

<sup>1</sup> **Classification: Biological Sciences**

<sup>2</sup> **Shaped to kill: The evolution of siphonophore tentilla  
3 for specialized prey capture in the open ocean**

<sup>4</sup> Alejandro Damian-Serrano<sup>1,‡</sup>, Steven H.D. Haddock<sup>2</sup>, Casey W. Dunn<sup>1</sup>

<sup>5</sup> <sup>1</sup> Yale University, Department of Ecology and Evolutionary Biology, 165 Prospect St.,  
<sup>6</sup> New Haven, CT 06520, USA

<sup>7</sup> <sup>2</sup> Monterey Bay Aquarium Research Institute, 7700 Sandholdt Rd., Moss Landing, CA  
<sup>8</sup> 95039, USA

<sup>9</sup> **0.0.1 ORCID: Alejandro Damian-Serrano: 0000-0002-2544-0489; Steven H.D.  
10 Haddock: 0000-0001-9420-4482; Casey Dunn: 0000-0003-0628-5150**

<sup>11</sup> <sup>‡</sup> Corresponding author: Alejandro Damian-Serrano. Address: 165 Prospect St., New Haven,  
<sup>12</sup> CT, 06511. Phone: (401) 441-9613. Email: alejandro.damianserrano@yale.edu.

<sup>13</sup> **Keywords**

<sup>14</sup> Siphonophores, tentilla, nematocysts, predation, specialization, character evolution

<sup>15</sup> **Abstract**

<sup>16</sup> For predators to specialize on different prey taxa, their apparatus for prey capture has to  
<sup>17</sup> evolve into a variety of forms. Specialization has been considered an evolutionary ‘dead-  
<sup>18</sup> end’ due to the constraints associated with these morphological changes. However, in  
<sup>19</sup> predators with modular structures used exclusively for prey capture, this may not be the  
<sup>20</sup> case. Siphonophores, a clade of colonial cnidarians, use tentilla (tentacle side branches armed  
<sup>21</sup> with nematocysts) exclusively for prey capture. Here we study how siphonophore specialists  
<sup>22</sup> and generalists evolve, and how their predatory specialization shapes morphological evolution.  
<sup>23</sup> To answer these questions, we: (1) measured 29 morphological characters of tentacles from  
<sup>24</sup> 45 siphonophore species, (2) mapped these data to a phylogenetic tree, and (3) analyzed  
<sup>25</sup> the evolutionary associations between morphological characters and prey type data from  
<sup>26</sup> the literature. Instead of a dead-end, we found that predatory specialists can evolve into  
<sup>27</sup> generalists, and that specialists on one prey type have directly evolved into specialists on  
<sup>28</sup> other prey types. Our results show that siphonophore tentillum morphology has strong  
<sup>29</sup> evolutionary associations with prey type, and suggest that shifts between prey types are  
<sup>30</sup> linked to shifts in the morphology, mode of evolution, and genetic correlations of tentilla  
<sup>31</sup> and their nematocysts. The evolutionary history of tentilla shows that siphonophores are a  
<sup>32</sup> unique example of ecological niche diversification via morphological innovation and evolution.  
<sup>33</sup> These findings contribute to understanding how specialization and morphological evolution  
<sup>34</sup> have shaped present-day food webs.

<sup>35</sup> **Significance Statement**

<sup>36</sup> Predatory specialization is often associated with the evolution of modifications in the mor-  
<sup>37</sup> phology of the prey capture apparatus. Specialization has been considered an evolutionary  
<sup>38</sup> ‘dead-end’ due to the constraints associated with these morphological changes. However,  
<sup>39</sup> in predators like siphonophores, armed with modular structures used exclusively for prey  
<sup>40</sup> 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

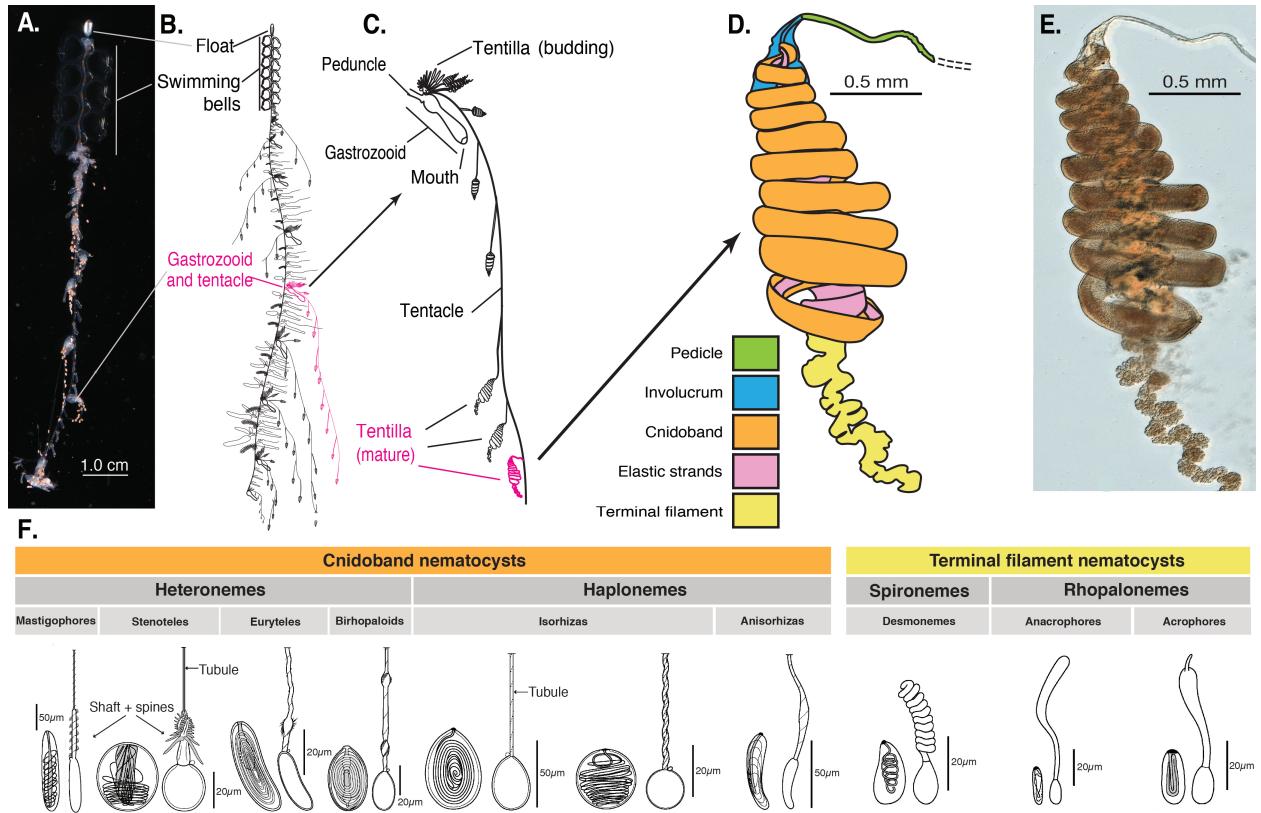


Figure 1: Siphonophore anatomy. A - *Nanomia* sp. siphonophore colony (photo by Catriona Munro). B, C - Illustration of a *Nanomia* colony, gastrozooid, and tentacle closeup (by Freya Goetz). D - *Nanomia* sp. Tentillum illustration and main parts. E - Differential interference contrast micrograph of 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  
109 inaccessibility of their oceanic habitat and the difficulties associated with the collection of  
110 fragile siphonophores. Thus, the morphological diversity of tentilla has only been characterized  
111 for a few taxa, and their evolutionary history remains largely unexplored. Contemporary  
112 underwater sampling technology provides an unprecedented opportunity to explore the  
113 trophic ecology (22) and functional morphology (23) of siphonophores. In addition, well-  
114 supported phylogenies based on molecular data are now available for these organisms (24).  
115 These advances allow for the examination of the evolutionary relationships between modern  
116 siphonophore form, function, and ecology. Our work builds upon previous pioneering studies  
117 that have explored the relationships between tentilla and diet, and showed that siphonophores  
118 are a robust system for the study of predatory specialization via morphological diversification.  
119 Purcell (25, 26) showed clear relationships between diet, tentillum, and nematocyst characters  
120 in co-occurring epipelagic siphonophores for a small subset of extant epipelagic siphonophore

<sup>121</sup> species.

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

## <sup>132</sup> Results

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

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

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

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

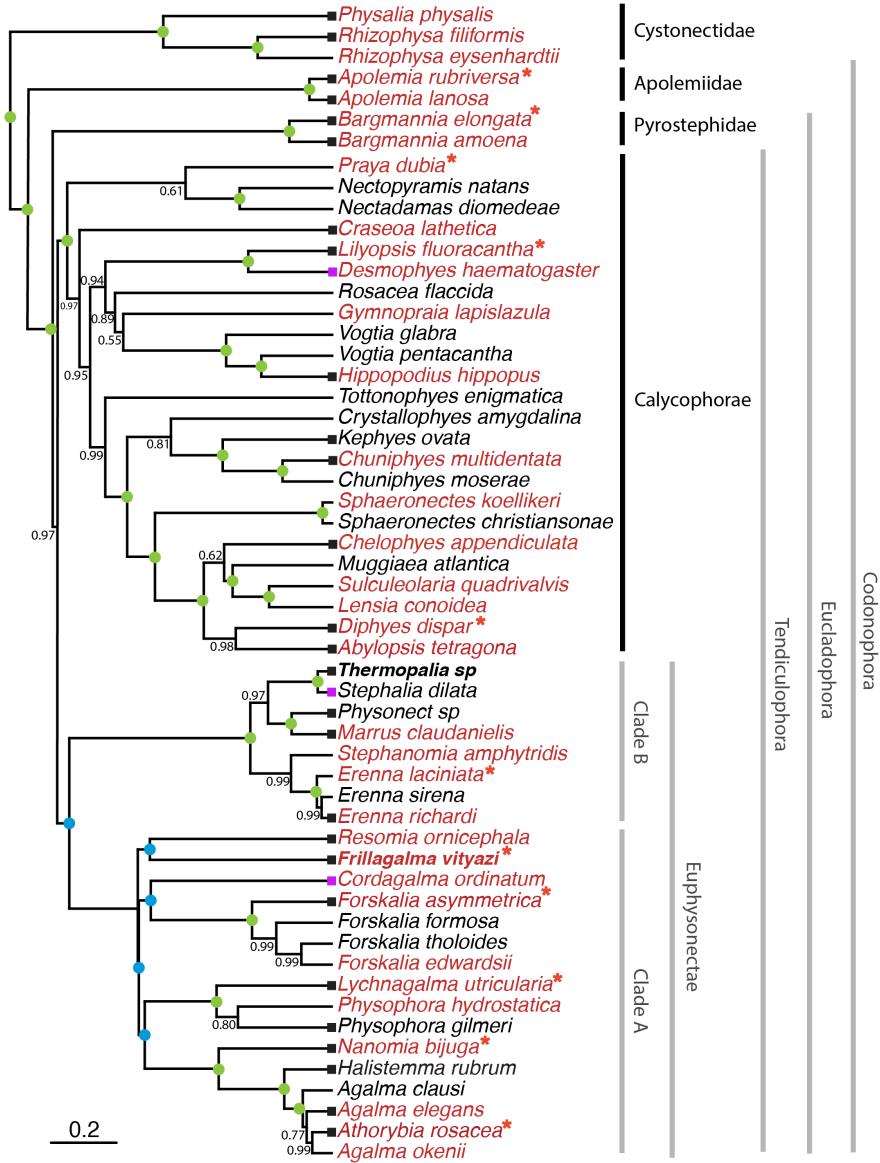


Figure 2: Bayesian time-tree inferred from 18S + 16S concatenated sequences and constrained to be congruent with a published transcriptome phylogeny. Branch lengths estimated using a relaxed molecular clock. Species names in red indicate replicated representation in the morphology data. All data were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

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

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

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

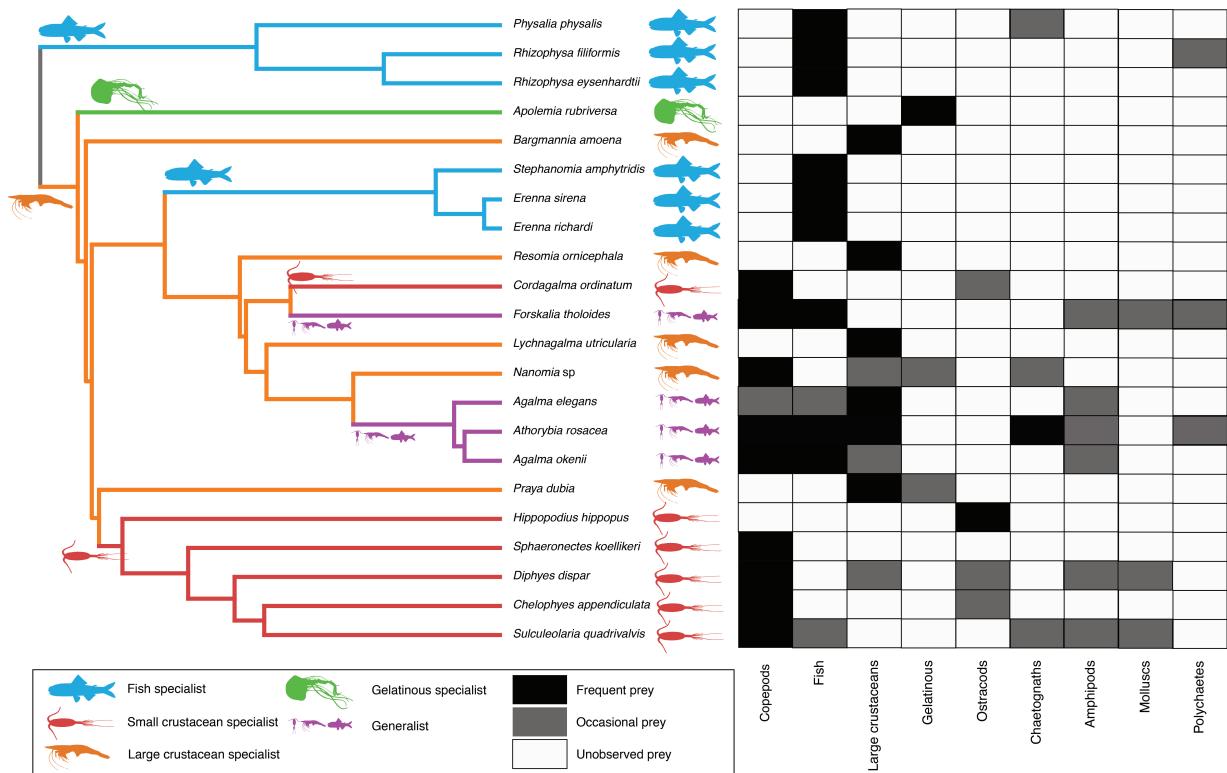


Figure 3: Left - Subset phylogeny showing the mapped feeding guild regimes that were used to inform the *OUwie* analyses. Right - Grid showing the prey items consumed from which the feeding guild categories were derived. Diet data were obtained from the literature review, available in the Dryad repository.

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 numbers, 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.

221 We tested some of the diet-morphology associations previously proposed in the literature  
222 (25, 26) for correlated evolution (Table 1). We found that most, such as heteroneme volume  
223 and copepod prey size, do show evidence for correlated evolution. The sole exception was the  
224 relationship between terminal filament nematocysts (rhopalonemes and desmonemes) and  
225 crustaceans in the diet. Analyses that do not take phylogeny into account do recover this  
226 correlation across the extant species studied, but it is not consistent with correlated evolution.  
227 The latter is likely a product of the larger species richness of crustacean-eating species with

228 terminal filament nematocysts, rather than simultaneous evolutionary gains.

229 Table 1. Table 1. Tests of correlated evolution between siphonophore morphological  
230 characters and aspects of the diet found correlated in the literature. We report the direction  
231 and significance of the evolutionary association, the number of taxa used for the analysis,  
232 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

233

234

235 Table 2. Discriminant analysis of principal components for the presence of specific prey  
236 types using the morphological data. Top quartile variable (character) contributions to the  
237 linear discriminants are ordered from highest to lowest. Logistic regressions and GLMs  
238 were fitted to predict prey type presence and selectivity respectively. The sign of the slope  
239 of each predictor is reported, marked with an asterisk if significant ( $p\text{-value} < 0.05$ ), and  
240 highlighted grey if it differs between prey presence in diet and prey selectivity. Pseudo- $R^2$   
241 (%) 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

242

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

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

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

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

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

285 (found in every other regime) to a positive correlation. This is consistent with Clade B  
286 haplonemes becoming rounder, more similar to Cystonect haplonemes specialized in fish prey  
287 penetration and envenomation. Gelatinous specialization, albeit appearing only once in our  
288 tree, also carries a unique signature in character rate correlation shifts, with an increase in  
289 the strength of the correlation between heteroneme shape and shaft width, consistent with  
290 the appearance of birrhopaloid nematocysts with swollen shafts that are likely effective at  
291 anchoring gelatinous tissue (see reference to Narcomedusae nematocysts in (26)).

## 292 Discussion

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

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

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

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

336 Siphonophores are an abundant group of zooplankton in oceanic ecosystems (41, 42).  
337 While little is known about siphonophore trophic ecology, what is known indicates that

<sup>338</sup> they occupy a central position in midwater food webs (22), serving as important trophic  
<sup>339</sup> intermediaries between smaller zooplankton and higher trophic level predators. Our findings  
<sup>340</sup> on the unique evolutionary history of siphonophore trophic specialization elucidate how they  
<sup>341</sup> arrived to play this fundamental role in the oceanic food web.

## <sup>342</sup> Conclusions

<sup>343</sup> Most studies on the evolution of predation have focused on vertebrate systems with an  
<sup>344</sup> integrated feeding apparatus serving multiple functions. This has led to a biased understanding  
<sup>345</sup> of the evolutionary outcomes of specialization, where extreme morphological evolution  
<sup>346</sup> constrains further shifts in their ecology. Siphonophores differ in many ways from commonly-  
<sup>347</sup> known predators, using modular weapons for prey capture (the tentilla) that are fully  
<sup>348</sup> decoupled from other structures and body functions. Our analysis of the evolutionary history  
<sup>349</sup> of dietary specialization and morphological change in these elusive animals has revealed  
<sup>350</sup> notable deviations from traditional expectations. While much of the feeding ecology literature  
<sup>351</sup> focuses on how predatory generalists evolve into predatory specialists, in siphonophores we  
<sup>352</sup> find predatory specialists can evolve into generalists, and that specialists on one prey type  
<sup>353</sup> have directly evolved into specialists on other prey types. We find that the character states,  
<sup>354</sup> evolutionary optima, and genetic correlations of many morphological characters have evolved  
<sup>355</sup> following these ecological shifts. We find that the relationships between form and ecology  
<sup>356</sup> hold across a large set of siphonophore taxa and characters. These findings are central to  
<sup>357</sup> understanding the evolutionary mechanisms driving the emergence of food web complexity.

## <sup>358</sup> Materials and Methods

<sup>359</sup> *Tentillum morphology* – The morphological work was carried out on siphonophore specimens  
<sup>360</sup> fixed in 4% formalin from the Yale Peabody Museum Invertebrate Zoology (YPM-IZ) collection  
<sup>361</sup> (accession numbers in Dryad repository). These specimens were collected intact across many  
<sup>362</sup> years of fieldwork expeditions, using blue-water diving (43), remotely operated vehicles

363 (ROVs), plankton net trawls, and human-operated submersibles. Tentacles were dissected  
364 from non-larval gastrozooids, sequentially dehydrated into 100% ethanol, cleared in methyl  
365 salicylate, and mounted onto slides with Canada Balsam or Permount mounting media.  
366 The slides were imaged as tiled z-stacks using differential interference contrast (DIC) on an  
367 automated stage at YPM-IZ (with the assistance of Daniel Drew and Eric Lazo-Wasem) and  
368 with laser point confocal microscopy using a 488 nm Argon laser that excited autofluorescence  
369 in the tissues. Thirty characters (defined in S1) were measured using Fiji (44, 45). We did not  
370 measure the lengths of contractile structures (terminal filaments, pedicles, gastrozooids, and  
371 tentacles) since they are too variable to quantify. We measured at least one specimen for 96  
372 different species (raw data available in Dryad). Of these, we selected 38 focal species across  
373 clades based on specimen availability and phylogenetic representation. Three to five tentacle  
374 specimens from each one of these selected species were measured to capture intraspecific  
375 variation.

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

389 Unconstrained ML and Bayesian phylogenies were congruent (S2,S5). Given the broader

390 sequence sampling of the transcriptome phylogeny, we ran constrained inferences (using  
391 both ML and Bayesian approaches, which produced fully congruent topologies (S4,S6)) after  
392 clamping the 5 nodes (S3) that were incongruent with the topology of the consensus tree in  
393 (24). This topology was then used to inform a Bayesian relaxed molecular clock time-tree  
394 in RevBayes, using a birth-death process (sampling probability calculated from the known  
395 number of described siphonophore species) to generate ultrametric branch lengths (S7-8).  
396 Scripts and tree files available in the Dryad repository.

397 *Feeding ecology* – We extracted categorical diet data for different siphonophore species  
398 from published sources, including seminal papers (4, 25, 32, 47–50), and ROV observation  
399 data (22, 51) with the assistance of Elizabeth Hetherington and C. Anela Choy (data available  
400 in Dryad repository). In order to detect coarse-level patterns in feeding habits, the data  
401 were merged into feeding guilds. For more details on how the diet data was curated and  
402 summarized into guilds, please see Supplementary Methods.

403 We also extracted copepod prey length data from (25). To calculate specific prey  
404 selectivities, we extracted quantitative diet and zooplankton composition data from (32),  
405 matched each diet assessment to each prey field quantification by site, calculated Ivlev's  
406 electivity indices (52), and averaged those by species (data available in Dryad repository).

407 *Statistical analyses* – We used a series of phylogenetic comparative methods to test the  
408 evolutionary hypotheses presented in this study. We reconstructed ancestral states using ML  
409 (R phytools::anc.ML (53)), and stochastic character mapping (R phytools::make.simmap) for  
410 categorical characters. For more details on the data wrangling prior to these analyses, please  
411 see the Supplementary Methods. R scripts available in the Dryad repository.

412 In order to study the evolution of predatory specialization, we reconstructed components  
413 of the diet and prey selectivity on the phylogeny using ML (R phytools::anc.ML). To identify  
414 evolutionary associations of diet with tentillum and nematocyst characters, we compared the  
415 performance of a neutral evolution model to that of a diet-driven directional selection model.  
416 First, we collapsed the diet data into the five feeding guilds mentioned above (fish specialist,

417 small crustacean specialist, large crustacean specialist, gelatinous specialist, generalist), based  
418 on which prey types they were observed consuming most frequently. Then, we reconstructed  
419 the feeding guild ancestral states using the ML function `ace` (package `ape` (54)), removing tips  
420 with no feeding data. The ML reconstruction was congruent with the consensus stochastic  
421 character mapping (S15). Then, using the package `OUwie` (55), we fitted an OU model with  
422 multiple optima and rates of evolution (OUM) matched to the reconstructed ancestral diet  
423 regimes, a single optimum OU model, and a BM null model, inspired by the analyses in (56).  
424 We then ranked the models in order of increasing parametric complexity (BM, OU, OUM),  
425 and compared the corrected Akaike Information Criterion (AICc) support scores (57) to the  
426 lowest (best) score, using a cutoff of 2 units to determine significantly better support. When  
427 the best fitting model was not significantly better than a less complex alternative, we selected  
428 the least complex model (S9). In addition, we calculated and reported the model adequacy  
429 scores using the R package *arbutus* (58).

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

441 We carried out a linear discriminant analysis of principal components (DAPC) using  
442 the `dapc` function (R `adegenet::dapc`) (59). This function allowed us to incorporate more  
443 predictors than individuals. We generated discriminant functions for feeding guild, and

<sup>444</sup> for the presence of copepods, fish, and shrimp (large crustaceans) in the diet (S10-13).  
<sup>445</sup> From these DAPCs we obtained the highest contributing morphological characters to the  
<sup>446</sup> discrimination (characters in the top quartile of the weighted sum of the linear discriminant  
<sup>447</sup> loadings controlling for the eigenvalue of each discriminant). In order to identify the sign of  
<sup>448</sup> the relationship between the predictor characters and prey type presence in the diet, we then  
<sup>449</sup> generated generalized logistic regression models (as a type of generalized linear model, or  
<sup>450</sup> GLM using R stats::glm) and phylogenetic generalized linear models (R phylolm::phyloglm)  
<sup>451</sup> with the top contributing characters (from the corresponding DAPC) as predictors (S14). We  
<sup>452</sup> also carried out these GLMs on the Ivlev's selectivity indices for each prey type calculated  
<sup>453</sup> from (32). In addition, we ran a series of comparative analyses to address hypotheses of diet-  
<sup>454</sup> tentillum relationships posed in the literature. Additional details on the DAPC optimization  
<sup>455</sup> are available in the Supplementary Materials.

## <sup>456</sup> **Supplementary Materials**

<sup>457</sup> Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.NNNN>  
<sup>458</sup> Supplementary Materials are available in [https://github.com/dunnlab/tentilla\\_morph/](https://github.com/dunnlab/tentilla_morph/)  
<sup>459</sup> Supplement\_forSupershort.pdf

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