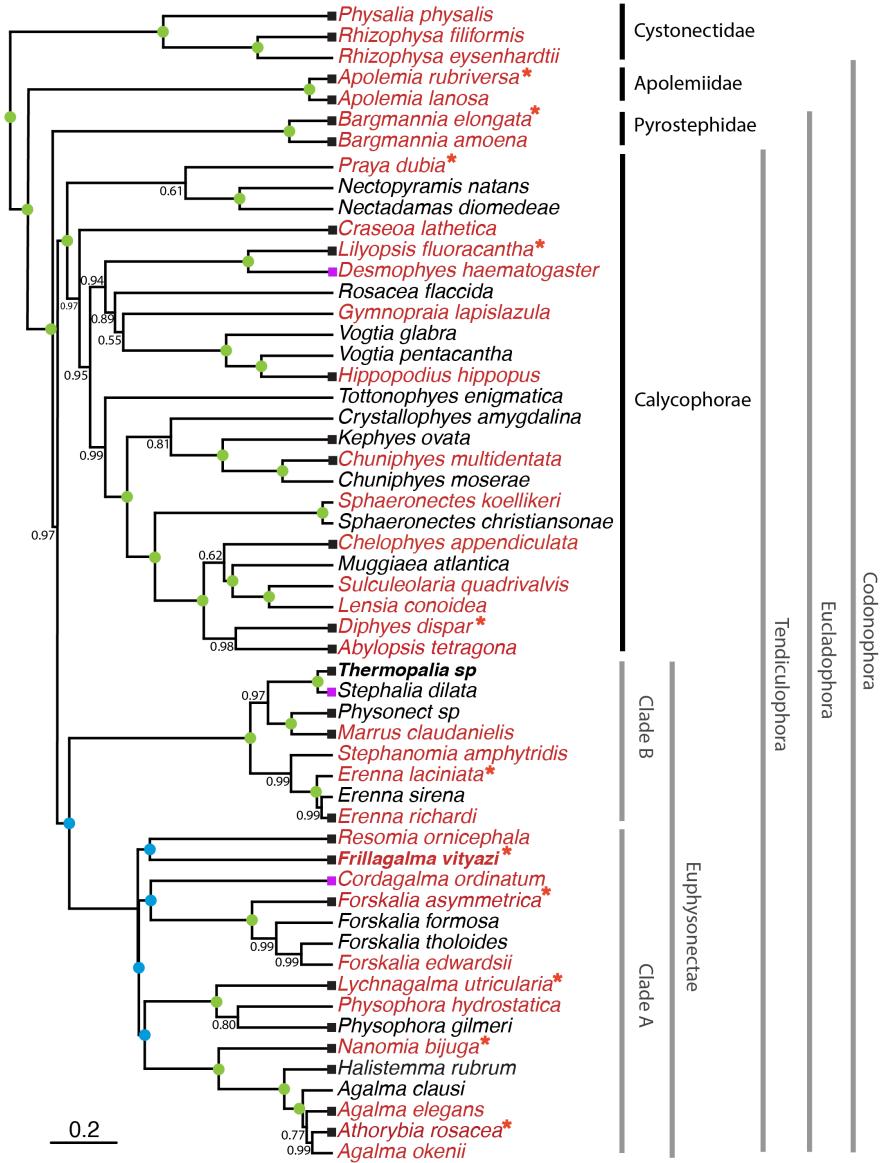


Figure 2: Bayesian time-tree inferred from 18S + 16S concatenated sequences and constrained to be congruent with a published transcriptome phylogeny. Branch lengths estimated using a relaxed molecular clock. Species names in red indicate replicated representation in the morphology data. All data was publicly available, apart from new sequences produced for *Thermopalria taraxaca* and *Frillaglma vityazi*. 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: in black for described taxonomic units, and in grey for operational phylogenetic designations.

<sup>156</sup> bearers". Tendiculophora are characterized by bearing rhopalonemes and desmonemes in the  
<sup>157</sup> terminal filament, having a pair of elastic strands, and developing proximally detachable  
<sup>158</sup> cnidobands. The etymology of this clade is derived from the Latin *tendicula* for "snare or  
<sup>159</sup> noose" and the Greek *phóros* for "carriers".

<sup>160</sup>       *Evolutionary associations between diet and tentillum morphology* – We reconstructed  
<sup>161</sup> the evolutionary history of feeding guilds using stochastic mapping on the new phylogeny.  
<sup>162</sup> Our reconstructions do not recover generalism as the ancestral siphonophore diet. None of  
<sup>163</sup> the transitions in diet are consistent with scenario 1 (specialists evolving from generalists).  
<sup>164</sup> Feeding guild specializations have shifted from an alternative ancestral state at least five  
<sup>165</sup> times, consistent with instances supporting scenario 2 (specialists evolving to feed on a  
<sup>166</sup> different resource). We also recover multiple independent origins of generalism from specialist  
<sup>167</sup> ancestors (Fig. 3). Large crustacean specialists evolve into generalists twice independently,  
<sup>168</sup> consistent with instances of scenario 3 (generalists evolving from specialists). This finding  
<sup>169</sup> is particularly compelling given in that it is the opposite of known biases in ancestral state  
<sup>170</sup> reconstruction. (28) found that such methods tend to infer higher transition rates toward the  
<sup>171</sup> more frequent state. In this case, that would lead to a bias for an increased rate of transition  
<sup>172</sup> from generalists (the rarer state across the tips) to specialists (the more common state across  
<sup>173</sup> the tips). We observe the opposite, indicating strong evidence that these generalists are  
<sup>174</sup> indeed a derived state.

<sup>175</sup>       To test whether measured morphological characters evolved in association with shifts in  
<sup>176</sup> feeding ecology, we analyzed the evolutionary history of each character on the phylogeny, with  
<sup>177</sup> the feeding guilds reconstructed on it as hypothetical selective regimes. We fit and compared  
<sup>178</sup> alternative evolutionary models for each continuous character. The models compared were the  
<sup>179</sup> Brownian Motion (BM) model of neutral divergent evolution (29), the Ornstein-Uhlenbeck  
<sup>180</sup> (OU) model of stabilizing selection around a single fitted optimum state (30, 31), and an OU  
<sup>181</sup> model with multiple optima corresponding to each reconstructed selective regime (feeding  
<sup>182</sup> guild). The model comparison shows that out of 30 characters, 10 show significantly stronger

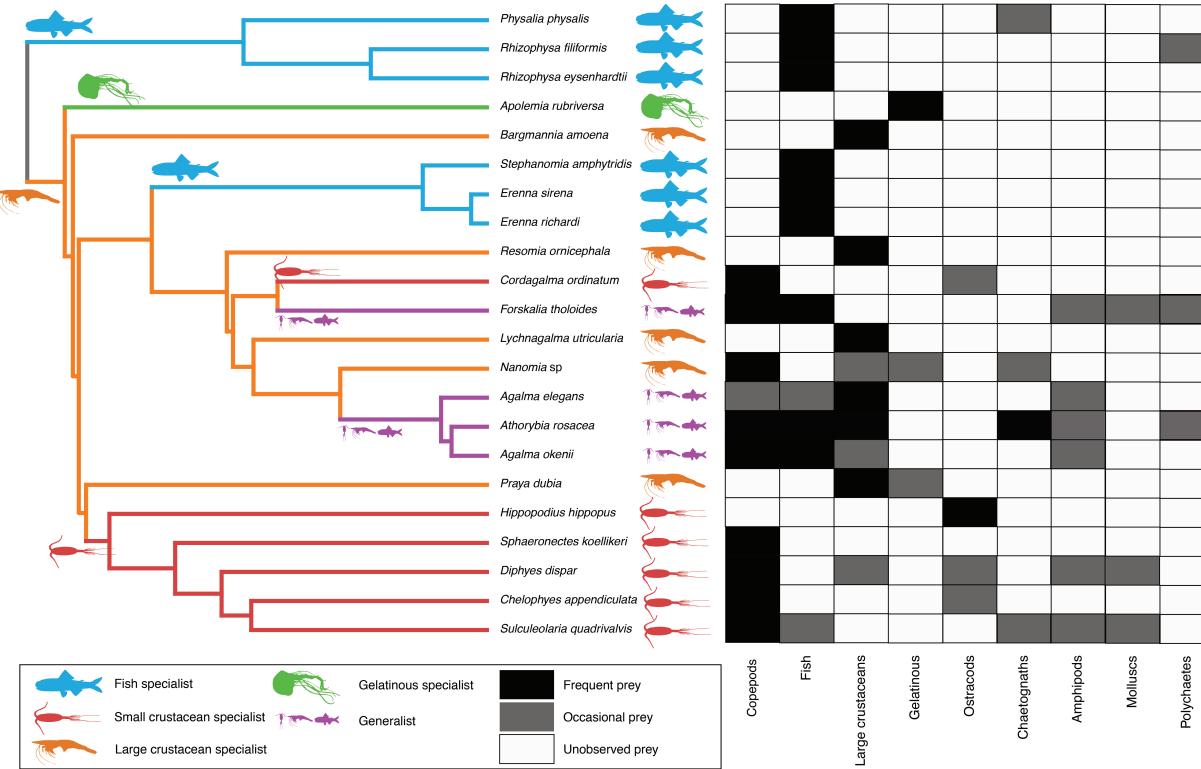


Figure 3: Left - Subset phylogeny showing the mapped feeding guild regimes that were used to inform the *OUwie* analyses. Right - Grid showing the prey items consumed from which the feeding guild categories were derived. Diet data were obtained from the literature review, available in the Dryad repository.

183 support for the diet-driven multi-optima OU model (S15). These characters include terminal  
 184 filament nematocyst size and shape, involucrum length, elastic strand width, and heteroneme  
 185 number. Most of these characters are found exclusively in Tendiculophora, thus this may  
 186 reflect processes that could be unique to this subtree. Five characters including cnidoband  
 187 length, cnidoband shape, and haploneme length show maximal support for a diet-driven  
 188 single-optimum OU model. The remaining 15 characters support BM (or OU with marginal  
 189 AICc difference with BM).

190 In order to investigate the associations between the evolutionary history of morphological  
 191 characters and specific prey types found in the diet, we used phylogenetic logistic regressions.  
 192 We found that several characters were significantly correlated with the gains and losses of  
 193 specific prey types (Fig. 3, right). Shifts toward ostracod presence in diet correlated with

194 reductions in pedicle width and total haploneme volume. Shifts to copepod presence in  
195 the diet were associated with reductions in haploneme width, cnidoband length and width,  
196 total haploneme and heteroneme volumes, and tentacle and pedicle widths. Consistently,  
197 transitions to decapod presence in the diet correlated with more coiled cnidobands (S21).  
198 Evolutionary shifts in these characters may have allowed the inclusion of these prey types in  
199 the diet.

200 In addition to studying correlations with prey type presence/absence, we also tested for  
201 correlations between morphological characters and shifts in prey selectivity using phylogenetic  
202 linear models. Prey selectivity values were calculated from (32) by contrasting the gut  
203 content frequencies to the corresponding environmental abundances of prey. We found that  
204 fish selectivity is associated with increased number of heteronemes per tentillum, increased  
205 roundness of nematocysts (desmonemes and haplonemes), larger heteronemes, reduced  
206 heteroneme/cnidoband length ratios, smaller rhopalonemes, lower haploneme surface area  
207 to volume ratio ( $SA/V$ ), and larger the cnidoband, elastic strand, pedicle and tentacle  
208 widths. Decapod-selective diets were associated with increasing cnidoband size and coiledness,  
209 haploneme row number, elastic strand width, and heteroneme number. Copepod-selective  
210 diets evolved in association with smaller heteroneme and total nematocyst volumes, smaller  
211 cnidobands, rounder rhopalonemes, elongated heteronemes, narrower haplonemes with higher  
212  $SA/V$  ratios, and smaller heteronemes, tentacles, pedicles, and elastic strands. Selectivity  
213 for ostracods was associated with reductions in size and number of heteroneme nematocysts,  
214 cnidoband size, number of haploneme rows, heteroneme numbers, and cnidoband coiledness.  
215 Heteroneme length and elongation also correlated negatively with chaetognath selectivity  
216 (S21). These results indicate that not only diet but also differential feeding selectivity has  
217 evolved in correlation with changes in the prey capture apparatus of siphonophores.

218 We tested some of the diet-morphology associations previously proposed in the literature  
219 (25, 26) for correlated evolution (Table 1). We found that most, such as heteroneme volume  
220 and copepod prey size, do show evidence for correlated evolution. The sole exception was the

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

226 Table 1. Table 1. Tests of correlated evolution between siphonophore morphological  
227 characters and aspects of the diet found correlated in the literature. We report the direction  
228 and significance of the evolutionary association, the number of taxa used for the analysis,  
229 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	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

230

231

232 Table 2. Discriminant analysis of principal components for the presence of specific prey  
233 types using the morphological data. Top quartile variable (character) contributions to the  
234 linear discriminants are ordered from highest to lowest. Logistic regressions and GLMs  
235 were fitted to predict prey type presence and selectivity respectively. The sign of the slope  
236 of each predictor is reported, marked with an asterisk if significant ( $p\text{-value} < 0.05$ ), and  
237 highlighted grey if it differs between prey presence in diet and prey selectivity. Pseudo- $R^2$   
238 (%) 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 <sup>2</sup> (%)
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

239

240 *Evolution of relationships between characters with diet – Phenotypic integration results*

241 in correlation patterns between morphological characters and their rates of evolution. To  
 242 study these patterns, we fit a set of evolutionary variance-covariance matrices (33). The  
 243 quantitative characters we measured from tentilla and their nematocysts are highly correlated.  
 244 The results indicate that the dimensionality (number of independent axes of variation) of  
 245 tentillum morphology is low, that many traits are associated with size, but that nematocyst  
 246 arrangement and shape are independent of it (S4). The variance-covariance matrices (S36-38)  
 247 are congruent with the abundant positive correlations observed among simple measurement  
 248 characters in S3. This analysis more clearly reveals the diagonal blocks that constitute  
 249 the evolutionary modules, such as the heteroneme block, the terminal filament nematocyst  
 250 block, and the cnidoband-pedicle-tentacle block. These results were not sensitive to the  
 251 transformation of inapplicable states and taxon sampling. These results indicate that  
 252 siphonophore tentilla and nematocysts are phenotypically integrated and co-evolve within  
 253 discrete evolutionary modules.

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

255 feeding guild regimes, we compared the rate covariance terms between characters across the  
256 subtrees occupied by the different feeding guild regimes (S41). We found that half (48%)  
257 of the character pairs presented significantly distinct correlation coefficients across different  
258 regimes (S39), indicating that the mode of phenotypic integration also shifts with trophic  
259 niche. When contrasting the regime-specific rate correlation matrices to the whole-tree  
260 matrix, we were able to identify the character dependencies that are unique to each predatory  
261 niche (S42). These results indicate that the evolutionary dependencies in these integrated  
262 modules are changing across the phylogeny, and evolving together with changes in prey type  
263 specializations.

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

<sup>282</sup> B, since cystonects lack tentacular heteronemes), and the surface area to volume ratio of  
<sup>283</sup> haploneme nematocysts switched from a strong negative relationship with cnidoband size  
<sup>284</sup> (found in every other regime) to a positive correlation. Gelatinous specialization, albeit  
<sup>285</sup> appearing only once in our tree, also carries a unique signature in character rate correlation  
<sup>286</sup> shifts, with an increase in the strength of the correlation between heteroneme shape and shaft  
<sup>287</sup> width, consistent with the appearance of birrhopaloid nematocysts with swollen shafts that  
<sup>288</sup> are likely effective at anchoring gelatinous tissue (see reference to Narcomedusae nematocysts  
<sup>289</sup> in (26)).

## <sup>290</sup> Discussion

<sup>291</sup> Several studies (12–16) have suggested that resource specialization is an irreversible dead-end  
<sup>292</sup> due to the constraints posed by extreme phenotypic specialization. Our results show that  
<sup>293</sup> this is not the case for siphonophores, where the prey type on which they specialize has  
<sup>294</sup> shifted at least 5 times. We find no support for any transitions from generalist to specialist  
<sup>295</sup> (scenario 1, as described in the Introduction). We do find support for at least 3 instances of  
<sup>296</sup> specialists switching from one prey type to another prey type, (scenario 2) and two switches  
<sup>297</sup> from specialist to generalist (scenario 3). This is consistent with the findings of recent studies  
<sup>298</sup> on phytophagous insects (19), where the rate of evolution from generalists to specialists is  
<sup>299</sup> comparable to the reverse, thus specialization does not limit further evolution. Our results  
<sup>300</sup> are also consistent with analyses of lepidopterans (21), where specialized resource switching  
<sup>301</sup> is the primary transition type while niche breadth remains fairly constant. The evolutionary  
<sup>302</sup> history of tentilla shows that siphonophores are an example of trophic niche diversification  
<sup>303</sup> via morphological innovation and evolution, which allowed transitions between specialized  
<sup>304</sup> trophic niches. In more familiar predators, the prey capture apparatus is well integrated in the  
<sup>305</sup> body (such as claws and jaws), leading to trade-offs and whole body adaptations to feeding  
<sup>306</sup> specialization. The extreme modularity of the siphonophore prey capture apparatus could  
<sup>307</sup> release them from the constraints typically imposed by adaptation to ecological specialization.

308 This evolutionary mechanism is particularly important in a deep open ocean ecosystem, which  
309 is a relatively homogeneous physical environment, where the primary niche heterogeneity  
310 available is the potential interactions between organisms (8).

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

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

334 Siphonophores are an abundant group of zooplankton in oceanic ecosystems (41, 42).

<sup>335</sup> While little is known about siphonophore trophic ecology, what is known indicates that  
<sup>336</sup> they occupy a central position in midwater food webs (22), serving as important trophic  
<sup>337</sup> intermediaries between smaller zooplankton and higher trophic level predators. Our findings  
<sup>338</sup> on the unique evolutionary history of siphonophore trophic specialization elucidate how they  
<sup>339</sup> arrived to play this fundamental role in the oceanic food web.

## <sup>340</sup> Conclusions

<sup>341</sup> Most studies on the evolution of predation have focused on vertebrate systems with a prey  
<sup>342</sup> capture apparatus (such as jaws and limbs) deeply integrated into their bodies, serving  
<sup>343</sup> multiple functions. This has led to a biased understanding of the evolutionary outcomes  
<sup>344</sup> of specialization, where extreme morphological evolution constrains further shifts in their  
<sup>345</sup> ecology. Siphonophores differ in many ways from commonly-known predators, using modular  
<sup>346</sup> weapons for prey capture (the tentilla) that are fully decoupled from other structures and body  
<sup>347</sup> functions. Our analysis of the evolutionary history of dietary specialization and morphological  
<sup>348</sup> change in these elusive animals has revealed notable deviations from traditional expectations.  
<sup>349</sup> While much of the feeding ecology literature focuses on how predatory generalists evolve  
<sup>350</sup> into predatory specialists, in siphonophores we find predatory specialists can evolve into  
<sup>351</sup> generalists, and that specialists on one prey type have directly evolved into specialists on  
<sup>352</sup> other prey types. We find that the character states, evolutionary optima, and genetic  
<sup>353</sup> correlations of many morphological characters have evolved following these ecological shifts.  
<sup>354</sup> Our extended morphological characterization shows that the relationships between form and  
<sup>355</sup> ecology hold across a large set of siphonophore taxa and characters. These findings are central  
<sup>356</sup> to understanding the evolutionary mechanisms driving the emergence of food web complexity.

## <sup>357</sup> Materials and Methods

<sup>358</sup> *Tentillum morphology* – The morphological work was carried out on siphonophore specimens  
<sup>359</sup> fixed in 4% formalin from the Yale Peabody Museum Invertebrate Zoology (YPM-IZ) collection

360 (accession numbers in Dryad repository). These specimens were collected intact across many  
361 years of fieldwork expeditions, using blue-water diving (43), remotely operated vehicles  
362 (ROVs), plankton net trawls, and human-operated submersibles. Tentacles were dissected  
363 from non-larval gastrozooids, sequentially dehydrated into 100% ethanol, cleared in methyl  
364 salicylate, and mounted onto slides with Canada Balsam or Permount mounting media.  
365 The slides were imaged as tiled z-stacks using differential interference contrast (DIC) on an  
366 automated stage at YPM-IZ (with the assistance of Daniel Drew and Eric Lazo-Wasem) and  
367 with laser point confocal microscopy using a 488 nm Argon laser that excited autofluorescence  
368 in the tissues. Thirty characters (defined in S5) were measured using Fiji (44, 45). We did not  
369 measure the lengths of contractile structures (terminal filaments, pedicles, gastrozooids, and  
370 tentacles) since they are too variable to quantify. We measured at least one specimen for 96  
371 different species (raw data available in Dryad). Of these, we selected 38 focal species across  
372 clades based on specimen availability and phylogenetic representation. Three to five tentacle  
373 specimens from each one of these selected species were measured to capture intraspecific  
374 variation.

375 *Siphonophore phylogeny* – While the main goal of this work is not to elucidate a novel  
376 phylogeny for Siphonophora, we did expand on the most recent transcriptome based phylogeny  
377 (24) to accommodate a larger taxon sampling. In order to do this, we ran a constrained analysis  
378 on an extensive 18S+16S dataset. The phylogenetic analysis included 55 siphonophore species  
379 and 6 outgroup cnidarian species (*Clytia hemisphaerica*, *Hydra circumcincta*, *Ectopleura*  
380 *dumortieri*, *Porpita porpita*, *Velella velella*, *Staurocladia wellingtoni*). The gene sequences we  
381 used in this study are available online (accession numbers in Dryad repository). Some of the  
382 sequences we used were accessioned in (27), and others we extracted from the transcriptomes  
383 in (24). Two new 16S sequences for *Frillagalma vityazi* (MK958598) and *Thermopalia* sp.  
384 (MK958599) sequenced by Lynne Christianson using the primers from (46) (read 3' to 5' F:  
385 TCGACTGTTACCAAAACATAGC , R: ACGGAATGAACCAAATCATGTAAAG) were  
386 included and accessioned to NCBI. Additional details on the phylogenetic inference methods

387 are available in the Supplementary Methods.

388 Given the broader sequence sampling of the transcriptome phylogeny, we ran constrained  
389 inferences (using both ML and Bayesian approaches, which produced fully congruent topologies  
390 (S8 and S10)) after fixing the 5 nodes that were incongruent with the topology of the consensus  
391 tree in (24). This topology was then used to inform a Bayesian relaxed molecular clock  
392 time-tree in RevBayes, using a birth-death process (sampling probability calculated from the  
393 known number of described siphonophore species) to generate ultrametric branch lengths  
394 (S11-12). Scripts 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, 47–50), and ROV observation  
397 data (22, 51) 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 (52), and averaged those by species (data available in the 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 (53)), 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` (54)), removing tips  
418 with no feeding data. The ML reconstruction was congruent with the consensus stochastic  
419 character mapping (S31). Then, using the package `OUwie` (55), 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 (56).  
422 We then ranked the models in order of increasing parametric complexity (BM, OU, OUM), and  
423 compared the corrected Akaike Information Criterion (AICc) support scores (57) to the lowest  
424 (best) score, using a cutoff of 2 units to determine significantly better support. When the best  
425 fitting model was not significantly better than a less complex alternative, we selected the least  
426 complex model (S15). In addition, we calculated and reported the model adequacy scores  
427 using the R package `arbutus` (58). To model the evolutionary associations between individual  
428 tentillum and nematocyst characters and the ability to capture particular prey types in the  
429 diet, we ran a series of phylogenetic generalized linear models (R `phylolm::phyloglm`) (S21).  
430 In addition, we ran a series of comparative analyses to address hypotheses of diet-tentillum  
431 relationships posed in the literature.

432 In order to study correlations between the rates of evolution between different characters,  
433 we fitted a set of evolutionary variance-covariance matrices (33) (R `phytools::evol.vcv`). For  
434 more details on the data wrangling preceding these analyses, please see Supplementary  
435 Methods. To test whether phenotypic integration changes across selective regimes determined  
436 by the reconstructed feeding guilds, we carried out character-pairwise variance-covariance  
437 analysis comparing alternative models (R `phytools::evolvcv.lite`), including those where  
438 correlations are the same across the whole tree and models where correlations differ between  
439 selective regimes (S42). Finally, we compared regime-specific variance-covariance matrices to  
440 the general matrix and to their preceding regime matrix to identify the changes in character

441 dependences unique to each regime (S43).

442 We carried out a linear discriminant analysis of principal components (DAPC) using  
443 the dapc function (R adegenet::dapc) (59). This function allowed us to incorporate more  
444 predictors than individuals. We generated discriminant functions for feeding guild, and  
445 for the presence of copepods, fish, and shrimp (large crustaceans) in the diet (S16-20).  
446 From these DAPCs we obtained the highest contributing morphological characters to the  
447 discrimination (characters in the top quartile of the weighted sum of the linear discriminant  
448 loadings controlling for the eigenvalue of each discriminant). In order to identify the sign  
449 of the relationship between the predictor characters prey type presence in the diet, we then  
450 generated generalized logistic regression models (as a type of generalized linear model, or  
451 GLM using R stats::glm) with the top contributing characters (from the corresponding DAPC)  
452 as predictors. We also carried out these GLMs on the Ivlev's selectivity indices for each prey  
453 type calculated from (32). Additional details on the DAPC optimization are available in the  
454 Supplementary Materials.

## 455 **Supplementary Materials**

456 Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.NNNN>  
457 Supplementary Materials are available in [https://github.com/dunnlab/tentilla\\_morph/](https://github.com/dunnlab/tentilla_morph/)  
458 Supplement\_forSupershort.pdf

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