

Evidence of carnivory for harvestmen in Belize based on stable nitrogen isotopes and fecal samples

M. H. Schaus, V. R. Townsend, Jr., S. A. Wiltshire and M. C. Metro: Department of Biology, Virginia Wesleyan University, 5817 Wesleyan Dr., Virginia Beach, VA, 23455 USA; E-mail: mschaus@vwu.edu

Abstract. Harvestmen are generally considered to be omnivores. In contrast to most other arachnids that are fluid feeders, harvestmen are capable of ingesting solid food particles and may consume invertebrates, small vertebrates, plant tissues, fungi, and detritus. However, for most species, quantitative diet data are lacking. Stable nitrogen isotope analysis provides a means to measure the contribution of these materials to the diet over the time scale of tissue turnover, and thus quantify trophic position. Examination of fecal material can provide additional evidence of recent feeding, which can further complement field observations and isotopic analyses. We investigated the diet of *Erginulus clavotibialis* (F.O. Pickard-Cambridge, 1905) (Arachnida, Opiliones, Cosmetidae) in western Belize, using stable isotope analysis and scanning electron microscopy of fecal samples. We also examined nitrogen isotopic ratios for carnivores (scorpions and spiders), herbivores (grasshoppers and katydids), and preliminary measures for two other harvestmen species, ants, and termites. *Erginulus clavotibialis* had $\delta^{15}\text{N}$ signatures similar to scorpions and spiders, indicating carnivory. Nymphs and adults (females and males) of *E. clavotibialis* did not differ significantly in their trophic level despite possessing chelicerae and pedipalps that differ substantially in relative size and morphology. Fecal samples contained considerable evidence of arthropod consumption, as fragments of legs and exoskeleton were evident. Our study provides an initial quantification of harvestman diet and documents that *E. clavotibialis* is primarily carnivorous.

Keywords: Opiliones, omnivory, feeding history, SEM

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Harvestmen (Arachnida, Opiliones) are abundant and diverse members of arthropod communities in many Neotropical forests (Townsend et al. 2010; Proud et al. 2012). They are generally considered to be omnivorous and opportunistic, with preferences for soft-bodied invertebrates (Acosta & Machado 2007). The diets of different species may include live or dead arthropods (Dixon & McKinlay 1989; Gnaspini 1996), earthworms (Halaj & Cady 2000), snails and slugs (Nyffeler & Symondson 2001), fruits (Machado & Pizo 2000), pollen (Castanho et al. 2012), fungi (Goodnight & Goodnight 1960), detritus (Pinto-da-Rocha 1996), guano (Bristowe 1949), and even live (Benson & Chartier 2010) or dead vertebrates (Castanho & Pinto-da-Rocha 2005). Inferences concerning diet and food preferences are typically derived from qualitative (Phillipson 1960; Gnaspini 1996) or quantitative (Halaj & Cady 2000) field observations, or from laboratory studies (Gnaspini 1996; Allard & Yeorgan 2005; Hvam & Toft 2008; Schaus et al. 2013). However, the diets of harvestmen in the laboratory and field can vary substantially (i.e., Edgar 1971; Gnaspini 1996; Santos & Gnaspini 2002). For most harvestmen species, quantitative diet data are either lacking or represent a snapshot in time, based on a limited number of field observations (Acosta & Machado 2007).

Multiple stable isotope analysis of diet provides a means of quantifying the feeding history of organisms integrated over the time scale of tissue turnover. Stable isotopes of C and N have often been used in food web analyses to quantify the carbon source ($\delta^{13}\text{C}$) and realized trophic level ($\delta^{15}\text{N}$) of consumers (Peterson & Fry 1987). However, this approach has not been used frequently to study arachnids in general, and harvestmen in particular (Potapov et al. 2022). For example, a review of plant consumption by spiders (Nyffeler et al. 2016) only included one study (Eggs & Sanders 2013) that used stable isotope analysis to document feeding on plants, out of over 140 articles reviewed. A follow up review article that examined herbivory and fungivory by both harvestmen and spiders (Nyffeler et al. 2023) only included one additional study (Hyodo et al. 2018) that used stable isotopes to document spiders eating plants.

We are familiar with only a few recent studies examining the diets of harvestmen using stable isotopes (Table 1). Most of these

investigations focused on large guilds of invertebrates (i.e., König et al. 2011; Korobushkin et al. 2014), or on linkages between terrestrial and aquatic systems (i.e., Gratton et al. 2008; Bartrons et al. 2015), and did not focus primarily on the dietary role of harvestmen. None of these studies were conducted in Neotropical forests (all were temperate or alpine) and this technique has generally been used by food web ecologists, not arachnologists. As one example of the utility of this approach, deHart & Strand (2012) observed modest shifts in $\delta^{13}\text{C}$ of harvestmen and spiders in temperate plots with the invasive plant garlic mustard (*Alliaria petiolate*), which has a different $\delta^{13}\text{C}$ signal compared to other plants in that area. They also observed that relatively small harvestmen (<9 mg) occupied a lower trophic position (approximately 2/3 of a trophic level lower), compared to that of larger species, but did not identify the different species of harvestmen included in the study (deHart & Strand 2012).

Likewise, in some ecological analyses of diet, fecal analysis has been used to document evidence of the consumption of specific food items (i.e., Dickman & Huang 1988; Trites & Joy 2005). Parts of many food items can pass through the gut incompletely digested, such as the exoskeleton of arthropods, fibrous plant tissue, and other less digestible materials. These items can be examined using conventional dissecting microscopy, but scanning electron microscopy (SEM) provides a means to examine these materials using a finer resolution. Whereas isotopic analysis integrates feeding history over the time scale of tissue turnover, fecal analysis indicates recent feeding on specific diet items. Thus, these two techniques can provide complimentary roles in diet analyses.

The goal of the present study was to quantify feeding in three species of Neotropical harvestmen using stable isotope and fecal analyses, with a focus on the trophic position of the cosmetid *Erginulus clavotibialis* (F.O. Pickard-Cambridge, 1905). This taxon is larger than any of the other opiloid species found in western Belize, enabling us to easily identify the nymphs, which are larger than nymphs of other species and the adults of most harvestmen in this area (Goodnight & Goodnight 1976, 1977). This facilitated a comparison of the diet of nymphs, females, and males for *E. clavotibialis*. This is especially relevant because there is considerable

Table 1.—Published studies of harvestmen that used stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) analysis of the diet. For each harvestman taxon, the family is listed using a footnote. Taxa are denoted “Opiliones” if the authors did not identify the species that were studied, or if they lumped all harvestmen together. Data on the harvestman taxa studied or the sample size in each study were obtained from the original articles or from supplementary data sets available online. The study focus is given as Large Guilds of Invertebrates (LGI), Aquatic-Terrestrial Linkages (ATL), Mammalian diet focus (Mam), Genetically Modified Crop Focus (GMC), Avian focus (Aves), or Arachnid focus (Arach). Conclusions regarding harvestman diets were categorized as Predaceous (P), Omnivorous (O), or Variable (V), for studies that reported $\delta^{15}\text{N}$ values.

Reference (Year) and Location	Harvestman Taxa Studied	Number of Harvestmen	Study Focus	Conclusions Regarding Harvestmen Diet Predaceous (P) Omnivorous (O) or Variable (V)
Ponsard & Arditi (2000) France	<i>Rilaena triangularis</i> * <i>Nemastoma lugubre</i> † <i>Anelasmacephalus cambridgei</i> ‡ <i>Trogulus sp.</i> ‡ <i>Odiellus spinosus</i> *	63	LGI	Presented $\delta^{15}\text{N}$ data lumped with other predators, all harvestmen categorized as predators in the appendix. (P)
Scheu & Falca (2000) Germany	<i>Lophopilio palpinalis</i> *	2	LGI	Somewhat enriched in $\delta^{15}\text{N}$, but not as much as most spiders. Small sample size for harvestmen. (O)
Gratton et al. (2008) Iceland	“Opiliones”	19	ATL	$\delta^{15}\text{N}$ values were consistently intermediate to spiders and prey species. (O)
Okuzaki et al. (2009) Japan	<i>Yuria pulcra</i> §	4	LGI	Enriched in $\delta^{15}\text{N}$ values and equivalent to spiders and other predatory soil arthropods. Small sample size for harvestmen. (P)
König et al. (2011) Austria	<i>Mitopus morio</i> * <i>Mitopus glacialis</i> * <i>Dicranopalpus gasteinensis</i> *	>15	LGI	<i>M. morio</i> had intermediate $\delta^{15}\text{N}$ values at 2 sites, and enriched $\delta^{15}\text{N}$ values at the 3 rd , <i>D. gasteinensis</i> was enriched in $\delta^{15}\text{N}$. (V)
Shaner & Macko (2011) Virginia, USA	“Opiliones”	13	Mam	$\delta^{15}\text{N}$ values intermediate to other arthropods, usually somewhat lower than spiders or other predators, when observed together. (O)
deHart & Strand (2012) Virginia, USA	Large vs. Small Harvestmen, plus Wolf Spiders	20	Arach	Harvestmen had $\delta^{15}\text{N}$ values intermediate to spiders and prey species. Larger harvestmen were 2/3 of a trophic level higher than smaller harvestmen. (V)
Korobushkin et al. (2014) Russia	“Opiliones”	10	LGI	Harvestmen varied in $\delta^{15}\text{N}$, ranging from equal to spiders to equivalent to prey species. (V)
Bartrons et al. (2015) Iceland	<i>Mitopus morio</i> *	19	ATL	<i>Mitopus</i> had a variable trophic position, predatory when relying on terrestrial foods (lower $\delta^{13}\text{C}$), and intermediate when relying on aquatic foods (less depleted in ^{13}C); categorized as a predator/detritivore. (V)
deHart et al. (2017) Virginia, USA	“Opiliones” plus 10 spider taxa	37	Arach	Harvestman $\delta^{15}\text{N}$ was equivalent or nearly equal to web weaving spiders, but was 2‰ lower than cursorial spiders. (Primarily P)
Manfrin et al. (2018) Germany	<i>Rilaena triangularis</i> * <i>Nelima semproni</i> ¶ <i>Phalangium opilio</i> *	57	ATL	Harvestmen had $\delta^{13}\text{C}$ values that were similar to spiders. In the treatment with artificial light, they ingested slightly less aquatic prey.
Hoekman et al. (2019) Iceland	“Opiliones”	244	ATL	Harvestmen showed marked $\delta^{13}\text{C}$ depletion in midge exclusion plots.
Rozanova et al. (2022) Russia	“Opiliones”	30	LGI	Harvestmen were enriched in $\delta^{15}\text{N}$ relative to the mean for arthropods, 1/2 trophic level lower than spiders in spruce forests, but equivalent to spiders in mixed forest. (V)
Caputi et al. (2022) Spain	“Opiliones”	40	GMC	$\delta^{15}\text{N}$ was enriched, but was 1/3 trophic level lower than spiders. Harvestmen decreased their reliance on insects that fed on maize over time. (O)
Meyhoff et al. (2022) Alberta, Canada	“Opiliones”	2	Aves	$\delta^{15}\text{N}$ was higher than the mean for predatory arthropods, but ~1.2‰ lower than spiders. Small sample size for harvestmen. (P)
This Study Belize	<i>Erginulus clavotibialis</i> # <i>Holovonones pilosus</i> # <i>Prionostemma sp.</i> 1¶	23	Arach	$\delta^{15}\text{N}$ was enriched for <i>Erginulus</i> and <i>Holovonones</i> , similar to scorpions and spiders. <i>Prionostemma</i> was mostly carnivorous. (Primarily P)

*Phalangidae.
†Nemastomatidae.
‡Trogulidae.
§Travuniidae.
¶Sclerosomatidae.
#Cosmetidae.

Table 2.—Mean stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) values and mean C and N content for harvestmen and other invertebrates observed in the study site.

	Sample Size	Mean $\delta^{13}\text{C}$	Mean %C	Mean $\delta^{15}\text{N}$	Mean %N
Harvestmen					
<i>Erginulus clavotibialis</i> male	6	-26.04	46.14	6.94	12.87
<i>Erginulus clavotibialis</i> female	6	-26.15	45.47	7.02	12.44
<i>Erginulus clavotibialis</i> nymph	6	-26.29	46.45	7.31	11.72
<i>Holovonones pilosus</i>	3	-26.50	45.58	7.14	13.05
<i>Prionostemma</i> sp. 1	2	-28.07	49.39	6.76	10.63
Other Arthropods					
Buthid Scorpion (<i>Nyssodesmus</i> sp.)	6	-25.75	49.10	7.09	11.58
Ctenid Spider (<i>Cupiennius</i> sp.)	1	-25.30	45.94	7.02	12.75
Grasshopper	2	-28.50	46.67	5.13	12.16
Katydid	4	-28.01	47.26	5.02	12.73
Ants	1	-30.27	49.23	3.24	9.88
Termites	1	-26.04	46.14	3.73	8.45

ontogenetic variation in pedipalp morphology between cosmetid nymphs and adults (Townsend & Enzmann 2018); nymphs have thinner, cylindrical pedipalps, whereas adults have larger, flattened, spoon-shaped pedipalps (Pagoti et al. 2019). In addition, the males have substantially larger chelicerae compared to females (Goodnight & Goodnight 1976), suggesting that males may feed upon different prey than females. While these morphological differences could be due to other reasons, such as sexual selection (Buzatto & Machado 2014; Solano-Brenes et al. 2018), it suggests that nymphs, females, and males may feed on different food items, potentially resulting in different trophic positions for each, and that isotopic and fecal samples of these harvestmen could reveal evidence of omnivorous diets.

METHODS

Samples for isotopic analysis were collected in the tropical seasonal forest reserve at Clarissa Falls Resort, Belize (17°06'56"N, 89°07'15"W). At this location, we have examined the ecology of harvestmen in prior investigations (Schaus et al. 2013; Albert et al. 2019) and much of the natural history of these species is known from studies by Goodnight & Goodnight (1976, 1977). In order to put the trophic position of harvestmen into the context of the local food web, samples of harvestmen and other invertebrates were collected for isotopic analysis. The species of harvestmen that were examined included the cosmetids *Erginulus clavotibialis* and *Holovonones pilosus* (Goodnight & Goodnight 1977) and the sclerosomatid *Prionostemma* sp. 1 Pocock 1903. We have observed at least 4 species of *Prionostemma* in Belize (Townsend and Schaus, unpublished data); this study focused on *Prionostemma* sp. 1, which has a large red dot on the posterior portion of the abdomen. We captured males, females, penultimate and antepenultimate nymphs for *E. clavotibialis*, and identified this species based on size and other morphological characteristics. Other invertebrates representing herbivores and carnivores were sampled from this location, to help put the diets of the harvestmen in a food web context. Additional taxa included specimens of scorpions and spiders (carnivores), orthopterans (herbivores), and termites and ants (fungivores) (Table 2). All invertebrates were collected by hand from the vegetation, the leaf litter, or beneath logs and rocks in forested habitats, using forceps, as needed.

Arthropods to be processed for isotopic analyses (Table 2) were housed separately by species in round, vented plastic containers

(15 cm diameter) lined with moist paper towels and provided water *ad libitum* for 24 hours, but not fed. Containers were kept out of direct sunlight in shade or a darkened room. This process allows these organisms to void the majority of the gut contents, so that the isotopic ratios would more accurately represent that of the organisms sampled and minimize any isotopic contribution of the ingested food, following the recommendation of Potapov et al. (2019). The specimens were then euthanized by freezing for at least 8 hrs, dried overnight at 60°C, ground to a fine powder using a mortar and pestle, and shipped to Washington State University's Stable Isotope facility, where they were analyzed for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, %C and %N using a continuous flow isotope ratio mass spectrometer (Delta PlusXP, Thermofinnigan, Bremen). Isotopic δ units are reported as a part per thousand difference from the reference standard, Vienna Pee Dee Belemnite for $^{13}\text{C}/^{12}\text{C}$ and atmospheric N for $^{15}\text{N}/^{14}\text{N}$; higher values indicate a relative enrichment of the heavier isotope (Peterson & Fry 1987). The $\delta^{13}\text{C}$ of consumers typically matches closely with their food items (0–0.5‰ enrichment), whereas $\delta^{15}\text{N}$ levels are typically enriched by 1.4–3.3‰ with each successive trophic level (Peterson & Fry 1987; McCutchan et al. 2003). Samples for *Prionostemma*, ants, and termites had to be pooled to ensure sufficient sample material for the isotopic analyses: three *Prionostemma* individuals of similar size were pooled per sample; many ants and many termites were pooled for the isotopic samples of each of those species.

Nitrogen isotopic signatures were compared statistically using ANOVA followed by a Tukey Post Test, with $\alpha = 0.05$ set as the level of significance. We first compared males, females, and nymphs ($n = 6$ for each group) of *E. clavotibialis* statistically, and then compared this species ($n = 18$), with the Orthoptera ($n = 6$) and the other arachnids ($n = 7$). *Holovonones*, *Prionostemma*, ants, and termites were excluded from the statistical analyses because of the small sample sizes, and because it was often necessary to pool several or all individuals collected into a single sample to have sufficient material for isotopic analysis. Where appropriate, the maximum contribution of plant materials to the diet was estimated using the 2-member mixing model described by Forsberg et al. (1993).

For fecal analysis, we captured adults ($n = 67$) and nymphs ($n = 29$) of *Erginulus clavotibialis* from two locations: Clarissa Falls, Cayo District, Belize 19–24 July 2018 and Las Cuevas Research Station (16°44'06"N, 88°59'09"W) in Chiquibul National Forest Reserve, Cayo District from 25–26 July 2018. Individuals were housed as described above, with nymphs, males, and females housed separately, and were held for 48–72 hrs. Each day, we checked the containers in the morning and again in the afternoon for the presence of fecal material. In total, we collected and examined 42 fecal samples (16 from nymphs, 26 samples from adults). When observed, feces were removed and preserved in 70% ethanol for observation using scanning electron microscopy (SEM). Fecal samples were dehydrated in a graded ethanol series and dried with hexamethyldisilazane (Nation 1983). We used forceps to break apart fecal pellets and spread the contents more evenly over the surface of aluminum stubs that were covered with graphite adhesives. We sputter-coated the samples with 10–20 nm of gold and examined them using a Hitachi S-3400 VP SEM (Tokyo, Japan) at accelerating voltages of 5–15 kV.

RESULTS

Males, females, and nymphs of *Erginulus clavotibialis* had similar mean $\delta^{15}\text{N}$ values (Table 2) that did not differ statistically

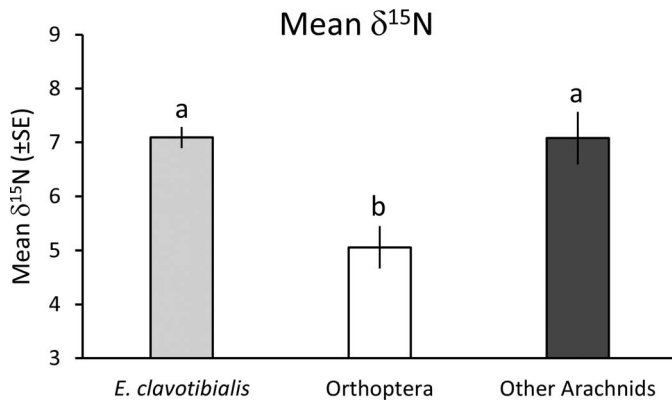


Figure 1.—Nitrogen Isotopic Ratios ($\delta^{15}\text{N}$) of *E. clavotibialis* (light gray bar), Orthoptera (white bar - katydids and grasshoppers) and other arachnids (dark gray bar - scorpions and spider). Overall, the different groups differed statistically ($P < 0.001$; $F_{2,28} = 10.477$), and different letters denote statistically significant differences ($P < 0.05$) among groups.

($P = 0.740$; $F_{2,15} = 0.3077$), indicating a similar trophic position. Thus, for all remaining analyses, all individuals of *E. clavotibialis* ($n = 18$) were grouped together. *Erginulus clavotibialis* had a mean $\delta^{15}\text{N}$ value of 7.09 (Fig. 1), which was similar to the mean $\delta^{15}\text{N}$ isotopic signatures of the carnivores (scorpions and spiders), but significantly elevated ($P < 0.001$; $F_{2,28} = 10.477$) $\sim 2\%$ ^{15}N over that of the herbivores, the katydids and grasshoppers (Table 2, Fig. 1), indicating that this species had a carnivorous diet. Our preliminary measures for the other harvestman taxa indicate that *Holovonones pilosus* had a $\delta^{15}\text{N}$ value similar to that of *Erginulus* (Table 2), and *Prionostemma* sp. 1 had a slightly lower mean $\delta^{15}\text{N}$ of 6.76 (Table 2). Data for *Holovonones* and *Prionostemma* should be interpreted cautiously, due to the small sample size. For *Holovonones*, we were only able to collect 3 individuals at this site, and for *Prionostemma*, it was necessary to pool three individuals of similar size to obtain sufficient material for each of the isotopic analyses, thus decreasing our sample size. The pooled samples of the leafcutter ants and of the termites had ^{15}N values lower than the other organisms observed in this study (Table 2), but these results are preliminary, due to the small sample size. Carbon isotope values were consistent among most of the harvestmen studied, with some relative depletion of ^{13}C in *Prionostemma* (Table 2).

For the fecal samples observed, all samples contained masses of bacteria and unidentifiable materials. However, we observed arthropod remains in the feces of both nymphs (Fig. 2) and adults (Fig. 3) of *E. clavotibialis*, and most of the fecal SEM samples we observed included at least some arthropod parts, including insects, lepidopterans, and other arachnids. It was difficult to quantify the proportion of the viewing field comprised of arthropod materials, or the proportion of the ingested food items that were more fully digested, and thus not present in the fecal samples, but these observations provide qualitative supporting evidence of arthropod feeding by *E. clavotibialis*. There was no clear difference in the diet composition of fecal samples taken from nymphs and adults, as fecal matter from both nymphs and adults contained a wide variety of arthropods, with no plant material observed (Figs. 2,3). While we did not observe plant or fungal material in the feces of this species, our subsequent studies of harvestmen in Virginia, U.S.A. have documented that plant and fungal materials can be observed in the feces (Townsend et al., unpublished data).

The lack of observed plant material in the feces, plus our isotopic data suggests that this species derives its nutrition primarily from animal sources: either live prey or scavenged food items.

DISCUSSION

The results of our study revealed that *E. clavotibialis* from western Belize is predominantly carnivorous (Fig. 1, Table 2). Our SEM observations of fecal samples also support the general observation that invertebrates comprise the major portion of the diet of *E. clavotibialis*. While this species may include other food items in an opportunistic manner, the realized trophic level observed was equivalent to that of other known carnivores, including spiders and scorpions. We found that adults (males and females) and nymphs had $\delta^{15}\text{N}$ values that did not differ significantly. This indicates that there was no major ontogenetic dietary shift in trophic level nor is there a sexual difference in trophic level for this species. It is possible that nymphs and adults could shift to feed on different invertebrates at the same trophic level (i.e., from feeding on one type of herbivore as nymphs to another herbivore as adults), as this technique integrates all nitrogen obtained, and would give similar values for differing organisms from the same trophic level. However, our fecal analyses did not observe dramatic differences in the diets of nymphs vs. adults either (Figs. 2,3) but did document that harvestmen feed on a variety of arthropods, including lepidopterans and other insects, and other arachnids, without evidence of plant feeding. The functional difference of the morphologically different mouthparts of juvenile and adult cosmetid harvestmen remains an unanswered question (Pagoti et al. 2019), which warrants further investigation.

Only a few studies have used stable isotope analysis to infer the trophic level of harvestmen (Table 1), and this study is the first to report the trophic level of tropical harvestmen using this technique. Some investigators have used stable isotope analysis to examine the trophic levels of a variety of soil invertebrates, but relatively few have primarily utilized this technique to quantify the diets of arachnids (Table 1; Nyffeler et al. 2016, 2023). König et al. (2011) examined the role of soil invertebrates, including two species of harvestmen in a glacier foreland soil food web and observed that the phalangiid harvestmen *Mitopus glacialis* (Heer, 1845) and *M. morio* (Fabricius, 1779) were trophically similar in the newly exposed site (deglaciated < 34 years), with both species functioning as intermediate predators. However, at an older site (deglaciated ~ 120 years) which did not have *M. glacialis*, *M. morio* functioned more as a top invertebrate predator, with an isotopic signal that was enriched $\sim 2\%$ ^{15}N ($2/3$ to one full trophic level) relative to the young and intermediate (deglaciated ~ 60 years) sites. DeHart et al. (2017) observed different trophic positions of harvestmen in different habitats. Harvestman $\delta^{15}\text{N}$ values were equivalent to web weaving spiders but lower than cursorial spiders in field habitats, whereas, in forested habitats, they held a trophic position approximately $1/3$ – $2/3$ of a trophic level lower than both types of spiders (deHart et al. 2017). The elevated $\delta^{15}\text{N}$ values of cursorial spiders could indicate a mix of herbivorous and carnivorous prey items. Our results are similar to the findings of several isotopic studies of harvestman diet (Table 1), as the taxa in this study were primarily carnivorous, with *Prionostemma* suggesting a small portion of its diet may have been derived from plant sources. Out of the 13 other original studies that reported harvestmen diet using stable nitrogen isotopes (Table 1), four studies categorized harvestmen as intermediate omnivores, four studies (plus the current study)

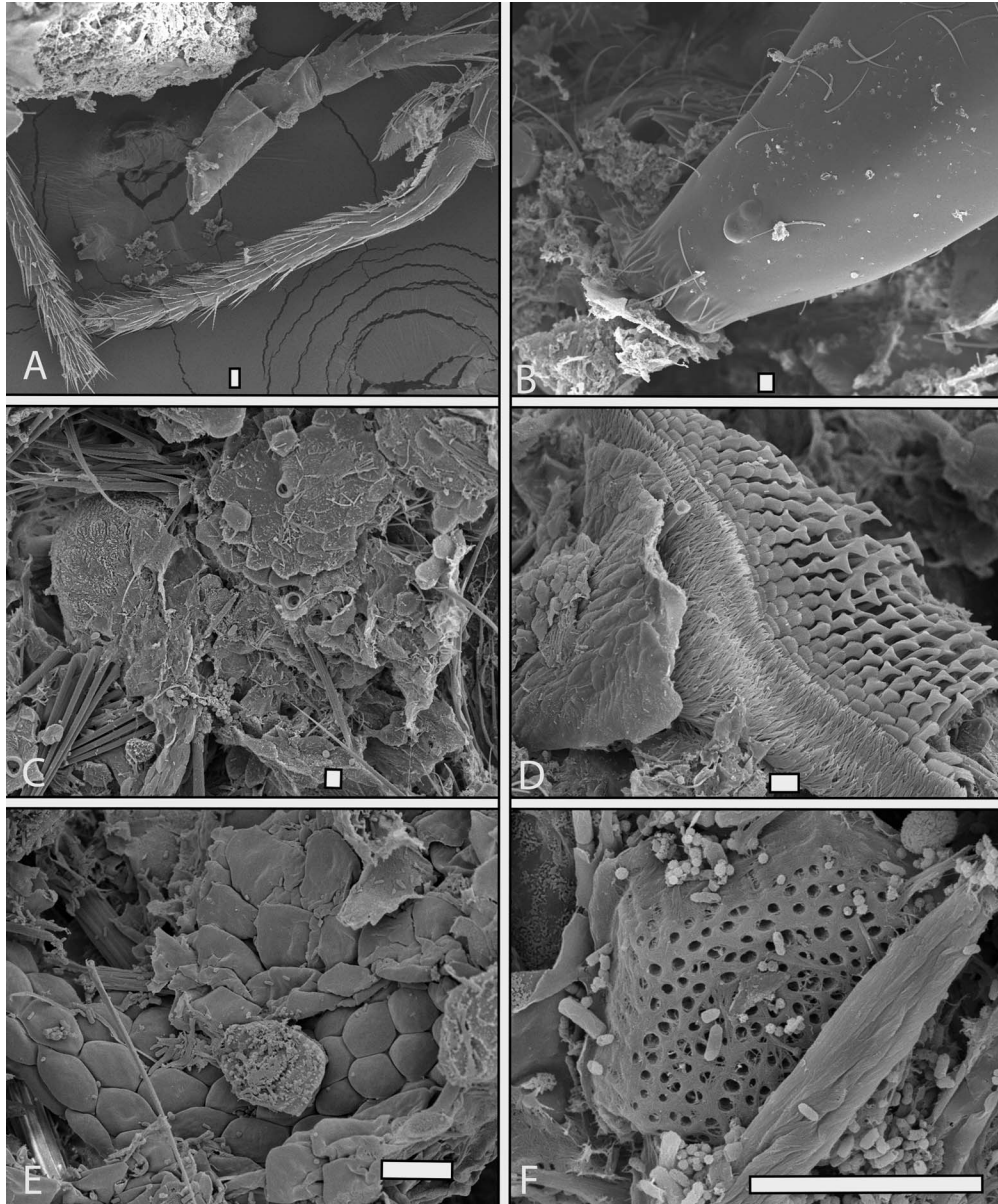


Figure 2.—SEM micrographs of fecal samples obtained from nymphs of *E. clavotibialis*. A. Distal segments of appendages from an unidentified arthropod. B. Part of the cephalothorax of an unidentified arachnid. C. Setae and cuticle of unidentified arthropod. D. Remnants of arthropod cuticle E. Part of the compound eye of an insect F. Inner surface of arthropod cuticle. Scale bars = 10 μ m.

categorized them as primarily carnivorous, and five studies had results suggesting that the trophic level of harvestmen can vary substantially among taxa or between locations.

Stable isotope analysis has also been used to document dietary shifts, including shifts between differing primary carbon sources or shifts in trophic level. Gratton et al. (2008) quantified the contribution of aquatic midges to the diet of terrestrial predators, including harvestmen, and observed that near lakes with a larger midge emergence, harvestman species demonstrated a shift in $\delta^{13}\text{C}$, reflecting greater reliance on aquatic midges, either via predation or via scavenging. Shaner & Macko (2011) quantified the isotopic signature of harvestmen as a part of a study examining the impact of experimental nutrient pulses (millet seeds and cicadas) on the diet of the white-footed mouse, *Peromyscus leucopus* (Rafinesque, 1818). Their

study observed that harvestmen had $\delta^{15}\text{N}$ values similar to other aboveground arthropods, and that the $\delta^{15}\text{N}$ values of mice shifted in response to both types of nutrient pulses, documenting greater carnivory when mice shifted to feed on cicadas (Shaner & Macko 2011). These changes in isotopic signals following dietary shifts are similar to the theoretical changes described by Potapov et al. (2019). In our study, we did not document shifts in trophic level with ontogeny, as nymphs and adult *E. clavotibialis* occupied equivalent trophic positions (Table 2).

Stable isotope analyses show great potential for quantifying feeding relationships in complex food webs, such as those that occur in tropical rain forests (Forsberg et al. 1993; Herrera et al. 2002). These techniques are especially useful in combination with observational and lab studies, because isotopic analyses integrate

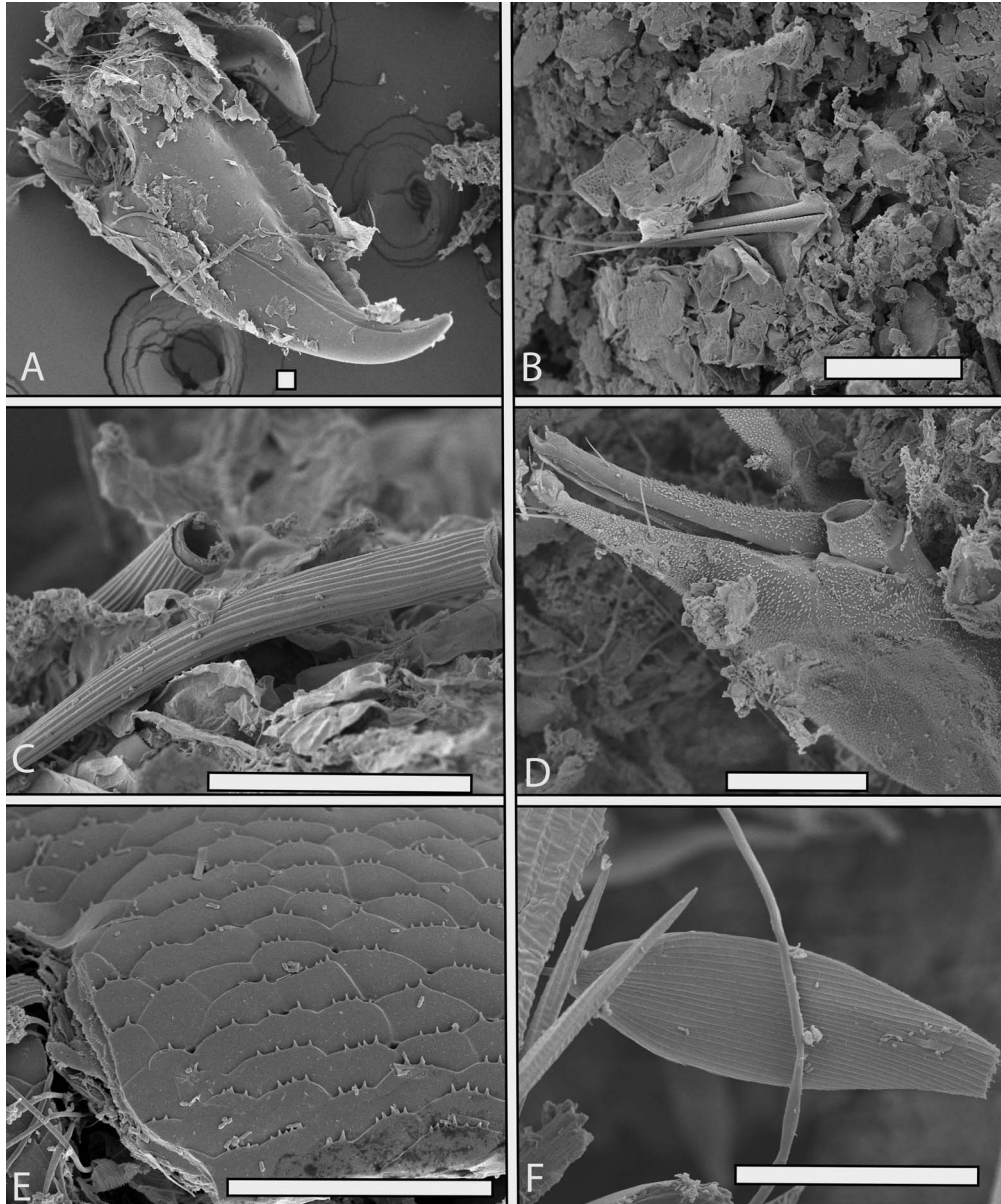


Figure 3.—SEM micrographs of fecal samples obtained from adults of *E. clavotibialis* (A-C are from females, D-F are from males). A. Arthropod mouthpart. B. Parts of an arthropod. C. Arthropod setae. D. Arthropod appendage. E. Arthropod cuticle. F. Lepidopteran scale. Scale bars = 30 μm .

feeding history and the food items actually assimilated, but do not identify specific prey items. Thus, a specific isotopic signature could reflect exclusive feeding on a particular diet item or feeding on multiple diet items that average out to that isotopic value. Likewise, occasional feeding on materials that are poorly digestible may not be reflected in an organism's isotopic signature because of the reduced assimilation of these diet items. Field observations, gut content analyses, and/or fecal studies can be more effective at identifying short term changes in diet, whereas isotopic analyses are more effective when investigating the integration of long-term feeding history (Beaudoin et al. 1999; Schaus et al. 2002). Ideally, a combination of techniques to analyze diet can work in a complementary manner. As an example, Herrera et al. (2002) used a combination of stable nitrogen isotope analysis and fecal studies to examine the protein source of five species of fruit-eating bats in tropical forests of Mexico.

Their study documented that the majority of the nitrogen came from fruits, even though this food source is lower in nitrogen compared to insects. Two species had individuals that supplemented their diet with insects, and this became more pronounced during the dry season, when fruits were scarcer. In the Herrera et al. (2002) study, stable isotope analyses were more effective at quantifying the variable proportion of the diet derived from plant vs. insect sources, although some fecal samples also documented insectivory, and the examination of feces allowed the investigators to distinguish between bats feeding on fruit vs. pollen. As in our study, a combination of these two approaches allowed for a more comprehensive description of the diet.

Likewise, the conclusions of laboratory and field studies of diet can differ substantially (i.e., Edgar 1971; Gnaspi 1996; Santos & Gnaspi 2002). Our earlier laboratory investigation of feeding by *E. clavotibialis* showed that this species is capable of

capturing and consuming live arthropods (termites), and that this species has a slight preference for fruit, at least in a laboratory setting (Schaus et al. 2013). In contrast, the stable isotope analyses in this study documented that *E. clavotibialis* relies most heavily on invertebrates in the field as the major component of the long-term diet. The availability of arthropod prey and fruits likely varies somewhat on a seasonal basis, but our results document that over the time scale of tissue turnover, this species fed primarily on arthropod prey (either live captured or scavenged) and was equivalent in trophic position to other carnivores (Fig. 1, Table 2). While this finding does not preclude the option for opportunistic foraging by this species on foods that are rich in sugars, lipids, or protein, it does suggest that this may not occur frequently in this species' natural habitat.

Studies utilizing stable isotopes are advantageous because they quantify the proportion of the diet derived from different sources and can be used to determine the *in situ* trophic position. This quantitative nature puts this approach at an advantage over haphazard or sporadic field observations of feeding, which are more of a snapshot of feeding at that time. Future studies could utilize these techniques and/or other analytical approaches (i.e., metabarcoding of gut contents) to quantify the diets of other harvestmen in the field. It is especially helpful to utilize a combination of approaches, where one approach (i.e., stable isotope analysis) can be supplemented by the results of the other approach (i.e., fecal analysis), in order to combine the relative advantages of each of the approaches. These techniques and other analytical approaches can contribute much to our understanding of the feeding ecology and natural history of tropical harvestmen, including how the diet can vary ontogenetically, seasonally, among species, and among habitats.

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