

Microhabitat use and behavior differ across sex-age classes in the scorpion *Brachistosternus ferrugineus* (Scorpiones: Bothriuridae)

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Abstract. Intra- and interspecific coexistence has been recorded in several species of scorpions, reflecting different levels of aggregation and sociability. Some species of scorpions avoid temporal or spatial overlap of their surface activities, which may differ depending on species, age group or gender, and thus reduce intra- and interspecific competition and predation. We examined the surface activity of males, females and juveniles (sex-age class) of the scorpion *Brachistosternus ferrugineus* (Thorell, 1876) in an area of Arid Chaco, and also its microhabitat preference and behavior by each sex-age class. The month-by-month activity of each sex-age class was different, but all the classes were observed each month. The most frequently used microhabitat was *soil* (64.8%), while *leaf litter* and *vegetation* were used in similar proportions. The behavior most frequently observed was *ambush* (68.3%), followed by *walking* and less frequently *feeding*, *doorkeeping* and *courting*. Each sex-age class performed one particular behavior with more frequency than the others. Analyzing combinations of microhabitat, behavior and sex-age class, we found the juveniles were associated with *feeding* on *vegetation*, males with *walking* on *leaf litter*, while females were related to *ambush* on *soil*. No marked temporal distribution between sex-age classes was observed. However, the spatial distribution and frequency of behaviors were highly dependent on developmental stage and sex. These differences may facilitate understanding of the coexistence of different age-sex classes of *B. ferrugineus*.

Keywords: Arachnids, intraspecific coexistence, surface activity, Arid Chaco

Scorpions are primarily solitary and sedentary arthropods. They are excellent predators, preying on a wide variety of populations of insects, spiders and even other scorpions, and in turn are prey, mostly of vertebrates (Williams 1987; McCormick & Polis 1990; Polis 1990; Brownell & Polis 2001). They occur in a variety of terrestrial habitats and may be divided into two general groups based on microhabitat preference: ground-dwelling species, which live in burrows or under surface debris such as rocks or logs, and arboreal species, found at various heights on the vegetation (Polis 1990; Brown & O'Connell 2000). In deserts and semiarid regions, where scorpions are common, the majority of species are ground-dwellers (Polis 1990; Polis & Yamashita 1991). During the day, these species are relatively inactive and remain in their shelter or burrow. Individuals emerge from these diurnal retreats shortly after sunset to forage or engage in other activities, usually remaining on the ground surface near the burrow or shelter (Polis 1979; Polis et al. 1985).

Intra- and interspecific coexistence has been recorded in several species of scorpions (McReynolds 2004, 2008; Kaltsas et al. 2009; Shehab et al. 2011; Lira and De Souza 2014), reflecting different levels of aggregation and sociability (Polis & Lourenço 1986; Polis 1990). Some species of scorpions avoid temporal or spatial overlap of their surface activities, which may differ depending on species, age group, gender or body size. This is probably due to the presence of conspecifics and heterospecifics in the environment, which would result in substantial competition for food and shelter resources and

may decisively influence habitat selection (Polis 1980, 1984; Due & Polis 1985; Polis & McCormick 1987; McReynolds 2008; Kaltsas et al. 2009; Lira et al. 2013). Habitat selection by an intermediate predator often means a balance between success in foraging and risk of predation (Murdoch & Sih 1978; Luttbegg & Schmitz 2000). Foraging success in scorpions may be associated with seasonal changes in prey availability (Polis 1980; Polis & McCormick 1986) and in the risk of predation by nocturnal predators, which may also be associated with the lunar cycle (Hadley & Williams 1968; Polis 1980; Polis et al. 1981; Nime et al. 2013). Thus, to increase predation success and to avoid being preyed upon, scorpions may modify their nocturnal activity or microhabitat use on nights with high illumination (Skutelsky 1996). For example, they may feed on trees or shrubs with high prey availability which, in turn, could provide protection against predators (McReynolds 2004).

The behavior and the substrate used by many species of scorpions are related, among other factors, with the sex-age class (Skutelsky 1996; Brown & O'Connell 2000; McReynolds 2004; Yamashita 2004). For example, walking behavior in several scorpion species is more associated with males than with females and juveniles (Polis & Farley 1979; Polis 1980; Yamashita 2004). Major displacement has even been observed in the males of many species, especially in the breeding season, as they actively search for females to mate with (Polis & Farley 1979; Polis & Sissom 1990; Araújo et al. 2010). Males of *Smeringurus mesaensis* (Stahnke, 1957) can travel more than 100 m in a night (with an average of 34.7 m), meanwhile, the females were observed up to one meter from their burrows (Polis & Farley 1979). Likewise, the behavior of climbing vegetation has been observed more frequently in juveniles than

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in adults of several species of scorpions (Bradley 1988; Polis 1990; Skutelsky 1996).

No previous studies have examined habitat use by *Brachistosternus ferrugineus* (Thorell, 1876) or its behavior in the months of activity. Only a few studies have addressed the ecology of scorpions in Arid Chaco regions (Acosta 1995; Nime et al. 2013, 2014). This lack is particularly surprising in view of the high biodiversity of such environments and the evidence that environmental change is transforming the ecology of arid regions, which are often predicted to be among the ecosystems most responsive to global climatic change (Hamerlynck et al. 2000; Whitford 2002).

The aims of this study were to analyze whether the surface activity of males, females and juveniles (sex-age class) of the scorpion *B. ferrugineus* varies from month to month in an area of Arid Chaco, and to examine whether each sex-age class has a preferred microhabitat and behavior. Our hypothesis is that sex-age classes of *B. ferrugineus* use different microhabitats and shows temporal displacement. The findings of this study on the ecology and behavior of this widespread and abundant species (Acosta 1995; Ojanguren-Affilastro 2005; Nime et al. 2013) are therefore important for understanding the processes of intra-specific coexistence and can contribute to a greater understanding of the structure of arthropod communities in the Argentine Arid Chaco.

METHODS

Study site.—The study was conducted in the Parque Provincial y Reserva Forestal Chancaní (Chancaní Reserve, 31°22'13.21" S 65°27'13.75" W, 4,960 ha). The reserve is located in the southernmost portion of the Chaco (Arid Chaco ecoregion, NT0701 in Olson et al. 2001) in Córdoba province, Argentina. Vegetation in the reserve is dry xerophilous woodland. The canopy is discontinuous and ~15 m high, dominated by *Aspidosperma quebracho-blanco* and *Prosopis flexuosa* trees. The shrub stratum (~4 m high) is thorny, dense, and almost continuous (Carranza et al. 1992; Cabido & Pacha 2002). The reserve supports forest stands that are close to climax conditions. The climate is highly seasonal, with a pronounced dry season. Annual rainfall averages 450 mm, concentrated during the summer (October–March). In the dry winter season (April–September), the water balance is negative, resulting in a soil humidity deficit. Mean annual temperature is 18 °C, with a mean value of 25 °C in the warmest month (January, with maximum temperatures reaching 45 °C during the day) and 10 °C in the coldest month (July) (Cabido & Pacha 2002).

There are four different ecological-type sites within the study site. (1) The *mature forest* site shows forest formations that are close to climax conditions, mainly with trees such as *A. quebracho-blanco* and *P. flexuosa*. The shrub layer is dominated by *Larrea divaricata*, *Mymozyanthus carinatus* and *Acacia furcatispina* (Carranza et al. 1992). (2) In December 1994, a high-intensity wildfire affected about 32,000 ha of Chaco forest, 230 ha of which are within the western boundaries of the Chancaní Reserve. Alongside the mature forest area, the fire generated a *secondary forest* area in which the vegetation is dense and homogeneous, dominated by high grasses (about 1 m tall) and shrubs about 2.5 m in height. In this area, young trees are common and dead trees

are still standing (Pelegrin & Bucher 2010). (3) The *Jarillal* site is located in the central area of the reserve. In the past, the area was used for the logging of large trees for fuel and charcoal. Currently, it is dominated by shrubs of the *Larrea* genus (“jarilla”); the area is not used for any activities and is recovering. (4) The *forest with livestock* is a private area facing the reserve that has been used for years for raising livestock, so the site is much degraded with low, scattered shrubs.

Study animal.—The genus *Brachistosternus* Pocock, 1893 is second in richness within the family Bothriuridae Simon, 1880, containing 43 species described so far (Rein 2015; Ojanguren-Affilastro et al. 2016). The species of this genus inhabit arid and semi-arid environments from northern Ecuador to southern Patagonia in Argentina. Within Argentina, *Brachistosternus* species are dominant in xeric environments of the west and south, where they form large populations and are generally more abundant than other sympatric species (Acosta 1995; Ojanguren-Affilastro 2005; Nime et al. 2013; Ojanguren-Affilastro et al. 2016). They are ground-dwelling, living in burrows or under surface debris such as rocks or logs (Ojanguren-Affilastro 2005). Most species of *Brachistosternus* are active during the summer (from November to April), with generally a more extended activity period than other bothriurid genera (Ojanguren-Affilastro 2005). *Brachistosternus ferrugineus* are small to medium size scorpions (males between 27 to 43 mm, females between 35 to 56 mm) (Ojanguren-Affilastro 2005). Most of the localities where *B. ferrugineus* have been collected in Argentina are within the Chaco phytogeographic province, where it is the most common species (Acosta 1995; Ojanguren-Affilastro 2005). This species lives at low or medium altitudes, from 100 to 1500 meters above sea level (Ojanguren-Affilastro 2005).

Experimental design.—Three sites were selected within the Chancaní Reserve representative of “mature forest” (Fig. 1a), “secondary forest” (Fig. 1b) and “Jarillal” (Fig. 1d). An additional site, “forest with livestock”, was added adjacent to the reserve (Fig. 1c) in order to cover the landscape’s heterogeneity.

At each site, 15 transects (50 × 6 m) were established, separated from each other by 70 m. Transects, one after the other, passed along and on the main roads in each sector, due to the difficulty of accessing and observing scorpions inside the dense forest.

Sampling consisted of walking along each transect with a portable ultraviolet (UV) lamp. Irradiation with UV causes scorpions to fluoresce, which enables all stages of the active scorpion to be easily detected in the dark (Honetschlager 1965).

Sampling lasted about 2 h after dusk (from 21:00 to 23:00), as these were the main activity hours of scorpions at the study site. Sampling was conducted for three nights a month at each site, always close to the new moon phase, to avoid the effect of moonlight on scorpion activity (Nime et al. 2013). Sampling was conducted during the periods November 2009 through January 2010 and November 2010 through February 2011. The sampling effort was the same at each site on each recording night (2 hours/night), but the months in which each site was sampled were not the same (Table 1). February was sampled in only one year (2010) but sampling was carried out

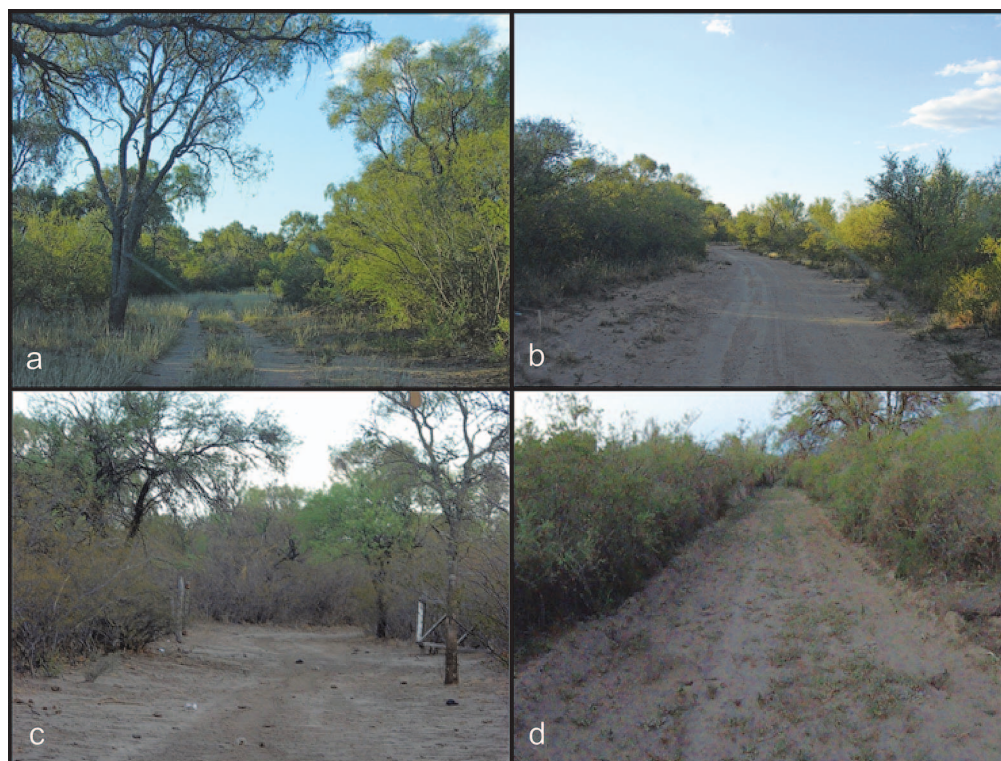


Figure 1.—Sampling sites within and outside the Chancaní reserve, Córdoba, Argentina. a. mature forest; b. secondary forest; c. forest with livestock; d. Jarillal.

in the other months in both seasons. Every month had the same number of sampling nights.

When a scorpion of *B. ferrugineus* species was located, it was observed and classified by sex-age class (male, female and juvenile). When necessary, scorpions were temporarily captured with forceps for identification. Every observation was made avoiding disturbances to other scorpions nearby. Specimens were not marked, since on a preliminary study the recovery of marked individual was too low (less than 2%). At the time of observation, we recorded the behavior of each scorpion and the microhabitat in which it was found. The behaviors recorded were *ambush*, *feeding*, *walking*, *doorkeeping* and *courting*. *Ambush* was when the individual was at rest, with extended pedipalps (waiting for prey) or resting with the appendages in contact with the body. We attempted to record both behaviors (ambush and rest) separately according to the position of the pedipalps, but if they perceived our vibrations when we approached, they moved their pedipalps to the body, so we prefer not to discriminate between these two behaviors. The scorpion was classified as *feeding* when it was feeding on

prey or when it had consumed the prey leaving only a small part between the chelicerae. The scorpion was classified as *walking* when it was observed moving. *Doorkeeping* behavior was when the animal was seen at the entrance of the burrow, either in ambush or entering. Finally, *courting* behavior is performed by scorpions prior to copulation (the male holding the female with their pedipalps), so only adults exhibit this behavior (Fig. 2).

The microhabitats considered were grouped into the following categories: *soil*, *leaf litter* and *vegetation*. *Soil* was considered when the individual was on bare ground, usually packed soil (Fig. 2). In *leaf litter*, the individual was on the leaf litter composed mainly of dry leaves (mostly from *A. quebracho-blanco* trees) and small branches. In *vegetation*, the individual was observed climbing vegetation, usually at the end or in the middle, not above 30 cm in height. Three scorpions were observed on cattle feces at the site with livestock, and this was considered as *leaf litter*.

We surveyed the availability of each microhabitat in the total area and in each site (with exception of jarillal). Three

Table 1.—Number of nights sampled for scorpions with UV light in the Chancaní reserve. * = no data.

Site	Nov.'09	Dec.'09	Jan.'10	Nov.'10	Dec.'10	Jan.'11	Feb.'11	Total
Mature forest	3	3	3	3	3	3	3	26
Secondary forest	3	3	3	3	3	3	3	26
Forest with livestock	*	3	3	3	3	3	3	18
Jarillal	*	*	*	3	3	*	*	6
Total	6	9	9	12	12	9	9	75



Figure 2.—*Brachistosternus ferrugineus* couple, male on the right and female on the left, courting on soil, in the secondary forest of Chancaní reserve, Córdoba, Argentina. Photo courtesy of María Eugenia Romero Lebrón.

quadrats (0.50×0.50 m) were placed to left, right and center of each transect (9 quadrats per transect) and the percentage (%) of vegetation, leaf litter and soil was recorded. The average of each microhabitat was then calculated at each site and for all of these.

Statistical analysis.—*Temporal distribution of the sex-age class:* Surface activity of scorpions (measured as the number of individuals active on the ground surface) of each sex-age class (males, females and juveniles) of the species *B. ferrugineus* and its variation between months were analyzed. Scorpion counts were modeled with a generalized linear mixed model (GLMM) using the glmer function of the lme4 library through the interface with R-packages (R Core Team 2013) implemented in InfoStat (Di Rienzo et al. 2013). We used a Poisson distribution (with a log-link function) because this is most appropriate for counts. The overdispersion was evaluated to ensure that the Poisson assumptions held. Sex-age class and month and their interaction were considered fixed effects. In order to take into account the repeated measures in months, site and transect were considered random effects. Significance level was set at 0.05. To compare means, we used the Fisher's least significant difference test (LSD).

Microhabitat preferences and behaviors associated with each sex-age class: First, contingency tables were used to evaluate if there were significant differences in proportions of microhabitat use by *B. ferrugineus* without discriminating by sex-age class. The same was done for the differences between proportions of behavior observed. Subsequently, a GLMM was performed to determine whether there were differences in proportions between classes within each microhabitat, using site, month and sex-age class as classification variables; sex-age class was selected as a fixed factor and site and month as random factors. The same analysis was performed for behavior.

Finally, a bi-plot obtained from multiple correspondence analyses (MCA) was performed to explore the associations between microhabitat, behavior and sex-age class. MCA takes multiple categorical variables and seeks to identify associa-

tions between levels of those variables. MCA allows exploring contingency tables by means of the decomposition of chi-square matrix. The contributions of each axis are indicated as a percentage of inertia (Abdi & Valentin 2007). The MCA results allow all the variables (behavior, microhabitat, sex-age class) to be observed together. Analyses were performed for the total sample and for each site separately to detect changes in associations due to the effects of site. Microhabitat, behavior and sex-age class were selected as classification criteria and frequency as the combination of these variables.

RESULTS

Temporal distribution of sex-age class.—We found a significant interaction between sex-age class and month for scorpion counts (Wald test, $\chi^2 = 25.60$, $P = 0.0003$). Due to the presence of the interaction, we performed a Fisher's LSD test for the combination of sex-age class and month. Females and juveniles had a higher surface activity than males in November. In January, the majority of active individuals that we saw were juveniles. In February, we observed more males and juveniles than females (Fig. 3). December showed no significant differences between the three classes. In general, the average number of active individuals of all three sex-age classes decreased from November through January, rising again in February (Fig. 3).

Microhabitat preference and behaviors associated with each sex-age class.—*Microhabitat availability:* Soil was the most abundant microhabitat in the study area (54.9%), while leaf litter (23.7%) and vegetation (21.3%) were similar in proportion. Soil was also the most abundant microhabitat in the three sampled sites (from 50.4 to 60.2%, see Table 2), followed by leaf litter in the mature forest site, and vegetation in the secondary forest. In the forest with livestock, there were similar percentages of both vegetation and leaf litter (Table 2).

Microhabitat preference: The results showed a significant difference between proportions of microhabitat used for total scorpions (Chi-square test, $\chi^2 = 702.32$, $P < 0.0001$). The most frequently used microhabitat for total scorpions was soil (64.8%), while leaf litter (18.1%) and vegetation (17.1%) were used in similar proportions, matching microhabitat availability. Table 3 shows the number of individuals of each sex-age class in different microhabitats at each site.

However, we detected significant differences between counts of sex-age classes in leaf litter and vegetation microhabitats (Table 4, Fig. 4). Males and females were significantly more abundant than juveniles in the leaf litter microhabitat. Juveniles were significantly more abundant in vegetation, followed by females and then the males (Table 4). No significant differences were found between the classes in the use of soil (Table 4).

Frequencies of behavior: The results showed a significant difference in proportions of observed behavior for total scorpions (Chi-square test, $\chi^2 = 3693.46$, $P < 0.0001$). Ambush behavior was the most frequently observed (68.3%), followed by walking (20.6%) and less frequently feeding (7.6%), door-keeping (2.7%) and courting (0.8%). Table 5 shows the number of individuals in each sex-age class performing different behaviors at each site.

In the analysis of sex-age classes and behaviors, significant differences were detected for some behaviors (Table 6, Fig. 5).

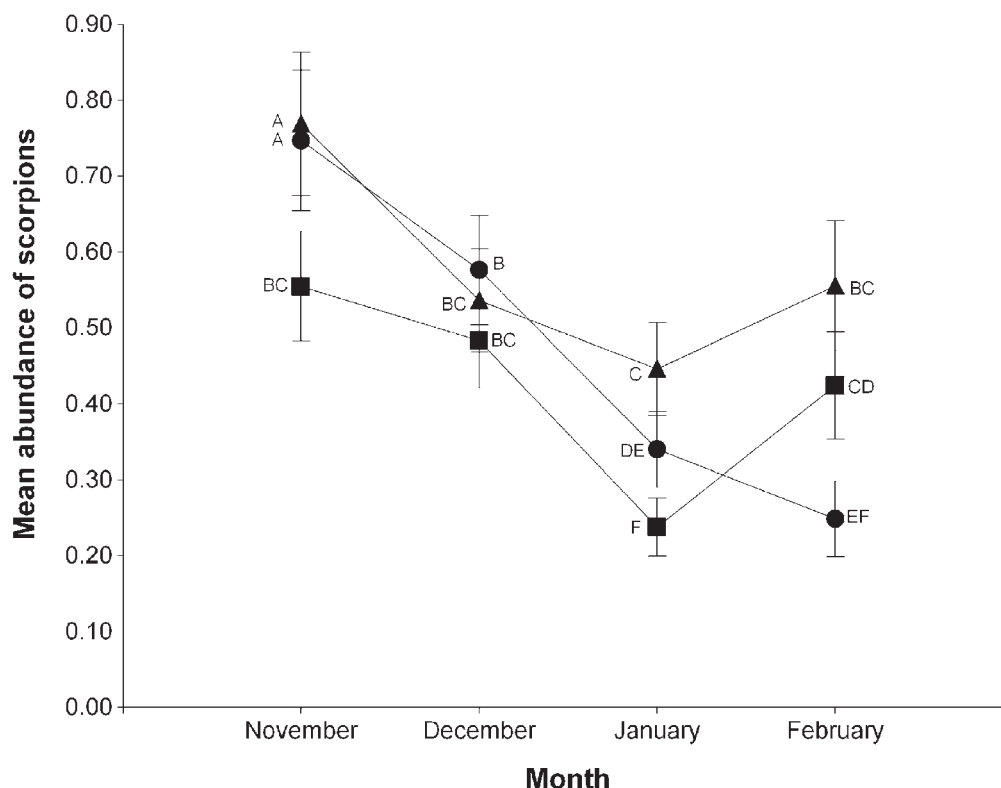


Figure 3.—Number of *Brachistosternus ferrugineus* scorpions, males (squares), females (circles) and juveniles (triangles), each month in the Chancaní reserve. Mean expressed as average number of scorpions by transect ($\pm$ SE). Common letters are not statistically different (Fisher's LSD test, $p > 0.05$).

Ambush behavior was performed more frequently by females and juveniles than males. Juveniles were observed *feeding* more frequently than adults (males and females), and male adults did so less frequently than females. Females were observed more *doorkeeping* than males and juveniles. Most of the time, the females were found with the metasoma outside the burrow, as if entering the burrow (in 93% of observations). *Courtship* behavior was observed in six pairs of scorpions.

Combination of microhabitat, behavior and sex-age class.—Significant differences were observed between proportions of microhabitats used and sex-age class (Chi-square test, $\chi^2 = 777.38$, $P < 0.0001$), and between behavior and sex-age class (Chi-square test, $\chi^2 = 3188.31$, $P < 0.0001$). The bi-plot obtained by multiple correspondence analyses, performed for the total sample using sex-age class, behavior and microhabitat, reveals that juveniles were more likely to be *feeding* on *vegetation* (Fig. 6). While the majority of males were observed *walking* on the *leaf litter*, the females were related to *ambush* on *soil* (Fig. 6). Even when the four sites were analyzed separately, the pattern observed was very similar.

Table 2.—Percentage of microhabitat available (mean $\pm$ SD) in three sampled sites of the Chancaní reserve.

Site	Soil	Leaf litter	Vegetation
Mature forest	50.4 $\pm$ 16.1	30.6 $\pm$ 14.4	18.7 $\pm$ 13.5
Secondary forest	53.9 $\pm$ 14.3	20.0 $\pm$ 12.9	26.0 $\pm$ 13.9
Forest with livestock	60.2 $\pm$ 14.3	20.6 $\pm$ 11.5	19.2 $\pm$ 8.6

DISCUSSION

Temporal distribution of sex-age class.—The surface activity of males, females and juveniles was higher in November, and declined over the months in all three classes. However, all sex-age classes remained active during all the months sampled. The decrease was more marked in females; they did not have the increase in February that was observed in the other classes (Fig. 3). This may be because, after getting pregnant, the

Table 3.—Counts of *Brachistosternus ferrugineus* occurrence in each microhabitat within each site in Chancaní reserve, during the period of sampling.

Site	Sex-age class	Soil	Leaf litter	Vegetation	Total
Mature forest	Males	126	43	15	184
	Females	108	41	47	196
	Juveniles	104	43	83	230
Secondary forest	Males	93	24	5	122
	Females	125	38	19	182
	Juveniles	118	12	54	184
Forest with livestock	Males	42	16	2	60
	Females	64	27	7	98
	Juveniles	73	7	20	100
Jarillal	Males	53	12	0	65
	Females	30	9	3	42
	Juveniles	56	5	7	68
Total		992	277	262	1531

Table 4.—Generalized linear mixed models. Relationship between the surface activity of males, females and juveniles of *Brachistosternus ferrugineus* and each microhabitat used in the Chancaní reserve. Average monthly scorpions $\pm$ standard error of the mean. Means with a letter in common are not significantly different (Fisher's LSD, $P > 0.05$).

Microhabitat	χ^2	P	Males	Females	Juveniles
Soil	2.12	0.3463	40.99 $\pm$ 6.83 A	42.68 $\pm$ 7.10 A	45.82 $\pm$ 7.59 A
Leaf litter	12.93	0.0016	12.92 $\pm$ 2.08 A	15.64 $\pm$ 2.42 A	9.11 $\pm$ 1.58 B
Vegetation	124.89	<0.0001	2.93 $\pm$ 0.74 C	10.12 $\pm$ 1.82 B	21.83 $\pm$ 3.48 A

females stay sheltered in their burrows (Mahsberg 2001; Kaltsas et al. 2008).

The higher surface activity for the three classes in November could be because, in this region of the southern hemisphere, scorpions generally begin to leave their shelters or burrows and surface during spring when temperatures begin rising (Warburg & Polis 1990; Ojanguren-Affilastro 2005; Yamaguti & Pinto-da-Rocha 2006; Nime et al. 2013) to begin feeding after the winter and probably find a partner. We did not observe marked differences in temporal distribution between sex-age classes, as exists in many species of scorpions to avoid intra- and interspecific competition and predation (Polis 1980, 1984; Due & Polis 1985; Polis & McCormick 1987).

Microhabitat preference and behaviors associated with each sex-age class.—The microhabitat most used in all classes of *B. ferrugineus* was *soil*, and *ambush* was the most common behavior. When we examined all sex-age classes of scorpions together, we found that microhabitats were used in proportion to their availability. On the another hand, the most common behavior expected was *ambush*, because active looking for prey is not common in scorpions (McCormick & Polis 1990) since it involves a significant expenditure of energy (Kaltsas et al. 2008). Kaltsas et al. (2008) found that the most common behavior (over 84%) in looking for food for all sex-age classes

of *Mesobuthus gibbosus* (Brulli, 1832) species was “sit-and-wait” (*ambush*), coinciding with our observations.

However, the correspondence analysis clearly demonstrated one combination of a behavior and microhabitat was associated with each sex-age class. Male scorpions were mostly seen *walking on leaf litter*, females were more likely to be seen engaging in *ambush* behavior on *soil*, and juveniles were associated with *feeding in vegetation*.

That *walking* behavior is more associated with males was also observed in the species *Centruroides vittatus* (Say, 1821) (Polis 1980; Yamashita 2004). Males of this species are more active (54.4% walking on the soil surface) than females (34.9%). Also, males had a greater displacement, with marked individuals being found many meters away from the initial site, while females were found a few meters from the site, even after several weeks (Yamashita 2004). This increased movement of males is observed mainly in the breeding season as they actively search for females to mate (Polis & Farley 1979; Polis & Sissom 1990; Araújo et al. 2010).

In the present study, *doorkeeping* behavior was performed more often by females than by males and juveniles. Similar results were observed in females of *M. gibbosus*; these fed and looked for prey at the entrance of their burrows (“*door-keeping*” strategy) more than males and juveniles, who were preferably near or far from their burrows (Kaltsas et al. 2008).

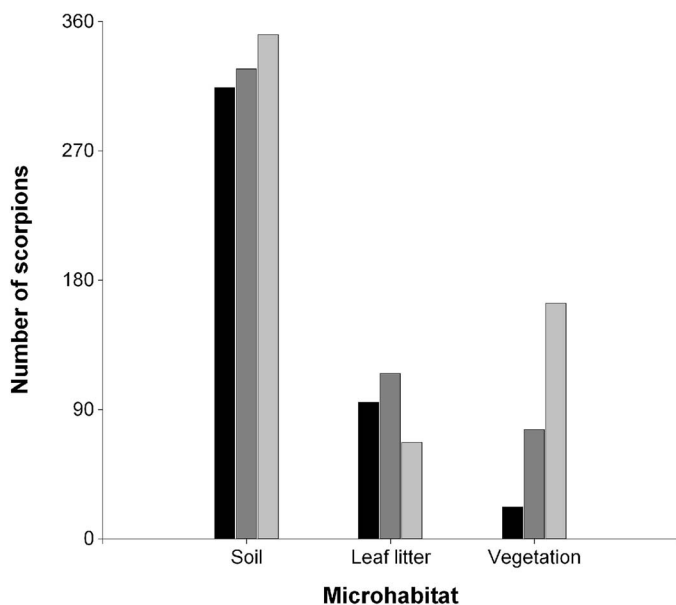


Figure 4.—Surface activity of males (black bars), females (dark grey bars) and juveniles (light grey bars) of *Brachistosternus ferrugineus* in different microhabitats in the Chancaní Reserve. Bars represent total number of scorpions observed.

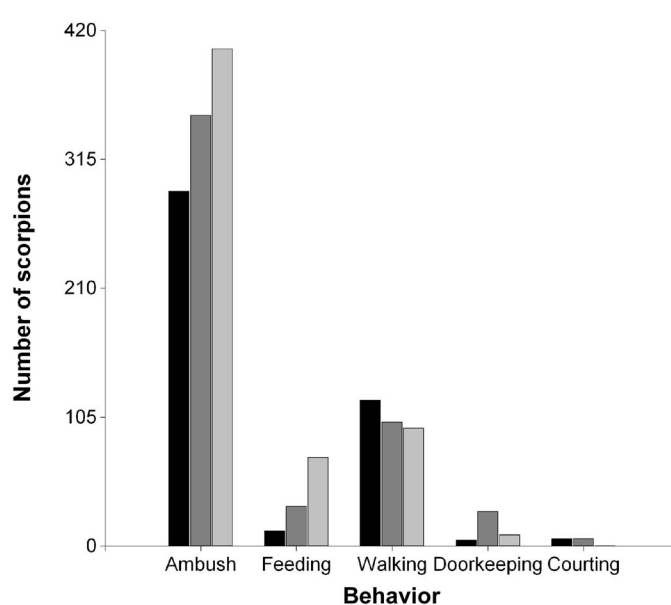


Figure 5.—Surface activity of males (black bars), females (dark grey bars) and juveniles (light grey bars) of *Brachistosternus ferrugineus* in the performing of different behaviors in the Chancaní Reserve. Bars represent total number of scorpions observed.

Table 5.—Counts of *Brachistosternus ferrugineus* performing each behavior in each study site of the Chancaní reserve.

Site	Sex-age class	Ambush	Feeding	Walking	Doorkeeping	Courting	Total
Mature forest	Males	114	9	57	1	3	184
	Females	144	11	31	7	3	196
	Juveniles	161	31	34	4	0	230
Secondary forest	Males	81	1	36	3	1	122
	Females	119	9	41	12	1	182
	Juveniles	125	22	36	1	0	184
Forest with livestock	Males	39	2	19	0	0	60
	Females	59	10	22	7	0	98
	Juveniles	65	15	17	3	0	100
Jarillal	Males	55	0	7	1	2	65
	Females	29	2	7	2	2	42
	Juveniles	54	4	9	1	0	68
Total		1045	116	316	42	12	1531

In the “doorkeeping” hunting strategy, the scorpion is located at the entrance to the burrow or refuge and waits for prey to approach (Benton 2001). Most females of *M. gibbosus* had recently mated. Therefore, staying close to their burrows is probably due to the maternal protective instinct (Mahsberg 2001; Kaltsas et al. 2008). Furthermore, foraging far from the burrow requires tolerance to adverse environmental conditions and inter- and intraspecific competition. In the open area, where males and juveniles of *M. gibbosus* feed mainly using the “sit and wait” strategy, temperature and relative humidity are comparatively lower, wind speed is higher and the moon is an important factor, while in the burrows of females, generally under the vegetation, environmental conditions are better (Kaltsas et al. 2008). Probably staying at the entrance is advantageous as there is a microclimate inside and environmental conditions are more favorable, and they have a shelter near to hide from predators. The females of *M. gibbosus* observed at the entrance of their burrows had only their pedipalps and sometimes their prosoma visible. In our study, the most common behavior of females of *B. ferrugineus* when doing doorkeeping, was at the entrance of their burrows with their metasoma outside, as if entering the burrow (in 93% of observations). The reasons why scorpions prefer a backwards position at the burrow entrance are unknown; they may have been entering in their burrows in flight after sensing the approach of collectors. Burrowing scorpions are very sensitive to vibrations in the ground (Warburg & Polis 1990).

We found the juveniles associated with feeding in vegetation. The behavior of climbing the vegetation has been previously observed in other species of scorpions (Williams 1970; Polis

1979; Bradley 1988; Cao 1993; Skutelsky 1996; Brown & O’Connell 2000; McReynolds 2004, 2008). In *Paruroctonus utahensis* (Williams, 1968), more juveniles than adults were observed in the vegetation (Bradley 1988). Juveniles of *Buthus occitanus* (Amoreux, 1789) were found in the bushes at a rate ten times higher than that of adults (Skutelsky 1996).

Although the reason for climbing behavior in the vegetation is unclear, there are some hypotheses such as decreasing the risk of predation, or increasing feeding success by foraging in an area with higher prey availability (Bradley 1988; Polis 1990; Brown & O’Connell 2000). The first hypothesis suggests that climbing is a behavior to avoid predation and assumes that the risk of predation is lower in vegetation than on the soil. Two observations are consistent with this hypothesis (Brown & O’Connell 2000). First, climbing has been seen generally in small species and juveniles of larger species (Polis 1979; Bradley 1988; Skutelsky 1996). In this study, *B. ferrugineus* is one of the smallest species in the area and the only one that we observed to climb vegetation. As these species or individuals probably have a wide range of predators that live on the soil (including larger intra- and interspecific scorpions; Polis & McCormick 1987), climbing could reduce meeting potential predators. Also, it was noted that *C. vittatus* moves on to vegetation during the phase of the moon with greater light intensity (50–100%) (McReynolds 2004). In open areas, scorpions are more visible to predators at night and so such a change in microhabitat use during the lunar cycle behavior may reduce the risk of predation when the illumination of the moon is high (McReynolds 2004). The present study could not test this, because sampling was always performed on moonless nights. Second, some species have been seen to take prey

Table 6.—Generalized linear mixed models. Relationship between the surface activity of males, females and juveniles of *Brachistosternus ferrugineus* in each behavior observed in the Chancaní reserve. Average monthly scorpions $\pm$ standard error. Means with a letter in common are not significantly different (Fisher’s LSD, $P > 0.05$).

Behavior	χ^2	<i>P</i>	Males	Females	Juveniles
Ambush	19.51	0.0001	38.48 $\pm$ 5.94 B	46.74 $\pm$ 7.12 A	53.93 $\pm$ 8.15 A
Feeding	49.33	<0.0001	1.53 $\pm$ 0.53 C	4.09 $\pm$ 1.08 B	9.19 $\pm$ 2.11 A
Walking	2.73	0.2548	11.67 $\pm$ 5.19 A	9.90 $\pm$ 4.42 A	9.41 $\pm$ 4.21 A
Doorkeeping	19.19	<0.0001	0.60 $\pm$ 0.30 B	3.24 $\pm$ 1.00 A	1.08 $\pm$ 0.44 B
Courting	9.73	0.0077	0.32 $\pm$ 0.26 A	0.32 $\pm$ 0.26 A	0 $\pm$ 0 B

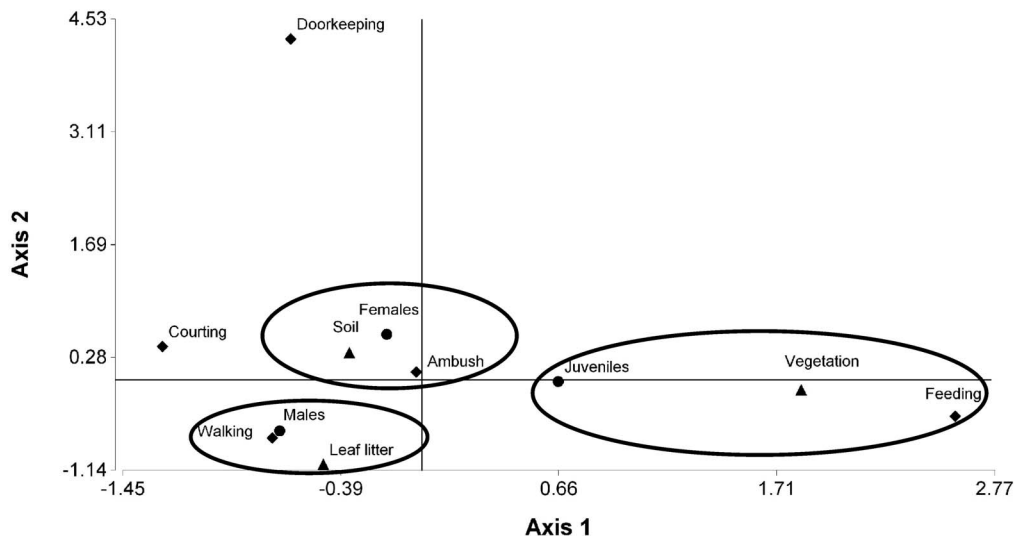


Figure 6.—Bi-plot obtained by multiple correspondence analysis. The principal axes represents the gradient in the relationship among the categorical variables sex-age class, behavior and microhabitat of *Brachistosternus ferrugineus*; categories close in the graph are more related.

captured on soil to vegetation before consumption, perhaps in order to reduce the chance of encountering a predator that might cause death or the loss of the prey while trying to escape (Polis 1979; Cao 1993; Brown & O'Connell 2000). In *Smeringurus mesaensis* this was age-specific behavior, with a significantly higher proportion of juveniles than of adults consuming prey in the vegetation (Polis 1979). In the present study, after the juveniles, females of *B. ferrugineus* were more frequently observed in the vegetation. The same occurs in *C. vittatus*, with females climbing more than males (Brown & O'Connell 2000; Yamashita 2004).

The second hypothesis suggests that prey abundance is higher in the vegetation, so feeding there would be more energy efficient (Bradley 1988; Polis 1990). However, although scorpions have been observed feeding while on vegetation, evidence of active foraging there is scarce (Polis 1990; Skutelsky 1996).

Possibly the vegetation, which creates a more complex environment than environmental deserts, offers juveniles more opportunities for hiding and reducing overlap with adults (Höfer et al. 1996). Habitat complexities reduce niche overlaps and may reduce the need of temporary displacement (Yamashita 2004). Based on our results, we hypothesize that the behavior of climbing into vegetation is performed by juveniles for the purpose of feeding without risk of losing the prey and avoiding being preyed on in turn. However, this hypothesis requires testing.

Our hypothesis that sex-age classes of *B. ferrugineus* use different microhabitats while showing temporal displacement is rejected; sex-age classes did use different microhabitats, but without temporal displacement. We conclude that the microhabitat use and behavior frequencies were highly dependent on developmental stage and sex. The differences observed may facilitate the age-sex class coexistence of *B. ferrugineus* and may reduce the need of temporary displacement due to the risk of predation and competition for feeding. Also, this species is the only one that uses vegetation in the study area, and this possibility of using different niches could

be one reason why *B. ferrugineus* is the most abundant and conspicuous species in the area, despite being physically one of the smallest (Nime et al. 2013). The findings of this study on the ecology and behavior of this widespread species are therefore important for understanding the processes of intra-specific coexistence and can contribute to a greater understanding of the structure of arthropod communities in the Argentine Arid Chaco, and to general knowledge of scorpion ecology.

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LITERATURE CITED

- Abdi, H. & D. Valentin. 2007. Multiple correspondence analysis. Pp. 651–657. In *Encyclopedia of measurement and statistics*. (N.J. Salkind, ed.). Sage Publications, Thousand Oaks, California.
- Acosta, L.E. 1995. The scorpions of the Argentinian Western Chaco II. Community survey in the Llanos District. *Biogeographica* 7:187–196.
- Araújo, C.S., D.M. Candido, H.F.P. Araújo, S.C. de Dias & A. Vasconcellos. 2010. Seasonal variations in scorpion activities (Arachnida: Scorpiones) in an area of Caatinga vegetation in northeastern Brazil. *Zoologia (Curitiba)* 27:372–376.
- Benton, T.G. 2001. Reproductive ecology. Pp. 278–301. In *Scorpion*

- Biology and Research. (P. Brownell & G.A. Polis, eds.). Oxford University Press, New York.
- Bradley, R.A. 1988. The influence of weather and biotic factors on the behavior of the scorpion (*Paruroctonus utahensis*). *Journal of Animal Ecology* 57:533–551.
- Brown, C.A. & D.J. O'Connell. 2000. Plant climbing behavior in the scorpion *Centruroides vittatus*. *American Midland Naturalist* 144:406–418.
- Brownell, P. & G.A. Polis. 2001. *Scorpion Biology and Research*. Oxford University Press, New York.
- Cabido, M. & M.J. Pacha. 2002. Vegetación y Flora de la Reserva Natural Chancaní. Agencia Córdoba Ambiente, Córdoba, Argentina.
- Cao, W. 1993. Coexistence of three species of desert scorpions by habitat selection. Ph.D. Dissertation, University of Arizona.
- Carranza, M.L., M.R. Cabido, A. Acosta & S.A. Paez. 1992. Las comunidades vegetales del parque Natural Provincial y Reserva Forestal Natural Chancaní, provincia de Córdoba. *Lilloana* 38:75–92.
- Di Rienzo, J.A., F. Casanoves, M.G. Balzarini, L. Gonzalez, M. Tablada & C.W. Robledo. 2013. InfoStat. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. Online at <http://www.infostat.com.ar>
- Due, D. & G.A. Polis. 1985. The biology of *Vaejovis littoralis* Williams, an intertidal scorpion from Baja California, Mexico. *Journal of Zoology, London* 207:563–580.
- Hadley, N.F. & S.C. Williams. 1968. Surface activities of some North American scorpions in relation to feeding. *Ecology* 49:726–734.
- Hamerlynck, E.P., T.E. Huxman, R.S. Nowak, S. Redar, M.E. Loik, D.N. Jordan et al. 2000. Photosynthetic responses of *Larrea tridentata* to a step-increase in atmospheric CO₂ at the Nevada Desert FACE Facility. *Journal of Arid Environments* 44:425–436.
- Höfer, H., E. Wollscheid & T. Gasnier. 1996. The relative abundance of *Brotheas amazonicus* (Chactidae, Scorpiones) in different habitat types of a central Amazon rainforest. *Journal of Arachnology* 24:34–38.
- Honetschlager, L.D. 1965. A new method for hunting scorpions. *Turtos News* 43:69–70.
- Kaltsas, D., I. Stathi & M. Mylonas. 2008. The foraging activity of *Mesobuthus gibbosus* (Scorpiones: Buthidae) in central and south Aegean archipelago. *Journal of Natural History* 42:513–527.
- Kaltsas, D., I. Stathi & M. Mylonas. 2009. Intraspecific differentiation of social behavior and selection in *Mesobuthus gibbosus* (Brulé, 1832) (Scorpiones: Buthidae). *Journal of Ethology* 27:467–473.
- Lira, A.F.A. & A.M. De Souza. 2014. Microhabitat use by scorpion species (Arachnida: Scorpiones) in the montane Atlantic Rain Forest, Brazil. *Revista Ibérica de Aracnología* 24:107–108.
- Lira, A.F.A., A.M. De Souza, A.A.C. Silva Filho & C.M.R. Albuquerque. 2013. Spatio-temporal microhabitat use by two cooccurring species of scorpions in Atlantic rainforest in Brazil. *Zoology* 116:182–185.
- Luttbeg, B. & O.J. Schmitz. 2000. Predator and prey models with flexible individual behavior and imperfect information. *American Naturalist* 155:669–683.
- Mahsberg, D. 2001. Brood care and social behavior. Pp. 257–277. *In* *Scorpion Biology and Research*. (P. Brownell & G.A. Polis, eds.). Oxford University Press, New York.
- McCormick, S.J. & G.A. Polis. 1990. Prey, predators and parasites. Pp. 294–320. *In* *The Biology of Scorpions*. (G.A. Polis, ed.). University Press, Stanford, California.
- McReynolds, C.N. 2004. Temporal patterns in microhabitat use for the scorpion *Centruroides vittatus* (Scorpiones: Buthidae). *Euscorpius* 17:35–45.
- McReynolds, C.N. 2008. Microhabitat preferences for the errant scorpion, *Centruroides vittatus* (Scorpiones, Buthidae). *Journal of Arachnology* 36:557–564.
- Murdoch, W.W. & A. Sih. 1978. Age dependent interference in a predatory insect. *Journal of Animal Ecology* 47:581–592.
- Nime, M.F., F. Casanoves & C.I. Mattoni. 2014. Scorpion diversity in two different habitats in the Arid Chaco, Argentina. *Journal of Insect Conservation* 18:373–384.
- Nime, M.F., F. Casanoves, D.E. Vrech & C.I. Mattoni. 2013. Relationship between environmental variables and surface activity of scorpions in the Arid Chaco ecoregion of Argentina. *Invertebrate Biology* 132:145–155.
- Ojanguren-Affilastro, A.A. 2005. Estudio monográfico de los escorpiones de la República Argentina. *Revista Ibérica de Aracnología* 11:75–241.
- Ojanguren-Affilastro, A.A., C. I. Mattoni, J.A. Ochoa, M.J. Ramírez, F.S. Ceccarelli & L. Prendini. 2016. Phylogeny, species delimitation and convergence in the South American bothriurid scorpion genus *Brachistosternus* Pocock 1893: Integrating morphology, nuclear and mitochondrial DNA. *Molecular Phylogenetics and Evolution* 94:159–170.
- Olson, D.M., E. Dinerstein, E.D. Wikramanayake, N.D. Burgess, G.V.N. Powell, E.C. Underwood et al. 2001. Terrestrial ecoregions of the world: a new map of life on earth. *Bioscience* 51:933–938.
- Pelegrin, N. & E.H. Bucher. 2010. Long-term effects of a wildfire on a lizard assemblage in the Arid Chaco forest. *Journal of Arid Environments* 74:368–372.
- Polis, G.A. 1979. Prey and feeding phenology of the desert sand scorpion *Paruroctonus mesaensis* (Scorpionida: Vaejovidae). *Journal of Zoology, London* 188:333–346.
- Polis, G.A. 1980. Seasonal patterns and age-specific variation in the surface activity of a population of desert scorpions in relation to environmental factors. *Journal of Animal Ecology* 49:1–18.
- Polis, G.A. 1984. Age structure component of niche width and intraspecific resource partitioning: Can age groups function as ecological species? *American Naturalist* 123:541–564.
- Polis, G.A. 1990. *The Biology of Scorpions*. Stanford University Press, Stanford, California.
- Polis, G.A. & R.D. Farley. 1979. Behavior and ecology of mating in the cannibalistic scorpion, *Paruroctonus mesaensis* Stahnke (Scorpionida: Vaejovidae). *Journal of Arachnology* 7:33–46.
- Polis, G.A. & W.R. Lourenço. 1986. Sociality among scorpions. Pp. 111–115. *In* *Proceedings of the 10th International Congress of Arachnology*. International Society of Arachnology, Barcelona, Spain.
- Polis, G.A. & S.J. McCormick. 1986. Patterns of resource use and age structure among species of desert scorpion. *Journal of Animal Ecology* 55:59–74.
- Polis, G.A. & S.J. McCormick. 1987. Intraguild predation and competition among desert scorpions. *Ecology* 68:332–343.
- Polis, G.A. & W.D. Sissom. 1990. Life History. Pp. 161–223. *In* *The Biology of Scorpions*. (G.A. Polis, ed.). Stanford University Press, Stanford, California.
- Polis, G.A. & T. Yamashita. 1991. The ecology and importance of predaceous arthropods in desert communities. Pp. 180–222. *In* *The Ecology of Desert Communities*. (G.A. Polis, ed.). University of Arizona Press, Tucson, Arizona.
- Polis, G.A., C.N. McReynolds & G.R. Ford. 1985. Home range geometry of the desert scorpion *Paruroctonus mesaensis*. *Oecologia* 67:273–277.
- Polis, G.A., W.D. Sissom & S.J. McCormick. 1981. Predators of scorpions: field data and a review. *Journal of Arid Environments* 4:309–326.
- R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Online at <http://www.R-project.org/>

- Rein, J.O. 2015. The Scorpion Files. Norwegian University of Science and Technology. Online at <http://www.ub.ntnu.no/scorpion-files/>
- Shehab, A.H., Z.S. Amr & J.A. Lindsell. 2011. Ecology and biology of scorpions in Palmyra, Syria. *Turkish Journal of Zoology* 35:333–341.
- Skutelsky, O. 1996. Predation risk and state-dependent foraging in scorpions: effects of moonlight on foraging in the scorpion *Buthus occitanus*. *Animal Behavior* 52:49–57.
- Warburg, M.R. & G.A. Polis. 1990. Behavioral responses, rhythms and activity patterns. Pp. 224–246. *In* The Biology of Scorpions. (G.A. Polis, ed.). University Press, Stanford, California.
- Whitford, W. 2002. Ecology of Desert Systems. Academic Press, London.
- Williams, S.C. 1970. Coexistence of desert scorpions by differential habitat preference. *Pan-Pacific Entomologist* 46:254–267.
- Williams, S.C. 1987. Scorpion bionomics. *Annual Review of Entomology* 32:275–295.
- Yamaguti, H.Y. & R. Pinto-da-Rocha. 2006. Ecology of *Thestylus aurantiurus* of the Parque Estadual da Serra da Cantareira, São Paulo, Brazil (Scorpiones, Bothriuridae). *Journal of Arachnology* 34:214–220.
- Yamashita, T. 2004. Surface activity, biomass, and phenology of the striped scorpion, *Centruroides vittatus* (Buthidae) in Arkansas, USA. *Euscorpius* 17:25–33.

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