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RESEARCH ARTICLE

Functional Ecology



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

Susan Kennedy^{1,2} | Jun Ying Lim^{3,4} | Joanne Clavel^{1,5} | Henrik Krehenwinkel^{1,6} |
1 2 Rosemary G. Gillespie¹

¹Department of Environmental Science, Policy and Management, University of California, Berkeley, California; ²Biodiversity and Biocomplexity Unit, Okinawa Institute of Science and Technology, Okinawa, Japan; ³Department of Integrative Biology, University of California, Berkeley, California; ⁴Institute of Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands; ⁵Centre National de la Recherche Scientifique, UMR 7533, LADYSS University of Paris, Paris, France and ⁶Department of Biogeography, Trier University, Trier, Germany

Correspondence

Susan Kennedy

Email: susanrkennedy@gmail.com

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Abstract

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

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their webs. Our findings indicate an important link between web structure, microhabitat preference and diet in the Hawaiian *Tetragnatha*.

KEYWORDS

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

1 | INTRODUCTION

Ecological niche partitioning, the process by which co-occurring populations divide the available niche space by exploiting different sets of resources (MacArthur, 1958; Schoener, 1974), is a common phenomenon in adaptive radiations (Emrich, Clare, Symondson, Koenig, & Fenton, 2014; Giménez et al., 2018; Losos, 1994; Losos et al., 2003; Schluter & McPhail, 1992; von Rintelen, Wilson, Meyer, & Glaubrecht, 2004). Among animals, trophic niche differentiation has been shown to be a primary driver in adaptive radiations such as the anoles (Losos, 2011), cichlid fish (Ford et al., 2016; Muschick, Indermaur, & Salzburger, 2012; Wagner, McIntyre, Buels, Gilbert, & Michel, 2009) and Galápagos finches (Grant, 1999; Mallarino et al., 2012). However, different attributes are used as proxies for trophic niche (e.g. beak shape in finches, leg length in lizards, pharyngeal jaw morphology in fish), and it is often difficult to assess which aspects of the multidimensional niche translate into meaningful trophic separation between co-occurring taxa. This information is nonetheless critical in order to understand the factors that define an adaptive radiation. Here, we test for trophic niche partitioning in an adaptive radiation of spiders, using multiple ecological and behavioural measures to provide a multifaceted view of the factors that reinforce ecological differentiation in this group.

The Hawaiian Islands present numerous opportunities for examining the ecological underpinnings of adaptive radiation (Shaw & Gillespie, 2016). This archipelago has extraordinarily high levels of endemism due to its extreme isolation from other landmasses (>4,000 km from the nearest mainland, Bennett & O'Grady, 2013; Goodman, Evenhuis, Bartošová-Sojková, & O'Grady, 2014), and, because this is coupled with a chronological arrangement of current high islands stretching from 0 to 6.3 mya (Lim & Marshall, 2017), it has been deemed a "natural laboratory" (Roderick & Gillespie, 1998; Simon, 1987). The physical isolation and ecological opportunities afforded by the formation of the Hawaiian Islands have promoted multiple well-known evolutionary radiations. Among arthropods, *Drosophila* flies (Magnacca & Price, 2015), *Megalagrion* damselflies (Jordan, Simon, & Polhemus, 2003), *Nesophrosyne* leafhoppers (Bennett & O'Grady, 2013), and *Mecaphesa* (Garb & Gillespie, 2009), *Ariamnes* (Gillespie, Benjamin, Brewer, Rivera, & Roderick, 2018) and *Tetragnatha* (Gillespie, 2004, 2016) spiders are well-established examples. In each instance, representatives of the lineage have evolved into multiple new phenotypes not found in mainland relatives. Furthermore, multiple species within each radiation coexist in the same habitat, raising the question of whether the observed novel ecomorphologies correspond to the exploitation of new ecological

niches and whether this results in partitioning of the available niche space.

Here, we test for trophic niche partitioning in a group of sympatric species belonging to an adaptive radiation of Hawaiian long-jawed orb-weaving spiders (*Tetragnatha*, Tetragnathidae). The genus *Tetragnatha* is globally distributed and largely homogeneous in morphology and ecology (Kaston, 1978). Most *Tetragnatha* species have long, slender bodies and long chelicerae (jaws), and spin orb webs near bodies of water (Gillespie, 1987). In the Hawaiian Archipelago, however, the genus has evolved numerous novel morphologies and behaviours, including the loss of web-building behaviour in one clade (the Spiny Leg clade, Gillespie, 1991, 2002), new morphologies such as stouter bodies, shorter jaws, humps or tubercles on the abdomen, and a variety of colorations, from brown to maroon to bright green to red. These innovations are presumed to reflect adaptations to allow specialization on particular elements of the dietary niche (Blackledge & Gillespie, 2004), while also serving to camouflage a given taxon on specific substrates (Gillespie, 2004). Earlier observational work suggests dietary differences among sympatric web-building *Tetragnatha* species (Blackledge, Binford, & Gillespie, 2003) and, on a broader scale, between the web-building spiders and the actively hunting Spiny Leg spiders (Binford, 2001). Furthermore, it was recently found that web-building *Tetragnatha* carry a different isotopic signature in their tissues from co-occurring Spiny Leg *Tetragnatha* on the Big Island of Hawai'i (Kennedy, Dawson, & Gillespie, 2018). With over 30 described species (Gillespie, 1991, 1992, 1994, 2002, 2003), many of which co-occur in overlapping ranges, the Hawaiian *Tetragnatha* spiders represent an ideal system in which to examine the ecological and behavioural dynamics of sympatric species in the context of adaptive radiation.

We characterize the niches of co-occurring Hawaiian *Tetragnatha* spiders to evaluate the niche axes that separate species and the extent to which these niche differences are reflected in dietary differences. For web-builders, we measure *web architecture*, the structure of the spiders' foraging tool, and *site choice*, the physical placement of webs in the habitat. For cursorial spiders, we already have detailed knowledge of microhabitat association (Gillespie, 2004). In order to determine the extent to which these ecological and behavioural differences translate into significant differences in the trophic niche, we use measurements of stable isotope ratios and analysis of gut contents to give both long-term and short-term indicators of differences in trophic repertoire between species. Stable isotopes are a well-established tool in food web studies (Cabana & Rasmussen, 1994; France, 1995; Peterson, Howarth, & Garritt, 1985): both nitrogen and carbon isotopes are

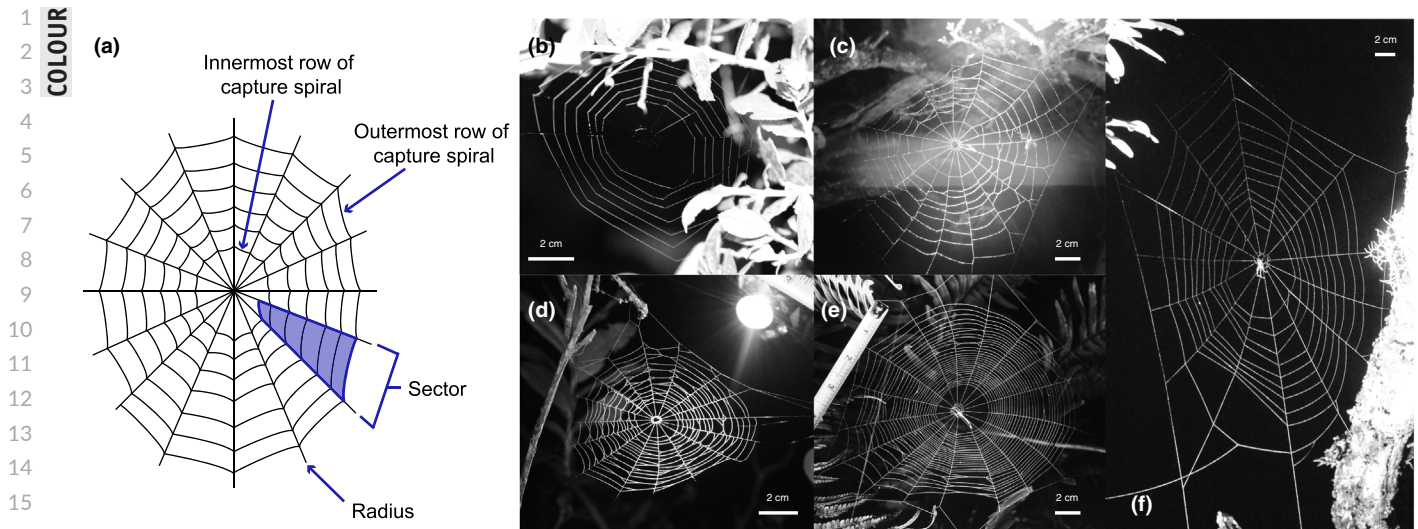


FIGURE 1 Web schematic (a) showing architectural components of a *Tetragnatha* web, and exemplar webs of the five study species. b = *T. acuta*, c = *T. eurychasma*, d = *T. filiciphilia*, e = *T. stellarobusta*, f = *T. trituberculata*

useful for assessing trophic position, as the ratio of heavier to lighter isotope ($\delta^{13}\text{C}$ or $\delta^{15}\text{N}$) tends to increase in a stepwise pattern with successive trophic levels (Post, 2002). For gut content analysis, we identify the prey of individual spiders to the order level, using a metabarcoding approach (Krehenwinkel, Kennedy, Pekár, & Gillespie, 2017). We hypothesize that hunting mode—web-building versus active foraging—plays a major role in shaping the trophic niche, and consequently, we expect significant differences in isotopic signature and gut content between the web-builders and the Spiny Leg spiders. Additionally, we hypothesize that sympatric species of web-building spiders exhibit finer-scale niche partitioning, manifested by differences in web architecture and site choice, which translate into dietary differences among species.

2 | MATERIALS AND METHODS

2.1 | Study system

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

Web-building *Tetragnatha* spin two-dimensional orb webs. A spiral of sticky silk, the “capture spiral,” is supported by several spokes, or “radii,” which converge into a central hub (Figure 1). The section of web between two adjacent radii is called a “sector.” *Tetragnatha* spiders are nocturnal, and generally spin a fresh web every night, then dismantle and eat the web (recycling the silk) every morning.

These webs are relatively delicate and sustain significant damage when they intercept prey, so spiders also generally dismantle their webs after each prey capture event.

2.2 | Sample collection

Sampling took place in The Nature Conservancy's Waikamoi Preserve, located on the windward slope of Haleakalā Volcano, East Maui. We sampled in the summers of 2013, 2014 and 2017, and in November 2016, at two different elevations: low (1,380–1,500 m: along the Maile Trail, an approximately 500-m distance between coordinates N20.80276°, W156.25460° and N20.79870°, W156.25279°) and high (1,800–1,950 m: along the Waikamoi Boardwalk, an approximately 350-m distance between coordinates N20.78055°, W156.22709° and N20.77806°, W156.22868°). This allowed us to capture data on nearly all *Tetragnatha* species that occur in Maui's wet forest, as, although several species are found throughout the region, two of the species (the web-building *T. filiciphilia* and the Spiny Leg *T. brevignatha*) are found primarily at lower elevations while the remaining species co-occur at higher elevations. Sample sizes by species, year and elevation are summarized in Table S1.

2.3 | Web measurements

For each web found, we collected the spider into a clean snap cap vial. We then dusted the web with cornstarch to enhance visibility and photographed it from a 90° angle with a ruler held in frame for scale. To evaluate microhabitat (site choice), we recorded the vegetation genus and functional “type” (fern, grass, shrub, tree or vine) to which the web was attached, and measured the web's height above the ground and angle from vertical (0° = vertical, 90° = horizontal). We measured webs from digital photos using IMAGEJ (National Institutes of Health, USA) and took the

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 ²)	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 ²)	CTL/CA
Silk density 2, SD2 (cm)	CA/MW

measurements summarized in Table 1 (see also Figure 1). Capture thread length (CTL), the total length of sticky capture spiral, was estimated using the large-scale formula of Heiling, Herberstein, and Spitzer (1998):

$$CTL = \sum ((T_i + T_s) / 2) \times x_i,$$

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

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

$$MW = 0.5 \times (D_u / (R_u - 1) + D_l / (R_l - 1)),$$

where D_u is the length of a designated "upper" sector, from innermost to outermost row of capture thread, R_u is the number of rows of capture thread in the "upper" sector, D_l is the length of a designated "lower" sector 180° from the "upper" sector, and R_l is the number of rows of capture thread in the "lower" sector.

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

2.4 | Stable isotope analysis

Spider specimens used for C and N stable isotope analysis were collected into separate snap cap vials and euthanized in a freezer. Individuals were then photographed up close using a Nikon D5200 with a macro lens, creating a digital voucher. The spiders were air-dried at room temperature, with clean beads of silica gel in each vial to accelerate drying and prevent decomposition. No baseline

samples (such as plant leaves or soil) were taken; only spider specimens were analysed for isotopic signature.

Individual spiders were homogenized with a mortar and pestle, and 1.5 mg of tissue per sample was weighed out into a 9 × 5 mm tin capsule. This amount was chosen to optimize the efficiency of the analysis based on the C:N ratio in spiders' tissues (S. Mambelli, pers. comm., 2014). When spiders weighed <2.5 mg, we used whole bodies without homogenization to prevent loss of material in the grinding process. We used whole bodies because of the limited amount of tissue, recognizing that this may bias isotopic values towards recently consumed meals due to the inclusion of the digestive tract in the sample. However, we do not expect any differences among species in terms of how recently the spiders fed before being collected; therefore, the inclusion of the digestive tract should not significantly bias the comparison of isotopic signatures among species. Samples were analysed for carbon and nitrogen content (per cent dry weight) and carbon and nitrogen stable isotope ratios by continuous flow dual isotope analysis on a CHNOS Elemental Analyzer (vario ISOTOPE cube; Elementar) connected to an IsoPrime100 mass spectrometer (Isoprime Ltd) at the Center for Stable Isotope Biogeochemistry at the University of California, Berkeley. The standard used for carbon was Vienna Pee Dee Belemnite, and the standard for nitrogen was air. The reference material NIST SMR 1547 (peach leaves) was used as a calibration standard. Long-term external precision based on reference material NIST 244 SMR 1577b (bovine liver) is 0.10‰ for carbon and 0.15‰ for nitrogen isotope analyses.

2.5 | Gut content analysis

Spider specimens used for gut content analysis were preserved in 95% ETOH in the field and subsequently kept frozen at -20°C until DNA extraction. Gut contents were identified using Illumina amplicon sequencing following Krehenwinkel et al. (2017). DNA was first extracted from crushed abdomens, using a Qiagen Genra Puregene kit (Qiagen) according to the manufacturer's protocol. PCR was performed using a Qiagen Multiplex kit, following the manufacturer's protocol, to amplify a 210-bp fragment of COI (Zeale, Butlin, Barker, Lees, & Jones, 2011). The primers had Illumina TruSeq tails attached to the 5' ends, which served as binding sites for barcoding primers added in a second round of indexing PCR (Lange et al., 2014). After indexing, samples were pooled together in equal amounts, diluted to 4 nM concentration, and sequenced on an Illumina MiSeq (Illumina) with V3 chemistry at UC Berkeley's QB3 (Berkeley, CA) and at the California Academy of Sciences' Center for Comparative Genomics (San Francisco, CA). Reads were demultiplexed, then merged using PEAR (Zhang, Kobert, Flouri, & Stamatakis, 2013), with a minimum overlap of 50 bp and minimum quality of Q20. Assembled reads were then quality filtered and converted to FASTA format using FastX Toolkit (Gordon & Hannon, 2010) with the threshold of at least 90% of sequences having at least Q30 quality. Sequences were clustered into OTUs (operational taxonomic units) at 97% similarity, and chimeras were removed, using USearch (Edgar, 2010). Prey OTUs were identified to the lowest possible taxonomic level using a BLAST search

(Altschul, Gish, Miller, Myers, & Lipman, 1990) against the National Center for Biotechnology Information (NCBI) database. OTU tables showing the number of sequence reads of each prey OTU for each specimen were built using USearch. All OTUs that matched family Tetragnathidae were discarded as likely belonging to the *Tetragnatha* spiders rather than the prey. Prey read counts of each specimen were then rarefied to equal coverage (500 reads per specimen) using the GUniFrac package (Chen, 2012) in R. To allow for more accurate comparisons of dietary diversity, each *Tetragnatha* species was randomly subsampled using a random number generator so that species were represented by equal numbers of individuals within each clade ($n = 5$ for web-building species, $n = 6$ for Spiny Leg species; Table S1).

2.6 | Data analysis

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

2.7 | Testing for relationships among niche parameters

To assess niche divergences between the different *Tetragnatha* species, we tested relationships between (a) web architecture and isotopic signature, (b) web architecture and gut contents, and (c) isotopic signature and gut contents. We first quantified the variability in web architecture and isotopic composition occupied by each species by constructing hypervolumes for both web and isotope variables. This approach quantifies the multidimensional space occupied by each species using multivariate density kernel estimation (Blonder, Lamanna, Violle, & Enquist, 2014). This is an improvement over convex hull methods, which are more sensitive to outlier points. Hypervolumes were calculated using the HYPERVOLUME package in R (Blonder et al., 2014). Web hypervolumes were constructed using loadings for the first two PCA axes as hypervolume calculations can be sensitive to collinear variables. Isotope hypervolumes were calculated using $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values.

We then calculated pairwise differences in web and isotope hypervolumes by computing the pairwise Jaccard overlap

between species hypervolumes. For two hypervolumes, A and B, hypervolume Jaccard overlap is calculated as follows: $(A \cap B) / (A \cup B)$. Values range from 0 (species are completely dissimilar) to 1 (species are completely similar). If web architecture were driving differences in isotopic signatures through a selective effect on diet, one would expect species with more similar webs to be more similar in isotopic composition (i.e. web hypervolume overlap should be positively correlated with isotope hypervolume overlap). We assessed the relationship between web hypervolume overlap and isotope hypervolume overlap using a Pearson's correlation test. For gut content data, we assessed the degree of pairwise dissimilarity in diet by calculating the Bray-Curtis dissimilarity between pairs of species in VEGAN (Oksanen et al., 2010). We used prey data at the ordinal level because order is expected to correspond to broad but important functional traits in arthropods, for example feeding style, life history and mobility. Using the hypervolume overlaps calculated as described above, we then tested for correlations among: (a) gut content and web architecture, and (b) gut content and isotopic signature, using a Pearson's correlation test. If web architecture were driving differences in gut content, or if gut content were driving differences in isotopic signature, then we would expect a negative correlation between gut content dissimilarity and hypervolume overlaps for web architecture and isotopic signature. Additionally, to determine whether diets differed significantly among species, we performed pairwise PERMANOVA between pairs of web-building and Spiny Leg species in VEGAN, using prey data at both the ordinal and OTU levels.

3 | RESULTS

3.1 | Web architecture

The first two axes in our principal component analysis of web architecture account for 87.3% of the total variation in the sample population (Figure 2, Table S2). The first axis, accounting for 52% of variation, is positively correlated with number of rows, followed closely by number of radii, capture thread length and silk density 2 (capture area divided by mesh width). Mesh width is negatively correlated with the first axis, but has a weaker association than the other variables. Thus, the first axis generally describes web density: as spiders increase in PC1, their webs have longer capture threads, more silk per unit area, larger numbers of radii and smaller mesh widths. The second PCA axis (35.3% of variation) is positively associated with capture area and mesh width, and negatively associated with silk density 1 (capture thread length divided by capture area). Thus, as spiders increase in PC2, their webs are larger and looser, with longer distances between adjacent rows of capture spiral.

We found significant interspecific differences in species position on PC1 (ANOVA $F = 32.74$; $df = 4,179$; $p < 0.005$) and PC2 (ANOVA $F = 47.15$; $df = 4,179$; $p < 0.005$; Figure 2, Table S3). *T. stelarobusta* was significantly higher in PC1 (web density) than all other species,

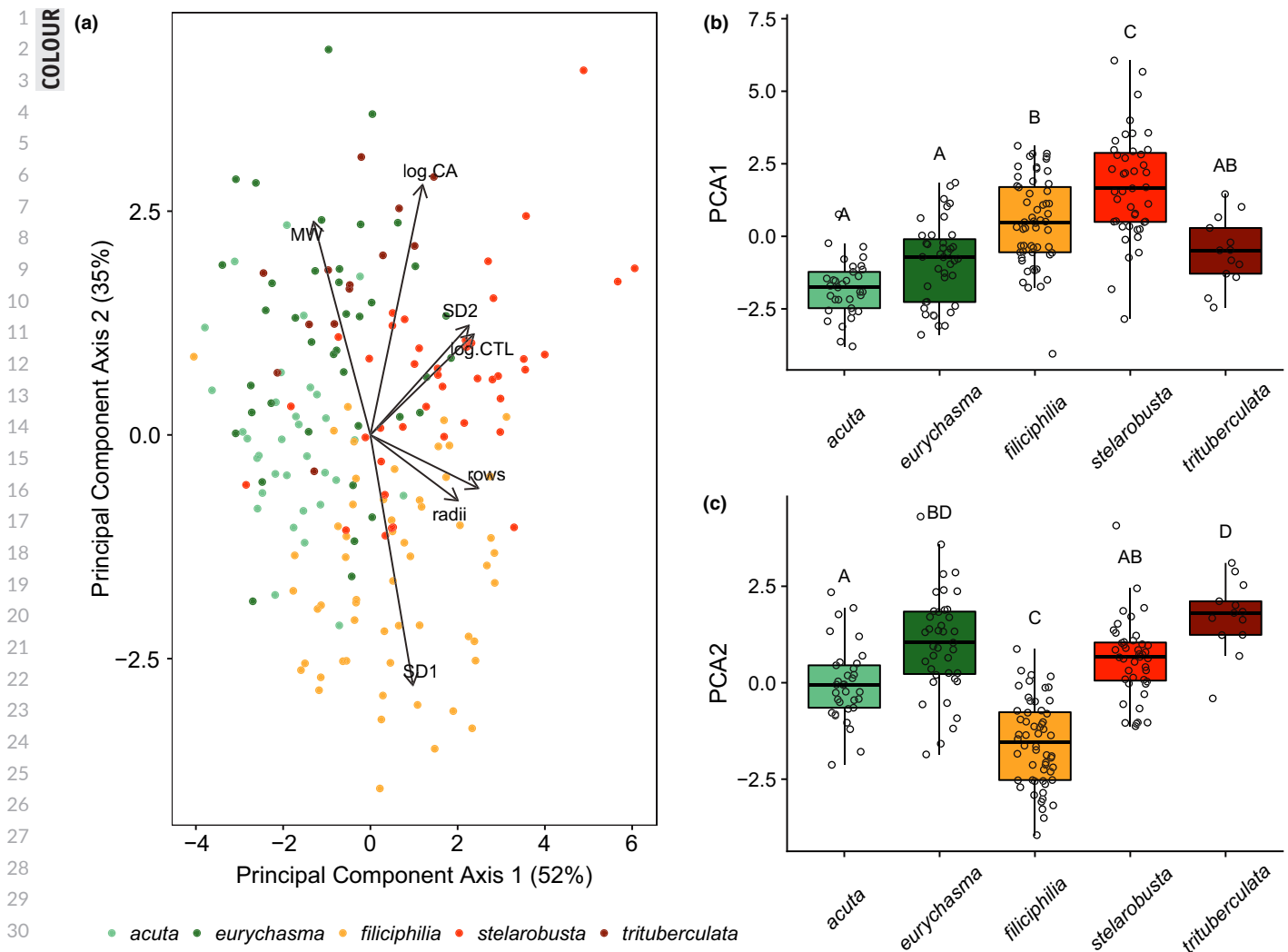


FIGURE 2 Multivariate analysis of web architectural values across species of *Tetragnatha*. Principal component axes 1 and 2 cumulatively explain 87% of variance. Arrows represent standardized loadings for each web architectural variable included in the analysis. Letters above boxplots indicate significant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

followed by *T. filiciphilia*. *T. acuta* and *T. eurychasma* had the lowest PC1 values. For PC2, *T. filiciphilia* had significantly lower values (i.e. smaller and denser webs) than the other species, and *T. trituberculata* had significantly higher values (larger and looser webs) than all species except *T. eurychasma* (Table S4).

3.2 | Site choice

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

Height and angle of webs differed significantly among species (height: ANOVA $F = 12.11$, $df = 4,179$, $p < 0.005$; angle: ANOVA

$F = 18.38$, $df = 4,179$, $p < 0.005$; Figure 3, Tables S5 and S6). *T. trituberculata* built significantly more vertical webs than others (mean = 13°), followed by *T. acuta* and *T. stelarobusta*. *T. filiciphilia* and *T. eurychasma* built obliquely oriented webs, and *T. filiciphilia* had the greatest deviation from vertical (mean = 51°). *T. trituberculata* built webs significantly higher than all other species (mean = 186 cm), while *T. stelarobusta* built webs significantly lower than all others (mean = 109 cm).

3.3 | Isotopic differences among species

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 $F = 5.50$; $df = 4,93$; $p < 0.05$) among web-building species (Figure 4, Tables S7 and S8). Using a post hoc Tukey's HSD test, we found that *T. filiciphilia* and *T. stelarobusta* were both significantly higher in $\delta^{15}\text{N}$ than the other three web-building species ($p < 0.05$). *T. stelarobusta* had significantly lower $\delta^{13}\text{C}$ values than all other web-building species except for *T.*

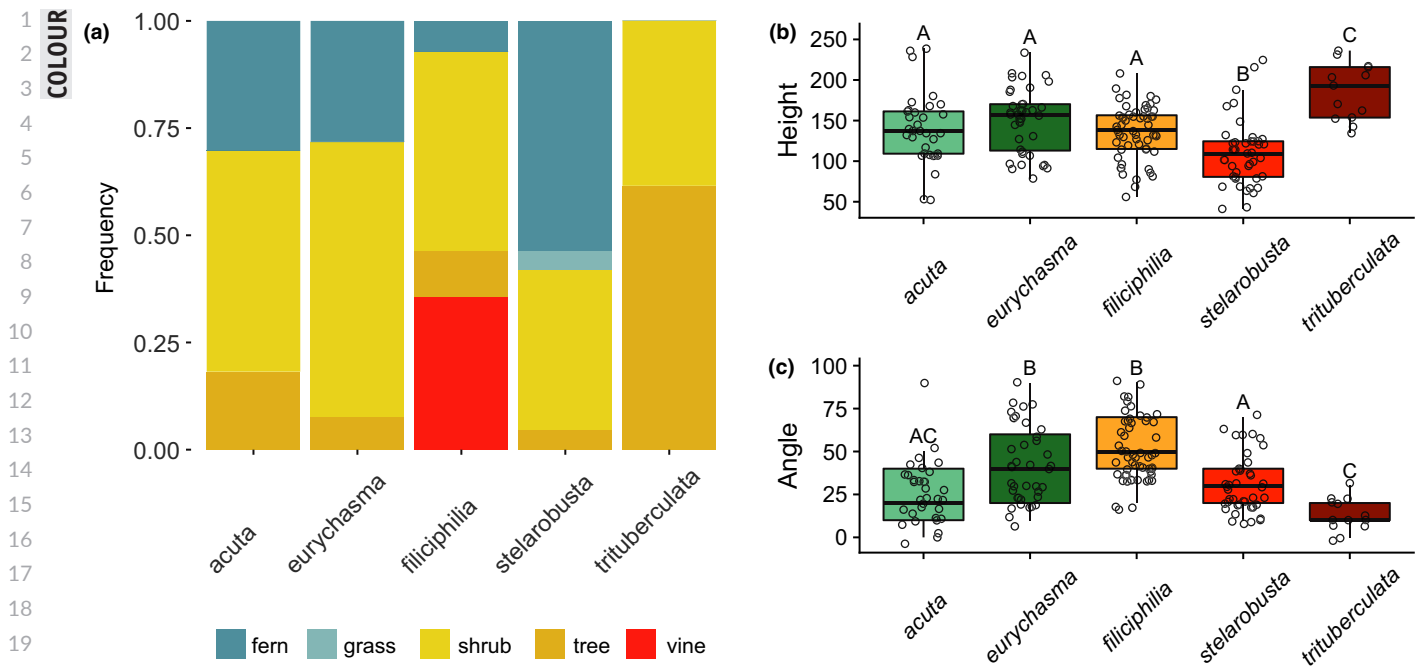
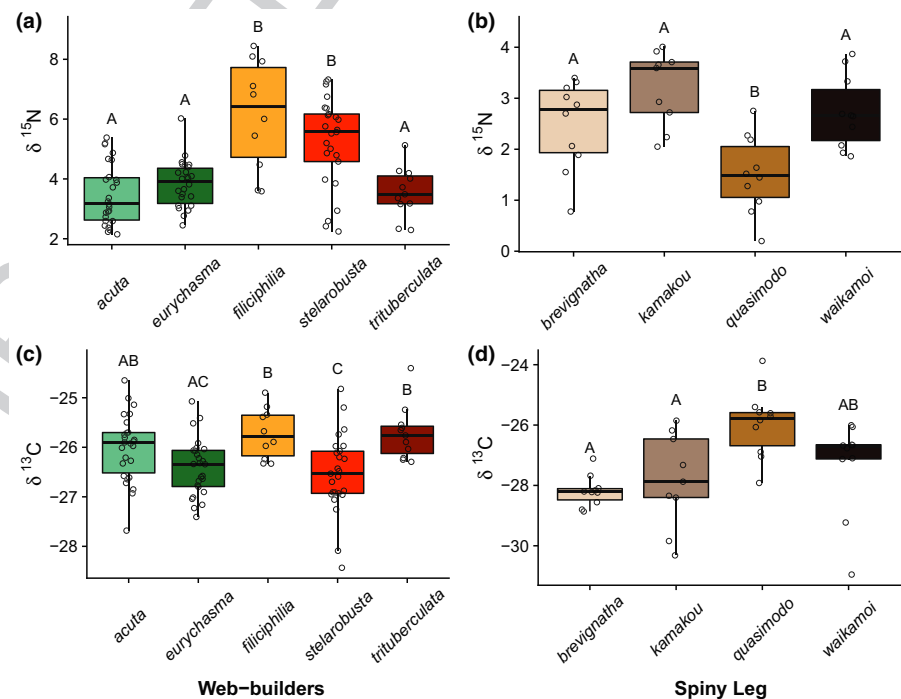


FIGURE 3 Site choice parameters of web-building *Tetragnatha* species. (a) Stacked bar plots of vegetation-level differences in web attachment site among species, (b) height (cm above-ground) and (c) angle of placement (0° = vertical, 90° = horizontal) of webs. Letters above boxplots indicate significant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



eurychasma, which had significantly lower $\delta^{13}\text{C}$ values than *T. trituberculata* and *T. filiciphilia*.

At the clade level, we found a highly significant difference between web-builders and Spiny Leg spiders for both $\delta^{15}\text{N}$ (ANOVA $F = 50.41$ $df = 1,135$, $p < 0.001$) and $\delta^{13}\text{C}$ (ANOVA $F = 37.24$, $df = 1,135$, $p < 0.001$), with web-builders falling higher than Spiny Leg spiders for both isotopic signatures (Figure 5).

3.4 | Gut content

Based on the numbers of sequences obtained from spiders' gut contents, each species showed a distinctive prey composition at the order level (Figure 6). On the whole, web-builders had more Diptera while Spiny Leg spiders had more Hemiptera and Hymenoptera in their guts, and both clades had substantial

contributions of Lepidoptera. Bray–Curtis dissimilarities showed moderate to high complementarity between most pairs of spider species within each clade (Tables 2 and 3), ranging from approximately 0.2–0.5. PERMANOVA showed significant differences in diet between most pairs of web-building species at both the ordinal and OTU levels of prey, while diets of Spiny Leg species largely did not differ significantly from one another (Tables S11–S14).

3.5 | Correlations among different measures of niche

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

4 | DISCUSSION

4.1 | Web architecture and site choice

We demonstrate significant interspecific differences in web architecture and site choice, that is foraging behaviour (Figures 2 and 3). The web-building species show strong differentiation in the structure of their webs, primarily in terms of size and silk density. Additionally, different species show associations with particular web attachment substrates and orientations in space. These web architectures and microhabitats may be tailored for the capture of specific assemblages of prey, which may be more effectively intercepted by particular web forms, and more abundant in particular microhabitats. That each species showed a specific web architecture and site

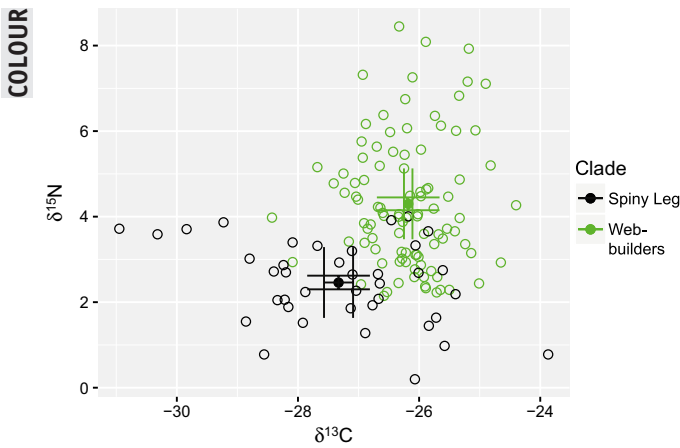


FIGURE 5 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in web-building versus Spiny Leg *Tetragnatha*

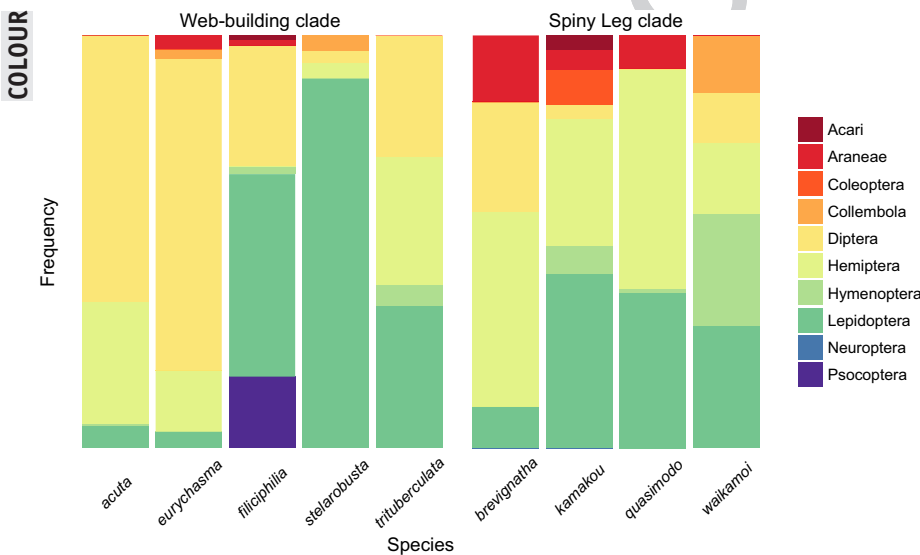


FIGURE 6 Prey orders detected by molecular gut content analysis in five species of web-building and four species of Spiny Leg *Tetragnatha*: per cent composition by spider species

	<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 2 Pairwise Bray–Curtis dissimilarities in gut content in web-building *Tetragnatha*

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

choice suggests inherent behavioural differences among species, possibly acting as a mechanism of trophic niche partitioning.

4.2 | Isotopic differences among species

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

A number of variables may have contributed to the spiders' isotopic signatures, particularly for $\delta^{13}\text{C}$. In addition to dietary information, $\delta^{13}\text{C}$ can also reflect subtle microhabitat differences such as variations in sun exposure (Li et al., 2007; Zimmerman &

Ehleringer, 1990) or humidity (Liu, Dahe, Shao, Chen, & Ren, 2003; Porter, Pisaric, Kokelj, & Edwards, 2009). Most isotopic studies find that $\delta^{13}\text{C}$ closely reflects the signature of an animal's food source and changes little from one trophic level to the next (Hobson, Barnett-Johnson, & Cerling, 2010). If this is the case in the Hawaiian *Tetragnatha*, then it is plausible that isotopic signatures of the prey's microhabitat—for example, sun-exposed canopy versus darker, more humid understory vegetation—will be borne up into the spiders' tissues. Further study on the isotopic signatures of putative prey from a variety of different microhabitats is needed in order to disentangle these potential effects.

For nitrogen, higher values of $\delta^{15}\text{N}$ are typically associated with higher trophic level, with standard discrimination rates of approximately 3.4 per mill with each successive trophic level (Post, 2002). We find significantly higher levels of $\delta^{15}\text{N}$ in *T. filiciphilia* and *T. stelarobusta* than in any other species (Figure 4), raising the possibility that these two species feed at a higher trophic level, for example by consuming predatory or parasitoid flies or wasps. However, diet is not necessarily the only factor affecting the stable isotope ratios in spiders' tissues; alternatively, the higher $\delta^{15}\text{N}$ in *T. stelarobusta* and *T. filiciphilia* may be related to the higher density of silk in these two species' webs. *T. filiciphilia* fell highest for "Silk Density 1," and *T. stelarobusta* highest for "Silk Density 2" (Figure 2). It is possible that the act of spinning and recycling (eating) silk contributes to the loss of the lighter ^{14}N isotope and ultimately the higher concentration of ^{15}N in a spider's tissues. If so, then this phenomenon could also at least partially account for the higher $\delta^{15}\text{N}$ that we found in web-builders compared with Spiny Leg spiders. While little is known about the impact of silk spinning and recycling on isotopes, other studies have found silk-recycling web spinners to have higher values of $\delta^{15}\text{N}$ than those that do not recycle their silk (Sanders, Vogel, &

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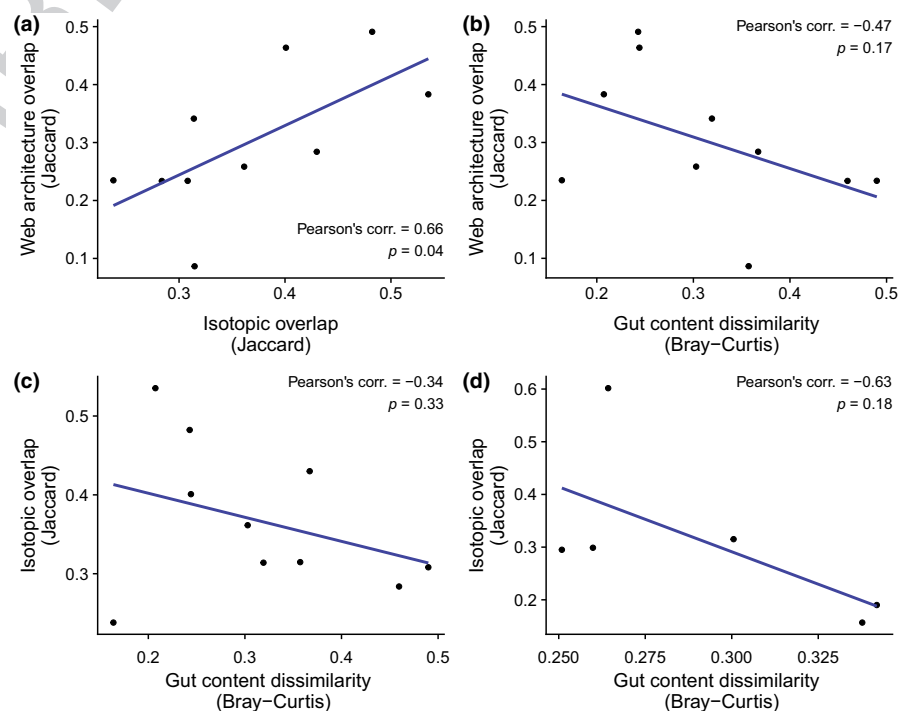


FIGURE 7 Correlation tests for niche hypervolume Jaccard overlaps and gut content Bray–Curtis dissimilarity. Top panel: (a) web hypervolume overlaps with isotopic hypervolume overlaps, (b) web hypervolume overlaps with gut content Bray–Curtis dissimilarity. Bottom panel: isotope hypervolume overlaps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

Knop, 2015). Indeed, a recent study of *Tetragnatha* on the Big Island of Hawai'i found the same pattern of higher $\delta^{15}\text{N}$ in web-builders than in members of the Spiny Leg clade (Kennedy et al., 2018). A deeper exploration of this phenomenon is called for, and experimental studies of the effects of silk recycling on spiders' isotopic signatures could be a promising future direction.

4.3 | Gut content

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

4.4 | Ecological and behavioural differences define trophic niche

A key finding was that ecological and behavioural differences are reflected in dietary differences, as inferred from the stable isotope signatures and taxonomic composition of gut contents of the spiders. This suggests that trophic niche partitioning plays an important role in the community ecological dynamics of Hawaiian *Tetragnatha* spiders. At the same time, we find marked trophic niche differences between the two major clades, possibly corresponding to their different hunting strategies (web-building vs. active hunting). We also find a significant correlation between web hypervolume and isotope hypervolume overlaps, indicating that web architecture has an effect on the types of prey consumed by a spider, with measurable impacts

on the spider's isotopic signature. Taken together, our results show that behavioural and ecological axes of the trophic niche are closely linked to the actual dietary composition of these spiders, supporting the hypothesis that co-occurring species have adapted feeding strategies that result in the exploitation of different sets of food resources.

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AUTHORS' CONTRIBUTIONS

S.K., J.C., J.Y.L., H.K. and R.G.G. conceived the ideas and designed methodology; S.K., J.C. and R.G.G. collected data; J.Y.L., S.K. and H.K. analysed data; S.K. and J.Y.L. led the writing of the manuscript.

DATA ACCESSIBILITY

Data deposited in the Dryad Digital Repository <http://doi.org/10.5061/dryad.29p63mb> (Kennedy, Lim, Clavel, Krehenwinkel, & Gillespie, 2019).

ORCID

Susan Kennedy  <https://orcid.org/0000-0002-1616-3985>

Jun Ying Lim  <https://orcid.org/0000-0001-7493-2159>

Henrik Krehenwinkel  <https://orcid.org/0000-0001-5069-8601>

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