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Experimental test of the mechanism underlying sexual segregation at communal roosts of harvestmen (*Prionostemma* spp.)

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Abstract

Closely related, ecologically similar species often roost in distinctly different habitats, and roosting patterns also vary within species in relation to sex, age and season. The causes of such variation are not well understood at either a proximate or ultimate level. We studied communal roosting in two congeneric species of *Prionostemma* harvestmen at a rainforest site in Nicaragua. Previous research showed that *Prionostemma* sp. 1 forms male-biased communal roosts in tree-root cavities, while *Prionostemma* sp. 2 forms communal roosts of variable but temporally stable sex ratios on spiny palms. Here, we investigate potential mechanisms underlying variation in roosting site choice between and within these syntopic species. First, we present the results of a field experiment designed to probe the mechanism underlying skewed roost sex ratios in *Prionostemma* sp. 2. Previous studies have suggested that these harvestmen use conspecific scent to locate communal roosts and that new roosts can be established via group translocation. Therefore, to test the hypothesis that skewed roost sex ratios in this species arise from sex differences in scent marks, we translocated single-sex groups of ca. 30 individuals to each of 20 previously unoccupied spiny palms. Female release sites attracted new recruits of both sexes, while male release sites attracted almost exclusively males. We infer that *Prionostemma* sp. 2 females preferentially roost in sites scent-marked by females and that this mechanism is sufficient to explain the skewed roost ratios. Further adding to knowledge of *Prionostemma* roosting behavior, we show that *Prionostemma* sp. 1 forms female-biased communal roosts on spiny palms, that some roosts contain both species, and that the species composition is stable on a time scale of at least 2 weeks. To the best of our knowledge, this study is the first experimental test of mechanisms underlying sexual segregation at communal roosts in any taxon.

KEYWORDS

communal roost, Opiliones, roosting aggregation, sex ratio, translocation experiment

1 | INTRODUCTION

Many animal species aggregate during periods of inactivity, a behavior pattern referred to as communal roosting (Beauchamp,

1999; Bijleveld, Egas, van Gils, & Piersma, 2010; Laughlin, Sheldon, Winkler, & Taylor, 2014). Communal roosts have been studied in primates (Anderson, 1998, 2000; Anson, Hammerschmidt, & Todt, 1992), bats (Foster & Kurta, 1999), birds (Beauchamp, 1999; Eiserer, 1984), insects (Devries, Schull, & Greig, 1987; Finkbeiner, Briscoe, & Reed, 2012; Grether & Switzer, 2000) and arachnids (Pereira,

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Elpino-Campos, Del-Claro, & Machado, 2004; Wade, Loaiza-Phillips, Townsend, & Proud, 2011).

Comparative studies of communal roosting generally classify species as exhibiting communal roosting behavior or not (Beauchamp, 1999), but there is also considerable variation in the behavior of species that form communal roosts (Laughlin et al., 2014). While some closely related species are found together in mixed aggregations, others roost in distinctly different habitats (Grether et al., 2014a; Kunz, 1982; Proud et al., 2012; Ruczynski & Bogdanowicz, 2005). In some species, individuals are highly faithful to particular roost sites, while in other species the location of communal roosts is stable but individuals move freely among roosts (reviewed in Laughlin et al., 2014). Roosting patterns can also vary within species, in relation to sex, age and season (Blanco & Tella, 1999; Donazar & Feijoo, 2002; Kunz, 1982; Pereira et al., 2004; Summers, Westlake, & Feare, 1986).

The causes of variation in communal roosting behavior are largely unstudied at either a proximate or ultimate level. Laughlin et al. (2014) showed, with an individual-based model, that a wide range of communal roosting patterns can be produced by varying the levels of individual roost site fidelity and conspecific attraction. The sensory mechanisms through which individuals assess suitable roosting sites and detect conspecifics are also likely to affect population-level roosting patterns and their ecological consequences (Grether, Levi, Antaky, & Shier, 2014b).

We investigated intra- and inter-specific variation in roost site choice of two syntopic species of harvestman (Eupnoi: Sclerosomatidae: Gagrellinae: *Prionostemma* Pocock, 1903) at a lowland rainforest site in Nicaragua. Following previous authors, we refer to these unnamed species as *Prionostemma* sp. 1 and *Prionostemma* sp. 2 (Grether et al., 2014a; Proud et al., 2012). Both species roost during the day (dawn to dusk) and leave the roosts to forage at night (Donaldson & Grether, 2007; Grether et al., 2014a). Individual site fidelity varies (Donaldson & Grether, 2007; Grether et al., 2014a), but roosting aggregations tend to form repeatedly in the same locations, with some sites being used for over a decade (Grether et al., 2014b; Teng, Dao, Donaldson, & Grether, 2012).

At our study site, *Prionostemma* sp. 2 usually forms loose roosting aggregations in spiny palms (*Bactris*, *Astrocaryum*) (Grether & Donaldson, 2007). The roost sex ratio varies and most roosts are consistently skewed toward one sex or the other (Grether et al., 2014a). Surprisingly, roosts with skewed sex ratios remained skewed in the same direction even after all of the animals were experimentally removed and the roosts were recolonized by new individuals (Grether et al., 2014a). A possible explanation is that the sexes differ in their roost site microhabitat preferences, but no microhabitat differences have been found between male-biased and female-biased roosts of this species (Grether et al., 2014a). An alternative hypothesis is that the sexes differ in the quantity or quality of scent-marking chemicals and that individuals of one or both sexes are preferentially attracted to same-sex scent marks (Grether et al., 2014a). Sexually dimorphic scent glands have been described in harvestmen (Murayama & Willemart, 2015; Willemart, Perez-Gonzalez, Farine, & Gnaspini, 2010), and *Prionostemma* sp. 2 have been observed rubbing against the substrate

at roosting sites, a possible scent-marking behavior (Donaldson & Grether, 2007). While the scent glands and chemicals have yet to be characterized, the results of field experiments strongly suggest that these animals use conspecific scent to locate communal roosts (Donaldson & Grether, 2007; Teng et al., 2012). One such experiment showed that new communal roosts can be established by translocating groups of harvestmen to previously unused spiny palms (Teng et al., 2012).

To test the hypothesis that skewed roost sex ratios in *Prionostemma* sp. 2 result from attraction to same-sex scent marks, we carried out single-sex group translocations. If attraction to same-sex scent marks is the mechanism underlying the skewed roost sex ratios, then, we predicted, one or both sexes should preferentially colonize same-sex release sites. We also took microhabitat measurements at release sites to further test the alternative hypothesis that skewed sex ratios arise from sex differences in roost site microhabitat preferences.

The other syntopic species, *Prionostemma* sp. 1, was previously found to form male-biased communal roosts in tree-root cavities (Grether et al., 2014a). Male-biased population sex ratios are quite rare in harvestmen, and thus it was inferred that females of this species tend to roost somewhere other than tree-root cavities (Grether et al., 2014a). Indeed, during the current study, we found female *Prionostemma* sp. 1 roosting in spiny palms, along with *Prionostemma* sp. 2. In addition to documenting this sex difference in roosting habitat, we provide the first description of mixed-species aggregations in *Prionostemma*. Specifically, we investigated whether the *Prionostemma* species composition at particular sites is variable or stable over time, whether the species segregate vertically in the spiny palms, and whether the presence of one species affects the vertical position of the other.

Thus, in this paper, we (i) use a field experiment to examine the underlying causes of roost sex ratio variation in *Prionostemma* sp. 2, (ii) characterize the sex difference in roosting habitat of *Prionostemma* sp. 1, and (iii) begin to elucidate the patterns and causes of variation in mixed-species *Prionostemma* roosting aggregations.

2 | MATERIALS AND METHODS

2.1 | Study area

We conducted this study at Refugio Bartola, a lowland tropical rainforest in southeastern Nicaragua (10.973°N, 84.339°W), during the beginning of the dry season from 3–16 February 2015. This private reserve is contiguous with Indio Maíz Biological Reserve (ca. 4500 km²). The climate is wet tropical, with about 4 m of rainfall per year, peak precipitation in June–August, and a dry season from February–April (Cody, 2000). Approximately 56 mm of rain fell at Refugio Bartola during the study period.

2.2 | Operational definitions

As in all previous publications on *Prionostemma* roosting behavior at this study site, we use the terms communal roost and roosting

aggregation interchangeably to refer to groups of two or more individuals resting in the same site (Donaldson & Grether, 2007; Grether & Donaldson, 2007; Teng et al., 2012; Grether & Donaldson, 2007; Teng et al., 2012; Grether & Donaldson, 2007; Grether, Levi et al., 2014). In the case of spiny palms, which tend to grow in clusters with overlapping fronds, we consider palms with trunks within 1 m of each other to belong to the same roost site. The rationale for this site definition is that harvestmen aggregations often span the fronds of multiple palms and individual harvestmen can easily move between them. In the case of tree cavities, we consider a single cavity to be a roost site. While some investigators have defined harvestman aggregations in terms of leg overlap (Machado & Macias-Ordóñez, 2007; Willemart & Gnaspini, 2004), in the case of *Prionostemma* communal roosts, such a criterion would be arbitrary and difficult to apply because roost sites can contain multiple indistinct subgroups and individuals often move while they are being counted.

2.3 | The causes of sex ratio variation in *Prionostemma* sp. 2 communal roosts – Translocation experiment

2.3.1 | Effects of same-sex scent on recruitment

In this experiment, single-sex groups of *Prionostemma* sp. 2 were translocated from existing roosting aggregations (source sites, $n = 12$) to sites without *Prionostemma* (release sites, $n = 20$). Release sites were *Bactris* spiny palm sites where we did not find either species of *Prionostemma* during daily surveys carried out for 2 or 3 days prior to the experiment and were farther than 20 m apart from each other and from all existing communal roosts. These sites were temporally paired (i.e., the first two sites to be used were paired, the second two sites were paired, etc.) and then randomly assigned, within pairs, to one of two treatments: male release (all founders, i.e., individuals translocated to the site, were male) or female release (all founders were female). We carried out 2–4 translocations per day from 4–10 February 2015. On a given day that a source site was used in the experiment, the *Prionostemma* were captured by hand; individuals that were too high to be captured initially were chased down using a wooden pole. Founders (i.e., *Prionostemma* sp. 2 captured for translocation) were given a paint mark (Marvy Decocolor, Uchida of America, Torrance, CA USA) on the dorsal surface of the abdomen indicating their sex and release site, and placed in single-sex mesh cage (Bioquip Products, Rancho Dominguez, CA USA) for transport to a release site. We placed approximately 30 harvestmen of a single sex at each release site (308 males, 28–33 per site, mean \pm SD = 30.8 ± 1.5 , $n = 10$ sites; 315 females, 29–33 per site, mean \pm SD = 31.5 ± 1.2 , $n = 10$ sites). To minimize desiccation and handling effects, translocations were carried out in the morning (0900–1100) and animals were held captive for <2 hrs. All *Prionostemma* sp. 1, and any *Prionostemma* sp. 2 in excess of the number needed for the translocation experiment, were left unmarked and released back to the source site from which they were taken. Some source sites were used on more than 1 day to obtain founders, and

some groups of founders included individuals from multiple source sites; previous research has shown that roost membership is quite fluid and that these harvestmen do not distinguish roost mates from non-roost mates (Donaldson & Grether, 2007; Grether & Donaldson, 2007).

We counted the number of marked founders (i.e., returning) and unmarked (i.e., recruited) *Prionostemma* of each sex and species at the release sites 1 day post-release and once again 2–7 days post-release. *Prionostemma* can easily be counted, but not sexed, without being captured. Thus, the animals were captured by hand, held temporarily in a mesh cage, sexed and then placed back in the same release site. Post-release surveys were conducted between 1400 and 1600 hrs, which is well before the animals begin leaving the roost to forage (approximately 1730 hr) (Grether & Donaldson, 2007). Because the animals were not marked individually, our analyses of the number of returning founders and new recruits are therefore based on minima. For example, if five unmarked harvestmen were found in the first post-release survey and seven were found in the second post-release survey, we used seven, not twelve, as the number of new recruits. It was not feasible for observers to be blind to the experimental treatments of the release sites.

2.3.2 | Effects of habitat characteristics on recruitment

No habitat characteristics have previously been found to correlate with the sex ratio of *Prionostemma* sp. 2 communal roosts (Grether et al., 2014a), but the size of the aggregations has been shown to correlate positively with spine density and negatively with canopy openness and air temperature (Teng et al., 2012). To test for effects of habitat variables on overall recruitment or sex-specific recruitment in the translocation experiment, we took seven habitat measurements at each release site, including the characteristics previously found to correlate with aggregation size and some additional characteristics that might affect recruitment, as follows:

1. Number of spiny palms in the cluster. Because harvestmen rarely use spiny palms less than 2 m in height, we only counted palms greater than 2 m in height (Grether & Donaldson, 2007; Grether et al., 2014b). Clusters were defined as described above (Operational definitions).
2. Spine density. As in previous studies, we counted spines within a 4-cm² wire quadrat placed along the trunk of the palm at 0.8, 1.15 and 1.55 m above the ground (Teng et al., 2012). Spine counts were made on all trunks in clusters of fewer than 6 palms and on half of the trunks in clusters of 6 or more palms; the mean spine density for each release site was used in the analysis.
3. Canopy openness. We measured forest canopy openness with a concave spherical densiometer (Forestry Suppliers Inc., Jackson, MS, USA) while standing close to the release site.
4. Spiny palm density. To estimate the density of spiny palms in the vicinity of each release site, we counted the number of spiny palms within a 5-m radius from the center of the release site.

5. Contact with surrounding vegetation. To measure the extent of contact with the surrounding vegetation, we counted the stems of all plants in contact with spiny palms in the cluster.
- 6,7. Air temperature and relative humidity. We measured air temperature and relative humidity with a Kestrel 3000 (Nielsen-Kellerman Co., Boothwyn, PA, USA) in the early afternoon (1300–1500) at eye level while standing next to the release site.

For consistency, one person took all measurements of each type.

2.4 | Sex differences in roosting habitat of *Prionostemma* sp. 1

To test for sex differences in roost site selection in *Prionostemma* sp. 1, we compared spiny palm source sites with sp. 1 ($n = 8$ spiny palm source sites) to buttressed tree-root cavities where we found roosting aggregations of sp. 1 ($n = 12$ cavities). We captured all of the harvestmen in the root cavities by hand, counted and sexed them and returned them to the same cavities (121 males, 29 females).

2.5 | Mixed-species *Prionostemma* roosting aggregations in spiny palms

Mixed-species roosts have not previously been described in *Prionostemma* and thus one of our goals is to provide a quantitative description of this phenomenon. We examine whether the species composition of the roosts was stable over the course of our study, whether the species segregated vertically within the spiny palms, and whether the presence of one species affects the vertical position of the other. We also test for species differences in roost sex ratio and vertical segregation between the sexes of both species.

For species composition and vertical segregation analyses, we combined data from the 12 source sites used in our translocation experiment with visual counts taken at 32 other roosting aggregations. Visual counts of mixed-species roosts were made between 0900 and 1600 hrs using a flashlight and binoculars (the animals were not captured and thus the sexes were not distinguished). Visual counts are undoubtedly less accurate than capturing all the animals by hand (as was done in the translocation experiment), but these harvestmen form relatively loose aggregations and while legs are often overlapping, the bodies are easy to distinguish (Figure 1). Whenever possible, two observers counted the harvestmen independently and compared the results, and if the independent counts did not match, both observers repeated independent counts until the difference was only 1 or 2 individuals. Based on this we are confident that the method is sufficiently accurate.

2.5.1 | Species difference in communal roost sex ratio

To test for a difference between the species in the sex ratio of communal roosts in spiny palms, we used the data collected at source sites (see above).

2.5.2 | Species composition stability

To examine the temporal stability of the species composition at particular sites, we made visual counts on two different days at 15 sites (a subset of the 32 sites that were not used in the translocation experiment). Repeat counts were made 1 to 13 days apart (mean \pm SD = 7.9 ± 4.6 days, $n = 15$ sites).

2.5.3 | Sex and species differences in roosting height

Prionostemma can be found from the base of the trunk to the crown of spiny palms. To test for a sex difference in vertical distribution within the spiny palms, we counted the number of males and females above and below 2 m, separately by species, at 10 of the 12 source sites (at two source sites we only captured harvestmen below 2 m, so these sites were excluded from the analysis). Although some source sites were used on multiple days in the translocation experiment, we only used data from the first count when testing for a sex difference in roosting height ($n = 8$ sites with species 1; $n = 10$ sites with species 2; $n = 10$ sites with both species).

To test for a species difference in roosting height, and for effects of one species on the roosting height of the other, we combined data from source sites with data from roosting sites where we made visual counts of the number of harvestmen of the two species above and below 2 m ($n = 27$ sites, a subset of the $n = 32$ sites where visual counts were made). To avoid confounding roosting height with possible species differences in roosting habitat, this analysis was restricted to sites where we found both species ($n = 8$ source sites, $n = 14$ other aggregation sites; $n = 22$ sites total). To test for effects of one species on the roosting height of the other, we compared sites where only one species was found to sites where both species were found.

2.6 | Statistical analysis

To analyze recruitment of new *Prionostemma* sp. 2 (i.e., unmarked individuals) to the release sites, we used negative binomial regression (NBR) with the number of recruits as the dependent variable, the sex of the recruits and experimental treatment (sex of the founders) as categorical predictor variables, and release site as a random-effects panel variable in `xtnbreg` (Stata 14.1, StataCorp, College Station, TX, USA). NBR is a generalized linear model that reduces to a Poisson regression if the data follow the Poisson distribution but which can also properly handle data that are over-dispersed relative to the Poisson distribution, as is often the case for counts (Hoffman, 2016). The NBR model described above allowed us to test for (i) effects of the experimental treatment (sex of the founders) on the number of recruits, (ii) a difference between the sexes in recruitment, and (iii) an interaction between the treatment and the sex of the recruits. The sex term tests for differences in the number of males and females, i.e., deviations of the sex ratio from 1:1. The treatment-by-sex interaction term tests for effects of the experimental treatment (sex of the founders) on the sex of the individuals recruited, and thus was of primary interest, given the goal of the experiment (see Introduction). Note

that if we had instead pooled the data across release sites, we could have used proportions tests for (i) and (ii) and a Chi-square test of independence for (iii), but sites with relatively high recruitment would have had a disproportionately large influence on the results (a form of pseudoreplication; Hurlbert, 1984). The NBR model (xtnbreg) allowed us to properly account for variation among release sites by including site as a random-effects term in the model. Post-estimation contrasts were used to dissect interactions between treatment and sex.

To test for effects of habitat characteristics on recruitment, we first reduced the seven habitat variables (see *Effects of habitat characteristics on recruitment*, above) to a smaller number of orthogonal variables using principal components (PC) analysis. We then calculated PC scores for the components with eigenvalues > 1 and entered the PC scores as continuous predictor variables in separate NBR models of the form described above. We tested for main effects of the PCs and both two- and three-way interactions between the PCs, sex of the recruits and treatment (sex of the founders). The PC-by-sex interactions test for effects of habitat characteristics on the sex ratio of recruits and thus were our primary interest.

To analyze return rates of founders (i.e., marked individuals released at the site), we used NBR with the number of returning founders as the dependent variable, treatment (sex of the founders) as the categorical predictor variable, and release site as a random-effects panel variable.

Although release sites were assigned to treatments at random, it was important to assess whether there were accidental microhabitat differences between treatments that could potentially have influenced recruitment. We therefore carried out a one-way MANOVA with all seven habitat variables (see *Effects of habitat characteristics on recruitment*, above) as the dependent variables and treatment (sex of the founders) as the categorical variable. We used the MANOVA model to test for a multivariate linear difference between treatments in microhabitat (equivalent to testing for a difference in Euclidian distance between treatments in multivariate space).

To test for sex differences in the roosting habitat of *Prionostemma* sp. 1., we used NBR with the number of harvestmen as the dependent variable, site type (root cavity or spiny palm) and sex (male or female) as categorical predictor variables, and roost site as a random-effects panel variable. A significant site type-by-sex interaction would indicate that the sexes differ in roosting habitat.

Prionostemma sp. 2 was found in more spiny palm roosts than was *Prionostemma* sp. 1 (see Results). To determine the probability of this occurring by chance, we used a two-by-two Fisher's exact test. To test for a species difference in abundance in the mixed-species roosts, we used NBR with the first count at each mixed-species roost as the dependent variable, species as a categorical predictor variable, and roost site as a random-effects panel variable.

To test for differences between the two *Prionostemma* species in the sex ratio in spiny palm roosts, we used NBR with the number of harvestmen as the dependent variable, species and sex as categorical predictor variables, and roost site as a random-effects panel variable. A significant species-by-sex interaction would indicate that the species differ in sex ratio in spiny palm roosts.

To characterize the stability (repeatability) of species composition in spiny palm roosts, we calculated the Spearman rank correlation between species composition (expressed as the proportion of species 1) at 15 sites on two different sampling dates. We also examined whether the difference in species composition at the same site on different dates depended on the number of days elapsed, by calculating the Spearman rank correlation between the number of days elapsed between repeat counts and the change in species composition. We computed *p*-values for Spearman rank correlations by permutation (permute, Stata 14.1).

To test for sex differences in roosting height in the spiny palm source sites (where we identified individuals to both species and sex), we constructed separate NBRs for each species and used the number of individuals as the dependent variable, sex and roost height (whether above or below 2 m) as categorical predictor variables, and roost site as a random-effects panel variable. A significant sex-by-roost height interaction would indicate that the sexes differ in roosting height.

To test for a species difference in roosting height across the full sample of spiny palm roosts where the harvestmen were identified to species, we used NBR with the number of individuals as the dependent variable, species and roost height (whether above or below 2 m) as categorical predictor variables, and roost site as a random-effects panel variable. A significant species-by-roost height interaction would indicate that the species differ in roosting height.

To examine whether the presence of *Prionostemma* sp. 1 affected the roosting height of *Prionostemma* sp. 2, we used NBR with the number of *Prionostemma* sp. 2 as the dependent variable, roost height and the presence or absence of sp. 1 as categorical predictor variables, and roost site as a random-effects panel variable. A significant interaction would indicate that the height of sp. 2 depends on the presence of sp. 1.

All reported *p*-values are two-tailed. Ranges, means and standard deviations are provided to facilitate comparisons with future studies.



FIGURE 1 Close-up view of a mixed-species communal roost of *Prionostemma* harvestmen in a spiny palm (*Bactris* sp.). The individual at the center is a female of *Prionostemma* sp. 2, and the individual in the upper left is a female of *Prionostemma* sp. 1. The other two individuals are males of *Prionostemma* sp. 2

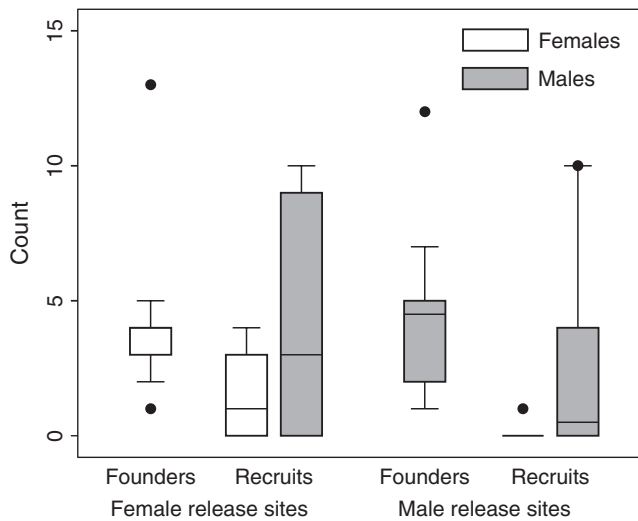


FIGURE 2 Translocation experiment results. Count (vertical axis): minimum number of *Prionostemma* sp. 2 found at the release sites. Founders: translocated harvestmen that returned to the site where they were released. Recruits: unmarked individuals. Box plots depict the median (horizontal line within the box), interquartile range (box), lower and upper adjacent values (whiskers), and outside values (dots). $N = 10$ sites in each treatment group

3 | RESULTS

3.1 | The causes of sex ratio variation in *Prionostemma* sp. 2 communal roosts – Translocation experiment

3.1.1 | Effects of same-sex scent on recruitment

New recruits were found in 12 (60%) of the release sites. As predicted by the same-sex chemical attraction hypothesis, the sex ratio among new recruits was affected by the sex of the founders (interaction between sex and treatment: $z = 2.03$, $p = .042$; Figure 2). Dissection of this interaction shows that the number of male recruits was not affected by the sex of the founders (treatment effect in males: $\chi^2 = 0.49$, $p = .48$), while female recruits were found almost exclusively in female release sites (treatment effect in females: $\chi^2 = 4.77$, $p = .029$). Overall, female release sites tended to attract more new recruits than did male release sites (treatment, $\chi^2 = 3.59$, $p = .058$) and more males were recruited than females (sex, $\chi^2 = 16.03$, $p = .0001$). The sex ratio of recruits was male-biased at both male release sites (sex at male release sites, $\chi^2 = 9.96$, $p = .0016$) and female release sites (sex at female release sites, $\chi^2 = 11.43$, $p = .0007$). In total, including founders and *Prionostemma* sp. 2 recruits, one female and 72 males (45 founders) were found in male release sites and 57 females (44 founders) and 40 males were found in female release sites.

Founders were captured at all 20 release sites (Figure 2) and invariably at the site where they were released. There was no significant difference between male release and female release sites in the return rate of founders (NBR, treatment: $z = -0.33$, $p = .74$; female release site range = 1–13, mean \pm SD = 4.3 ± 3.3 , $n = 10$; male release site range = 1–12, mean \pm SD = 4.7 ± 3.2 , $n = 10$).

TABLE 1 Habitat characteristic principal component loading matrix

Variable	PC1	PC2	PC3
No. of spiny palms in cluster	0.29	0.32	0.55
Canopy openness	0.17	0.56	-0.21
Spine density	0.15	0.31	-0.65
Air temperature	0.56	-0.23	0.30
Relative humidity	-0.57	0.19	0.21
No. of stems in contact	0.43	-0.24	-0.24
No. of spiny palms in 5 m	0.18	0.59	0.20
Eigenvalue	2.18	1.84	1.13

3.1.2 | Effects of habitat characteristics on recruitment

There was no multivariate difference between male and female release sites in habitat characteristics (MANOVA, Pillai's trace = 0.4519, $F_{7,12} = 1.41$, $p = .29$), confirming that our random assignment of sites to treatments did not result in accidental microhabitat differences between treatments.

Principal Components Analysis of the seven habitat variables yielded three PCs with eigenvalues >1 , cumulatively accounting for 73.7% of the variance (Table 1). There were no significant linear effects of the PCs on *Prionostemma* sp. 2 recruitment (NBR, all $p \geq 0.3$) and no significant PC by treatment, PC by sex, or PC by treatment by sex interactions (all $p \geq 0.2$, $n = 20$ release sites). Thus, recruitment of *Prionostemma* sp. 2 to the release sites was not affected by variation in the habitat characteristics we measured.

3.2 | Sex differences in roosting habitat of *Prionostemma* sp. 1

We checked 80 cavities in trees with buttress roots and found aggregations of *Prionostemma* sp. 1 in 12 (15%) of the cavities. The *Prionostemma* sp. 1 roosting aggregations in root cavities and spiny palms did not differ in size (NBR, site type, $\chi^2 = 0.13$, $p = .72$) but differed in sex ratio (interaction between site type and sex, $z = 6.61$, $p < .001$; $n = 8$ spiny palm source sites; $n = 12$ root cavity sites). Dissection of this interaction shows that root cavity roosts were strongly biased toward males (sex in cavity roosts: $\chi^2 = 22.69$, $p < .001$; male range = 2–52, mean \pm SD = 9.8 ± 14.0 ; female range = 0–11, mean \pm SD = 2.4 ± 2.9 , $n = 12$), while spiny palm roosts were strongly biased toward females (sex in spiny palm roosts: $\chi^2 = 24.99$, $p < .001$; male range = 0–6, mean \pm SD = 2.6 ± 2.2 ; female range = 1–76, mean \pm SD = 16.1 ± 24.8 , $n = 8$).

3.3 | Mixed-species *Prionostemma* roosting aggregations in spiny palms

3.3.1 | Frequency and abundance

At spiny palm roosts, *Prionostemma* sp. 2 were found more frequently and in greater abundance than *Prionostemma* sp. 1. The majority of

roosting aggregations in spiny palms contained both *Prionostemma* species (26 of 44; 59.1%), but *Prionostemma* sp. 1 was found in significantly fewer roosts (28 of 44; 63.6%) than was *Prionostemma* sp. 2 (42 of 44; 95.4%; Fisher's exact test, $p < .001$). In the mixed-species roosts, across all visits, the number of *Prionostemma* sp. 1 ranged from 1 to 82, while the number of *Prionostemma* sp. 2 ranged from 1 to 356. Using only data from the first visit to each site, we found significantly more *Prionostemma* sp. 2 (mean \pm SD = 42.5 ± 57.2) than *Prionostemma* sp. 1 (12.8 ± 17.5) in the mixed-species roosts (NBR, species, $z = 3.69$, $p < .001$, $n = 26$). The proportion of *Prionostemma* sp. 2 in the mixed-species roosts ranged from 0.091 to 0.995 (mean \pm SD = 0.71 ± 0.27 , $n = 26$).

3.3.2 | Species difference in communal roost sex ratio

The sex ratio in mixed-species communal roosts in spiny palms differed between the species (NBR, interaction between species and sex: $z = -2.62$, $p = .009$, $n = 8$ source sites). Across all of the source sites, the sex ratio was female biased in *Prionostemma* sp. 1 (NBR, sex in sp. 1: $z = 4.35$, $p < .001$; mean proportion female \pm SD = 0.82 ± 0.16 , $n = 8$ source sites) and male biased in *Prionostemma* sp. 2 (sex in sp. 2: $z = -3.34$, $p < .001$; mean proportion female \pm SD: 0.33 ± 0.14 ; $z = -3.34$, $n = 10$ source sites).

3.3.3 | Species composition stability

Over the 2-week period, some roost sites consistently attracted more *Prionostemma* sp. 1, while others attracted more *Prionostemma* sp. 2. At 15 roost sites where we counted the number of *Prionostemma* on multiple days, there was a strong positive correlation between the proportion of *Prionostemma* sp. 1 present on the first and last days ($r_s = .86$, $n = 15$ sites, $p = .004$; Figure 3). Across the range of time intervals between repeat counts (1–13 days), there was no significant correlation between the change in species composition and the number of days elapsed between repeat counts ($r_s = -.51$, $n = 15$ sites, $p = .09$). Thus, the species composition at particular roosting sites was relatively stable during our study.

3.3.4 | Sex and species differences in roosting height

We found no difference between the sexes in the number of individuals roosting above 2 m at source sites in either species (NBR, interaction between sex and roost height, species 1: $z = 0.45$, $p = .66$, $n = 8$ source sites; species 2: $z = -0.93$, $p = .35$, $n = 10$ source sites).

In the mixed-species roosts, *Prionostemma* sp. 1 roosted lower than *Prionostemma* sp. 2 (NBR, interaction between species and roost height: $z = 2.77$, $p = .006$, $n = 22$ roosts with both species). Across all of the sites where either species was found, *Prionostemma* sp. 1 showed no tendency to roost above or below 2 m (NBR, roost height: $z = 0.49$, $p = .62$, $n = 24$ roosts with species 1), while *Prionostemma* sp. 2 preferentially roosted above 2 m (NBR, roost height, $z = 3.64$, $p < .001$, $n = 35$ roosts with species 2). At mixed-species roosts, the

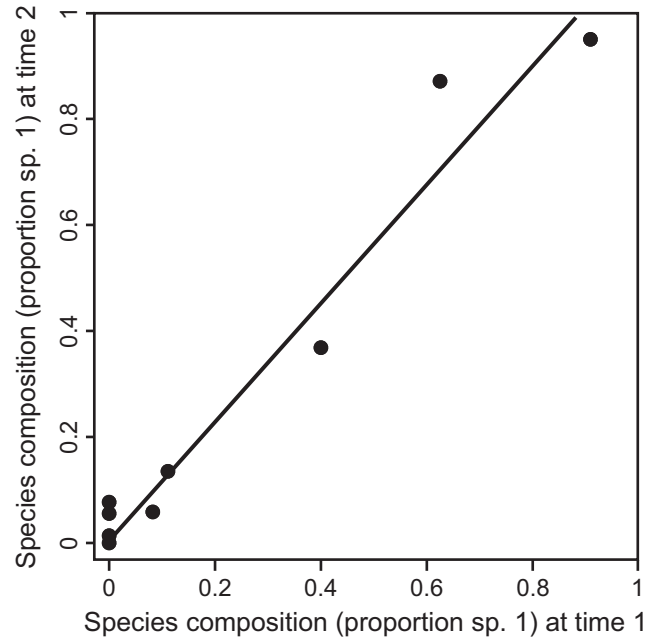


FIGURE 3 Consistency in species composition over two sampling times at spiny palm roosting aggregation sites. Times 1 and 2 refer to two different days on which harvestmen were counted. $N = 15$ sites. Zero *Prionostemma* sp. 1 were found at 7 sites and thus seven points are overlapping at (0, 0)

average proportion of individuals roosting above 2 m was 0.58 ± 0.41 (mean \pm SD) for *Prionostemma* sp. 1 and 0.80 ± 0.29 for *Prionostemma* sp. 2 ($n = 22$ sites). Comparing the mixed-species roosts ($n = 22$) to roosts where *Prionostemma* sp. 1 was not found ($n = 13$), *Prionostemma* sp. 2 did not roost higher (or lower) in the absence of *Prionostemma* sp. 1 (NBR, interaction between roost height and presence of species 1: $z = 1.66$, $p = .1$). We lacked sufficient data to test for an effect of *Prionostemma* sp. 2 on the roost height of *Prionostemma* sp. 1, because sp. 2 was found in all but two roosting sites.

We found no evidence that the species were attracted or repelled by each other. The number of individuals of the two species in the aggregations was not significantly correlated, whether the analysis included all roosts (Spearman $r_s = -.017$, $p = .91$, $n = 44$) or only roosts where both species were found ($r_s = .22$, $p = .28$, $n = 26$). Thus, the number of *Prionostemma* sp. 1 in a roost appears to be independent of the number of *Prionostemma* sp. 2, and vice versa.

4 | DISCUSSION

4.1 | The causes of sex ratio variation in *Prionostemma* sp. 2 at communal roosts – Translocation experiment

We carried out a field experiment to test the hypothesis that skewed sex ratios in the communal roosts of *Prionostemma* sp. 2 are maintained by attraction to same-sex scent cues. Our results indicate that females were almost exclusively attracted to female release sites, while males were attracted to both male and female release sites

(Figure 2). A possible proximate explanation is that the sexes differ in scent-marking chemicals and that females, but not males, preferentially roost in sites marked with female scent. Sexually dimorphic scent-marking glands have been reported in other species of harvestmen (Murayama & Willemart, 2015). Other arachnids are known to distinguish between male and female scent (Cerveira & Jackson, 2013; Cross & Jackson, 2013), and there is evidence that females of the harvestman species *Leiobunum vittatum* can distinguish between female and male scent marks (Willemart & Hebets, 2012).

An alternative explanation for the observed sex-biased recruitment that does not require sex differences in scent-marking chemicals is that females (the larger sex) deposit larger amounts of scent and that males are more likely to find sites that are weakly marked with conspecific scent (i.e., male release sites). Consistent with this explanation, female release sites tended to attract more new recruits than did male release sites, and the sex ratio of new recruits was male biased at both female and male release sites (Figure 2). In other species of harvestmen, and arachnids generally, males tend to be more mobile than females (Foelix, 1996; Willemart & Gnaspini, 2004). Grether et al. (2014a) reported that after experimental removals of *Prionostemma* sp. 2, the post-recolonization roost sex ratio was more male biased than the sex ratio prior to removal, which is consistent with males being faster to colonize new sites.

In summary, skewed roost sex ratios in *Prionostemma* sp. 2 could result from (i) sex differences in scent-marking chemicals and attraction to same-sex scent in females, (ii) sex differences in the quantity of scent deposited and higher mobility in males, or a combination of these two mechanisms.

Why might females benefit from roosting with females or avoiding roosting with males? Roosting aggregations in *Prionostemma* and other tropical harvestmen probably function to provide some protection from predators (e.g., dilution or confusion effects, Grether & Donaldson, 2007; Willemart & Gnaspini, 2004; Chelini, Willemart, & Gnaspini, 2012), but this seems unlikely to account for sex differences in roosting behavior. Rather, the sex differences seem more likely the result of sexual conflict and/or behavior associated with reproduction. In some other communally roosting taxa, males and females segregate during the reproductive period (Bloss, Acree, Bloss, Hood, & Kunz, 2002; Bouchard, 2001). Female harvestmen might benefit from avoiding roost sites with an abundance of males because of sexual harassment and/or male-male aggression (see Chelini et al., 2012; Fowler-Finn, Triana, & Miller, 2014; Machado & Macias-Ordóñez, 2007; Willemart & Gnaspini, 2004). We have not observed aggression, mate guarding, egg laying or any other behaviors related to reproduction at *Prionostemma* sp. roosts, but our study and all previous studies of *Prionostemma* roosting behavior were carried out during the dry season, and reproduction probably occurs during the wet season. Based on year-to-year stability of the locations of the roosting aggregations, combined with evidence that the aggregations form in the same locations repeatedly because of attraction to conspecific scent marks (Teng et al., 2012), we suspect that *Prionostemma* roosting aggregations form year round. Thus, it seems possible that the sex differences in roosting behavior that we observed during the dry season are by-products of reproductive behavior during the wet season, but this remains to be investigated.

4.2 | Sex differences in roosting habitat of *Prionostemma* sp. 1

In *Prionostemma* sp. 1, we found a striking sex difference in roosting habitat: males roost primarily in buttressed tree-root cavities, while females roost primarily in spiny palms. Partial sexual segregation at roost sites has also been reported in damselflies (Switzer & Grether, 2000) and bats (Bloss et al., 2002; Bouchard, 2001; Encarnacao, Kierdorf, Holweg, Jasnoch, & Wolters, 2005; Law & Anderson, 2000; Levin, Roll, Dolev, Yom-Tov, & Kronfeld-Shcor, 2013) and appears to have multiple causes. In the greater mouse-tailed bat (*Rhinopoma microphyllum*), sexual segregation at roosts appears to be a by-product of sex differences in foraging habitat (Levin et al., 2013). In Eastern forest bats (*Vespadelus pumilus*) and Daubenton's bats (*Myotis daubentonii*), female-biased roosts form during the breeding and maternity season in locations that have ideal microhabitat conditions for offspring rearing (Encarnacao et al., 2005; Law & Anderson, 2000). In other species of bats, females may prefer to roost with familiar female roost mates over unknown females and males to avoid social parasitism (Bloss et al., 2002; Bouchard, 2001).

4.3 | Mixed-species *Prionostemma* roosting aggregations in spiny palms

We found that the ratio of the two *Prionostemma* species at particular spiny palm roosts was stable over a 2-week period (Figure 3), despite the low site fidelity of individuals (Donaldson & Grether, 2007). While some aggregation sites consistently attracted more *Prionostemma* sp. 1, others attracted more *Prionostemma* sp. 2. This implies that sites vary in attractiveness to the two species. One possible explanation is that the species differ in microhabitat preferences. While previous studies have failed to identify microhabitat characteristics that predict whether a spiny palm is occupied by *Prionostemma* (Donaldson & Grether, 2007), some microhabitat variables (e.g., canopy cover) are predictive of the size of the aggregations (Teng et al., 2012). Whether microhabitat variables are predictive of the *Prionostemma* species composition remains to be investigated. Another possible explanation for the stable species composition is that the species differ in their scent-marking chemicals and are preferentially attracted to conspecific scent (Chelini et al., 2012; Donaldson & Grether, 2007; Grether & Donaldson, 2007). *Prionostemma* sp. 1 were found at several of the release sites in our translocation experiment, which suggests that the scent marks are not entirely species specific. Nevertheless, the scent-marking chemicals might differ sufficiently between species to account for the stable species composition. In addition to isolating and identifying the scent-marking chemicals, this hypothesis could be tested with a translocation experiment analogous to the one reported here but where the species of the founders is the experimental treatment. Stable species composition in mixed-species roosts has also been reported in birds (Martinez & Gomez, 2013).

In spiny palm roosts that contained both species, *Prionostemma* sp. 2 were usually more numerous than *Prionostemma* sp. 1 and

Prionostemma sp. 2 tended to roost higher than *Prionostemma* sp. 1. Based on comparing sites with and without *Prionostemma* sp. 1, we found no evidence that the roost height of *Prionostemma* sp. 2 was affected by the presence of *Prionostemma* sp. 1. We were unable to make the reverse comparison because too few roost sites lacked *Prionostemma* sp. 2. Competitive displacement seems unlikely *a priori* because we observed no aggressive interactions, but this hypothesis could be tested by selectively removing *Prionostemma* sp. 2 and monitoring the roost heights of *Prionostemma* sp. 1 over time. We found no difference between the sexes in the number of individuals roosting above 2 m at source sites in either species.

Prionostemma harvestmen have proven to be especially tractable subjects for field experiments on communal roosting behavior (Donaldson & Grether, 2007; Teng et al., 2012; Grether-Aller et al., 2014; Grether, Levi et al., 2014). Indeed, the translocation experiment reported here appears to be the first experimental test of the mechanisms underlying sexual segregation at communal roosts in any taxon. Nevertheless, much remains to be learned about communal roosting behavior in *Prionostemma*. Future studies should aim to (i) isolate and identify the scent-marking chemicals; (ii) test for sex- and species-specificity in the scent-marking chemicals; and (iii) determine whether roosting aggregations form during the breeding season, and if so, whether interactions within or between the sexes affect roost site selection.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL APPROVAL

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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