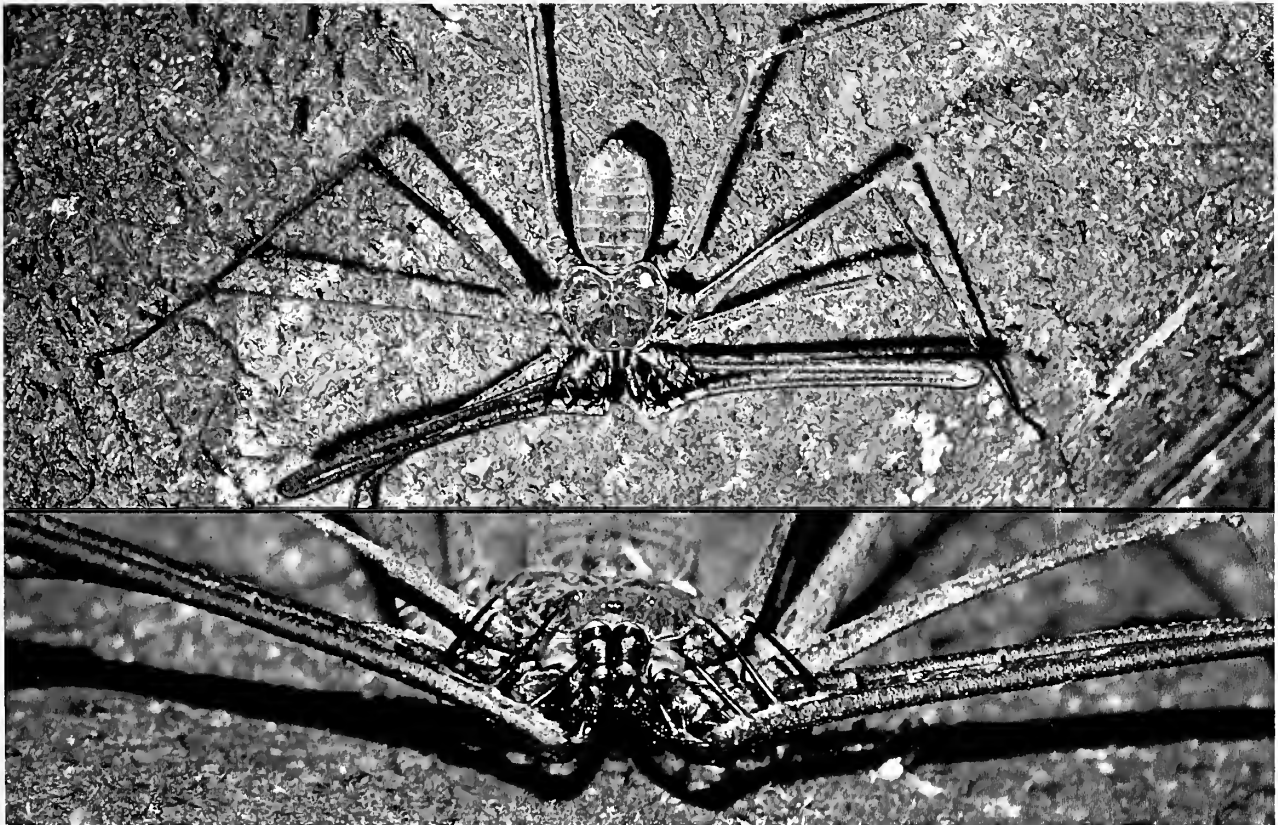


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Cover photo: Two views of an adult male *Heterophrynus batesii* (Amblypygi) from Tiputini Biodiversity Station outside Yasuni National Park, Orellana Province, Ecuador (see page 1). Photos by Kenneth J. Chapin.

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The behavioral ecology of amblypygids

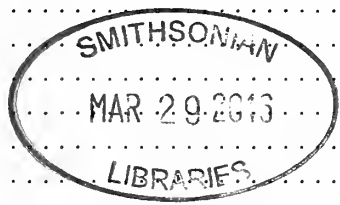
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Abstract. Arachnologists have uncovered tantalizing details about amblypygid behavioral ecology—the study of the fitness consequences of their behavior. Thus, it is the aim of this review to position Amblypygi as a useful system in which to investigate the principles of animal behavioral ecology. We synthesize amblypygid habitat preference and navigation modalities; predator, prey, parasite, parasitoid, cannibal, and commensal interactions; resource contests and territoriality; mating systems and mate choice; parental investment and sociality; and genetics and genomics as they relate to behavioral ecology. We present ideas for future research in each of these areas and discuss future directions for Amblypygi behavioral ecology research as they relate to four areas of behavioral ecology: adaptation, evolutionary history, mechano-sensory control of behavior, and behavioral development. We conclude by identifying several avenues of Amblypygi behavioral ecology that we think have the highest potential for transformative discoveries.

Keywords: *Damon*, cannibalism, fitness, *Heterophrynus*, microhabitat preference, navigation, *Paraphrynus*, *Phrynus*, tailless whip scorpions, territoriality, whip spiders

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1. FORWARD

“Whip spiders are bizarre yet spectacular animals... Their appearance is so horrible that many of the local people are extremely afraid of them and consider them to be poisonous and dangerous. Even the first European scientists had similar views.... To me, however, these creatures do not appear horrible; rather they are extremely fascinating.”

– Peter Weygoldt (2000, p. 9)

Scientists have long been intrigued by the behavior of animals. Behavioral ecologists and animal behaviorists in

particular are interested in the fitness consequences of behavior (Birkhead & Monaghan 2010); in how selection pressures ranging from an organism’s abiotic environment to its inter- and intraspecific interactions ultimately shape morphology and behavior. The history of these fields shows that scientists are drawn toward the study of evolutionary paradoxes that animals reveal through their behavior. For example, classic questions in ethology and behavioral ecology include why eusocial insects forgo reproduction (Hamilton 1964), why some birds display such vibrant plumage (Fisher 1915; Zahavi 1975) or why male lions kill cubs (Parker 1979). We posit that amblypygids (Class Arachnida, Order Amblypygi) offer exceptional

behavioral phenomena whose further study could contribute substantially to our understanding of animal behavior and its evolutionary relationships with morphology and sensory physiology.

The new century has seen the proliferation of tantalizing behavioral studies on amblypygids (Fig. 1), providing a solid foundation from which future work can build. For example, researchers have discovered that some amblypygid species exhibit initial levels of sociality (Rayor & Taylor 2006), individual-level recognition (Walsh & Rayor 2008), tactile learning (Santer & Hebets 2009a), and intraspecific behavioral divergence (Chapin 2015; Fig. 1). Further, research on the physiology, neuroanatomy, and sensory biology of Amblypygi (reviewed in Santer & Hebets 2011a) has opened the doors for investigations connecting proximate and ultimate mechanisms (*sensu* Tinbergen 1963), improving our understanding of the evolution of specialized sensory systems and associated behavior. The purpose of this review is to synthesize and outline the history of Amblypygi behavior research as a means of stimulating future work on these remarkable organisms. It is our view that Amblypygi are both fascinating organisms and excellent study systems for many questions in behavior, especially those addressing evolutionary relationships between sensory systems, neurophysiology, and complex behavior, as well as those focused on the role of the environment in behavioral divergence.

2. INTRODUCTION TO THE ORDER

2.1 Diversity and evolutionary relationships.—To date, within the arachnid order Amblypygi, there are approximately 151 extant named species divided among 17 genera and 4 families (Harvey 2002, 2003; Beccaloni 2009; ITIS 2015; Fig. 1). Amblypygi form a monophyletic group with Schizomida (short-tailed whip scorpions) and Thelyphonida (vinegaroons) called the Pedipalpi. These three orders together with Araneae form the clade Tetrapulmonata (Shultz 1990, 2007; Wheeler & Hayashi 2005; Regier et al. 2010). Fossil Amblypygi date to 312 mya, placing their divergence from Araneae prior to the Upper Carboniferous (Dunlop 1994, 2011; Dunlop & Martill 2002).

2.2 External morphology.—Amblypygids have dorso-ventrally flattened bodies, orthognathous chelicerae, raptorial pedipalps, and a front pair of extremely elongate legs (Fig. 2). The front legs are not used for locomotion, but serve as specialized sensory structures adorned with thousands of sensory organs (reviewed in Santer & Hebets 2011a). All legs, including the antenniform first pair, autotomize at the patella-tibia joint using muscles specialized for this purpose. Similar to some spiders and contrary to all harvestmen (Gnaspini & Hara 2007), amblypygid limbs regenerate with ecdysis (Weygoldt 1984, 2000). Amblypygi have eight ocelli (except some troglomorphic forms) in three groups (two lateral, one medial) and all species are nocturnal (Beck & Pabst 1969; Beck 1972; Weygoldt 2000).

2.3 Sensory physiology and neuroanatomy.—A recent in-depth review of amblypygid sensory physiology provides both an overview and specific details regarding the current understanding of amblypygid sensory physiology and how their unique sensory adaptations may be related to their natural history and behavior (Santer & Hebets 2011a). Briefly, distinct sensory organs on the legs of amblypygids can detect a variety of substrate-borne and airborne chemical and mechanical cues (Beck et al. 1974, 1977; Foelix et al. 1975; Foelix & Troyer

1980; Hebets & Chapman 2000a; Santer & Hebets 2008, 2009a, b, 2011a, b) including near-field particle velocity (Robert & Hoy 2007; Santer & Hebets 2008, 2011b) and substrate texture (Santer & Hebets 2009a). Details of these sensory structures and their putative functions were reviewed in Santer & Hebets (2011a). Amblypygids also possess giant interneurons that connect receptor cells to the central ganglia allowing for an extremely fast pathway of information (Foelix & Troyer 1980; reviewed in Foelix & Hebets 2001, Spence & Hebets 2006). This pathway seems important for several context-specific roles in Amblypygi behavior (reviewed in Santer & Hebets 2011a).

The central ganglia of amblypygids also include the largest mushroom bodies of any arthropod, relative to their body size (Strausfeld et al. 1998). Mushroom bodies are higher brain centers located in the first brain segment of all arthropods and their common ancestors (Kenyon 1896; Strausfeld et al. 2006; Brown & Wolff 2012; Strausfeld 2012; Wolff et al. 2012). In insects, they are important in contextual information processing, learning, and memory (fruit flies: de Belle & Heisenberg 1994; Zars et al. 2000; Pascual & Preat 2001; Heisenberg 2003; honey bees: Erber et al. 1980, Menzel 2001; cockroaches: Mizunami et al. 1998). Compared to insects, lobes of amblypygid mushroom bodies are extraordinarily large and elaborately folded and are hypothesized to be associated with complex behavior such as multisensory integration during homing (Hebets et al. 2014a, b).

2.4 Reproductive physiology and life history.—Males transfer sperm to females using a spermatophore that they attach to the substrate for females to pick up with sclerotized claspers (gonopods) on their genitalia (Weygoldt 2000). Oviposition occurs weeks to months later, after which females carry eggs inside an eggsac located on the ventral surface of their opisthosoma (Fig. 3c; Weygoldt 2000). Young hatch from eggs after about three months. Young molt, emerge from the eggsac, and move to the dorsal surface of the mother's opisthosoma (Fig. 3d) before molting again, after which they are free-living. The amblypygid lifespan is not well known, but larger species can live over 10 years in captivity (Weygoldt 2000). They are the only order of arachnid in which all species exhibit post-ultimate molts. Across the Arachnida, post-ultimate molts occur in only a few spider groups (Kraus & Kraus 1988; Coddington & Levi 1991; Vetter 2011), and usually only females continue to molt. They occur in tropical through temperate regions throughout the world, where they represent primary and secondary predators (Beccaloni 2009).

3. ENVIRONMENTAL INTERACTIONS

3.1 Microhabitat use and preference.—Amblypygi are found throughout tropical and subtropical zones across all continents (Fig. 1). Species distributions are generally limited to latitudes where freezing temperatures are rare. Within this climate zone, amblypygids inhabit a variety of ecosystem types, from tropical wet forests to xeric deserts, caves, and island environments. Like many other organisms, amblypygids show preferences for specific microhabitats (Table 1). In common across studied Amblypygi is the occupation and defense of small areas around a single refuge. Refuges typically follow a shape suitable to accommodate the dorso-ventrally flattened amblypygid body form such as in cracks and crevices in cave walls (Chapin 2015) or in tropical tree buttresses (Hebets 2002; Dias &

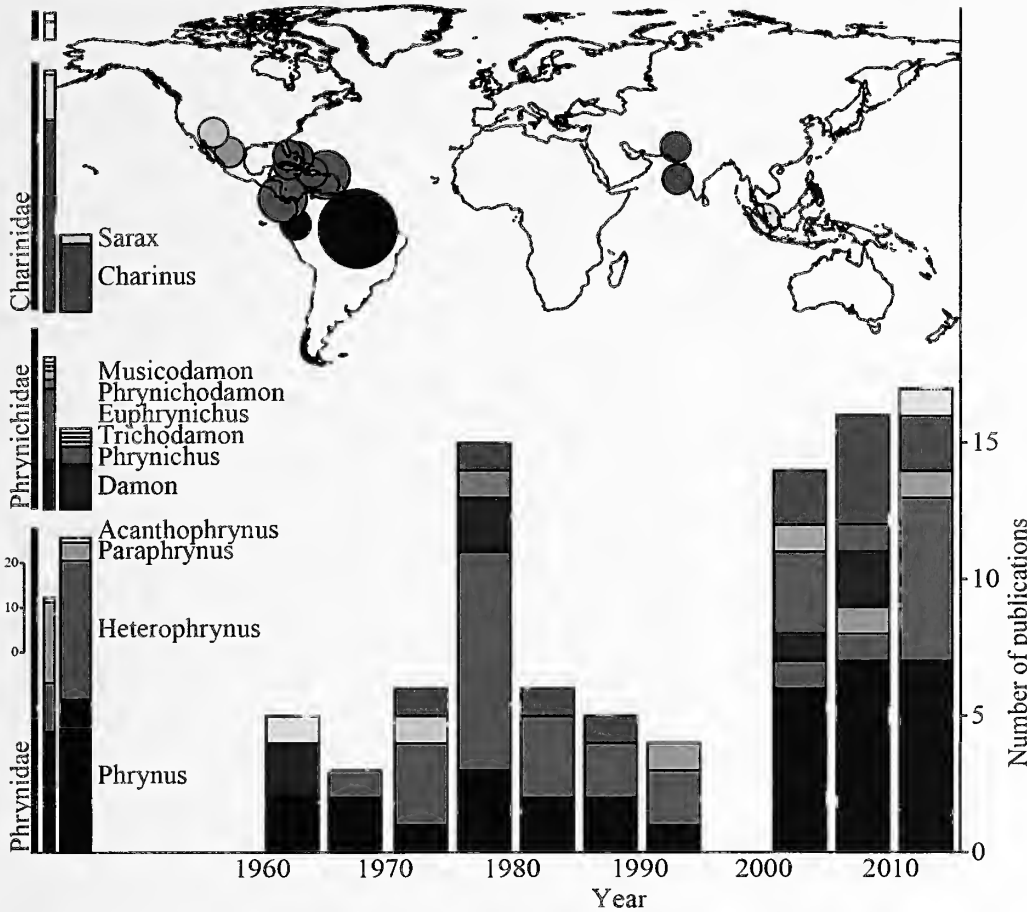


Figure 1.—Summary of behavioral ecology-related research using Amblypygi species as study organisms. Left bars: species richness (thin bars) compared to publication richness (thick bars) by genus. Right bars: Number of publications per half decade by genus. Map: Location of field studies. Circle color and size indicate genus studied and number of publications.

Machado 2006; Carvalho et al. 2012; Chapin 2014), under debris like rocks and logs (Fowler-Finn & Hebets 2006), in appropriated abandoned burrows (Weygoldt 2000), in bracts of bromeliads (Jocque & Giupponi 2012), or even in termite mounds (Carvalho et al. 2011). Refuges house individuals during daylight hours and provide shelter from predators.

Most research on Amblypygi habitat preference has been limited to species dwelling in and on tree trunks—a common microhabitat of forest amblypygids (Table 1). These species

show preferences for large, buttressing trees with refuges in abandoned burrows under bark, under leaf litter piles, or in crevices created by decay or buttressing (Hebets 2002; Dias & Machado 2006; Carvalho et al. 2012; Chapin 2014, 2015). Thus, tree-dwelling species appear to rely on large, old growth trees for territory formation (Chapin 2014). As such, selective logging negatively impacts population sizes (Bloch & Weiss 2002). Intriguingly, seasonal variation in microhabitat preference appears to occur in some species, perhaps explained by

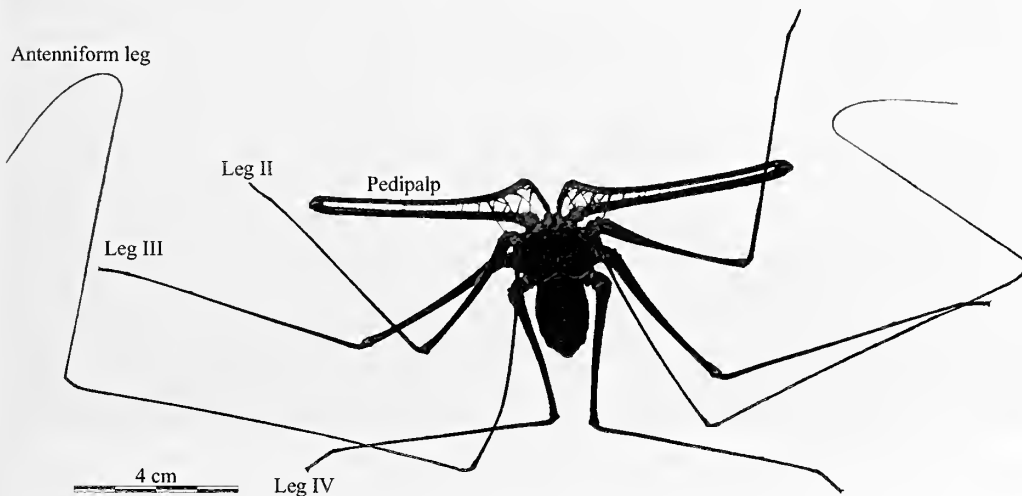


Figure 2.—Image of *Heterophrynus batesii*, indicating main appendages. Antenniform legs are used for sensing the environment and communication, not walking. The pedipalps act as the main prey capture appendages. The main body is divided into two segments: The anterior prosoma and posterior opisthosoma. Amblypygi do not produce silk or venom. In this species, pedipalp length is longer in males (shown). Photograph by K.J. Chapin.

Table 1.—Amblypygi microhabitat preference studies. While informative, current research is limited to New World tropical and subtropical forests.

Species	Habitat	Microhabitat	Study
<i>Heterophrynus batesii</i>	Wet tropical forest	Large, complex, and buttressing trees	Chapin 2014
<i>Phrynus longipes</i>	Wet subtropical forest	Large trees, variation in tree species with season	Curtis & Bloch 2014
<i>Heterophrynus longicornis</i>	Wet tropical forest	Trees with burrows at bases, not tree size	Porto & Peixoto 2013
<i>Heterophrynus longicornis</i>	Wet tropical forest	Areas with abundance of large trees and termite nests	Carvalho et al. 2012
<i>Heterophrynus longicornis</i>	Wet tropical forest	Large trees with burrows at base	Dias & Machado 2006
<i>Phrynus longipes</i>	Wet subtropical forest	Lower density in anthropogenically-disturbed forests	Bloch & Weiss 2002
<i>Phrynus pseudoparvulus</i>	Wet tropical forest	Large trees with high moss cover	Hebets 2002

prey abundance, competition, or ontogeny (Curtis & Bloch 2014)—a finding worthy of future research. Finally, recent research investigated behavioral variation across habitats. In *Phrynus longipes* (Pocock, 1894) of Puerto Rico, eave populations exhibit distinct, environment-specific variation in activity level, vigilance, hunting behaviors, and aggression relative to epigeal (surface-dwelling) conspecifics (Chapin 2015). The selection pressures or behavioral plasticity that promote this variation are yet unknown.

While habitat preference is relatively well studied among some Amblypygi species (Table 1), the costs and benefits of habitat preferences remain unresolved. Preferred microhabitats should provide limiting resources like prey, predator defense, or access to mates. Indeed, some differences in site fidelity occur across sexes with females demonstrating higher site fidelity than males (Hebets 2002), but the reason for this is unclear. Laboratory or semi-natural experiments that manipulate the potential benefits afforded to microhabitat holders could reveal putative adaptive value of microhabitat preference. Further, the field research has been biased toward only a few genera and localities (Fig. 1), with a notable underrepresentation of old world ecosystems. Research on microhabitat preferences

of additional amblypygid species across distinct ecosystems, combined with analyses of costs and benefits associated with microhabitat preferences, are needed to obtain a general understanding of the relationship between amblypygids and the ecosystems in which they occur.

3.2 Navigation.—Given that studied amblypygid species tend to reside in refuges that they reliably occupy over various time frames (Hebets 2002; Dias & Machado 2006; Carvalho et al. 2012; Porto & Peixoto 2013; Chapin 2014, 2015; Curtis & Bloch 2014; Chapin & Hill-Lindsay 2015), it may not be surprising that residents are capable of finding their way back to these refuges—a phenomenon known as homing. The distances over which amblypygids have been shown to home, in combination with the complex habitats through which they travel (e.g., lowland tropical rainforests), make these feats quite impressive. Similar homing activities have been studied extensively in the wolf spider *Lycosa tarantula* (Linnaeus, 1758) (Ortega-Escobar 2002, 2011; Reyes-Alcubilla et al. 2009; Ortega-Escobar & Ruiz 2014) and the wandering desert spider *Leucorchestris arenicola* Lawrence, 1962 (Norgaard et al. 2003, 2007, 2008, 2012; Norgaard 2005). Like many of their arthropod relatives (e.g., ants, bees, crabs, etc.; reviewed in Cheng

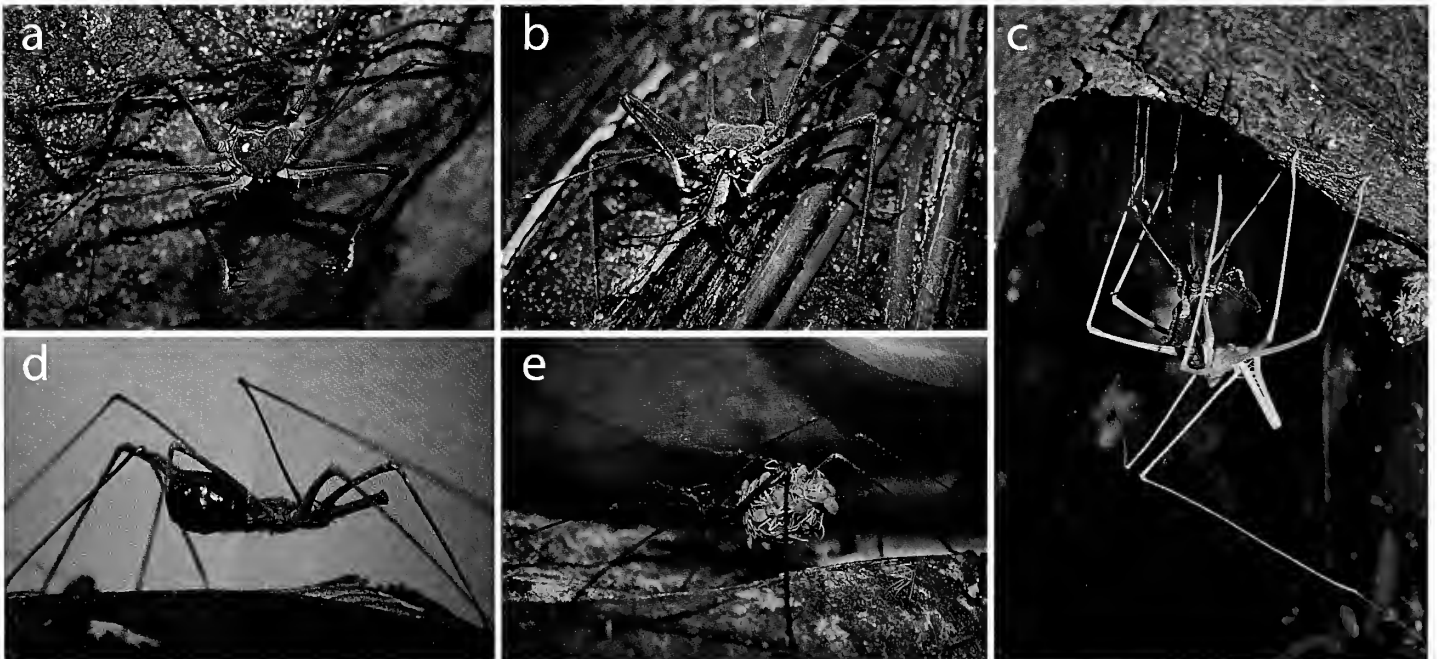


Figure 3.—Photographs of the natural history of the amblypygid *Heterophrynus batesii* in Amazonian Ecuador, (a) awaiting prey (b) feeding on a *Nephila* sp. spider, (c) engaging in ecdysis, (d) carrying an eggsack, and (e) carrying offspring. Photographs by K.J. Chapin.

2006, 2012), homing in *L. tarantula* involves path integration, or the constant updating of a homeward vector during an outward route (Ortega-Escobar 2002; Reyes-Alcubilla et al. 2009; Ortega-Escobar & Ruiz 2014); and in both *L. tarantula* and *L. arenicola*, nocturnal navigation appears to be based on vision (Ortega-Escobar 2002, 2011; Norgaard et al. 2007, 2008, 2012; Reyes-Alcubilla et al. 2009; Ortega-Escobar & Ruiz 2014). In contrast to their spider relatives, however, neither path integration nor vision appears necessary for amblypygid homing, though more work is needed to further explore the putative role of vision (Hebets et al. 2014a, b).

Physical displacement studies that moved individuals up to 10 m in a tropical rainforest understory found that amblypygids can return to their home refuge within three nights, many of them returning after only one (Beck & Görke 1974; Hebets et al. 2014a). Their successful homing in the face of physical displacement demonstrates that path integration is not critical for nightly homing. It is important to note, however, that path integration may nonetheless be important in establishing a working knowledge of an unfamiliar environment (e.g., during learning walks; Norgaard et al. 2012). In addition to successfully homing, radio telemetry experiments have demonstrated that the return paths of displaced amblypygids are often not direct—they include stopovers at additional refuges along the way (Hebets et al. 2014a). This observation is intriguing and suggests that amblypygids might possess a more general working knowledge of their local microhabitat (e.g., cognitive maps; Hebets et al. 2014a). Future work involving long-term tracking of individuals or long-term observations of amblypygids in a novel environment may shed light on the degree to which prior knowledge of an environment influences homing ability.

Though many nocturnal arthropods appear to exhibit visually-guided navigation behavior (Cheng 2006, 2012), olfaction has long been suggested as important in amblypygid navigation. Indeed, work by Beck & Görke (1974) suggested an olfactory mechanism in amblypygid homing. More recent field displacement studies using *Phrynus pseudoparvulus* Armas & Viquez, 2002 with occluded olfactory or visual capacities further support the role of olfaction and potentially vision in amblypygid homing. Sensory-occluded individuals were less successful in homing compared to sensory intact individuals (Hebets et al. 2014b). Importantly, the methods used to occlude olfactory capacities involved either clipping of the distal tip of the antenniform legs or physically covering the distal tip of the antenniform legs with nail polish. Both methods undoubtedly influence mechanosensory perception as well, making it impossible to rule out the importance of tactile information. Laboratory trials have also demonstrated that the amblypygid *Phrynus marginemaculatus* C.L. Koch, 1840 can learn tactile cues to navigate to a retreat (Santer & Hebets 2009b) and this additional modality may also be important in amblypygid navigation. Future work should focus on determining the relative importance of distinct sensory information (e.g., olfactory, visual, tactile) and their probable interactions on amblypygid navigation.

The size of arthropod mushroom bodies has been hypothesized to reflect their spatial navigation strategies (Jacobs 2012), and amblypygids are well-known for their enlarged mushroom bodies (Strausfeld 1998). In the visually guided

desert ant, *Cataglyphis bicolor* (Fabricius, 1793), researchers were able to demonstrate a remarkable increase in mushroom body size during the extremely short lifespan (ca. 6 days), and argued that the need for higher-level navigational requirements might drive their observed increase in neuropile volume (Kuhn-Buhlmann & Wehner 2006). Subsequent comparative work on the desert ant *Cataglyphis fortis* (Forel, 1902) and *Melophorus bagoti* Lubbock, 1883 further suggest that species-specific navigational capacities are influenced by environment-dependent characteristics such as habitat complexity (e.g., presence or absence of landmarks; Buehlmann et al. 2011). For example, *C. fortis*, which is found in a more featureless natural habitat than *M. bagoti*, tends to rely more on vector-based navigational strategies while *M. bagoti* can use landmark-guided navigation (Buehlmann et al. 2011). Research that focuses on diverse arthropod taxa, such as amblypygids, could greatly enhance our understanding of the links between such navigation strategies and selection pressures imposed by environmental complexity. Additionally, the demonstrated navigational capacities of amblypygids and their possible relationship with multi-sensory integration and enlarged mushroom bodies makes them another putative model system for a more general understanding of the neural mechanisms underlying complex navigation.

4. INTERSPECIFIC INTERACTIONS

4.1 Predators and prey.—Amblypygids act as secondary and tertiary consumers of the ecosystems in which they occur. The amblypygid diet is varied and seemingly opportunistic, comprised mainly of primary consumer arthropods, especially Orthoptera and Blattodea (Table 2). Amblypygids have also been observed feeding on sphingid and noctuid moths, orb weaving spiders (Fig. 3b), the scorpion *Centruroides gracilis* (Latreille, 1804), *Anolis* lizards, hummingbirds, and crayfish captured from streams (Table 2). Interestingly, some species seem quite adept at aerial or aquatic prey capture (Hebets & Chapman 2000b; Hebets 2002; Ladle & Velander 2003). Individuals hunt prey using sit-and-wait tactics and are commonly seen with open pedipalps awaiting prey (Fig. 3a). Species inhabiting vertical environments (e.g., tree trunks, cave walls) are most often seen facing down (Weygoldt 2000; Hebets 2002; Chapin 2014), possibly for efficiency in prey capture (Fig. 3a), though this remains to be tested.

Amblypygids fall prey to large lizards and small mammals, including bats (Table 3). Field studies recorded lycosid spiders (Chapin 2011) and scorpions (Hebets 2002; Teruel & Toledo 2014) preying on amblypygids (Table 3). Thus, many Amblypygi species engage in symmetrical intraguild predation by preying on species that are both competitors for prey and potential predators (Polis et al. 1989; Holt & Polis 1997). Intraguild predation has structural effects on ecosystems in which it occurs, including the reduction of predators when prey of lower trophic levels are scarce (Polis & McCormick 1987). This highlights the potentially important role of amblypygids in the trophic structure of ecosystems in which they are abundant. Further, amblypygids engage in a special case of symmetric intraguild predation: cannibalism. Cannibalism rates vary across species, from being quite rare to up to 20% of interactions ending in cannibalism during laboratory trials (Weygoldt 2000; Pinto-da-Rocha et al. 2002; Torres-Contreras et al. 2015;

Table 2.—Known prey of amblypygids delineated by vertebrate and invertebrate taxa.

Prey	Predator	Citation
Invertebrates		
Harvestmen (Opiliones)	<i>Phrynus pseudoparvulus</i>	Hebets 2002
Scorpion (<i>Centruroides gracilis</i>)	<i>Paraphrynus cubensis</i>	Forcelledo & Armas 2014
Spiders (Araneae)	<i>Phrynus pseudoparvulus</i>	Hebets 2002
Giant golden orb-weaver spider (Nephilidae)	<i>Heterophrynus batesii</i>	Chapin 2011
Crickets and katydids (Orthoptera)	<i>Heterophrynus batesii</i> , <i>Phrynus pseudoparvulus</i>	Hebets 2002, Chapin 2011
Cockroaches (Blattodea)	<i>Phrynus longipes</i> , <i>Phrynus pseudoparvulus</i>	Hebets 2002, Chapin 2015
Moth (Lepidoptera)	<i>Phrynus longipes</i>	Hebets 2002
Sphingid moth (Sphingidae)	<i>Heterophrynus batesii</i> , <i>Phrynus pseudoparvulus</i>	Beck & Görke 1974
Freshwater prawn (<i>Macrobrachium</i> sp.)	<i>Heterophrynus cheiracanthus</i>	Ladle & Velandar 2003
Millipedes (Myriapoda)	<i>Phrynus pseudoparvulus</i>	Hebets 2002
Vertebrates		
Antillean crested hummingbird (<i>Orthorhyncus cristatus</i>)	<i>Phrynus longipes</i>	Owen & Cokendolpher 2006
Anoline lizards (<i>Anolis</i> sp.)	<i>Phrynus longipes</i>	Reagan 1996
Goldenscale anole (<i>Anolis nitens chrysolepis</i>)	<i>Heterophrynus longicornis</i>	Kok 1998

Chapin & Hill-Lindsay 2015). It is unclear, however, how prevalent cannibalism is under natural conditions. Most cannibalism among amblypygids is size-structured or assumed so, such that ontogenetically asymmetric predation is the norm (Persson et al. 2004). Some amblypygid species, however, also cannibalize as an resolution to contests (Chapin 2015). In such cases, cannibalism is most likely to occur among size-matched contest opponents rather than size-asymmetric pairs.

Intraguild predation and cannibalism offer interesting avenues to understand the dynamic relationships within an ecosystem. In particular, how prey choice, including cannibalism, changes with individual condition might reveal the fitness costs that may disfavor cannibalism in times of plenty. Further, cannibalistic behavior under laboratory conditions is often a consequence or by-product of agonistic interactions. Thus, contests can impact the ecology of environments in which they occur. More aggressive populations should have higher rates of cannibalism due to the escalation of agonistic interactions. To date, most information on both predators and prey of amblypygids comes from opportunistic field observations, and more quantitative assessments of the role of amblypygids in ecosystem trophic structures are needed.

4.2 Parasites and parasitoids.—New instances of amblypygid parasites and parasitoids continue to be documented as the number of field studies on these fascinating creatures increases (Fig. 4). For example, Armas & Trueba (2003) found

a prostigmatan mite infesting *Phrynus kennidae* Armas & González, 2002. The mite was described as an ectoparasite on other arthropods as well, indicating that it is not host-specific. A mite of the genus *Odontacarus* Ewing, 1929 was similarly found parasitizing the amblypygid *Charinus brasiliensis* Weygoldt, 1972 in southeastern Brazil (Gonçalves-Souza et al. 2014) and an unidentified Brachyceran fly has been documented to parasitize the abdominal lumen of adult *Heterophrynus batesii* (Jorya & Rojas 2013). Several *P. marginemaculatus* died from mite infestations under laboratory conditions (Rayor & Taylor 2006).

Parasitoids are also known to use Amblypygi as hosts. The parasitoid chloropid fly (*Pseudogaurax* sp.) parasitizes the eggsacs of *P. pseudoparvulus* and *Paraphrynus laevifrons* (Pocock, 1894) (Viquez & Armas 2009). It is believed that eggs are laid on the eggsacs of females and the fly larvae consume the Amblypygi eggs upon hatching. The larvae then pupate on the female's opisthosoma before emerging. Parasites and parasitoids of wild amblypygids are probably more common than reported due to the lack of field research on the order (Gonçalves-Souza et al. 2014). In fact, recent field work on *P. laevifrons* in Costa Rica has identified multiple parasitized females in close proximity (Fig. 4; Tyler Corey personal observation). Research into parasite- and parasitoid-host relationships does not yet exist in amblypygids, yet is surely a fruitful avenue for future research. Studies of host-specificity and parasite defense could highlight the role of amblypygids in their ecosystems.

Table 3.—Known predators of amblypygids delineated by vertebrate and invertebrate taxa.

Predator	Prey	Citation
Invertebrates		
Lycosid spider (Araneae: Lycosidae)	<i>Heterophrynus batesii</i>	Chapin 2011
<i>Alayotityus sierramaestrae</i> Armas, 1973 (Scorpionida: Buthidae)	<i>Phrynus damonidaensis</i>	Armas et al. 2013
<i>Rhopahurus junceus</i> (Herbst, 1800) (Scorpionida: Buthidae)	<i>Phrynus pinarensis</i>	Teruel & Toledo 2014
<i>Centruroides edwardsii</i> (Gervais, 1843) (Scorpionida: Buthidae)	<i>Phrynus whitei</i>	Armas 1995
<i>Phrynus longipes</i> (Amblypygi: Phrynidae)	<i>Phrynus hispaniolae</i>	Armas & Ramírez 1989
Vertebrates		
White-throated round-eared bat (<i>Lophostoma silvicolum</i>)	Amblypygi sp.	Reid 1997
Common coqui frog (<i>Eleutherodactylus coqui</i>)	<i>P. longipes</i>	Stewart & Woolbright 1996
Bronze coqui frog (<i>Eleutherodactylus richmondi</i>)	<i>Phrynus longipes</i>	Stewart & Woolbright 1996
Cuban solenodon (<i>Solenodon cubanus</i>)	<i>Paraphrynus robustus</i>	Armas 1987
Asian water monitor (<i>Varanus salvator</i>)	<i>Stygophrynus dammermani</i>	Dammerman 1948

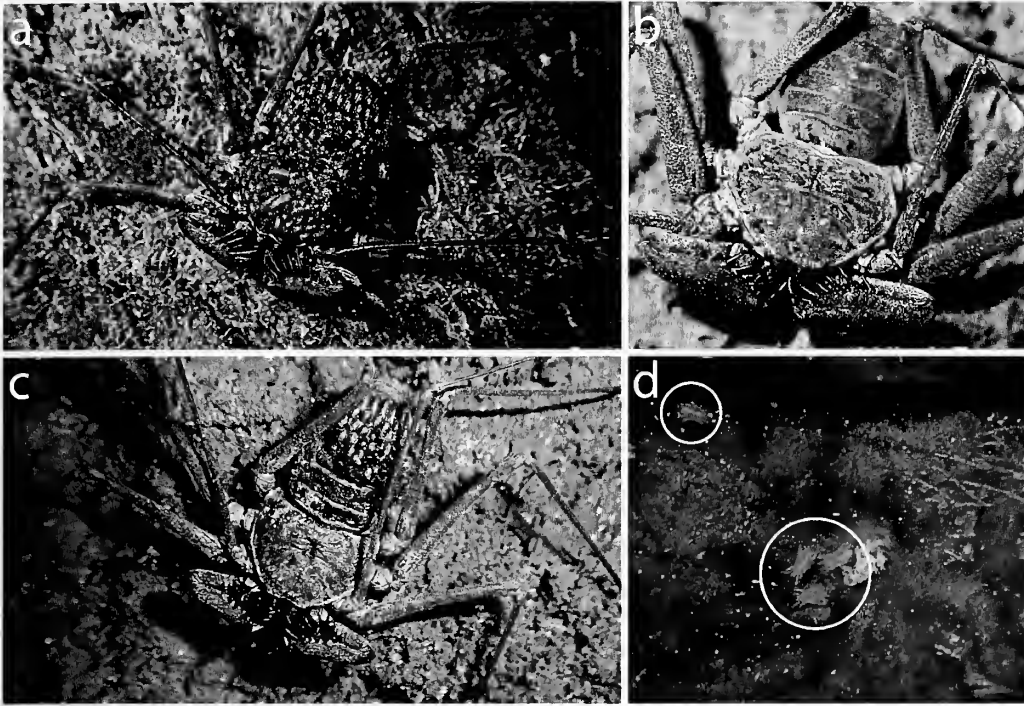


Figure 4.—Ectoparasites of some Amblypygi species. (a, c) The amblypygid *Paraphrynus laevifrons* in Costa Rica with parasitoid eggs. The parasitoid species is yet to be identified. Photo by Tyler Corey. (b) Amblypygi sp. with mites. Photo by Glauco Machado. (d) Microscopy image of the ventral opisthosoma of *Phrynus longipes* with mites in association with intersclerite membranes (top circle) and spiracles (bottom circle). Photo by K.J. Chapin.

The literature does not report any information on potential bacterial or fungal relationships among Amblypygi. Field observations of cave-dwelling *Phrynus longipes* in Puerto Rico, however, have noted a white substance covering the cuticle of some individuals (E.A. Hebets personal observation). While this substance was not identified, the possibility of fungus- or bacterial-amblypygid relationships remains a real possibility.

4.3 Commensalism.—Amblypygids interestingly share the active burrows of several other animals, including birds, mammals, scorpions, ants, and termites (Weygoldt 2000; G. Machado personal communication). Termite mounds appear to provide both shelter and a food source for *Damon medius* (Herbst, 1797) (Weygoldt 2000), the blind *Paracharon caecus* Hansen, 1921, and *Heterophrynus longicornis* (Butler, 1873) (Carvalho et al. 2011). Amblypygids are also found in ant nests. *Charinus quinteroi* Weygoldt, 2002 and *Charinus platnicki* (Quintero, 1986) both associate with ant nests and both have reduced eyes (Weygoldt 2000). Of particular note is the neotropical species *Phrynus gervaisii* (Pocock, 1894), which was found to occupy nearly half of the nests of *Paraponera clavata* (Fabricius, 1775)—the particularly toxic bullet ant (Schmidt et al. 1984)—on Barro Colorado Island, Panama (LeClerc et al. 1987). Follow-up observations on *Phrynus pseudoparvulus* in Costa Rica, however, found the presence of amblypygids to be independent of the presence of *P. clavata* nests (E.A. Hebets unpublished data), suggesting that the presence of amblypygids in these nests might reflect opportunistic refuge use as opposed to any special relationship between amblypygids and ants. Regardless, numerous observations highlight the potential for amblypygids to share the homes of other animals and in these cases, amblypygids seem to be able to avoid the antipredator defenses of their host nests. The potential for interesting interspecific interactions, including undescribed commensalisms and mutualisms, exists between amblypygids and the hosts of

shared nests. We see a combination of field and laboratory studies exploring first, refuge choice associated with animal burrows and second, the costs and benefits of this choice as particularly interesting areas for future study.

5. INTRASPECIFIC INTERACTIONS

5.1 Contests and territoriality.—Several lines of evidence suggest that territoriality is common throughout the order Amblypygi. Under laboratory conditions, amblypygids exhibit territory defense, such that territory holders are more likely to win contests than intruders (Chapin & Hill-Lindsay 2015). In the field, putative territories with resident Amblypygi removed are recolonized by smaller conspecifics (Porto & Peixoto 2013), indicating that large animals might exclude smaller would-be usurpers. Further, field observations have recorded site associations lasting for months with several species exhibiting high site fidelity (Beck & Görke 1974; Hebets 2002; Chapin 2011; Hebets et al. 2014a, b). Taken together, these findings show that territoriality occurs in at least some Amblypygi species.

Contests within select amblypygid species are well-described (Weygoldt 1969, 1974a, b, 1977a, b, 2000; Fowler-Finn & Hebets 2006; Santer & Hebets 2008; Chapin 2015). Interactions appear quite ritualized and stereotyped as they frequently follow a regular sequence of behaviors supposedly adapted for communication (e.g., Santer & Hebets 2008). For example, when presented with an opponent, individuals generally engage in a series of pedipalp and antenniform leg movements that may escalate to physical aggression in a regular sequence (Santer & Hebets 2008). Injuries during contests range from nonexistent (Weygoldt 1977a; Fowler-Finn & Hebets 2006; Santer & Hebets 2008) to frequent death and cannibalism (Chapin 2015). While specifics of agonistic interactions vary across species, most species studied to date include vibrations and fencing with antenniform legs in addition to pedipalp movements in

agonistic displays (Weygoldt 2000; Santer & Hebets 2008; Chapin 2015). Detailed work combining high speed videography and electrophysiology demonstrated that amblypygids communicate with air particle displacement (near-field sound), detected by opponent trichobothria (Santer & Hebets 2008, 2011b). Amblypygids were the first arthropod where filiform hairs were confirmed to function in communication (Santer & Hebets 2008, 2011b), demonstrating their potential for novel contributions to animal communication more broadly.

Contest outcome is predicted by proxies for contestant resource holding potential (RHP), or the absolute fighting ability of animals (Parker 1974). Laboratory trials show that size, body condition, antenniform leg movement, and body raising predict RHP and contest outcome (Fowler-Finn & Hebets 2006; Santer & Hebets 2008; Chapin & Hill-Lindsay 2015). Further, males of many species have elongated pedipalps as compared to females (Weygoldt 2000; Chapin 2011, 2014), which might play a role in territory contests. Available data to date suggest a predominant role of mechano-sensory stimuli (i.e., generated from probing and antenniform leg vibrations; Santer & Hebets 2008) in agonistic interactions while the roles of vision or olfaction remain less clear (Santer & Hebets 2011a). That being said, olfaction may be important for territory recognition (Chapin & Hill-Lindsay 2015).

Territorial behavior typically reflects the need to defend a valuable resource (Briffa & Hardy 2013), but the exact resource and its putative value have not been directly identified in amblypygids. Individuals most likely benefit from territorial behavior by securing retreats used for predator defense, which might be particularly valuable for females during maternal care. How territoriality might differ across sexes is unclear; both sexes engage in territory contests (Chapin 2015), but some research suggests that females show higher site fidelity than males (Hebets 2002). Further, females of at least one species, *P. marginemaculatus*, are less likely to escalate to more risky behaviors than males in laboratory conditions (Fowler-Finn & Hebets 2006).

5.2 Mating systems and mate choice.—Reproductive behavior is the best studied area of amblypygid biology, but all observations published to date have been conducted in the laboratory (Alexander 1962a, b; Klingel 1963; Weygoldt 1969, 1970, 1972, 1974a, b, 1977a, b, 1995, 1996, 1997, 1999a, b, 2002, 2003, 2005, 2006, 2007; Weygoldt & Hoffmann 1995; Peretti 2002; Weygoldt et al. 2010). Survivorship beyond reproduction provides no fitness benefits in most arthropods because they have no post-ultimate molt and often only reproduce one or a few times before death (Triplehorn & Johnson 2005; Beccaloni 2009). Amblypygids, however, spend most of their lives as adults and post-ultimate ecdysis probably increases adult survivorship by providing opportunities for injury recovery, limb regeneration, parasite shedding, and even sperm rejection. Furthermore, growth seems indeterminate (Chapin 2011)—a rare trait among arachnids. This may result in older individuals having both increased fecundity and greater resource holding potential, allowing for higher reproduction and more mating opportunities. This effect may be promoted by the sexual dimorphisms found in most species, which become more pronounced as they age (Chapin 2011).

Amblypygids mate by indirect sperm transfer via a sclerotized spermatophore. Courtship lasts from one to eight hours

and the pre-copulative ritual involves a varied repertoire of behavior (Weygoldt 2000). This includes male antenniform leg vibrations, jerking motions, petting with special bristles involved in antenniform leg cleaning, extending pedipalps, angling pedipalps at the trochanter, and stroking the female's distal pedipalp with the male's chelicerae. Behavior and timing are thought to be species specific, and might play a role in interspecies copulation avoidance (Weygoldt 2000). That being said, spermatophore and gonopod morphology are probably greater barriers to interspecies copulation than behavior, as is thought to be the case with other arachnid groups (Eberhard 1985; Huber 2002). Spermatophore deposition takes about five minutes in observed species. The female collects spermatozoa by lifting the genital operculum and grasping with gonopods, which are articulating sclerotized structures for taking spermatozoa packets (Weygoldt 2000). In laboratory settings, the pair mates multiple times, sometimes over several weeks (Weygoldt 2000).

Mating systems have not been explicitly studied in any species of amblypygid. Across the order, polygyny (males mating with multiple females) is expected because females invest in egg guarding by carrying eggsacs attached to the ventral abdomen and young on their dorsum, thereby freeing males to mate with other females but precluding females from mating again until offspring are free-living. It could be the case, however, that females mate multiply before producing egg clutches. As mentioned previously, females offered a single male in laboratory conditions often mate multiply (Weygoldt 2000). In natural conditions, however, this may be realized as multiple mating with separate males. Alternatively, males may mate guard and mate multiply to ensure clutch-wide paternity. Weygoldt (2000) proposed that multiple mating episodes might stimulate reproductive physiology, ensure fertilization, ensure paternity, overcome possible genetic defects occurring as sperm ages, or enable sperm competition. Other researchers have suggested that female defense polygyny (*sensu* Emlen & Oring 1977) seems likely (Weygoldt 2000). Monogamy via parental care is suspected in at least one amblypygid species (*H. longicornis*; Weygoldt 1977a), however, mate guarding might be a better explanation for male presence. Lastly, parthenogenesis likely occurs in at least four species of Amblypygi, as evidenced by female-only populations or captive reproduction by virgin females (Armas 2000, 2005; Weygoldt 2005, 2007; Seiter & Wolff 2014). Given the relatively small number of observations on species-specific reproductive behavior, we lack a comprehensive overview of amblypygid mating system diversity and thus have an incomplete understanding of the selection pressures that might lead to variable mating systems.

5.3 Parental investment and sociality.—Amblypygi have altricial development and substantial parental investment. Following sperm acquisition and gestation, female amblypygids lay a clutch of eggs that adhere to their ventral opisthosoma where embryos develop externally before emerging. Offspring emerge from the eggsac and climb to the opisthosoma dorsum, after which they molt and are thereafter free-living. Amblypygid clutch sizes range from about 10 to 90 eggs depending on the species, of which only a portion will hatch into free-living offspring (Gray & Robinson 1986; Weygoldt 2000; K.J. Chapin personal observation). Survivorship has not been tracked in any naturally-occurring amblypygid populations, but the

Table 4.—Amblypygi genomic and transcriptomic studies.

Loci	Citations
nDNA	
28S	Wheeler & Hayashi 1998; Giribet & Ribera 2000; Prendini et al. 2005; Mallatt & Giribet 2006; Pepato et al. 2010; Arabi et al. 2012; Esposito et al. 2015
Actin 5S	Vink et al. 2008
EF-1 α , EF-2, Pol II	Regier & Shultz, unpublished data
H3	Prendini et al. 2005; Esposito et al. 2015
mtDNA	
12S, 16S	Prendini et al. 2005; Esposito et al. 2015
18S	Wheeler & Hayashi 1998; Giribet & Ribera 2000; Prendini et al. 2005; Mallatt & Giribet 2006; Pepato et al. 2010; Arabi et al. 2012
CO1	Prendini et al. 2005; Arabi et al. 2012; Esposito et al. 2015
ATP6, ATP8, COX1, COX2, COX3, ND2	Hassanin et al. 2005
mtDNA genome	Fahreïn et al. 2009; Masta 2008
mRNA	
56 mRNA sequences	Regier et al. 2010
reduced representation transcriptome	Borner et al. 2014
HcA-HcG	Rehm et al. 2012

greatest mortality is likely experienced early in life. That being said, cannibalism is more common among adults than juveniles, at least in some species (Chapin & Hill-Lindsay 2015; see 4.1 Predators and prey).

Social behavior beyond maternal care is suggested to exist for a few species (Weygoldt 1977a; Rayor & Taylor 2006; Chapin 2011). For example, field observations suggest that *Heterophrynus longicornis* occur in family groups of a mated pair and their offspring (Weygoldt 1977a) with observed juveniles as old as the fourth or fifth instar. Adults of this species were almost never found singly or in larger groups, but it remains unclear whether or not groups were of related animals (Weygoldt 1977a). Later research on *H. longicornis* found that multiple individuals are sometimes found in association, but the benefits that group living might afford remain unknown (Dias & Machado 2006; Carvalho et al. 2012). Similarly, laboratory observations found that immature *Phrynus marginemaculatus* from Florida and *Damon diadema* (Simon, 1876) from Kenya and Tanzania associated with each other and their mothers for as long as a year in captivity (Rayor & Taylor 2006), but field observations of these phenomena remain undocumented. Research on group-living *Heterophrynus batesii* (Butler, 1873) in Ecuador found that groups occurred on larger, more complex trees with more leaf litter when compared to the same microhabitat variables of random trees in the environment (Chapin 2011, 2014). It is clear that increased resources allow for larger groups. Given that the co-occurrence of individuals in this species is related to microhabitat characteristics, it remains unknown whether aggregations result from resource abundance, an emergent benefit provided by group living, or both.

The observations of group living and the suggestion of potential sociality in amblypygids continues to intrigue biologists,

and sociality is certainly not unknown among their close relatives (Shivashankar 1994; Machado 2002; Rayor & Taylor 2006; Lubin & Bilde 2007; Del-Claro & Tizo-Pederso 2009). It remains important, however, to explore the costs and benefits of group living beyond increased access to resources. While one might imagine several ways in which group living might be beneficial to amblypygids (e.g., foraging, defense, or reproduction), these possibilities must be directly assessed in the field. For example, amblypygid groups may benefit from predator defense or offspring food sharing, or grouping may be important for a mating system like harems (i.e., mate guarding multiple females). Nonetheless, the possibility of individual recognition, potentially quite long life spans, and monogamy in at least one amblypygid species (proposed as an essential starting point for sociality; Hughes et al. 2008; Boomsma 2009; Boomsma et al. 2011; but see Nonacs 2011) certainly establishes a plausible foundation for some level of sociality among amblypygids.

5.4 Genetics and genomics.—Genetic research on amblypygids is largely limited to universal markers typically used for phylogenetics (Table 4). Masta (2008) and Fahrein et al. (2009) sequenced Amblypygi mitochondrial genomes and more recently, approximately 2 million basepairs of nuclear DNA were sequenced for *H. batesii* (K.J. Chapin unpublished data). Additionally, the chromosomes of two species have been mapped with cytogenetic techniques. *Heterophrynus longicornis* and *D. medius* have $2n = 66$ and 70 chromosomes with homomorphic sex chromosomes (Vitková et al. 2005; Paulaneto et al. 2013). Most recently, Amblypygi have been instrumental in understanding the biogeography of Caribbean islands (Esposito et al. 2015). Research on *Phrynus* sp. from Puerto Rico and surrounding islands revealed exceptional levels of endemism at island, geologic region, and cave scales, thereby presenting a multilevel model for phylogeography (Esposito et al. 2015). How behavioral variation might be both impacted by, or contribute to, genetic isolation has yet to be investigated.

6. CONCLUSION & FUTURE DIRECTIONS

Researchers have documented fascinating behavioral phenomena across several Amblypygi species. These findings, coupled with the unique neurophysiology and life histories of amblypygids, have opened several avenues for future research. Indeed, our current knowledge raises many unanswered questions. For example, much remains to be explored in regards to amblypygid relationships with their abiotic and biotic environment. While we recognize that site fidelity, homing, and agonistic contests are indicative of territoriality, resource defense has not been tested. Which resources promote the evolution of territoriality in amblypygids? How does territorial behavior interact with the mating systems and potential sociality of amblypygids? Similarly, how does the spatial distribution of resources, including potential mates, influence navigational demands and how might this select for increased navigational capacities?

A handful of field studies have enabled glimpses into potentially interesting predatory behavior, but how specialized are amblypygids as predators? Some species are known to have the capacity to breathe underwater (Hebets & Chapman 2000b) and others have been observed feeding on aquatic prey (Ladle & Velander 2003). Do amblypygids have special

adaptations that allow them to forage underwater? Similarly, amblypygids can catch moths in flight. Does their giant interneuron system underlie this incredible capacity, and what role do trichobothria play? Further, how important are food resources in determining spatial distributions and carrying capacities?

We know very little about major survival challenges of amblypygids in the wild. What are their main predators? Do they have special adaptations or behavior associated with predator avoidance? Leg autotomy is common across amblypygid species as a strategy to avoid predation. How does autotomy affect other areas of life history, like mating success or territoriality? How often does cannibalism occur and is it a major selective agent? Recent field observations suggest that parasitoids of amblypygids may be much more common than previously appreciated. Are these parasitoids specialists of amblypygids, and how has parasite selection pressure affected amblypygid behavioral ecology?

Intraspecific interactions also offer opportunities for valuable additions to behavior research. While the reproduction of select amblypygid species has been studied in the laboratory, few field studies exist on intersexual interactions or variation across species. We know nothing about the level of mate preference that either males or females might exhibit. Males produce large, sometimes quite complex, spermatophores with refractory periods that may last several days. This pattern hints at male mate choice, which could make amblypygids an important research system contributing knowledge to this relatively understudied phenomenon (relative to female mate choice). We know nothing of whether females or males might mate multiply in the field—with either the same or different mates. Given that amblypygids continue to molt throughout their lives, can females molt as a means to dispose of unwanted sperm? If so, cryptic female mate choice could be a driver of sexual selection. Further, behavioral variation between juvenile and adult male and female behavioral repertoires remains unstudied. Despite this, juveniles experience different predators and prey, including cannibalism risks, which might have important effects on behavioral evolution. Understanding when in development male and female behaviors diverge may elucidate important mating system details across Amblypygi species.

Social behavior among amblypygids remains a fruitful avenue for research. Preliminary studies, combined with field observations, raise the distinct possibility that at least some amblypygid species exhibit basic levels of sociality via both fraternal and egalitarian pathways (Weygoldt 1977; Rayor & Taylor 2006; *sensu* Bourke 2011; Chapin 2014). If so, amblypygids could provide an excellent system for studying the initial transition from a solitary to social life history and the costs and benefits thereof. This is especially true given the aggressive and sometimes cannibalistic inclinations of the ancestors of putative social species.

Amblypygid learning abilities are remarkable (Santer & Hebets 2009a, 2011a) but poorly understood. The functional value of learning in nature and the full repertoire of learning abilities across amblypygid species remain unknown. The unique combination of Amblypygi sensory modalities and learning and memory abilities would make amblypygids a great addition to the diversity of learning research across animals.

While much amblypygid research has been driven by curiosity regarding their unusual morphology and neurophysiology, much remains to be done to link these with amblypygid behavior. Santer and Hebets (2011a) provide an excellent starting point for understanding the relationship between amblypygid neurophysiology and behavior but major questions remain unanswered. We still know little about the function of amblypygid giant interneurons or enlarged mushroom bodies, yet future work connecting this neuroanatomy to complex behavior will provide fundamental insights into neural mechanisms underlying behavior. Following from this proximate view of behavior, little is known about development in amblypygids.

Finally, amblypygid species are often discussed *en masse* and variation across species is poorly understood. Understanding behavioral variation across species and populations under different suites of selection pressures will allow for a broader understanding of amblypygid behavior and evolution (Chapin 2015). The continued combination of field research around the globe (Fig. 1) with semi-natural or laboratory manipulative experiments will be the best approach for increasing our understanding of these incredible animals.

Amblypygi research has been tantalizingly suggestive of Amblypygi exceptionalism and has built the requisite foundation for behavioral ecology research. Amblypygids have remarkable neuroanatomy and sensory biology; curious conspecific interactions ranging from cannibalism and territoriality to extended parental care and sociality; and a litany of community interactions that make them an important component of the ecosystems in which they occur. It is our hope that amblypygids and their researchers continue to contribute to our understanding of how ecologies shape the evolution of behavior and become seated at the leading edge of behavioral research.

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REVIEW

Plant-eating by spiders

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Abstract. Spiders, a group of predominantly insectivorous predators, occasionally use plant food to supplement their insect prey. In the current review, we tracked down 95 reported incidents of spiders feeding on plant food under natural conditions. Globally, >60 spider species representing ten families have been observed feeding on plant materials from over 20 plant families. Cursorial spiders including the families Anyphaenidae, Clubionidae, Eutichuridae, Salticidae, Thomisidae, and Trachelidae dominate among the spiders feeding on plant food (>80% of reported incidents). Spiders feed on a wide diversity of plant-derived products including floral nectar, extrafloral nectar, stigmatic exudate, plant sap, honeydew, seeds, Beltian bodies, Müllerian bodies and pollen (originating from very different plant types such as coniferous and deciduous trees, herbaceous plants and shrubs, annual weeds, grasses, climbing plants, orchids, carnivorous plants, and ferns). Furthermore, spiders have been shown to consume fungal spores in laboratory trials. Supplementary feeding on plant materials by spiders was shown to be global in extent and widespread across spider taxa, plant taxa and plant materials; however, the extent to which the different categories of plant food contribute to the spiders' diet and how this may affect their behavior and life history is still largely unexplored. This review is expected to lay a foundation for future research on this topic.

Keywords: Food bodies, honeydew, nectar, nutritional ecology, plant-derived food, pollen

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1. INTRODUCTION

Spiders are a speciose arthropod group with a wide variety of ecological niches, yet they have been conventionally described as

predators feeding almost exclusively on live insects (Whitcomb 1974; Sunderland et al. 1986; Young & Edwards 1990). More recent studies broadened our understanding of spider foraging

behaviors and diet composition, including observations of spiders feeding on prey such as crustaceans, earthworms, gastropods, and a variety of small vertebrates (Symondson et al. 2002; Brooks 2012; Nyffeler & Knörnschild 2013; Nyffeler & Pusey 2014). There is also a growing set of reports of spiders feeding on static foods such as arthropod eggs and dead insects (Pfannenstiel 2008; Vetter 2011; Peng et al. 2013; Vickers et al. 2014). Captive spiders have fed on unusual foods such as sausage, banana, grapes (Decae 1986; Schultz & Schultz 2009) and soy milk (Amalin et al. 1999, 2001), and have been successfully raised on artificial diets composed of milk and egg yolk mixtures (Peck & Whitcomb 1968; Amalin et al. 1999, 2001). Spiders readily imbibe sugar water (Witt 1971; Lanza 1988), and have been observed visiting hummingbird feeders (Stoaks 2009).

Reports of spiders feeding on plant products in nature first appeared in recent decades. Smith & Mommsen (1984) reported that the webs of immature cribellate orb-weavers are pollen collectors and that the pollen grains adhering to the sticky threads are unintentionally ingested along with the old silk material when the spiderlings are recycling their webs. Pollard et al. (1995) as well as Taylor & Foster (1996) documented cases of spider nectarivory from North America and Central America. Since those early reports, there have been many more discoveries of spiders feeding on plant materials (e.g., Vogeley & Greissl 1989; Ruhren & Handel 1999; Jackson et al. 2001; Blüthgen & Reifenth 2003) including a remarkable case of a salticid, *Bagheera kiplingi* Peckham & Peckham, 1896, that derives the majority of its nutrition from plant resources (Meehan et al. 2009). So far information on plant-eating spiders has been widely scattered throughout the literature and a comprehensive review covering this topic was missing. To close this gap, a systematic review on plant-eating spiders was conducted and the insights from this research are presented here.

2. METHODS

An extensive bibliographic search was carried out to locate reports on spiders feeding on plant materials. The search was based largely on the Thomson-Reuters database, Web of Science (all data bases), Google Scholar, Google Books, and ProQuest Dissertations & Theses. In addition to this, an internet search for information on this topic was conducted, and authors of internet reports on spider phytophagy were contacted to obtain additional information on their observations. Furthermore, biology books and journals not included in the large data bases were hand-searched and arachnologists and field biologists with known interest in spider phytophagy were contacted to request unpublished reports on this topic. Reports of feeding on fungi as a type of vegetarian food (in contrast to food of animal origin) were included despite the fact that fungi, taxonomically, do not belong to the plant kingdom.

With the exception of two records relating to orb-weavers assessed by stable isotope analyses (see Eggs & Sanders 2013), the core data used in this paper are based on direct observation in the field (see Appendix 1). The problem with laboratory feeding studies is that they are in many cases not reliable. For instance, spiders have been considered to be nectarivorous based on the observation that they drink sucrose solutions in laboratory tests (e.g., Vogeley & Greissl 1989; Pollard et al. 1995; Jackson et al. 2001; Milne 2010; Wu et al. 2011; Pfannenstiel & Patt 2012). However, according to Lundgren (2009), a wide variety

of predaceous arthropods tested in the lab all accepted sucrose solutions, thus such studies cannot be considered to be conclusive evidence for nectarivory. Other researchers reported that spiders are nectarivorous because of positive cold anthrone test results (Taylor & Pfannenstiel 2008; Chen et al. 2010), but these wild-caught spiders may have acquired a high sugar content indirectly by preying on well-fed nectarivorous insects (Wilder 2011). Despite all these concerns, experimental laboratory studies provide valuable supportive evidence for the field observational studies reviewed in this paper (Appendix 1) and therefore are included in the discussion.

A total of 95 records of plant-feeding by spiders was gathered (Appendix 1). Eighty-two (86%) of these were previously reported in the scientific literature and roughly 20% included photographic documentation of phytophagy. Nomenclature of spiders follows Platnick (2014).

3. RESULTS

Feeding on plant food by spiders is global in extent, as such behavior has been reported from all continents except Antarctica. Spiders have been observed to feed on a wide variety of different types of plant products, with floral nectar and extrafloral nectar dominating (~75% of all reported incidents). We classified the reported incidents of plant feeding into five major groups: 1. Leaf feeders, 2. Sugar feeders, 3. Pollen feeders, 4. Seed feeders, and 5. Spore feeders (see Lundgren 2009). In addition to this, we briefly mention the possibility that plant food in the guts of herbivores eaten by spiders will be ingested unintentionally along with the dissolved prey tissue.

3.1 Spiders as leaf feeders.—Spiders cannot ingest solid particles, so by ‘leaf feeders’ we mean both a) instances of spiders biting off or otherwise removing pieces of leaves and extra-orally enzymatically dissolving them prior to ingesting, or b) piercing leaves with their chelicerae to extract plant sap. We hereby view feeding on Beltian bodies and Müllerian bodies, respectively, as a special case of leaf-feeding because these small oval-shaped multicellular structures are produced on the leaflet tips or on leaf petioles (Rickson 1976; Meehan 2009).

3.1.1 Feeding on Beltian bodies: A major case of a leaf feeder is that of *Bagheera kiplingi*, a salticid spider reported to depend largely on plant food (Fig. 1A, B; Jackson 2009; Meehan et al. 2009). *Bagheera kiplingi* has been collected from southeastern Mexico to northwestern Costa Rica, coincident with the range of the Central American ant acacias (*Vachellia* spp., Fabaceae) on which it is apparently an obligate resident (Meehan et al. 2009; Scully 2012; Eric Olson, pers. obs.). The well-studied ant-plant mutualism leads to the production of extrafloral nectar and peculiar lipid and protein-rich food bodies of high nutritional value (i.e., ‘Beltian bodies’) harvested by *Pseudomyrmex* spp. ants (Pseudomyrmecinae), which in return patrol the foliage and stems acting as ‘plant bodyguards’. The spider *B. kiplingi* (5–6 mm adult body length) exploits this mutualism in several ways, but most strikingly by harvesting Beltian bodies (Fig. 1A). *Bagheera kiplingi* individuals of all ages from neonates to adults, and of both sexes, pluck Beltian bodies from leaflet tips. An adult or large subadult spider can consume a single food body in less than four minutes and on one occasion an adult female consumed 36 Beltian bodies in a single feeding episode, representing 10% of the food bodies on one acacia leaf (Eric Olson, pers. obs.). That particular spider specimen had

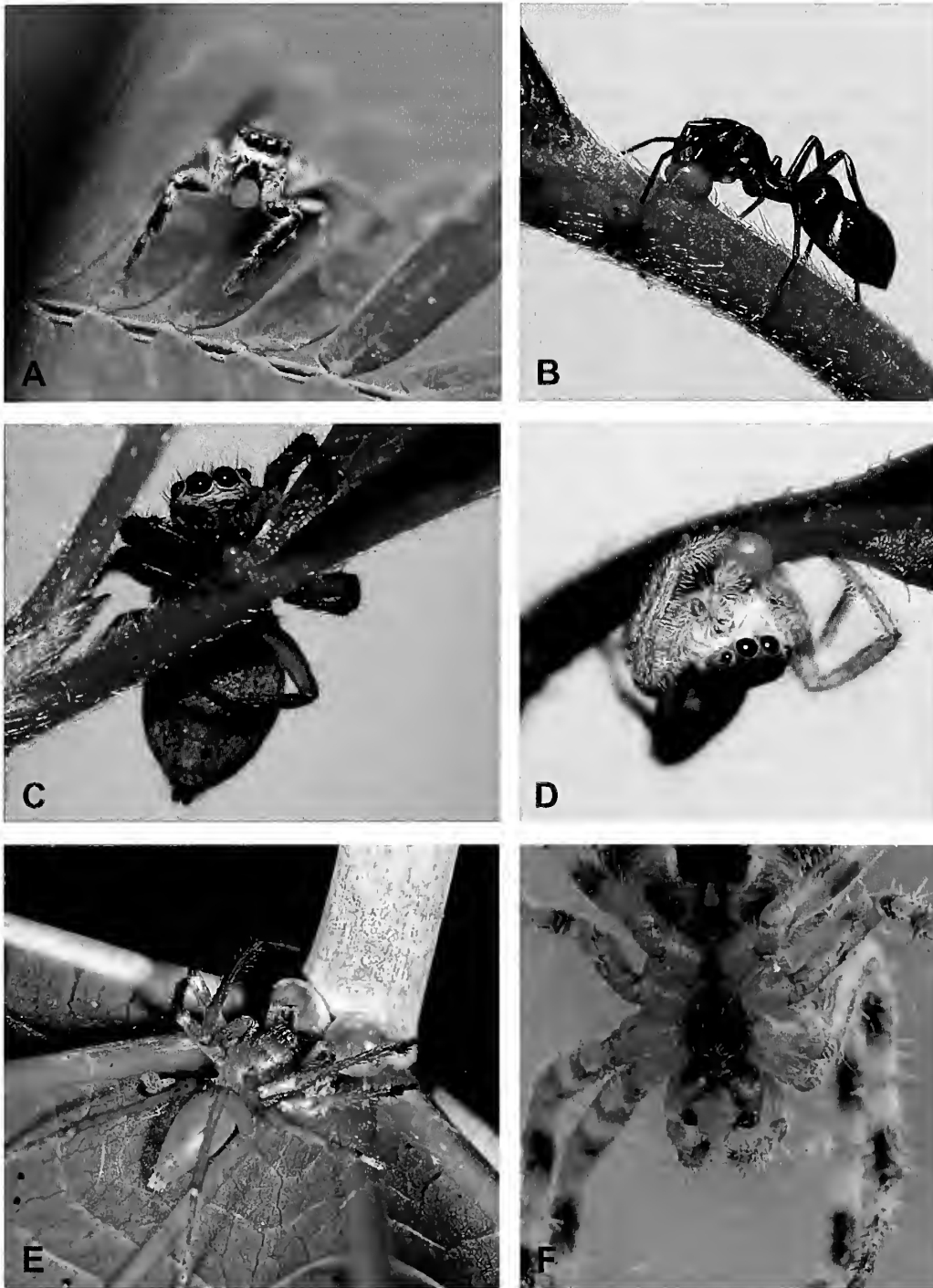


Figure 1.—Plant-eating by spiders. A. Juvenile *Bagheera kiplingi* consuming a Beltian body on a *Vachellia collinsii* plant in Akumal, Mexico (photo by E. J. Scully); B. Adult female salticid spider *Peckhamia* sp. drinking nectar at an extrafloral nectary of *Prunus* sp. (photo by D. E. Hill); C. Adult female *Maevia inclemens* (Walckenaer, 1837) feeding at extrafloral nectaries of *Prunus* sp. (photo by D. E. Hill); D. Male *Heutzia mitrata* imbibing nectar from an extrafloral nectary of a *Prunus* shrub (photo by D. E. Hill); E. *Hibaua velox* feeding at paired extrafloral nectaries of a castor bean plant in Vero Beach, FL (photo by W. A. Foster); F. Adult female *Araneus diadematus* digesting a pollen package left behind by an escaping wild bee on the edge of a field near Munich, Germany (photo by C. Ludy).

been kept in the laboratory for several days without food and was then released onto a plant with a weak ant colony, where it was able to feed with impunity (Eric Olson, pers. obs.). At first glance it may appear that this is quite a large quantity of food. However, the weight of a single Beltian body ≤ 1 mm in length is very low (~ 0.5 mg fresh weight / food body) and harvesting three dozen Beltian bodies results in an amount of food of only ~ 18 mg fresh weight which is the equivalent of one small insect prey.

In addition to Beltian bodies, *B. kiplingi* regularly take nectar from their host plants' abundant extrafloral nectaries,

consume ant larvae, which the spiders seize from worker ants as the ants are transferring brood between thorns, and occasionally kill minute dipterans and other insect prey. Insect prey is scarce on vigorously defended ant-acacias, and plant-derived food made up $>90\%$ of the recorded foraging events at the Mexico location. In Costa Rica, the proportion of plant material was lower ($\sim 60\%$) and that of insect prey (*Pseudomyrmex* larvae and small dipterans) higher ($\sim 40\%$) (Meehan et al. 2009).

During a seven-year salticid inventory in Costa Rica, 48 salticid species were encountered (N = 1,174 individuals) but,

apart from *B. kiplingi*, none of these salticid spiders was witnessed feeding on Beltian bodies, indicating that the use of this type of food by *B. kiplingi* might be unique among salticid spiders (Eric Olson, pers. obs.). This notwithstanding, researchers from Villanova University, USA, are currently investigating whether additional salticid species might be found that feed on Beltian bodies (Eastburn 2014).

3.1.2 Feeding on Müllerian bodies: A second example of consumption of leaf material by spiders has been reported from the Rio de Janeiro area, Brazil, where a nocturnal running spider of the genus *Clubiona* Latreille, 1804 (Clubionidae) was seen feeding on food bodies produced by *Cecropia* trees (Urticaceae) (Andrade 1981; Jolivet 1988). The food bodies on the leaf petioles of *Cecropia* trees (termed 'Müllerian bodies') are small (~0.3 mm x 1.0 mm) and are composed primarily of the carbohydrate glycogen but also contain lipids, proteins, and amino acids (Rickson 1973). *Cecropia* trees are also inhabited by ants in many environments, most commonly *Azteca* spp. (Dolichoderinae), some species of which are obligate *Cecropia* inhabitants that (at least in some settings) defend *Cecropia* trees against some herbivores (e.g., Vasconcelos and Casimiro 1997). Müllerian bodies are harvested by resident *Azteca* ants and are a significant food source for their larvae (Sagers et al. 2000). To date, *Clubiona* spiders have been spotted on only a few occasions consuming Müllerian bodies (Andrade 1981; Jolivet 1988) and the relative importance of these food bodies in spider nutrition remains unknown.

3.1.3 Feeding on plant sap: A third example of spiders as leaf feeders was reported by Stejskal (1976) from a mango plantation (Anacardiaceae) in eastern Venezuela, where immatures and adults of an unspecified species in the genus *Anelosimus* Simon, 1891 (Theridiidae) were observed biting with their chelicerae into mango leaves (also see Fowler & Levi 1979; Agnarsson 2006). The species in question was later identified by Agnarsson (2005) to be *Anelosimus rupununi* Levi, 1956. After piercing the epidermis on the leaves' underside, the spiders were imbibing plant sap (Stejskal 1976). The spiders were also observed preying on insects, as is common behavior in *Anelosimus* spp. (Levi 1972; Fowler & Levi 1979; Vollrath & Rohde-Arndt 1983). Spiders in this genus appear often to be food-limited which might explain the need of *A. rupununi* to supplement its insect diet by additionally imbibing plant sap (see Vollrath & Rohde-Arndt 1983). In this Venezuelan study, *A. rupununi* was found exclusively on mango, citrus, and coffee trees (Stejskal 1976) which fits observations from Trinidad and Surinam (Levi 1972). Fowler & Levi (1979) suggested that Stejskal's studies should be followed up in order to confirm the unusual leaf piercing behavior of these spiders.

A study by Hajer (1988) conducted under laboratory conditions seems to confirm Stejskal's observation that certain spiders occasionally pierce leaves to gain access to plant sap. While using *Philodendron scandens* K. Koch & Sello (family Araceae) as a plant substrate to breed *Nigma flavescens* (Walckenaer, 1830) (Dictynidae), this author witnessed destruction of the leaf surface down to the mesophyll level. Hajer (1988) concluded that "the content of the plant cells had been apparently used by nymphs of 1st instar as a source of food".

Indoor observations by David Hill on three North American salticid spiders provide further evidence for spiders feeding on plant sap. This researcher repeatedly witnessed adults of

Pelegrina galathea (Walckenaer, 1837), *Hentzia mitrata* (Hentz, 1846), and *Maevia inclemens* (Walckenaer, 1837) biting with their chelicerae into leaves of *Rubus* sp. or *Prunus* sp. (Hill 2009; David Hill, pers. comm.). Each time after leaves had been pierced, plant sap exuded from the bite holes. The spiders were seen lowering their mouthparts to the leaf surface, apparently sipping from the liquid with pumping movements (Hill 2011; David Hill, pers. comm.). Furthermore, an immature *Phidippus audax* (Hentz, 1845) was observed feeding on small droplets of crystalline exudate on the upper surface of a leaf under laboratory conditions (David Hill, pers. comm.). Consumption of crystallized materials, such as dried sugar solutions, by predaceous arthropods has been reported previously in the literature (Bartlett 1962; Lundgren 2009).

3.2 Spiders as sugar feeders.—Animals are defined as 'sugar feeders' when they feed on liquids with a high sugar content (Lundgren 2009). This type of feeding encompasses consumption of sugary plant-derived products such as floral nectar, extrafloral nectar, and honeydew. The stigmatic exudate produced by the flowers of Winteraceae trees is included in this category of plant products due to its functional similarity to floral nectar (Richards 1997).

3.2.1 Feeding on floral nectar: Jumping spiders (Salticidae) from >30 different species have been witnessed imbibing nectar from floral nectaries in the wild (Jackson et al. 2001; Lim & Li 2004; Cross & Jackson 2009). Likewise, drinking of floral nectar has been observed in nocturnal running spiders of the families Anyphaenidae, Clubionidae, Eutichuridae, and Trachelidae (Taylor & Foster 1996; Taylor 2004; Suetsugu et al. 2014). Furthermore, there are reports of flower-dwelling thomisid spiders in the subfamily Thomisinae (genera *Misumena*, *Misumenoides*, and *Thomisus*) imbibing nectar from the flowers of a variety of plants (Vogelei & Greissl 1989; Pollard 1993; Pollard et al. 1995; Morse 2007). During such incidents, the spiders were seen pushing their mouthparts deep into flowers to drink nectar, similar to the way nectar-drinking insects feed (Pollard 1993; Taylor & Foster 1996; Jackson et al. 2001; Suetsugu et al. 2014). According to Jackson et al. (2001) the spiders sometimes "positioned their chelicerae around flowers and inserted their fangs". Usually the spiders visit multiple flowers in succession, spending a few seconds up to a few minutes at each flower (Pollard et al. 1995; Taylor & Foster 1996; Suetsugu et al. 2014). In extreme cases, a single spider may visit 60–80 flowers in one hour (Pollard et al. 1995). Quite a number of studies suggest that chemical cues, both olfactory and gustatory, are used to detect and locate nectar sources (Taylor 2004; Patt & Pfannenstiel 2008, 2009; Cross & Jackson 2009). Spiders are attracted to flower fragrances such as eugenol (Krell & Krämer 1998; Dodson et al. 2013).

3.2.2 Feeding on extrafloral nectar: There are reports from many parts of the world of salticid spiders drinking nectar from the extrafloral nectaries of a variety of different plants (Fig. 1B–D; Edmunds 1978; Douglas 1983; Ruhren & Handel 1999; Blüthgen & Reifenrath 2003; Cross & Jackson 2009; Hill 2011; Soren & Chowdhury 2011; Nahas et al. 2012; Hill & Edwards 2013; Mondal et al. 2013). For the ant acacia specialist *B. kiplingi*, that frequently visits nectaries, extrafloral nectar may be more important as a source of moisture than of sugar, at least during dry periods (Eric Olson, pers. obs.).

However, in other spider species, extrafloral nectar has been implicated as an important source of energy that increases their lifespan (Taylor & Foster 1996; Nelson 2004; Taylor & Bradley 2009; Taylor & Pfannenstiel 2009). Drinking of extrafloral nectar has also been witnessed in spiders of the families Anyphaenidae, Eutichuridae, Linyphiidae, Oxyopidae, Thomisidae, and Trachelidae (Fig. 1E; Taylor & Foster 1996; Chen et al. 2010; Nahas et al. 2012; Nico Blüthgen, pers. comm.; Marc Milne, pers. comm.). In many cases, in order to get access to extrafloral nectaries, the spiders have to break through the ant-plant bodyguard defenses (Soren & Chowdhury 2011; Nico Blüthgen, pers. comm.).

That the spiders actually consume nectar has been demonstrated by testing field-collected spiders for fructose (a sugar contained in nectars) using cold-anthrone tests. In such studies it was shown that ~20–30% of all field collected spiders tested positive for fructose, which is indicative for nectarivory (Taylor & Pfannenstiel 2008; Chen et al. 2010).

3.2.3 Feeding on honeydew: Honeydew is a plant-derived sugary fluid excreted by phloem-feeding insects (e.g., coccids and aphids; order Homoptera) after passing through their digestive tracts (Douglas 2006; Lundgren 2009). Consumption of this energy-rich food source has been observed in two species of salticid spiders, *Myrmarachne foenisex* Simon, 1910 and *M. melanotarsa* Wesolowska & Salm, 2002, both of which live in close association with ants of the genus *Crematogaster* (Myrmicinae). Such unusual feeding behavior by *Myrmarachne* spp. has been witnessed in Africa, where the spiders were observed imbibing honeydew while tending scale insects (coccids) alongside ants (Collart 1929; Salm 2005; Jackson et al. 2008).

Furthermore, there are anecdotal reports from Europe of web-building spiders imbibing droplets of honeydew (Bristowe 1941; Kirchner 1964; Stephan Scheurer, pers. comm.). It has been witnessed numerous times that linyphiids and araneids did imbibe droplets of honeydew falling down on their webs from pine trees, *Pinus sylvestris* L. (Stephan Scheurer, pers. comm.). These incidents always occurred in September at a time when the aphids *Schizolachnus pineti* (Fabricius, 1781) and *Cinara pinea* (Mordvilko, 1895) were excreting honeydew while sucking on pine needles. Bristowe (1941) stated that a sheet-web spider (*Linyphia triangularis* (Clerck, 1757)) "...suck drops of honeydew raining down on its web from overhanging trees, but the flavor of this was evidently distasteful because this was always followed by the spider running to the edge of its web to wipe its mouth on a leaf..." Honeydew sometimes contains plant-derived defensive chemicals such as alkaloids or cardenolides, originating from the scale insects' host plants, which can give it a bitter flavor or even render it toxic (Lundgren 2009). The effects of unpalatable or toxic honeydew on predators are still largely unexplored.

3.2.4 Feeding on stigmatic exudate: A spider of the genus *Thwaitesia* O. Pickard-Cambridge, 1881 (Theridiidae) was seen drinking stigmatic exudate in an open flower of *Drimys granadensis* L.f. (Marquinez et al. 2010). As with floral nectar, the glittering stigmatic exudate of this flower functions to attract insect pollinators (Gottsberger et al. 1980). Stigmatic exudate is rich in lipids and amino acids, but has a low sugar content compared to floral nectar (Frame 2003). Whether the incident reported by Marquinez et al. (2010) was merely

a chance event or a regular occurrence remains to be further investigated.

3.3 Spiders as pollen feeders.—Pollen production can potentially provide spiders with a supplementary food source available in large quantities (Peterson et al. 2010). For example, pollen production amounts to ~100–300 kg fresh weight per ha per year in deciduous forests and to ≤460 kg per ha per year in grassland (Smart et al. 1979; Saito et al. 1991, 2006). Feeding on pollen by spiders can take place in different ways. Firstly, plant-dwelling cursorial spiders encounter pollen grains or pollinia while wandering over flowers (Jennings et al. 1989; Nyoka & Ferguson 1999). In particular, thomisids, salticids, and clubionids have been witnessed consuming pollen grains (Pollard et al. 1995; Nelson & Jackson 2011; Suetsugu et al. 2014). This has also been confirmed for cutichurids under laboratory conditions (Pfannenstiel 2012). Cursorial spiders were reported feeding on pollen from plants of the families Apiaceae, Asteraceae, Malvaceae, and Orchidaceae (Appendix 1). A special case is given when clubionid or thomisid spiders are biting the pollinia off orchid flowers (Suetsugu et al. 2014).

Pollen may also become available to spiders after being transported to the spiders' webs by pollen-carrying insects (Cook et al. 1981; Beardsell et al. 1986; Craig & Ebert 1994, Ludy 2004). Craig & Ebert (1994) found that a high percentage of pollen-carrying wild bees intercepted in webs of large orb-weaving spiders, were able to escape leaving the pollen they were carrying behind. These authors suggest that the use of left-behind pollen as a concentrated source of nutrients may play a beneficial role in the nutritional ecology of some species of orb-weaving spiders. For example an adult orb-weaving spider, *Araneus diadematus* Clerck, 1757 consumed a package of pollen grains after it was left behind by an escaping wild bee temporarily entangled in the spider's web (Fig. 1F; Ludy 2004). A similar case was also reported for an adult female orb-weaver, *Gasteracantha cancriformis* (Linnaeus, 1758) (Araneidae) eating pollen from the corbicula of a captured bee's leg (Gregory 1989).

Pollen transported via air currents can also get trapped in the sticky threads of aerial webs (Smith & Mommsen 1984; Linsken et al. 1993; Del Fiol et al. 2007). Such airborne particles become highly electrostatically charged when moving through the air, which enhances their likelihood of entrapment in spider webs (Ortega-Jimenez & Dudley 2013; Vollrath & Edmonds 2013). As already pointed out in the Introduction, the pollen grains are later unintentionally ingested when the spider eats them along with its old web (recycling of web proteins) prior to constructing a new web (Breed et al. 1964; Peakall 1971; Smith & Mommsen 1984; Townley & Tillinghast 1988; Levi 1997; Blackledge et al. 2009). Unintentional pollen feeding is difficult to observe with the naked eye under field conditions because it frequently occurs during the night (Cloudsley-Thompson 1987); however, this type of pollen feeding could be indirectly proven by means of laboratory techniques (Smith & Mommsen 1984; Ludy 2004; Eggs & Sanders 2013). Smith & Mommsen (1984) have shown in laboratory experiments that the life expectancy of second instar *A. diadematus* spiderlings with access to birch pollen doubled compared to starving spiderlings, providing evidence that the spiderlings did indeed gain nutrition from the trapped pollen grains during the web recycling process. Birch pollen used in this study is

considered high quality pollen because of its high protein content (Roulston et al. 2000). Similarly Eggs & Sanders (2013) have shown by means of a stable isotope analysis that *A. diadematus* spiderlings did unintentionally ingest nutrients from spruce and pine pollen trapped in the spiderlings' webs. In the case of pine pollen, however, this must be viewed with great caution because pine pollen is considered to be low quality pollen with little nutritional value due to low protein content (compare Knop & Hoy 1983; Carrel et al. 2000; Roulston et al. 2000).

Finally, web-building spiders such as linyphiids and theridiids, which do not recycle their webs (Benjamin & Zschokke 2003, 2004; Blackledge et al. 2009), likewise intercept airborne pollen in their webs. Such spiders have been witnessed feeding on pollen grains after plucking them from the web (Meissle & Romeis 2009; Peterson et al. 2010). The behavior of plucking pollen grains from the web and directly eating them (intentional pollen feeding) does sometimes appear in orb-weavers as well (Ludy 2004).

3.4 Spiders as seed feeders.—Apart from plant pollen, tiny airborne plant seeds get caught in spider webs (Dale 1989). For example, hundreds of the minute airborne seeds of the orchid *Cymbidium bicolor* Lindl. (<1mm Ø; Swamy et al. 2004) are sometimes trapped in orb-webs (Anonymous 2010) and it is safe to assume that they are unintentionally ingested along with the old web during the recycling process. There is an anecdotal report by Berland (1933) of an orb-weaving spider, *Neoscona adianta* (Walckenaer, 1802)(Araneidae), sucking a grass seed (Poaceae). This is, so far, the only published report of an instance of seed feeding by spiders. In the laboratory researchers succeeded in raising spiders on a liquid diet manufactured from the large seeds of the soybean *Glycine max* (Fabaceae) (Amalin et al. 1999, 2001).

3.5 Spiders as spore feeders.—In addition to pollen and minute seeds, airborne spores from many different fungal families (e.g., Botryosphaeriaceae, Davidiellaceae, Helotiaceae, Massariaceae, Microascaceae, Nectriaceae, Phragmidiaceae, Pleosporaceae, Trichocomaceae, Trichosphaeriaceae, and Venturiaceae) are blown by wind into spider webs (Smith & Mommsen 1984; Linskens et al. 1993; Bera et al. 2002; Del Fiol et al. 2007; Quamar & Chauhan 2011). The spores collected from webs represent some of the most common fungal genera one would expect to find in air (and which can be identified easily; Kathie Hodge, pers. comm.). As far as is known to us, feeding on fungal material by spiders has never been witnessed in the field; but it has been proven in laboratory experiments that fungal spores sticking to the viscid threads of orb-webs of *A. diadematus* were ingested along with the old web (unintentional spore feeding) just prior to the construction of a new web (Smith & Mommsen 1984). Furthermore, feeding on fungal material by a linyphiid spider was witnessed in laboratory feeding trials (Sunderland et al. 1996). The digestive fluid of spiders does contain the enzyme chitinase needed to dissolve and digest the chitinous spore cell wall of fungi and this explains why spiders are capable of digesting fungal spores (Mommsen 1978a, 1980; Smith & Mommsen 1984). It is still unknown whether unintentional ingestion of fungal spores might benefit spiders. Preliminary feeding experiments conducted by Smith & Mommsen (1984) using spores of *Cladosporium herbarum* (Pers.) Link (1816) suggest that the spores

from this fungus were of no nutritive value to the tested spiders. The suitability of fungal spores in a spider diet is furthermore questionable due to their content of toxic secondary metabolites (Smith & Mommsen 1984; Lundgren 2009). When second instar spiderlings of the orb-weaving spider *A. diadematus* were offered *Cladosporium* spores as potential food, the webs were rebuilt less frequently compared to a control group of starving spiderlings suggesting that the *Cladosporium* spores were either unpalatable or deleterious, so that the spiderlings tried to avoid exposure to them by reducing the frequency of web building (Smith & Mommsen 1984).

Whether cursorial spiders, which encounter fungal spores while wandering over plants, also consume this type of food has not yet been explored. Encountering fungal spores is definitely not without risk for spiders, because there are ~50–100 species of highly specialized fungal pathogens (in particular in the family Cordycipitaceae) whose spores are lethal once they attached themselves to the spiders' integument (Evans 2013). Fungal pathogens cause high mortality in populations of cursorial and web-building spiders in the tropics (Evans & Samson 1987; Gonzaga et al. 2006).

3.6 Spiders feeding on plant material present in the guts of herbivorous prey.—Spiders must also ingest plant material present in the guts of herbivorous prey. For example, linyphiid spiders eating aphids (e.g., Harwood et al. 2004) will also be consuming the undigested (plant sap) and digested (honeydew) plant material within the aphids. Herbivorous prey are therefore likely to be a major source of plant material consumed by most spider species. The evolution of digestive enzymes that can break down this material may have made spiders pre-adapted to feeding on plants directly (for a discussion on spider digestive enzymes see also Mommsen 1978a,b,c,d, 1980; Taylor 2004; Orón-Tamayo et al. 2013).

4. DISCUSSION

4.1 How frequent are incidents of plant feeding?—Feeding on plant materials has been reported so far more frequently from warmer areas (~80% of all reported incidents originating from locations between 40°N and 40°S). This might be due to the fact that a larger number of the reports relate to nectar consumption which has its core distribution in the warmer areas between latitude 0–34° where plants secreting copious nectar are widespread (Pemberton 1998; Chamberlain & Holland 2009) and where nectar can be more easily imbibed due to its lower viscosity at higher temperatures (Lundgren 2009; Nicolson et al. 2013). The most northern occurrence of nectar consumption by spiders refers to a location in Maine (~44° N latitude) and there this type of feeding is apparently scarce (Morse 2007).

4.2 Which spider groups are engaged in plant feeding?—Overall, >60 spider species representing ten araneomorph families have been reported to be engaged in phytophagy under natural conditions (Appendix 1). Six families of cursorial spiders combined, all belonging to the two-clawed *Dionycha* clade (i.e., Anyphaenidae, Clubionidae, Eutichuridae, Salticidae, Thomisidae, and Trachelidae), were attributable to >80% of the reported incidents of phytophagy, whereas cursorial spiders of the three-clawed *Lycosoidea* clade (i.e., Oxyopidae) constituted ~1%. In most cases, spiders reported feeding on plant food were small (including a high percentage of immatures; Smith

& Mommsen 1984; Vogelei & Greissl 1989; Pollard et al. 1995; Jackson et al. 2001; Blüthgen & Reifenrath 2003; Soren & Chowdhury 2011; Pfannenstiel 2012).

One of the most prominent group of spiders engaged in phytophagous activities was Salticidae which accounted for ~60% of all documented incidents (Fig. 1A–D). More than 40 species of salticid spiders, representing a dozen different subfamilies, were observed feeding on plant-derived food in the wild (Appendix 1). The members of this spider family utilize more different types of plant materials as food (including floral nectar, extrafloral nectar, honeydew, plant sap, pollen, and Beltian bodies) than any other spider group. This fits our idea that most salticid species have broad diets and highly flexible foraging patterns (Nyffeler 1999). Furthermore, as mentioned earlier, this family includes the only confirmed species of a specialized plant-eating spider (*B. kiplingi*) known so far (Meehan et al. 2009). Another noticeable group of plant eaters are the group of ‘nocturnal runners’ including the families Anyphaenidae, Clubionidae, Eutichuridae, and Trachelidae (15% of the witnessed incidents; Fig. 1E), whereas flower-dwelling thomisid spiders in the subfamily Thomisinae made up ~11%. The nocturnal runners, composed of fast-moving species, are considered to be the nocturnal counterpart of the diurnal salticids in as much as both spider groups are plant-dwelling cursorial hunters with excellent running and jumping capabilities (Gertsch 1979; Taylor & Foster 1996). But otherwise the two groups differ in many ways from each other, anatomically and behaviorally. A striking difference is the fact that the salticids have excellent vision, whereas the eyesight of the group of nocturnal runners is rudimentary (Jackson et al. 2001). In both groups feeding on plant materials seems to be wide-spread although it must be said that the feeding habits of the nocturnal runners are still largely unknown (Taylor & Foster 1996). The significance of the nocturnal runners as nectar feeders has probably been underestimated in this paper. Though exact data are missing, it can already at this stage be postulated that many of the >8,000 described species belonging to these three spider groups might be engaged in nectarivory at some time of their life history (also see Jackson & Pollard 1996; Jackson et al. 2001; Taylor 2004).

The data presented in this paper suggest that web-building spiders are rather insignificant as consumers of plant food (~13% of the witnessed incidents) compared to the cursorial hunters. However, in reality, pollen feeding by web-building spiders (and in particular orb-weavers) may be more common than shown in this study. Except for a few species hiding in eaves and houses (Smithers 2005; Yoder et al. 2009), most cribellate orb-weaving spiders can be expected to trap pollen and other aerial phytoplankton in their webs when it is available. Cribellate orb-weaver species (with the exception of a small minority of species in the subfamily Cyrtophorinae) all recycle their old web more or less daily prior to rebuilding a new web (Breed et al. 1964; Peakall 1971; Townley & Tillinghast 1988; Levi 1997; Blackledge et al. 2009). Following this logic, we hypothesize that a large percentage of the >4,000 species of cribellate orb-weaver species might derive some nutrients from trapped pollen grains at some point of their life cycle. However, we must admit that it is still unknown what role unintentional pollen feeding plays in the orb-weavers’ energy budget (also see Eggs & Sanders 2013).

4.3 Can spiders survive feeding on only plant food?—The question of whether spiders can survive on a diet of only plant matter was studied in the laboratory using *Bagheera kiplingi* by exclusively offering Beltian bodies as food (Eric Olson, pers. obs.). Although this food was readily accepted, all spiders kept strictly on a Beltian body diet perished after one to several weeks, and always before molting to the next instar, which implies that even this spider, considered by some to be a specialized plant feeder, is incapable of surviving on an exclusively plant diet. These are difficult experiments to perform, however, because the maturation of Beltian bodies is likely to be interrupted by severing Beltian body-bearing leaves from the plant. Results in Mexico, where the spiders feed almost exclusively on Beltian bodies, suggest that when allowed to feed from intact foliage, *Bagheera kiplingi* spiders can survive on a near-exclusive diet of plant material (Meehan 2009; Meehan et al. 2009).

Whether spiders can survive feeding on only plant food was also investigated by offering them an exclusively pollen diet under laboratory conditions. Smith & Mommsen (1984) and Vogelei & Greissl (1989) found that spiderlings of *Araneus diadematus* and *Thomisus onustus* Walckenaer, 1805 kept on an only pollen diet were unable to molt. In these studies, the spiderlings were fed with pollen originating from birch (Betulaceae) and asterids (Asteraceae), respectively. Deficiencies of essential amino acids (e.g., tyrosine) in pollen grains are thought to be responsible for the inability of spiderlings to molt when offered an exclusively pollen diet (Smith & Mommsen 1984; Vogelei & Greissl 1989; Mondal et al. 1998; Praz et al. 2008).

A similar trend was found when spiders were offered an exclusively nectar diet. Anyphaenid and thomisid spiders that received nectar but no prey did not molt in a regular manner compared to insect-fed spiders, and this might be explained by a requirement for protein-rich food in order to molt (Vogelei & Greissl 1989; Taylor 2004). Nectar is known to have a low protein content (Lundgren 2009).

Still another confirmation comes from experiments carried out by Amalin et al. (1999) who fed the spider *Hibana velox* (Becker, 1879) on a ‘soybean diet’ on the one hand and on a ‘milk + egg yolk diet’ on the other. These authors compared the development of the spiders raised on the two diets and found that spiders that were fed an exclusively soybean diet were significantly smaller than those that were fed a milk + egg yolk diet. In another study, Amalin et al. (2001) found that spiders reared on a soybean diet underwent fewer molts compared to spiders reared on a milk + egg yolk mixture. The authors suggested that the delayed development and lower molting success of spiders raised exclusively on a soybean diet might have been caused by some nutritional deficiency (e.g., cholesterol deficiency typical of soybean; Amalin et al. 1999).

It should be pointed out, however, that many generalist predators do better on a diverse diet than they do when restricted to just one prey type (Harwood et al. 2009). This is because predators may gain different nutritional benefits from consuming different types of prey (Mayntz et al. 2005) and/or can dilute prey toxins through eating a mixture of different prey. For example, many linyphiid spiders eat aphids, but when restricted to an aphid-only diet suffer from higher mortality and lower reproductive rates (Harwood et al. 2009). It is not surprising, therefore, that generalist spiders restricted in the laboratory to

a single type of plant food are subject to similar detrimental factors.

5. CONCLUDING REMARKS

Our review reveals that spiders from ten families have been documented to feed on plant products under natural conditions. But evidence based on cold anthrone tests and laboratory feeding studies suggests that spiders from even more families may occasionally consume plant food (e.g., Hajer 1988; Chen et al. 2010; Milne 2010). In the future, the question of whether spiders are eating plants might be tested by means of stable isotopes, or via PCR, using plant (or fungal) primers to detect these materials in their guts. Although this approach would provide evidence of many direct trophic links between spiders and plants there would also be the problem of the indirect links discussed previously, where the plant material was in fact ingested unintentionally through predation on herbivores with plant DNA in their guts. Further work would be necessary to determine the origin of the plant material detected. Pekár & Toft (2015) in a recent review hold the view that alternative foods such as plant materials “often appear to be only marginal foods for spiders and further evidence is required”.

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Appendix 1.—Ninety-three records of spider phytophagy based on visual observation and two records (*) based on stable isotope analyses.

Spider family/species	Food type	Plant family/species	Source
Anypheidae			
<i>Arachosia</i> sp.	Extrafloral nectar	Vochysiaceae / <i>Qualea multiflora</i>	Nahas et al. (2012)
<i>Hibana similaris</i> (Banks, 1929)	Extrafloral nectar	Anacardiaceae / <i>Anacardium occidentale</i>	Taylor & Foster (1996)
<i>Hibana velox</i> (Becker, 1879)	Floral nectar	Asteraceae / <i>Eupatorium serotinum</i>	Taylor & Foster (1996)
	Extrafloral nectar	Euphorbiaceae / <i>Ricinus communis</i>	Taylor & Foster (1996)
Unidentified	Floral nectar	Combretaceae / <i>Terminalia catappa</i>	Taylor & Foster (1996)
Araneidae			
<i>Araneus diadematus</i> Clerck, 1757	Pollen*	Pinaceae / <i>Picea abies</i> , <i>Pinus sylvestris</i>	Eggs & Sanders (2013)
	Pollen	Asteraceae? / Unidentified	Ludy (2004)
<i>Gasteracantha cancriformis</i> (Linnaeus, 1758)	Pollen	Unidentified	Gregory (1989)
<i>Neoscona adianta</i> (Walckenaer, 1802)	Seed	Poaceae / Unidentified	Berland (1933)
Unidentified	Honeydew	Pinaceae / <i>Pinus sylvestris</i>	Stephan Scheurer, pers. comm.
Clubionidae			
<i>Clubiona</i> sp. 1	Müllerian bodies	Urticaceae / <i>Cecropia lyratiloba</i>	Jolivet (1988)
<i>Clubiona</i> sp. 2	Floral nectar	Orchidaceae / <i>Neottianthe cucullata</i>	Suetsugu et al. (2014)
<i>Clubiona</i> sp. 2	Pollen	Orchidaceae / <i>Neottianthe cucullata</i>	Suetsugu et al. (2014)
Eutichuridae			
<i>Cheiracanthium mildei</i> L. Koch, 1864	Floral nectar	Asteraceae / <i>Eupatorium serotinum</i>	Taylor & Foster (1996)
	Extrafloral nectar	Euphorbiaceae / <i>Ricinus communis</i>	Taylor & Foster (1996)
Linyphiidae			
<i>Ceratinopsis interpres</i> (O. P.-Cambridge, 1874)	Extrafloral nectar	Sarraceniaceae / <i>Sarracenia purpurea</i>	Marc Milne, pers. comm.
<i>Linyphia triangularis</i> (Clerck, 1757)	Honeydew	Unidentified	Bristowe (1941)
Unidentified	Honeydew	Pinaceae / <i>Pinus sylvestris</i>	Stephan Scheurer, pers. comm.
Oxyopidae			
<i>Oxyopes macroscelides</i> Mello-Leitão, 1929	Extrafloral nectar	Vochysiaceae / <i>Qualea multiflora</i>	Nahas et al. (2012)
Salticidae			
<i>Afraflacilla</i> sp. 1	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Afraflacilla</i> sp. 2	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Asemonea murphyae</i> Wanless, 1980	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Bagheera kiplingi</i> Peckham & Peckham, 1896	Beltian bodies	Fabaceae / <i>Vachellia</i> spp.	Meehan et al. (2009) Scully (2012); Eric Olson, pers. obs.
	Extrafloral nectar	Fabaceae / <i>Vachellia</i> spp.	Meehan et al. (2009); Eric Olson, pers. obs.
<i>Bavia aereiceps</i> Simon, 1877	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Chrysilla lauta</i> Thorell, 1887	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Cosmophasis estrellaensis</i> Barrion & Litsinger, 1995	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Cosmophasis micarioides</i> (L. Koch, 1880)	Floral nectar	Unidentified	Jackson et al. (2001)
	Extrafloral nectar	Flagellariaceae / <i>Flagellaria indica</i>	Andrea Lim, pers. comm.
<i>Cosmophasis umbratica</i> Simon, 1903	Floral nectar	Rubiaceae / <i>Ixora javanica</i>	Lim & Li (2004)
<i>Cyrba algerina</i> (Lucas, 1846)	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Cytaea frontalis</i> (Thorell, 1881)	Extrafloral nectar	Convolvulaceae / <i>Merremia peltata</i> ; Euphorbiaceae / <i>Rockinghamia</i> sp.	Blüthgen & Reifenrath (2003); Nico Blüthgen, pers. comm.
<i>Cytaea</i> sp.	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Diolenius</i> sp.	Extrafloral nectar	Euphorbiaceae / <i>Flagellaria indica</i>	Nico Blüthgen, pers. comm.
<i>Epeus hawigalboboguttatus</i> Barrion & Litsinger, 1995	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Eris</i> sp.	Extrafloral nectar	Fabaceae / <i>Chamaecrista nicticans</i>	Ruhren & Handel (1999)
<i>Euryattus</i> sp.	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Evarcha culicivora</i> Wesolowska & Jackson, 2003	Floral nectar	Euphorbiaceae / <i>Ricinus communis</i> ; Verbenaceae / <i>Lantana camara</i>	Cross & Jackson (2009)
	Extrafloral nectar	Euphorbiaceae / <i>Ricinus communis</i>	Cross & Jackson (2009)
	Pollen	Malvaceae / <i>Hibiscus</i> sp.	Nelson & Jackson (2011)
<i>Goleba puella</i> (Simon, 1885)	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Harmochirus brachiatus</i> (Thorell, 1877)	Floral nectar	Unidentified	Jackson et al. (2001)

Appendix 1.—Continued.

Spider family/species	Food type	Plant family/species	Source
<i>Heliophanillus fulgens</i> (O. P.- Cambridge, 1872)	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Heliophanus debilis</i> Simon, 1901	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Hentzia mitrata</i> (Hentz, 1846)	Extrafloral nectar	Rosaceae / <i>Prunus</i> sp.	Hill (2011)
<i>Hentzia palmarum</i> (Hentz, 1832)	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Heratemita alboplagiata</i> (Simon, 1899)	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Hyllus gulosus</i> (Simon, 1877)	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Lepidemathis sericea</i> (Simon, 1899)	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Lyssomanes viridis</i> (Walckenaer, 1837)	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Metaphidippus</i> sp.	Extrafloral nectar	Fabaceae / <i>Chamaecrista nicticans</i>	Ruhren & Handel (1999)
<i>Myrmarachne assimilis</i> Banks, 1930	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Myrmarachne bakeri</i> Banks, 1930	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Myrmarachne foenisex</i> Simon, 1910	Honeydew	Unidentified	Collart (1929)
<i>Myrmarachne legon</i> Wanless, 1978	Extrafloral nectar	Rubiaceae / <i>Morinda lucida</i>	Edmunds (1978)
<i>Myrmarachne lupata</i> (L. Koch, 1879)	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Myrmarachne melanotarsa</i> Wesolowska & Salm, 2002	Honeydew	Unidentified	Jackson et al. (2008)
<i>Myrmarachne</i> sp.	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Natta horizontalis</i> Karsch, 1879	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Peckhamia</i> sp.	Extrafloral nectar	Unidentified	David Hill, pers. comm.
<i>Pelegrina proterva</i> (Walckenaer, 1837)	Extrafloral nectar	Dennstaedtiaceae / <i>Peridium aquilinum</i>	Douglas (1983)
<i>Phidippus</i> sp. 1	Extrafloral nectar	Dennstaedtiaceae / <i>Peridium aquilinum</i>	Douglas (1983)
<i>Phidippus</i> sp. 2	Extrafloral nectar	Dennstaedtiaceae / <i>Peridium aquilinum</i>	Douglas (1983)
<i>Phintella aequipes</i> (Peckham & Peckham, 1903)	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Phintella piatensis</i> Barrion & Litsinger, 1995	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Phintella vittata</i> (C. L. Koch, 1846)	Extrafloral nectar	Malvaceae / <i>Urena lobata</i>	Soren & Chowdhury (2011)
<i>Portia fimbriata</i> (Doleschall, 1859)	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Siler semiglaucus</i> (Simon, 1901)	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Tauala lepidus</i> Wanless, 1988	Floral nectar	Unidentified	Jackson et al. (2001)
<i>Xenocytaea</i> sp.	Floral nectar	Unidentified	Jackson et al. (2001)
Unidentified	Extrafloral nectar	Convolvulaceae / <i>Ipomoea pes-caprae</i>	Mondal et al. (2013)
Unidentified	Extrafloral nectar	Vochysiaceae / <i>Qualea multiflora</i>	Nahas et al. (2012)
Theridiidae			
<i>Anelosimus rupununi</i> Levi, 1956	Plant sap	Anacardiaceae / <i>Mangifera indica</i>	Stejskal (1976)
<i>Anelosimus vittatus</i> (C. L. Koch, 1836)	Pollen	Rosaceae / <i>Potentilla</i> sp.	Brian Valentine, pers. comm.
<i>Thwaitesia</i> sp.	Stigmatic exudate	Winteraceae / <i>Drimys granadensis</i>	Marquez et al. (2010)
Thomisidae			
<i>Diaea</i> sp.	Extrafloral nectar	Euphorbiaceae / <i>Macaranga</i> sp.	Nico Blüthgen, pers. comm.
<i>Ebrechtella tricuspidata</i> (Fabricius, 1775)	Extrafloral nectar	Unidentified	Chen et al. (2010)
<i>Misumena vatia</i> (Clerck, 1757)	Floral nectar	Asteraceae / <i>Solidago</i> spp.	Morse (2007)
<i>Misumenooides formosipes</i> (Walckenaer, 1837)	Floral nectar	Apiaceae / <i>Daucus carota</i> ; Asteraceae / <i>Cichorium intybus</i> , <i>Solidago</i> spp.; Lamiaceae / <i>Ocimum basilicum</i>	Pollard (1993); Pollard et al. (1995); Jane Marlow, pers. comm.
<i>Misumenops</i> sp.	Pollen	Apiaceae / <i>Daucus carota</i>	Pollard et al. (1995)
Unidentified	Extrafloral nectar	Vochysiaceae / <i>Qualea multiflora</i>	Nahas et al. (2012)
Unidentified	Pollen	Asteraceae / <i>Rudbeckia hirta</i>	Jane Marlow, pers. comm.
Trachelidae			
<i>Trachelas similis</i> F. O. P.-Cambridge, 1899	Floral nectar	Asteraceae / <i>Eupatorium serotinum</i>	Taylor & Foster (1996)
	Extrafloral nectar	Euphorbiaceae / <i>Ricinus communis</i>	Taylor & Foster (1996)
<i>Trachelas volutus</i> Gertsch, 1935	Floral nectar	Asteraceae / <i>Eupatorium serotinum</i>	Taylor & Foster (1996)
	Extrafloral nectar	Euphorbiaceae / <i>Ricinus communis</i>	Taylor & Foster (1996)

Importance of vegetation structure to the assembly of an aerial web-building spider community in North American open grassland

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Abstract. Spatial and temporal heterogeneity of suitable habitat in grasslands can promote species and functional diversity in arthropods, including responses by ubiquitous web-building spiders. A field experiment in tallgrass prairie habitat was performed to examine the response in abundance and web-type richness of aerial web-building spiders to changes in the availability of structure for web placement (vegetation architecture). To test the hypothesis that vegetation structure contributes directly to the web-builder abundance and web-type richness in open grasslands, we increased vegetation structure by adding dead woody stems of a common shrub along transects in each of three watersheds that differed in burn histories and existing habitat structure. Aerial web-building spiders were visually censused before and after the manipulations, at which time we recorded web-orientation, height, web-type, and the presence/absence of the spider associated with a web. Over the duration of the study, a total of seven web-type groups were encountered, of which medium-sized orb weavers were the most abundant web-building group across all watersheds. In general, higher spider abundances of orb-building spiders were observed in sections with added structure compared to the non-manipulated sections. However, reduced richness of web types was found on the manipulated sections of transects, suggesting that the architecture provided by woody stems does not provide sufficient and appropriate web-anchoring structure for the full range of web-building spiders groups in tallgrass prairie.

Keywords: Web types, heterogeneity–biodiversity hypothesis, habitat structure, Konza, tallgrass prairie

Spiders are ubiquitous, generalist and functionally important arthropod predators in terrestrial ecosystems, including grasslands, where they can reach high levels of local species richness and abundance (Diehl et al. 2013; Malumbres-Olarte et al. 2013). In grasslands, interactions among fire, ungulate grazing, plant species diversity, and climate are key drivers determining habitat structure. In turn, consumers respond to bottom-up processes that promote significant spatial and temporal heterogeneity of habitat structure (Bonte et al. 2000; Fuhlendorf & Engle 2001, 2004; Joern 2005). An overarching hypothesis here is that the overall abundance and species diversity of consumer communities increases with increasing spatial heterogeneity of critical habitat attributes, including the overall variability in vegetation architecture and plant species diversity (Dennis et al. 1998; Fuhlendorf & Engle 2001; Fuhlendorf et al. 2006; Jimenez-Valverde & Lobo 2007; Allouche et al. 2012).

Habitat structure affects species interactions in spider communities in multiple ways (Jones & Syms 1998). More precisely, vegetation complexity has been recognized as an important factor influencing species presence, richness and composition of spider communities (Jimenez-Valverde & Lobo 2007). Spiders can partition habitat at fine scales, facilitating the presence of different hunting strategies, where different prey species are susceptible to different hunting strategies (Schmitz & Suttle 2001; Wise 2006; Malumbres-Olarte et al. 2013). Microhabitat partitioning has been documented in web-building spiders where webs differ in placement height, orientation, or type depending on vegetation structure (Enders 1974; Brown 1981; Schmitz & Suttle 2001; Wise 2006). Structural complexity of the habitat can also lead to reduced spider mortality by providing refuges from predation or by influencing intraguild interactions (Finke & Denno 2002; Malumbres-Olarte et al. 2013). Thus, understanding habitat characteristics that affect web placement can reveal whether and how the structural complexity of the habitat can modulate spider community assembly,

species abundance and overall functional diversity (Robinson 1981; Bultman & Uetz 1982; Jimenez-Valverde & Lobo 2007; Diehl et al. 2013). In this sense, non-trophic effects on spider communities associated with habitat heterogeneity in plant architecture act as a “bottom-up template” for structuring spider assemblages (Halaj et al. 2000).

Open grasslands are dominated by non-woody vegetation, where graminoids comprise about 80% of the above ground vegetation biomass, while forbs often comprise about 80% of the plant species diversity (Knapp & Seastedt 1998; Joern & Laws 2013). Variation in vegetation architecture among habitats could result in different spider assemblages across the landscape. Web builders are a diverse group of predators representing ~ 60% of the North American spider fauna (Young & Edwards 1990), and web builders in US agricultural fields accounted for ~ 44% of the arachnid species richness associated to these ecosystems. These web-building spiders are mainly represented by the families Tetragnathidae, Araneidae, Linyphiidae, Theridiidae, and Dictynidae (Nyffeler & Sunderland 2003). However, web-building spider guilds are uncommon in North American open grasslands, seemingly because of a paucity of structure on which to anchor webs (Baldissera et al. 2004; Podganski et al. 2013). Grassland ecotones with woody vegetation along waterways or woodlots often exhibit sharp boundaries characterized by rapid changes in habitat structural complexity, vegetation height, and dominant plant cover. The functional composition of spider communities also changes routinely along this structural gradient, where guilds of web builders are common at the woody end of the gradient but nearly absent in open grasslands (Baldissera et al. 2004).

We experimentally added woody structure in open grassland habitat to examine how changes in structural complexity affected aerial-web-builder abundance and the richness of web types. Our primary hypothesis states that the availability of structure for web placement limits density, richness of both

species and web types, and the distribution of web-building spider guilds in open grassland. We focused first on how web density and web-type richness responded to the newly added structures for web anchoring (woody vegetation: manipulation) in open grasslands. Second, we asked how increasing the distance at which structures for web anchoring are available relative to riparian woodland edges affects web density and web-type richness. If the availability of structures for web placements limits web-building spiders in open grassland, we predicted: (a) a greater density of web-building spiders and web-type richness will be found in areas with increased diversity and availability of web-anchoring structure, regardless of burn history at a local scale (300 m² transects) and distance from grassland-woodland edge; (b) the density of web-building spiders will be higher in watersheds with a history of lower burn frequency because the availability of structures for web placement increase with increasing time since the last burn; c) the distance from a riparian wood stand edge is expected to negatively influence the abundances or web densities of web-building spiders along transects to which we added structure for web placement; and (d) aerial web-builder abundances within manipulated sections will be higher than those in non-manipulated sections of these transects regardless of distance from edge. Web-type richness will be affected primarily by the availability of web-anchoring structures with lower richness on non-manipulated sections of the open grassland.

METHODS

Study site.—This study was conducted at the Konza Prairie Biological Station (KPBS) located in the Flint Hills grassland of north-eastern Kansas, 10 km south of Manhattan (39° 05' N, 96° 35' W). KPBS is a 3487 ha native tallgrass prairie preserve that experiences a highly variable US continental climate consisting of wet, hot summers and dry, cold winters (Knapp & Seastedt 1998; Joern 2005). KPBS is a protected research area with long-term, landscape-level treatments that manipulate prescribed fire (1, 2, 4 and 20 year frequencies) and large ungulate grazing. Mean annual precipitation is 835 mm, most of which falls during the growing season. Steep-sloped terrain overlain by shallow soils and limestone benches unsuitable for cultivation characterize the topography of the site. The KPBS flora includes a mixture of more than 600 species (Towne 2002), including warm-season and cool-season grasses, legumes, and other forbs. The vegetation cover of KPBS is dominated predominately by perennial warm-season C₄ grasses although forb species contribute more than 80% of the plant diversity (Towne 2002). Prescribed burns in the watershed used in this study were initiated in 1972 (Knapp & Seastedt 1998; Collins & Calabrese 2012). Vegetation structure and species diversity has diverged over time, resulting in watersheds that vary from largely open grassland habitat with little woody cover to those with extensive invasion from woody vegetation in watersheds subjected to low fire frequency treatments (Briggs et al. 2002; Ratajczak et al. 2012) and along the riparian areas (Knight et al. 1994; Collins & Calabrese 2012). Woody plant encroachment is characterized by increased woody and forb cover at the expense of grass cover (Wilcox & Huang 2010), providing an ideal structurally complex and spatially heterogeneous habitat for web-building spiders.

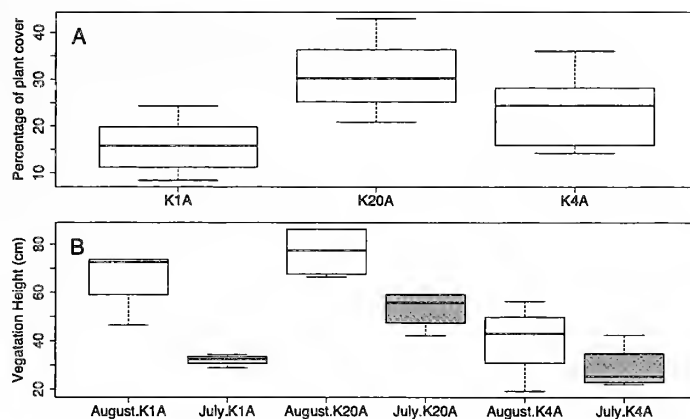


Figure 1.—(A) Comparison of the percent cover of plants with complex architecture (forbs and woody vegetation) among watersheds at Kings Creek basin. (B) Comparison of the vegetation height of the grass layers between early July (shaded) and mid-August (open). The box plot elements represent the following: box vertical dimension = interquartile range; horizontal line = median; whiskers = the minimum and maximum values.

Our study was conducted on three ungrazed watersheds (K1A, 113.9 ha; K4A, 53.16 ha; K20A, 83.13 ha) with fire frequency histories of 1, 4 and 20 years fire return intervals, respectively. All three watersheds were burned in spring 2013 just weeks prior to commencing this experiment, resulting in similar understory vegetation layers in all three watersheds. However, woody plant and forbs vegetation cover still differed among watersheds (ANOVA, $F_{2,18} = 25.3$, $P < 0.0001$; Fig. 1A). Vegetation height differed among watersheds (ANOVA, $F_{2,18} = 16.2$, $P < 0.0001$) and among the early (June) and late (August) part of the growing season (ANOVA, $F_{2,18} = 28.1$, $P < 0.0001$; Fig. 1B), reflecting the legacies of burn history. Vegetation in these watersheds ranged from an open grass canopy in K1A with little woody vegetation to abundant woody shrub islands (a mixture of *Prunus* species, *Cornus drummondii*, and other shrub species) in watershed K20A; watershed K4A was intermediate in woody structure and consists primarily of open grass canopy with incursions of shrub islands.

Study design.—To test the hypothesis that available structure for web placement limited the abundance and richness of web types in open grassland, woody structure was added along sections of the transects. In each of the three watersheds (K1A, K4A and K20A), we established four transects (100 m long by 3 m wide) beginning at the transition edge between a riparian woody stand near King's Creek and open grassland. We placed one end of all transects at the edge of woody riparian vegetation because we expected this transition zone to serve as a source of web-building spiders for recruitment into open grasslands if a response occurred. This scheme also allowed us to determine whether distance from the edge of a woody stand affected aerial-web-builder recruitment into open grasslands when woody structure is added at different distances from this transition zone.

We manipulated habitat structure by adding woody stems of *Cornus* (dogwood) (experimental treatment) to randomly selected, 25 m sections for each 100 m long transect during July 1–4. The rest of each transect (75 m) served as a control. Two aluminum wires were stretched along five, evenly

Table 1.—Web-building spider functional groups at KPBS and diversity associated with each functional group. Sample specimens were collected on August 2013 and identified to genus with the exception of the Dictynidae, which were no longer active in the field at this time of the year.

Web morphology	Family	Genus	Web size (cm ²)	
			Min.	Max.
Small-orb	Araneidae	<i>Micrathena</i>	4	< 50
Medium-orb	Tetragnathidae Araneidae	<i>Tetragnatha Acanthepeira, Argiope, Cyclosa, Larimioides, Mangora</i>	> 50	740
Large-orb	Araneidae	<i>Neoscona</i>	> 740	3239
Funnel-web	Agelenidae	<i>Agelenopsis</i>	9.43	2842
Doily-sheet-web	Linyphiidae	<i>Frontinella</i>	5	506
Irregular-all-direction-web	Theridiidae	<i>Euryopis, Theridion</i>	55	566
Irregular-mesh-web	Dictynidae		4	12

distributed fence posts (top and bottom) along the 25 m spans to serve as support structure for the dead *Cornus* stems. Stems that ranged in height between 1.5–2 m were collected nearby. To obtain a canopy diameter of approximately 60 cm, groups of two to three stems were tied to the wires approximately every 2.5 meters. Upon installation, the *Cornus* stems were immediately and carefully examined for the presence of spiders; none were detected during this installation phase. Transects were left unchecked for eight days to allow naturally dispersing spiders to colonize the structures before the first of three post manipulation censuses was conducted in the second week of July.

Sampling the spider community.—Web builders are a diverse group of predators representing ~ 60% of the North American spider fauna (Young & Edwards 1990). They are uncommon in North American open grasslands relative to other spider groups, seemingly because of a paucity of structure on which to anchor webs (Baldissera et al. 2004; Podgaiski et al. 2013). Aerial web-building spiders were visually censused up to 1.5 m away on each side of the 100 m long transects; each transect was spaced 50 meters from the neighboring transects and censused five times during a three month period – two times in June before the habitat structure manipulation, and three times after the addition of woody structure (second and fourth week of July and third week of August). Visual censuses are effective for counting spiders with conspicuous webs, and spiders remain undisturbed in the study area and can be found repeatedly throughout the study period (Lubin 1978). Reference samples for each species were taken during our last survey in August to identify families and species, and to define their respective web types. For each web, we recorded the orientation (vertical, diagonal, horizontal or no clear orientation), height above the ground, web type (orb, bowl, sheet, funnel, threads without a clear structure), two measurements of web diameter to estimate area for prey capture in the web, and the presence or absence of the spider.

In the field, we used web structure to identify webs to family if spiders were not present on the web (Halaj et al. 1998; Uetz et al. 1999; Eisman et al. 2010). Family identity was confirmed on site for webs with resident spiders. Irregular, hackled silk around the heads of flowers, branches and dead stalks were classified as Dictynidae and sheet webs as Linyphiidae. Even though Linyphiidae is a very diverse family of sheet web builders in the US, representatives of this family tend to be less common (usually < 25% of total spider individuals)

(Nyffeler & Sunderland 2003). Because we sampled only aerial webs in this study while most linyphiids build their webs near the ground, we only found individuals of the genus *Frontinella* F.O. Pickard-Cambridge, 1902 as representatives of this family. Thus, the linyphiids are likely underrepresented in our surveys. All funnel webs were considered Agelenidae, and irregular cobwebs were classified as members of Theridiidae. Vertical and diagonally oriented orb webs were classified as Araneidae if the web had a closed center hub while webs with an open center hub were designated as Tetragnathidae.

We measured 740 webs from six families and 12 genera over a three-month period. Following Uetz et al. (1999) and Eisman et al. (2010), we divided the web-builder spider community into five web-type groups: Orb web builders, Funnel web builders, Doily-sheet web builders, Irregular-all-directions web builders and Irregular-mesh web builders. Because of the large variation in orb-web diameters, we further divided this web-type group into three size classes, small (< 50 cm²), medium (> 50–740 cm²), and large (> 740 cm²), using as a reference a subset of data from those orb-weaving spiders that were consistently identified to genus, species or morpho-species (Table 1).

Habitat structure.—Vegetation along the transects was measured twice during this study, once in early July before manipulating habitat structure and once in late August during the last spider survey. Habitat structural complexity (canopy structure: vegetation median height, mean height and number of vegetation layers) was measured using a modified point sampling technique (Joern 1979). A modified Robel pole was constructed using a 1.85 m copper tube (diameter 1.25 cm) demarcated with a scale of 5 cm increments. Canopy structure (habitat structural complexity) along transects was measured by taking measurements every 5 m along these transects (21 points per transect). At each point, the pole was placed within the vegetation perpendicular to the ground and the number of vegetation hits touching the pole in each 5 cm segment was recorded. “Vegetation median height” per transect was estimated as the average of the median for 21 sampling points along each transect. The “number of vegetation layers” at each site was based on vegetation touches in each 5 cm increment interval on the Robel pole. Values for the number of vegetation layers ranged from 1 to 37 layers per site, and at least two hits in a layer were required for a height interval to be considered a layer. “Vegetation cover composition” was estimated using a 0.1 m² quadrat, where cover was classified as open soil, litter, grass, forbs, or woody

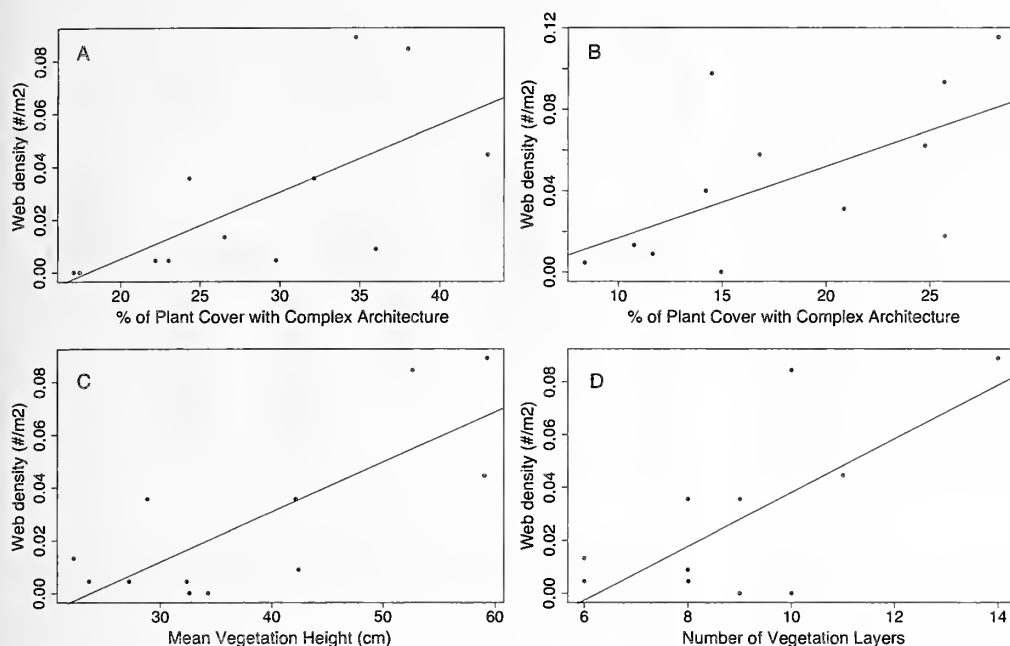


Figure 2.—Relationships between web density and percent plant cover in July (A) and in August (B). Relationships between web density and mean vegetation height (C) or number of vegetation layers (D) in July.

plant. Following the Daubenmire cover class method, the percentage of each vegetation cover class was scored as: 0 = 0%, 1 = 1–5%, 2 = 6–25%, 3 = 26–50%, 4 = 51–75%, 5 = 75–95%, 6 = 96–100% within each quadrat. Vegetation structure was scored three times in each 5-meter transect segment for a total of 60 estimates for each transect during each of the two vegetation sampling periods. Midpoints of cover classes were used in quantitative comparisons of samples and subsequent analyses (Daubenmire 1959). Because we were interested in how aerial-web-building spider abundance and the richness of web types responded to the availability of vegetation with complex architecture, we combined the values of forb and woody vegetation to obtain a better estimate of cover of plants with complex architecture.

Statistical analyses.—Because watersheds differed in the percentage cover of plants with complex architecture and complexity of habitat structure, linear regression analyses were performed separately for July and August to assess whether web density increased as habitat complexity increased from open grassland to habitats with extensive invasion from woody vegetation in the non-manipulated sections of the transects. For these analyses, we used vegetation surveys from July and August, the first of the two July spider surveys, and the August spider survey. We only used the web density values from the non-manipulated sections of these transects to avoid web-density bias effects driven by the addition of woody vegetation in the manipulated sections of these transects. Predictor variables to assess web density responses were: percentage of plants with complex architecture, Robel median, vegetation height, and number of vegetation layers.

We performed a 3-way analysis of variance (ANOVA) with repeated measures to determine the effects of adding habitat structure to aerial-web-builder density and richness of web types with respect to distance from riparian woodland edge. The response variables measured were: web density and richness of web types. We also explored the individual responses of the small-orb weaver and medium-orb weaver groups

to our habitat manipulation as these were the numerically dominant groups in our samples and commonly found in the experimental structures. The predictor variables were: stem manipulation (addition of woody vegetation vs. control), distance of 25 m experimental sections of these transects from the woodland edge (four levels: 0, 25, 50, 75 m) and survey (1–5) corrected for repeated measures per transects. Transects were treated as independent experimental units and the location of manipulated sections (0–25, 25–50, 50–75, 75–100 m) along transects were assigned randomly. Spider density and richness were calculated for each 25 m section of these transects for each survey period. Statistical analyses were performed using R 3.1.2 (R Core Team 2013) package *vegan* (Oksanen et al. 2011).

RESULTS

Spider responses to gradients in vegetation structure.—Web density was positively related to an increase in percentage cover of plants with complex architecture, forbs and woody vegetation, both in July and August (Linear regression, $F_{1,10} = 7.82$, $R^2 = 0.38$, $P = 0.02$; $F_{1,10} = 5.53$, $R^2 = 0.29$, $P = 0.04$, respectively; Fig. 2A, B). The density of aerial web builders increased with increased vegetation height (Linear regression, $F_{1,10} = 15.51$, $R^2 = 0.57$, $P = 0.003$; Fig. 2C), and with an increase in the number of vegetation layers (Linear regression, $F_{1,10} = 9.76$, $R^2 = 0.44$, $P < 0.01$; Fig. 2D) in early July. But these relationships were not significant in the late August survey (Linear regression, $F_{1,10} = 0.5$, $R^2 = 0.05$, $P = 0.49$; $F_{1,10} = 2.72$, $R^2 = 0.14$, $P = 0.13$, respectively). These predictor variables suggest that web-builder density increased along a gradient of habitat structural complexity and web-anchoring availability driven by forbs and woody vegetation. No significant response was seen between web density and vegetation median height in either July or August surveys (Linear regression, $F_{1,10} = 1.71$, $R^2 = 0.06$, $P = 0.22$; $F_{1,10} = 0.67$, $R^2 = 0.06$, $P = 0.43$, respectively). Results are consistent with the hypothesis that a greater abundance of aerial web-building spiders is found in areas with greater availability

of web-anchoring structure associated with increased structural complexity.

The richness of web types varied among the watersheds with K20A showing the greatest response in abundance and richness of web-anchoring structures. For example, Funnel-web-builder groups were more abundant than expected because of the abundance of web-anchoring structure close to the ground (dead woody stems), which was lacking in the other two watersheds. Due to the woody vegetation type selected for the manipulation, we did not influence the abundance and/or distribution of this group. Our results also showed that the Irregular-mesh-web builders (Dictynidae) were only present during the early part of this study, disappearing in late June (Supplemental material 1: online at <http://dx.doi.org/10.1636/P14-58.s1>), just prior to habitat manipulation. Thus, our habitat manipulation potentially influenced density and distribution of five out of seven web types groups of web-building spider found at KPBS.

Web-building spider responses to increases in availability of web anchors in open grasslands.—In general, we found that web density was higher on the manipulated sections of the transects. We found that distance from riparian-woodland edge affected aerial-web-builder density, where web density decreased with increased distance from woodland edge (ANOVA, $F_{1,219} = 5.3$, $P = 0.02$). Even though the manipulated section of these transects generally had higher web density, we found a strong interaction for manipulation-by-distance from the woodland edge (ANOVA, $F_{1,219} = 4.0$, $P = 0.048$; Fig. 3C). Web density in the manipulated sections decreased as the distance from the riparian-woodland edge increased, even though they still showed higher web densities than those seen in the non-manipulated sections of the transects. Results indicate that woody vegetation is a limiting resource for aerial web-building spiders in open grasslands systems. Of the five groups of web builders, only the density of the small-orb and medium-orb weaver groups (Fig. 3A, B) responded positively to the addition of woody web-anchoring structure (*Cornus* stems) in open grasslands (ANOVA, $F_{1,221} = 8.0$, $P = 0.005$; $F_{1,221} = 6.0$, $P = 0.015$, respectively) regardless of distance from woodland edge or sampling period after manipulation.

To determine whether aerial web-building spider density responded quickly to structure availability, we compared densities from our second pre-manipulation (late June) survey to our first survey after manipulation (early July), which was conducted eight days later. Habitat manipulation led to a quick positive effect on small-orb weaver density (ANOVA, $F_{1,77} = 5.2$, $P = 0.026$); higher densities of this spider were observed in manipulated sections of transects when compared to non-manipulated sections, regardless of distance from wood stand edge.

Results showed differences in web-type richness among manipulated and non-manipulated sections of these transects (ANOVA, $F_{1,221} = 5.5$, $P = 0.02$). Web-type richness increased in the manipulated sections of these transects as the growing season progressed (ANOVA, $F_{1,221} = 5.4$, $P = 0.02$; Fig. 4) compared to the non-manipulated sections.

DISCUSSION

The habitat heterogeneity hypothesis posits that the abundance and diversity of arthropod species will respond positively

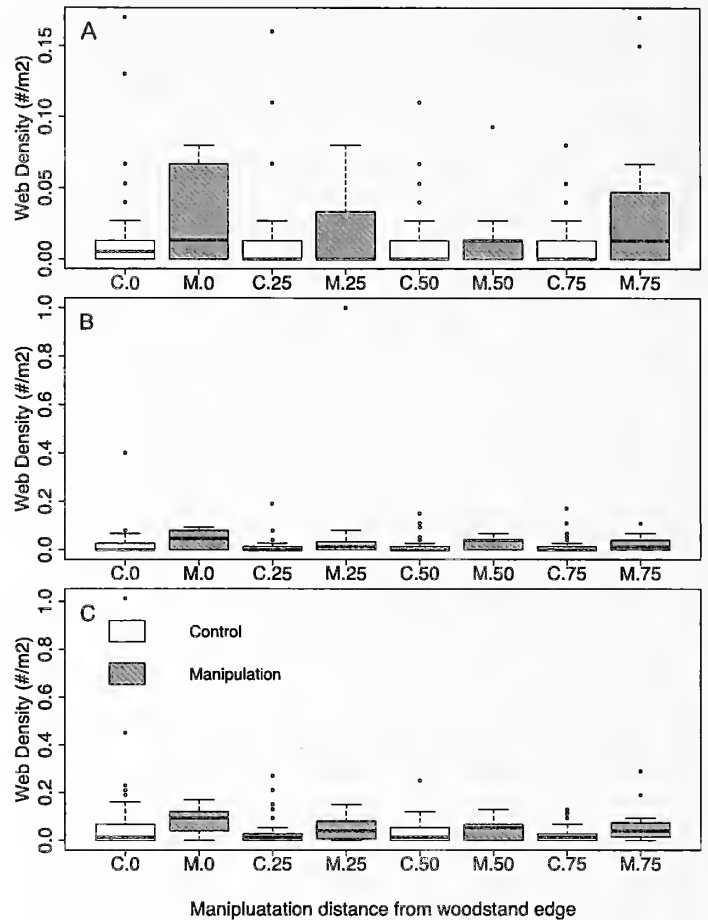


Figure 3.—Comparison of open grassland web-builder spider density among various distances from a riparian woodland edge at Konza Prairie Biological Station, Kansas. (A) Small-orb weavers, (B) medium-orb weavers, (C) total web density (all web types groups combined). The box plot elements represent the following: box vertical dimension = interquartile range; horizontal line = median; whiskers = minimum and maximum values; circular points = maximum observations 1.5(IQR) above the 75th percentile.

to increases in the spatial heterogeneity of plant species richness and vegetation architecture (Dennis et al. 1998; Fuhlendorf & Engle 2001; Fuhlendorf et al. 2006; Jimenez-Valverde & Lobo 2007; Allouche et al. 2012). It is well recognized that fire-grazing interactions in grasslands modulate habitat heterogeneity with great effects on the abundances, diversity and trophic complexity of consumer assemblages through bottom-up regulation mediated by plants (Joern 2005; Joern & Laws 2013). Thus, habitat structural complexity and local plant architecture diversity in particular are important factors influencing terrestrial arthropod diversity (Dennis et al. 1998; Halaj et al. 2000; Joern 2005; Cobbold & MacMahon 2012; Joern & Laws 2013), abundance, and community dynamics at multiple levels (Langellotto & Denno 2004). For example, intensive grazing by sheep in Hungarian grassland led to the loss of spider species sensitive to habitat disturbance and increased the representation of common and disturbance-tolerant species (Szinétár & Samu 2012).

In this study, we experimentally tested the proposition that availability of physical structure for web placement can limit aerial web-building spider communities in open grassland. We

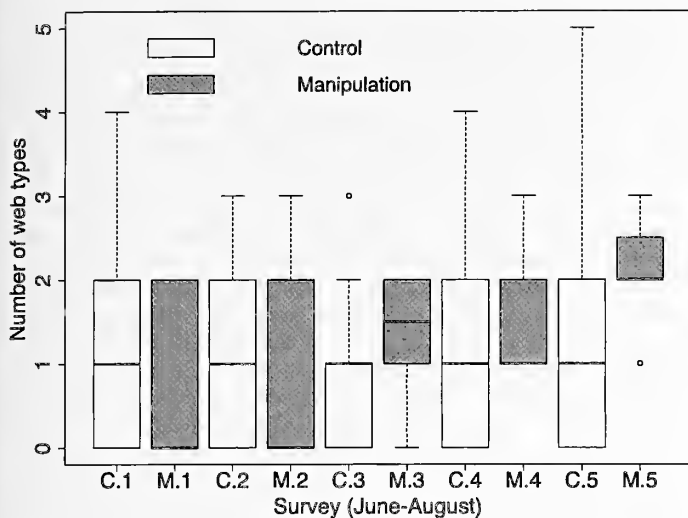


Figure 4.—Comparison of richness of web types among various distances from riparian woodland edge. The box plot elements represent the following: box vertical dimension = interquartile range; horizontal line = median; whiskers = minimum and maximum values; circular points = maximum observations 1.5(IQR) above the 75th percentile.

did so by increasing woody structure in the herbaceous-shrub layer at various distances from riparian woodland edge in three watersheds. Distributions of web-building spiders are directly linked to the spatial configuration of woody vegetation in their habitat due to web-anchoring requirements; both experimental and observational studies indicate the tight relationship between spider abundance and habitat structure (Halaj et al. 1998; Rypstra et al. 1999; Diehl et al. 2013). Vegetation examined among the three watersheds in this study ranged from an open grass canopy with little woody vegetation to watersheds with significant levels of woody vegetation encroachment and thus a significant gradient of habitat complexity. Indeed, our results for both July and August show that web-builder density increased as the percentage cover of forbs and woody vegetation increased from open grassland to grassland encroached by woody vegetation. In July, web-builder density increased with increasing number of vegetation layers as vegetation height increased, thus increasing the possibility of habitat partitioning by web-building spiders. The highest web densities along this habitat structure gradient were observed on watershed K20A, a watershed with a history of low burn frequency and a high accumulation of woody vegetation.

Several lines of evidence from our experiment suggest that the availability of web-anchoring structure limits web-building spiders in open tallgrass prairie. We found critical responses by aerial-web-building spiders to regions where web-anchoring structures were added. (a) There was a greater density of aerial-web-building spiders and richness of web types in manipulated areas at the transect scale (300 m² transect) towards the end of the growing season. (b) Web density on the manipulated section decreased with increasing distance from the riparian woodland edge, even though it was higher overall than in non-manipulated sections. (c) After *Cornus* stems were added, web-type richness increased over time until, by the end of the study, it was higher than in the control sections. Similar results were found by Toti et al. (2000), where species richness of aerial

web builders increased from spring to fall in grass habitat at the Great Smoky Mountains National Park. However, some transects in that study had control sections that consistently maintained high web types richness over the entire duration of the study, and the response was driven mostly by naturally high abundances and richness of web anchoring structures. (d) Finally, the addition of web-anchoring structure led to an increased density of small-orb and medium-orb web-builder groups in a watershed under frequent burn treatments, watersheds characterized by the paucity of woody vegetation cover. Even though small-orb and medium-orb densities were higher at the manipulated section than on the control sections at all distances from the riparian woodland edge as predicted, we also observed a decrease in the density of these two groups of web builders overall. Such results suggest that other factors such as species dispersal ability could affect their distribution on the habitat.

Web-building spider species differ in how webs are positioned within the vegetation, outcomes driven by differences in web type and web structure (e.g., spacing of mesh, web size, height of web placement, and the sizes of prey captured). Such variability in web placement constraints facilitates the assembly of web-builder diversity in habitats that are architecturally and floristically diverse (Richardson & Hanks 2009). Differences in the richness of web types seen among watersheds were associated with differences in habitat structural complexity and underlying variation in cover with complex architecture. Generally, we observed that web-type richness was higher in the manipulated sections of these transects even with exceptions from a few control sections from regions associated with dense, woody stands (e.g., *Cornus* shrub islands and/or diverse mixture of woody plant and forbs). Such sample sites with high overall structure could maintain high levels of web-type richness through the entire duration of the study. Such hotspots of web-type richness were found on all four transects of K20A watershed and one transect of K4A. We note that these hotspots not only supported diverse web-type richness but they also showed a higher diversity of web-building spiders species per web-type group than in samples from non-hotspot points along the same transect. Unfortunately, we could not directly analyze species richness in this study due to difficulty in identifying all species in the field and the lack of watershed replication in this study.

Habitat structure and vegetation architecture affect the spatial distribution of spiders with different web types (Colebourn 1974). For example, while our habitat manipulation using *Cornus* stems positively affected small-orb and medium-orb density at various distances from the riparian woodland edge, it did not influence the funnel-weavers, a common group of web builders at KPBS. The experimental design did not provide the appropriate web-anchoring structure close to the ground for assessing responses by funnel-weavers (Agelenidae) that were only found in open grasslands in zones with accumulation of woody stems near the ground in K20 and one transect in the K1A watershed. Even though, they were the most abundant web-building spiders found along the King's Creek basin in KPBS during the summer of 2012, Agelenidae densities dropped dramatically at the transition zone from riparian woodlands into open grassland (J.E. Gómez, unpublished data). Also, we could not address responses of the mesh-weaver group (Dictynidae) to increases

of web-anchoring structure in open grasslands as this group was only active early in the growing season and their abundance dropped by the end of June before our manipulation was conducted.

Even though vegetation structure is widely recognized as a key determinant resource of spider community composition, the exact mechanism for its influence is unknown and other indirect effects such as microclimate and prey availability may play important roles (Jimenez-Valverde & Lobo 2007). Initial colonization by spiders may be relatively quick because even large web spinners are capable of aerial dispersal at immature stages (Gibson et al. 1992). Our experiment demonstrates that spider density responded quickly to an increase in the availability of woody structure as new *Cornus* stems were colonized by multiple types of web-building spiders within a period of only eight days, and spider density was consistently higher on the manipulated sections when compared to the non-manipulated sections of these transects from mid- to late summer. We found that overall density responses of aerial-web builders to increased availability of web-anchoring structures were largely driven by the small-orb weavers and to a lesser extent by the medium-orb weavers. It makes sense that orb-weavers were the first colonizers (Blamires et al. 2007) as orb weavers occupy a wide range of habitats and accordingly there is a great diversity in both web architecture and behaviors among genera. For example, *Nephila* Leach, 1815 and *Tetragnatha* Latreille, 1804 build large webs in open habitats while *Argiope* Audouin, 1826 builds smaller webs among low, dense (closed) vegetation (Blamires et al. 2007). This increases the possibility that at least one species within an orb weaver web-type group could benefit from the architecture of *Cornus* stems. We conclude that the abundance and architecture of web anchoring structures limit aerial-web-builder density, distribution and richness of web type in the open grassland studied here.

Results from this study offer further support for the notion that structurally complex habitats provide a wider selection of web-attachment sites and thus increased habitat suitability for web-builder spiders in open grasslands in North America. Responses are facilitated by increased opportunities for spatial partitioning as the architectural complexity of the habitat increases (Robinson 1981), and responses likely operate at multiple scales.

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The effects of habitat size and quality on the orb-weaving spider guild (Arachnida: Araneae) in an Atlantic Forest fragmented landscape

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Abstract. Fragmentation of natural habitats is considered one of the greatest threats to the maintenance of global biodiversity. In this study, we tested the importance of forest patch size and vegetation structure on the richness, diversity, abundance, and composition of the orb-weaving spider guild in an area of the Atlantic Forest (State of São Paulo, Brazil). We sampled 16 sites, grouped into the following categories composed of four sites each: continuous mature forest, continuous secondary forest, large (52–175 ha) secondary forest fragments, and small secondary forest fragments (14–28 ha). The richness ranged from 29 to 55 species per site, but was unrelated to forest size or vegetation structure. The communities from the continuous mature forests were more abundant and less diverse than those from the other categories, but this was due to a few dominant species. The changes in composition were related to the vegetation structure, suggesting that this variable is more important to the composition of orb-weaving communities than the size of the forest patch. Overall, the results indicate that the orb-weaving spider community in this region, even in the fragments, is still rich and diverse, which may be attributable to some characteristics of spiders, such as generalist behaviour and a good dispersal capacity. Nonetheless, our results also highlight the importance of continuous areas, especially those with mature vegetation that harbor a characteristic orb-weaving community that can serve as a source for the fragments.

Keywords: Araneidae, biodiversity, bioindicator, fragmentation, habitat loss, orb-weavers

The destruction of natural habitats is considered the main factor responsible for the biodiversity crisis (Dirzo & Raven 2003), and a common consequence of those human-induced changes are fragmented landscapes with forest remnant patches inserted into a deforested matrix. The biodiversity crisis is especially critical in the tropics due to the alarming rate of land clearing and the fact that tropical forests are the most species-rich terrestrial biome (Laurance 2007).

The two main impacts of fragmentation are habitat loss and alteration of the forest remnants (Fahrig 2003; Laurance et al. 2011), as the vegetation in forest fragments is usually more degraded than that of larger forested areas. Because of their larger edge to core habitat ratio, fragments are more exposed to edge effects (Murcia 1995) and are more vulnerable to other anthropogenic disturbances, such as logging, hunting, grazing, and fires (Laurance et al. 2011). Forest fragments may also consist of secondary vegetation simply because they can be formed through natural re-growth after the clearing and abandonment of the land.

Most of the knowledge on this subject was originally based on vertebrate groups (Turner 1996; Zuidema et al. 1996), but attention to invertebrate communities in fragments has been increasing, with a particular focus on insect taxa (Didham et al. 1996; Tscharrntke et al. 2002; Nichols et al. 2007). Most recently, spiders have also been investigated. The majority of work has been aimed at assessing the effects of patch size on community richness (Abensperg-Traun et al. 1996; Miyashita et al. 1998; Bolger et al. 2000; Gibb & Hochuli 2002; Floren & Deeleman-Rheinold 2005; Major et al. 2006; Kapoor 2008), a common research subject, because it represents a direct measure of the impact of habitat loss on diversity (Debinski & Holt 2000).

The richness of spider communities is not usually related to patch size, but positive (Abensperg-Traun et al. 1996; Miyashita et al. 1998) or even negative (Bolger et al. 2000)

relationships have been reported, suggesting a complex response. Vegetation quality is another factor that may have a significant influence on spider communities. The characteristics of the vegetation, especially its spatial structure, are among the most important factors for spider communities (Wise 1993; Malumbres-Olarte et al. 2013), and communities from diverse forest types may differ in richness (Pinkus-Rendón et al. 2006; Lo-Man-Hung et al. 2008) and, more frequently, in composition (Chen & Tso 2004; Floren & Deeleman-Rheinold 2005; Cabra-Garcia et al. 2010; Raub et al. 2014).

Some of the studies that investigated the effects of patch size on spider communities also verified the effects of the vegetation structure, but those factors have never been tested independently. For example, Kapoor (2008) reported differences in the composition of spider communities from larger and smaller fragments, but the former also had more preserved vegetation than the latter. A similar situation was found by Gibb & Hochuli (2002) in a study with large and small fragments under different disturbance regimes.

Our aim was to assess the importance of patch size and vegetation structure on the richness, diversity, abundance, and community composition of orb-weaving spiders by comparing the fauna of four different categories of habitats: continuous mature forest, continuous secondary forest, and large and small fragments of secondary forest. The use of spider communities in ecological studies is recommended due to their diversity and abundance, as well as for their unquestionable ecological importance as top predators among the invertebrates (Coddington et al. 1991; New 1999; Gerlach et al. 2013). Like all web-building spiders, orb-weaving spiders also seem to be particularly sensitive to vegetation structure, because the availability of attachment points for webs is considered one of the most important resources for these groups (Wise 1993).

This study was conducted in a fragmented Atlantic Forest landscape. This biome occupies a large area on the coast of

eastern Brazil (5–30°S). It is considered a biodiversity hotspot (Myers 1988; perhaps even the “hottest” according to Laurance 2009) because it combines high levels of biological richness and endemism with equally high levels of deforestation and human threat. Currently, only about 12% of the forest remains, and more than 83% of the remnants are composed of small fragments (< 50 ha; Ribeiro et al. 2009) that are still under heavy anthropic pressure because the ecosystem is located on the Brazilian east coast, the most developed and densely populated region of the country. Thus, the Atlantic Forest unfortunately represents a very good example of a fragmented ecosystem, and the study of the impact of this process over its biological communities is urgent and of fundamental importance.

METHODS

Study area.—Our sampling sites were located in the Reserva Florestal do Morro Grande (RFMG), a forest reserve, and in nearby forest fragments in the municipalities of Cotia and Ibiúna (State of São Paulo, Brazil; 23° 35' S to 23° 50' S; 46° 45' W to 47° 15' W). The RFMG covers 10,000 ha, but it is connected with other large forested areas at its southern edge, and together they are considered a continuous area. The altitude varies from 850 to 1,100 meters above sea level, and the natural vegetation is classified as ‘montane ombrofilous forest’ (Veloso et al. 1991). Its climate (Köppen climate classification: Cwa; Köppen 1948) is subtropical, characterized by a dry winter (mean temperature < 18° C) and a warm, rainy summer (mean temperature > 22° C).

We selected sixteen sampling sites, eight within the RFMG and eight forest fragments (Fig. 1). Four sites in the RFMG were composed of secondary vegetation at an intermediate to advanced stage of regeneration (Metzger et al. 2006), and four had mature vegetation. The fragments were also composed of secondary vegetation at an intermediate/advanced stage of regeneration (Uezu et al. 2005). The sites were divided into four categories (Table 1): continuous mature forest (hereafter called CM 1 to 4), continuous secondary forest (CS 1 to 4), large fragments (LF 1 to 4; 52–175 ha), and small fragments (SF 1 to 4; 14–28 ha). The average distance between one site and its nearest surveyed neighbor was 1561 m (SD = 474 m, range = 864–2395 m) and did not differ among the habitat categories (ANOVA, $F_{3,12} = 0.3$, $P = 0.842$). The entire landscape area, including all of the sampling sites, will be referred to as Caucaia, the popular name of that region.

Sampling.—We captured the spiders by manual nocturnal sampling and preserved them in 70% ethanol. We searched in logs, vegetation, and several other microhabitats in the soil and understory, from the leaf litter up to 2 m high. In all areas, we sampled for one hour along a 30 m long transect oriented perpendicularly to a main trail, always located at least 50 m from the forest edge, and 30 m from other transects. We sampled each of the 16 sites for two nights, one in December 2002, and one in March 2003. Sampling teams were composed of four collectors, each of whom investigated three transects per night for totals of 24 transects per site and 384 transects for all sites combined.

Voucher specimens were deposited in the Museu de Zoologia and the Laboratório de Artrópodes do Instituto Butantan. Immature individuals were discarded, while the adults were separated into morphospecies and identified to the lowest

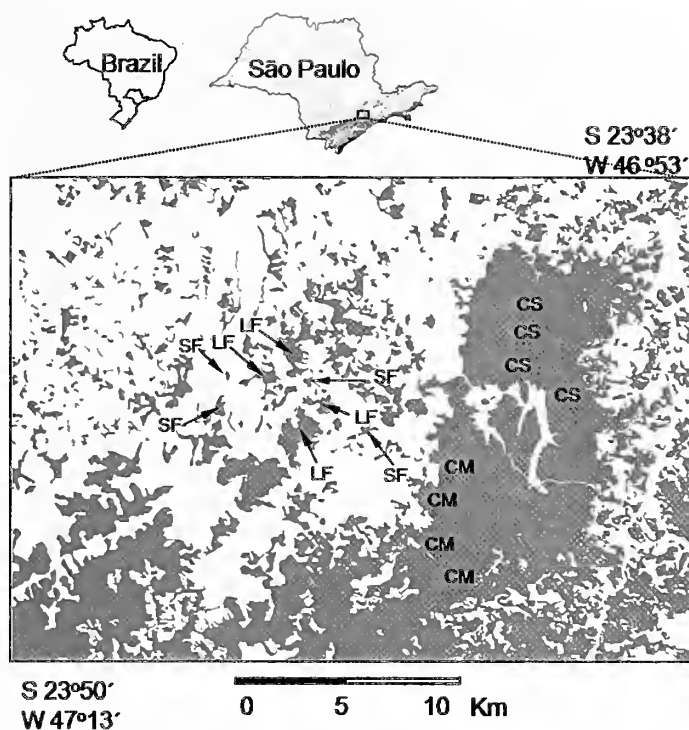


Figure 1.—Location and map of the study area. Forested areas are highlighted in grey, and the eight fragments sampled are indicated by arrows. CM, continuous mature sites; CS, continuous secondary forest sites; LF, large fragments; and SF, small fragments.

possible level. Thus, all of the results and analyses concern only adult individuals.

Forest characterization.—We described the forest structure by measuring the foliage density and stratification, which are good indicators of the tropical forest regeneration stage (DeWalt et al. 2003) and level of forest disturbance (Malcolm 1994). We used a modification of the method described in

Table 1.—The richness, abundance, diversity index $\exp(H')$ and rarefied richness (Sraref) for the orb-weaving spider communities investigated in December 2002 and March 2003. The size (ha) of the fragments is in parentheses. CM, continuous mature sites; CS, continuous secondary forest sites, LF, large fragments; SF, small fragments; and $\exp(H')$, exponential of the Shannon index (H').

Sites	Richness	Abundance	$\exp(H')$	Sraref
CM 1	50	311	23.10	30.55
CM 2	44	256	21.13	27.90
CM 3	40	309	13.69	23.44
CM 4	35	266	13.23	22.12
CS 1	41	189	22.42	29.44
CS 2	49	216	31.34	34.55
CS 3	54	165	31.81	38.32
CS 4	46	205	33.11	36.16
LF 1 (52.2)	45	152	30.66	35.66
LF 2 (175.1)	48	153	32.56	37.23
LF 3 (53.1)	29	88	19.12	29
LF 4 (99.4)	31	128	18.19	27.51
SF 1 (14.1)	37	133	20.76	30.41
SF 2 (18.7)	49	249	21.84	29.47
SF 3 (28.8)	38	126	24.93	32.45
SF 4 (14.1)	38	202	16.74	25.81

Malcolm (1995). At each site, we established two parallel 165-m long lines separated by 20 m. Each line comprised 12 stations, one every 15 m. At each station, a 4-m pole was used to establish an imaginary 150-mm diameter vertical column. We used a telemeter to measure the height of the inferior and superior limits of all foliage that stretched along the imaginary column to calculate the length in meters occupied by the foliage in five strata (0–1, 1–5, 5–10, 10–15, >15 m). For each site, we calculated the mean foliage length in each stratum for the 24 sampling stations. For a more detailed description of this method, see Pardini et al. (2005).

Data analysis.—We calculated the richness and abundance at each of the 16 sampled sites. To minimize the differences in richness due to differences in the numbers of individuals, two diversity measures were also used, the rarefied richness (S_{raref}) and the exponential of the Shannon index [$\exp(H')$; Jost 2006]. We also calculated the proportion of singletons (species represented by just one individual) for each site. A one-way ANOVA was used to test for differences in these parameters between the four habitat categories, and a Tukey test was employed when significant differences were detected. When there was no homogeneity of variances, we performed a Kruskal-Wallis test, with rank-transformed data.

Simple linear regression analysis was used to test the influence of the vegetation structure on the richness, diversity, proportion of singletons, abundance, the abundance of three dominant species, and the abundance of the community excluding those dominant species. To treat the vegetation structure as a continuous variable, we performed a Principal components analysis (PCA) (Fig. 2) using the foliage density measures for the five strata in the 16 sites in a correlation matrix (centered and standardized per species) using the package CANOCO for Windows 4.0 (ter Braak & Smilauer 1998). The first axis explained 54.8% of the variation and formed a gradient in which the lower values represented the sites with a taller canopy and more opened understory, characteristics of mature vegetation, while the higher values represented areas with the opposite characteristics. The scores from the first axis were used to perform the regressions.

We treated patch size as a categorical variable, with three levels: continuous area, large fragments, and small fragments. To measure the effect of patch size independently from that of the vegetation, we used the residuals of the regressions performed with the vegetation structure and the same community parameters cited above. To compare the categories, we performed two *t*-tests utilizing the orthogonal contrast procedure (Montgomery 2001). This procedure allows choosing a number of comparisons (*k*; contrasts) equal to the number of categories minus 1 (in this case, there are 3 categories, and *k* = 2). The total variance is partitioned among the contrasts, which are thus independent and do not increase the probability of a type-1 error. The first *t*-test compared the eight continuous areas (those located in the continuous forest) with the eight forest fragments, while the second compared the large and the small fragments. ANOVA, *t*-test, and regressions were performed with Statistica Software, version 6.0 (StatSoft 2003).

The similarity of the sampled sites was studied using a detrended correspondence analysis (DCA). The DCA shows the sites in a two-dimensional plot based on the similarity of their fauna, as well as the species used in the analysis based

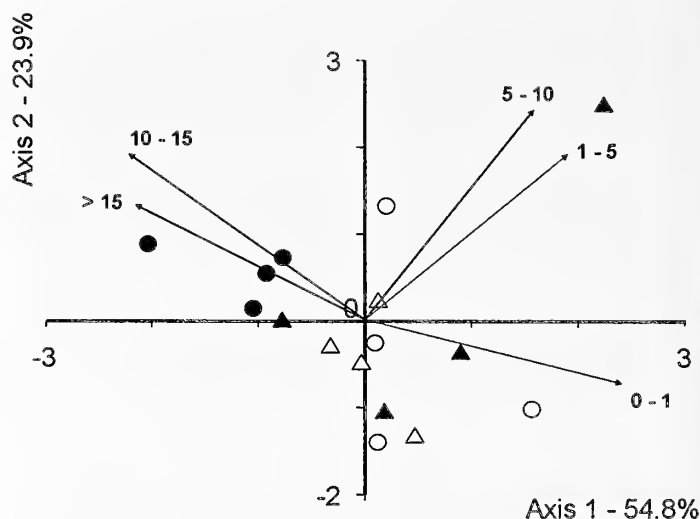


Figure 2.—Principal components analysis of the vegetation structure for the 16 sampled sites in the Reserva Florestal do Morro Grande and fragments in Cotia and Ibiúna, SP. The placement of the sites is based on the foliage density in five different strata of the forest. Numbers associated with the arrows represent the height (m) of the different strata of the vegetation. Black circles, continuous mature (CM) forest sites; black triangles, continuous secondary (CS) forest sites; white circles, large fragment (LF) sites; white triangles, small fragment (SF) sites.

on their distribution among the sites. Because species with low abundance that occur at only a few sites may not be very informative, only the 43 most abundant species (those represented by at least 20 individuals) were included in the analysis, which was performed with MVSP (Multivariate Statistical Package), version 3.1 (Kovach Computing Services 2000). A Mantel test was performed to test whether the changes in the spider community compositions at each site were related to the geographic distance between them. We calculated the similarity of the faunas with the Bray-Curtis index, and the resulting matrix was compared with a site-distance matrix. Three tests were performed, one for the sites in the RFMG, one for the fragments, and one for all sites combined. These tests were based on 5,000 permutations and were performed with the PAST (Paleontological Statistics) software (Hammer & Harper 2009).

Finally, we performed an indicator species analysis (ISA; Dufrêne & Legendre 1997) to see the association between the same 43 most abundant species and the sites sampled in more detail. ISA calculates an indicator value (IndVal) based on the frequency, relative abundance, and exclusivity with which a species occurs at the sites of a given category and then tests if it significantly differs from random based on a Monte Carlo permutation ($n = 1000$). The higher the frequency and exclusivity of a distribution within a given category, the higher will be the IndVal of a species, which range from 0 (the absence of a category) to 100 (exclusively present in one category and occurring at all sites). Those species whose distributions were evaluated as significant ($P < 0.05$) were considered indicator species.

The ISA also allows partitioning of the sites into different typologies. For example, we could divide the sites according to the vegetation type [CM sites \times (CS sites + Fragments)] or forest patch size [(CM + CS sites) \times Fragments] and compare

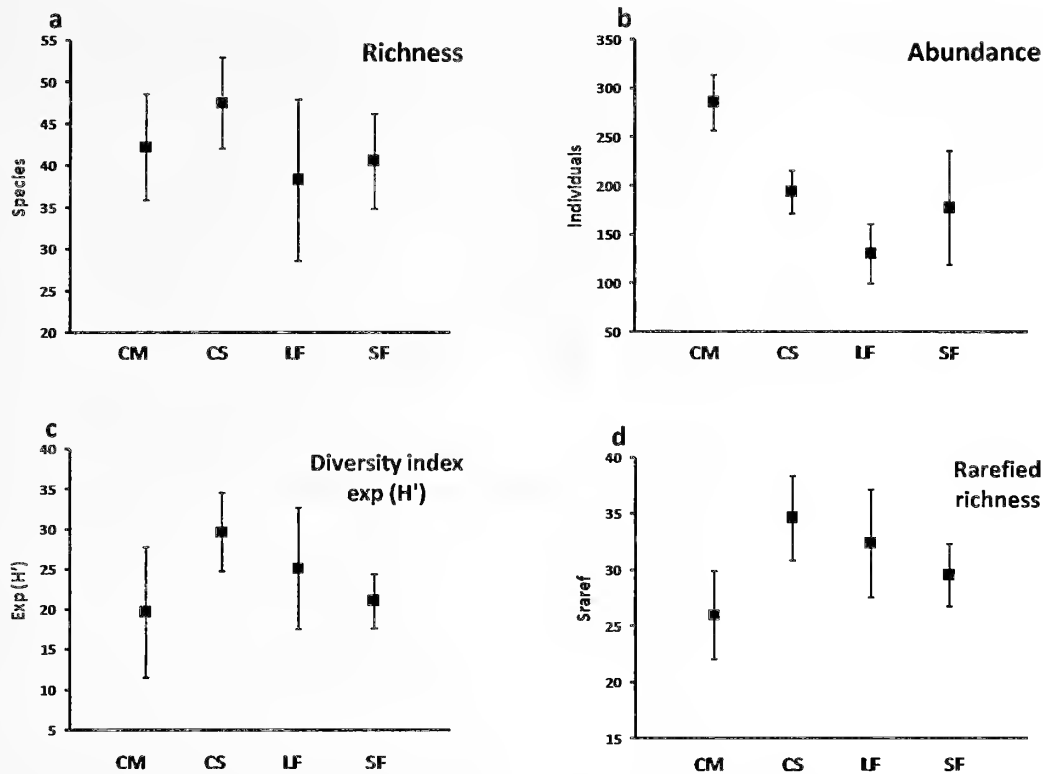


Figure 3.—Comparison of mean (standard deviation indicated by whiskers) of the orb-weaving spider communities in the four site categories for the following parameters: (a) richness; (b) abundance; (c) diversity index $\exp(H')$; and (d) rarefied richness. CM, continuous mature forest sites; CS, continuous secondary forest sites; LF, large fragments; and SF, small fragments.

the results to determine which typology was more adequate to our data. Dufrêne & Legendre (1997) suggested that the sum of the IndVals for all species for each typology could be used as a criterion to reveal the best arrangement of the sites, as a higher total IndVal for a given typology means that more species were selected as indicator species, and/or that the IndVal of the indicator species was higher. Similarly, a species can also be assigned as an indicator for different typologies, and in this case, we consider the one in which it attains its maximum IndVal as the most appropriate to its distribution.

We analysed the species distribution among our sites under three different typologies. First, we separated the sites according to the type of vegetation, mature or secondary (including the CS sites and the fragments). Second, we opposed the sites in the RFMG (CM + CS sites), i.e., continuous forest sites, versus the fragments. For the third partition, we divided the sites into three categories, CM, CS, and fragments, to check whether a more detailed typology would be more appropriate for the distribution of the species. The ISA was performed with the “Ind Val” function of the “labdsv” 1.6-1 (Roberts 2013) package in the software R (R Core Team 2014).

For all the analyses, the result of the two sampling campaigns (December and March) were summed and considered together.

RESULTS

For all of the sites, we collected 12,683 orb-weaving spiders, 3,148 adults and 9,535 immatures. The adults belonged to 121 species and 8 families (a full species list is available as supplemental material online at <http://dx.doi.org/10.1636/P15-19.s1>,

and for a community description, see Nogueira et al. 2006). All differences related to patch size presented below refer to comparisons between the continuous sites and the fragments, as none of the comparisons between the two classes of fragments was significant.

Richness.—The number of species in the sampled sites varied from 29 to 54 (Table 1, Fig. 3), but there were no significant differences between the four categories (ANOVA, $F = 1.3$, $P = 0.33$). The richness was unrelated to the patch size (Linear regression, $R^2 = 0.06$, $P = 0.36$) or vegetation structure (t-test, $t = -0.3$, $P = 0.78$).

Abundance.—The abundance per site ranged from 88 to 311 individuals (Table 1, Fig. 3), and significantly differed between the categories (ANOVA, $F = 11.9$, $P < 0.01$). A Tukey test revealed that the CM sites had significantly more adults than the other three site categories.

The abundance was related to both patch size and vegetation structure. It was higher in sites with mature vegetation (Linear regression, $R^2 = 0.45$, $P < 0.01$), as well as in the continuous sites relative to the fragments (t-test, $t = 307.9$, $P < 0.01$). However, in both cases, the results were influenced by a few dominant species. *Micrathena sanctispiritus* Brignoli, 1983 (Araneidae) and *Chrysometa ludibunda* (Keyserling, 1893) (Tetragnathidae) were associated with continuous sites (t-test, $t = 2.6$, $P < 0.05$ and $t = 2.2$, $P < 0.05$, respectively), and *M. sanctispiritus* and *M. nigrichelis* Strand, 1908 were positively correlated with mature forests (Linear regression, $R^2 = 0.27$, $P < 0.05$ and $R^2 = 0.34$, $P < 0.05$, respectively). In both cases, when the species were removed from their respective analyses, the relationships became much weaker (continuous

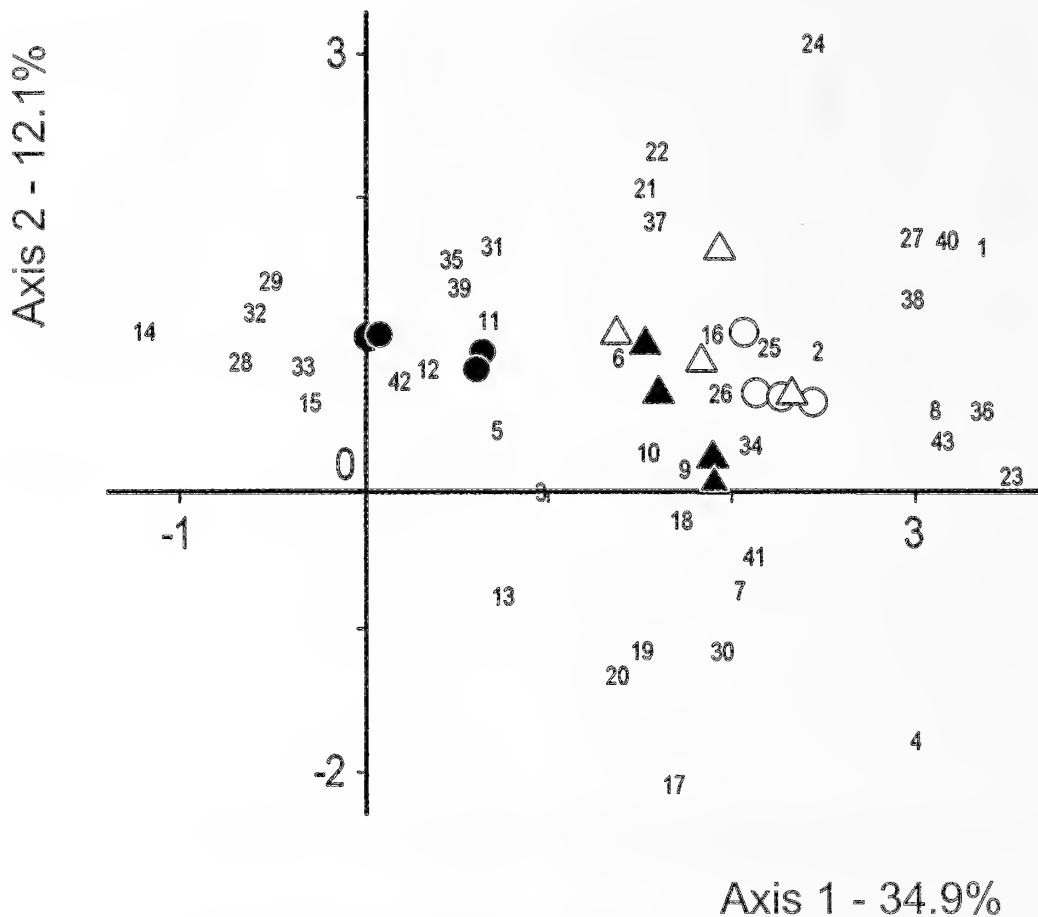


Figure 4.—Scatterplot from the detrended correspondence analysis performed for the 16 sites sampled in the Reserva Florestal do Morro Grande and fragments in Cotia and Ibiúna, SP (December 2002 and March 2003). The arrangement of the sites is based on the similarity of their fauna. The arrangement of the 43 species used in the analysis is based on the similarity of their distribution among the sites. Species are represented by numbers. Black circles, continuous mature (CM) forest sites; black triangles, continuous secondary (CS) forest sites; white circles, large fragment (LF) sites; and white triangles, small fragment (SF) sites.

sites \times fragments: t-test, $t = 1.9$, $P = 0.07$ and Linear regression, $R^2 = 0.235$, $P = 0.0566$, respectively).

Diversity.—The diversity index $\exp(H')$ and the rarefied richness varied significantly between the categories ($\exp(H')$: ANOVA, $F = 3.6$, $P < 0.05$; Sraref: $F = 3.7$, $P < 0.05$; Table 1, Fig. 3). In both cases, the diversity of the CM sites was lower than that of the other categories and was significantly lower than that of the CS sites. However, the variation observed was unrelated to the patch size ($\exp(H')$: t-test, $t = 0.3$, $P = 0.77$; Sraref: $t < 0.1$, $P = 0.95$) or vegetation structure ($\exp(H')$: Linear regression, $R^2 = 0.01$, $P = 0.71$; Sraref: $R^2 = 0.03$, $P = 0.49$).

The lower diversity of the CM sites is a consequence of the high abundance of the two dominant species associated with *M. nigrichelis* and *M. sanctispiritus*, although the reasons for this association are not clear. Nonetheless, if we exclude the dominant species from the analyses, the differences in diversity are no longer significant (ANOVA. $\exp(H')$: $F = 1.1$, $P = 0.38$; Sraref: $F = 1.3$, $P = 0.32$),

The proportion of singletons ranged from 15 % to 51 %, but the differences between the categories were negligible (ANOVA, $F = 0.9$, $P = 0.47$). The variation in this parameter was also unrelated to vegetation structure (Linear regression, $R^2 = 0.19$, $P = 0.09$) or patch size (t-test, $t = -1.5$, $P = 0.32$).

Composition and indicator species.—The DCA showed a site grouping pattern based on the vegetation type (Fig. 4). This division occurred in the first axis, which explained 34.9 % of the variation. The four continuous sites with mature vegetation (CM 1–4) were grouped together and separated from those with secondary vegetation. The placement of the secondary vegetation sites formed a gradient, where those in the continuous area (CS 1–4) and three of the four small fragments were closer to the CM sites than the four large fragments and the remaining fourth small fragment. The second axis explained only 12.1 % of the variation and did not seem to reveal any particular trends concerning the patch size or vegetation structure.

The Mantel test revealed a significant correlation between the similarity and distance matrices for all of the sites combined (Mantel test, $r = 0.40$; $P < 0.01$) and for the sites in the RFMG (Mantel test, $r = 0.81$; $P < 0.01$), but not for the fragments alone (Mantel test, $r = 0.29$; $P = 0.08$). However, we believe the positive results may be attributable to a characteristic of our study area.

In the RFMG, the CM sites are located in the southern part of the reserve, while the CS sites are in the northern part (Fig. 1), which means that sites with similar vegetation were closer to each other than to sites with different vegetation. Therefore, in the RFMG we had two factors, distance and

Table 2.—Species selected as significant indicators by an indicator species analysis for the three different typologies classifications. The indicator values (IndVal) of the species for each typology category are shown with the *p*-values in brackets. Bold font represents the maximum IndVal. M and CM, continuous mature forest sites; S, secondary forest sites; C, continuous forest sites; F and FR, fragments

Species	Vegetation type		Forest area size		3 categories		
	M	S	C	F	CM	CS	FR
<i>Miagrammopes</i> sp.1	93.5 (<0.01)		62.5 (<0.05)		82.8 (<0.01)		
<i>Micrathena nigricheles</i>	92.8 (<0.01)		72.2 (<0.05)		89 (<0.01)		
<i>Wagneriana iguape</i>	87.2 (<0.01)		85.7 (<0.01)		74.7 (<0.01)		
<i>Micrathena sanctispiritus</i>	78.5 (<0.05)		78.1 (<0.05)		61.6 (<0.05)		
<i>Chrysometa ludibunda</i>	92.1 (<0.01)		95.3 (<0.01)		81.3 (<0.01)		
<i>Chrysometa boraceia</i>	79.9 (<0.05)		82.1 (<0.01)				
<i>Chrysometa cambara</i>	100 (<0.01)				100 (<0.01)		
<i>Mangora blumenau</i>	94.1 (<0.01)				88.1 (<0.01)		
<i>Testudinaria gravatai</i>	81.2 (<0.05)				69.6 (<0.05)		
<i>Micrathena triangularis</i>	73.1 (<0.05)				72.1 (<0.05)		
<i>Azilia histrio</i>	77.4 (<0.05)						
<i>Cyclosa fililineata</i>			77.2 (<0.05)			71.9 (<0.01)	
<i>Araneus vincibilis</i>						62.5 (<0.05)	
<i>Acacesia graciosa</i>		83.3 (<0.05)		73.1 (<0.05)			
<i>Mangora ramirezi</i>		75 (<0.05)		95 (<0.01)			90.5 (<0.01)
<i>Testudinaria lemniscata</i>		73.2 (<0.05)		86.8 (<0.01)			76.6 (<0.01)
<i>Wagneriana dimastophora</i>		96.6 (<0.01)					
<i>Ogulnius</i> sp.2		83.3 (<0.05)					

vegetation type, acting synergistically over the faunal similarity, which would explain the very high correlation coefficient obtained for the RFMG sites. This situation also influenced the results of the test performed for the sites combined, although the correlation became much weaker because the similarity in the samples from the fragments did not correspond as closely to distance, as shown by the Mantel test for the fragments alone. This last result is the most meaningful, because it shows that when only distances are considered, the correlation with faunal similarity is insignificant, providing evidence that the spiders are not spatially limited in the fragmented landscape we studied.

The ISA assigned 18 species as indicators for some categories in at least one of the typologies analysed (Table 2). The genus *Micrathena* Sundevall, 1833 was the most associated with mature forest, with three species designated as significant indicators and reaching a maximum IndVal in the vegetation-type partition. The genus *Chrysometa* Simon, 1984 also had three species designated as significant indicators of mature forest, but two of them, *C. ludibunda* and *C. boraceia* Levi, 1986, had a larger IndVal for the second typology, signalling that those species were more associated with continuous forest areas than with vegetation type. It is also noteworthy that some genera had species associated with different vegetation types, such as the araneid genera *Mangora* O. Pickard-Cambridge, 1889, *Testudinaria* Taczanowski, 1879, and *Wagneriana* F.O. Pickard-Cambridge, 1904.

Partitioning the sites according to vegetation type proved to be the most appropriate for our data, as it yielded the largest value for all of the parameters investigated: the number of indicator species, proportion of indicator species (in relation to the richness of the category), average IndVal, number of indicator species with their maximum IndVals, and the total sum of the IndVals (Table 3). In contrast, partitioning the sites according to the area size (continuous sites × fragments) produced the least number of indicator species and lowest total IndVal sum.

DISCUSSION

Our results indicate that the diversity in most sites is high and that vegetation structure is much more important to orb-weaving spider communities than the size of the habitat. While the fragments and continuous areas had similar diversity values, all of the important differences observed (diversity measures, abundance, and composition) were related to the vegetation type. Nevertheless, as most of those differences were heavily influenced by a few dominant species, we can also state that overall, the orb-weaving communities from different sampling sites were quite similar, especially in the sites with the same kinds of vegetation.

The influence of the vegetation on the results was expected, as there are several examples in the literature of its importance for spiders. The composition of the community seems to be particularly sensitive to changes in the vegetation structure. Differences in community composition are often found when comparing the fauna of secondary and mature forests or forests under different disturbance regimes (Chen & Tso 2004; Floren & Deeleman-Rheinold 2005; Rego et al. 2007; Kapoor 2008; Cabra-Garcia et al. 2010; Baldissera et al. 2012; Maya-Morales et al. 2012; Raub et al. 2014), which indicates some degree of habitat specificity. Not surprisingly, the ISA results revealed that the partition of the sites by vegetation type had the best fit to our data for all of the parameters available.

Although the biology of Neotropical orb-weaving species is still poorly known, some recent studies from the southern Atlantic Forest with spider species lists from fragments and other kinds of forests may offer the possibility of a comparison with our results. Baldissera et al. (2004) sampled web-spiders in forest interiors, forest edges, and pastures, and the species from the genera *Miagrammopes* O. Pickard-Cambridge, 1870 (Uloboridae) and *Micrathena* were more abundant in the forest interior. A species of *Miagrammopes* also preferred forest interiors over edges in a study on the diversity of spiders in riparian

Table 3.—Results of the indicator species analysis (ISA) for the three different typologies classifications. Categories: M and CM, continuous mature forest sites; S, secondary forest sites; C, continuous forest sites; F and FR, fragments. ISA parameters: S, richness; NIS, number of significant indicator species; %IS, proportion of indicator species/richness; Average IV, average indicator value (IndVal) of the species selected as significant indicators; Max IV; number of species reaching the maximum IndVal; and Total IV, sum of the IndVals for all species selected as significant indicators.

Typology	Category	S	NIS	%IS	Average IV	Max IV	Total IV
Vegetation type	M	38	11	28.9	86.35	9	9.50
	S	42	5	11.9	82.29	3	4.11
	Total	43	16	37.2	85.08	12	13.61
Forest area size	C	43	7	16.3	79.02	3	5.53
	F	40	3	7.5	84.95	2	2.55
	Total	43	10	23.3	80.8	5	8.08
3 categories	CM	38	9	23.7	79.93	1	7.19
	CS	41	2	4.9	67.19	1	1.34
	FR	40	2	5.0	83.55	0	1.67
	Total	43	13	30.2	78.53	2	10.21

forests (Rodrigues et al. 2014), which seems to be in accordance with our findings.

Raub et al. (2014) recently provided a list of spider genera identified as indicators of old-growth and secondary forest based on a study of the Atlantic Forest in southern Brazil, and the genera *Mangora* (Araneidae) and *Miagrammopes* were considered associated with old-growth forests. In our study, one species of each of these genera also showed a preference for mature forests. However, another species of *Mangora* was associated with the secondary forests and fragments, while the distribution of the remaining three species of the genus was more random. This represents further evidence that the response of orb-weaving spiders to the environment may be specific, and that an analysis at higher taxonomic levels may not be accurate enough to characterize habitat preferences.

Further studies are necessary to improve knowledge of the habitat preferences of orb-weaving spider species in the Atlantic Forest and to assess the consistency of the response of species to the environment, which may reveal potential biological indicators. The list presented in this study (Table 2) represents another contribution for this purpose, and the use of ISA or other analyses of habitat preference should be stimulated. The genus *Miagrammopes* could receive more attention, as it appears to be repeatedly associated with mature forest or forest interiors.

In contrast to what was observed for composition, our results suggest that richness is less variable in forested habitats. Indeed, most of the studies cited above reported a similar number of species for the different types of forests being compared (Blanco-Vargas et al. 2003; Chen & Tso 2004; Floren & Deeleman-Rheinold 2005; Baldissera et al. 2008; Cabra-García et al. 2010; Prieto-Benítez & Méndez 2011; Raub et al. 2014; Rodrigues et al. 2014), although there may be some exceptions (Pinkus-Rendón et al. 2006; Maya-Morales et al. 2012). Significant differences in spider richness or diversity usually occur when communities from very different environments are compared (i.e., open field or agro-ecosystems versus forest), and a greater number of species is always found in the structurally more complex habitat (Fowler & Venticinque 1995; Baldissera et al. 2004; Banks et al. 2007). However, unlike most of these studies, we found significant differences in the diversity measures between the treatments, as the orb-weaving communities from the CM sites were less diverse

than those in areas with secondary vegetation, especially the CS sites. Nevertheless, as already mentioned, this result is directly influenced by two dominant species associated with CM sites, and if they are excluded from the analysis the CM and CS sites does not present significant differences anymore, indicating that the structure of the remaining community is similar to that observed for the others categories.

The persistence of a rich and diverse spider community in the fragments signals that habitat reduction and the isolation of the remnants are not affecting the orb-weaving communities in a significant way. The resilience of spiders to these impacts, also recorded earlier in similar studies (Gibb & Hochuli 2002; Major et al. 2006; Kapoor 2008), may be attributable to some characteristics of these animals. Most spiders are generalist predators, and although predators may be more sensitive to environmental disturbance than other trophic groups (Didham et al. 1998; Davies et al. 2000; Ryall & Fahrig 2005), animals with generalist behaviours are usually less affected by fragmentation than species with more specialized requirements (Didham et al. 1996; Tschardt et al. 2002; Fonseca et al. 2009). The small size of the animals may also be an advantage, as small species have smaller space requirements than large species and are thus less susceptible to fragmentation (Henle et al. 2004; Laurance et al. 2011).

Finally, dispersal capacity is considered a key feature for survival in fragments (Tschardt et al. 2002; Moir et al. 2005). Spiders are usually very good dispersers, and several species can cross from hundreds of meters to several kilometres through an act known as ballooning (Bell et al. 2005). Although ballooning has traditionally been observed and studied in open habitats, recent work has demonstrated the ballooning propensity of several forest spider species in temperate forests, especially web-building spiders (Larivée & Buddle 2011).

In a recent study conducted in fragments in southern Brazil, Baldissera et al. (2012) partitioned the compositional variation of the web-spider communities into environmental and spatial components and concluded that the web-spider meta-community was not limited by dispersal in the study landscape. An absence of spatial legacy was also reported for spiders in Spain due to the lack of a significant correlation between their composition and geographic distance (Jiménez-Valverde et al. 2010), as was observed in our results for the fragments. Finally, the

capacity of orb-weaving spiders to colonize new habitats was highlighted in a study of the effects of fragmentation on canopy spiders in Borneo (Floren et al. 2011) because the relative importance of the orb-weaving guild increased in isolated fragments, suggesting they were more successful in the re-colonization process than other spider guilds.

Reviewing our results, we can conclude that the orb-weaving spider communities in Caucaia have not suffered a very large impact from the processes of habitat reduction and isolation occurring in the region. Despite some compositional changes due to the vegetation structure, their richness and diversity remain high, even in the fragments. However, those optimistic statements must be taken with care, and some caveats concerning the universality of our observations should be mentioned.

First, this region of Caucaia represents a best-case scenario with regard to forest conservation at the landscape level. The fragments studied are located within a region that still possesses a 37% forest cover (Uezu et al. 2005) in addition to the RFMG, a large forest patch (10,000 ha). This suggests that the whole landscape may still be functionally connected for a group with good dispersal capacities. Moreover, the fragments sampled are relatively large, ranging from 14 to 175.1 ha. Ribeiro et al. (2009) showed that the remaining Atlantic Forest is scattered in more than 245,000 fragments and that 83.4% of them are smaller than 50 ha, meaning that at least half of the fragments in Caucaia are larger than the vast majority of Atlantic Forest fragments.

Indeed, in the few studies reporting a negative relationship between forest patch size and spider species richness (Miyashita et al. 1998; Floren & Deeleman-Rheindold 2005; Floren et al. 2011), the fragments sampled were considerably smaller than the fragments in Caucaia (< 6.5 ha). Those studies also demonstrated that the presence of large forested areas in the landscape could prevent (or help to recover) the decrease in richness/diversity of spider communities by acting as a source for the fragments' fauna, which highlights the importance of the RFMG in our study area.

The second point is that, as discussed above, orb-weaving spiders seems to be less sensitive to the disturbances related to fragmentation than other groups. For instance, a study conducted at the same sites on harvestmen (Arachnida-Opliones) depicted a much more negative situation, with a sharp decrease in richness from the continuous sites to the fragments, as well as from the sites with mature vegetation to those with secondary vegetation (Bragagnolo et al. 2007). This vulnerability was attributed to the narrow microclimatic requirements and the poor dispersal ability of these animals. Therefore, the same fragments that provide shelter to a diverse spider community may not be adequate for other components of the biota, even for other invertebrate groups.

Finally, one last question must be addressed. The communities from all of the sites sampled were characterized by a large number of rare species (average proportion of singletons and standard deviation was $37.9 \pm 9.6\%$). Although the proportion of singletons in the fragments was similar to that observed in the continuous areas, rare species are considered one of the most sensitive groups to fragmentation and other disturbances, mainly due to their low population numbers (Tscharrntke et al. 2002; Henle et al. 2004). This suggests that, in the longer term,

the persistence of these rare species in the fragments may be threatened.

Nevertheless, as a final conclusion, our study showed that fragments, even as small as 14 ha, still support a rich and diverse orb-weaving spider community and are therefore valuable for the conservation of local biodiversity. The large-sized reserves in the Atlantic Forest, such as RFMG, are very important, for its role as a source, and especially for usually harbouring sites composed of mature vegetation. As observed for many groups (Gibson et al. 2011), our mature forest sites presented a distinct fauna, and in this sense, may be considered irreplaceable for the maintenance of orb-weaving spider diversity in the Atlantic Forest.

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Niche differentiation of two sibling wolf spider species, *Pardosa lugubris* and *Pardosa alacris*, along a canopy openness gradient

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Abstract. Phylogenetic niche conservatism can cause strong interspecific competition among closely related species leading to competitive exclusion from local communities or meta-communities. However, the coexistence of close relatives is often reported. One of the most frequent mechanisms mediating such coexistence is resource partitioning. Here, we investigated the niche differentiation of two sibling spider species, *Pardosa alacris* C.L. Koch, 1833 and *P. lugubris* Walckenaer, 1802, along a canopy openness gradient. We further investigated differences in body size as an additional axis for niche partitioning. We explored niche partitioning along the canopy openness gradient at eight locations. In each afforested location, 60-m-long transects were established consisting of five pitfall traps placed at regular 15-m intervals along the gradient. We measured the body size of individuals of both species collected at the gradient's extremes. We found that the two *Pardosa* species occurred syntopically but had clearly differentiated spatial niches along the canopy openness gradient. *Pardosa lugubris* displayed a preference for closed canopies in dense forest habitats and its abundance gradually decreased as the canopy opened while the opposite was the case for *P. alacris*. The two species also differed in body size. Each species was larger at its preferred gradient extreme than was the other species. The coexistence of the two *Pardosa* species was mediated mainly by spatial niche partitioning. Body size differences may represent another axis for niche partitioning.

Keywords: Coexistence, resource partitioning, spatial niche, body size, Lycosidae

Interspecific competition is considered to be one of the main ecological and evolutionary forces driving species composition and abundances of meta-communities (Chase & Leibold 2003). Classical niche theory predicts that two species with the same niche but having distinct competitive abilities cannot coexist at equilibrium when the resources are limited because the weaker competitor would be excluded (Gause 1934; Hardin 1960; Violle et al. 2011). In other words, each species needs to be limited more by itself than by heterospecifics in order to coexist (Chesson 2000). The most frequently stated mechanism that mediates coexistence is resource partitioning. In such cases, species differ in the resources that they use (e.g., diet, micro-habitat, light). Alternatively, species may differ in the time or place of their use of common resources (Chesson 2000).

Interspecific competition is considered to be rare or weak in spiders (Wise 1993). In closely related spiders, however, it can sometimes be sufficiently strong as to cause niche heterogenization, niche shifts, and even competitive exclusion (e.g., Spiller 1984; Nyffeler et al. 1986; Herberstein 1998; Marshall & Rypstra 1999; Miyashita 2001; Michalko & Pekár 2014). Interspecific competition is also one of the main forces that led to the adaptive radiation of tetragnathids on the Hawaiian Islands (Gillespie 2005).

Resource partitioning has been documented as the mechanism most frequently mediating the coexistence of spiders. With respect to trophic niches, spiders can utilize different prey types or sizes as an effect of different hunting strategies and/or body sizes (Olive 1980; Michalko & Pekár 2015). Temporal niche partitioning can be mediated by different diurnal activities or phenology (Tretzel 1954, 1955; Herberstein &

Elgar 1994; Herberstein 1997). Spatial niche partitioning plays a very important role for spiders and can occur at a very fine scale (Scheidler 1989; Cumming & Wesłowska 2004). Spider assemblages can be vertically or horizontally stratified due to different physiological tolerances, body sizes, and hunting strategies as well as distinct primary defence mechanisms (Richman et al. 1995; Wagner et al. 2003; Cumming & Wesłowska 2004; DeVito et al. 2004).

Pardosa lugubris Walckenaer, 1802 and *P. alacris* C.L. Koch, 1833 (Lycosidae) are sibling species belonging to the *lugubris* species group. The *lugubris* group consists of four species in Central Europe: *P. lugubris*, *P. alacris*, *P. saltans* Töpfer-Hofmann, 2000 and *P. baehrorum* Kronstedt, 1999 (Töpfer-Hofmann et al. 2000). *Pardosa baehrorum* differs from the other three in habitat requirements as it lives in *Salix* grown around the Danube River. The other three species occur in very similar environmental conditions, but *P. saltans* is a western European species, while *P. lugubris* and *P. alacris* are distributed across the whole of Europe (Töpfer-Hofmann et al. 2000; Nentwig et al. 2015). Here, we focus on the latter two species, which are the most common species in deciduous forests in Central and Eastern Europe.

Both of the studied species are very similar and only males can be distinguished reliably on the basis of their morphological characters (Kronstedt 1992; Töpfer-Hofmann et al. 2000). Moreover, these two species often occur sympatrically (Buchar 1999; Buchar & Růžička 2002; Bryja et al. 2005; Košulič & Hula 2011). This raises the important question of how such coexistence is possible. It is hardly possible that the coexistence is mediated by trophic niche partitioning as the diets of

Table 1.—Characteristics of individual study sites located across South Moravia in the Czech Republic and abundances of *Pardosa alacris* and *P. lugubris* in each studied location.

Location	District	Latitude	Longitude	Altitude (m)	Age (years)	<i>P. alacris</i> / <i>P. lugubris</i>
Kuntínov	Břeclav	48°57'12.52"N	16°50'11.65"E	280	100–120	110 / 69
Jesličky	Břeclav	48°56'35.02"N	16°50'19.26"E	245	80–100	65 / 107
Kurdějov	Břeclav	48°58'58.93"N	16°46'4.41"E	305	90–110	18 / 34
Nedánov	Břeclav	48°56'43.20"N	16°50'55.71"E	325	90–110	104 / 76
Vranovice	Brno	48°57'25.56"N	16°35'46.07"E	205	100–120	72 / 57
Milovice	Mikulov	48°50'55.90"N	16°41'34.68"E	215	100–120	63 / 63
Mutěnice	Hodonín	48°52'51.23"N	17° 4'49.07"E	180	110–130	75 / 59
Dubňany	Hodonín	48°53'30.18"N	17° 7'10.71"E	204	110–130	114 / 85

Pardosa spiders generally overlap considerably (Suwa 1986). Moreover, the phenology of *Pardosa* spiders is also very similar (Tretzel 1954; Suwa 1986; Moring & Stewart 1994; Buchar 1999; Nentwig et al. 2015). Available data suggest that *P. lugubris* and *P. alacris* might have differentiated their niches along the light gradient but this idea had never been tested adequately. In the present study, we investigated the two sibling species' microhabitat preferences by establishing 60-m-long transects of pitfall traps along the canopy openness gradient at eight afforested locations. We also measured the sizes of the spiders as an additional dimension for niche partitioning. We hypothesized that the coexistence of *P. lugubris* and *P. alacris* is mediated mainly by niche differentiation along the light gradient.

METHODS

Studied species.—We studied two species of the genus *Pardosa* (Lycosidae): *P. alacris* and *P. lugubris*. They are closely related species of the *lugubris* group and have very similar morphological and ecological characteristics. Only adult males can be distinguished according to the shape of their tegular apophyses and the colour of their cymbium, while adult females of the two species are almost identical (Töpfer-Hofmann & von Helversen 1990; Kronstedt 1992, 1999). The two species also differ in courtship behaviour (Vlček 1995; Töpfer-Hofmann et al. 2000; Chiarle et al. 2013). These species have Eastern Palearctic (*P. alacris*) and Palearctic (*P. lugubris*) distributions (World Spider Catalog 2015). Both of the studied species prefer deciduous forests (except dense beech forest), forest-steppe areas, and forest edges but without any specific environmental characteristics (Buchar & Růžička 2002). They belong to the ground hunters guild and employ a sit-and-move hunting strategy (Samu et al. 2003; Cardoso et al. 2011). *Pardosa alacris* and *P. lugubris* are very common in the Czech Republic and frequently have sympatric occurrence (Buchar 1999; Buchar & Růžička 2002).

Study area.—The study was conducted at eight locations across South Moravia at the northern-most edge of Pannonia in the Břeclav, Brno, Mikulov, and Hodonín districts (Table 1). All study locations were afforested with *Quercus robur*, *Q. petraea*, and *Carpinus betulus* as the main tree species with a mixture of xerothermophilous *Q. pubescens* (*Q. pubescens*–*Q. petraea* plant communities). All study sites had similar altitudes and comparable forest growth ages (former coppiced forests) and are now not actively managed or disturbed by humans (Table 1).

Data collection.—We established 60-m-long transects reflecting the canopy openness gradient in each of eight forest stands. Each trap location differed in light density from the most open

and sparse canopy (forest steppe clearings) to the most closed and dense canopy (dense forest habitat). Each transect consisted of five pitfall traps placed at regular intervals. Each pitfall trap consisted of a plastic cup (9 cm in diameter, 15 cm long) sunk so as to be flush with the soil surface and filled with 4% formaldehyde solution as a killing and preserving agent. In total, there were eight transects and each level of canopy openness was therefore represented eight times. Spiders were collected from 12 May to 14 July 2012. Adult males were used for analyses as they can be easily determined and collected during the early summer period (Kronstedt 1992, 1999). We selected this period because it is when both species reach the peak of their activity in Central Europe and it could be expected that all specimens would be adult (Buchar 1999). All examined material was deposited in the public collection of the Mendel University, Faculty of Forestry and Wood Technology in Brno. The numbers of collected specimens in each studied locality are shown in Table 1 and the raw data can be found in Supplemental 1 (online at <http://dx.doi.org/10.1636/M15-46.s1>).

The light volume gradient was calculated using imaging software (GAP Light Analyzer, version 2.0) for extracting canopy structure and gap light transmission indices from true-colour fisheye photographs. Each fisheye photograph was taken from ground level around the pitfall traps during material collection (14 July 2012). The date was selected because the canopy is the densest during this period.

Statistical analyses.—All analyses were performed within the R environment (R Development Core Team 2015) with R packages “geopack” (Højsgaard et al. 2006) and “nlme” (Pinheiro et al. 2015). Niche overlap (NO) was calculated using kernel density estimation (see Geange et al. 2011). The index has values ranging from 0 (no overlap) to 1 (complete overlap). We performed null modelling to test whether the two *Pardosa* species occupied significantly distinct niches because deviance from 1 may be caused purely by chance (Geange et al. 2011). Because we collected the data at eight locations, we calculated NO for each location separately and present here mean NO and its standard error. We performed 1000 permutations for each location. We employed the Bonferroni correction to minimize the probability of type I errors and we considered the significance level to be $\alpha = 0.00625$ for distinct niche occupancy. To investigate the change in the spatial distribution of the two *Pardosa* species along the canopy openness gradient, we calculated for each location the proportions of individuals that were caught in each trap. The changes in proportions along the openness gradient were studied using generalized

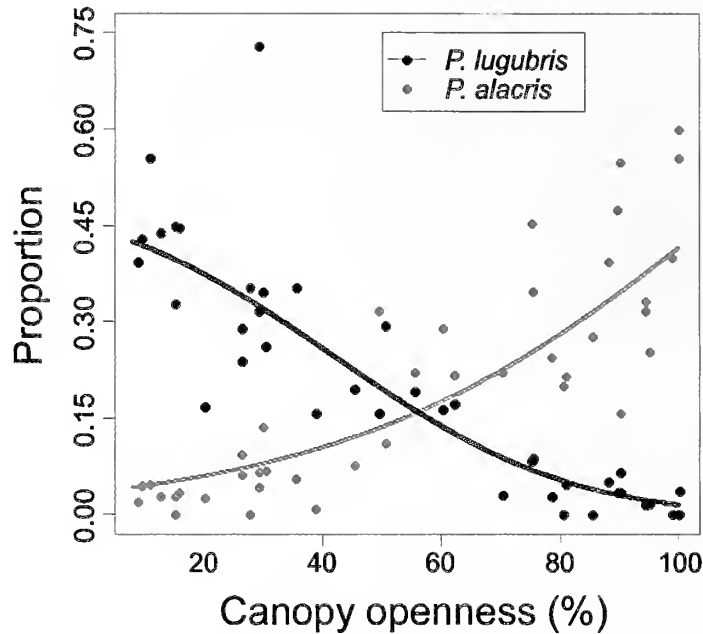


Figure 1.—Relationship between canopy openness and relative abundances of two *Pardosa* species.

estimating equations (GEE) with binomial error structure and logit link (GEE-b) as an extension of generalized linear models for correlated data. GEE was used because the samples were taken at eight different localities (Pekár & Brabec 2012). Because the traps in each locality were situated at regular 15-m intervals, we used the 'AR1' correlation structure where the correlation between each two traps decreases with increasing distance between the traps. The *Pardosa* species' niche width along the canopy openness gradient was estimated using the Shannon diversity index (H), which was calculated for each location separately. Because there were five traps per locality, the index can reach values from 0 to 1.61. The degree of specialisation depends on the choice of a researcher (Futuyma & Moreno 1988). We therefore defined the stenovalence as $H = 0-0.54$, oligovalence as $H = 0.55-1.07$ and euryvalence as $H = 1.08-1.61$. Niche width was compared using linear mixed effects models (LMEs) where species and location acted as fixed and random effects, respectively.

To explore whether the distribution of the two *Pardosa* species could be explained also by interspecific competition, we employed a static approach for calculating the coefficients of competition from census data where species abundances are regressed against one another (Pfister 1995; Fox & Luo 1996). Significant negative slope parameters then indicate (not prove) the presence of interspecific competition. However, as negative relationships may arise from distinct requirements of environmental conditions rather than from interspecific competition, environmental conditions need to be incorporated into the model's predictor (Pfister 1995; Fox & Luo 1996). Species abundances should be also standardized as estimations of competitive coefficients are dependent on population variance and thus could produce statistical artefacts (Fox & Luo 1996). Competition coefficients were studied using LMEs where the standardized abundances of the potential competitor and canopy openness acted as fixed effects and location acted as a random effect. We used the 'varIdent' and

'varExp' variance functions to deal with heteroscedasticity (Pekár & Brabec 2012).

To compare size differences, we selected 8–10 individuals from each species from six locations ($n = 117$). Individuals were selected so that both gradient extremes (open and closed canopy) were represented by 4–5 individuals. Individuals within these groups were selected randomly without replacement. We compared the sizes of the two species using a GEE with gamma error structure and log link (GEE-g) since size can be considered as having the gamma distribution and the data were auto-correlated (Pekár & Brabec 2009, 2012). Species, habitat type according to canopy openness, and the interaction between these two factors acted as the explanatory variables while location acted as a grouping variable. We used an 'exchangeable' correlation structure because the relationship among individuals within the grouping variable was blocked (Pekár & Brabec 2012).

RESULTS

In total, 1171 adult spiders from the *P. lugubris* group were collected. Out of these, 621 specimens belonged to *P. alacris* and 550 specimens belonged to *P. lugubris* (Table 1).

The two *Pardosa* species occupied distinct environmental niches (mean $NO = 0.27$, $SE = 0.11$, null model, 1000 permutations, $P < 0.001$). The proportions of *P. alacris* increased (GEE-b, $\chi_1^2 = 43.9$, $P < 0.001$, Fig. 1) and those of *P. lugubris* decreased (GEE-b, $\chi_1^2 = 196$, $P < 0.001$, Fig. 1) with canopy openness. Both species were euryvalent (*P. alacris*: $H = 1.23$, $SE = 0.06$; *P. lugubris*: $H = 1.18$, $SE = 0.06$) and we were not able to reject the null hypothesis for niche width differences between the two species (LME, $F_{1,7} = 0.4$, $P = 0.55$).

Pardosa lugubris abundance had a significant positive effect on *P. alacris* abundance (LME, $F_{1,29} = 43$, $P < 0.0001$, slope parameter $\beta = 0.8$), but *P. alacris* abundance did not have a significant effect on *P. lugubris* abundance (LME, $F_{1,29} = 2.7$, $P = 0.11$, slope parameter $\beta = 0.9$).

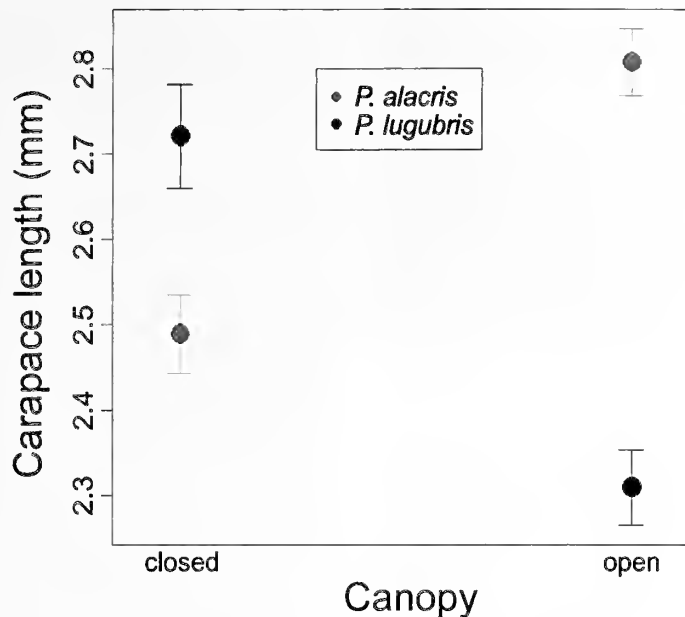


Figure 2.—Size comparison between two *Pardosa* species from two habitats with distinct levels of canopy openness. Points are means and line segments are SE.

There was a significant difference in carapace length between the two species (GEE-g, $\chi^2_1 = 5.6$, $P = 0.0184$, Fig. 2) as well as significant interaction between species and habitat type (GEE-g, $\chi^2_1 = 44.9$, $P < 0.0001$, Fig. 2). *Pardosa alacris* was larger in clearings than it was in forests (contrasts, $P = 0.0002$) while the opposite was true for *P. lugubris* (contrasts, $P < 0.0001$). In clearings, *P. alacris* was larger than *P. lugubris* (contrasts, $P < 0.0001$), while the opposite was true in forests (contrasts, $P < 0.0001$). *Pardosa alacris* from clearings was larger than was *P. lugubris* from forests (contrasts, $P < 0.0001$).

DISCUSSION

In the present study, we studied the spatial niche partitioning between two sibling *Pardosa* species, *P. lugubris* and *P. alacris*. We found that even though these lycosids are euryvalent and occur syntopically, they had clearly distinguished niches along the canopy openness gradient. The relative abundance of *P. alacris* gradually increased with openness while the opposite was true for *P. lugubris*. Thus, niche differentiation along the canopy openness gradient mediated the coexistence of these two species in meta-communities. Horizontal stratification is a frequently reported mode of niche differentiation among wolf spiders and even *Pardosa* species. For example, Suwa (1986) found that four *Pardosa* species occurring in Hokkaido in Japan were clearly separated in space. Species with similar habitat preferences are separated geographically and occur allotopically in overlapping areas. In contrast, species with distinct habitat preferences evince high geographical overlap. Moring & Stewart (1994) found that six *Pardosa* species that occur syntopically had clearly segregated spatial niches among five distinct habitats that represented successional plant zones along a stream.

It is unlikely that current interspecific competition is the process behind the distribution pattern of *P. alacris* and *P. lugubris* since no significant negative relationship between their abundances

was observed after taking environmental factors into account. Instead, there was a positive relationship between their abundances when *P. alacris* abundance acted as an explanatory variable. This positive relationship suggests that these species respond similarly to some landscape features. The distribution pattern is therefore most likely influenced by distinct physiological tolerances, but this assumption needs to be tested.

There was an interesting pattern in size distribution between the two *Pardosa* species. Conspecific males from suitable conditions were larger than were heterospecifics for which the conditions were less suitable. *P. lugubris* males were larger than *P. alacris* males under closed canopies, while *P. alacris* males were larger under open canopy conditions. This pattern may have been caused by several not mutually exclusive factors. Smaller or less competitively capable males might have been pushed by intraspecific interactions (competition for mates, food, etc.) into less favourable conditions. Interspecific interactions might also have contributed to this pattern. Nevertheless, the size difference between the two lycosids could also represent an additional axis for niche differentiation, e.g., trophic niche. Although the *Pardosa* spiders utilize very similar prey types, body size differences can lead to trophic niche partitioning with respect to prey size (Suwa 1986; Michalko & Pekár 2015).

In summary, we found that the coexistence of the two sibling species, *Pardosa alacris* and *P. lugubris*, is mediated by spatial niche separation. Although both species can occur syntopically, *P. alacris* preferred open canopies while *P. lugubris* preferred closed canopy habitats. The distribution of the two *Pardosa* species is most probably caused by distinct requirements for environmental conditions. The two lycosid spiders differed in body sizes, which could represent an additional axis for niche partitioning. Nevertheless, it should be noted that our results have a rather local relevance and apply only to the studied region. The distribution pattern of the *Pardosa* species from the *lugubris* group can differ among various regions. Therefore,

further study is needed to explore how the overall coexistence of these species is mediated at their common distribution range.

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Laboratory refugia preferences of the brown widow spider, *Latrodectus geometricus* (Araneae: Theridiidae)

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Abstract. During collecting episodes in urban southern California, brown widow spiders, *Latrodectus geometricus* C. L. Koch, 1841, were observed to reside most often in specific locations such as acute angles within patio furniture or on the underside of railings. In this laboratory study, we tested several variables to determine which factors brown widow females may use for web site selection. All tests were conducted with corrugated cardboard refugia in enclosed dome-shaped insect cages. Brown widows were found to significantly prefer: 1) 30° angles compared to 60° or 90° angles, 2) cavities of 75 and 100 mm depth compared to 25 or 50 mm depth, 3) cavities lined with single-faced corrugated cardboard compared to smooth surfaces, and 4) refuges that had previously housed a brown widow compared to similar but previously unoccupied refugia. These data provide unusually detailed information about microhabitat selection by an urban pest, with possible implications for the pest control industry for eradication or for home owners to avoid envenomations.

Keywords: Synanthropy, urban entomology, urban pest

The brown widow spider, *Latrodectus geometricus* C. L. Koch, 1841, is non-native to North America (Garb et al. 2004). Its first North American establishment was in Florida in 1935 (Pearson 1936) where it still exists. In the early 21st century, *L. geometricus* greatly expanded its range, colonizing the southeastern Gulf Coast states (Brown et al. 2008) and southern California (Vineent et al. 2008). In southern California and possibly elsewhere, the brown widow spider has become a major nuisance pest such that it is now one of the most common spiders found around homes and parks in urban areas.

Field collections by Vetter et al. (2012a,b) enabled the authors to reliably identify locations where brown widows would be found. Spiders appeared to prefer refuges on the undersides of solid horizontal cover with acute angles being favored over obtuse angles (Fig. 1). This predilection for tight spaces has allowed the brown widow to preferentially infest the undersides of inexpensive plastic patio furniture, which has many crisscrossing, vertical supports to strengthen seats or tables, offering a multitude of refuge sites. The undersides of curved rims on the margins of plant pots and recessed handles on plastic trash cans are also preferred brown widow habit. Additionally, brown widows were plentiful under wooden picnic tables with the legs producing a right angle attachment to the tabletop but not so common under smooth concrete tables with gently curved leg supports. Situations like these put brown widow females in close association with humans, possibly increasing the potential of accidental envenomation.

Studies of refugia preferences of pests such as cockroaches (Koehler et al. 1994) have aided in developing control measures to construct traps to manage populations. In the laboratory, Vetter & Rust (2008) showed that reclusive spiders (*Loxosceles reclusa* Gertsch & Mulaik, 1940, *L. laeta* (Nicolet, 1849)) preferred vertically oriented refugia rather than horizontal ones and preferred refugia containing silk of previous congeners over refugia without silk, but had no definitive preference for cavity size among the sizes that were tested. Strope (2010)

offered various refugia placed in leaf litter where *Loxosceles gaucho* Gertsch, 1967 showed preference for refugia with acute angular internal walls rather than walls that were obtuse, right-angled or smoothly curved. Carrel (2015) demonstrated in the laboratory that spiderlings of *Kukulcania hibernalis* (Hentz, 1842) shifted to different sized holes drilled in wooden dowels as they grew over a 24-week period. Here we examine the preferences of female brown widow spiders for refugia characteristics in laboratory assays. Although this research may lend itself to practical use for pest control, it was performed primarily to determine whether brown widows assess structures for refuge suitability and show preference for some locations over others, and if so, whether general selection criteria could be identified.

METHODS

Spiders.—Brown widow spiders were collected from urban areas in spring and early summer in 2014 and 2015 (Orange County) and the city of Riverside (Riverside County), both in southern California, USA. Spiders were maintained individually in 163-ml plastic food cups (soufflé cups, First Street, Amerifoods Trading Co., Los Angeles, California) with plastic lids. A Y-shaped piece of fiberboard (2 mm wide with two legs of 35 mm length and one leg of 52 mm length) was placed inside the cup to act as a substrate for web attachment. Spiders were offered a mealworm (*Tenebrio molitor*) every 5 to 14 days, an amount that was sufficient to prevent spider death from starvation. We only tested subadult or mature females for refuge preferences.

General assay specifications.—Spiders were individually tested in insect cages (Fig. 2; Bug Dorm, BD2120F MegaView Science, Taiwan). The spiders were tested in the University of California Riverside Insectary and Quarantine Facility in a room with a south-facing window covered with unwaxed brown paper. The window did not receive direct sunlight. Average room temperature was 24.9 ± 0.4 °C and relative humidity was 39.0 ± 7.7 %. Lights were turned on when setting up an experiment, releasing spiders, checking spider position or



Figure 1.—A railing at a condominium complex showing the preference of the brown widow for acute vs. obtuse angles. In this picture, at least 17 egg sacs were collected from within the acute angle.

cleaning out cages for the next set of trials. A small piece of cardboard was placed on the bottom of the insect cage (to protect the thin plastic bottom) and a wooden dowel was placed on the cardboard such that it held cardboard refugia being tested at the top of the dome by wedging it in place (Fig. 2). This



Figure 2.—The insect cage (60 × 60 × 65 cm) with a wooden dowel positioned on a piece of cardboard at the bottom which forcibly wedges the test cardboard at the top, flush against the mesh such that the brown widow could not choose the space at the top of the insect cage. To initiate the assay, a cup containing the spider was uncapped and placed at the base of the dowel, near twilight.

tight-fitting design also prevented spiders from seeking refuge in the small space above the test cardboard shape. Therefore, when deployed, the open portion of the cardboard refugia faced downward and the spider could elect to crawl up the dowel and use one of three sites for refugia: the cardboard cavity, the vertical folds of the insect cage or the interface of the upper cardboard and mesh. Experimental cardboard refugia were constructed with white glue (Elmer's School Glue, Elmer's Products, Inc., Westerville, Ohio). In addition, when pieces of cardboard refugia required stapling, the cardboard was stapled from the inside of the strip so the piercing ends of metal were outside the cavity and then flattened with pliers in case the staples influenced site selection; this preference was examined in the first assay.

In all tests except the second pre-occupancy assay (see below), spiders were released around twilight by uncapping and placing the open cup near the base of the dowel without forcing the spider out of the cup. This allowed spiders to leave the cup on their own accord during their nocturnal activity period. In most assays, eight spiders were tested at one time, if there were sufficient numbers. To minimize disturbance, the location of the spider in the cardboard was detected using a flashlight and a telescoping mirror. Both the spiders and the cardboard refugia were used only once in a particular assay even if spiders did not take refuge within the test structure. When an individual assay was terminated, the wooden dowels and inside walls of the insect cages were wiped with a dry paper towel to remove noticeable silk.

The assays are presented here sequentially. However, in order to maximize the use of spiders before senescence reduced the pool of specimens, often the different categories of assays were run consecutively, building up sample size contemporaneously, as opposed to completing one assay before starting the next one. Therefore, information determined in one assay, which might appear useful in the design of subsequent assays, often was not available for us to incorporate at the time of initial assay undertaking.

Angle preference assay.—A 25-mm × 500-mm strip of corrugated cardboard was configured into a geometric shape with leg lengths of approximately 9, 15.3 and 18 cm, and a small length of overlap for stapling, creating a triangle with 30°, 60° and 90° angles (Fig. 3). The stapled overlapping end was distributed evenly among angles in case staple texture influenced refugia choice. The triangle was glued to a 22.5-cm square piece of corrugated cardboard. One angle was placed pointing toward one edge and the other two angles toward the side edges in equal numbers such that north and south angles were equally represented in the samples tested for each of the three angle categories. The cardboard square with the triangle facing downward was wedged against the roof of the insect cage with a wooden dowel (19-mm diameter × 56-cm length) (Fig. 2).

Cages were checked daily for three consecutive days to determine the location of the spider, although because of site fidelity the first day's position was sufficient for analysis. Data were collected on whether a spider chose an angle in the cardboard triangle, which angle was chosen, and whether the chosen angle had staples. In this assay, 48 spiders were tested.

Depth preference and site fidelity assay.—In this assay, spiders were offered a cardboard box with four compartments of

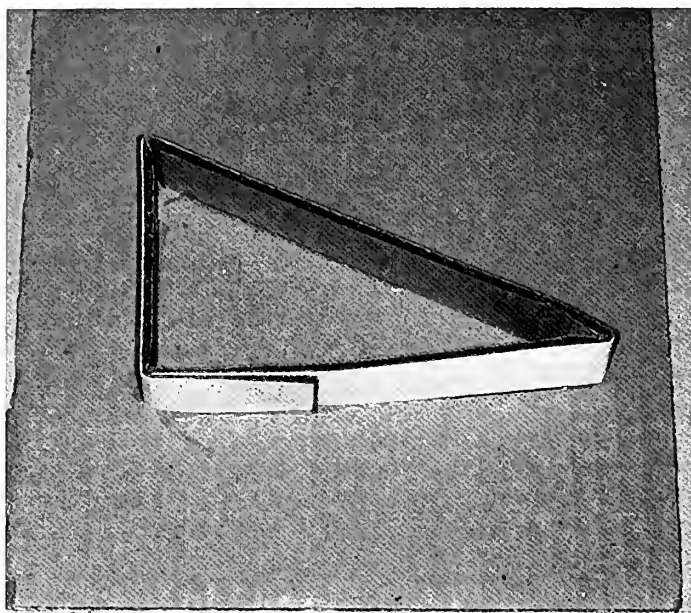


Figure 3.—The triangle glued to a cardboard square. The square was inverted when put in place with the opening of the triangle facing downward.

depths of 25, 50, 75 and 100 mm (Fig. 4). Cardboard shipping boxes for fluorescent light bulbs were obtained from a local lighting company. The internal cross-sectional dimensions of the boxes were approximately 140 × 185 mm. Boxes were cut into 100 mm sections with a table saw, a piece of cardboard was glued to the bottom and a cardboard cross was inserted in the box to form four subequal cavities of approximately 60-mm width × 90-mm length. U-shaped cardboard inserts were then individually cut and slid into cavities to produce depths of 25, 50, and 75 mm (Fig. 4) and were held in place by snugness of fit without glue. The 100-mm depth cavity required no insert. The order of the depths was randomized starting in the lower left hand corner, moving clockwise in order

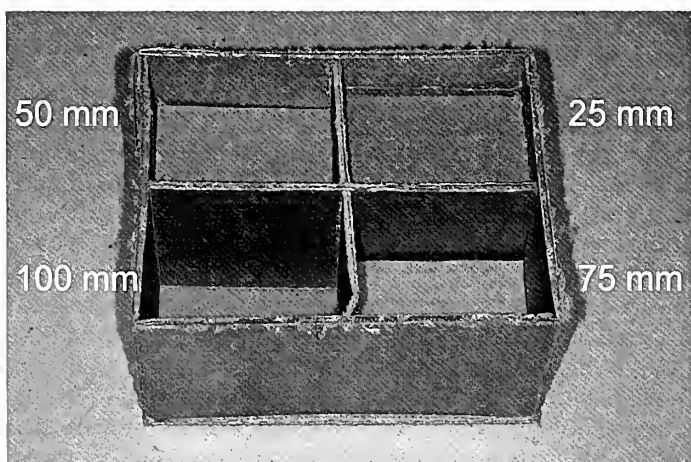


Figure 4.—A 100 mm deep box, separated into four sections with cardboard inserts placed in three of the sections of depths of 25, 50 and 75 mm. Only the flat bottom of the inverted U-shaped insert is visible. The box was inverted when put in place with the opening of the box facing downward.

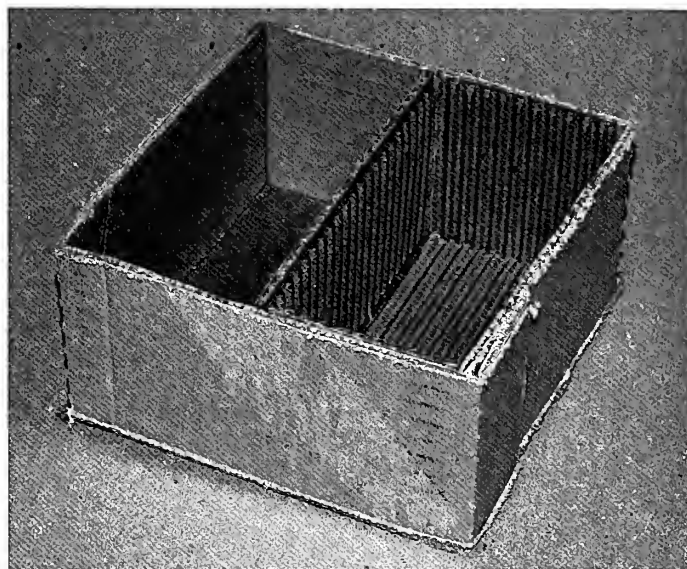


Figure 5.—A 75 mm deep box, bisected with one cavity lined with single-faced corrugated cardboard. The box was inverted when put in place with the opening of the box facing downward.

that one depth would have randomly chosen depth cavities as adjacent neighbors from box to box. To effect the test, the box was secured against the roof of the insect cage with a wooden dowel (19-mm diameter × 46-cm length) which was placed at the middle of the box resting on the cross-section of the cross insert so that the spider had equal chance of accessing any of the four cavities. In this assay, 36 spiders were tested.

To test site fidelity more thoroughly, in this depth assay spiders were checked every day for a week to determine if any changes in daily position occurred, either from one cavity to another or from the initial chosen corner to another within a cavity.

Surface roughness assay.—We tested whether brown widows preferred a rough or smooth cardboard surface on the inside of their cavity. Fluorescent lighting boxes with a 150-mm square cross section were cut into 75-mm sections and a cardboard bottom was glued to each. A cardboard divider created two

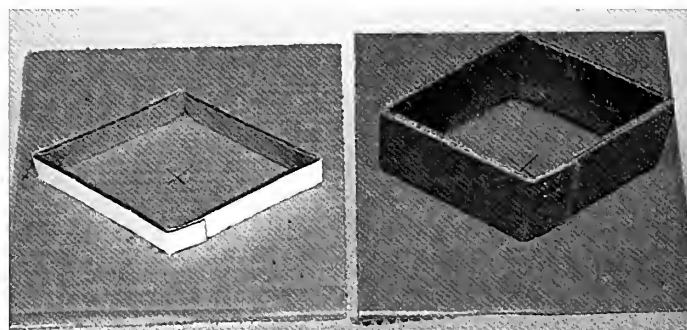


Figure 6.—The parallelogram of 60° and 120° angles and 25-mm height in the first previous-occupancy assay (left), which was placed at the top of the insect cage. The squares were inverted when put in place with the opening of the parallelogram facing downward. In the second assay, the walls of the parallelogram were increased to 75 mm (right) and the parallelogram was placed directly over the cup with the spider, ensuring that the spider would only make a choice within the parallelogram interior.

subequal cavities of 70-mm width \times 140-mm length. Pieces of single-faced B-flute corrugated cardboard (2-mm high corrugations at 11 corrugations per 7 cm of length) were used to line the bottom and walls of one of the cavities with the corrugations facing outward, creating a rugose surface (Fig. 5). The other cavity was left unaltered with smooth walls and bottom. The completed paired cavities were wedged into the roof of the insect cage with a wooden dowel (19-mm diameter \times 49-cm length). The cavity with corrugations was equally oriented at the four cardinal compass points successively between runs. Spiders were checked every day for three days. In this assay, 48 spiders were tested.

Pre-occupancy assay #1.—Spiders were tested to see if prior occupancy by another brown widow would influence choice. A 25-mm wide strip of cardboard was formed into a parallelogram with two 60° and two 120° angles. Staples were placed in one of the 120° angles such that the two 60° angles would be identical (Fig. 6, left). The length of each leg was 11.5 cm. Results from earlier tests indicated that the spiders would preferentially choose one of the 60° angles. The parallelogram was arranged such that the 60° angles alternated between north-south or east-west orientation in successive runs on top of the same dowel used in the angle preference assay. In order for a cardboard refugium to be used by the second cohort of spiders, the first occupant needed to be found in the same location for each of the 7 days such that only one location should show signs of occupancy. Refugia were checked for 7 consecutive days with no spider in a refugium switching position from the first day. On the morning of the seventh day, spiders were gently forced out with a probe, while attempting to minimally disrupt the web structure. The cardboard refuge was repositioned in the same orientation as in the first portion of the assay. A second set of spiders was then released into evacuated test arenas at dusk. The position of the second cohort of spiders was recorded for 7 consecutive days. In this portion of the assay, 32 spiders were tested in the first cohort with 15 being tested in the second cohort.

Preoccupancy assay #2.—The premise of the above experiment was repeated, however, to increase the percentage of spiders choosing a location in the cardboard refugium in this assay in comparison to the previous assay, the width of the strip of cardboard was increased from 25 to 75 mm (Fig. 6, right) and instead of having the cardboard refugia at the top of a dowel, the cup with the spider was opened and the cardboard refugia was placed directly over the cup located on the floor of the insect cage. This allowed the spider to leave the 60-mm tall cup and choose an angle inside the 75-mm high refugium. The spiders were checked each day for the following week, forced out on the seventh morning, their location noted, and the refugium was rotated 180° degrees. Rotation helped control for unknown environmental factors other than prior occupancy that may affect choice. The cups containing the second set of spiders were then uncapped and the cardboard refugia placed over open cups, once again forcing the spider to make a choice inside the refugia. Twenty spiders were initially tested with 18 choosing an angle (16 always in the same location in a 60° angle each day, qualifying these for the second half of the assay) to which a second cohort of 16 spiders was subsequently exposed. The second cohort of spiders was checked for choice the next day.

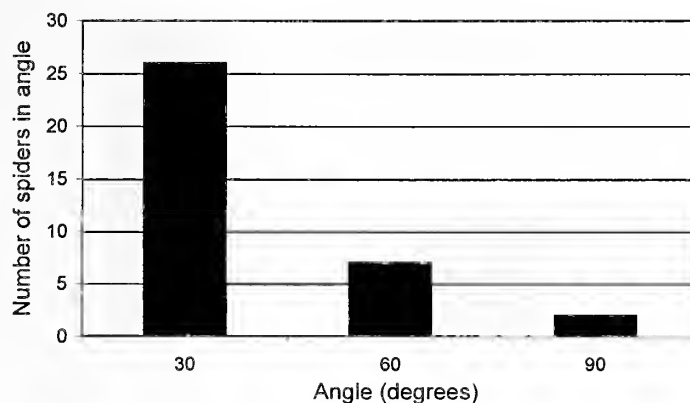


Figure 7.—Preference of brown widows for angles of 30°, 60° and 90°.

Statistics.—A Chi-square Goodness of Fit Test at the 0.05 level of significance was performed on all assays to determine refugia preferences. Data were analyzed using the statistical package SAS.

RESULTS

Angle preference assay.—In the angle preference assay, 35 of the 48 brown widows chose a refugium in the cardboard triangle with a significant preference for 30° angles ($\chi^2 = 27.49$, $df = 2$, $P < 0.001$; Fig. 7); the remaining 13 settled elsewhere in the insect cage. The angle with the staple was chosen 13 of 35 times (37.1%) indicating that presence of the staple had no influence on the choice of angle ($\chi^2 = 0.22$, $df = 1$, $P = 0.63$).

Depth preference and site fidelity assay.—In the depth preference assay, 28 of 36 spiders chose one of the cavities in the cardboard box with the remaining eight choosing folds of the insect cage. Spiders choosing cavities preferred a depth of 75 and 100 mm depth over 25- and 50-mm depths ($\chi^2 = 17.43$, $df = 3$, $P = 0.001$) (Fig. 8).

In the site fidelity assessment, all 28 spiders that chose a cavity in the cardboard, remained in the same depth and even in the same corner within that cavity for each of the seven days that they were checked. Likewise, the eight spiders that chose a location other than inside the cardboard box occupied the same location every day that they were checked.

Surface roughness assay.—In the surface roughness assay, 48 spiders were tested of which 37 chose a cavity in the cardboard. The cavity lined with corrugations was preferred nearly 3:1

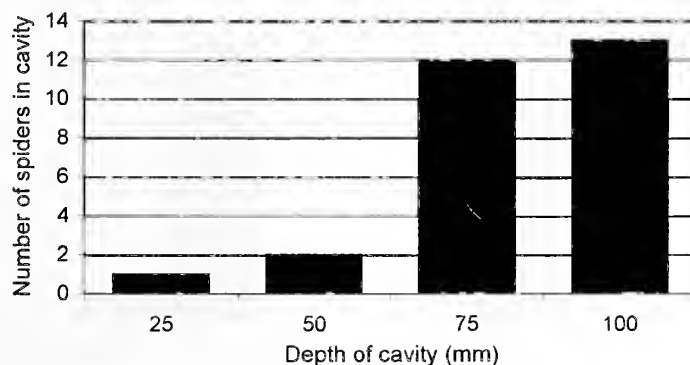


Figure 8.—Preference of brown widows for depths of 25, 50, 75 and 100 mm.

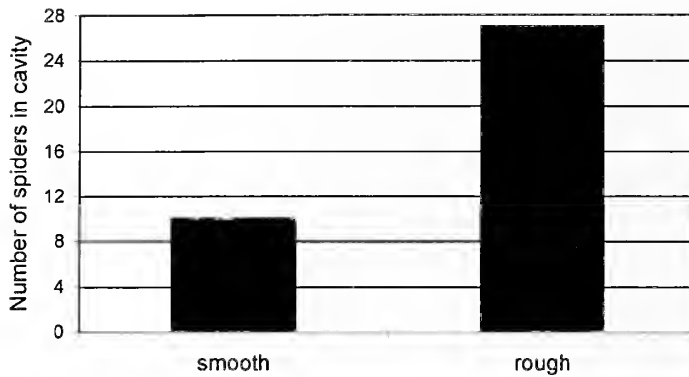


Figure 9.—Preference of brown widows for rough vs. smooth surfaced cardboard.

over the smooth-walled cavity ($\chi^2 = 7.81$, $df = 1$, $P = 0.005$; Fig. 9).

Pre-occupancy assay #1.—Of the 32 spiders of the first cohort that were tested, only 15 chose the cardboard refugia with 13 choosing 60° angles and two choosing 120° angles ($\chi^2 = 8.07$, $df = 1$, $P = 0.005$). Regarding the 13 pre-occupied 60° angles, the second cohort of spiders chose the pre-occupied angle 12 times and one spider chose the uninhabited 60° angle, a significant difference ($\chi^2 = 9.31$, $df = 1$, $P = 0.002$) (Fig. 10). Of the remaining two choices where the 120° angles were chosen by the first set of spiders, the second cohort chose 60° angles instead of choosing the pre-occupied obtuse angle. Even though only 15 of 32 spiders (47%) chose cardboard refugia in the first round, all 15 spiders in the second round that were tested on previously-inhabited refugia chose one of the angles in the cardboard.

Pre-occupancy assay #2.—In the second assay of 20 spiders, 17 chose a 60° angle, one chose a 120° angle ($\chi^2 = 14.22$, $df = 1$, $P < 0.001$), and two did not leave their cups. Of the 17 that chose a 60° angle, 16 were in the same location for each of the seven days that they were checked. One spider moved from one 60° angle to the other on day 2 and then moved back on day 7; this refugium was not tested in the second cohort release nor was the refugium where the 120° angle was chosen. The second cohort of 16 spiders chose the previously occupied 60° angle 14 times with one spider choosing the

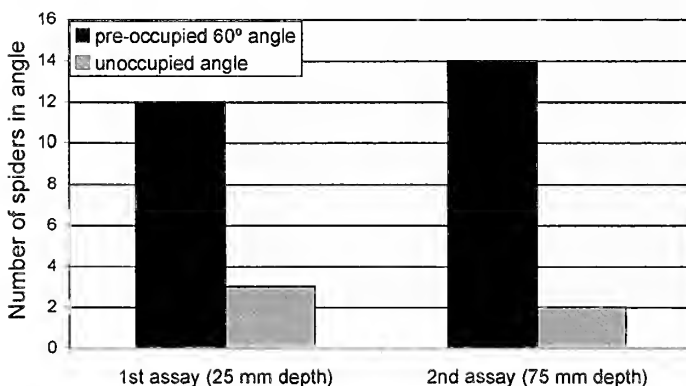


Figure 10.—Preference of brown widows for previously occupied refugia.

unoccupied 60° angle and another choosing a 120° angle ($\chi^2 = 9.0$, $df = 1$, $P = 0.003$; Fig. 10).

DISCUSSION

Brown widows demonstrated a distinct preference for refugia with acute angles, deep cavities, rough surfaces and previously occupied by a conspecific. Experimental spiders appeared to examine their environment and actively made decisions as to where to establish refugia. An extensive body of research exists regarding habitat selection in spiders, mostly performed in natural settings, examining the biotic and abiotic factors that spiders find attractive for web sites (e.g., reviews in Janetos 1986 and Gillespie 1986). Most of these studies involved localized ecological aspects such as complexity or height of vegetation, proximity to surface water, or availability of silk attachment sites (e.g., Gillespie 1987; Herberstein 1997). Some studies involved manipulation of the environment by removal or enhancement of vegetation (e.g., McNett & Rypstra 2000) whereas others introduced experimental structures into an environment to investigate how spiders would use physical enhancements (e.g., Robinson 1981; Stropa 2010). Fischer & Vasconcellos-Neto (2005) reported on *Loxosceles intermedia* Mello-Leitão, 1934 and *L. laeta* in synanthropic situations in Brazil and found the spiders had a preference for placing their retreats on rough surfaces such as paper, wood and construction materials rather than plastic or metal. Riechert (1976) reported on the importance of various natural environmental features for web site acceptance of the desert spider, *Agelenopsis aperta* (Gertsch, 1934).

In this current study, the brown widow's choice of the smallest acute angle in the triangle assay reflects what is observed in urban environments. However, in the absence of easily accessible alternatives, brown widows will exploit 90° angles on the undersides of picnic tables and other locations because a 90° angle is a common by-product of human-built structures. Brown widows typically make long, thin conical retreats that would fit snugly into a 30° angle. The choice of deeper cavities was somewhat unexpected because, in collections for earlier studies (Vetter et al. 2012a, b), great variation in web sites was noticed in the field, including some that were extremely exposed such as the underside of wrought iron railings where, when backlit by the sun, the entire silhouette of the spider could be detected (R. S. V., pers. obs.). Rough surfaces might be easier for fastening webbing and thus provide a stronger conical retreat. However, spiders easily attached silk to smooth surfaces in the other assays. The attractiveness of sites previously occupied by conspecifics in brown widows parallels similar behavior in other web-spinning spider species (Leborgne & Pasquet 1987; Hodge & Storfer-Isser 1997; Vetter & Rust 2008). Spider silks are proteins with metabolic costs associated with their production (Craig et al. 1999); some spiders, such as araneids, reingest their webs (Janetos 1986). Such a behavioral preference for pre-occupied retreats should enable conservation of silk proteins. This behavior also may explain the few occasions when Vetter et al. (2012a, b) discovered up to 35 brown widow egg sacs in one location. The extreme number of egg sacs is likely the work of more than one female, although brown widow females can oviposit more than 20 egg sacs in a lifetime (Bouillon & Lekie 1961; Heeres 1991).

Brown widows are synanthropic as they are often found around homes and urban areas as opposed to more natural environments (Baerg 1954; Lamoral 1968; Vetter et al. 2012b; Marie & Vetter 2015). Our findings might be useful information for the pest control industry. Knowing where brown widows may be more likely to choose habitats for making a retreat could focus pest management efforts. Our information on web site preferences may also reduce envenomation risk for the general public by increasing awareness of the spider's presence on patio furniture or other yard structures (e.g., fence railings, looped door handles).

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Phenology of the Sonoran desert-endemic populations of *Homalonychus selenopoides* (Araneae: Homalonychidae)

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Abstract. *Homalonychus selenopoides* Marx, 1891 is endemic to the Sonoran Desert in Mexico and the southwestern United States. Despite its wide distribution in the desert, nothing is known about its phenology. For this study, we collected 464 immature and adult spiders and 67 egg sacs during late 2007 and throughout 2008. In the field, the life cycle is annual and phenology is markedly seasonal. Egg sacs and 2nd instar spiderlings occur in the field only from April–July, juveniles throughout the year, penultimate spiders from September–April, and adults of both sexes from November–April. Temperature and juvenile frequency were positively correlated ($r^2 = 0.9374$; $P < 0.0001$), but temperature and adult frequency were negatively correlated ($r^2 = -0.8904$; $P < 0.0001$). These spiders are active in winter and this is when the adults mature and mate. The phenology model proposed fits the winter-mature pattern of Aitchison (1984). Reproductive diapause governs the seasonal development of this species, which is possibly semelparous and univoltine.

Keywords: Homalonychids, seasonality, winter-mature, winter-active, reproductive diapause

In temperate zones, significant temporal changes occur in environmental conditions during the year. These seasonal changes in abiotic and biotic factors are periodic and predictable, and individuals of any population must adapt their life cycles to these changes (Roff 1992). Thus, periodicity of seasonally changing environments can synchronize the animals' developmental stages or activities to favorable times of the year (Schaefer 1977), or to help them escape from an unfavorable time in the same year (Krebs 1978). Spiders, similar to other arthropods, also coordinate their development with shifts in the environment using cues such as photoperiod, temperature, and availability or quality of food.

Phenology is the study of seasonal changes in population composition in relation to their environment (Jackson 1978). It helps us to understand how life history characteristics are shaped by natural selection. Although since the 1970s there has been increasing interest in the phenology of spiders (Jackson 1978), the life history and lifespan of few spider species have been investigated (Hallas 1989; Foelix 1996; Rybak 2007). The phenology of spiders has been documented in both field and laboratory studies, but phenology studies conducted in the lab poorly approximate natural conditions (Toft 1976). A comprehensive picture of the life history of a spider species in its natural habitat should include monitoring its activity throughout the year (Aitchison 1984).

Homalonychus selenopoides Marx, 1891 is a spider endemic to the arid regions of the North American southwest, including the desert plains of Sonora, México (Roth 1984; Crews & Hedin 2006). Recently, there has been great interest in determining different aspects of behavior (Domínguez & Jiménez 2005, Alvarado-Castro & Jiménez 2011), morphology (Duncan et al. 2007; Crews 2009), systematics, and phylogenetics (Crews & Hedin 2006; Miller et al. 2010) of homalonychid spiders, but there are still several aspects that are worthy of study.

The phylogenetic position of Homalonychidae remains elusive; a relationship with Lycosoidea (Domínguez & Jiménez 2005) and Zodariioidea (Miller et al. 2010) has been proposed,

among others. The morphological and biogeographical distinctiveness of these spiders suggest a long history of phylogenetic isolation (Crews & Hedin 2006). They use sand to powder their bodies, to build their eggsacs, and to build threads of silk and sand (Alvarado-Castro & Jiménez 2011). All of these features, besides the spiders' existence confined almost exclusively to the desert, make these spiders interesting subjects of study.

Here we describe the phenology of *H. selenopoides* in the field and provide hypotheses about its functional role in survivorship as well as the evolutionary significance of the natural history of these enigmatic spiders. We provide information about the strong seasonality of the different development stages; we also provide hypotheses about the relationship of these stages to the climate and biotic factors. Additionally, our results can be a solid basis for adequate planning of laboratory and field studies of this species.

METHODS

Study organism.—*Homalonychus* Marx, 1891 is a monotypic genus, the only representative of the Homalonychidae, and it includes only two species, *H. selenopoides* and *H. theologus* Chamberlin, 1924 (Roth 1984; Crews & Hedin 2006). The first species ranges in the Sonoran Desert from central Sonora northward to the Grand Canyon, including southwestern Arizona, southern Nevada, and southern California. A few populations have also been observed west of the Colorado River in the northern Mojave Desert. *Homalonychus theologus* ranges from the tip of the Baja California Peninsula northward to southwestern California and the southern end of Nevada (Roth 1984; Crews & Hedin 2006). We studied a population of *H. selenopoides* located at the southern tip of its distribution (Fig. 1). Aspects of taxonomy, distribution, morphology, habitat, and behavior of *Homalonychus* are summarized by Alvarado-Castro & Jiménez (2011).

Study area.—We collected spiders and egg sacs in the channel bed and sloping sides of the El Macapul arroyo and

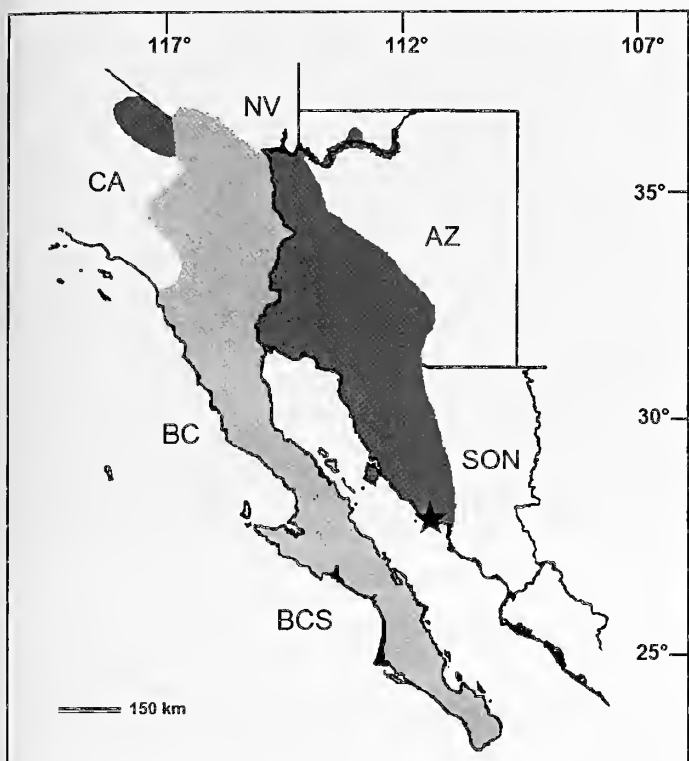


Figure 1.—Regional map, including distribution of *Homalonychus selenopoides* (dark gray) and *Homalonychus theologus* (light gray) (adapted from Crews & Hedin 2006). The collection area of *H. selenopoides* is also indicated (★). Abbreviations: SON, Sonora; BCS, Baja California Sur; BC, Baja California; AZ, Arizona; CA, California; NV, Nevada.

surrounding area (El Macapul) (27.9833° N, 111.0377° W and 28.02° N, 111.0508° W), including the entrance to Cañon de Nacapule, both north of San Carlos, Sonora, in the southern extreme of the Sierra El Aguaje. Climate, vegetation, and soil of the study area have been described in Alvarado-Castro & Jiménez (2011). We also collected some spiders inside the Cañon de Nacapule (Nacapule) (28.0155° N, 111.0541° W) and near Estero El Soldado (El Soldado) (27.9663° N, 110.9925° W) (Fig. 2).

We took weather information from CONAGUA station # 76256 (Observatorio “La Bola”), 23 km ESE from the study site, in Empalme, Sonora (27.9638° N, 110.8083° W). We used this information to construct a climate graph of the study region (1980–2008) (Fig. 3) for comparison with field data about phenology and to analyze the relationship between temperature and abundance of juveniles and adults.

Terminology.—We used the term “juvenile” to describe spiders from the 3rd instar to the antepenultimate stage prior to the maturing molt and the term “penultimate” to refer to the penultimate instar prior to the maturing molt. Because the mating and oviposition periods occur in different seasons in *H. selenopoides*, we refer to the reproductive season as when oviposition occurs (Schaefer 1977).

Determination of the developmental stage.—To verify the developmental stages of the spiders found in the field, we transported all of them to the laboratory at the Universidad Estatal de Sonora in Hermosillo, Sonora. Based on external morphology, we identified the following developmental stages

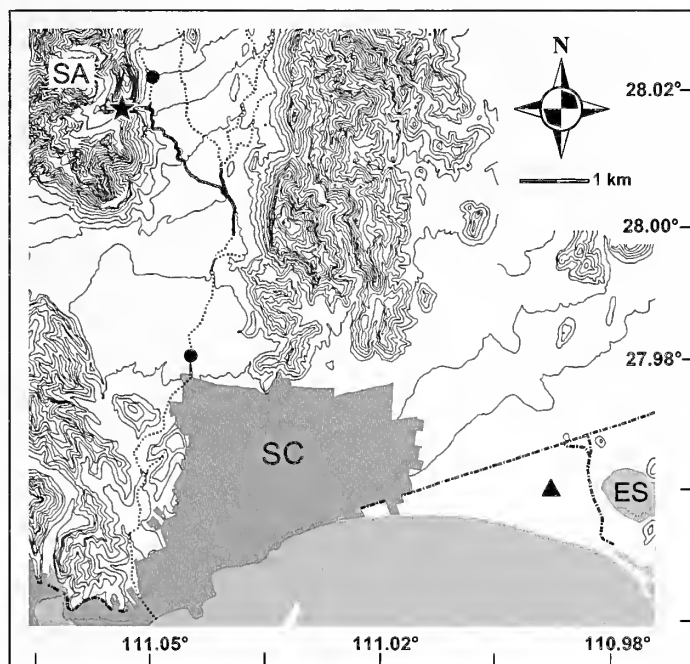


Figure 2.—Study area showing the collection sites of *Homalonychus selenopoides* specimens: Nacapule (★), El Soldado (▲) and El Macapul (section of arroyo highlighted with black). Two sites with unique collections are also indicated (●). Abbreviations: SC, San Carlos; SA, Sierra el Aguaje; ES, Estero el Soldado.

of the specimens: 2nd instar spiderlings, juveniles, penultimate spiders, and adults. The 2nd instar spiderlings were identified by their size; penultimate males were identified by the swollen pedipalp tarsi; and the penultimate females by the presence of epigynal lobes, which although poorly developed, were microscopically visible. Also, we identified the penultimate females easily when they matured after one molt in the lab. For this, the spiders were reared as described by Alvarado-Castro & Jiménez (2011).

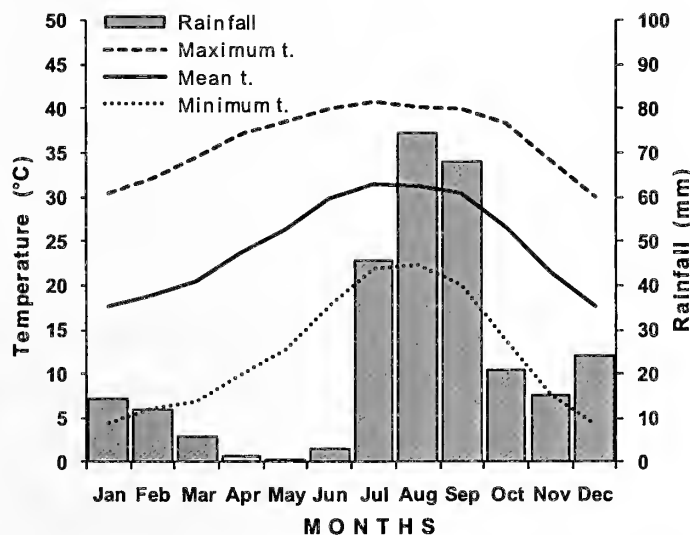


Figure 3.—Climate graph of the study region (“Empalme”, 29 years), including average rainfall and temperatures that occurred from 1980–2008.

Table 1.—Number of spiders and egg sacs of *Homalonychus selenopoides* found in each collection area by collection method. Percentages of spiders and total egg sacs are included in parentheses.

Collection areas	Hand collection		Pitfall trapping
	Spiders	Egg sacs	Spiders
El Macapul	437 (94%)	67 (100%)	14 (3%)
Nacapule	0 (0%)	0 (0%)	1 (< 1%)
El Soldado	9 (2%)	0 (0%)	3 (< 1%)

Phenology.—Field collection was conducted from November 2007–November 2008 to determine the frequency of each life stage of *H. selenopoides* throughout the year. We hand collected 3–5 days per month for a total of 43 collection days and also carried out pitfall trapping.

Pitfall trapping: We placed 10 pitfall traps each at El Macapul, Nacapule, and El Soldado. Each trap was constructed with one 2-liter plastic soda bottle, measuring 18 cm in height and 11 cm in diameter. The internal chamber contained ethylene glycol as the lethal agent. In each trapping month, we activated the traps for 14 days, and inactivated them the following 14 days.

Diurnal hand collections: Collections were made by 3–5 participants. We collected adult and immature spiders from under stones, dry cattle dung, wood, bricks, cardboard, or sheet metal; the egg sacs were found under rocks and dry cattle dung. We placed live spiders individually in plastic containers and transported them to our laboratory. Male and female voucher specimens were preserved in 75% ethanol and were deposited in the CIBNOR Arachnological and Entomological Collection (CARCIB) in La Paz, Baja California Sur, Mexico.

RESULTS

Collected spiders.—During our study we collected a total of 464 spiders and 67 egg sacs of *H. selenopoides* of which 451 spiders (97.2%) were at El Macapul and 13 spiders (2.8%) at the other collection sites (Table 1). Of these, in 13 months of pitfall trapping we caught only 18 *H. selenopoides*, which represented 3.9% of the total specimens we captured in the study area; most of these spiders were also caught at El Macapul (Table 1).

Phenology.—We collected 47 second instar spiderlings, 212 juveniles, 64 penultimate males, 22 penultimate females, 25 adult males, and 94 adult females. The presence and number of these stages of development changed during the year (Table 2).

The field life cycle of the population of *H. selenopoides* studied is annual. We found egg sacs from April–July with 21, 13, 27 and six egg sacs, respectively; most of them (91%) were

found during the months with lower rainfall (April–June) (Fig. 3). Egg sacs were usually found individually, although rarely we found two or three under the same shelter. All egg sacs were found hanging from the lower surface of the object (stone or cattle dung) that formed the ceiling of the shelter (Fig. 4). Second instar spiderlings were recorded from April–July. Juveniles were present almost all year round, but early juveniles were absent from December–January. Juveniles were scarce from November–April but were more numerous from May–October, reaching 100% of the collections in August. Penultimate spiders of both sexes were collected from September–April. Both sexes matured starting from November (Table 2). Females were present from November–May (only one female was collected in May), and males were present from November–April (Table 2), the cooler months of the year (Fig. 3). Juveniles comprised 100% of the collections from June–October with some 2nd instar spiderlings and penultimate instars at the beginning and end of this period, respectively (Table 2). The general phenology pattern is summarized in Fig. 5.

The relative abundance or the percentage of each developmental stage during the year had a partially overlapping unimodal distribution (Fig. 6), except for the penultimate stage, which had an irregular trend. Although outdated for a month, during the warmer months (March–November) the relative abundance of adults and abundance of penultimate spiders were positively correlated (linear regression, $r^2 = 0.9231$; $P < 0.0001$) whereas during the cooler months (December–February), no correlations were found. We found that the average temperature and the relative abundances of juveniles were positively correlated using a quadratic regression because of the high coefficient of regression ($r^2 = 0.9374$; $P < 0.0001$) whereas the average temperature and abundance of adults were negatively correlated using a linear regression ($r^2 = -0.8904$; $P < 0.0001$) (Fig. 7).

DISCUSSION

In this study, we provide relevant information about the basic ecology of *H. selenopoides*.

Pitfall trapping.—Even though pitfall traps have been the most widely employed sampling method for ground-dwelling arthropods, particularly cursorial spiders (Schmidt et al. 2006), we trapped only a few specimens of *H. selenopoides*. Thus, pitfall traps were useful only to confirm the mating period. We propose that mating occurs in winter, when we most often found both males and females. There is a consensus that the presence of adult spiders, particularly males, in pitfall traps

Table 2.—Number of *Homalonychus selenopoides* collected per month from November 2007 to November 2008, including their developmental stage and egg sacs. Numbers of egg sacs in italics are not added in totals per month.

Stage	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
Egg sacs	0	0	0	0	0	21	13	27	6	0	0	0	0
2 nd Instar	0	0	0	0	0	10	17	16	4	0	0	0	0
Juveniles	4	5	0	5	6	10	17	33	27	40	34	19	12
Penultimate ♂♂	8	3	8	9	10	1	0	0	0	0	5	12	8
Penultimate ♀♀	0	1	3	2	7	1	0	0	0	0	1	6	1
Males	1	9	5	0	2	1	0	0	0	0	0	0	7
Females	7	11	27	21	8	12	1	0	0	0	0	0	7
Total/month	20	29	43	37	33	35	35	49	31	40	40	37	35



Figure 4.—Egg sac of *Homalonychus selenopoides* in the field, attached to the underside of a rock.

indicates the peak of copulation period (Toft 1976; Aitchison 1984).

Phenology.—The phenology of the studied population of *H. selenopoides* is markedly seasonal, correlated in part with large seasonal weather fluctuations of the study region. This has also been reported for some salticid spiders (Jackson 1978).

Crews & Hedin (2006) mention a time of development and longevity of *Homalonychus* females of up to five years in the lab. Although it is known that these traits are different in the field, we were surprised that the *H. selenopoides* life cycle in the field was annual, as in most species of spiders in temperate regions (Gertsch 1979; Foelix 1996). However, the majority of temperate-region spiders have spring-summer reproduction and overwinter as immatures (Kiss & Samu 2002; Rybak 2007). There are different proposed pattern types of life cycle or phenology (e.g., Toft 1976; Schaefer 1977; Aitchison 1984) based on duration of the life cycle, overwintering stage, seasonal peaks of activity, and reproductive period or seasonality of adults. During the winter collections, we did not observe dormancy in adults (or in penultimate spiders or juveniles). Because we infer that adults mate during winter, the phenology model proposed fits the Winter-mature pattern of Aitchison (1984) (“Winter-reif” of Tretzel 1954). Our model also partially

fits the type V phenology of Schaefer (1977) (Stenochronous, Winter-active species), but in *H. selenopoides* the true reproduction (oviposition) occurs exclusively in spring.

In temperate latitudes, winter-active spiders are uncommon and represent only 9% of all the spider species studied (Schaefer 1977). In turn, only 15% of the winter-active spiders correspond to the “Winter-mature” category, but the full life cycle is known in only a few species. Although these “winter mature” spiders also mate in winter (Toft 1976; Schaefer 1977; Aitchison 1984), this does not agree with our findings with *H. selenopoides* with respect to seasonality of the other life cycle stages (e.g., overwinter stage, oviposition period, spiderlings emergence) most likely because of severe weather conditions. A more relevant comparison would be with other winter-mature desert spiders of annual cycle, but we found no published accounts.

Our model coincides with the months of sporadic collection of juveniles, males, females, and egg sacs reported by Chamberlin (1924), Roth (1984), and Vetter & Cokendolpher (2000) for *H. selenopoides*. Our study is also corroborated by sporadic collections of this species made from 2012–2015, except that we found a few egg sacs in February 2012 and 2015, when the previous winters were relatively warm.

Although similarities with the phenology of *H. theologus* could be expected, we did not find them. Domínguez-Linares (2002) found *H. theologus* females in October and two peaks of early juveniles, one in December and another in May–July. Additionally, Roth (1984) also reported males and females of *H. theologus* collected in October. Differences in phenology between both species of *Homalonychus* are feasible because the areas where they were studied are at different latitudes with different subclimates (García 1973; INEGI 2002), which in turn have consequent effects on vegetation and predator/prey abundance, as well as different temperatures yielding different growth rates.

Although *H. selenopoides* females constructed some egg sacs sequentially in our laboratory, we assume that this species is semelparous in the field. If we consider that females are monandrous (Alvarado-Castro & Jiménez 2011), reassuming their sedentary lifestyle after pairing, and that we rarely found egg sacs and females in the same shelter, we speculate that most females construct only one egg sac in their lifetime in the field. The risk of complete batch loss of the egg sacs in *Homalonychus* may be very low because females construct the egg sacs inside the same

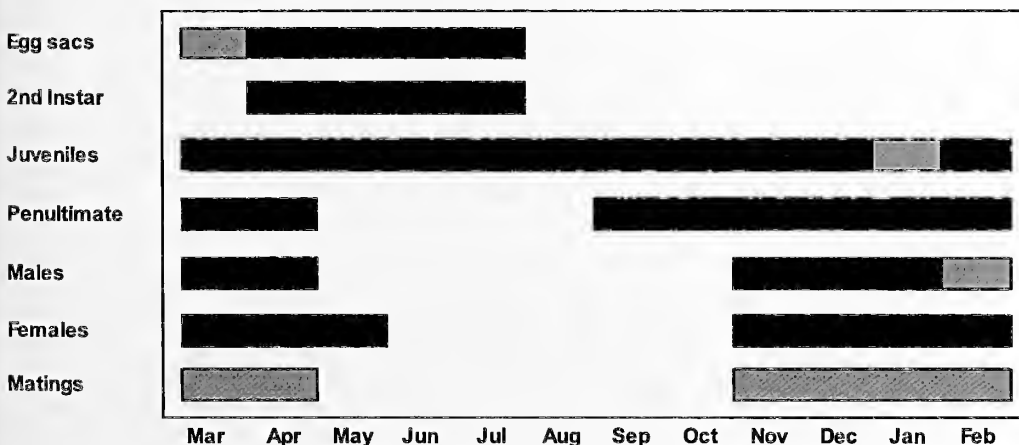


Figure 5.—Phenology model proposed for *Homalonychus selenopoides*. Hypothetical occurrence highlighted in gray.

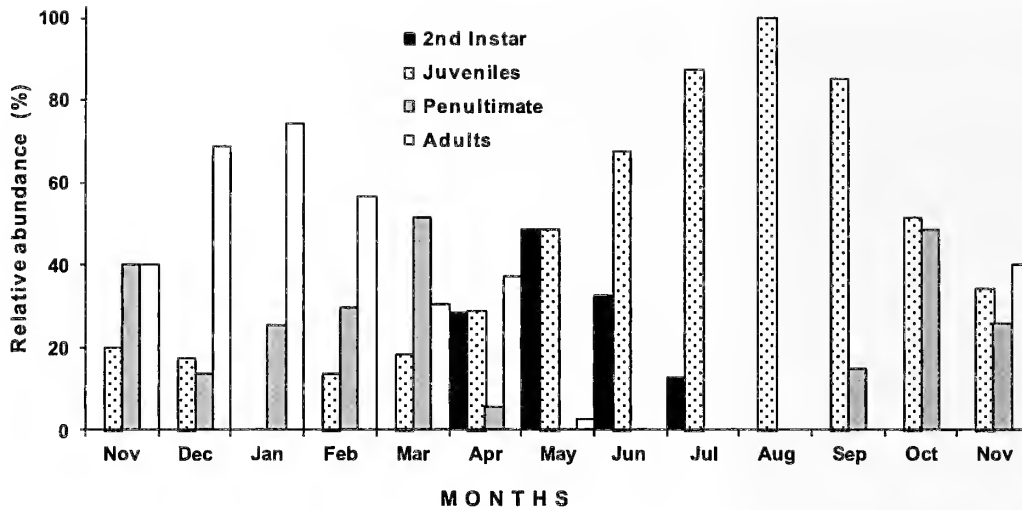


Figure 6.—Seasonal distribution of developmental stages of *Homalonychus selenopoides* during thirteen collection months. Relative abundance is the percentage of the total individuals collected per month.

refuges they occupy, and the elaborate structure of the egg sac provides significant protection of the eggs (Alvarado-Castro & Jiménez 2011). Thus, the conditions of the models of Murphy (1968) and Charnov & Schaffer (1973) that predict iteroparity do not seem to fit with *H. selenopoides*.

If *H. selenopoides* is semelparous, it is possible that it is univoltine like other desert spiders that have a single generation per year (Boulton & Polis 1999). Despite the potential for iteroparity and greater longevity, the one-year, one-egg sac pattern occurs in many desert arthropods due to the costly egg production and limited longevity in desert ecosystems (Polis 1991).

A season with lower temperatures is important in synchronizing the life cycles among individuals in the same population. In spiders, similar to insects, developmental cycles are controlled by photoperiod and temperature (Lees 1955; Schaefer 1977); also, these cycles can be partly defined by certain mechanisms of dormancy (Schaefer 1977). The photoperiod in our lab was natural, and the temperature, humidity, and

food availability were different between the laboratory and the field; however, the females started to construct their egg sacs simultaneously in both environments at the beginning of the spring. Thus, we infer that most likely the photoperiod controls the start of the oviposition period in *H. selenopoides*. Ultimately, changes in photoperiod are related to the evolution of physiological responses associated with diapause (Zimmermann & Spence 1998).

We propose that reproductive diapause (Schaefer 1977) of females governs the seasonal development of *H. selenopoides*. Diapause plays a central role for synchronizing the development of many univoltine arthropods (including spiders) with seasonally changing environments. Diplochronous spiders that mate in autumn and lay eggs in spring have reproductive diapauses. This is induced by short days, with inhibition of the development of the female reproductive system and the deposition of yolk. With long days, diapause is averted (facultative diapause) (Schaefer 1977).

We found egg sacs in the field only during spring and early summer. Some conjectures can explain this trait. Oviposition coincides with the start of insect abundance and increased temperatures that favor faster development of embryos and juvenile spiders. Moreover, these juveniles will have a long warm period (spring–autumn) to mature. Because laying eggs occurs only in the months of the lowest or no rainfall, we suggest that, additionally, this may be a strategy of ecological escape from the summer monsoon. Egg sacs may be vulnerable to surface water runoff because they are constructed with silk and sand at ground level (Alvarado-Castro & Jiménez 2011).

The absence of *H. selenopoides* males in the field from May is explained by their short lifespan. Males were less than one third as abundant as females in the field, which suggests their higher mortality, in line with the higher mortality rate of males reported by Rybak (2007) for *Bathyphantes simillimus* (now *B. eumensis* L. Koch, 1879) (Linyphiidae). Dominguez-Linares (2002) reported longevity in the laboratory of only 76 days for *H. theologus*. Because females began to mature in the field in November and they virtually disappear by May, we assumed that females had a catastrophic mortality during spring. Indeed, spring is the season with less precipitation in our study area. The cost of egg production reduces longevity of

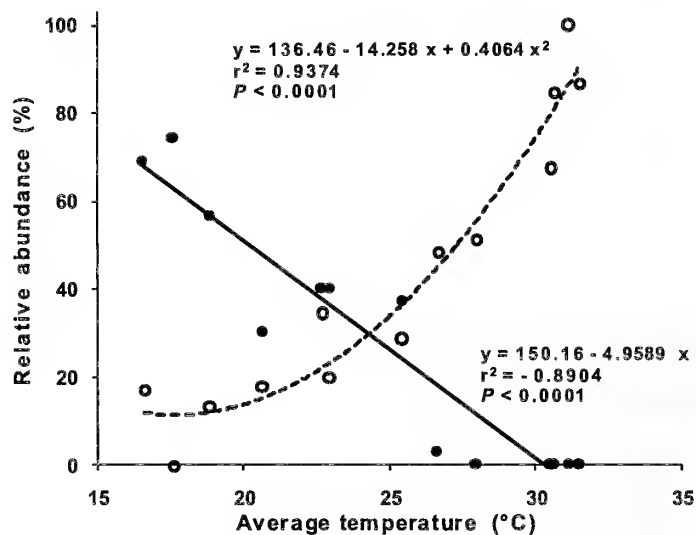


Figure 7.—Relationship between average temperatures and relative abundance of *Homalonychus selenopoides* juveniles (—○—) and adults (—●—) in the field.

arthropods in desert ecosystems (Polis & Yamashita 1991). In the desert spider *Diguetia mojavea* Gertsch, 1958 (Diguettidae), an inverse relationship was found between adult density and egg sac density, and females were not found after the oviposition period (Boulton & Polis 1999).

Adult activity in winter can be another strategy of ecological escape. In the field, many females had an enlarged opisthosoma and were clumsy to escape or did not flee. Thus, it seems reasonable that adult females that may be unable to avoid predators occur during winter when predators (i.e., other spiders, scorpions, solifugids, centipedes, and lizards [Cloudsley-Thompson 1983, 1995; Punzo 2003]) are hibernating or are less active (Muma 1974; Crawford et al. 1975; Polis 1979; Baltosser & Best 1990; Prentice 1997; Punzo 2003). This would explain the inverse relationship between the abundance of adults and the temperature, because the higher the temperature, the higher the predator activity. If this phenological feature reduces the possibility of an encounter with predators, then it is an adaptive anti-predator strategy of the primary type (Cloudsley-Thompson 1995). Other adaptive advantages of winter active or winter-mature spiders have been suggested by Jackson (1978) and Huhta & Viramo (1979).

Concluding remarks.—It would be useful to know the phenology of the most northern populations of *H. selenopoides* (e.g., those south of the Great Basin where the temperatures are colder in winter) to compare with our results. It would help to understand how climate shapes the phenology of this species, as well as how their populations respond to achieve inclusive fitness in different extreme environments. Additionally, a study of *H. theologus* life history strategies compared with that of *H. selenopoides* could be productive. It seems there are important differences in the phenology of both species, and our understanding of the evolution of these differences can likely be clarified by this kind of study.

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Three new species of the Mexican harvestman genus *Chapulobunus* (Opiliones: Stygnopsidae)

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Abstract. Three new species of the genus *Chapulobunus* Goodnight & Goodnight, 1946, are described: *Chapulobunus asper* sp. nov., *Chapulobunus psilocybe* sp. nov., and *Chapulobunus regiomontano* sp. nov. from the states of San Luis Potosí, Oaxaca, and Nuevo León, in Mexico, respectively. Additionally, an identification key to the five known species of the genus is provided.

Keywords: Taxonomy, Laniatores, Sierra Madre Oriental.

<http://zoobank.org/References/urn:lsid:zoobank.org:pub:676FCD17-7BAA-4B60-96E9-5A16FE1748F1>

The harvestman genus *Chapulobunus* Goodnight & Goodnight, 1946, and its type species *Chapulobunus unispinosus* Goodnight & Goodnight, 1946, were originally described in the family Phalangodidae, subfamily Stygnopsinae (Goodnight & Goodnight 1946). Subsequently, Goodnight & Goodnight (1953) considered this genus a junior synonym of the genus *Karos* Goodnight & Goodnight, 1944 (at that time allocated in the subfamily Phalangodinae), without providing any justification. Recently, *Chapulobunus* was resurrected from the synonymy with *Karos*, based on a cladistic analysis using morphological data (Cruz-López & Francke in press). In the same work, the genus was rediagnosed, the type species was redescribed, the male genitalia were illustrated and described for the first time, and the second species of the genus was described: *Chapulobunus poblano* Cruz-López & Francke, in press.

Phylogenetically, the genus belongs to the *Karos* genus-group, which is characterized by the following: the presence of lateral clear areas at the level of mesotergal area I, occasionally clear areas present on corners of area V and free tergites, ocularium displaced from the frontal margin, small chelicerae, cheliceral dentition in males homogeneous, without sexual dimorphism in cheliceral size and pedipalpal armature, pedipalpal femur with one meso-apical setiferous tubercle, ventral plate of penis slender, not differentiated from the truncus, and base of follis exposed (Cruz-López & Francke in press).

The sister group of *Chapulobunus* is the monotypic *Montabunus* Goodnight & Goodnight, 1945. These genera share a similar male genitalia pattern, differing primarily in the external morphology, *Montabunus* with an unarmed dorsum and mid-bulge of scutum small and level with mesotergal area I (see Cruz-López & Francke in press for phylogenetic details).

The genus is easily distinguished from other stygnopsids by the presence of a remarkably robust body, with lateral margins of scutums broadly convex, with a wide spine in the middle of mesotergal area II, with a prominent middle spine on area III; areas III and IV slightly fused, area III invading and dividing in two area IV; legs IV sexually dimorphic: males have femur or/and patella with ventral armature, stronger than on females; and pars distalis of the male genitalia medially swollen in dorsal/ventral views, lateral macrosetae forming an unique irregular row with several setae, three or more pairs of ventral

microsetae and lateral projections of glans pointed distally (Cruz-López & Francke in press).

The species of *Chapulobunus* have been found in pine forests in the Sierra Madre Oriental in Mexico (Figs. 1–4), with altitudinal range restricted between ca. 1600 and 2250 meters. Occasionally these are found under rocks, in small hollows (Fig. 5), but usually they are found under or inside of decomposing tree stumps (Fig. 6). They are commonly found forming small monospecific aggregations of three to six individuals (pers. obs.).

Herein, based on new material collected during recent field work in the region, three new species are described. Further, an identification key is provided to separate the five species currently in this genus.

METHODS

All material examined is deposited in the Colección Nacional de Arácnidos (CNAN), at UNAM, Mexico, and Texas Tech University (TTU), USA. Drawings were made in Photoshop CS5 software, using assembled photographs to delineate the structures. Microphotographs were taken using a Hitachi S-2460N Scanning Electronic Microscope. All plates were edited using the previously mentioned version of Photoshop. In addition to visual color approximations, the universal color guide Pantone® was used to ascertain the coloration of the new species. Morphological nomenclature follows Cruz-López & Francke (in press), pedipalpal armature follows Acosta et al. (2007), structures related to ozopores (lateral pegs) follows Gnaspini & Rodrigues (2011), and setal nomenclature of male genitalia according to Kury & Villarreal (2015). Height/width of the ocularium, height of spine III/height of spine II, height of spine III/height of ocularium ratios were calculated. Measurements of pedipalps are: trochanter/femur/patella/tibia/tarsus/claw, and legs are trochanter/femur/patella/tibia/metatarsus. All measurements are in mm. Distribution map was generated using gvSIG 1.11.0-RC1 software.

TAXONOMY

Family Stygnopsidae Sørensen, 1932

Genus *Chapulobunus* Goodnight & Goodnight, 1946

Chapulobunus Goodnight & Goodnight 1946:1; Cruz-López & Francke in press:000 (status revalidated).



Figures 1–6.—Habitats and microhabitats of species of *Chapulobunus*. 1. Habitat of *Chapulobunus asper* new species, pine forest from Buena Vista, near Ahuacatlán, Municipio de Xilitla, San Luis Potosí; 2. Habitat of *Chapulobunus poblano* Cruz-López & Francke in press, pine forest from Estación de microondas Tomaquillo, Municipio de Zacapoaxtla, Puebla; 3. Habitat of *Chapulobunus regiomontano* new species, pine forest from road to Mina la Huiche, Municipio de Galeana, Nuevo León; 4. Habitat of *Chapulobunus unispinosus* Goodnight & Goodnight 1946, pine forest from La Mojonera, Municipio de Zacualtipan, Hidalgo; 5. Microhabitat of *Chapulobunus regiomontano* new species, the red arrow indicates the aggregation of several specimens in a small hollow; 6. Microhabitat of *Chapulobunus unispinosus* Goodnight & Goodnight, 1946, decomposing logs.

Karos [in part]: Goodnight & Goodnight 1953:20 (generic synonymy transfer); Goodnight & Goodnight 1973: 83; Kury 2003: 238 (catalogue).

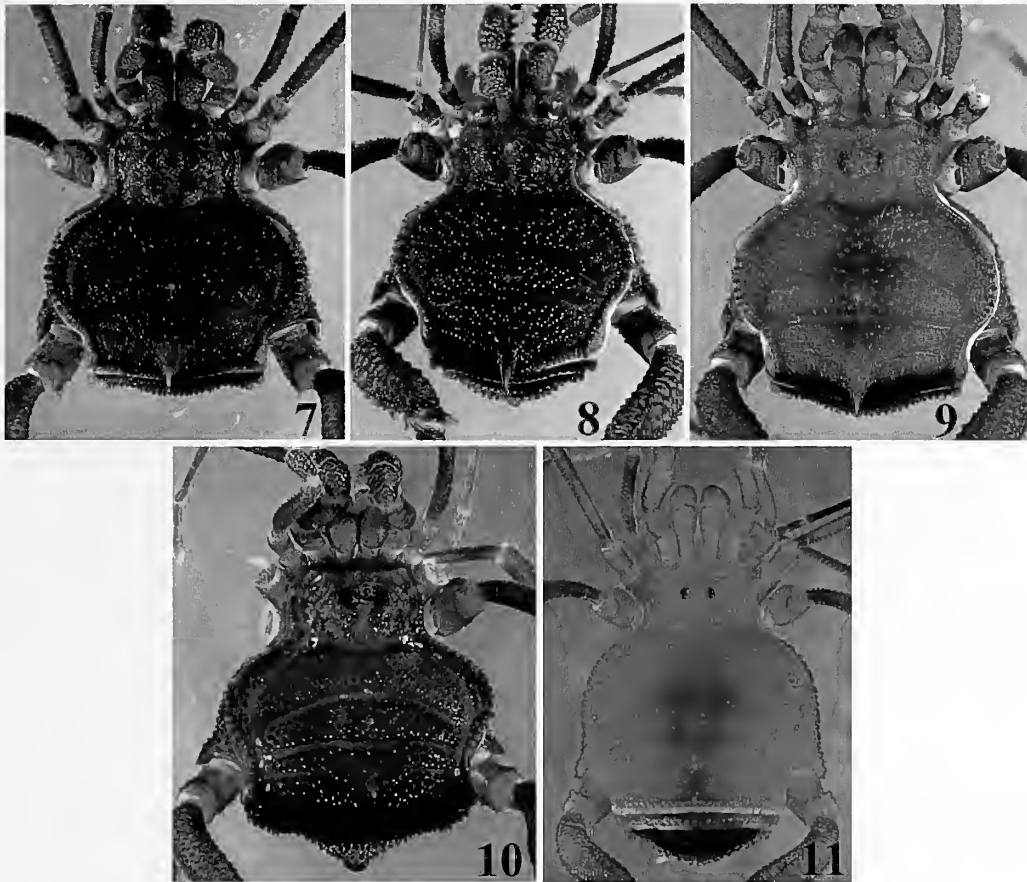
Type species.—*Chapulobunus unispinosus* Goodnight & Goodnight, 1946, by original designation.

Taxonomic considerations.—Cruz-López & Francke (in press) named the macro and microsetae of the penis according to their position. They recognized the lateral rows of macrosetae, ventral rows of microsetae and parastylar setae, this last term taken from Cokendolpher (2004). Recently, Kury & Villarreal (2015) proposed the first homology hypothesis in penial setation in Gonyleptoidea. These authors recognized five setal groups in a broad taxa sampling of many gonyleptoid families. Regarding Stygnopsidae, Kury & Villarreal detected these setal groups in *Hoplobunus boneti* (Goodnight & Goodnight, 1942), *Karos* sp. and the three genera of *Paramitraceras* genus-group (Cruz-López & Francke in press).

Here, we adopted the chaetotaxy nomenclature proposed by Kury & Villarreal (2015) and described the male genitalia of the new species with their terminology. On this, we had problems trying to detect any B setae in *Chapulobunus*. Kury & Villarreal (2015:6) said: "...A and B are mostly associated with each other...sometimes it is difficult to tell which one is B owing to their similar conformation, but often MS B are smaller...". Examining species of *Chapulobunus*, we do not find evidence to recognize any B setae, only *Chapulobunus psylocibe* new species exhibits a basalmost small seta, which could be interpreted as B. But to maintain consistency in the setal nomenclature in the genus, we consider that only groups A, C, D and E are present in *Chapulobunus*. Sometimes the C and A groups are easily distinguishable, such is the case of *C. unispinosus*, *C. poblano* (Cruz-López & Francke in press: Figs. 31, 34) and *Chapulobunus asper* new species (Figs. 21–23). However in *C. psylocibe* new species and *Chapulobunus regiomontano* new species, C and A setal groups are contiguous. These are recognizable only if the penis is seen dorsally or ventrally.

KEY TO SPECIES OF *CHAPULOBUNUS*

1. Ocularium blunt, with one small spine over each eye (Fig. 15). Anal plate in males without central bulge (Figs. 14, 38)
2. Ocularium spiniform, pointed apically, without one small spine over each eye (Figs. 25, 27). Anal plate in males with central bulge (Figs. 10, 26) 4



Figures 7–11.—Male dorsal habitus of all species of *Chapulobunus*. 7. *Chapulobunus unispinosus* Goodnight & Goodnight 1946 from La Mojonera, Municipio de Zacualtipán, Hidalgo; 8 *Chapulobunus poblano* Cruz-López & Francke in press, holotype; 9. *Chapulobunus asper* new species, holotype; 10. *Chapulobunus psilocybe* new species, holotype; 11. *Chapulobunus regiomontano* new species, holotype. These figures are not at the same scale.

- 2. Spines of mesotergal areas II and III absent (Figs. 36, 37). Ventral tubercles of femur IV on males foliose (Figs. 42, 43) *Chapulobunus regiomontano* sp. nov.
- Spines of mesotergal areas II and III present, long (Figs. 13, 25). Ventral tubercles of femur IV on males spiniform (Figs. 18, 19, 30, 31) 3
- 3. Males with ventral tubercles on femur IV similar in size (Cruz-López & Francke in press, Fig. 30A). Ocularium semi-triangular in frontal view, height/width ratio of ocularium 0.75 (Cruz-López & Francke in press, Fig. 29C). Penis with two pairs of D setae and two pairs of A setae (Cruz-López & Francke in press, Fig. 31) *Chapulobunus unispinosus*
- Males with ventral tubercles on femur IV dissimilar in size, apical longer than basal (Figs. 18, 19). Ocularium blunt in frontal view, height/width ratio of ocularium 0.50. Penis with three or four pairs of D setae and three pairs of A setae (Figs. 21–23) *Chapulobunus asper* sp. nov.
- 4. Height spine III/II ratio 3. Ocularium in frontal view triangular (Fig. 27). Males with ventral tubercles of femur IV small, homogeneous in size. Penis with apical margin of pars distalis concave (Figs. 33–35) *Chapulobunus psilocybe* sp. nov.
- Height spine III/II ratio 5. Ocularium in frontal view spiniform (Cruz-López & Francke in press, Fig. 32C). Males with ventral tubercles of femur IV heterogeneous, small and large. Penis with apical margin of pars distalis convex (Cruz-López & Francke in press, Fig. 34) *Chapulobunus poblano*

***Chapulobunus asper* new species**

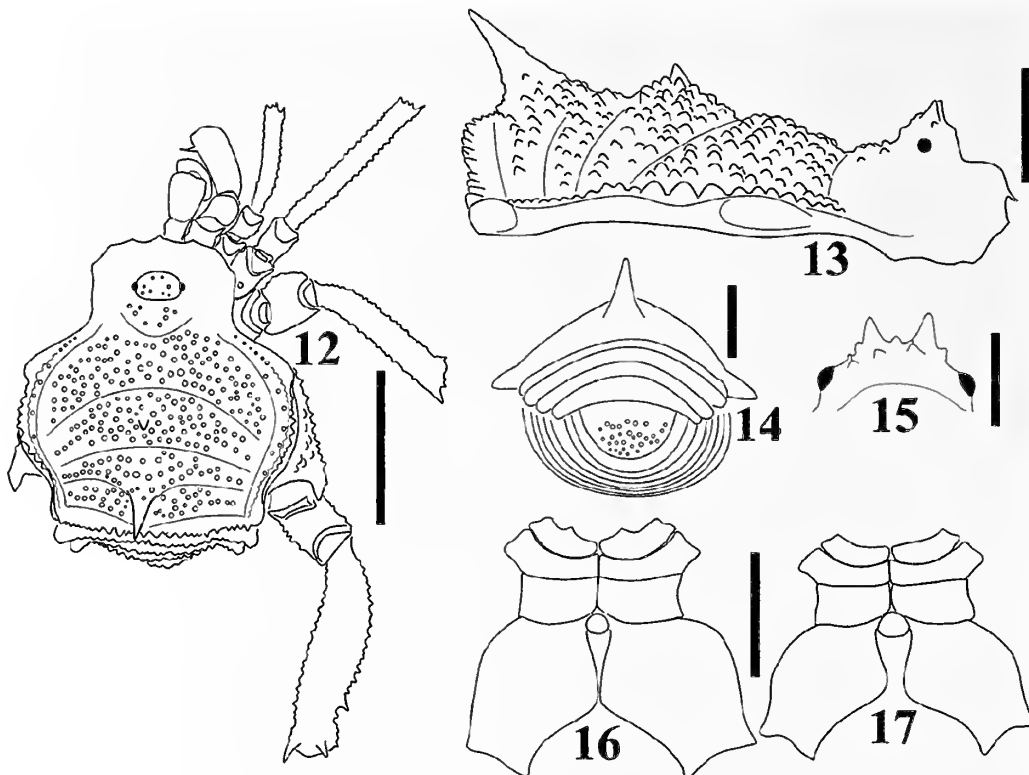
<http://zoobank.org/NomenclaturalActs/urn:lsid:zoobank.org:act:30BFA258-4EB2-4A4C-A681-934CA0674758>
(Figs. 9, 12–23)

Material examined.—*Holotype*: MEXICO: San Luis Potosí: male, Buena Vista, near Ahuacatlán (21°20'26.340"N, 99°05'

55.247"W), Municipio de Xilitla, 6 May 2011, O. Francke, A. Valdez, J. Cruz, G. Contreras and R. Monjaraz (CNAN-T0838).

Paratypes: MEXICO: San Luis Potosí: 1 male and 1 female, same locality and data (CNAN-T0839).

Other material: MEXICO: San Luis Potosí: 1 incomplete female DNA voucher from same locality and same data (DNA-Op0043).



Figures 12–17.—*Chapulobunus asper* new species, male holotype and female paratype. 12. Male habitus in dorsal view; 13. Male habitus in lateral view; 14. Male habitus in posterior view; 15. Male ocularium in frontal view; 16. Male habitus in ventral view; 17. Female habitus in ventral view. Scale bars: Figs. 12, 16, 17 = 2.5 mm; Figs. 13, 14 = 1.0 mm; Fig. 15 = 0.6 mm.

Etymology.—The specific name comes from the Latin *asper*, an adjective which means rough, in reference to the external appearance in this species.

Diagnosis.—This species can be differentiated from *C. poblano* and *C. psilocybe* by the absence of a central bulge in the anal plate in males (Fig. 14) and the ocularium lacks a median spine (Fig. 15). It differs from *C. regiomontano* by the ventral armature of leg IV, which is spiniform in *C. asper* (Figs. 18, 19). This species is very similar to *C. unispinosus*, but differs in: a) the height/width ratio of the ocularium in *C. asper* is 0.50, in *C. unispinosus* is 0.75, and b) the penis in *C. unispinosus* has two pairs of D setae, but on *C. asper* has three or four pairs (Fig. 21).

Description.—*Male holotype*: Measurements: scutum length 5.75, scutum maximum width 5.05, pedipalp 0.80/1.70/1.05/1.30/1.10/0.75, legs I 0.70/2.10/0.85/1.75/2.15, II 0.80/3.30/1.10/2.75/3.05, III 1.15/2.90/1.25/2.50/3.00, IV 1.50/4.35/1.50/4.05/4.40.

Dorsum: Mesotergum densely covered by rounded tubercles, these tubercles have one minute apical seta. Carapace with few tubercles, located posterior to ocularium. Ocularium height/width ratio = 0.5, dorsally covered by spiniform tubercles, two of these over each eye sharper. Pegs of the lateral row rounded, in a continuous line. Central spine of mesotergal area II small, height of central spine of area III = 1.00, height spine III/II ratio = 5, height spine III/ocularium ratio = 1.49, spine III pointing posteriorly. Six to seven extra lateral pegs, on widest part of scutum. Lateral clear areas in three regions: a) on the widest region of scutum, teardrop-shaped; b) posterior to first, not projected, with numerous slots; c) on the corners

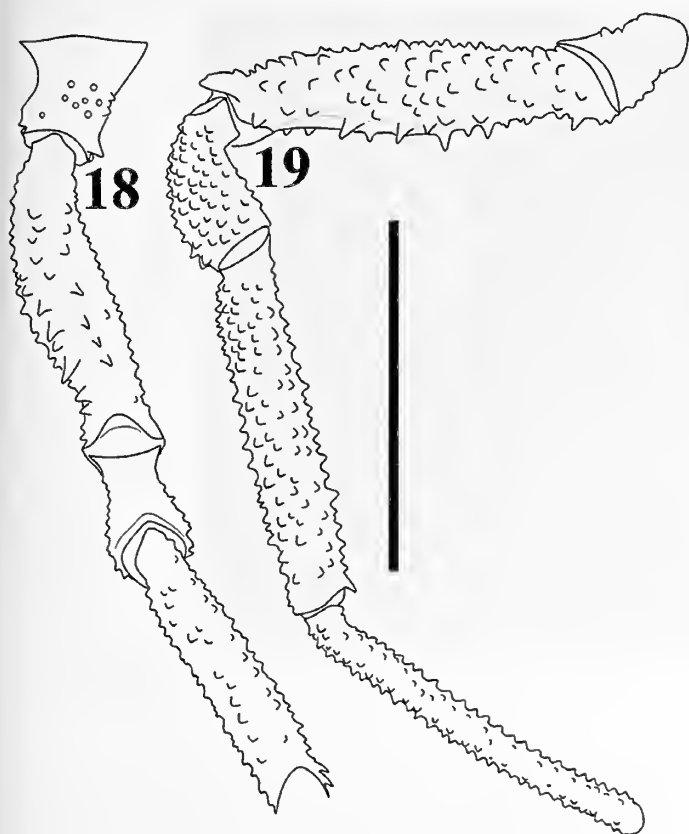
of area V, rounded and projected (Figs. 12–15, 20). Free tergites covered by tubercles similar to the dorsum. Corners of free tergites I and II with clear, rounded projections.

Venter: Covered similarly to dorsum. Stigmatic region plus sternum forming inverse “T” with the shaft constrained in the middle, arms curved posteriorly (Fig. 16). Coxae IV very large, with dorso-ectal apophysis. Genital operculum rounded, small, between anterior portions of coxae IV. Free sternites and anal plate covered by tubercles similar to dorsum.

Chelicera: Small, bulla not developed; fixed finger with five small teeth; movable finger with numerous small teeth, forming a serrula.

Pedipalp: Trochanter globular. Femur cylindrical; dorsally covered by minute tubercles, ventrally with five setiferous tubercles forming a row; one setiferous tubercle on meso-apical portion. Patella cylindrical, ornate with small tubercles, with three setiferous tubercles mesally. Tibia cylindrical, dorsally covered by small tubercles, with iiiII tubercles on ectal side ($4 > 5 > 2 > 1 = 3$), iili on mesal side ($1 = 3 > 4 > 2$). Tarsus smooth dorsally, conical, with IIIi setiferous tubercles on both sides ($1 = 2 = 3 > 4$). Claw slightly curved, shorter than tarsus.

Legs: Trochanter to tibia of legs covered by small tubercles, similar to dorsum; metatarsus and tarsus covered by small tubercles and setae. Legs III and IV stronger than I and II. All femora with two dorso-apical spines, retrolateral slightly larger than prolateral. Femur II with two irregular, ventral rows of spiniform tubercles. Metatarsus IV slightly curved. Trochanter III globose, bigger than the rest, with one dorsal spine mesally (Figs. 18, 19). Trochanter IV cylindrical, with one ventro-apical spine. Tarsal count 4(2):6(3):6:6.



Figures 18 and 19.—*Chapulobunus asper* new species, male holotype. 18. Trochanter to tibia IV in ventral view; 19. Trochanter to metatarsus IV in ectal view. Scale bar = 1.5 mm.

Genitalia: Pars distalis swollen in the middle, apical margin convex, irregular. Follis wide basally; latero-apical projections spiniform, pointed; inner side of follis, around the stylus, covered by small spiniform projections. Setae C group forming a mesoapical longitudinal row of five setae. Setae A group forming an irregular longitudinal row of three setae, the medium slightly ventrally, the basalmost setae slightly separated from the other two. Setae C and A groups visibly separated. Setae D group formed by three/four setae, basal to follis. Setae E group formed by two longitudinal rows of microsetae, at lateral margins of pars distalis. The two apical E setae close to each

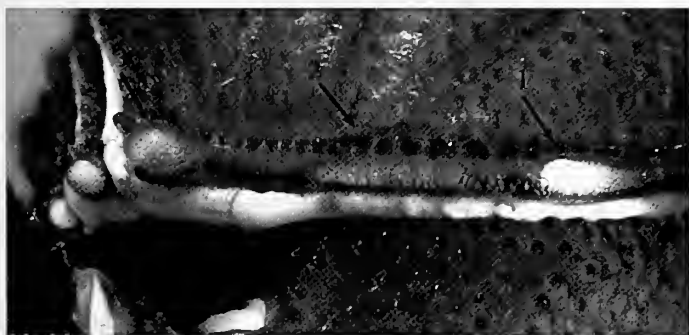


Figure 20.—*Chapulobunus asper* new species, male holotype. Detail of clear lateral areas of scutum. Numbered arrows indicate the clear areas.

other, at the base of apical margin. The two basalmost E setae in the middle of par distalis (Figs. 21–23).

Color: In alcohol, this species is dark brown (Black 5 2X), but under the light of the microscope appears lighter (PMS 4625) (Fig. 9). Apical portion of legs is slightly lighter. Tips of dorsal spines II and III, and lateral clear areas are yellow brown (PMS 100).

Female paratype: Measurements: scutum length 5.40, scutum maximum width 4.65, pedipalp 0.75/1.45/0.95/1.10/1.05/0.70, legs I 0.65/1.90/0.80/1.47/1.95, II 0.65/2.95/1.05/2.42/2.67, III 1.00/2.67/1.12/2.15/2.55, IV 1.10/3.95/1.45/3.25/3.75. Differs from the male only in the less sharp ventral armature of femur IV, femur and tibia IV weaker, and shaft of the inverse “T” on venter slightly constrained in the middle (Fig. 17).

Distribution.—This species is known only from the type locality (Fig. 48).

Remarks.—When the specimens were found, they showed thanatosis behavior, which has been reported in the two previously known species of the genus (Cruz-López & Francke in press). The unusual asymmetry in the number of D setae, and variation in the position of the E microsetae are common features in specimens of this genus (also observed in *C. unispinosus* and *C. psilocybe*); unfortunately, a large series of male specimens is not available to analyze these peculiar variations in detail.

Chapulobunus psilocybe new species

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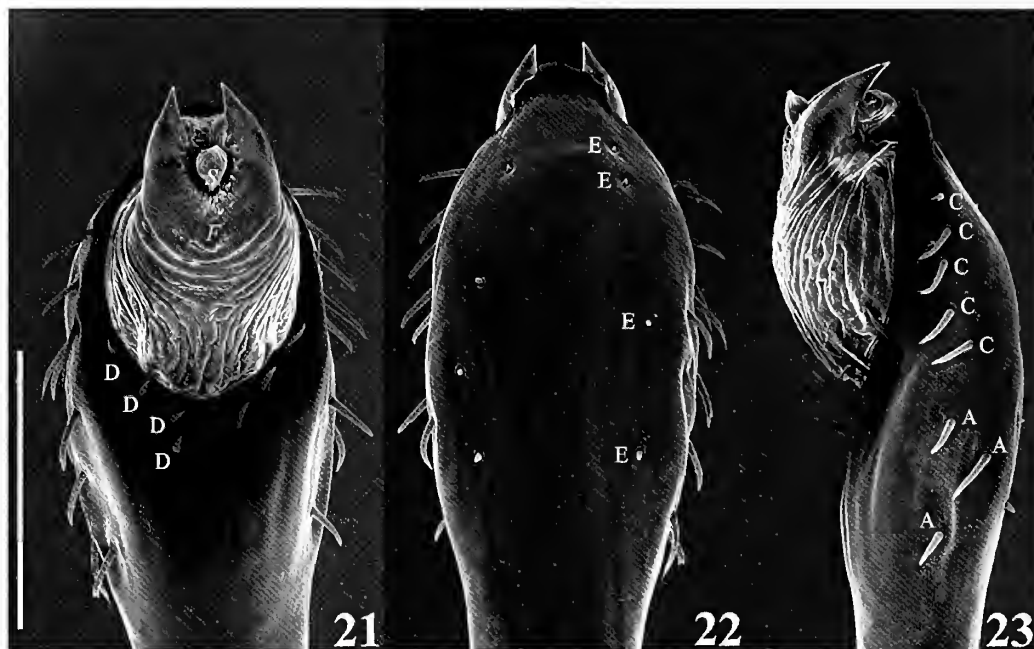
(Figs. 10, 24–35)

Material examined.—**Holotype:** MEXICO: *Oaxaca*: male: 4 km W. of Puerto de la Soledad (18°10'31.540"N, 97°00'17.207"W), Municipio de Huautla de Jiménez, 10 December 2011, G. Contreras, J. Mendoza, E. Hijmensen and E. Goyer, (CNAN-T0840).

Other material examined: MEXICO: *Oaxaca*: 1 incomplete female DNA voucher, same locality and data (DNA-Op0069); 1 female, same locality, except 11 September 2010, O. Francke, A. Valdez, D. Barrales and J. Cruz.

Etymology.—The specific name is a noun in apposition. The word *Psilocybe* is a generic epithet in mushrooms (Fungi). The species of that genus are well known for their hallucinogenic effects. The type locality is located at the municipality of Huautla. This municipality is well known because some local healers and shamans (e.g., the most popular, María Sabina) use these mushrooms in sacred rituals. The name refers to the co-existence of the mushrooms and this small stynopsid harvestman.

Diagnosis.—This species differs from *C. asper*, *C. regiomontano*, and *C. unispinosus* by the presence of a central bulge on anal plate on males (Figs. 10, 26), and the presence of a median spine on the ocularium in both sexes (Fig. 27). It differs from *C. poblano* in that *C. psilocybe* has a) ocularium triangular in frontal view (Fig. 27), the median spine is contiguous with the lateral margins of ocularium, whereas on *C. poblano* the ocularium is completely spiniform in frontal view; b) *C. poblano* has the ventral armature on femur IV well developed, sharp, whereas on *C. psilocybe* femur IV lacks ventral armature (Figs. 30, 31); and c) the apical margin of the penis is very concave (Figs. 33, 34), whereas in *C. poblano* it is convex, forming a rounded apex.

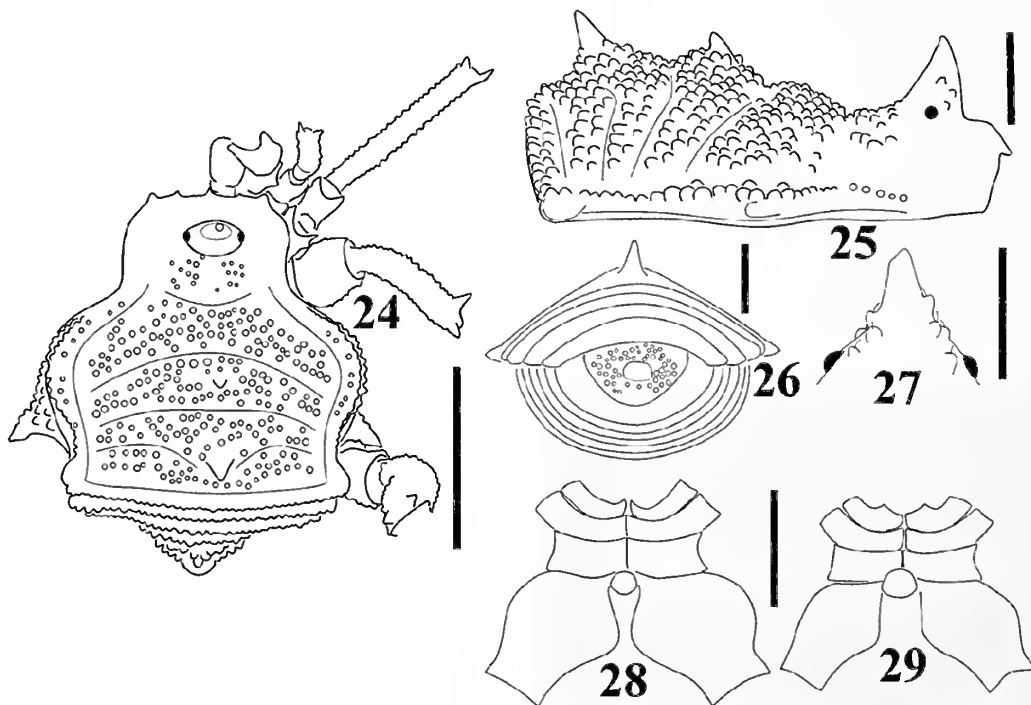


Figures 21–23.—*Chapulobunus asper* new species, male paratype, genitalia. 21. Dorsal view; 22. Ventral view; 23. Lateral view. Scale: 100 μ m. S = stylus, F = follis, D, E, C and A indicate setal groups.

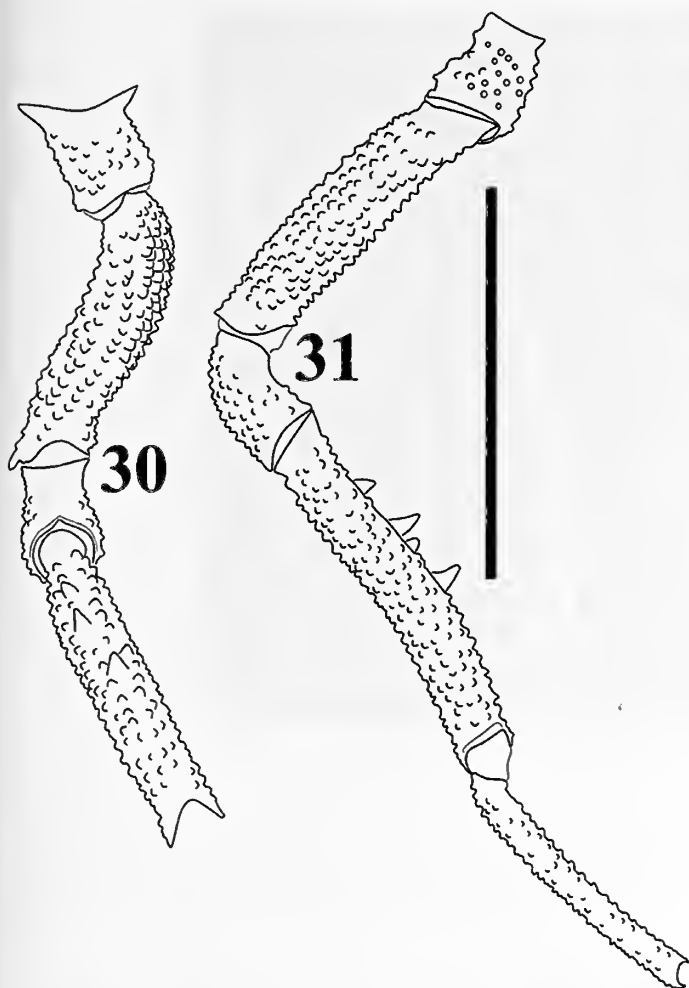
Description.—*Male holotype*: measurements: scutum length 3.97, scutum maximum width 3.95, pedipalp 0.60/1.25/0.75/0.95/0.75/0.55, legs I 0.35/1.70/0.75/1.35/1.40, II 0.65/2.47/0.85/2.17/1.70, III 0.90/2.30/0.90/2.00/2.25, IV 1.00/2.80/1.15/3.25/2.75.

Dorsum: covered by rounded tubercles throughout. Carapace with tubercles only on posterior portion. Ocularium

height/width ratio = 0.9, triangular-shaped in frontal view, dorsally forming an acute spine. Lateral pegs forming a discontinuous row, pegs similar to the dorsal tubercles. With eight to ten extra lateral pegs, some bifurcate. Central spine of area II small, height of central spine of dorsal area III = 0.45, height spine III/II ratio = 3, height spine III/ocularium ratio = 0.60, spine III pointing backwards. The first and second portions of



Figures 24–29.—*Chapulobunus psilocybe* new species, male holotype and female. 24. Male habitus in dorsal view; 25. Male habitus in lateral view; 26. Male habitus in posterior view; 27. Male ocularium in frontal view; 28. Male habitus in ventral view; 29. Female habitus in ventral view. Scale bars: Figs. 24, 28, 29 = 2.0 mm; Figs. 25, 26 = 0.5 mm; Fig. 27 = 0.7 mm.



Figures 30 and 31.—*Chapulobunus psilocybe* new species, male holotype. 30. Trochanter to tibia IV in ventral view; 31. Trochanter to metatarsus IV in ectal view. Scale bar = 1.0 mm.

lateral clear areas are not projected beyond cuticular surface, contiguous with numerous slots; third clear area rounded and projected, on the corners of area V (Figs. 24–28, 32). Free tergites covered by rounded tubercles. Corners of free tergites I and II with very small, clear, rounded projections.

Venter: covered similarly to dorsum. Stigmatic region plus sternum forming inverse “T,” shaft of “T” slightly constrained in the middle, arms curved posteriorly (Fig. 28). Coxae IV very large; with small, dorso-ectal apophyses. Genital operculum rounded. Free sternites and anal plate covered by tubercles similar to dorsum, central portion of anal plate with noticeable rounded bulge (Fig. 26).

Chelicera: Small, bulla not developed; fixed finger with three small teeth, movable finger with a serrula.

Pedipalp: Trochanter globular, with small dorsal bulge. Femur cylindrical; dorsally covered by small tubercles; ventrally with one row of five setiferous tubercles, all of similar size, with one setiferous tubercle on meso-apical side. Patella ornate with small tubercles, mesal side with two setiferous tubercles. Tibia dorsally covered by small tubercles, with iIII tubercles on ectal side ($2 = 3 > 1$), the basalmost separate from the other two, liIII on mesal side ($3 > 1 = 4 > 2$). Tarsus conical, without

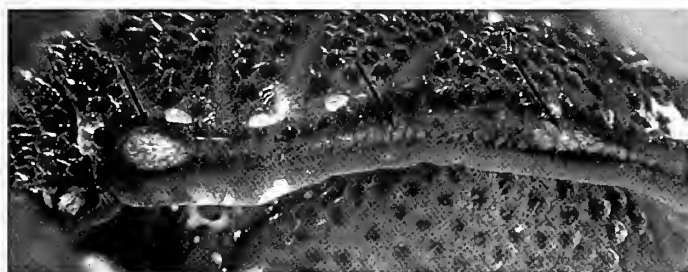


Figure 32.—*Chapulobunus psilocybe* new species, male holotype. Detail of clear lateral areas of scutum, holotype. Numbered arrows indicate the clear areas.

ornamentation, with III setiferous tubercles on both sides ($1 = 2 = 3$). Claw slightly curved, shorter than tarsus.

Legs: All segments except metatarsi and tarsi ornate with rounded tubercles, metatarsi and tarsi covered by small tubercles. Legs III and IV slightly stronger than legs I and II. Femora II to IV with two dorso-apical spines, retrolateral slightly larger than prolateral. Femur IV ornate with small, rounded tubercles similar in size. Tibia with ventral row of non-contiguous spiniform tubercles, heterogeneous in size. Metatarsus IV slightly curved (Figs. 30, 31). Trochanter III globose. Trochanter IV cylindrical, covered by small tubercles. Tarsal count 4(2):5(3):6:6.

Genitalia: Pars distalis swollen, apical margin concave, lateral apex irregular and dentate. Follis robust, latero-apical projections conical, slightly curved, very robust, inner side covered by small spiniform projections. Setae C group forming a longitudinal row of six macrosetae, the two basalmost at the same level. Setae A group forming a longitudinal row of four setae, the middle pair close to each other, the basalmost smaller. Setae D group with one/two pairs of macrosetae, basal to follis. Setae E group with three pairs of microsetae, the two more apical in a horizontal position, the basalmost slightly displaced to the middle of pars distalis. (Figs. 33–35).

Color: In alcohol this species is reddish brown (PMS 1545), under light of the microscope seems PMS 1525 color (Fig. 10). Apical portion of legs is slightly lighter. Tips of dorsal spines II and III, and lateral clear areas are yellowish (PMS 125).

Female: measurements: scutum length 4.05, scutum maximum width 3.65, pedipalp 0.52/1.25/0.75/0.95/0.75/0.55, legs I 0.42/1.60/0.75/1.25/1.27, II 0.57/2.25/0.95/1.85/1.60, III 0.87/1.95/0.80/1.97/1.82, IV 0.95/2.70/0.97/2.65/2.70. Differs from the male in ventral armature of tibia IV, all ornamentation is formed by small rounded tubercles; absence of the central bulge on anal plate, and venter with shaft of inverse “T” very wide (Fig. 29).

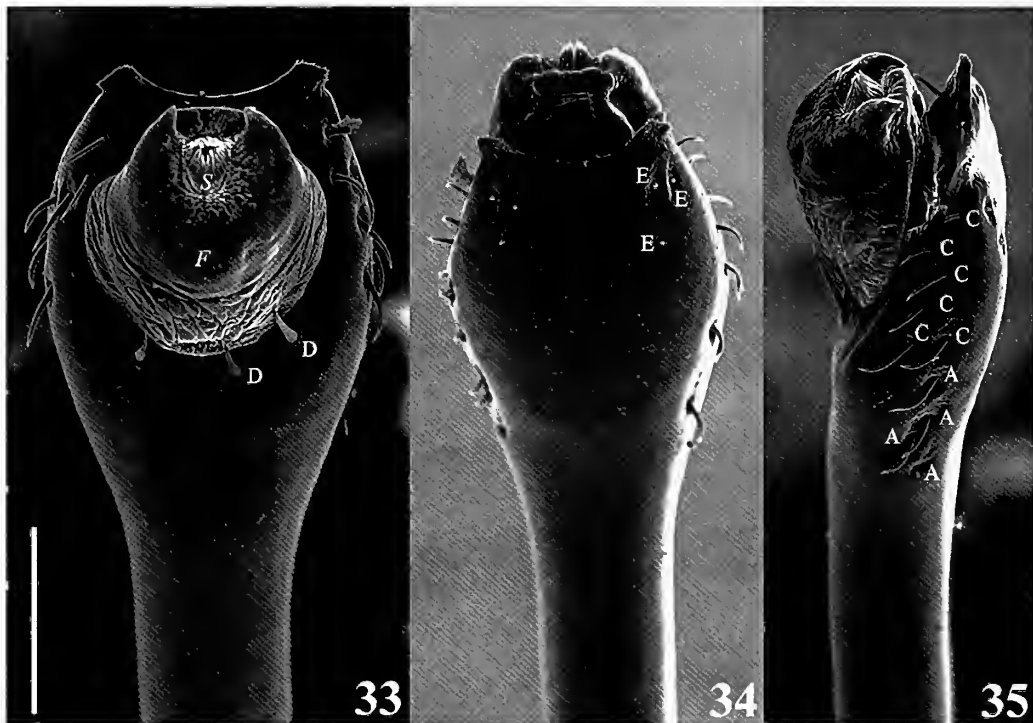
Distribution.—Only known from the type locality (Fig. 48).

Remarks.—As in its other congeners, this species showed similar defensive behavior (thanatosis) when first observed in the field. The holotype was found in aggregation with three undetermined *Hoplobunus* sp. (Stygnoptidae).

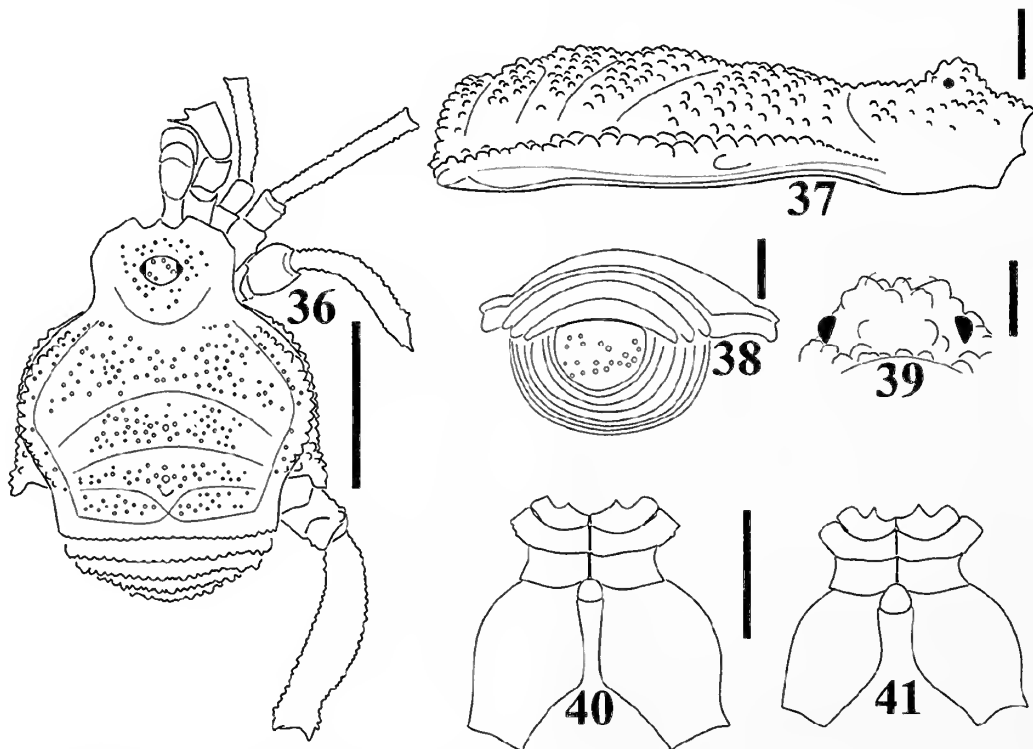
Chapulobunus regionmontano new species

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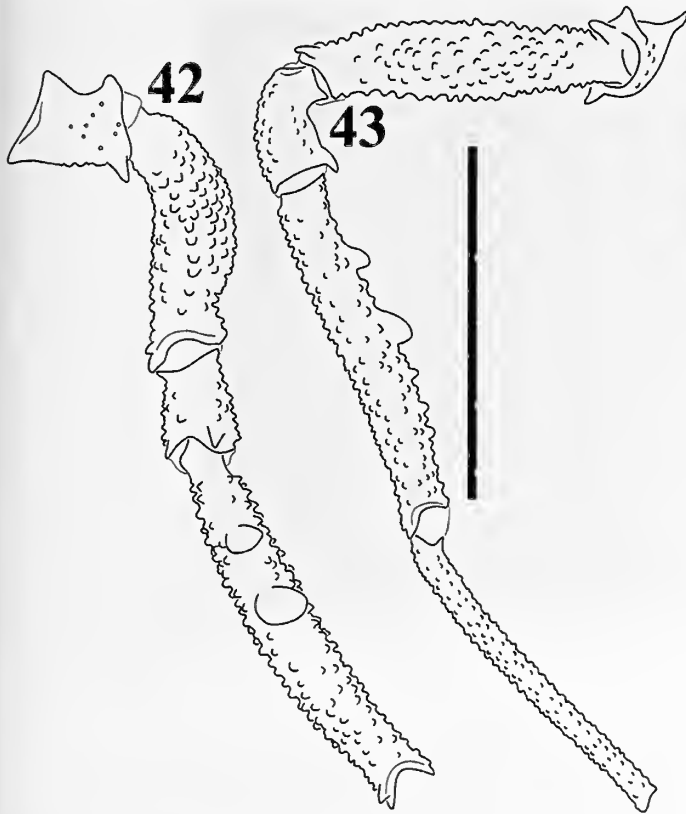
(Figs. 11, 36–47)



Figures 33–35.—*Chapulobunus psilocybe* new species, holotype, male genitalia. 33. Dorsal view; 34. Ventral view; 35. Lateral view. Scale: 100 μ m. *S* = stylus, *F* = follis, *D*, *E*, *C* and *A* indicate setal groups.



Figures 36–41.—*Chapulobunus regiomontano* new species, male holotype, female paratype. 36. Male habitus in dorsal view; 37. Male habitus in lateral view; 38. Male habitus in posterior view; 39. Male ocularium in frontal view; 40. Male habitus in ventral view; 41. Female habitus in ventral view. Scale bars: Figs. 36, 40, 41 = 2.0 mm; Figs. 37, 38 = 1.0 mm; Fig. 39 = 0.5 mm.



Figures 42 and 43.—*Chapulobunus regiomontano* new species, male holotype. 42. Trochanter to tibia IV in ventral view; 43. Trochanter to metatarsus IV in ectal view. Scale bar: 0.8 mm.

Material examined.—*Holotype*: MEXICO: *Nuevo León*: male: 2 km W. of 18 de Marzo (24°54'04.320"N, 100°11'24.899"W), Municipio de Galeana, 28 November 2013, O. Francke, A. Valdez, J. Cruz, D. Barrales and A. Guzmán (CNAN-T0841).

Paratypes: MEXICO: *Nuevo León*: 1 male, 3 female, same locality and data as holotype (CNAN-T0842).

Other material: MEXICO: *Nuevo León*: 1 male and 1 incomplete female, DNA vouchers (DNA-Op0093 and DNA-Op0094), 2 km on road to Mina la Huiche (24°41'33.719"N, 100°03'19.908"W), Municipio de Galeana, 28 November 2013, O. Francke, A. Valdez, J. Cruz, D. Barrales and A. Guzmán.

Etymology.—The people from the capital of the state of Nuevo León, Monterrey, Mexico are called *regios* or *regiomontanos*. The specific epithet is used as a noun in apposition and is masculine in gender.

Diagnosis.—This species is easily distinguished from the other four because the ventral tubercles on tibia IV are foliose (Figs. 42, 43), whereas on the others they are spiniform. Additionally, the median spine is absent on mesotergal areas II and III on *C. regiomontano* (Fig. 37).

Description.—*Male holotype*: measurements: scutum length 3.50, scutum maximum width 3.35, pedipalp 0.47/1.00/0.60/0.80/0.60/0.45, legs I 0.45/1.35/0.55/1.15/1.35, II 0.50/2.15/0.75/1.70/1.72, III 0.62/1.77/0.75/1.67/1.95, IV 0.70/2.57/0.85/2.75/2.80.

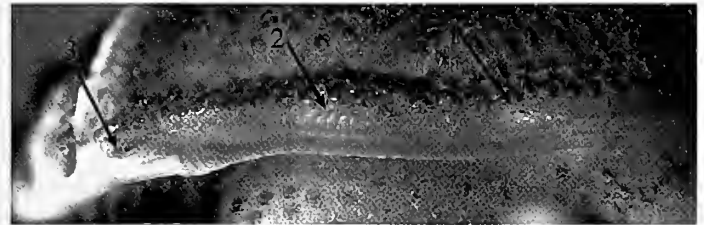


Figure 44.—*Chapulobunus regiomontano* new species, male holotype. Detail of clear lateral areas of scutum, holotype. Numbered arrows indicate the clear areas.

Dorsum: Entire dorsum covered densely by rounded tubercles. Ocularium small, height/width ratio = 0.2, apical spines minute. Lateral pegs rounded, in a discontinuous line. Central spines of areas II and III absent, with only two vestigial, blunt tubercles. Extra lateral pegs formed by 11 to 12 spiniform tubercles, along lateral margin, between carapace and area II. Lateral clear areas in three portions: first, on middle of lateral margin, teardrop-shaped, inconspicuous and slightly projected; second with numerous slots, discrete; third on corners of area V, small, rounded and slightly projected. Free tergites covered by tubercles similar to dorsum. Corners of free tergites I and II with small, clear rounded projections (Figs. 36–39, 44).

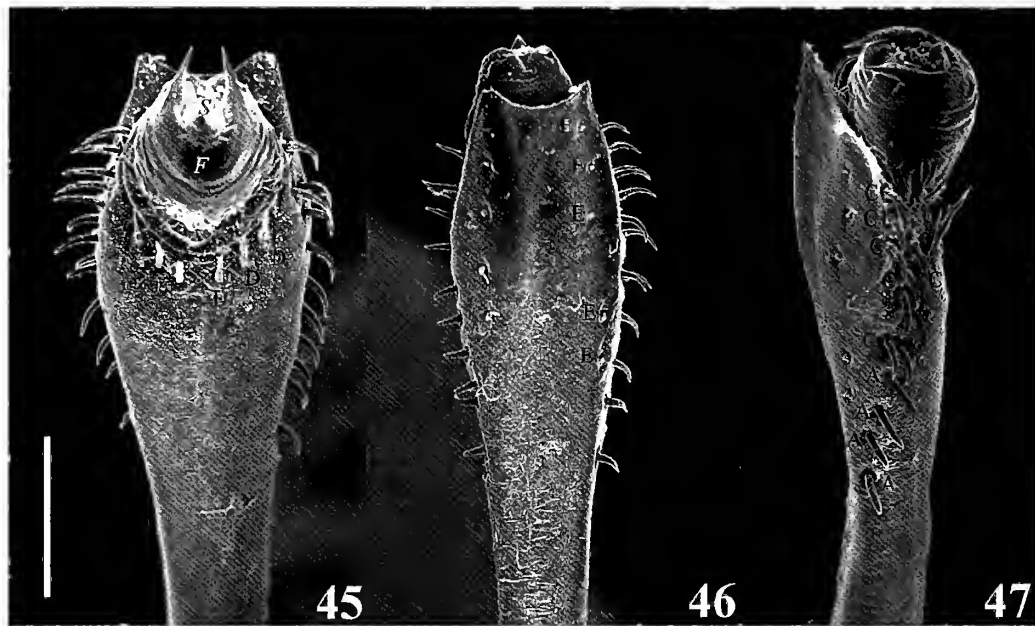
Venter: covered similarly to dorsum. Shaft of ventral inverse "T" slightly constrained in the middle, arms strong, curved posteriorly (Fig. 40). Coxae IV large, with dorso-ectal bifurcated apophysis. Genital operculum small, located between anterior portions of coxae IV. Free sternites and anal plate covered by small rounded tubercles.

Chelicera: Bulla not developed; fixed finger with three small teeth in the meso-apical portion; movable finger with a serrula.

Pedipalp: Trochanter globular, with small dorsal bulge. Femur dorsally covered by minute tubercles, ventrally with six setiferous tubercles in a row, the basalmost larger than the others; with one setiferous tubercle on meso-apical portion. Patella ornate with small tubercles, with three setiferous tubercles mesally. Tibia cylindrical, dorsally covered by small tubercles, with IIII tubercles on ectal side (4 > 5 > 2 > 3 > 1), IIII on mesal side (1 = 3 > 2 = 4). Tarsus smooth dorsally, conical, with IIII setiferous tubercles on both sides (1 = 2 = 3 > 4). Claw shorter than tarsus.

Legs: all segments covered by rounded tubercles, except metatarsus and tarsus that are covered by small tubercles and setae. Legs III and IV slightly stronger than I and II. Femora III and IV with two dorso-apical spines, both very small, retrolateral slightly larger than prolateral. Femur IV covered by tubercles throughout, without ventral armature. Tibia IV curved, ventrally with two mesal, foliose tubercles (Figs. 42, 43). Metatarsus IV slightly curved. Trochanter III globose, but slightly bigger than other trochanters. Trochanter IV cylindrical, with one dorsal and one ventral spiniform tubercles. Tarsal count 4(2):6(3):6:6.

Genitalia: Pars distalis slightly swollen, apical margin concave, lateral apex rounded. Follis wide basally; latero-apical projections tall, spiniform; inner side of follis, around the stylus, covered by small spiniform projections. Setae C group formed by longitudinal row of eight macrosetae, the median and basalmost setae forming subgroups of two or three setae. Setae A group forming a longitudinal row of four macrosetae, the



Figures 45-47.—*Chapulobunus regiomontano* new species, male paratype genitalia. 45. Dorsal view; 46. Ventral view; 47. Lateral view. Scale: 100 μ m. *S* = stylus, *F* = follis, *D*, *E*, *C* and *A* indicate setal groups.

basalmost slightly smaller and separated from the remaining *A* setae. Setae *D* group with three macrosetae, at the base of follis. Setae *E* group with two longitudinal rows of five microsetae, near to lateral margin of pars distalis. The three apical *E* setae separated from the two basalmost pairs (Figs. 45-47).

Color: In alcohol, this species is brown (PMS 1535), under light of the microscope matches PMS 160 color (Fig. 11).

Apical portion of legs slightly lighter. Tips of dorsal spines II and III, and lateral clear areas are yellowish (PMS 106).

Female paratype: measurements: scutum length 3.47, scutum maximum width 3.17, pedipalp 0.47/0.97/0.60/0.81/0.60/0.45, legs I 0.35/1.30/0.50/1.05/1.20, II 0.47/1.82/0.65/1.55/1.65, III 0.55/1.70/0.67/1.40/1.65, IV 0.65/2.25/0.72/2.22/2.45. Differs from the male in having the shaft of inverse

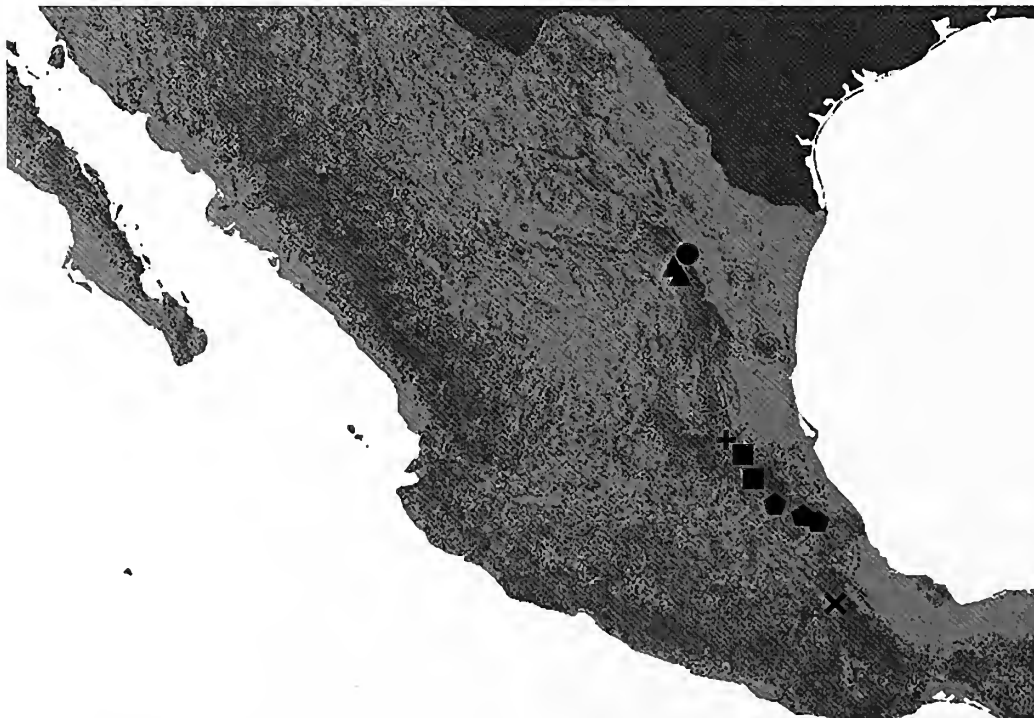


Figure 48.—Distribution map of the five known species of the genus *Chapulobunus*. ■: *Chapulobunus unispinosus* Goodnight & Goodnight, 1946; pentagons: *Chapulobunus poblano* Cruz-López & Francke, in press; +: *Chapulobunus asper* new species; ✱: *Chapulobunus psilocybe* new species; triangles: *Chapulobunus regiomontano* new species; circle: *Chapulobunus* aff. *regiomontano*.

“T” shorter and wider, and tibia IV without ventral foliose tubercles (Fig. 41).

Distribution.—Known only from the type locality and one nearby locality (Fig. 48).

Remarks.—The type species of the genus, *C. unispinosus* from Chapulhuacán, Hidalgo state, was described based only on females (Goodnight & Goodnight 1946). Subsequently, Goodnight & Goodnight (1973) examined one male and one female (TTU-Z 60,734) from “Chorros de Agua, E Rayones, 21 km WSW. of Montemorelos, Nuevo León,” and described the male but these specimens were considered erroneously as *C. unispinosus* (Cruz-López & Francke in press). These specimens were examined by Cruz-López & Francke (in press) and for the present work; unfortunately, the material is poorly preserved and the male genitalia have been lost. Externally, these two specimens are very similar to *C. regiomontano* (both from central mountains in Nuevo León), but have very small central spines on areas II and III, and on the male tibia IV has five ventral foliose tubercles. Without fresh material of both sexes from that locality, it is difficult to determine if they are conspecific with *C. regiomontano* or if they represent a new species.

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Losing legs and walking hard: effects of autotomy and different substrates in the locomotion of harvestmen in the genus *Prionostemma*

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Abstract. Autotomy, the strategy of voluntarily releasing a leg during an encounter with a potential predator or in agonistic interactions between conspecifics, is common in animals. The potential costs of this behavior have been scarcely studied. In addition, locomotion and substrate-dependent performance might be affected by autotomy. We did a comparative and observational study to investigate whether losing legs affects the escape speed and trajectory of harvestmen in the genus *Prionostemma* Pocock, 1903 (Eupnoi: Sclerosomatidae) on different substrates: soil (the least roughened), smooth bark and mossy bark (the most roughened) in a tropical premontane forest in Costa Rica. We observed that 71% of the individuals found were missing at least one leg. Harvestmen, regardless of leg condition, walked faster and made fewer turns in their trajectory in the soil. While climbing, they were faster on smooth bark than in moss. On all substrates, autotomized individuals were slower and had a more erratic trajectory than intact ones. The type of missing legs (sensory or locomotor) had no influence on the speed or trajectory. We experimentally induced autotomy and found that walking speed on soil decreases if individuals lose a leg. Our findings confirm that losing legs affects locomotion, and we provide novel insights on how locomotion in these harvestmen depends on surface roughness. Our data suggest that moss could be a type of substrate that requires more elaborate skills in balance, orientation and texture recognition than smooth bark.

Keywords: Costa Rica, Eupnoi, Opiliones, Sclerosomatidae

A wide variety of animals display defense mechanisms in which a body part or limb is voluntarily released. For instance, geckos (Congdon et al. 1974), lizards (Bellairs & Bryant 1985), scorpions (Mattoni et al. 2015) and sea snails (Lewin 1970) self-amputate their tail when they feel threatened; while some crickets (Bateman & Fleming 2005) and spiders (Eisner & Camazine 1983) release a leg, a strategy known as autotomy. Autotomy enhances survival because the detached member can distract a predator allowing the animal to escape (Maginnis 2006). However, it may have direct costs in locomotion and the biomechanical performance of animals.

Current information about the effect of autotomy on the locomotion of animals suggests long-term costs in locomotion (Bateman & Fleming 2005; Maginnis 2006; Combes et al. 2010). For example, tail loss in European wall lizards (*Podarcis muralis*) increased running speed and distance traveled but decreased their arboreal performance (Brown et al. 1995). Wrinn & Uetz (2008) studied the effect of autotomy in the North American wolf spiders *Schizocosa ocreata* (Hentz, 1844) and found a reduction in prey capture rates. Harvestmen or “daddy long-legs” (Arachnida: Opiliones) of two *Leiobunum* species from North America (*L. nigripes* Weed, 1887 and

L. vittatum Say, 1821) showed reduction in walking and climbing speed, as well as in foraging ability, after losing up to three legs (Guffey 1999; Houghton et al. 2011). In *Holmbergiana weyenberghii* Holmberg, 1876 from Uruguay, individuals missing one sensory leg walked and climbed a log slower than individuals lacking locomotor legs or individuals with all of their legs (Escalante et al. 2013). These findings suggest that lacking sensory legs may confer greater costs in orientation, balance and substrate recognition.

Based on the different biophysical properties of the environment in which animals are found (Spagna et al. 2007), a substrate-dependent change in performance is expected. Substrate roughness can affect the locomotor performance or walking speed of cursorial animals (Spagna et al. 2007; Spence et al. 2010). Spagna et al. (2007) experimentally tested how differences in a two-dimensional terrain surface affected the running speed and locomotor performance in spiders (*Hololena adnexa* Chamberlin & Gertsch, 1929), crabs (*Ocypode quadrata* Fabricius, 1787) and cockroaches (*Periplaneta americana* Linnaeus, 1758). The authors found that animals could attain high speed even when a substantial percentage of the terrain was experimentally removed (they removed up to 90% of the

surface contact area in wire mesh), altering the probability of support and consequently, the stability and speed. All these arthropods did this by changing the orientation of their legs and using leg spines to improve contact with the surface (Spagna et al. 2007). In addition, a recent study showed that a species of beetle (*Dendroctonus ponderosae* Hopkins, 1902) presented better grip walking on smooth bark than on rough bark of pine trees (Ferrenberg & Mitton 2014). Therefore, complex, intricate and three-dimensional substrates are expected to impose greater locomotor costs. However, this hypothesis remains untested. In our study, we investigated the effects of substrate complexity in natural conditions on locomotor performance of harvestmen that either had or had not suffered an autotomy event. Substrate complexity will ultimately have important consequences for biomechanical performance and fitness.

To achieve this, we tested three substrates commonly used by these harvestmen: soil, smooth bark and mossy bark. These substrates could be located in a scale of roughness with soil presenting the least roughness, smooth bark being located in the middle of the roughness scale, and mossy bark as the most roughened substrate.

Harvestmen are ideal organisms to study the effects of autotomy in locomotion because (1) autotomy is common (Roth & Roth 1984; Guffey 1999; Gnaspini & Hara 2007; Houghton et al. 2011), probably because harvestmen voluntarily release legs during a predator encounter or in male-male interactions (Macías-Ordoñez 1997; Fleming et al. 2007); and (2) they are not able to regenerate their legs (Kury 2000; Gnaspini & Hara 2007). In sclerosomatid harvestmen, legs from pair I, III and IV are used for locomotion while the longest legs corresponding to pair II are primarily (but not exclusively) used for sensory functions (Shultz & Pinto-da-Rocha 2007; Willemart & Chelini 2007; but see Willemart et al. 2009).

Here we investigate two undescribed species of *Prionostemma* Pocock, 1903 in southwestern Costa Rica. Our research questions were: (1) How frequent is autotomy in these species? (2) Do the number and type of missing legs affect locomotion? and (3) Does locomotion of harvestmen differ in substrates with different complexity? For question two, we predicted that individuals with an incomplete number of legs would walk and climb more slowly than individuals with a complete (eight) number of legs, and individuals lacking locomotor legs would walk slower than individuals lacking sensory legs. We used two subsets of individuals captured in the wild: (a) individuals with variable numbers of legs, to test the effects in wild caught autotomized individuals, and (b) an experimental subset of intact individuals in which autotomy was induced, to test the immediate effect of losing legs on locomotor performance. For question three, we expected a change in substrate-dependent performance (both in speed and trajectory) in the substrates on which they are frequently found in the field (Escalante, Dominguez and Gómez-Ruiz, unpublished data). Our experimental design allowed us to test whether there is a greater cost of being in a rough substrate, such as moss (where the studied species commonly group and roost during the day; Escalante, Domínguez and Gómez-Ruiz, unpublished data), because a non-smooth surface could hinder the escape response of all harvestmen regardless of the number of missing legs.

METHODS

Study site.—We performed trials in Las Cruces Biological Station, San Vito de Coto Brus, Puntarenas province, Costa Rica (8° 47' N, 82° 57' W), between 20–22 January 2012; and between 19–21 January 2013. The site is a 300 ha pre-montane wet forest reserve (elevation 1200 m; air temperature 13–26 °C; 4000 mm annual rainfall).

Field observations and experimental design.—We captured advanced juveniles and adult *Prionostemma* spp. harvestmen by hand along the Río Java, Melissa and Jungle trails. We did not determine the sex of individuals because this could have stressed them and affected their behavior. A total of 218 individuals were located mostly on tree trunks at 1–2 m height. Manipulation and observations were made during the day (0900–1500 hrs). We placed individuals in plastic cubic containers (30 x 15 x 15 cm) with wetted cotton, leaves and branches for a minimum of 20 min before trials to acclimate.

We recorded the number and type of missing legs prior to capture. If a leg was lost during capture we did not consider that individual for the study. We conducted two different types of trials, the first with animals that had experienced autotomy naturally, and the second in which we induced autotomy.

For our first trials, we emulated an escape event from a predator, by holding the harvestmen and releasing it in a substrate in which it could escape by either running or walking. We studied if under these conditions the locomotor performance on different substrates varied according to the quantity and type of legs. The substrates were: (1) soil, a circular area on the ground of 1 m in diameter previously cleared of leaf litter and branches, (2) mossy bark and (3) smooth bark. The latter two substrates were 90 x 30 cm quadrats on a vertical trunk with or without moss, each on a different tree of the same species that were 10 m apart. The same tree was used several times because we did not notice any behavioral indication that harvestmen were marking the substrate with chemical cues. We consider that our substrate categories reflect different roughness and complexity levels although this was not measured quantitatively.

We gently handled the harvestman by holding them by more than two legs to avoid inducing autotomy, and then carefully placed them in the middle of the experiment area, following Smith et al. (2012) and Escalante et al. (2013). Then, we measured the distance walked from the starting point to the edge of the arena, or to the place where they stopped for more than 10 s. We also measured the time spent walking in order to calculate their speed. Regardless of the substrate, we use escape speed to refer to walking (soil) or climbing (moss or bark) no matter the direction (horizontal or vertical) of the movement (Escalante et al. 2013). As another indication of their escape performance, we estimated their trajectory by measuring the number of times an individual changed its direction, and divided the number of changes of direction by the distance walked. We considered a greater number of turns to be associated with poor performance, as suggested by Escalante et al. (2013), under the assumption that maintaining a straight line during escape would be beneficial. We calculated the speed of 142 individuals, and the trajectory in 104 individuals. Due to logistic reasons, a subset of individuals only walked in the soil, whereas another subset was tested in both moss and bark, and we randomly alternated which substrate they climbed

first. We did not find an effect of order while alternating the substrate (moss or bark) on which harvestmen walked first (speed: Mann-Whitney $U = 1880.0$, $P = 0.83$; trajectory: $U = 1844.0$, $P = 0.70$). Smith et al. (2012) found leg length to be important for locomotion when comparing species of different families. Therefore, to test whether leg length affects the speed or trajectory, we measured the length (± 0.1 mm) of one leg IV of 74 individuals.

In a second subset of trials, we experimentally tested the effect of autotomy. First, those individuals were tested for walking performance on soil, as described above. Then we induced leg autotomy on 23 eight-legged harvestmen by carefully holding them upside down by the femur of a given leg, following Houghton et al. (2011). One leg of the pair II (sensory leg) was released in 10 individuals while one from pair I (locomotor leg) was released in 13 individuals. Five minutes after autotomy, we took a second measure of their walking speed on soil.

Statistical analysis.—All the analyses were conducted with STATISTICA 8.0 (StatSoft, Inc. 2007). To test whether the length of leg IV affects the speed or trajectory of individuals in the mossy bark and smooth bark substrate, we conducted simple linear regressions. We found no significant effect of leg length on speed ($r^2 = -0.08$, $P = 0.38$, $n = 124$), or trajectory ($r^2 = 0.05$, $P = 0.62$, $n = 104$), thus, we did not include leg length in further analyses of locomotor performance.

Our two measures of harvestmen locomotion (escape speed and trajectory; see above) allowed us to investigate the overall effect of autotomy. Although we found a significant negative correlation between these two variables ($r = -0.45$; $F_{1/102} = 27.29$; $P < 0.001$), we present results of both because we consider these measurements to provide a more integrative description of their locomotion. Also, both variables may elucidate the locomotor constraints that autotomized individuals experience depending on the substrate type.

To investigate the factors that influence escape speed and trajectory, we constructed models with three predictor variables: number of legs, type of missing legs (if any), and substrate where each individual walked. The type of missing leg included three categories: (a) individuals with all of their legs, (b) individuals lacking at least one locomotor leg, and (c) individuals lacking at least one sensory leg. Individuals lacking a combination of leg types (sensory and locomotor) were not considered for the trials. The substrate included three categories: soil, smooth bark, and mossy bark. We performed generalized linear models (GLM) to evaluate the effect of the type and number of missing legs and the type of substrate on speed and trajectory. These response variables were analyzed using two GLMs with normal error distribution and an identity link function. Data were tested for normality and heteroscedasticity, showing that assumptions were not violated for speed or for trajectory. For speed, data followed a normal distribution in the QQplot but Shapiro Wilks test was marginally significant ($W = 0.97$, $P = 0.05$). Also, the variance between the groups seems to be homogeneous when inspected both visually and analytically (Levenne test $F = 1.28$, $P = 0.23$). For trajectory, data seemed to follow a normal distribution in the QQplot and also in Shapiro Wilks test ($W = 0.97$, $P = 0.48$). Also, the variance between the groups seemed to be homogeneous

when inspected both visually and analytically (Levenne test $F = 1.39$, $P = 0.16$).

To test the effect of induced autotomy, we analyzed the speed before and after autotomy with a paired t-test since the differences between before and after were normally distributed (Shapiro Wilks test $W = 0.91$, $P = 0.14$). Additionally, to test if the type of missing legs affects speed immediately after autotomy we calculated the differences in the pairs (speed after and before autotomy) and carried out a t-test on the differences comparing the speed between the two treatments (lost sensory leg and lost locomotor leg).

Prionostemma is a taxonomically unresolved genus, and many papers have reported behavioral and ecological data without being able to identify them to the species level (Wade et al. 2011; Proud et al. 2012; Smith et al. 2012; Teng et al. 2012; Grether et al. 2014a, b). In our trials, we used two morphospecies (red and black: Escalante, Dominguez and Gómez-Ruiz, unpublished data), potentially two new species. However, because they did not differ in leg IV length ($T_{120} = 0.69$; $P = 0.49$) or walking speed or trajectory (walking speed: $T_{153} = -0.46$; $P = 0.64$, trajectory: $T_{100} = 0.15$; $P = 0.88$), we grouped the data of both morphospecies for our analyses.

RESULTS

We found that 29% of 157 individuals had all of their legs, 29% of the autotomized individuals lacked at least one leg from pair II (sensory legs), and a lower frequency of individuals lacked locomotor legs (pair I: 17%, pair III: 10% and pair IV 15%). These percentages are non-exclusive, meaning that individuals could be missing legs from more than one pair. Lacking a leg from pair II was more frequent than expected by chance ($X^2_3 = 21.98$, $P < 0.001$). We also found harvestmen unevenly distributed in various substrates in the field; from a total of 62 groups, 68% were in mossy trees and lianas, 13% on smooth bark, 2% on the soil, and the remaining in foliage ($X^2_4 = 91.4$, $P < 0.0001$). Additionally, we randomly chose 22 trees in which we found *Prionostemma*, and 14 (64%) of them had extensive moss cover.

Escape speed and trajectory.—Escape speed was affected by the number of legs (Fig. 1) and the type of substrate, but not by the type of missing leg (Fig. 2). Autotomized individuals had slower speed than intact individuals (GLM: Wald $X^2 = 5.93$, $df = 1$, $P = 0.01$, Fig. 1A). Also, individuals walked faster on the soil than on smooth bark, and climbed slower on the mossy bark substrate (GLM: Wald $X^2 = 21.07$, $df = 2$, $P < 0.001$, Fig. 1A). When analyzing the type of missing leg, the pattern was less clear. Lacking sensory or locomotor legs had no effect on speed (GLM: Wald $X^2 = 1.81$, $df = 2$, $P = 0.40$, Fig. 2A). Also, there was no interaction between the type of substrate and the type of missing legs on their escape speed (GLM: type of missing legs*substrate type speed [GLM: Wald $X^2 = 8.79$, $df = 4$, $P = 0.07$]).

Trajectory was also affected by the number of legs and the type of substrate (Fig. 1B), but not by the type of missing leg (Fig. 2B). Autotomized individuals made more turns in their trajectory than intact individuals (GLM: Wald $X^2 = 4.73$, $df = 1$, $P = 0.03$, Fig. 1B). Individuals made more turns per distance on mossy bark or smooth bark than on soil (GLM: Wald $X^2 = 47.66$, $df = 2$, $P < 0.001$, Fig. 1B). The number of turns did not differ with the type of missing leg (sensory

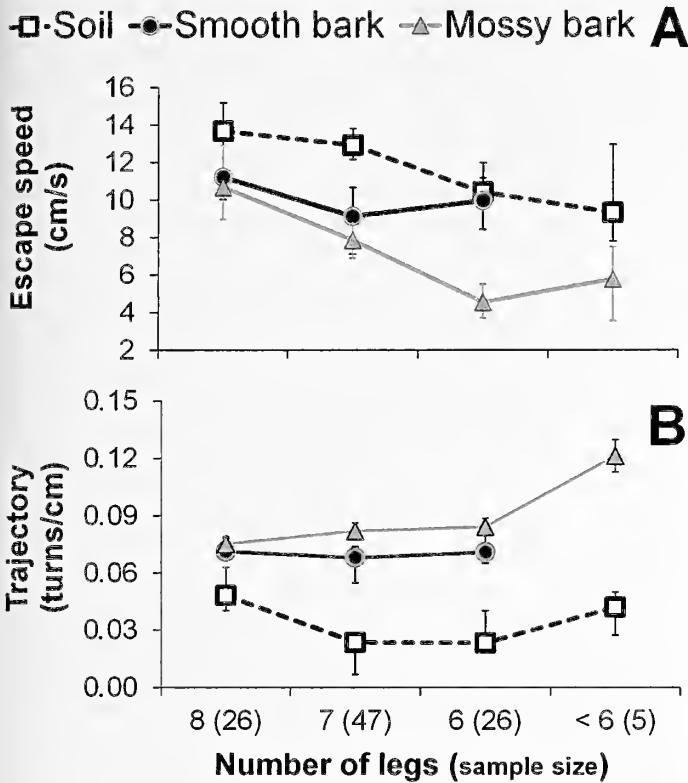


Figure 1.—(A) Escape speed mean (\pm standard error) and (B) trajectory (\pm standard error) of *Prionostemma* spp. on different substrates, according to the number of legs. The sample size for each category is included in parenthesis.

versus locomotor legs) (GLM: Wald $X^2 = 3.45$, $df = 2$, $P = 0.178$, Fig. 2B), and there was no interaction between the type of legs and the type of substrate (GLM: Wald $X^2 = 5.14$, $df = 4$, $P = 0.28$, Fig. 2B).

Pairwise comparisons, considering all individuals with and without a complete set of legs ($n = 65$), revealed that individuals climbed slower and with a more erratic trajectory (more turns) on mossy bark (mean velocity: 7.28 cm/s, mean trajectory: 0.08 turns/cm) and smooth bark (mean velocity: 9.12 cm/s, mean trajectory: 0.07 turns/cm) than on soil (mean velocity: 12.09 cm/s, mean trajectory: 0.03 turns/cm; Tukey tests $P < 0.05$) (Fig. 1).

Overall, harvestman speed decreased on average by 21% following autotomy of a leg (paired t-test = 2.33, $df = 22$, $P = 0.03$, Fig. 3). Regarding the type of leg missing, recently autotomized individuals that had lost a locomotor leg showed a greater overall change in their escape speed (mean = 6.45 cm/s) than the ones that lost a sensory leg (mean = 0.63 cm/s) (Fig. 4), although our test was marginally significant (t-test conducted with the differences before–after autotomy = 2.07, $df = 21$, $P = 0.054$).

DISCUSSION

In this study we found that harvestmen had greater speed on the soil, and climbed faster on smooth bark than on mossy bark. Furthermore, autotomized individuals were slower than intact individuals, regardless of which leg was lost. Our finding that individuals climbing on mossy bark and autotomized individuals had more erratic trajectories, demonstrates that harvestmen exhibit both substrate-dependent and autotomy-

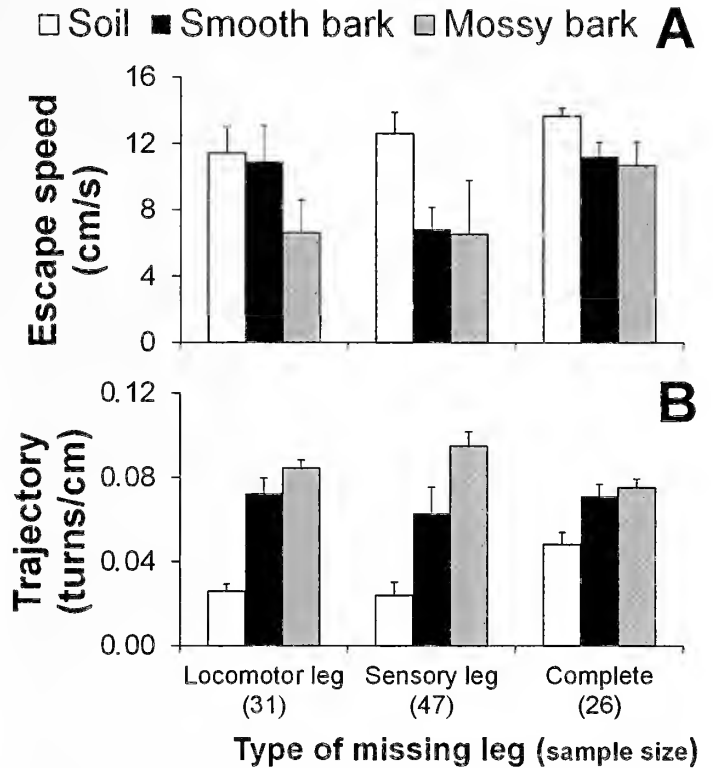


Figure 2.—(A) Escape speed mean (\pm standard error) and (B) trajectory (\pm standard error) of *Prionostemma* spp. on different substrates, according to the type of legs missing. The sample size for each category is included in parenthesis.

dependent trajectory patterns. We also found that missing legs (of any type) influenced their locomotion across all three substrates tested. All together, these results provide novel insights into the potential effect of substrate type on the locomotion performance of invertebrates. Appropriate substrate-dependent performance is likely to affect predatory avoidance

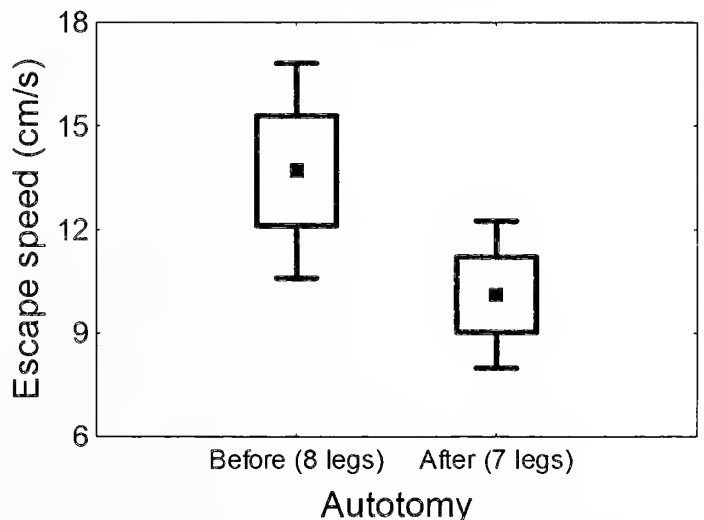


Figure 3.—Escape speed of *Prionostemma* spp. on soil before and after inducing autotomy; individuals had eight and seven legs in the trials, respectively. The same individuals were tested in both contexts, in a paired design. Box plots depict the mean (black square), standard error (box), and maximum and minimum values (whiskers).

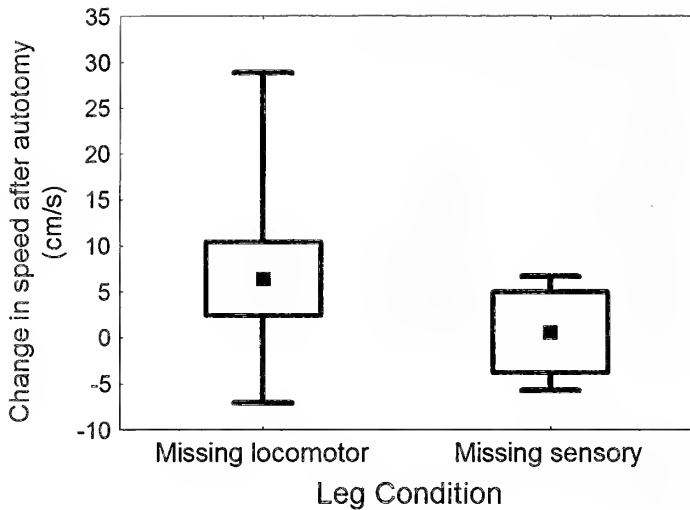


Figure 4.— Change in speed of *Prionostemma* spp. on soil after autotomy, depending on the type of leg lost. The change in speed was calculated as the difference between the speed before the experiment and the speed 5 minutes after we induced autotomy of one leg. Box plots depict the mean (black square), standard error (box), and maximum and minimum values (whiskers).

and escape success. Our results suggest that arthropods have differential performance according to different substrates, and, although speculative, we consider that this could mediate their substrate use and/or selection.

Surprisingly, our results show that *Prionostemma* spp. had worse locomotor performance on the substrate they most frequently use to group and roost during the day (mossy bark) compared with the other substrates. The complex three-dimensional structure of the mossy bark substrate might represent a more challenging surface to navigate compared to the smooth bark and soil substrates (see Spagna et al. 2007). Moreover, the potential benefits of thermoregulation and crypsis of the mossy bark substrate might be compromised if harvestmen acquire a poorer locomotor performance. However, to date information of this kind remains unknown. By studying microhabitat selection in intact and autotomized individuals and expanding on the potential benefits of the grouping sites, research will yield important insights on the costs of autotomy for animals.

Slower speed in the preferred substrate likely indicates a cost in terms of their ability to escape from predation events in the mossy bark. Thus, these harvestmen may rely primarily on their grouping ability and crypsis as anti-predatory tactics (Gnaspini & Hara 2007). Nonetheless, the predation pressure on the substrates in which they roost is unknown. *Prionostemma* spp. and other sclerosomatid harvestmen are likely to experience predation attempts during the day while roosting in either mossy bark or smooth bark substrates of trees. Additionally, they will encounter predators at night while foraging on the foliage and on the ground (soil substrate). Hence, we are confident that our trials represent biologically relevant situations in which *Prionostemma* spp. attempt to escape a predator by autotomizing legs. In our trials, we tested harvestmen in two different biomechanical contexts: horizontal and vertical locomotion; thus, it is possible that the effect of gravity in vertical substrates could partially explain why individuals walked

slower in the trees (either mossy bark or smooth bark trials) than on the soil. However, because there were also differences between the two vertical substrates (mossy bark and smooth bark), we do not consider gravity to be a confounding factor. Further studies should emphasize the possible effects of gravity by repeating these tests with all three substrates on the same plane. Furthermore, individuals made more turns per distance on mossy bark or smooth bark than on soil. This could be the result of orientation facilitated by gravity on vertical surfaces. A trade-off could be mediating locomotor performance. On the one hand, on vertical surfaces harvestmen could be investing more energy in looking for a previously used path by other individual through the detection of chemical cues (Willemart et al. 2009) or looking for leaves, twigs or branches to grab on, especially giving the prehensile tarsal capacities sclerosomatid harvestmen have (Guffey et al. 2000). On the other hand, while walking on horizontal surfaces, in this case the soil, escape to the nearest vertical surface would be prioritized, aiming to navigate in more complex three-dimensional environments wherein escape or crypsis is favored.

Our finding that autotomy was associated with a more erratic trajectory could be due to either a mechanical by-product of lacking legs, or a behavioral strategy to compensate for that loss. The differences in trajectory between intact and autotomized individuals suggest a cost to balance in individuals with different number of legs on each side of their body. Body symmetry can play an important role: having more legs on one side of the body could alter their balance by decoupling the hexapod-like alternating gait performed by sclerosomatid harvestmen (Sensenig & Shultz 2006) while walking. In the case of a behavioral strategy, a zigzagging locomotion may confuse a potential predator and increase the harvestman's probability of escape (Gnaspini & Hara 2007).

So far, little research has focused on the effect of autotomy in harvestmen and the few studies have uncovered different patterns. Previous findings indicate that leg loss causes a reduction in walking and climbing speed (Houghton et al. 2011), and foraging ability (Guffey 1999). Sensenig & Shultz (2006) showed that sensory legs do not participate directly in locomotion. In our study, we did not find any effect of leg type or leg IV length on locomotion, in contrast to the findings of Escalante et al. (2013). Potential differences in social structure and grouping behaviors, as well as environmental factors such as the roosting site, predation pressure or foraging efficiency could account for the differences found between studies.

Our experiments demonstrated that harvestmen travel at different speeds depending on the number of legs present. When we compared the escape speed of seven legged-individuals (after removing a sensory or locomotor leg) versus their initial speed, we found that a decrease in walking speed occurred, and we found partial support to the fact that losing a locomotor leg is more costly since it conveys a greater decrease in speed, contrary to Escalante et al. (2013). Overall, losing a leg seems to have an immediate effect on locomotion. Although we did not repeatedly test intact individuals to account for any variation they may exhibit in walking speeds, prior to inducing autotomy, our experimental results showed a clear pattern (a decrease in walking speed after losing a leg). The negative effect of experimentally induced autotomy on locomotion coupled with our finding that 42% of individuals lost at least a

locomotor leg prior to their capture for this study, highlights the prevalence and potentially great cost that autotomy has in locomotion, especially in the escape response.

Another finding was that lacking a sensory leg was more frequent than expected by chance. This frequent loss can be attributed to the fact that the long sensory legs are used to palpate and to obtain information about the surroundings. Spagna et al. (2007) noted that arthropods get mechanical feedback from the substrates in which they walk, which could be associated with efficient performance. Consequently, if harvestmen lose their sensory legs, they would be more exposed to a predator attack. In addition to the escape response from predators, harvestmen may also lose the legs of pair II more often simply because they are the longest, and the trochanter-femur joint must be under tension and thus, could be easier to break. The costs of losing certain legs could be asymmetrical. We found fewer individuals without legs from pair III (10%), and many individuals missing leg II and leg I (29% and 17%, respectively). In this study we induced autotomy to a leg I or II in order to have an appropriate comparison and sample size, and also because those were the two most frequently lost legs. However, the findings concerning leg I should not be extrapolated to the other locomotor legs (III and IV). The asymmetry in autotomy might suggest differential costs of losing legs I, III and IV. For instance, during the alternate tripod gait, leg III of one side moves simultaneously with legs I and IV from the other side (Sensenig & Shultz 2006). If any leg III is to be lost, the coordination and performance of the locomotion would potentially be more affected than if a leg I or IV were lost. So, according to our results, leg III is lost less frequently but this could be because it is the most expensive to lose because it may be more crucial for walking than is a leg I or IV, or simply because it is the shorter and less accessible (Shultz & Pinto-da-Rocha 2007). Future research should focus on the importance of each leg in locomotion biomechanics, as well as to test how willing harvestmen are to release legs from each pair (our preliminary observation suggests asymmetry in that trait also), in order to elucidate the context-specific costs of autotomy.

Additionally, further research on the effects of autotomy on sexual behavior is needed in order to understand the fitness consequences of this anti-predatory tactic. Fowler-Finn et al. (2014) found that the access to mating by males of the North American sclerosomatid *L. vittatum* was mediated by their courting behavior, particularly tapping and grabbing the female's leg II with legs III. Therefore, missing legs can have negative consequences for Eupnoi harvestmen. However, another sclerosomatid (*Nelima paessleri* Roewer, 1910) was seen mating when both male and female were missing legs (Escalante, unpublished data). Future research can provide specific information on how the fine scale details of the courtship, mating, and sperm transfer are affected by autotomy, in both sexes. Even though autotomized harvestmen can mate, probably the rate of mating success, the time spent in courting and other reproductive tasks are compromised.

In summary, impaired locomotor performance likely translates into decreased survivorship or decreased ability to forage/capture prey or escape from predators. Harvestmen seem to use autotomy to increase their survival (Gnasplini & Hara 2007); however, our findings implicate a tradeoff cost in their escape speed. Our research contributes to the understanding

of the prevalence of autotomy in *Prionostemma* spp. harvestmen and of how the number of missing legs and the substrate type affect locomotion. By comparing the speed and trajectory of individuals with different numbers of legs in various natural environments, this study provides the first description of the effect of substrate in locomotion performance.

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SHORT COMMUNICATION

Black bears feed on harvestmen (Opiliones) in northwestern Mexico

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Abstract. We report on the feeding by black bears on harvestmen (Opiliones). Two scats of black bears from Sonora, Mexico each had over 50 bodies of a species of *Leiobunum* C.L. Koch, 1839 collected in localities separated by 5 km. Microsatellite analysis indicated that the scats were from different individuals. That all harvestmen were found in only two of the 180 scats sampled suggested that the two bears fed on harvestmen while in aggregations. Because black bears are probably minimally affected by defensive compounds and would presumably not actively search for individual harvestmen, black bear predation on harvestmen, if frequent enough, would be a factor selecting against aggregative behavior in these arachnids in the study area. Further research is needed on the aggregation behavior and other natural enemies of harvestmen in northwestern Mexico.

Keywords: Opiliones, predation, black bears, Mexico

Harvestmen (Opiliones) are a well-known order of Arachnida found in almost all terrestrial ecosystems. Although generally not considered a numerically dominant taxon within the arthropods, Opiliones are relatively common and can have diverse communities, especially in tropical habitats (Proud et al. 2012), as well as having many and complex interactions with other organisms (Cokendolpher & Mitov 2007). Of these, the predators of harvestmen include many reported arthropods and vertebrates comprising many bird species, reptiles and small to medium sized (< 20 kg) mammals (Cokendolpher & Mitov 2007).

In the present paper, we report on the predation of aggregations of harvestmen by black bears (*Ursus americanus*). This finding was first made during a study of the population genetics and ecology of black bears in the state of Sonora in northwestern Mexico, and was previously cited in a list of prey items (Sierra Corona et al. 2005), although no mention or discussion of Opiliones was included in the text. The study site is one of the southernmost populations of this large carnivore, located within the “El Pinito” Ranch in the Sierra de San Luis, Sonora, Mexico, with the northwestern corner located at 31.20° N and 109.94° W and the southeastern corner at 31.12° N and 108.82° W. Sampling was carried out during April–November of 2002. A grid of camera traps was established to identify and determine population structure and density of black bears, and scats were collected and analyzed. Scats were used to determine feeding habits, as well as provide genetic sequences to identify individuals. These genetic sequences were obtained by amplifying and sequencing 10 microsatellites previously used for black bears (G10B, G10H, G10L, G10M, G1A, G10J, G1D, G10O, CXX20, and G10X). The software program Cervus 3.0 (Marshall et al. 1998) was used to determine individual identity, attributing the same individual identification only to scats with identical alleles. All samples of Opiliones are stored as vouchers in the Zoological Collection of the Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro, Querétaro, Mexico.

Two of a total of 180 black bear scats were found with fragments of harvestmen: the first with 62 bodies (prosoma + opistosoma, without legs) on 2 July 2002, and the second with 123 bodies on 10 July 2002. All bodies were identified as a single species of *Leiobunum* C.L. Koch, 1839 based on the key by Morales (1980) and through comparisons with species descriptions (Goodnight & Goodnight 1942, 1945, 1946, 1947). A species determination was impossible to make due

both to how distorted the body parts were (Fig. 1) and to the lack of keys for this speciose genus in northern Mexico. Both scats were found in oak-pine vegetation and were separated by 5 km. Analysis of microsatellites from the scats indicated that they were from different individuals. Varas (2010) determined that the population of black bears at the study site was composed of a minimum of 33 individuals at the time of the study.

That all fragments were concentrated in two scats suggests that both black bear individuals fed on aggregations of this *Leiobunum* species. Aggregation of harvestmen is common for many species and has been reported for 10 species of the genus *Leiobunum* (Cokendolpher 1988; Machado & Macías-Ordóñez 2007). Within the study site, large aggregations were frequently observed along dry river canyons. It is unknown whether bears eat harvestmen only as the result of chance encounters with aggregations or whether bears initiate active, directed searches for the aggregations under certain conditions.

Of the many hypotheses to explain why harvestmen aggregate, improved defense against natural enemies is frequently proposed. Machado & Macías-Ordóñez (2007) group the defensive hypotheses into three categories based on the possible differences in predation rates of individuals alone or in aggregation. These include: 1) increased concentrations of scent gland defensive secretions in aggregations, 2) augmentation of escape capabilities aided by alarm signals communicated within aggregations, and 3) reduced probabilities of being encountered by a predator due to dilution effects of aggregations when compared to more dispersed populations. Given the large size of black bears and the lack of adaptations to capture small prey, the cost-benefit ratio for bears feeding on individual harvestmen is almost certainly not favorable. Feeding on large aggregations however may result in a net gain of energy and/or nutrients relative to the energy expended. As for defensive compounds, those of Opiliones probably have little effect on such a large carnivore given the variety of other arthropods with noxious chemical defenses that are reported as food items (Rodríguez-Martínez et al. 2008; López-González et al. 2009). These arguments suggest that predation by black bears on Opiliones in northern Sonora would be a factor selecting for non-aggregative behavior in harvestmen if the rate of bear predation is not balanced by other factors favoring aggregation. More studies are needed to investigate the densities of harvestmen in the study region, the periods and locations



Figure 1.—Fragments of *Leioibunum* sp. from a scat of black bear (*Ursus americanus*) from Sonora, Mexico.

of aggregative behavior, and the frequency of predation on aggregated Opiliones by black bears.

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SHORT COMMUNICATION

The South African scorpion *Pseudolychas ochraceus* (Hirst, 1911) (Scorpiones: Buthidae) can reproduce by parthenogenesis

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Abstract. Of all scorpion species described to date, only a small fraction are known to reproduce without fertilization by a male, instead producing offspring by parthenogenesis. Here we show that isolated females of the buthid *Pseudolychas ochraceus* (Hirst, 1911) are capable of parthenogenetic reproduction and we provide data on the postembryonic growth of this species.

Keywords: Arachnida, scorpions, asexual reproduction, postembryonic and embryonic development

Sexual reproduction is the predominant reproductive strategy in multicellular eukaryotes and as such the vast majority of animal species develop from a zygote produced by the fusion of a female and a male gamete (Williams 1975). Parthenogenesis, the development of offspring from unfertilized eggs, occurs in diverse invertebrate and vertebrate taxa, albeit at a low frequency (White 1978). With the exception of mites in which various types of parthenogenetic reproduction are known to occur and parthenogenesis is prevalent in certain families (Oliver 1971; Sabelis 1985), asexual reproduction is quite rare in other arachnid orders (Bell 1982). Parthenogenesis is known to occur in several species of spiders (Edwards et al. 2003), harvestmen (Tsurusaki 1986), amblypygids (Armas 2000; Weygoldt 2005, 2007; Seiter & Wolff 2014) and scorpions (review in Francke 2008; Lourenço 2008), and is strongly suspected to occur in some species of pseudoscorpions (Dashdamirov & Golovatch 2005), schizomids (Nedvěd et al. 2011; Zawierucha et al. 2013) and palpigrades (Christian & Christophoryová 2013) based on the observation of all-female populations or a pronounced scarcity of males. Although the majority of claims about parthenogenetic reproduction in scorpion species were proven by experimental evidence, some are based on field observations or uneven sex distributions in samples of collected species alone (Francke 2008; Lourenço 2008). During a revision of the scorpion genus *Pseudolychas* Kraepelin, 1911, Prendini (2004) observed only five males in 115 preserved specimens of *Pseudolychas ochraceus* (Hirst, 1911) and suggested that this species might be capable of asexual reproduction. By rearing captive-born female specimens under isolation until maturation and observing that these gave birth to an all-female brood without being inseminated, we show that females of *P. ochraceus* from a population located in the vicinity of Onderstepoort, Pretoria, Gauteng Province in South Africa are capable of parthenogenetic reproduction.

All specimens were collected near Onderstepoort, Pretoria, Gauteng Province in South Africa and were found under stones, bark and leaf litter in humid microhabitats. Captive-born specimens were kept in isolation as soon as they left their mother's back after reaching the second instar. Specimens were kept in plastic terraria of different sizes using standard methods. The enclosures contained a 1 cm deep layer of soil-sand mixture and pieces of bark for the specimens to hide among. Food consisted of nymphs of *Acheta domesticus* (Linnaeus, 1758). All specimens were reared under the same conditions ($29 \pm 1^\circ\text{C}$, $35 \pm 5\%$ relative humidity and 16:8 h L:D photoperiod) and fed in intervals of seven days. Dead specimens were stored in ethanol (70% solution)

and deposited in the Natural History Museum Vienna, Austria (NHMW 27605). Specimens were studied, measured, and photographed with a stereomicroscope (Leica M205A) equipped with a Leica DFC420 camera. Digital images were processed using Adobe Photoshop® 8.0 to optimize contrast features of the micrographs. The specimens were identified using the key by Prendini (2004) and the original first description by Hirst (1911). The sex was determined by the presence or absence of enlarged first pectinal teeth, a dimorphic trait observable in all postembryonic stages of this species (Fig. 1).

Our results derived from five wild-caught second instar females. They were reared in isolation in captivity. One of them reached maturity (F0) and gave birth to two female offspring (F1), which after reaching adulthood gave birth to all-female litters of 9 and 15 offspring (F2), respectively. Three F2 specimens reached adulthood, and produced three all-female litters of one, two and three offspring. One female gave birth after 217 days following final ecdysis ($n = 1$). The first instar specimens molted an average of 12 days after birth and consecutive moltings took place after 95 days (instar III), 135 days (instar IV), 205 days (instar V) and 248 days (instar VI, adult) of postembryonic growth ($n = 2$).

In a critical review, Francke (2008) proposed that the parturition of a captive isolated female collected immature in the wild is the minimal evidence required to conclude that a scorpion species is parthenogenetic. Furthermore, Francke (2008) discusses previous claims of the occurrence of parthenogenesis in scorpion species and presents arguments why establishment of parthenogenesis should not be based on parturition of immature wild-caught specimens alone. Two key arguments are a reported case of a post-parturition molt in *Tityus uruguayensis* Borelli, 1901 (Toscano-Gadea 2001) and the common occurrence of iteroparity (Polis & Sissom 1990), which could cast doubt on the establishment of parthenogenesis by the parturition of specimens that were not entirely raised under conditions of isolation. Although other post-parturition molts have never been observed in scorpions, further confirmation of such an event would have serious implications on establishing parthenogenesis based on parturition of wild caught specimens. Therefore, we follow the more stringent criteria proposed by Francke (2008) to confirm the previously suspected parthenogenesis in *P. ochraceus* (Prendini 2004): raising captive born females to maturity in isolation and showing that these can reproduce without being inseminated. Following these criteria, parthenogenesis was previously established for seven buthid and one hormurid scorpion species to

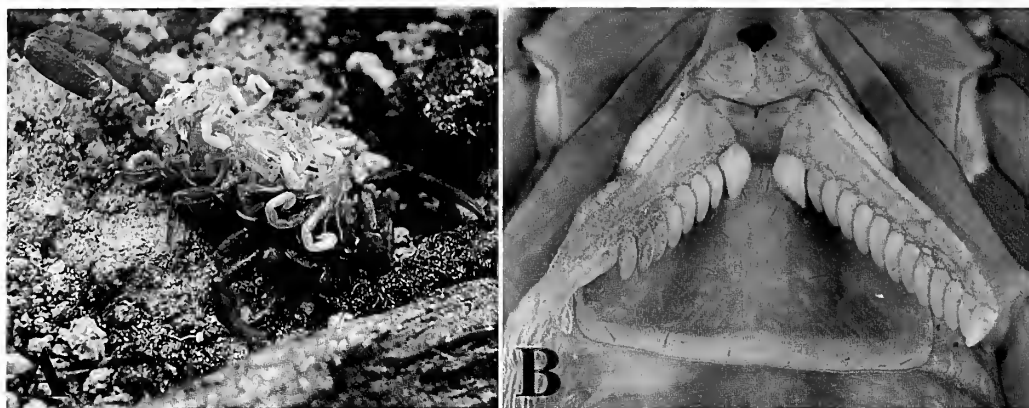


Figure 1.—*Pseudolychas ochraceus* specimen in captivity. A. Adult female with second instar offspring. B. Pectinal area with the elongated first pecten, characteristic of females. Photos by Jonas Wolff.

which we now add *P. ochraceus* as the eighth buthid scorpion of which parthenogenetic reproduction was shown by parturition of a captive-born female raised in isolation (Tab. 1) We note that the description of parthenogenesis in *Tityus stigmurus* (Thorell, 1876) by Ross (2010) leaves little room to question the ability of this species to reproduce parthenogenetically, however, the females used in this study were only isolated in a subadult state.

Based on our data, we cannot say whether the *P. ochraceus* specimens collected at Onderstepoort for our study belong to an all-female population reproducing exclusively by parthenogenesis. However, Prendini (2004) points out that most of the preserved specimens available in museum collections (of which ~96% are female) were collected in suburban habitats of the major South African cities of the Gauteng conurbation where this species is abundant. In the regions outside of the cities less influenced by human activities this species is more rarely found and in some cases was never recorded. This could indicate that sexually reproducing populations of *P. ochraceus*, in which females could be facultatively parthenogenetic, predominantly occur in sparsely sampled non-urban habitats, whereas asexual all-female populations are present in suburban habitats; a population distribution pattern

Table 1.—Scorpion species in which the capability to reproduce by parthenogenesis was established by fulfillment of the criterion that a captive-born female raised to adulthood in isolation gives birth. Within Buthidae, species are given in chronological order by record year.

Species	Record of parthenogenesis
Hormuridae Laurie, 1896	
<i>Liocheles australasiae</i> (Fabricius, 1775)	Yamazaki & Makioka (2004)
Buthidae (Koch, 1837)	
<i>Tityus serrulatus</i> Lutz & Mello, 1922	Matthiesen (1962)
<i>Tityus uruguayensis</i> Borelli, 1901	Zolessi (1985)
<i>Tityus trivittatus</i> Kraepelin, 1898	Toscano-Gadea (2004)
<i>Hottentotta hottentotta</i> (Fabricius)	Lourenço & Ythier (2007)
<i>Hottentotta caboverdensis</i> Lourenço & Ythier, 2006	Lourenço, Ythier & Cloudsley-Thompson (2007)
<i>Tityus neblina</i> Lourenço, 2008	Lourenço & Cloudsley- Thompson (2010)
<i>Tityus confluens</i> Borelli, 1899	Seiter (2012)
<i>Pseudolychas ochraceus</i> (Hirst, 1911)	This study

which would represent a case of geographic parthenogenesis as described by Vandel (1928).

This explanation is further substantiated by the observation that parthenogenetic reproduction facilitates dispersal by human activities, as only one specimen has to be introduced to a new habitat to found a new population. Many spiders introduced to European greenhouses are parthenogenetic and populations of introduced schizomids and palpigrades apparently exclusively consist of females (Nedvěd et al. 2011; Christian & Christophoryová 2013; Zawierucha et al. 2013). A well-known example for the potential of fast and effective dispersal of a parthenogenetic scorpion is the South American buthid species *Tityus serrulatus* (Lutz & Mello, 1922), which originally occurred only in a restricted region of the Brazilian state Minas Gerais but nowadays has widely spread to cities throughout the country (Lourenço & Cloudsley-Thompson 1996). Therefore, facilitated dispersal of parthenogenetic reproducing populations is an attractive explanation of why only females of *P. ochraceus* were collected in cities situated in regions where this species does not occur naturally. It would be interesting to test whether females from presumably all-female populations can reproduce with males from sexual populations to learn whether parthenogenesis in these populations is obligate or facultative.

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SHORT COMMUNICATION

Ecology and biogeography of the endemic scorpion *Euscorpium carpathicum* (Scorpiones: Euscorpiidae): a multiscale analysis

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Abstract. We present a first analysis of the ecology and potential distribution of *Euscorpium carpathicum* (Linnaeus, 1767), a scorpion species endemic to southern Romania, and report on the overwintering habitat selection of this species. Using field data, literature review, species distribution modelling, and habitat selection models, we document the broad scale distribution and ecology of *E. carpathicum*, as well as habitat selection in the foothills of the Curvature Carpathians, including exclusive microhabitat selection of riverine clay banks. In contrast with other species of the genus that inhabit cracks in cliffs or walls, *E. carpathicum* has adapted to cracks in clay.

Keywords: Carpathian scorpion, habitat selection, potential distribution, overwintering ecology, temperate region

Scorpions are a diverse group of invertebrates, with over 1,200 described species. They inhabit all main terrestrial habitats, reaching a maximum species richness in subtropical areas (23–38° latitude) (Hadley 1972; Polis 1990). Distinguishable from this general distribution pattern in hot and dry areas (Cloudsley-Thompson 1962, 1975) are scorpions that inhabit temperate regions (especially taxa in the family Euscorpiidae). For example, several species are found in humid and even cold environments, including mountain tops such as the Alps, Balkans, and Pindos, up to 2500 m above sea level (Fet 2010).

Euscorpium is a European genus of scorpions that comprises at least 20 species (Fet & Soleglad 2002; Fet et al. 2003; Vignoli et al. 2005). Many of the currently accepted species are former subspecies of *E. carpathicum* (Linnaeus, 1767) recently elevated to species status (Fet & Soleglad 2002). *Euscorpium carpathicum* (the Carpathian Scorpion) is the first described species of *Euscorpium* and is endemic to the foothills and mountains of southern Romania (Bunescu 1959; Fet & Soleglad 2002). The ecology of *E. carpathicum* has not been studied yet, most characteristics being extrapolated from its more studied relatives such as *E. italicus* (Herbst, 1800) or *E. flavicaudis* (De Geer, 1778) (Benton 1991a, b, 1992; Colombo 2006).

Unlike scorpions from warmer areas, those in the genus *Euscorpium* adapted their life cycle to withstand extreme seasonal variations from dry, hot summers to cold, snowy winters. Despite the obvious importance of understanding the overwintering ecology of temperate region scorpion species, data regarding this aspect are scarce or missing (as for all species of *Euscorpium*).

In the current paper, we provide an updated overview on the distribution of *E. carpathicum* based on previously published and newly collected data, create a general climatic tolerance profile and map of the climatically suitable areas for the species using occurrence and climatic data, as well as broaden our understanding of ecological mechanisms of temperate region scorpions in regards to overwintering behavioral ecology.

Current distribution.—Distribution data for *E. carpathicum* were obtained from three different sources: literature records (Calinescu 1956; Bunescu 1959; Fet & Soleglad 2002; Fet et al. 2002), field surveys by IG and ASO from 2008 to 2012 in Romania, and personal

communications with other biologists. We included in our analysis records with geographic coordinates, if available at a spatial uncertainty < 1 km; localities with spatial uncertainty > 1 km were either validated through field surveys or discarded from the analysis. Of the compiled records, 78.4% were previously known and 21.6% were new records. Our final dataset comprised 60 spatially unique occurrence records (Fig. 1).

The distribution of *E. carpathicum* records is clearly clustered as shown by Moran's I test ($z = 22.4$, $P < 0.0001$) and by GetisOrdGi* spatial statistic ($z = 9.8$, $P < 0.0001$), which indicates that the species has a restricted distribution (Fig. 1). We identified two isolated distribution hotspots, one in the foothills of the Curvature Carpathians, and another one in the Banat Mountains. We used minimum convex polygons to calculate the Extent of Occurrence (EoO) of the entire occurrence dataset and for the two clusters of occurrence points (distribution hotspots). The total EoO of *E. carpathicum* was 29,540 km². Due to the presence of a 160 km gap without scorpion observations between the two hotspots, the total EoO of the species decreased by 36.1% after summing the EoO of the two hotspots.

In order to summarize the habitat types in the proximity of *E. carpathicum* occurrences, we created a 5 km buffer around each distribution point and calculated the percentage of habitat type using Corine Biotopes 2000 map (CLC2000). The habitat matrix in which *E. carpathicum* were recorded is mostly composed of broad-leaved forests (41.95 %), pastures (10.39 %), and rural areas (6.16 %), suggesting that the species inhabits regions with moderate anthropogenic impacts. All analyses were done in ArcGIS 10 (ESRI 2011).

Potential distribution.—We downloaded climatic data from the WorldClim database (Hijmans et al. 2005), at a spatial resolution of 1 km to match the spatial uncertainty of occurrence data. We used a jackknife analysis to measure the importance of the variables, increasing model accuracy and reducing the initial set of 19 bioclimatic variables to a subset of seven variables that each contributed > 5% to model accuracy gain (Table 1). We used Pearson correlation to check for significant correlations among the remaining seven bioclimatic variables (as suggested by Fielding & Haworth 1995) and found no significant correlation ($r > 0.7$).

Table 1.—Variables used for generating the Maxent species distribution model, indicating the percent contribution to accuracy gain of the final model.

Variable	Final model
Precipitation of Warmest Quarter	35.7
Min Temperature of Coldest Month	15.1
Max Temperature of Warmest Month	14.3
Temperature Annual Range	12.8
Isothermality	10.2
Precipitation Seasonality	6.4
Precipitation of Coldest Quarter	5.6

We used Maxent version 3.3.3k (Phillips et al. 2006; Phillips & Dudik 2008) to estimate the potential distribution of the target species from species' occurrence records and environmental data; in evaluating the model performance, random test percentage was set to 25% of the occurrences, and the maximum number of background points was set to 10,000. Because Maxent produces continuous-type predictions, we used a 10% omission error of the training occurrence dataset as threshold to generate a binary prediction (presence/absence).

The model was evaluated using Area Under the Curve (AUC) of the Receiver Operating Characteristic (ROC) calculated in Maxent (Phillips et al. 2006; Phillips & Dudik 2008) and partial AUC (Peterson et al. 2008), calculated using partial-ROC application. In partial ROC we ran 100 iterations in which test occurrence data were bootstrapped to create a null distribution for comparison with our observed value of pAUC using a 10% omission error. We also calculated the omission error, representing the testing occurrence data predicted absent by the binary prediction.

The predictive power of our Maxent model for *E. carpathicus*, as measured by AUC (training AUC = 0.861 and testing AUC = 0.881), as well as partial AUC (mean of 1.34, SD = 0.07, $P < 0.05$) was high. Additionally, the model omitted only 6% of the testing occurrence

points. The variables that most contributed to model accuracy gain were precipitation of warmest quarter, minimum temperature of coldest month, and maximum temperature of warmest month, indicating a sensitivity of the species to the amount of precipitation during dry seasons, and to low temperatures during winter months.

Our model found Banat Mountains and Curvature Carpathian climatically suitable for *E. carpathicus* (Fig. 1). In addition, most of the foothills of Western and Eastern Carpathian Mountains, the Moldavian and Getic Plateaus, and parts of Transylvania were also predicted to be suitable (Fig. 1). On the southern side of the Danube River, the climatically suitable area was limited to the Iron Gates Canyon.

Microhabitat and overwintering site selection.—We conducted a field study in the foothills of the Curvature Carpathians in Buzau County in close proximity of Valea Nucului village. Due to the lack of ecological data on *E. carpathicus* and because previously published records only referred to its general distribution (locality records), we selected three types of habitat to survey in the studied area, based on the distance from a stream and general exposure: riparian habitat, meadow, and forest, each one with an approximate area of 2 km². Each habitat was surveyed three times, at ten sample points, with approximately six hours allocated for each survey; we did not find any scorpions in forests or meadows, suggesting a strong association between the scorpions and riparian habitats.

For each surveyed point, we recorded the following variables: presence of bushes, leaf cover, type of soil, slope, distance to the stream, and number, age (juvenile or adult), and sex of individuals found at each location. We used the First Sight Point methodology to characterize the microhabitats (clay, bushes, slope, leaf, aspect, and distance from stream) where we first observed an individual, regardless of the animal remaining at or leaving the site (see Bombi et al. 2009 for more details). We located a total of 20 scorpions during our surveys.

We used General Linear Models (GLM) to explain the overwintering habitat selection by *E. carpathicus* juveniles and adults and an information-theoretic approach to evaluate the relative support of the different models obtained (Anderson & Burnham 2002; Mazerolle 2006). As a result, we selected three of the computed GLMs that

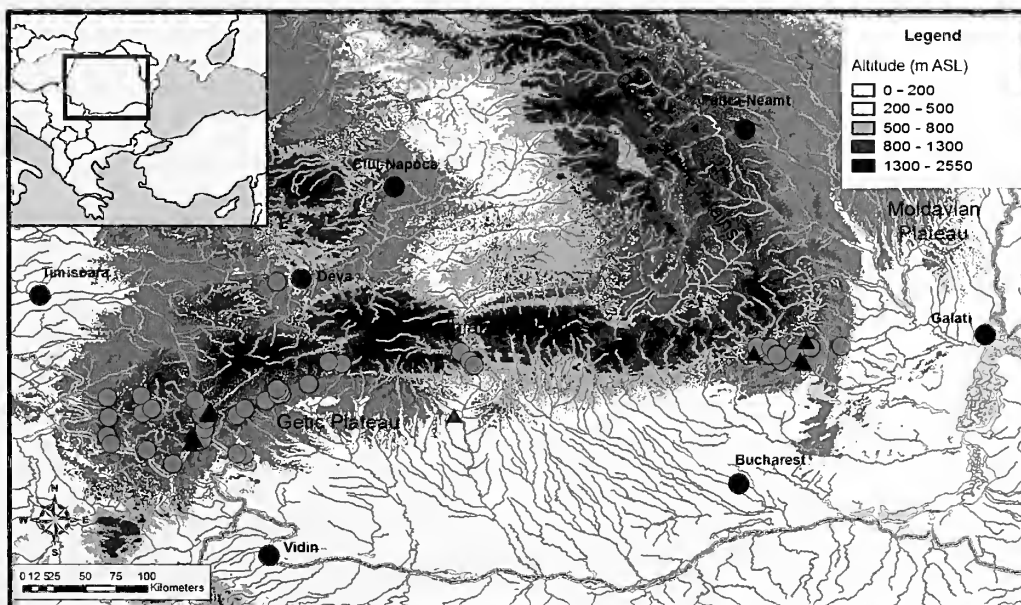


Figure 1.—Occurrence points and climatically suitable areas of *E. carpathicus*. Yellow points show sites with known *E. carpathicus* literature records and triangles show sites with new records, found for the first time during surveys reported here. The light red background color represents climatically suitable area for *E. carpathicus*, as predicted by the Maxent model; elevation is shown in shades of gray.

received the lowest Akaike Information Criterion (AIC) score (e.g., Gagné & Dayton 2002). We also calculated the AIC weights (w), which represent the probability of a model to be the best from the entire set of candidate models (Anderson & Burnham 2002). All statistical analyses were run in R 2.14 using “MuMIn”, “MASS”, and “bbmle” packages.

Our GLM results showed that the most important habitat variable for both ontogenetic stages of *E. carpathicus* is the presence of clay substratum which was retained in all of the best selected GLMs. Additionally, according to AIC weights, the probability that this variable is the most important was 0.785.

Except for the variable clay, in case of the adults, all GLM models obtained similar AIC values ($\Delta AIC = 1.6$) and presented almost no variability with respect to model variance and weights. Based on these results, we consider that adult *E. carpathicus* randomly select overwintering microhabitats but only in areas where clay substratum is present. For all adults, the best model according to AIC suggested an interaction between presence of bushes and clay. This model explained 81% of the variance of the data (Nagelkerke's R^2), and the probability that this was the best model was 0.80. All other variables only appeared in various combinations with the variable clay across the two age groups. In the case of juveniles, the best-generated GLM models suggest a selection of microhabitats, specifically an interaction between the presence of clay and leaves. This model had an AIC weight of 0.199 and explained 100% of variance of the data (Nagelkerke's R^2).

Euscorpium carpathicus is an endemic species that is found only in Romania, known from records from the Banat Mountains, in the vicinity of the city of Deva, a few isolated locations along the Olt River, and in the foothills of the Curvature Carpathians (Calinescu 1956; Bunescu 1959; Fet & Soleglad 2002). The distribution of this species is divided in two clusters, situated 160 km apart (Fig. 1). Despite our field surveying efforts, we were unable to locate scorpions within this gap. Furthermore, the habitat considered suitable for scorpions (broad-leaf forests, rocks, human settlements, etc.) is continuous along the 160 km long gap, thus we could not find any obvious reason for the absence of scorpions. Fet et al. (2002) suggested that this disjunct distribution might be the result of human influence or relict distribution. However, the Maxent estimated potential distribution of *E. carpathicus*, based on climatic factors, delineated two clusters, thus we propose that climatic conditions are responsible for the absence of scorpions within the gap region.

According to our Maxent model, only very small climatically suitable areas exist south of the Danube River. This finding suggests not only that the Danube River is a geographic barrier for dispersal, but also that the climatic conditions are not favorable for *E. carpathicus* south of the river. Northwards, most likely limitations are related to biology of the species (such as dispersal or perhaps food availability) and remain unknown. Further research on the species' biology is required for a better understanding of its distribution patterns in the northern part of its current range.

The microhabitat models suggest that clay substratum is the most important factor in predicting the presence of scorpions during the winter and this result can be explained by the scorpions' use of clay for shelter. *Euscorpium* species are known for selecting small cracks and holes in the walls and bare rocks (e.g., Benton 1992, Colombo 2006). *Euscorpium carpathicus* shares these preferences for cracks and holes but selects a different substrate, the clay banks in riparian areas. The presence of bushes as the second most important variable for *E. carpathicus* presence is explicable because bushes can provide warmer microhabitats in river valleys during winter. Therefore, by selecting riparian habitats with bush cover, *E. carpathicus* could attain a higher survival rate during the winter.

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SHORT COMMUNICATION

Courtship and the use of pheromones by *Pholcus manueli* (Araneae: Pholcidae)

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Abstract. Most web spiders use chemical and vibratory cues for intersexual communication prior to mating. Little is known regarding the behavior of the web spider, *Pholcus manueli* Gertsch, 1937. In the laboratory, we examined the behavior of males in female webs. Some webs were washed and allowed to air dry. Both washed and unwashed webs were tested with and without females. Overall, male behaviors were similar to courtship reported for other pholcid species and their frequency was related to male size. Web washing delayed the initiation of activity, reduced abdomen flexes, and increased the likelihood of pedipalp expansion and aggression. The presence of females caused males to initiate movement sooner and to increase silk pulling behavior. These results suggest that courtship is multimodal and that the vibratory dialog between males and females works along with chemical communication in this species. Hopefully, these data will enhance future studies of sexual selection in pholcids.

Keywords: male behavior, chemical communication, mating behavior

Spider silk is a particularly apt vehicle for information transmission and is known to augment visual and airborne signals with complex chemical, vibratory, and tactile information (Gaskett 2007; Uhl & Elias 2011). Many web spiders have poor vision and, as such, rely heavily on their web to help them identify prey, predators, and potential mates (Huber 2005; Gaskett 2007; Uhl & Elias 2011). It is particularly common for female spiders to attach pheromones to the silk, which provides males with information regarding the females' location, quality, and receptivity; often these pheromones are sufficiently potent to stimulate initial courtship activity (Gaskett 2007; Uhl & Elias 2011; Schulz 2013). These early phases of courtship serve to reduce potential aggression or cannibalism while allowing females to assess features of male quality (Schneider & Lubin 1998; Roberts & Uetz 2004; Huber 2005; Schulz 2013). Thus, characterizing the behavior of males in the presence of females and the pheromonal signals that are associated with the webs of females is an important first step in understanding the reproductive biology of any species.

Male and female cellar spiders (Araneae: Pholcidae) engage in a sophisticated dialog that includes air and silk-borne pheromones (Xiao et al. 2009, 2010; Hoefler et al. 2010) as well as body, leg, and pedipalp movements that affect silk tension (Bartos 1998; Uhl 1998; Huber 1999) and auditory signals produced by stridulation (Peretti et al. 2006). The courtship and mating behavior of *Pholcus phalangioides* (Fuesslin, 1775) has been well documented; pheromones in the webs of females elicit male orientation and signaling behaviors (Bartos 1998; Uhl 1998; Hoefler et al. 2010). Specific aspects of these signals along with prior experience, mating status, and body size of both sexes influence mating success (Schäfer & Uhl 2002, 2005; Schäfer et al. 2008; Uhl et al. 2005; Hoefler et al. 2010). Recent work with *Pholcus beijingensis* Zhu & Song, 1999 identifies many courtship behaviors analogous to those of *P. phalangioides* (Xiao et al. 2009, 2010). In addition, *P. beijingensis* is one of the few species where specific details about the structure and function of the pheromones deployed by both females and males are documented (Xiao et al. 2009, 2010). Much less is known about the courtship behavior and potential use of pheromones in other members of the genus *Pholcus*. Specifically, we have not been able to find any published studies on the behavior or ecology of *Pholcus manueli* Gertsch, 1937. The goal of this study was to document male behavior in female *P. manueli* webs in hopes of identifying key courtship activities. In addition, we examined the potential

importance of pheromones and body size in influencing male courtship activity in a female web.

Pholcus manueli were collected from various buildings and barns in Butler County, Ohio USA. Once in the laboratory, the spiders were housed individually in translucent cylindrical plastic cups (23 cm diameter, 16 cm high) and held in an environmental room at 25° C on a 13:11 L:D cycle. Adult spiders were fed two crickets, *Acheta domestica* (each 0.6 cm), per week for a period of at least three weeks prior to testing. Our preliminary observations suggested that both adult males and females mate multiple times and seem to be universally eager to mate when they have been held in isolation for at least two weeks and the females have not produced egg sacs. Each male spider was randomly assigned to a female to populate each of the following treatments: 1) female in a web ($n = 15$), 2) female in a washed web ($n = 12$), 3) an empty female web ($n = 14$), and 4) an empty washed female web ($n = 12$).

Experimental webs were spun by adult female spiders. One day after feeding, each female was placed into a new cup (23 cm diameter, 28 cm high) lined with a hardware cloth cylinder (16 cm diameter, 24 cm high). The spiders place the bulk of their web attachment points on this cylinder and, as such, we could use it to relocate the web with minimal destruction. After one week, females were removed from webs. We measured the length of the tibia-patella of the right first leg with a digital micrometer attached to a stereomicroscope (0.01 mm accuracy) and held them in vials (1.5 cm diameter, 3 cm high). Males were removed from their webs, measured, and then returned to their home web until experimentation.

For treatments that required washing, we removed the hardware cloth cylinder holding the web and sprayed it six times with 95% ethanol from various angles using a hand-held plant sprayer. The cylinder was then placed in a new cup, covered with a 0.1 cm mesh lid, and placed under a fume hood for 24 h so that the ethanol and any volatile components of the pheromones could evaporate. We moved unwashed webs from container to container in a similar fashion but we did not spray them and we held them sealed cups for the 24 h period to minimize pheromone loss.

For trials with females, the female was reintroduced into her own web and allowed to acclimate for 3 min. Males were then introduced into webs for all treatments, and the experimental container was placed under a video camera in a closed booth that fed to a video recorder

Table 1.—Descriptions of the male behaviors that were quantified and their loadings for the top three principal components.

Male behavior	Description	PC1 (31.0%)	PC2 (17.8%)	PC3 (13.2%)
Web deposits	The male adds silk to the female's web	0.55	-0.30	-0.19
Abdominal flexes	The male raises and lowers his abdomen at a high rate	0.29	-0.29	-0.61
Silk tastes	Male opens his chelicerae and appears to place his mouth on female silk	0.84	-0.23	0.16
Web taps	Male strikes the web or the female's leg repeatedly with one of his legs	0.90	-0.07	0.12
Searches	Male rotates the first pair of legs in large circular swivels	0.55	0.44	-0.30
Silk pulls	Male uses one of his first pair of legs to pull or pluck a strand of silk	0.08	0.86	0.02
Aggression	Male jerks toward the female with chelicerae spread wide	-0.13	-0.43	0.50
Pedipalp extension	The male unfolds one or both pedipalps to full length	0.50	0.28	0.52

located in an adjacent room. The behavior of the male was recorded for 30 min or until copulation or cannibalism started to occur. We reviewed a number of videos and identified eight distinctive behaviors (Table 1). We then watched all videos and recorded the initiation and duration of male activity as well as the frequency with which he performed each of the eight behaviors (Table 1).

We compared the time to initiate and the duration of male activity using two-way ANOVAs with female presence or absence, web washed or not, and the interaction as factors. The rest of the behaviors were combined in a Principal Components Analysis (PCA). Principal Components (PCs) with eigenvalues greater than one were retained and compared using two-way ANOVAs. For the 27 trials that included both males and females, we determined whether they differed in size using a matched pairs t-test. We also examined the relationship between size of the male or female and their behavioral repertoire by calculating Pearson product-moment correlation coefficients (r) between male or female size and each of the retained principal components.

All males initiated activities in trials but it took males significantly longer to start moving when they were in washed webs with no female present (Table 2, Fig. 1A). In all cases, males stopped activity before the 30 min trial was over but their activities lasted significantly longer when a female was present (Table 2, Fig. 1B). The patella-tibia length of males (7.95 ± 0.10 mm), our indicator of body size, was significantly larger than that of females (7.17 ± 0.08 mm) ($t = 6.69$, $P < 0.0001$).

Three PCs with eigenvalues greater than one emerged from the PCA and together they explained 62.1% of the variation in behavior (Table 1). Male size was correlated with PC1 ($r = 0.28$, $P = 0.04$), which captured 31% of the variation in the data. There were strong loadings of web tasting and web taps (>0.75) and moderate loadings of web deposits, searches, and pedipalp extension with PC1 (>0.50) (Table 1). PC1 was not affected by the presence of the female or silk washing (Table 2). Neither PC2 nor PC3 was correlated with male or female size (all $P > 0.5$). PC2 captured 17.8% of the variation in behavior; it reflected high levels of web pulling (the only item with a loading >0.75) (Table 1). Female presence but not silk washing affected PC2 (Table 2, Fig. 1C). PC3 captured 13.2% of the variation in behavior; it incorporated the likelihood of male aggression and pedipalp extension and was inversely related to the frequency with which males performed abdominal flexes (Table 1). Both female presence and silk washing were related to PC3 (Table 2); PC3 was highest in washed webs with females and lowest in unwashed webs without females (Fig. 1D).

Spiders use a variety of sensory channels to communicate and our results verify that communication in *P. manueli* is multi-modal (Hebets & Papaj 2005). While males delayed activity in empty washed webs, they responded quickly when females were present (Fig. 1A), presumably because they detected females through vibratory communication. In addition, reducing pheromones by washing the webs increased the

likelihood of aggression and the unfolding of pedipalps (PC3, Fig. 1D). The union of these two behaviors in one PC seems counterintuitive because pedipalp extension is considered a precursor to copulation in *P. beijingensis* (Xiao et al. 2009) but aggression would seem to signal mate rejection, the opposite response. This particular combination may reflect the male's uncertainty as to the exact status and receptivity of the female precisely because the pheromones are altered by our washing treatment. Notably PC3 values for males in washed and unwashed webs converge when females are present (Fig. 1D), which suggests that females' signals clarify the situation for males.

The array of activities we observed in male *P. manueli* is similar to courtship behaviors reported for other *Pholcus* species. Specifically female presence had a strong impact on PC2 which reflects the frequency of silk pulls, a behavior featured in the courtship repertoire of both *P. phalangioides* and *P. beijingensis* (Bartos 1998; Xiao et al. 2009). Thus, it would seem that this behavior plays an important role in sexual communication prior to mating. Specifically, this silk pulling seems to initiate a conversation with the female and the subsequent exchange that occurs between the two spiders may ultimately reveal the receptivity of the female. The abdomen flex, negatively associated with PC3, is another behavior featured in the courtship of both *P. phalangioides* and *P. beijingensis* (Bartos 1998; Xiao et al. 2009). The fact that PC3 was lower (i.e., that abdominal flexes were higher) in unwashed webs provides evidence that our washing treatment

Table 2.—Statistical analyses for courtship and principal components derived from the behaviors recorded. Non-significant interactions were eliminated from analyses.

Model features	Df	Test statistic	P
Initiation of activity			
Model	3,48	F = 3.84	0.0153
Female presence		t = 2.22	0.0314
Silk washing		t = 1.77	0.0832
Female * washing		t = 2.03	0.0483
Duration of activity			
Model	2,50	F = 3.28	0.0461
Female presence		t = 2.15	0.0367
Silk washing		t = 1.39	0.1694
PC1			
Model	2,50	F = 0.44	0.6464
Female presence		t = 0.77	0.4420
Silk washing		t = 0.52	0.6085
PC2			
Model	2,50	F = 4.40	0.0174
Female presence		t = 2.97	0.0046
Silk washing		t = 0.09	0.9257
PC3			
Model	2,50	F = 9.59	0.0003
Female presence		t = 3.11	0.0030
Silk washing		t = 3.13	0.0029

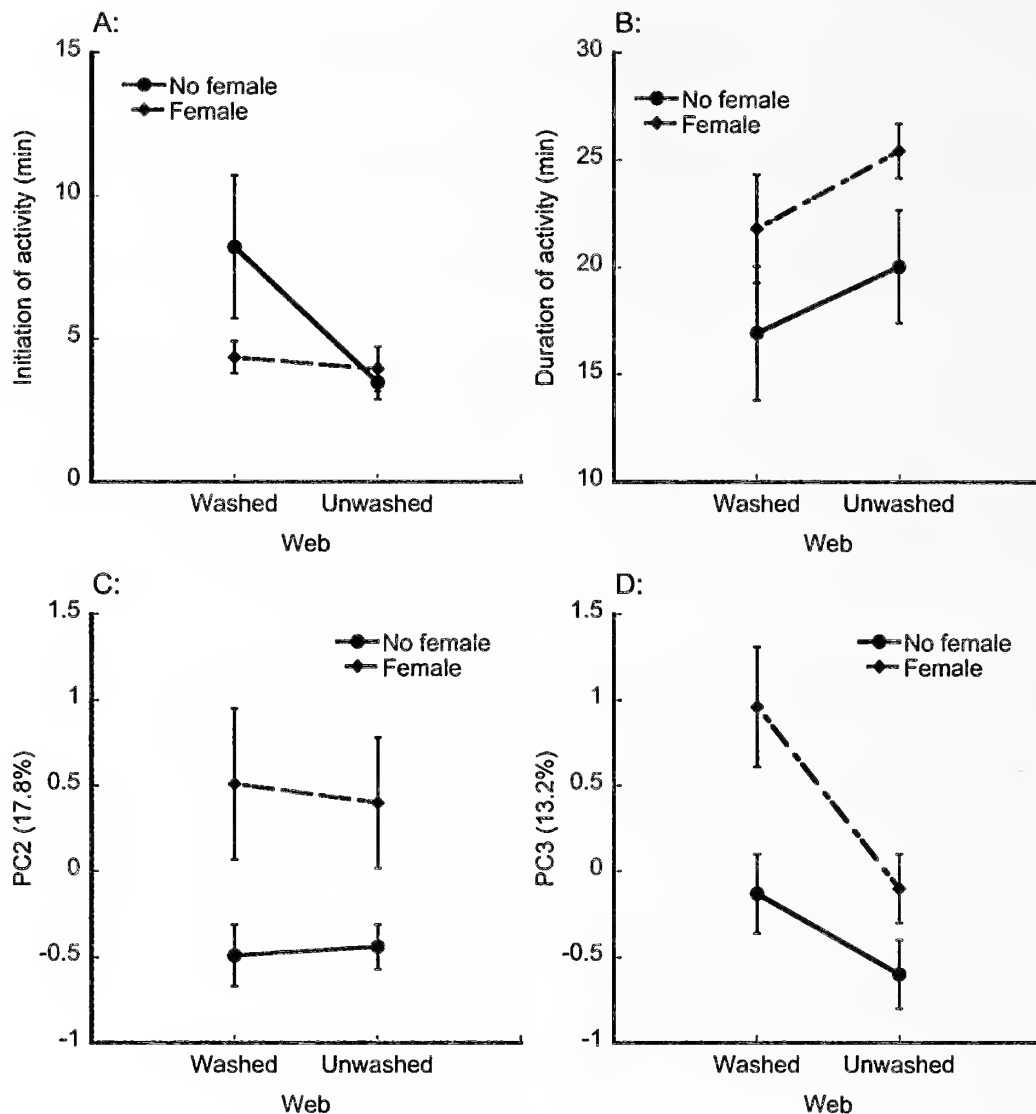


Figure 1.—Male behavioral metrics that were affected by either web washing, the presence of a female, or both. A: Time to initiate activity, B: Duration of activity, C: PC2 (see Table 1 for loadings), D: PC3 (see Table 1 for loadings). Statistical analyses are presented in Table 2.

influenced the normal course of courtship interactions in *P. manueli*. Males also seemed to engage in this behavior more intensely when no female was present (Fig. 3D). Thus, this activity may be an early courtship signal that helps males find females within the web, especially in situations where the chemical information is not clear. Males, with uncertain information in washed webs, may be more anxious to communicate to female web owners that they are potential mates and not prey items.

Intriguingly, many of the behaviors were unaffected by the presence of a female or web washing; PC1 captured most of the behavioral variation that we observed and was tied to the greatest diversity of behaviors. High values of PC1 indicate that males engaged in frequent silk tastes and web taps, behaviors that suggest animals added to their sensory input through gustatory (tastes) and vibratory (taps) channels. In addition, PC1 was moderately associated with web deposits, which would be a means by which males could communicate with females via pheromones (Xiao et al. 2010). PC1 was also associated with searching behavior, where males rotate their legs widely in a manner that expands their sensory range. Taken together, the suite of behaviors reflected by PC1 seems to be general information gathering. However, as males spend more time in the web, they are able to gather more evidence regarding

the location, size, and receptivity of females, all of which are important to mate solicitation and mating success. In webs with no females, males cease this activity sooner (Fig. 1B), but when females are present, they continue longer (Fig. 1B) and alter their behavior patterns (Fig. 1C, 1D).

Interestingly, the activities captured by PC1 increased with male size but we did not uncover any impact of female size on male behavior across treatments. If our female *P. manueli* responded to larger males with more encouragement, then those males may have been stimulated to intensify aspects of courtship captured by PC1. On the other hand, pre-copulatory sexual cannibalism is a much higher risk for small males (Wilder & Rypstra 2008) and, as a result, small males may approach more cautiously than their larger counterparts when on the webs of strange females. Only further study will clarify the details of the early dialog between males and females of this relatively unknown species as they enter courtship and attempt to mate.

The pheromones of *P. beijingensis* have two components that work primarily when they are present in a 2:1 ratio (Xiao et al. 2009). Our results suggest that *P. manueli* is not likely to be as sensitive to a specific blend of compounds. The ethanol and air treatment that we developed affected male activities but did not eliminate distinctive

behaviors that are key features of courtship documented for other *Pholcus* species. If a precise ratio of compounds were necessary for *P. manueli*, we would have expected our web washing to disrupt that balance and, even if our approach did not remove the pheromones, the disruption would have resulted in larger impacts of web washing on male behavior. It is the case that we had hoped to use more comprehensive washing technique for these initial experiments with a greater diversity of solvents but the webs were too fragile to withstand repeated treatments and we wanted the integrity of the webs to remain so that the females were housed in relatively normal intact webs during trials. Nevertheless, these results set the stage for the more sophisticated behavioral and chemical assays necessary to demonstrate the specific nature of the chemical signals.

Here we provide documentation of the array of behaviors performed by male, *P. manueli*, in females' webs. Our data suggest that both chemical and vibratory communication are important as males and females interact prior to mating. This study identifies a few behaviors that are more or less sensitive to chemical signals as well as those that seem to be directed at actual females. Further study of this species may help us generalize the detailed studies that have been conducted on congeners.

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SHORT COMMUNICATION

Loading the male pedipalps: sperm induction in a subsocial spider

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Abstract. Detailed descriptions of sperm induction are rare in the literature. *Anelosimus viera* Agnarsson, 2012 (Theridiidae) is a Uruguayan subsocial spider that lives in communal nests. This paper describes the sperm induction of this species under laboratory conditions. We collected spiders in Montevideo, Uruguay, from different trees and placed them in thirty male and female pairs in Petri dishes to observe the occurrence of sperm induction. We witnessed twenty-two males performing sperm induction after copulation. Sperm web characteristics and description of the male's behavior during sperm induction are described.

Keywords: *Anelosimus viera*, sperm web building

Spiders have a variety of reproductive strategies with particular characteristics making them an excellent model for the study of sexual selection (Eberhard 2004). Disconnection between the male pedipalps (copulatory organ) and the sexual glands is a feature unique among spiders. As a consequence, the male must build a sperm web and load his pedipalps with sperm before encountering the female. The sperm web varies in size and shape depending on the family of spider (Foelix 2011). The male deposits a droplet of semen on the web and fills his pedipalps (Foelix 2011). In most Araneomorph spiders the sperm web is a small structure. It may be reduced to a single thread (e.g., that of *Scytodes* Latreille, 1804). Alternatively, it could have a triangular or trapezoidal shape (e.g., *Tetragnatha* Latreille, 1804 and *Theridion* Walckenaer, 1805, respectively), with the induction process taking little time. In contrast, theraphosid spiders and the Araneomorph spider *Kukulcania hibernalis* (Hentz, 1842) build relatively large sperm webs and the entire induction process may take several hours, making it easier to observe (Costa & Pérez-Miles 2002; Barrantes & Ramírez 2013).

Although sperm induction is common, reports in the literature are relatively scarce, from descriptions of sperm webs (Dominguez & Jiménez 2005) and partial observations of the induction process (Fraser 1987), to single observations of the entire process (Levi 1967; Jackson & Macnab 1991). Detailed and rigorous descriptions of the induction process are needed to compare among different species so we can increase our knowledge of this important process.

Anelosimus viera Agnarsson, 2012 (Theridiidae) is a Uruguayan subsocial spider. Individuals live in communal nests in evergreen trees. The mother cares for young, and the juveniles show a lack of conspecific aggression. Males mature earlier and consistently disperse, resulting in inbreeding avoidance, while females mature asynchronously and may or may not disperse from the natal nest (Viera et al. 2007). Males court females using vibrations induced by plucking silk threads and by touching the female until she adopts the copulation posture with the ventral side upwards, body axis approximately inclined 45° and facing down, and copulation occurs (Albo et al. 2007). Copulation lasts one hour on average (Viera & Albo 2008). During mating trials, we observed several incidents of males performing sperm induction after copulation. Here we describe the sperm induction process of *A. viera*, including sperm web construction, sperm emergence and sperm induction itself, under laboratory conditions.

Juveniles of *A. viera* were collected from several nests of different trees in Montevideo, Uruguay (34°54'48"S, 56°10'2"W) during June 2014. In the laboratory, individuals were bred, isolated in plastic Petri dishes (5.5 cm diameter x 1.5 cm height) and maintained with moistened cotton wool. All spiders were fed *ad libitum* with *Drosophila* spp. (Diptera). Individuals were checked daily under a stereo microscope (Nikkon type 102; 10 x ocular) and the recently molted females were housed in smaller Petri dishes (3.3 cm diameter x 1.0 cm height), where the subsequent observations took place.

After 48 h of molting, each virgin adult female was exposed to a male from a different nest. Thirty trials were followed using a video camera (Sony DCRA-C151, 2.7" Wide Hybrid LCD, Handycam Station Supplied, 12 x optical zoom, 800 x digital zoom) during 2 h. When an individual did not engage in sexual behavior during the first 15 min or mating did not occur during the first 30 min, the trial was excluded. Each individual was used only once. We described the units of male sperm web construction, sperm emergence and sperm induction, and recorded durations and frequency. The sperm web was photographed and the area of it was measured using an image processing and analysis program (ImageJ). Voucher specimens were deposited in the Entomology Collection of the Faculty de Sciences, Montevideo, Uruguay.

Twenty-eight of the thirty trials resulted in mating. In 22 of these trials, males performed sperm induction after copulation. The latency post-mating was 22.33 ± 7.10 min. No male performed sperm induction just before mating.

The males, which were suspended with the ventral side up in the females' webs, built a scaffolding of the sperm web consisting of two nearly parallel threads located at each lateral side of the abdomen and connected to the female's web. After that, males placed their legs II and IV on the parallel threads and positioned legs III where the bridge between parallel threads would be built. Legs I remained on the female web, and males began to lay a swath of threads between the parallel threads using the following movements: (1) laid a line with the end of the abdomen (spinnerets) on one of the parallel threads near the prosoma end; (2) lifted the abdomen and first put it on the center area of the same thread, then on the most distal end in relation to the prosoma; (3) repeated the same abdominal movements on the other parallel thread; (4) once again lifted the abdomen, laying a line on the first parallel thread near the first foothold, and repeating this abdominal sequence a total of 19.64 ± 3.00 times (Fig. 1). The end result was a horizontal hourglass-shaped web (Fig. 2). The process of

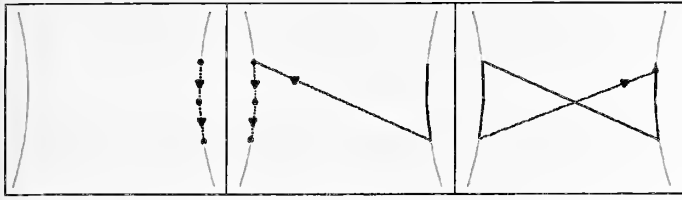


Figure 1.—Sperm web construction sequence. The threads (dotted and solid black lines) are laid between the scaffolding (gray lines). This sequence is repeated several times. The black points are the places where the silk threads are attached.

sperm web building lasted on average 36.64 ± 9.18 s. The mean area of the sperm web was 0.28 ± 0.11 mm².

Once the sperm web was finished, males deposited sperm. They made small legs flexions at the femur-patella articulation, with higher intensity of leg IV, and moved their abdomens up and down. They then continued the up-and-down abdominal movements, increasing the frequency and diminishing the amplitude without bending the legs, until a drop of sperm emerged and was later deposited on the thin middle portion of the sperm web. This process lasted 35.32 ± 11.09 s on average.

After sperm deposition, males moved backwards, holding the sperm web with their legs in the same position so the pedipalps could reach the sperm drop. The drop was deposited on the upper side of the web and was absorbed through to the same side with fast vibratory movements alternating between one pedipalp and then the other. This was done 10.45 ± 3.42 times on average (Fig. 2). The time they spent with each pedipalp on the sperm drop increased as the behavior progressed. Most males withdrew most of the sperm after approximately 3.28 ± 1.04 min, except in two cases where males left part of the sperm in their sperm web. The entire process of sperm induction (sperm web building + sperm drop + filling up the pedipalps) took an average of 4.47 ± 1.38 min. When induction was completed some males placed their pedipalps into their mouthparts and shook their abdomens once or twice but they did not consume the sperm web. (To view video of sperm web construction, sperm drop deposition, and loading the palps, see <http://dx.doi.org/10.1636/J15-57.s1>)

Because sperm induction is essential for successful copulation, it is surprising that there are few studies describing it in detail. In this species, this behavior always took place shortly after copulation.

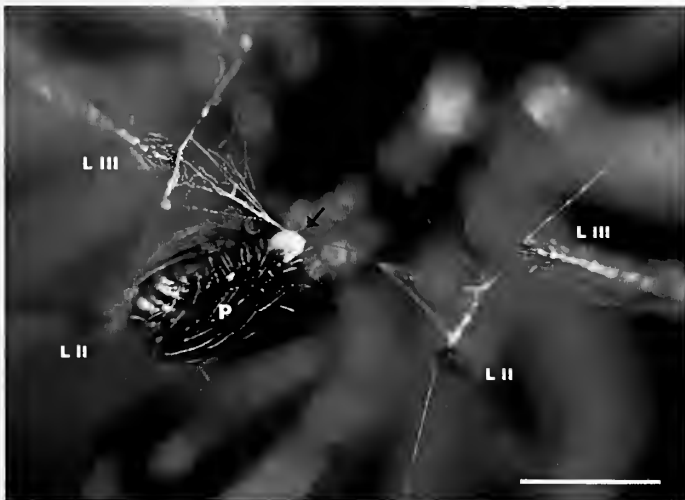


Figure 2.—Male loading one of his pedipalps with sperm from the sperm web. P: pedipalp; L II: leg II; L III: leg III. The arrow points to the sperm drop. Scale = 0.5 mm.

The small size of the sperm web in *A. vierae*, as in most araneomorph spiders (Foelix 2011), represents a low cost in time and effort, in contrast to mygalomorph spiders, which build a large sperm mat involving a significant cost for males (Costa & Pérez-Miles 2002). Also, the short induction duration of *A. vierae* implies low costs relative to most other spider species, in which many minutes, but usually less than half an hour, are required to perform it (Gertsch 1979). The position of the legs prior to sperm web construction is similar to the position of several species of the same family like *Theridion* sp., where the parallel threads are held by legs II and IV, and the bridge is spread by legs III (Knoflach 1998). The horizontal position of the sperm web by the males of *A. vierae* is similar to that commonly observed in other spiders (Foelix 2011). However, its hourglass shape differs from the common triangular or square shape described for other Araneomorph spiders (Gertsch 1979; Foelix 2011) or the trapezoidal shape seen in *Theridion* (Knoflach 1998).

As in many spider families, intensive movements of the abdomen just before ejaculation indicate the activity of the epigastric glands that support the building of the sperm web (Knoflach 1998). These intensive movements were seen in *A. vierae* and could imply the same activity of the epigastric glands. Filling the pedipalps with sperm corresponds to the direct form of sperm induction observed in web-building spiders (Foelix 2011). This process is characterized by a male extending his pedipalp on the same side of the sperm web as the droplet of sperm and contacting the droplet, which absorbs the sperm (Jackson & Macnab 1991). The fact that males performed sperm induction behavior after mating suggests that their pedipalps are loaded with sperm prior to mating. Thus, it may be the case that males load their pedipalps immediately after they molt into adults, as is the case with most spiders (Austad 1984; Michalik & Rittschof 2011). Under laboratory conditions, we observed similar sperm induction characteristics in *A. jabaquara* Levi, 1956, and it also occurred after mating (unpublished results).

Here, males did not copulate again with the same female, but another study reported that males of *A. vierae* made two or three reloads of pedipalps and remated with the same female (Viera et al. 2007). By remating with the same female, males can reduce the probability that she mates with another male, thus reducing the risk of direct sperm competition (Alcock 1994). This makes sense because in this species the first and second males that copulate with a female have similar percentages of paternity (Lorieto et al. 2010). Another option for males is to completely fill the female's spermathecae with sperm. However, the pedipalps may not be able to support all the sperm needed to do so, thus requiring the male to load them more than once. In the future, it would be interesting to evaluate the capacity of female's spermathecae compared with the capacity of the male's pedipalps.

Although the physical cost of sperm induction may be low, there may be other types of associated costs. First, males of *A. vierae* fight for access to females and the loser remains as a satellite male (Albo et al. 2007). When the winner leaves the female to engage in the sperm induction maneuvers, the satellite male could take advantage and copulate with the abandoned female. Females are permissive, tolerant, and accept re-matings with the same male and with others (Viera et al. 2007). Secondly, the brief sexual season of *A. vierae* can represent a pressure on males to load their pedipalps with sperm immediately after mating, because males may seek to copulate with many females over a short period of time (Viera et al. 2007). Future studies are required to investigate how often and under which circumstances (e.g., in the presence of one, two or three females, or other males) the males reload the pedipalps with sperm.

Given that *A. vierae* is a subsocial spider, this study is important for future comparative studies, and also for investigating how sociality may have evolved in spiders via reduced conspecific aggressiveness.

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SHORT COMMUNICATION

Allometry of locomotor organs and sexual size dimorphism in the mygalomorph spider *Grammostola rosea* (Walckenaer, 1837) (Araneae, Theraphosidae)

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Abstract. Although sexual size dimorphism is a widely observed phenomenon in nature, the selective forces that led to it are still controversial. Here we study sexual dimorphism in the static allometry of the legs of a large ground spider, *Grammostola rosea* (Walckenaer, 1837). We found that this species has a moderate sexual size dimorphism and males have longer legs relative to body size than females, similar to other ground spiders. We propose that male mate searching behavior may be a relevant factor in the genesis of this phenomenon. The longer extremities in males with respect to mass than in females would lead to an optimization of the costs associated with locomotion, because males have smaller masses and longer legs than the females both in absolute terms and relative to body mass.

Keywords: Allometry, sexual dimorphism, spiders, Mygalomorphae

Gender specific differences in locomotor structures have usually been attributed to a more active behavior of one sex, typically males (Gasnier et al. 2002). However, as we discuss below, gender-specific elongation of limbs may not only be associated with locomotion (Framenau 2005).

Ground-living spiders are less sexually dimorphic in size than web-building species, which has been attributed to their differing reproductive and foraging strategies (Prenter et al. 1999). There is evidence for sexual dimorphism in locomotor structures in ground-living spiders (Gasnier et al. 2002; Framenau 2005). Montgomery (1910) reported that males have relatively longer legs than females, suggesting that this is a result of the nomadic behavior of males after attaining sexual maturity. This idea is supported by studies on the locomotor activity of wolf spiders, in which males were the more active sex (Framenau et al. 1996; Framenau 2005), but see Aisenberg et al. (2010) for a counterexample. Morphometric data on leg length in wolf spiders showed comparatively longer legs for males than females only after the final molt, suggesting its significance in reproductive behavior such as searching for mates (Framenau 2005).

Although sexual dimorphism has been reported in mygalomorph spiders (Calderon et al. 1990; Costa & Pérez-Miles 2002; Santos 2007) to our knowledge there are no allometric studies of locomotor organs in these species. As in wolf spiders, adult males of mygalomorph spiders may be expected to show longer legs than females as a consequence of positive allometry. The aim of this study is to compare the allometric relationships between leg length and body mass in both sexes of the spider *Grammostola rosea* (Walckenaer, 1837) (Theraphosidae), a wandering mygalomorph spider in which the male actively searches for females during the reproductive season.

Like other Theraphosidae, *G. rosea* is a large, wandering mygalomorph spider with sexual size dimorphism (Costa & Pérez-Miles 2002). It inhabits mainly arid and semi-arid regions in the lowlands near mountain environments of sclerophyllous forest and Mediterranean scrubland, i.e., habitats characterized by cold, wet winters and hot, dry summers. It is a species of terrestrial habitats; it may be found in areas of low vegetation or in specific areas on slopes or soft ground, where it builds burrows up to 45 cm deep (Canals et al. 2007; Alfaro et al. 2013).

Fifty adults individuals, 24 females (16.93 ± 2.37 g; mean \pm SD) and 26 males (10.10 ± 1.19 g) were captured in Colina, north of Santiago, Chile ($33^{\circ}11'S$, $70^{\circ}40'W$). The individuals were taken to the laboratory and kept in individual terraria (24 x 12 x 10 cm) at 25 °C with a 12h:12h L:D photoperiod and water *ad libitum*. The spiders were fed weekly with five larvae of *Tenebrio molitor* to maintain the body weight at capture, based on estimations of Canals et al. (2012).

Each spider was immobilized via a dorso-ventral compression elastic device applied to the prosoma of the spider. Each leg and the palps were extended manually and fixed with staples in the femur. Then each spider and a reference mark of 50 mm were photographed with a NIKON D70 camera. The image file was analyzed with a morphometric software (ImageJ 1.47b software[®]), measuring the body length and the total length of each leg and palp of the right side considered as the sum of the lengths of the segments, the dorsal area of the prosoma and the dorsal area of the opistosoma.

Data were analyzed with R-software, considering a significance level $\alpha = 0.05$. For all variables, normality and homoscedasticity were tested with the Kolmogorov-Smirnov and Levene tests, respectively. Difference in body mass between sexes was analyzed with Student's *t* test for independent samples. Potential regressions ($y = ax^{\beta}$ or equivalently $\log(y) = \log(a) + \beta \log(x)$, with x and y as the independent and the dependent variables, respectively) were performed for body length, prosoma area, opistosoma area, and leg length (L1–L4) with respect to body mass for each sex. To compare the allometric relationships of the sexes, an ANCOVA was used to test for homogeneity of slopes with body mass as a co-variable. The allometric exponents (β) were compared with those expected by isometry with Student *t* tests: $t_{n-2} = (\beta - E(\beta))/SE(\beta)$, with the expected value $E(\beta) = 1/3$ for lengths and $E(\beta) = 2/3$ for areas. As all tests were repeated seven times (one time for each variable), the Bonferroni correction for *P*-value was used.

To study multivariate sex differences, comparisons of L1 to L4 (but standardized (L1_s to L4_s) by the cube root of the body mass) were performed with variance analysis for repeated measures (legs). Also, principal components and discriminant analyses were performed to find variables that explain the total variability and the sexual dimorphism, respectively.

Table 1.—Morphological characteristics (Value columns) and allometric relationships among different morphological variables and body size in males and females of the spider *Grammostola rosea*. M_b is the body mass, L1–L4 are length of legs 1 to 4, BL, PSA and OPA are body length, prosoma area and opisthosoma area, respectively. ** indicates a P -value $\ll 0.001$ for sexual differences. F is the F -test with $H_0: b = 0$, b the regression coefficient, and P the P -value of the allometric relationship. Also, the P -value for homogeneity of slopes between sexes in ANCOVA test is shown (P_{sh}), and * indicates differences with the expected values by isometry (1/3 for lengths and 2/3 for areas) in the student t -test.

Variable	Males				Females				P_{sh}
	Value	$F_{(1,24)}$	b	P	Value	$F_{(1,22)}$	b	P	
M_b (g)	$10.10 \pm 1.19^{**}$				16.93 ± 2.37				
BL (cm)	$4.25 \pm 0.22^{**}$	34.82	0.34	<0.001	5.06 ± 0.30	30.34	0.33	<0.001	0.92
PSA (cm ²)	$6.13 \pm 0.28^{**}$	36.3	0.61	<0.001	5.82 ± 0.24	13.44	0.41*	0.001	0.18
OPA (cm ²)	$5.80 \pm 0.22^{**}$	18.03	0.83	<0.001	5.39 ± 0.20	14.26	0.64	0.001	0.46
L1 (cm)	$5.37 \pm 0.25^{**}$	34.18	0.3	<0.001	5.08 ± 0.17	21.31	0.22*	<0.001	0.24
L2 (cm)	$6.44 \pm 0.26^{**}$	23.03	0.33	<0.001	6.13 ± 0.19	20.3	0.19*	<0.001	0.08
L3 (cm)	$2.89 \pm 0.26^{**}$	30.23	0.33	<0.001	3.71 ± 0.33	14.25	0.16*	0.001	0.04
L4 (cm)	$2.54 \pm 0.36^{**}$	30.49	0.25	<0.001	4.54 ± 0.68	8.47	0.12*	0.008	0.04

A clear sexual size dimorphism was found in all variables and all variables showed positive correlation with body mass in both sexes (Table 1). The allometric exponents were similar between the sexes except for L3 and L4, which showed marginal differences. The relationships of all variables with body mass were isometric in males, while females showed negative allometry in all variables except body length and the area of the opisthosoma.

Sexual differences in body length and prosoma area disappeared when body mass was used as covariate ($F_{1,47} = 0.003$, $P = 0.96$ and $F_{1,47} = 0.012$, $P = 0.91$, respectively). However, opisthosoma area showed differences which were not explained by body mass ($F_{1,47} = 8.04$, $P = 0.007$).

There were differences in the standardized lengths of legs L1_s to L4_s ($F_{3,141} = 861.9$, $P \ll 0.001$) and between sexes ($F_{1,47} = 15.2$, $P < 0.001$); an interaction between leg length and sex was found ($F_{3,141} = 8.4$, $P < 0.001$) (Fig. 1).

Principal components analysis of the standardized variables (by $M_b^{1/3}$ in the case of lengths and $M_b^{2/3}$ in the case of areas) showed that the first two components explained 81.4% of the total variance. The first axis was affected positively by L1_s to L4_s and prosoma area and negatively by the opisthosoma area, and the second axis was mainly explained by body length. Discriminant analysis showed a complete separation between males and females (Λ -Wilks = 0.024, $F_{8,40} = 202.98$, $P \ll 0.001$) with 100% correct classification. The discriminant phenetic axis was supported only by differences in L2_s (Λ -Wilks = 0.027, $F_{8,40} = 5.84$, $P = 0.02$) and L4_s (Λ -Wilks = 0.028, $F_{8,40} = 6.89$, $P = 0.01$).

Sexual size dimorphism is common in spiders (Moya-Laraño et al. 2002, 2009; Brandt & Andrade 2007). Our results showed a clear sexual size dimorphism in *G. rosea*, though not extreme because the body mass of males was 59.66% that of females, while in some web spiders of the Theridiidae family, males have 1% of the female body mass. This result agrees with those reported in other ground spiders (Gasnier et al. 2002; Framenau 2005), but is lower than those reported for orb web spiders with sexual size dimorphism (Hormiga et al. 2000).

Males of *G. rosea* showed an interesting isometry in all variables, indicating that an increment in body mass is accompanied by a proportional increment of all locomotor body parts. In contrast, the females only showed isometric growth in the opisthosoma area, while all other variables showed a negative allometric growth. This may be a consequence of favoring the development of the reproductive system instead of developing the locomotor system during the ontogeny of females. Although the abdomen size should be associated with increased fitness, this was decoupled with the growth of the legs, indicating that the females do not encounter selective pressures favoring increased

locomotor efficiency. The opisthosoma area of the females was larger than that of males (standardized by body mass) which may be a consequence of the presence of the large reproductive system in females compared to that of males. The large body mass of the females results in greater force on the legs and could explain the shortening of the limbs relative to that of males. Also, the body mass of females could explain the more pronounced negative allometry in legs 3 and 4, because the biomechanics of spider locomotion may be composed by two successive quadrupeds in series, being the second L3-R3-L4-R4 (L and R, left and right) (Biancardi et al. 2011) and these two legs support the torque caused by the weight of the large abdomen of the female.

These results and those of multivariate analysis show a robust sexual size dimorphism in this species; males have smaller size, longer legs and a shorter opisthosoma than do females. Sexual size dimorphism may come about by different combinations of factors and selective pressures: i) by an increase only in the size of the female, for example as a consequence of a correlation between body size and clutch size as postulated by the fecundity hypothesis in other spider species (Head 1995; Prenter et al. 1999); ii) by the reduction of male size only, which is postulated by several hypotheses (Ghiselin 1974; Reiss 1989; Vollrath & Parker 1992; Elgar & Fahey 1996; Moya-Laraño et al. 2002, 2009; Grossi & Canals 2015); iii) by an increase in female size and a decrease in male size; and iv) by an increase or decrease of the size in both sexes but at different rates. Hormiga et al. (1995, 2000) in a phylogenetic analysis with 80 genera of spiders showed that the monomorphic trait in spiders is a primitive condition and that sexual size dimorphism is a derived character which in some cases has been inverted; on more than five occasions in one family (Araneae, Argiopidae).

Sexual differences in locomotor organs in spiders may be favored both by selection on male mate-searching behavior and by natural selection of female movements in relation to foraging and oviposition (Framenau & Hebets 2007). However, sexual differences in locomotor organs may not be related to an advantage in locomotion. For example, elongated legs in males has been attributed to direct male competition for mates in the water strider *Gigantometra gigas* (Tseng & Rowe 1999) and megalopodine beetles (Eberhard & Marin 1996), in male courtship displays in wolf spiders (Kronstedt 1990; Framenau & Hebets 2007) and to reduce the risk of sexual cannibalism in some orb-web spiders because females may either fail to detect very small males, or ignore them as potential prey items. (Elgar et al. 1990).

Sexual dimorphism in locomotor structures seems widespread in ground living spiders (Calderon et al. 1990; Gasnier et al. 2002; Framenau 2005). For example, Gasnier et al. (2002) reported that males had higher area than females because they have longer legs relative to the

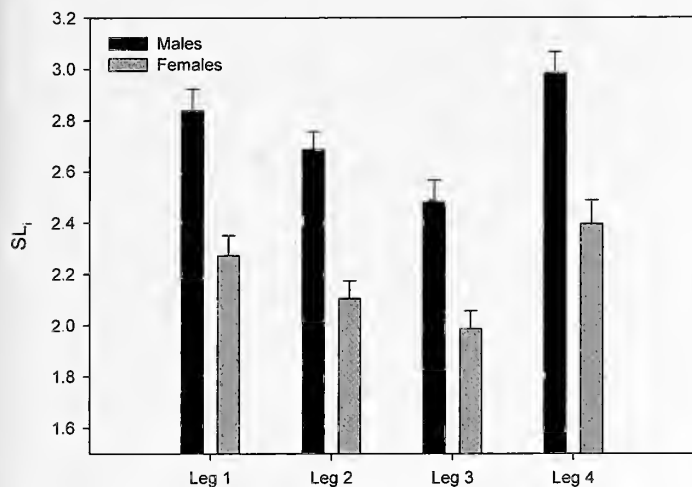


Figure 1.—Standardized length of legs (SL_i , $i = 1-4$) of males and females of the spider *Grammostola rosea*.

prosoma length than females in the spiders *Phoneutria* spp. and *Ctenus* spp. (Ctenidae). Framenau (2005) reported that in the wolf spider *Venatrix lapidosa* (McKay, 1974) which has sedentary females, males had comparatively longer legs than females, while in *Artoria* sp. which has vagrant females, there was no gender-specific difference in relative leg length. This suggests that leg length is associated with the locomotor activity of spiders and provides further evidence that limb elongation in males mainly arises due to indirect male competition for mates.

From an energetic point of view, male spiders with longer legs may have increased locomotor efficiency (Ghiselin 1974; Grossi & Canals 2015). Long legs are related to high speed, which may be determinant in opportunities to copulate, especially in the case of Theraphosidae in which walking males may be concentrated in time and space and have a short lifespan compared to that of females (Costa & Pérez-Miles 2002). High speed and low energy expenditure or cost of transport should be favored by natural selection. Pendulum mechanics show the advantages of long legs in spiders and their relationship to high speed, especially in climbing and bridging spiders. Small size is related to low cost of transport (Grossi & Canals 2015). Thus small size compensated by long legs should be the expected morphology for a fast and mobile male spider, agreeing with our results with *G. rosea*.

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