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1 **Title**

2 Spider webs, stable isotopes and molecular gut content analysis: Multiple lines of evidence support  
3 trophic niche differentiation in a community of Hawaiian spiders

4 **Running title**

5 Trophic niche differentiation in a community of Hawaiian spiders

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## 23 Abstract

- 24 1. Adaptive radiations are typically characterized by niche partitioning among their constituent  
25 species. Trophic niche partitioning is particularly important in predatory animals, which rely on  
26 limited food resources for survival.
- 27 2. We test for trophic niche partitioning in an adaptive radiation of Hawaiian *Tetragnatha* spiders,  
28 which have diversified *in situ* on the Hawaiian Islands. We focus on a community of nine species  
29 belonging to two different clades, one web building and the other actively hunting, which co-  
30 occur in wet forest on East Maui. We hypothesize that trophic niches differ significantly both  
31 1) among species within a clade, indicating food resource partitioning, and 2) between the two  
32 clades, corresponding with their differences in foraging strategy.
- 33 3. To assess niches of the spider species, we measure a) web architecture, the structure of the  
34 hunting tool, and b) site choice, the physical placement of the web in the habitat. We then test  
35 whether differences in these parameters translate into meaningful differences in trophic niche by  
36 measuring c) stable isotope signatures of carbon and nitrogen in the spiders' tissues, and d) gut  
37 content of spiders based on metabarcoding data.
- 38 4. We find significant interspecific differences in web architecture and site choice. Importantly, these  
39 differences are reflected in stable isotope signatures among the five web-building species, as well  
40 as significant isotopic differences between web-builders and active hunters. Gut content data  
41 also show interspecific and inter-clade differences. Pairwise overlaps of web architecture between  
42 species are positively correlated with overlaps of isotopic signature.
- 43 5. Our results reveal trophic niche partitioning among species within each clade, as well as between  
44 the web-building and actively hunting clades. Based on the correlation between web architecture  
45 and stable isotopes, it appears that the isotopic signatures of spiders' tissues are influenced by  
46 architectural differences among their webs. Our findings indicate an important link between web  
47 structure, microhabitat preference and diet in the Hawaiian *Tetragnatha*.

## 48 Keywords

49 adaptive radiation, ecological divergence, Hawaii, niche partitioning, trophic niche

## 50 Introduction

51 Ecological niche partitioning, the process by which co-occurring populations divide the available  
52 niche space by exploiting different sets of resources (MacArthur, 1958; Schoener, 1974), is a com-  
53 mon phenomenon in adaptive radiations (Schluter & McPhail, 1992; Losos, 1994; Losos *et al.*, 2003;  
54 von Rintelen *et al.*, 2004; Emrich *et al.*, 2014; Giménez *et al.*, 2018). Among animals, trophic niche  
55 differentiation has been shown to be a primary driver in adaptive radiations such as the anoles (Losos,  
56 2011), cichlid fish (Wagner *et al.*, 2009; Muschick *et al.*, 2012; Ford *et al.*, 2016), and Galápagos finches  
57 (Grant, 1999; Mallarino *et al.*, 2012). However, different attributes are used as proxies for trophic niche  
58 (e. g. beak shape in finches, leg length in lizards, pharyngeal jaw morphology in fish), and it is often  
59 difficult to assess which aspects of the multidimensional niche translate into meaningful trophic sepa-  
60 ration between co-occurring taxa. This information is nonetheless critical in order to understand the  
61 factors that define an adaptive radiation. Here, we test for trophic niche partitioning in an adaptive  
62 radiation of spiders, using multiple ecological and behavioral measures to provide a multifaceted view  
63 of the factors that reinforce ecological differentiation in this group.

64 The Hawaiian Islands present numerous opportunities for examining the ecological underpinnings  
65 of adaptive radiation (Shaw & Gillespie, 2016). This archipelago has extraordinarily high levels of  
66 endemism due to its extreme isolation from other landmasses ( $> 4,000$  km from the nearest mainland,  
67 Bennett & O’Grady, 2013; Goodman *et al.*, 2014), and, because this is coupled with a chronological  
68 arrangement of current high islands stretching from 0 – 6.3 mya (Lim & Marshall, 2017), it has been  
69 deemed a “natural laboratory” (Simon, 1987; Roderick & Gillespie, 1998). The physical isolation and  
70 ecological opportunities afforded by the formation of the Hawaiian Islands have promoted multiple  
71 well-known evolutionary radiations. Among arthropods, *Drosophila* flies (Magnacca & Price, 2015),  
72 *Megalagrion* damselflies (Jordan *et al.*, 2003), *Nesophrosyne* leafhoppers (Bennett & O’Grady, 2013),  
73 and *Mecaphesa* (Garb & Gillespie, 2009), *Ariamnes* (Gillespie *et al.*, 2018) and *Tetragnatha* (Gillespie,  
74 2004, 2016) spiders are well established examples. In each instance, representatives of the lineage have  
75 evolved into multiple new phenotypes not found in mainland relatives. Furthermore, multiple species  
76 within each radiation coexist in the same habitat, raising the question of whether the observed novel  
77 ecomorphologies correspond to the exploitation of new ecological niches and whether this results in  
78 partitioning of the available niche space.

79 Here, we test for trophic niche partitioning in a group of sympatric species belonging to an adaptive  
80 radiation of Hawaiian long-jawed orbweaving spiders (*Tetragnatha*, Tetragnathidae). The genus *Tetra-*  
81 *natha* is globally distributed and largely homogeneous in morphology and ecology (Kaston, 1978). Most

82 *Tetragnatha* species have long, slender bodies and long chelicerae (jaws), and spin orb webs near bod-  
83 ies of water (Gillespie, 1987). In the Hawaiian Archipelago, however, the genus has evolved numerous  
84 novel morphologies and behaviors, including the loss of web-building behavior in one clade (the Spiny  
85 Leg clade, Gillespie 1991, 2002), new morphologies such as stouter bodies, shorter jaws, humps or  
86 tubercles on the abdomen, and a variety of colorations, from brown to maroon to bright green to red.  
87 These innovations are presumed to reflect adaptations to allow specialization on particular elements  
88 of the dietary niche (Blackledge & Gillespie, 2004), while also serving to camouflage a given taxon  
89 on specific substrates (Gillespie, 2004). Earlier observational work suggests dietary differences among  
90 sympatric web-building *Tetragnatha* species (Blackledge *et al.*, 2003) and, on a broader scale, between  
91 the web-building spiders and the actively hunting Spiny Leg spiders (Binford, 2001). Furthermore, it  
92 was recently found that web-building *Tetragnatha* carry a different isotopic signature in their tissues  
93 from co-occurring Spiny Leg *Tetragnatha* on the Big Island of Hawai'i (Kennedy *et al.*, 2018). With  
94 over 30 described species (Gillespie, 1991, 1992, 1994, 2002, 2003), many of which co-occur in over-  
95 lapping ranges, the Hawaiian *Tetragnatha* spiders represent an ideal system in which to examine the  
96 ecological and behavioral dynamics of sympatric species in the context of adaptive radiation.

97 We characterize the niches of co-occurring Hawaiian *Tetragnatha* spiders to evaluate the niche  
98 axes that separate species, and the extent to which these niche differences are reflected in dietary  
99 differences. For web-builders, we measure *web architecture*, the structure of the spiders' foraging tool,  
100 and *site choice*, the physical placement of webs in the habitat. For cursorial spiders, we already have  
101 detailed knowledge of microhabitat association (Gillespie, 2004). In order to determine the extent to  
102 which these ecological and behavioral differences translate into significant differences in the trophic  
103 niche, we use measurements of stable isotope ratios and analysis of gut contents to give both long-term  
104 and short-term indicators of differences in trophic repertoire between species. Stable isotopes are a  
105 well-established tool in food web studies (Peterson *et al.*, 1985; Cabana & Rasmussen, 1994; France,  
106 1995): both nitrogen and carbon isotopes are useful for assessing trophic position, as the ratio of heavier  
107 to lighter isotope ( $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$ ) tends to increase in a stepwise pattern with successive trophic levels  
108 (Post, 2002). For gut content analysis, we identify the prey of individual spiders to the order level,  
109 using a metabarcoding approach (Krehenwinkel *et al.*, 2017). We hypothesize that hunting mode – web-  
110 building versus active foraging – plays a major role in shaping the trophic niche, and consequently,  
111 we expect significant differences in isotopic signature and gut content between the web-builders and  
112 the Spiny Leg spiders. Additionally, we hypothesize that sympatric species of web-building spiders  
113 exhibit finer-scale niche partitioning, manifested by differences in web architecture and site choice,  
114 which translate into dietary differences among species.

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## 115 **Materials and methods**

### 116 **Study system**

117 We focused on a community of nine co-occurring *Tetragnatha* species in a native montane forest of East  
118 Maui, which contains some of the highest species richness among the Hawaiian islands (Lim & Marshall,  
119 2017; Nishida *et al.*, 1992). Five of the species are web-builders; the remaining four are active hunters  
120 in the Spiny Leg clade of *Tetragnatha*, all of whose members hunt without the use of a web (Gillespie,  
121 1991). All nine species differ visibly from one another in their morphology, and are therefore easily  
122 identifiable in the field (Gillespie, 1991, 1992, 1994).

123 Web-building *Tetragnatha* spin two-dimensional orb webs. A spiral of sticky silk, the “capture  
124 spiral,” is supported by several spokes, or “radii,” which converge into a central hub (Fig. 1). The  
125 section of web between two adjacent radii is called a “sector.” *Tetragnatha* spiders are nocturnal, and  
126 generally spin a fresh web every night, then dismantle and eat the web (recycling the silk) every  
127 morning. These webs are relatively delicate and sustain significant damage when they intercept prey,  
128 so spiders also generally dismantle their webs after each prey capture event.

### 129 **Sample collection**

130 Sampling took place in The Nature Conservancy’s Waikamoi Preserve, located on the windward slope  
131 of Haleakalā Volcano, East Maui. We sampled in the summers of 2013, 2014 and 2017, and in November  
132 2016, at two different elevations: low (1380 – 1500 m: along the Maile Trail, an approximately 500-  
133 m distance between coordinates N20.80276°, W156.25460° and N20.79870°, W156.25279°) and high  
134 (1800 – 1950 m: along the Waikamoi Boardwalk, an approximately 350-m distance between coordinates  
135 N20.78055°, W156.22709° and N20.77806°, W156.22868°). This allowed us to capture data on nearly all  
136 *Tetragnatha* species that occur in Maui’s wet forest, as, although several species are found throughout  
137 the region, two of the species (the web-building *T. filiciphilia* and the Spiny Leg *T. brevignatha*) are  
138 found primarily at lower elevations while the remaining species co-occur at higher elevations. Sample  
139 sizes by species, year and elevation are summarized in Table S1 in Supporting Information.

### 140 **Web measurements**

141 For each web found, we collected the spider into a clean snap cap vial. We then dusted the web with  
142 cornstarch to enhance visibility and photographed it from a 90° angle with a ruler held in frame for  
143 scale. To evaluate microhabitat (site choice), we recorded the vegetation genus and functional “type”

144 (fern, grass, shrub, tree or vine) to which the web was attached, and measured the web's height above  
145 the ground and angle from vertical ( $0^0$  = vertical,  $90^0$  = horizontal). We measured webs from digital  
146 photos using ImageJ (National Institutes of Health, USA), and took the measurements summarized in  
147 Table 1 (see also Fig. 1). Capture thread length (CTL), the total length of sticky capture spiral, was  
148 estimated using the large-scale formula of Heiling *et al.* (1998):

$$149 \quad CTL = \sum((T_l + T_s)/2) * x_i$$

150 where, for a given sector  $i$  of the web,  $x_i$  is the number of rows of capture thread,  $T_l$  is the length of  
151 the outermost (longest) row of capture thread, and  $T_s$  is the length of the innermost (shortest) row of  
152 capture thread.

153 Mesh width (MW) was estimated using the following formula (Blackledge *et al.*, 2003):

$$154 \quad MW = 0.5 * (D_u/(R_u - 1) + D_l/(R_l - 1))$$

155 where  $D_u$  is the length of a designated "upper" sector, from innermost to outermost row of capture  
156 thread,  $R_u$  is the number of rows of capture thread in the "upper" sector,  $D_l$  is the length of a designated  
157 "lower" sector  $180^0$  from the "upper" sector, and  $R_l$  is the number of rows of capture thread in the  
158 "lower" sector.

159 Capture area of webs was measured directly from the image, and included only the area of the  
160 web that was covered by sticky silk. Radii were counted, and the mean number of rows of sticky silk  
161 was calculated for each sector. Using the primary measurements of capture thread length, mesh width  
162 and capture area, we calculated two metrics of silk density: silk density 1 = capture thread length /  
163 capture area, silk density 2 = capture area / mesh width.

## 164 **Stable isotope analysis**

165 Spider specimens used for C and N stable isotope analysis were collected into separate snap cap vials  
166 and euthanized in a freezer. Individuals were then photographed up close using a Nikon D5200 with  
167 a macro lens, creating a digital voucher. The spiders were air-dried at room temperature, with clean  
168 beads of silica gel in each vial to accelerate drying and prevent decomposition. No baseline samples  
169 (such as plant leaves or soil) were taken; only spider specimens were analyzed for isotopic signature.

170 Individual spiders were homogenized with a mortar and pestle, and 1.5 mg of tissue per sample  
171 was weighed out into a 9 x 5 mm tin capsule. This amount was chosen to optimize the efficiency of  
172 the analysis based on the C:N ratio in spiders' tissues (S. Mambelli, pers. comm., 2014). When spiders  
173 weighed less than 2.5 mg, we used whole bodies without homogenization to prevent loss of material in

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174 the grinding process. We used whole bodies because of the limited amount of tissue, recognizing that  
175 this may bias isotopic values toward recently consumed meals due to the inclusion of the digestive tract  
176 in the sample. However, we do not expect any differences among species in terms of how recently the  
177 spiders fed before being collected; therefore, the inclusion of the digestive tract should not significantly  
178 bias the comparison of isotopic signatures among species. Samples were analyzed for carbon and  
179 nitrogen content (percent dry weight) and carbon and nitrogen stable isotope ratios by continuous  
180 flow dual isotope analysis on a CHNOS Elemental Analyzer (vario ISOTOPE cube, Elementar, Hanau,  
181 Germany) connected to an IsoPrime100 mass spectrometer (Isoprime Ltd, Cheadle, UK) at the Center  
182 for Stable Isotope Biogeochemistry at the University of California, Berkeley. The standard used for  
183 carbon was Vienna Pee Dee Belemnite, and the standard for nitrogen was air. The reference material  
184 NIST SMR 1547 (peach leaves) was used as a calibration standard. Long-term external precision based  
185 on reference material NIST 244 SMR 1577b (bovine liver) is 0.10 ‰ for carbon and 0.15 ‰ for nitrogen  
186 isotope analyses.

### 187 **Gut content analysis**

188 Spider specimens used for gut content analysis were preserved in 95 % ETOH in the field, and subse-  
189 quently kept frozen at -20<sup>0</sup> C until DNA extraction. Gut contents were identified using Illumina ampli-  
190 con sequencing following [Krehenwinkel \*et al.\* \(2017\)](#). DNA was first extracted from crushed abdomens,  
191 using a Qiagen Genra Puregene kit (Qiagen, Valencia, CA, USA) according to the manufacturer's  
192 protocol. PCR was performed using a Qiagen Multiplex kit, following the manufacturer's protocol, to  
193 amplify a 210-bp fragment of COI ([Zeale \*et al.\*, 2011](#)). The primers had Illumina TruSeq tails attached  
194 to the 5' ends, which served as binding sites for barcoding primers added in a second round of indexing  
195 PCR ([Lange \*et al.\*, 2014](#)). After indexing, samples were pooled together in equal amounts, diluted to  
196 4 nM concentration, and sequenced on an Illumina MiSeq (Illumina, San Diego, CA, USA) with V3  
197 chemistry at UC Berkeley's QB3 (Berkeley, CA) and at the California Academy of Sciences' Center  
198 for Comparative Genomics (San Francisco, CA). Reads were demultiplexed, then merged using PEAR  
199 ([Zhang \*et al.\*, 2013](#)), with a minimum overlap of 50 bp and minimum quality of Q20. Assembled reads  
200 were then quality filtered and converted to FASTA format using FastX Toolkit ([Gordon & Hannon,](#)  
201 [2010](#)) with the threshold of at least 90 % of sequences having at least Q30 quality. Sequences were  
202 clustered into OTUs (operational taxonomic units) at 97 % similarity, and chimeras were removed,  
203 using USearch ([Edgar, 2010](#)). Prey OTUs were identified to the lowest possible taxonomic level using  
204 a BLAST search ([Altschul \*et al.\*, 1990](#)) against the National Center for Biotechnology Information



205 (NCBI) database. OTU tables showing the number of sequence reads of each prey OTU for each speci-  
206 men were built using USearch. All OTUs that matched family Tetragnathidae were discarded as likely  
207 belonging to the *Tetragnatha* spiders rather than the prey. Prey read counts of each specimen were  
208 then rarefied to equal coverage (500 reads per specimen) using the GUniFrac package (Chen, 2012) in  
209 R. To allow for more accurate comparisons of dietary diversity, each *Tetragnatha* species was randomly  
210 subsampled using a random number generator so that species were represented by equal numbers of  
211 individuals within each clade ( $n = 5$  for web-building species,  $n = 6$  for Spiny Leg species; Table S1).

## 212 Data analysis

213 All statistical analysis was done in R v 3.5.0 (R Core Development Team 2018). We performed principal  
214 component analysis (PCA) on web architecture variables (Table 1) using the FactoMineR package  
215 (Lê *et al.*, 2008). Capture area and capture thread length were first log-transformed to obtain a normal  
216 distribution, and variables were scaled to unit variance so that variable loadings would be more directly  
217 comparable. After testing our data for any violations of assumptions of normality, we performed a  
218 one-way ANOVA to test for differences among species along axes PC1 and PC2, followed by a post-  
219 hoc Tukey's Honest Significant Difference (HSD) test controlling for multiple comparisons. To test  
220 for differences in site choice (web attachment microhabitat), we performed a non-parametric Fisher's  
221 exact test on the number of times each species had been observed building webs on different vegetation  
222 groups (fern, grass, shrub, tree or vine). For web height and angle and stable isotope signatures, we  
223 performed a one-way ANOVA followed by a Tukey's HSD test to identify pairwise differences among  
224 species.

## 225 Testing for relationships among niche parameters

226 To assess niche divergences between the different *Tetragnatha* species, we tested relationships between  
227 1) web architecture and isotopic signature, 2) web architecture and gut contents, and 3) isotopic  
228 signature and gut contents. We first quantified the variability in web architecture and isotopic compo-  
229 sition occupied by each species by constructing hypervolumes for both web and isotope variables. This  
230 approach quantifies the multi-dimensional space occupied by each species using multivariate density  
231 kernel estimation (Blonder *et al.*, 2014). This is an improvement over convex hull methods, which are  
232 more sensitive to outlier points. Hypervolumes were calculated using the HYPERVOLUME package  
233 in R (Blonder *et al.*, 2014). Web hypervolumes were constructed using loadings for the first two PCA  
234 axes as hypervolume calculations can be sensitive to collinear variables. Isotope hypervolumes were

235 calculated using  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values.

236 We then calculated pairwise differences in web and isotope hypervolumes by computing the pairwise  
237 Jaccard overlap between species hypervolumes. For two hypervolumes, A and B, hypervolume Jaccard  
238 overlap is calculated as follows:  $(A \cap B) / (A \cup B)$ . Values range from 0 (species are completely  
239 dissimilar) to 1 (species are completely similar). If web architecture were driving differences in isotopic  
240 signatures through a selective effect on diet, one would expect species with more similar webs to be  
241 more similar in isotopic composition (i.e., web hypervolume overlap should be positively correlated  
242 with isotope hypervolume overlap). We assessed the relationship between web hypervolume overlap  
243 and isotope hypervolume overlap using a Pearson's correlation test.

244 For gut content data, we assessed the degree of pairwise dissimilarity in diet by calculating the Bray-  
245 Curtis dissimilarity between pairs of species in VEGAN (Oksanen *et al.*, 2010). We used prey data at  
246 the ordinal level because order is expected to correspond to broad but important functional traits in  
247 arthropods, e.g. feeding style, life history and mobility. Using the hypervolume overlaps calculated as  
248 described above, we then tested for correlations among 1) gut content and web architecture and 2)  
249 gut content and isotopic signature, using a Pearson's correlation test. If web architecture were driving  
250 differences in gut content, or if gut content were driving differences in isotopic signature, then we  
251 would expect a negative correlation between gut content dissimilarity and hypervolume overlaps for  
252 web architecture and isotopic signature. Additionally, to determine whether diets differed significantly  
253 among species, we performed pairwise PERMANOVA between pairs of web-building and Spiny Leg  
254 species in VEGAN, using prey data at both the ordinal and OTU levels.

## 255 Results

### 256 Web architecture

257 The first two axes in our principal component analysis of web architecture account for 87.3 % of the  
258 total variation in the sample population (Fig. 2, Table S2). The first axis, accounting for 52 % of  
259 variation, is positively correlated with number of rows, followed closely by number of radii, capture  
260 thread length and silk density 2 (capture area divided by mesh width). Mesh width is negatively  
261 correlated with the first axis, but has a weaker association than the other variables. Thus, the first axis  
262 generally describes web density: as spiders increase in PC1, their webs have longer capture threads,  
263 more silk per unit area, larger numbers of radii, and smaller mesh widths. The second PCA axis (35.3  
264 % of variation) is positively associated with capture area and mesh width, and negatively associated

265 with silk density 1 (capture thread length divided by capture area). Thus, as spiders increase in PC2,  
 266 their webs are larger and looser, with longer distances between adjacent rows of capture spiral.

267 We found significant interspecific differences in species position on PC1 (ANOVA  $F = 32.74$ ; d.f. =  
 268 4,179;  $p < 0.005$ ) and PC2 (ANOVA  $F = 47.15$ ; d.f. = 4,179;  $p < 0.005$ ) (Fig. 2, Table S3). *T. stelarobusta*  
 269 *busta* was significantly higher in PC1 (web density) than all other species, followed by *T. filiciphilia*.  
 270 *T. acuta* and *T. eurychasma* had the lowest PC1 values. For PC2, *T. filiciphilia* had significantly lower  
 271 values (i.e. smaller and denser webs) than the other species, and *T. trituberculata* had significantly  
 272 higher values (larger and looser webs) than all species except *T. eurychasma* (Table S4).

### 273 Site choice

274 Spider species had non-random differences in the vegetation group on which their webs were built  
 275 (Fisher's exact test,  $\chi^2 = 105.89$ , 2000 replicates,  $p < 0.005$ ; Fig. 3). *T. trituberculata* was mostly found  
 276 on trees (*Myrsine*, *Melicope*), and to a lesser extent on shrubs (*Vaccinium*, *Rubus*, *Leptecophylla*). *T.*  
 277 *stelarobusta* occurred mostly on ferns (*Dryopteris*, *Diplazium*, *Sadleria*) and shrubs. *T. filiciphilia* was  
 278 the only species found on vines, specifically *Alyxia oliviformis*.

279 Height and angle of webs differed significantly among species (height: ANOVA  $F = 12.11$ , d.f. =  
 280 4,179,  $p < 0.005$ ; angle: ANOVA  $F = 18.38$ , d.f. = 4,179,  $p < 0.005$ ; Fig. 3, Table S5, Table S6). *T.*  
 281 *trituberculata* built significantly more vertical webs than others (mean =  $13^\circ$ ), followed by *T. acuta*  
 282 and *T. stelarobusta*. *T. filiciphilia* and *T. eurychasma* built obliquely oriented webs, and *T. filiciphilia*  
 283 had the greatest deviation from vertical (mean =  $51^\circ$ ). *T. trituberculata* built webs significantly higher  
 284 than all other species (mean = 186 cm), while *T. stelarobusta* built webs significantly lower than all  
 285 others (mean = 109 cm).

### 286 Isotopic differences among species

287 There were significant differences in  $\delta^{15}\text{N}$  (ANOVA  $F = 15.01$ ; df = 4,93;  $p < 0.05$ ) and  $\delta^{13}\text{C}$  (ANOVA  
 288  $F = 5.50$ ; df = 4,93;  $p < 0.05$ ) among web-building species (Fig. 4, Table S7, Table S8). Using a  
 289 post-hoc Tukey's HSD test, we found that *T. filiciphilia* and *T. stelarobusta* were both significantly  
 290 higher in  $\delta^{15}\text{N}$  than the other three web-building species ( $p < 0.05$ ). *T. stelarobusta* had significantly  
 291 lower  $\delta^{13}\text{C}$  values than all other web-building species except for *T. eurychasma*, which had significantly  
 292 lower  $\delta^{13}\text{C}$  values than *T. trituberculata* and *T. filiciphilia*.

293 At the clade level, we found a highly significant difference between web-builders and Spiny Leg  
 294 spiders for both  $\delta^{15}\text{N}$  (ANOVA  $F = 50.41$  d.f. = 1,135,  $p < 0.001$ ) and  $\delta^{13}\text{C}$  (ANOVA  $F = 37.24$ ,

295 d.f. = 1,135,  $p < 0.001$ ), with web-builders falling higher than Spiny Leg spiders for both isotopic  
296 signatures (Fig. 5).

### 297 **Gut content**

298 Based on the numbers of sequences obtained from spiders' gut contents, each species showed a distinc-  
299 tive prey composition at the order level (Fig. 6). On the whole, web-builders had more Diptera while  
300 Spiny Leg spiders had more Hemiptera and Hymenoptera in their guts, and both clades had substan-  
301 tial contributions of Lepidoptera. Bray-Curtis dissimilarities showed moderate to high complementarity  
302 between most pairs of spider species within each clade (Tables 2, 3), ranging from approximately 0.2  
303 – 0.5. PERMANOVA showed significant differences in diet between most pairs of web-building species  
304 at both the ordinal and OTU levels of prey, while diets of Spiny Leg species largely did not differ  
305 significantly from one another (Tables S11, S12, S13, S14).

### 306 **Correlations among different measures of niche**

307 Web architecture hypervolume overlaps were significantly correlated with isotopic hypervolume over-  
308 laps (Pearson's product moment correlation coefficient = 0.66,  $p = 0.04$ , Fig. 7). Gut content Bray-  
309 Curtis dissimilarities showed a negative but non-significant relationship with web hypervolume overlaps  
310 (Pearson's coefficient = -0.47,  $p = 0.17$ ) and with isotopic hypervolume overlaps in web-builders (Pear-  
311 son's coefficient = -0.34,  $p = 0.33$ ) and in Spiny Leg spiders (Pearson's coefficient = -0.63,  $p = 0.18$ ).

## 312 **Discussion**

### 313 **Web architecture and site choice**

314 We demonstrate significant interspecific differences in web architecture and site choice, i.e. foraging  
315 behavior (Figs 2, 3). The web-building species show strong differentiation in the structure of their  
316 webs, primarily in terms of size and silk density. Additionally, different species show associations  
317 with particular web attachment substrates and orientations in space. These web architectures and  
318 microhabitats may be tailored for the capture of specific assemblages of prey, which may be more  
319 effectively intercepted by particular web forms, and more abundant in particular microhabitats. That  
320 each species showed a specific web architecture and site choice suggests inherent behavioral differences  
321 among species, possibly acting as a mechanism of trophic niche partitioning.

## 322 Isotopic differences among species

323 The isotopic data showed significant differences both among species and between the two major clades  
324 (Figs 4, 5). These results should be regarded with some caution due to potentially confounding effects  
325 of different collecting years and elevations (see Table S1), and because no baseline data (e.g. isotopic  
326 signatures of plant leaves or soil) were taken. Nevertheless, significant interspecific and inter-clade  
327 differences in isotopic signature persist when datasets are controlled for year and elevation (Fig. S1,  
328 Fig. S2). Interestingly, another recent study found that spiders' diets do not change over elevational  
329 gradients despite differences in the prey community over these gradients (Eitzinger *et al.*, 2019), though  
330 isotopic differences due to climatic variation may still occur between different elevations.

331 A number of variables may have contributed to the spiders' isotopic signatures, particularly for  
332  $\delta^{13}\text{C}$ . In addition to dietary information,  $\delta^{13}\text{C}$  can also reflect subtle microhabitat differences such as  
333 variations in sun exposure (Zimmerman & Ehleringer, 1990; Li *et al.*, 2007) or humidity (Liu *et al.*,  
334 2003; Porter *et al.*, 2009). Most isotopic studies find that  $\delta^{13}\text{C}$  closely reflects the signature of an  
335 animal's food source, and changes little from one trophic level to the next (Hobson *et al.*, 2010). If  
336 this is the case in the Hawaiian *Tetragnatha*, then it is plausible that isotopic signatures of the prey's  
337 microhabitat – e.g. sun-exposed canopy versus darker, more humid understory vegetation – will be  
338 borne up into the spiders' tissues. Further study on the isotopic signatures of putative prey from a  
339 variety of different microhabitats is needed in order to disentangle these potential effects.

340 For nitrogen, higher values of  $\delta^{15}\text{N}$  are typically associated with higher trophic level, with standard  
341 discrimination rates of approximately 3.4 per mill with each successive trophic level (Post, 2002). We  
342 find significantly higher levels of  $\delta^{15}\text{N}$  in *T. fliciphilia* and *T. stelarobusta* than in any other species  
343 (Fig. 4), raising the possibility that these two species feed at a higher trophic level, e.g. by consuming  
344 predatory or parasitoid flies or wasps. However, diet is not necessarily the only factor affecting the stable  
345 isotope ratios in spiders' tissues; alternatively, the higher  $\delta^{15}\text{N}$  in *T. stelarobusta* and *T. fliciphilia*  
346 may be related to the higher density of silk in these two species' webs. *T. fliciphilia* fell highest for  
347 “Silk Density 1,” and *T. stelarobusta* highest for “Silk Density 2” (Fig. 2). It is possible that the act  
348 of spinning and recycling (eating) silk contributes to the loss of the lighter  $^{14}\text{N}$  isotope and ultimately  
349 the higher concentration of  $^{15}\text{N}$  in a spider's tissues. If so, then this phenomenon could also at least  
350 partially account for the higher  $\delta^{15}\text{N}$  that we found in web-builders compared with Spiny Leg spiders.  
351 While little is known about the impact of silk spinning and recycling on isotopes, other studies have  
352 found silk-recycling web spinners to have higher values of  $\delta^{15}\text{N}$  than those that do not recycle their silk  
353 (Sanders *et al.*, 2015). Indeed, a recent study of *Tetragnatha* on the Big Island of Hawai'i found the

354 same pattern of higher  $\delta^{15}\text{N}$  in web-builders than in members of the Spiny Leg clade (Kennedy *et al.*,  
355 2018). A deeper exploration of this phenomenon is called for, and experimental studies of the effects  
356 of silk recycling on spiders' isotopic signatures could be a promising future direction.

### 357 Gut content

358 Our analyses of gut content data showed pronounced differences in taxonomic composition of diets  
359 among the spider species (Fig. 6). Sample sizes were small ( $n = 5$  for web-building species,  $n = 6$   
360 for Spiny Leg species; see Table S1), and the results should therefore not be assumed to reflect the  
361 full spectrum of these species' diets. Moreover, the *T. filiciphilia* samples were collected in a different  
362 year from the other species, which could have a confounding effect given that prey may fluctuate  
363 in their availability between years and seasons. However, while likely incomplete, our results appear  
364 to agree with previous studies on the diets of Hawaiian *Tetragnatha* spiders. Blackledge *et al.* (2003)  
365 found a greater proportion of tipulids (order Diptera) than other taxa in the webs of *T. eurychasma*,  
366 suggesting that the relatively large mesh width in this species may be well suited to intercepting  
367 the slender-bodied tipulids; Binford *et al.* (2016) also found Tipulidae in the jaws of  $> 50\%$  of *T.*  
368 *eurychasma* sampled. Our own findings also show Diptera occupying a very high proportion -  $75\%$  - of  
369 the diet of *T. eurychasma*. *T. stelarobusta* has been recorded catching mostly lepidopterans (Binford,  
370 2001; Blackledge *et al.*, 2003; Binford *et al.*, 2016); Blackledge *et al.* (2003) suggested that the high silk  
371 density of *T. stelarobusta* webs could facilitate the capture of moths, which might otherwise escape  
372 by shedding scales from their wings. Indeed, our gut content data show the highest proportion of  
373 Lepidoptera occurring in the diets of the species that spin the most silk-dense webs: *T. stelarobusta*  
374 ( $89.5\%$ ) and *T. filiciphilia* ( $49\%$ ). The parallels between previous findings and our own results suggest  
375 an important connection between the physical structure and function of spiders' webs, i.e. that specific  
376 web architectures may be attuned for capturing certain prey taxa.

### 377 Ecological and behavioral differences define trophic niche

378 A key finding was that ecological and behavioral differences are reflected in dietary differences, as  
379 inferred from the stable isotope signatures and taxonomic composition of gut contents of the spiders.  
380 This suggests that trophic niche partitioning plays an important role in the community ecological  
381 dynamics of Hawaiian *Tetragnatha* spiders. At the same time, we find marked trophic niche differences  
382 between the two major clades, possibly corresponding to their different hunting strategies (web-building  
383 versus active hunting). We also find a significant correlation between web hypervolume and isotope

384 hypervolume overlaps, indicating that web architecture has an effect on the types of prey consumed  
385 by a spider, with measurable impacts on the spider's isotopic signature. Taken together, our results  
386 show that behavioral and ecological axes of the trophic niche are closely linked to the actual dietary  
387 composition of these spiders, supporting the hypothesis that co-occurring species have adapted feeding  
388 strategies that result in the exploitation of different sets of food resources.

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## 401 **Authors' contributions**

402 SK, JC, JYL, HK and RGG conceived the ideas and designed methodology; SK, JC and RGG collected  
403 data; JYL, SK and HK analyzed data; SK and JYL led the writing of the manuscript.

## 404 **Data accessibility**

405 Data deposited in the Dryad Digital Repository: <http://doi.org/10.5061/dryad.29p63mb>, (Kennedy *et al.*,  
406 2019).

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Table 1: Definition of web architecture metrics

| Web architectural trait                   | Description  |
|---|--|
| Capture thread length, CTL (cm)           | Total length of capture spiral in the web                    |
| Capture area, CA (cm <sup>2</sup> )       | Two-dimensional area covered by capture spiral               |
| Mesh width, MW (cm)                       | Average distance between two adjacent rows of capture spiral |
| Radii                                     | Number of supporting spokes (radii)                          |
| Rows                                      | Mean number of rows of capture spiral                        |
| Silk density 1, SD1 (cm/cm <sup>2</sup> ) | CTL / CA   |
| Silk density 2, SD2 (cm)                  | CA / MW  |

Table 2: Pairwise Bray-Curtis dissimilarities in gut content in web-building *Tetragnatha*

|                       | <i>acuta</i> | <i>eurychasma</i> | <i>filiciphilia</i> | <i>stelarobusta</i> | <i>trituberculata</i> |
|-----------------------|--------------|-------------------|---------------------|---------------------|-----------------------|
| <i>acuta</i>          | 0.234        | NA                | NA                  | NA                  | NA                    |
| <i>eurychasma</i>     | 0.243        | 0.175             | NA                  | NA                  | NA                    |
| <i>filiciphilia</i>   | 0.490        | 0.460             | 0.338               | NA                  | NA                    |
| <i>stelarobusta</i>   | 0.302        | 0.319             | 0.367               | 0.196               | NA                    |
| <i>trituberculata</i> | 0.207        | 0.244             | 0.357               | 0.164               | 0.069                 |

Table 3: Pairwise Bray-Curtis dissimilarities in gut content in Spiny Leg *Tetragnatha*

|                    | <i>brevignatha</i> | <i>kamakou</i> | <i>quasimodo</i> | <i>waikamoi</i> |
|--------------------|--------------------|----------------|------------------|-----------------|
| <i>brevignatha</i> | 0.164              | NA             | NA               | NA              |
| <i>kamakou</i>     | 0.260              | 0.242          | NA               | NA              |
| <i>quasimodo</i>   | 0.338              | 0.342          | 0.246            | NA              |
| <i>waikamoi</i>    | 0.251              | 0.264          | 0.301            | 0.244           |

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Figure 1: Web schematic (a) showing architectural components of a *Tetragnatha* web, and exemplar webs of the five study species. b = *T. acuta*, c = *T. eurychasma*, d = *T. filiciphilia*, e = *T. stelarobusta*, f = *T. trituberculata*.

Figure 2: Multivariate analysis of web architectural values across species of *Tetragnatha*. Principal component axes 1 and 2 cumulatively explain 87 % of variance. Arrows represent standardized loadings for each web architectural variable included in the analysis. Letters above boxplots indicate significant differences among species.



Figure 3: Site choice parameters of web-building *Tetragnatha* species. a) Stacked bar plots of vegetation-level differences in web attachment site among species, b) height (cm above ground), and c) angle of placement ( $0^{\circ}$  = vertical,  $90^{\circ}$  = horizontal) of webs. Letters above boxplots indicate significant differences among species.

Figure 4:  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  across species of a) web-building and b) Spiny Leg *Tetragnatha*. Letters above boxplots indicate significant differences among species.

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Figure 5:  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in web-building versus Spiny Leg *Tetragnatha*.

Figure 6: Prey orders detected by molecular gut content analysis in five species of web-building and four species of Spiny Leg *Tetragnatha*: percent composition by spider species.

Figure 7: Correlation tests for niche hypervolume Jaccard overlaps and gut content Bray-Curtis dissimilarity. Top panel: a) web hypervolume overlaps with isotopic hypervolume overlaps, b) web hypervolume overlaps with gut content Bray-Curtis dissimilarity. Bottom panel: isotope hypervolume overlaps with gut content Bray-Curtis dissimilarity in c) web-builders and d) Spiny Leg spiders.

## 565 Supplementary Tables

Table S1: Sample sizes of each *Tetragnatha* species included in each data set, broken down by season/year of sampling and elevation. "Web" = web architecture and site choice dataset, "Iso" = stable isotope dataset, "Gut" = gut content dataset. "IsoSupp" samples are unidentified Spiny Leg species collected in summer 2014 from the Waikamoi boardwalk, used to compare isotopic signatures of web-builders versus Spiny Leg spiders while controlling for season/year and elevation. Numbers in parentheses indicate overlapping samples used in both web architecture/site choice and stable isotope datasets from 2014. In Elevation column, "high" = 1800-1950 m (Waikamoi boardwalk between coordinates N20.78055°, W156.22709° and N20.77806°, W156.22868°), "low" = 1380-1500 m (Maile trail between coordinates N20.80276°, W156.25460° and N20.79870°, W156.25279°).

| Dataset | Species                  | Clade        | Elevation (m) | Season/Year | # of samples |
|---------|--------------------------|--------------|---------------|-------------|--------------|
| Web     | <i>T. acuta</i>          | web-building | high          | Summer 2013 | 11           |
| Web     | <i>T. acuta</i>          | web-building | high          | Summer 2014 | 22(22)       |
| Web     | <i>T. eurychasma</i>     | web-building | high          | Summer 2013 | 16           |
| Web     | <i>T. eurychasma</i>     | web-building | high          | Summer 2014 | 23(19)       |
| Web     | <i>T. filiciphilia</i>   | web-building | low           | Summer 2013 | 56           |
| Web     | <i>T. stelarobusta</i>   | web-building | high          | Summer 2013 | 17           |
| Web     | <i>T. stelarobusta</i>   | web-building | low           | Summer 2013 | 7            |
| Web     | <i>T. stelarobusta</i>   | web-building | high          | Summer 2014 | 19(16)       |
| Web     | <i>T. trituberculata</i> | web-building | high          | Summer 2013 | 4            |
| Web     | <i>T. trituberculata</i> | web-building | high          | Summer 2014 | 9(9)         |
| Iso     | <i>T. acuta</i>          | web-building | high          | Summer 2014 | 26(22)       |
| Iso     | <i>T. eurychasma</i>     | web-building | high          | Summer      | 26(19)       |
| Iso     | <i>T. filiciphilia</i>   | web-building | low           | Fall 2016   | 10           |
| Iso     | <i>T. stelarobusta</i>   | web-building | high          | Summer 2014 | 20(16)       |
| Iso     | <i>T. stelarobusta</i>   | web-building | low           | Fall 2016   | 5            |
| Iso     | <i>T. trituberculata</i> | web-building | high          | Summer 2014 | 11(9)        |
| Iso     | <i>T. brevignatha</i>    | Spiny Leg    | low           | Fall 2016   | 10           |
| Iso     | <i>T. kamakou</i>        | Spiny Leg    | high          | Fall 2016   | 9            |
| Iso     | <i>T. quasimodo</i>      | Spiny Leg    | high          | Fall 2016   | 10           |
| Iso     | <i>T. waikamoi</i>       | Spiny Leg    | high          | Fall 2016   | 10           |
| Gut     | <i>T. acuta</i>          | web-building | high          | Fall 2016   | 5            |
| Gut     | <i>T. eurychasma</i>     | web-building | high          | Fall 2016   | 5            |
| Gut     | <i>T. filiciphilia</i>   | web-building | low           | Summer 2017 | 5            |
| Gut     | <i>T. stelarobusta</i>   | web-building | high          | Fall 2016   | 5            |
| Gut     | <i>T. trituberculata</i> | web-building | high          | Fall 2016   | 5            |
| Gut     | <i>T. brevignatha</i>    | Spiny Leg    | low           | Fall 2016   | 6            |
| Gut     | <i>T. kamakou</i>        | Spiny Leg    | high          | Fall 2016   | 6            |
| Gut     | <i>T. quasimodo</i>      | Spiny Leg    | high          | Fall 2016   | 6            |
| Gut     | <i>T. waikamoi</i>       | Spiny Leg    | high          | Fall 2016   | 6            |
| IsoSupp | unidentified             | Spiny Leg    | high          | Summer 2014 | 13           |

Table S2: Principal component analysis of web architectural characters

|   | PC1   | PC2    | PC3    | PC4    | PC5    | PC6    | PC7    |
|---|-------|--------|--------|--------|--------|--------|--------|
| <i>Eigenvalues</i>                      | 3.643 | 2.468  | 0.464  | 0.234  | 0.13   | 0.053  | 0.009  |
| <i>Proportion of variance explained</i> | 0.52  | 0.353  | 0.066  | 0.033  | 0.019  | 0.008  | 0.001  |
| <i>Variable loadings</i>                |       |        |        |        |        |        |        |
| log.CA                                  | 0.239 | 0.559  | -0.018 | -0.028 | 0.337  | -0.032 | -0.717 |
| log.CTL                                 | 0.475 | 0.225  | 0.115  | 0.101  | 0.548  | -0.215 | 0.595  |
| MW                                      | -0.26 | 0.477  | -0.407 | 0.683  | -0.206 | -0.02  | 0.172  |
| SD1                                     | 0.195 | -0.56  | -0.007 | 0.563  | 0.12   | -0.466 | -0.316 |
| SD2                                     | 0.452 | 0.245  | 0.266  | -0.072 | -0.711 | -0.392 | 0.021  |
| radii                                   | 0.402 | -0.147 | -0.855 | -0.281 | -0.079 | -0.013 | 0.016  |
| rows                                    | 0.496 | -0.119 | 0.139  | 0.348  | -0.131 | 0.762  | -0.04  |

Table S3: Pairwise differences in web architecture PCA Axis 1 among species of web-building *Tetrag-natha*. \* = Tukey multiple-comparisons adjusted p-value < 0.05.

|                       | <i>acuta</i> | <i>eurychasma</i> | <i>filiciphilia</i> | <i>stelarobusta</i> |
|-----------------------|--------------|-------------------|---------------------|---------------------|
| <i>eurychasma</i>     | 0.89         | NA                | NA                  | NA                  |
| <i>filiciphilia</i>   | 2.286*       | 1.396*            | NA                  | NA                  |
| <i>stelarobusta</i>   | 3.509*       | 2.619*            | 1.223*              | NA                  |
| <i>trituberculata</i> | 1.27         | 0.38              | -1.016              | -2.239*             |

Table S4: Pairwise differences in web architecture PCA Axis 2 among species of web-building *Tetrag-natha*. \* = Tukey multiple-comparisons adjusted p-value < 0.05.

|                       | <i>acuta</i> | <i>eurychasma</i> | <i>filiciphilia</i> | <i>stelarobusta</i> |
|-----------------------|--------------|-------------------|---------------------|---------------------|
| <i>eurychasma</i>     | 1.053*       | NA                | NA                  | NA                  |
| <i>filiciphilia</i>   | -1.532*      | -2.586*           | NA                  | NA                  |
| <i>stelarobusta</i>   | 0.634        | -0.419            | 2.167*              | NA                  |
| <i>trituberculata</i> | 1.749*       | 0.695             | 3.281*              | 1.114*              |

Table S5: Pairwise differences in web height (site choice) in cm among species of web-building *Tetrag-natha*. \* = Tukey multiple-comparisons adjusted p-value < 0.05.

|                       | <i>acuta</i> | <i>eurychasma</i> | <i>filiciphilia</i> | <i>stelarobusta</i> |
|-----------------------|--------------|-------------------|---------------------|---------------------|
| <i>eurychasma</i>     | 10.213       | NA                | NA                  | NA                  |
| <i>filiciphilia</i>   | -4.325       | -14.538           | NA                  | NA                  |
| <i>stelarobusta</i>   | -30.532*     | -40.746*          | -26.207*            | NA                  |
| <i>trituberculata</i> | 46.017*      | 35.804*           | 50.343*             | 76.55*              |

Table S6: Pairwise differences in web angle (site choice) among species of web-building *Tetrag-natha*. 0° = perfectly vertical, 90° = perfectly horizontal, \* = Tukey multiple-comparisons adjusted p-value < 0.05.

|                       | <i>acuta</i> | <i>eurychasma</i> | <i>filiciphilia</i> | <i>stelarobusta</i> |
|-----------------------|--------------|-------------------|---------------------|---------------------|
| <i>eurychasma</i>     | 16.503*      | NA                | NA                  | NA                  |
| <i>filiciphilia</i>   | 24.654*      | 8.15              | NA                  | NA                  |
| <i>stelarobusta</i>   | 4.637        | -11.866*          | -20.017*            | NA                  |
| <i>trituberculata</i> | -12.984      | -29.487*          | -37.637*            | -17.621*            |

Table S7: Pairwise differences in mean  $\delta^{15}N$  among species of web-building *Tetragnatha*. \* = Tukey multiple-comparisons adjusted p-value < 0.05.

|                       | <i>acuta</i> | <i>eurychasma</i> | <i>filiciphilia</i> | <i>stelarobusta</i> |
|-----------------------|--------------|-------------------|---------------------|---------------------|
| <i>eurychasma</i>     | 0.350        | NA                | NA                  | NA                  |
| <i>filiciphilia</i>   | 2.678*       | 2.328*            | NA                  | NA                  |
| <i>stelarobusta</i>   | 1.743*       | 1.393*            | -0.935              | NA                  |
| <i>trituberculata</i> | 0.079        | -0.271            | -2.599*             | -1.664*             |

Table S8: Pairwise differences in mean  $\delta^{13}C$  among species of web-building *Tetragnatha*. \* = Tukey multiple-comparisons adjusted p-value < 0.05.

|                       | <i>acuta</i> | <i>eurychasma</i> | <i>filiciphilia</i> | <i>stelarobusta</i> |
|-----------------------|--------------|-------------------|---------------------|---------------------|
| <i>eurychasma</i>     | -0.396       | NA                | NA                  | NA                  |
| <i>filiciphilia</i>   | 0.286        | 0.682*            | NA                  | NA                  |
| <i>stelarobusta</i>   | -0.512*      | -0.116            | -0.798*             | NA                  |
| <i>trituberculata</i> | 0.294        | 0.691*            | 0.009               | 0.806*              |

Table S9: Pairwise differences in mean  $\delta^{15}N$  among species of Spiny Leg *Tetragnatha*. \* = Tukey multiple-comparisons adjusted p-value < 0.05.

|                  | <i>brevignatha</i> | <i>kamakou</i> | <i>quasimodo</i> |
|------------------|--------------------|----------------|------------------|
| <i>kamakou</i>   | 0.724              | NA             | NA               |
| <i>quasimodo</i> | -0.973*            | -1.697*        | NA               |
| <i>waikamoi</i>  | 0.244              | -0.480         | 1.217*           |

Table S10: Pairwise differences in mean  $\delta^{13}C$  among species of Spiny Leg *Tetragnatha*. \* = Tukey multiple-comparisons adjusted p-value < 0.05.

|                  | <i>brevignatha</i> | <i>kamakou</i> | <i>quasimodo</i> |
|------------------|--------------------|----------------|------------------|
| <i>kamakou</i>   | 0.349              | NA             | NA               |
| <i>quasimodo</i> | 2.198*             | 1.849*         | NA               |
| <i>waikamoi</i>  | 0.867              | 0.518          | -1.331           |

Table S11: Pairwise results of PERMANOVA testing for differences in taxonomic composition of gut contents among species of web-building *Tetragnatha*. Table shows p-values for data analyzed at the taxonomic level of order. \* = p-value < 0.05.

|                       | <i>acuta</i> | <i>eurychasma</i> | <i>filiciphilia</i> | <i>stelarobusta</i> |
|-----------------------|--------------|-------------------|---------------------|---------------------|
| <i>eurychasma</i>     | 0.442        | NA                | NA                  | NA                  |
| <i>filiciphilia</i>   | 0.169        | 0.042*            | NA                  | NA                  |
| <i>stelarobusta</i>   | 0.011*       | 0.009*            | 0.113               | NA                  |
| <i>trituberculata</i> | 0.423        | 0.033*            | 0.286               | 0.012*              |

Table S12: Pairwise results of PERMANOVA testing for differences in taxonomic composition of gut contents among species of Spiny Leg *Tetragnatha*. Table shows p-values for data analyzed at the taxonomic level of order. \* = p-value < 0.05.

|                  | <i>brevignatha</i> | <i>kamakou</i> | <i>quasimodo</i> |
|------------------|--------------------|----------------|------------------|
| <i>kamakou</i>   | 0.213              | NA             | NA               |
| <i>quasimodo</i> | 0.338              | 0.686          | NA               |
| <i>waikamoi</i>  | 0.181              | 0.536          | 0.188            |

Table S13: Pairwise results of PERMANOVA testing for differences in taxonomic composition of gut contents among species of web-building *Tetragnatha*. Table shows p-values for data analyzed at the OTU level, with prey DNA sequences clustered at 97 % similarity. \* = p-value < 0.05.

|                       | <i>acuta</i> | <i>eurychasma</i> | <i>filiciphilia</i> | <i>stelarobusta</i> |
|-----------------------|--------------|-------------------|---------------------|---------------------|
| <i>eurychasma</i>     | 0.878        | NA                | NA                  | NA                  |
| <i>filiciphilia</i>   | 0.01*        | 0.014*            | NA                  | NA                  |
| <i>stelarobusta</i>   | 0.008*       | 0.013*            | 0.013*              | NA                  |
| <i>trituberculata</i> | 0.55         | 0.446             | 0.009*              | 0.011*              |

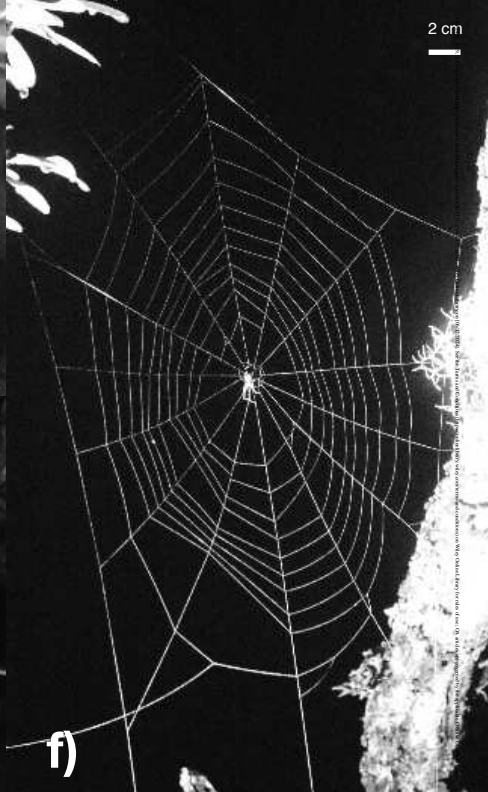
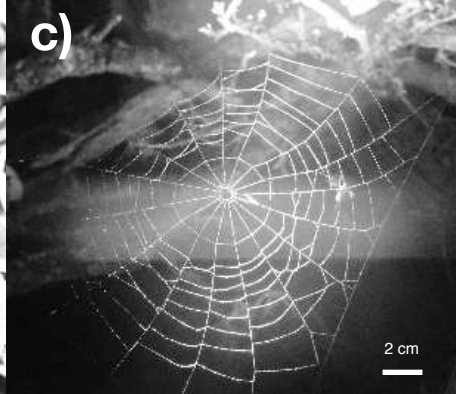
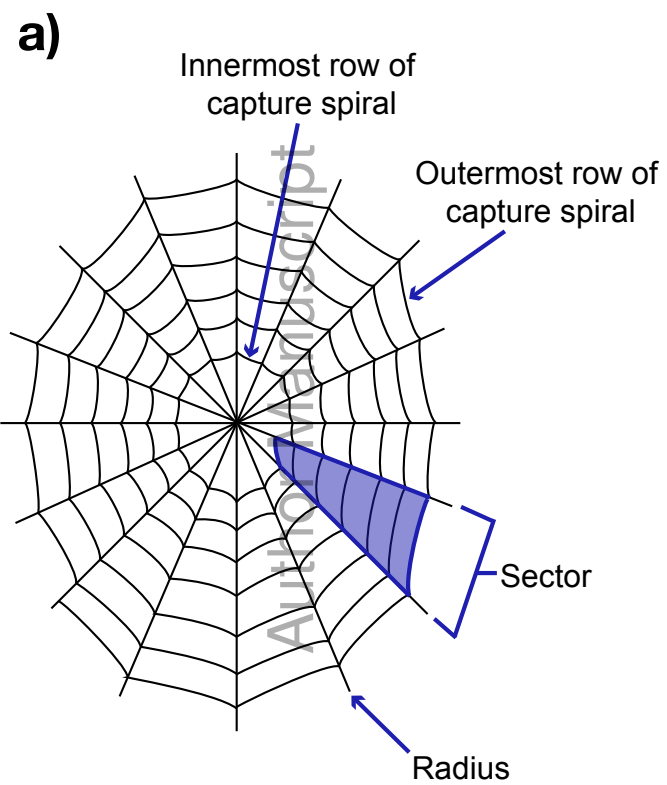
Table S14: Pairwise results of PERMANOVA testing for differences in taxonomic composition of gut contents among species of Spiny Leg *Tetragnatha*. Table shows p-values for data analyzed at the OTU level, with prey DNA sequences clustered at 97 % similarity. \* = p-value < 0.05.

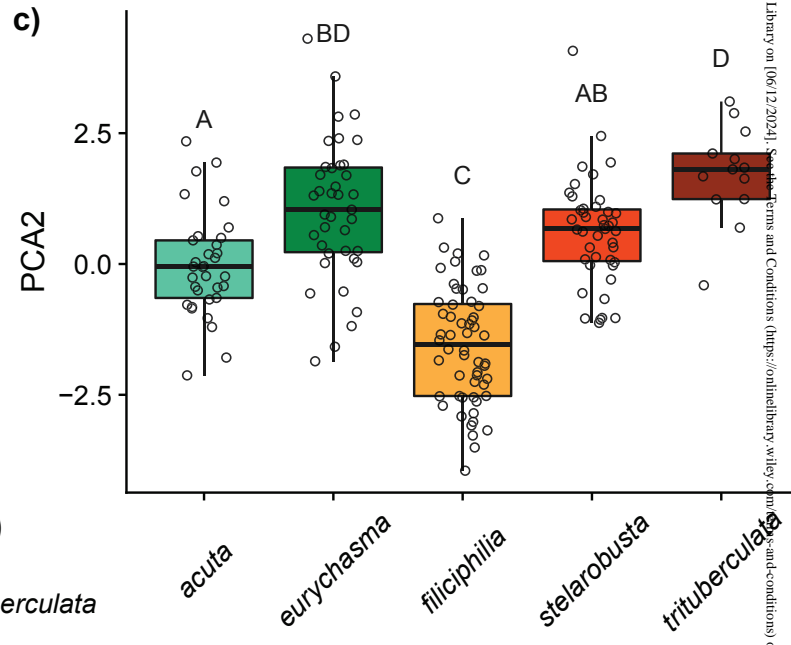
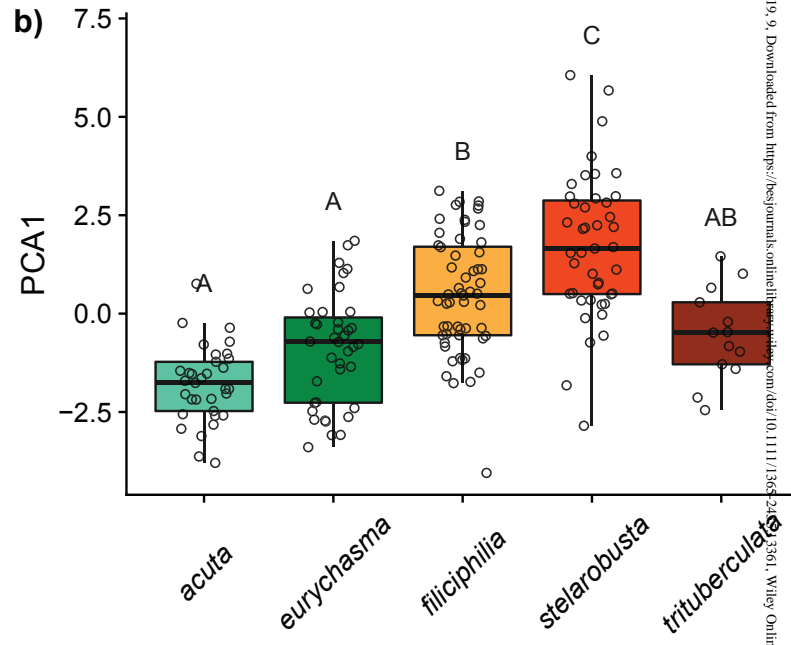
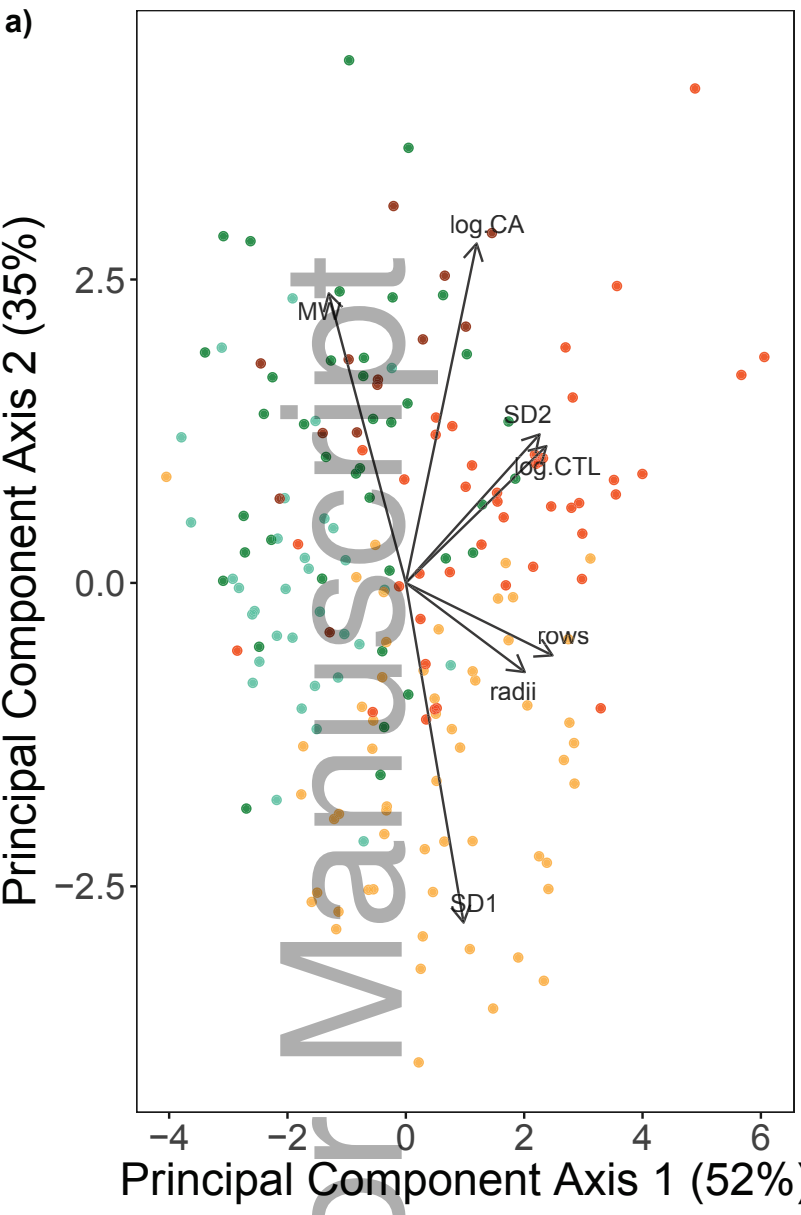
|                  | <i>brevignatha</i> | <i>kamakou</i> | <i>quasimodo</i> |
|------------------|--------------------|----------------|------------------|
| <i>kamakou</i>   | 0.092              | NA             | NA               |
| <i>quasimodo</i> | 0.107              | 0.828          | NA               |
| <i>waikamoi</i>  | 0.003*             | 0.128          | 0.059            |



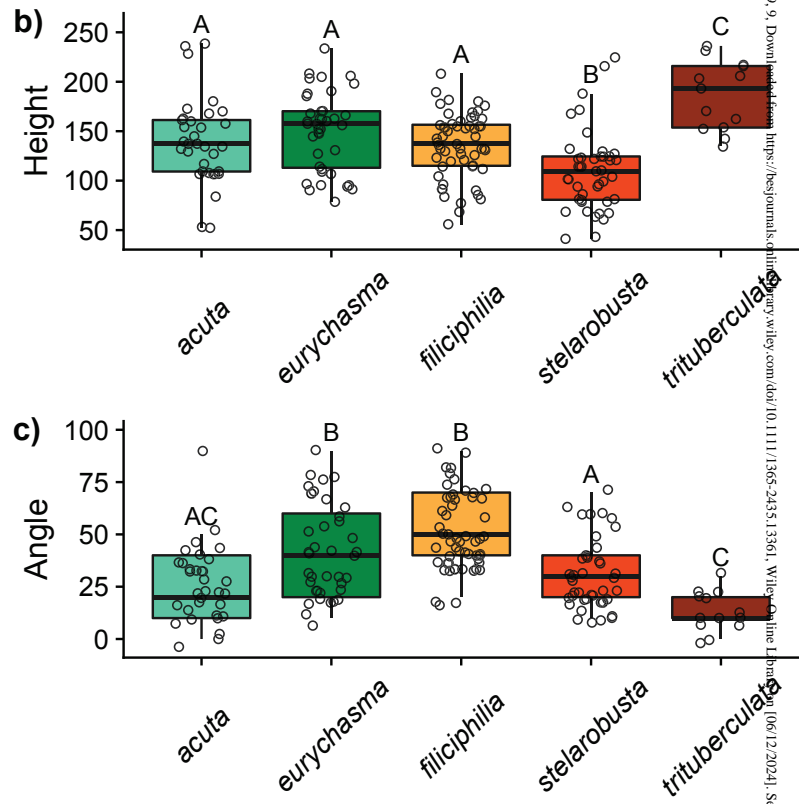
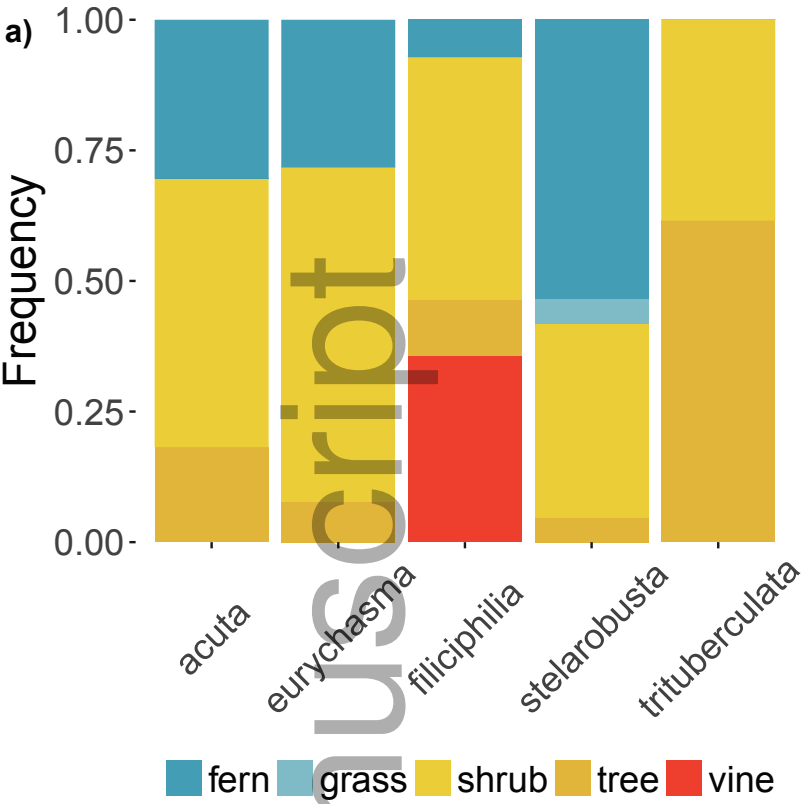
Figure S1: Isotopic signatures of *Tetragnatha* species controlled for sampling year, season and elevation. a), c): Web-builders from the Waikamoi boardwalk (1800-1950 m, between coordinates N20.78055°, W156.22709° and N20.77806°, W156.22868°), summer 2014; b), d) Spiny Leg species from the Waikamoi boardwalk, fall 2016. Letters above boxplots indicate significant difference among species.

Figure S2: Isotopic signatures of web-building versus Spiny Leg *Tetragnatha*, controlled for season/year (summer 2014) and sampling elevation (1800-1950 m, Waikamoi boardwalk, between coordinates N20.78055°, W156.22709° and N20.77806°, W156.22868°). Letters above boxplots indicate significant differences. The significant difference in  $\delta^{15}\text{N}$  between the clades persists when the dataset is controlled for elevation and season/year ( $p < 0.001$ ), but  $\delta^{13}\text{C}$  is no longer significantly different between the clades ( $p = 0.65$ ).

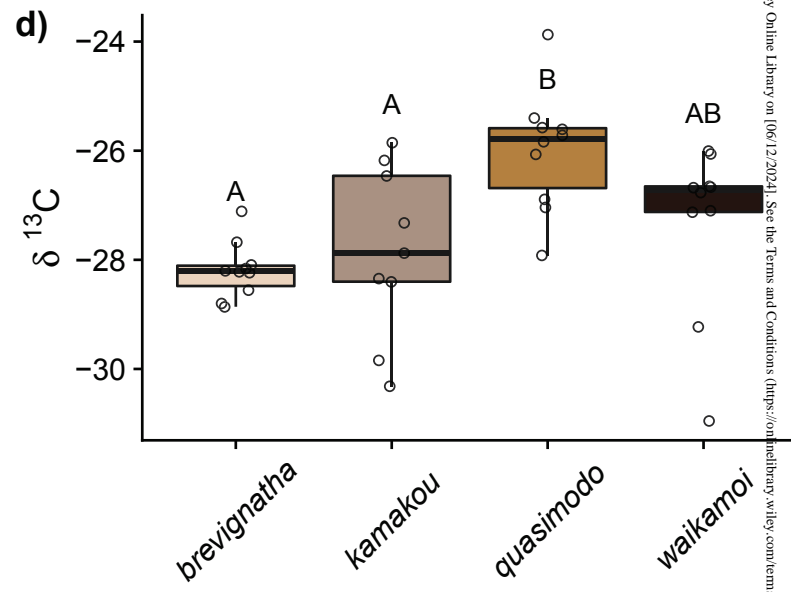
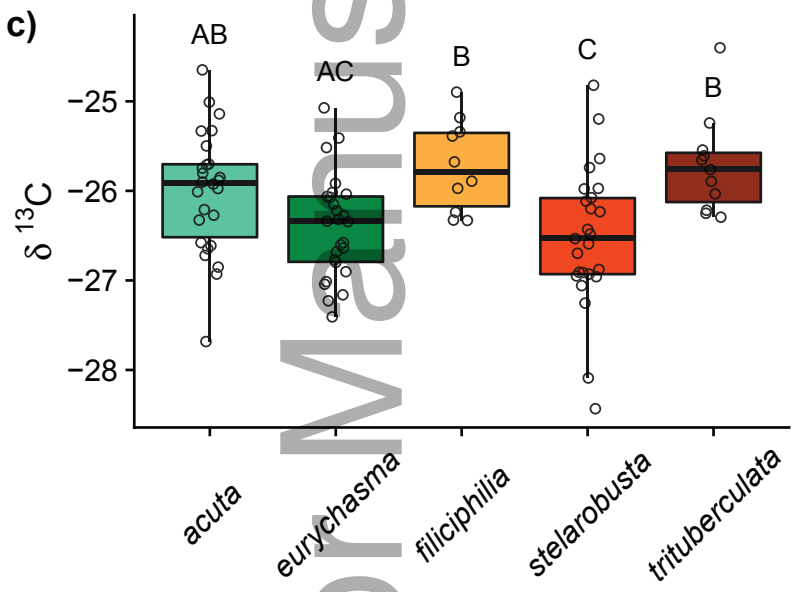
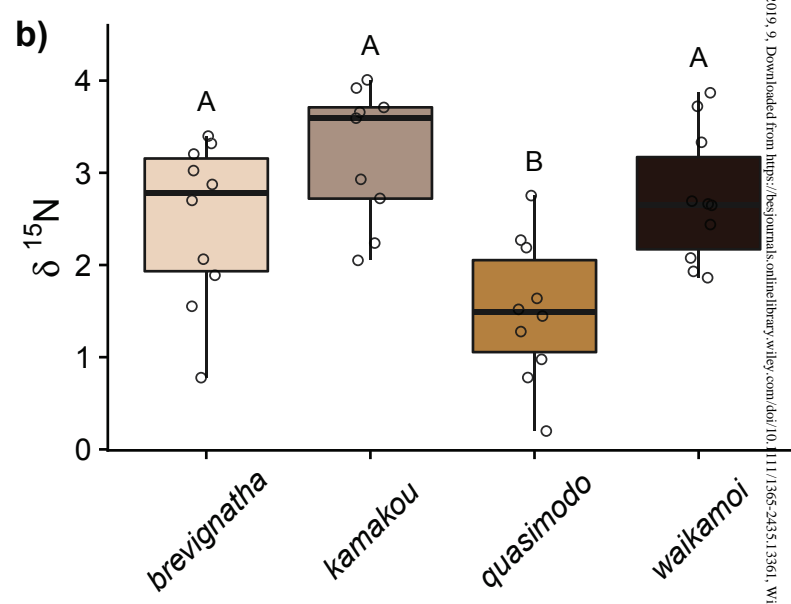
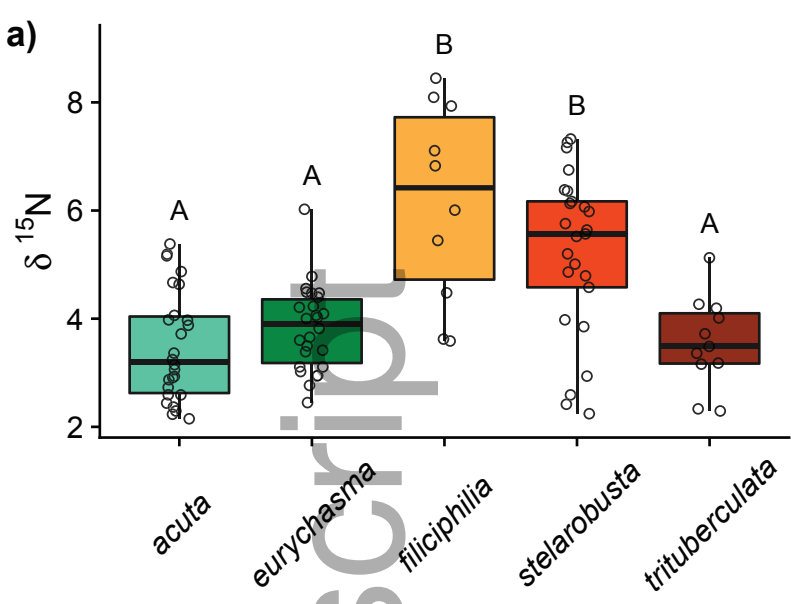




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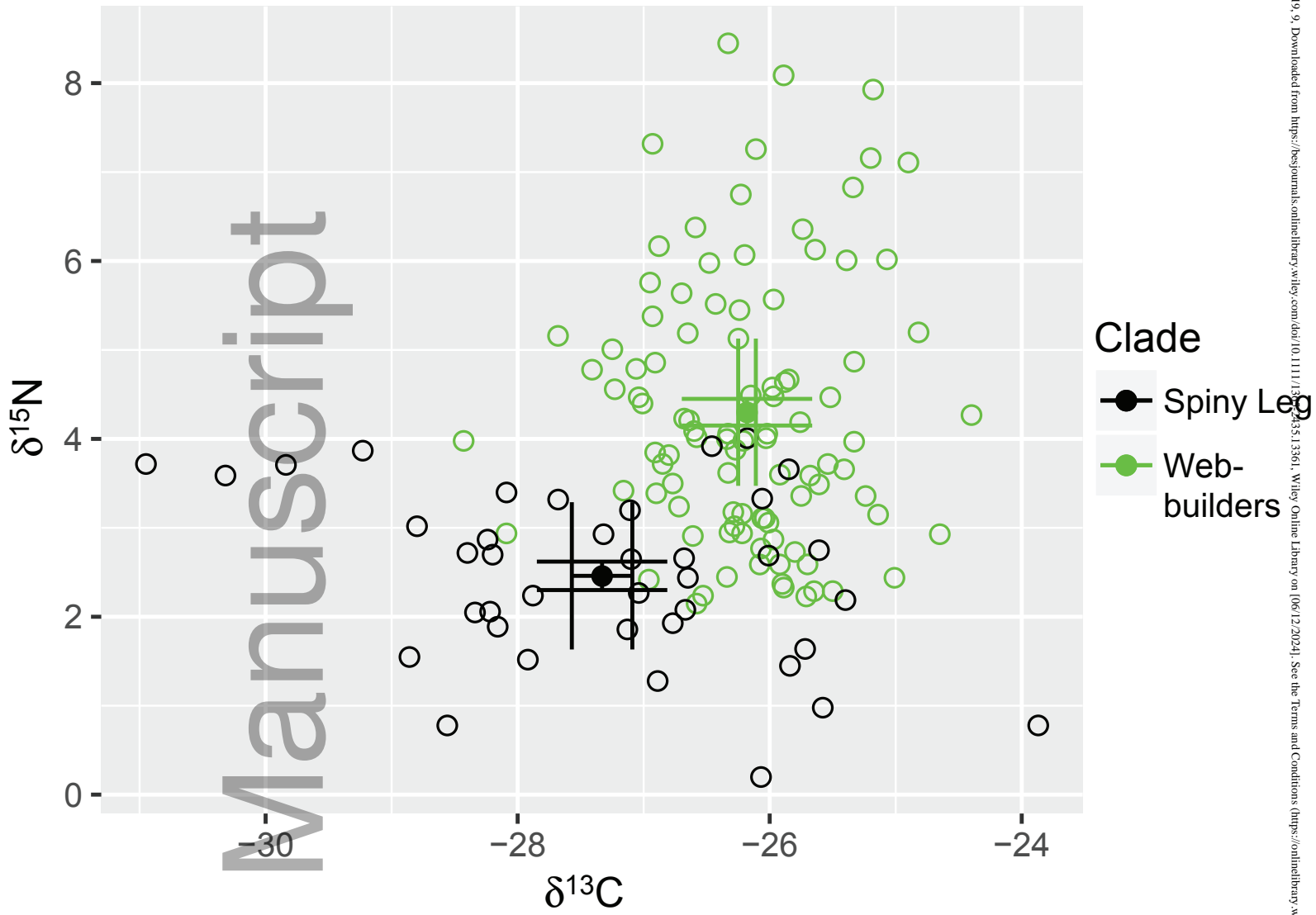
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**Web-builders**

**Spiny Leg**

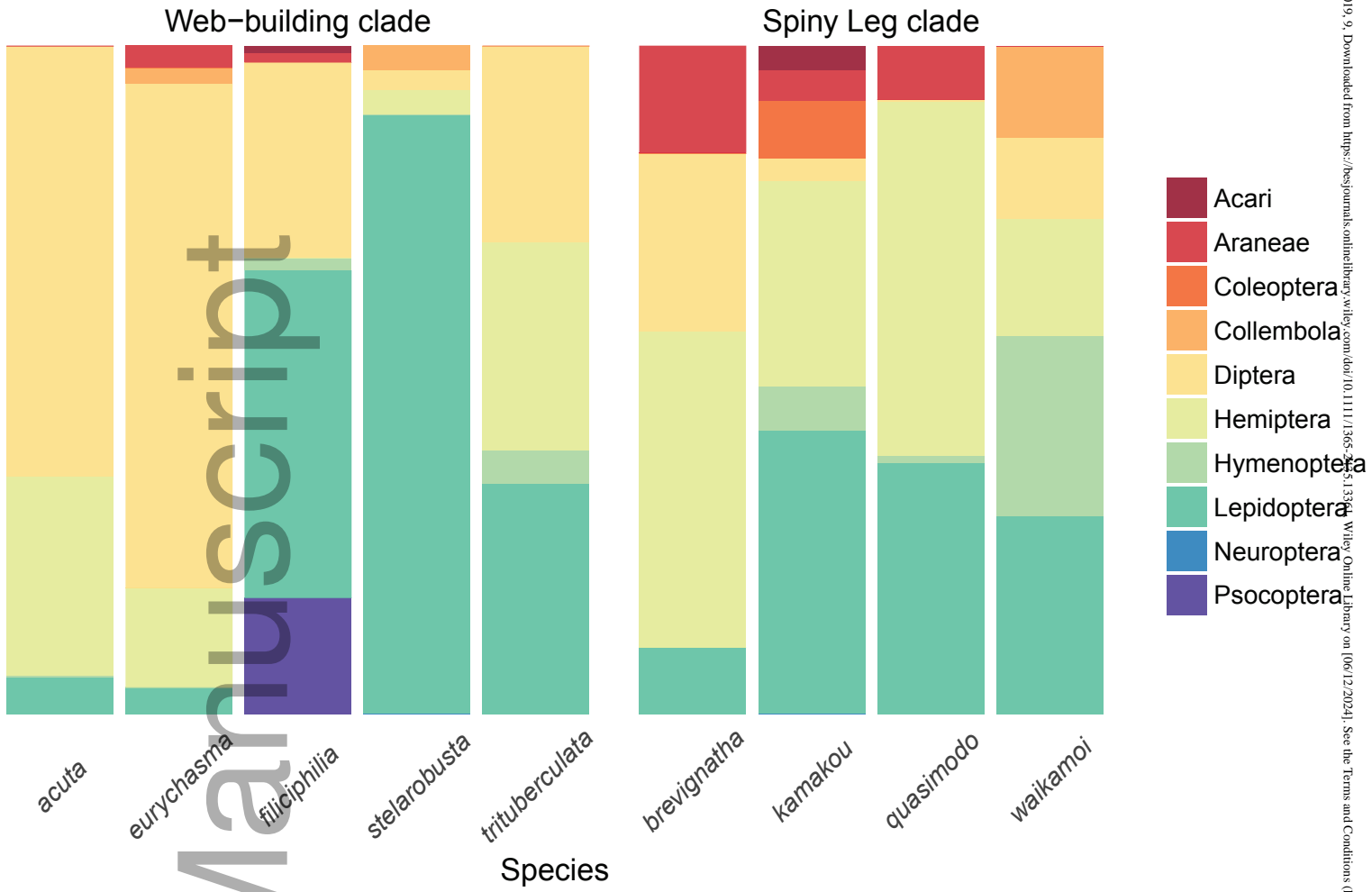
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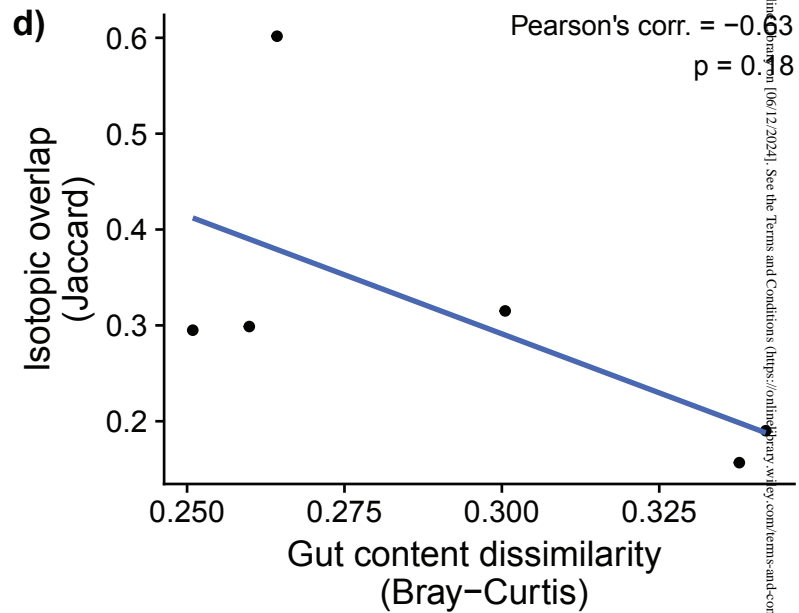
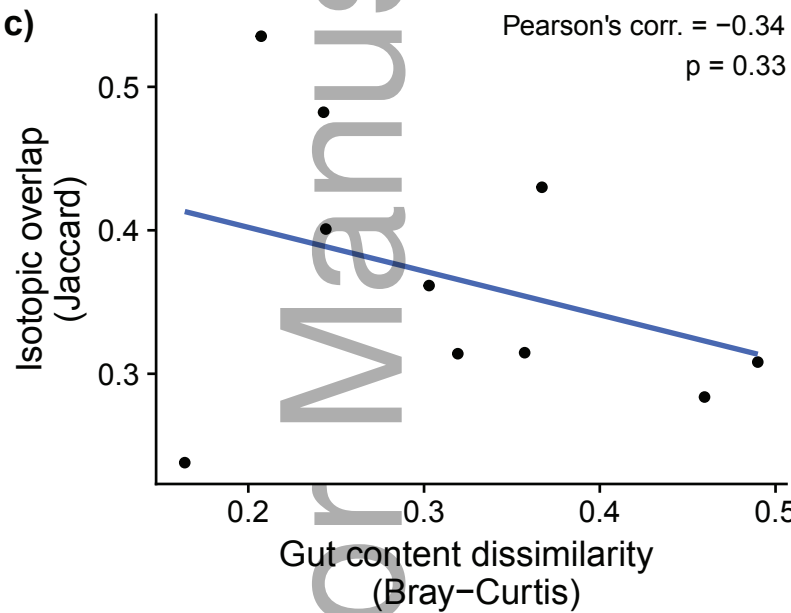
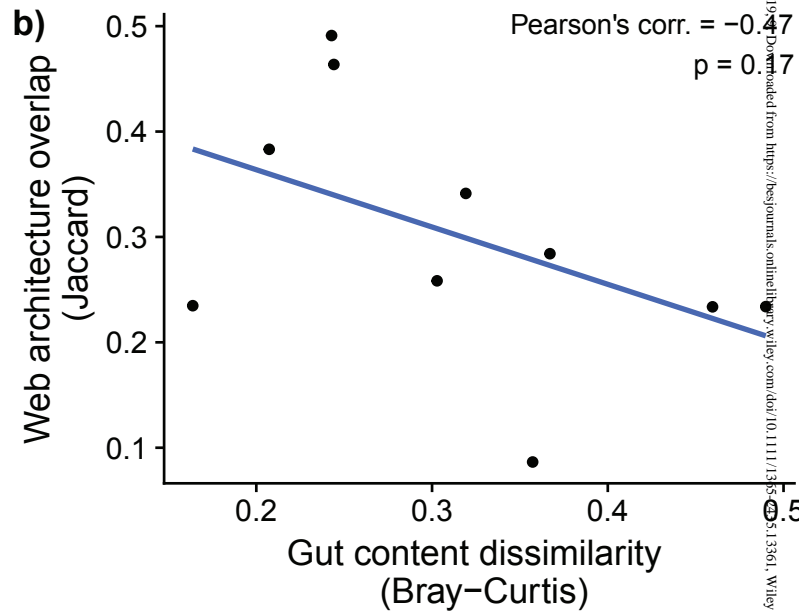
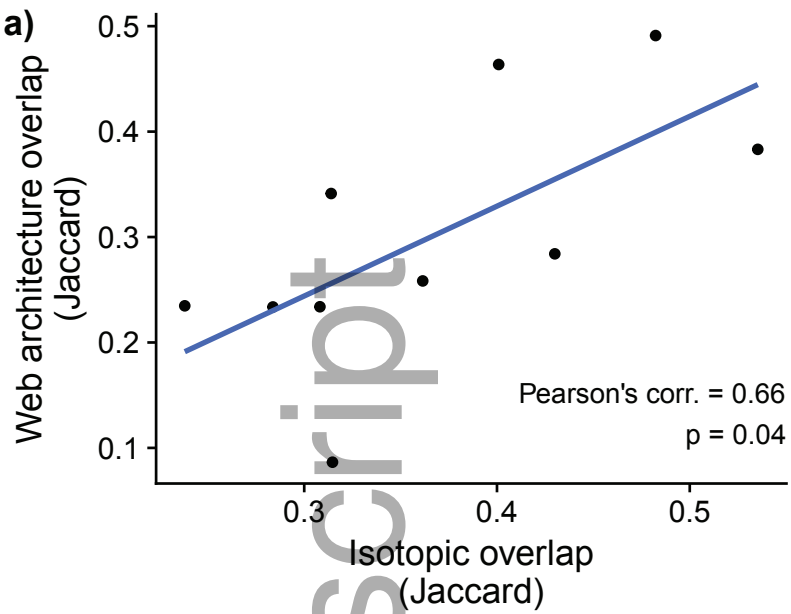
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