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Lacking sensory (rather than locomotive) legs affects locomotion but not food detection in the harvestman *Holmbergiana weyenberghi*

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Abstract: The ability to release a leg when forced by predators or during agonistic interactions is widespread and frequent in arthropods. Despite immediate benefits, losing legs may affect locomotion, sensory performance, reproduction, and fitness. The costs of autospasy in arachnids have been scarcely addressed. Therefore, we tested the hypothesis that the number and type of self-amputated legs (sensory or locomotive) affect locomotion and food detection speeds in the harvestman *Holmbergiana weyenberghi* (Holmberg, 1876) (Sclerosomatidae). With field surveys in a subtropical forest in Uruguay we found that 35% of individuals lacked at least one leg, and sensory legs (second pair) were the most frequently lost. In an indoor setup, we found that individuals missing one sensory leg walked and climbed a trunk slower than individuals lacking a locomotive leg (first, third, or fourth pair), or compared with those with eight legs. Lacking legs did not affect the food detection speed. Additionally, larger individuals with eight legs had greater walking and climbing speeds. Therefore, losing sensory legs affects locomotion in these harvestmen and may confer costs in orientation, balance, and substrate recognition. Finally, we compared our results with the different patterns reported for the effect of autospasy in other harvestman species.

Key words: autospasy, climbing speed, daddy long-legs, Opiliones, Sclerosomatidae, Uruguay, walking speed.

Résumé : La capacité de se départir d'une patte pour échapper à un prédateur ou durant des interactions agonistiques est répandue et fréquente chez les arthropodes. Si elle présente des avantages immédiats, la perte de pattes peut avoir une incidence sur la locomotion, la performance sensorielle, la reproduction et l'aptitude. Les coûts de l'autospasie chez les arachnides ont rarement été examinés. Nous avons donc testé l'hypothèse selon laquelle le nombre et le type de pattes (sensorielles ou locomotrices) auto-amputées auraient une incidence sur les vitesses de locomotion et de détection de la nourriture chez l'opilion *Holmbergiana weyenberghi* (Holmberg, 1876) (Sclerosomatidae). Des enquêtes sur le terrain dans une forêt subtropicale de l'Uruguay ont révélé que 35 % des individus avaient au moins une patte manquante, les pattes sensorielles (deuxième paire) étant celles qui manquaient le plus fréquemment. Dans une installation intérieure, nous avons constaté que les individus qui avaient perdu une patte sensorielle marchaient et escaladaient un tronc moins rapidement que les individus ayant perdu une patte locomotrice (première, troisième ou quatrième paire) ou ayant huit pattes. La perte de pattes n'avait pas d'incidence sur la vitesse de détection de la nourriture. En outre, les individus plus imposants à huit pattes marchaient et grimpaient plus rapidement que les plus petits individus. La perte de pattes sensorielles a donc une incidence sur la locomotion chez ces opilions et pourrait avoir des coûts en ce qui concerne l'orientation, l'équilibre et la reconnaissance du substrat. Nous avons comparé nos résultats aux résultats déjà publiés sur les effets de l'autospasie chez d'autres espèces d'opilions. [Traduit par la Rédaction]

Mots-clés : autospasie, vitesse d'escalade, faucheurs, opilions, Sclerosomatidae, Uruguay, vitesse de marche.

Introduction

Autospasy is the ability of certain animals to lose voluntarily an appendage in response to an external stimulus (Roth and Roth 1984; Fleming et al. 2007). It has been widely cited for vertebrates such as salamanders and lizards, as well as invertebrates such as crustaceans, arachnids, insects, and echinoderms (Roth and Roth 1984; Johnson and Jakob 1999). Self-amputation of a leg, an arm, a tail, or any other body part can help distract a predator, escape from a predator's grasp, and (or) get free from traps (Fleming et al. 2007). Furthermore, autospasy could also be advantageous during male intrasexual contests for female access (Macías-Ordóñez 1997). Though this mechanism seems beneficial as an antipredatory tactic or to prevent capture once an animal is attacked during intrasexual competition, lacking an appendage can have high costs on individual performance (Riechert 1988; Guffey 1998; Amaya et al. 2001; Taylor et al. 2008). Activities such as locomotion, foraging, mate search, courtship, copulation, and detection

of and evasion from predators could be negatively affected by limb loss. However, information on these topics is scarce.

In arachnids, the frequency of limb loss is variable among species, but is often high (Roth and Roth 1984; Uetz et al. 1996; Punzo 1997; Guffey 1999; Taylor et al. 2008; Houghton et al. 2011). Leg autospasy is known to occur in spiders (Johnson and Jakob 1999; Brueseke et al. 2001; Taylor et al. 2008; Wrinn and Uetz 2008) and harvestmen (Guffey 1998, 1999; Houghton et al. 2011; Smith et al. 2012), and tail loss has been recently described for the first time in a scorpion species (Mattoni et al. 2011). Though autospasy seems to be widespread in arachnids, very few studies have investigated its costs and results are variable and frequently conflicting when compared among species (Riechert 1988; Guffey 1998; Johnson and Jakob 1999; Amaya et al. 2001; Brueseke et al. 2001; Houghton et al. 2011). Though juvenile spiders are able to regenerate lost legs during molt (Fleming et al. 2007; Foelix 2011), harvestmen appendages cannot be regenerated, even if they are lost in early instars (Gnasplini and Hara 2007). Autospasy in harvestmen occurs in the

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suborder Eupnoi when individuals separate a leg when it is retained by an external source, and this happens at the trochanter-femur junction or near the base of the femur (Roth and Roth 1984). The effects of leg autospasy in harvestmen have been studied in a few species of Sclerosomatidae (Guffey 1999; Houghton et al. 2011). In the harvestman genus *Leiobunum* C.L. Koch, 1839, leg loss occurs in 50% of the individuals and most frequently involves leg II (Guffey 1998). In *Leiobunum nigripes* Weed, 1887 and *Leiobunum vittatum* (Say, 1821), leg loss has a negative effect both in locomotion speed and in prey detection when the harvestmen possesses five legs, but harvestmen with six or seven legs do not show negative effects (Guffey 1999). In another *Leiobunum* species, Houghton et al. (2011) found that harvestmen with five, six, or seven legs perched on lower vegetation and walked slower compared with those with eight legs. In a species of the harvestman genus *Prionostemma* Pocock, 1903, a Sclerosomatidae from Costa Rica, 50% of the individuals found in aggregations lacked one or more legs; however, 70% of solitary individuals lacked legs, suggesting that aggregations could provide a more effective defense against predators in this species (Quesada 2011). The second legs of harvestmen function as antennae and are specialized sensory organs (Pinto-da-Rocha et al. 2007), so not only the number of amputated legs but also which legs are lost could be determining the real costs of amputation in terms of future abilities in locomotion, mating, foraging success, and predation avoidance.

Holmbergiana weyenberghi (Holmberg, 1876) (Sclerosomatidae) is a solitary nocturnal harvestman that inhabits areas with high levels of humidity, near riversides, and along the coastlines of Argentina and Uruguay (Capocasale and Gudynas 1993; Aisenberg et al. 2011). During the day the individuals hide among the vegetation or under fallen leaves or logs. They become very active during summer nights, when they leave their refuges to forage and to search for mates (Aisenberg et al. 2011). Leg autospasy has been reported for this species (Aisenberg et al. 2011). However, no details on the frequencies and types of lost legs, or its associated costs, are available for this species. The objective of the present study was to register the frequencies and characteristics of leg loss under natural conditions, as well as to examine the effect of leg autospasy in *H. weyenberghi* on locomotive speeds. We tested the hypothesis that the number and type (sensory or locomotive) of missing legs affect the walking, climbing, and food detection speeds in this harvestman species. Under this hypothesis, we predicted that individuals lacking legs would have lower horizontal and vertical locomotion speeds, especially when locomotive legs are missing. Additionally, we expected that autospasy of leg II would lead to lower food detection speed.

Materials and methods

Study area

We conducted this study in La Quebrada de los Cuervos, Treinta y Tres province, Uruguay (32°55'34.3"S, 54°27'40.6"W; elevation 124 m). The site is a ravine that extends along the Yerbal Chico stream in Sierra del Yerbal. The Quebrada de los Cuervos is a 4400 ha protected area that includes natural grasslands, rocky hills, and a subtropical riparian forest running along the Yerbal stream (Evia and Gudynas 2000; Prigioni et al. 2011). In this forest, *H. weyenberghi* is abundant and easily found during the summer. During the day individuals hide under rocks, beneath rock crevices, and trunks. After dusk, they wander around over the leaf litter searching for food and mating (Aisenberg et al. 2011).

Field observations

Five researchers performed hand capture using head lamps and collected all large juveniles and adult individuals of *H. weyenberghi* found walking or perching at the vegetation along a linear transect 500 m long and 5 m wide, drawn along the riparian forest that runs along the edge of Yerbal stream. The samplings occurred

between the hours of 2100 and 2400 on 31 October 2012. For each individual, we recorded (i) the activity the individual was carrying out when it was captured (walking, bobbing, descending from a log, or perching), (ii) the substrate where it was found, and (iii) if the harvestman was found alone or in a group. As with most Eupnoi harvestmen, *H. weyenberghi* can perform bobbing behavior, in which cluster or solitary individuals vibrate their body upside down rapidly (Gnasparini and Hara 2007). The information about activity patterns of *H. weyenberghi*, although it was not in direct relation with our objective and hypothesis, provided useful information about their natural history and behavior, and served to understand the environment and substrates these harvestmen face. Afterwards, all the individuals were placed in plastic cylindrical containers with wet cotton, leaf litter, and small branches to provide a refuge and acclimatize them for indoor trials.

Indoor observations

We carried out indoor observations the next day (1 November 2012), from the hours of 1000 to 2000, in a dark room with 57% relative humidity and 22.8 °C. On 2 November 2012, after the observations had concluded, we released all the individuals to the same transect from where they had been collected. The harvestmen in the experiments were cared for in accordance with the *Guide to the Care and Use of Experimental Animals*, Vol. 1, of the Canadian Council on Animal Care. We randomly chose 99 individuals to perform the observations, and with the remaining 107 individuals that had been collected, we registered only the type and quantity of missing legs. We performed all the observations in the dark room using a head lamp that illuminated always in the same direction, pointing from the back of the individual placed at the start point.

We created two experimental groups for our observations: group I (a, b) and group II. The order of the trials in groups Ia, Ib, and II was randomly assigned and half of the harvestmen were first exposed in group I and half in group II, and then they were exchanged. In group I, the order of the trials was also interchanged: half of the harvestmen were first exposed in horizontal substrate and then in vertical substrate, and the other half were first exposed in vertical substrate and then in the other one. We waited for 15 min after each trial to expose the individual again.

In group Ia, we conducted observations of walking speed in a one gallon plastic container (30 cm long × 23 cm wide × 15 cm high) placed horizontally on a table, containing a 5 cm layer of soil as substrate, to test the locomotion speed in a natural substrate. Following similar methods as in Smith et al. (2012), we introduced each individual gently on the test arena, grabbing it from more than three legs to prevent autospasy. We placed every individual in a predetermined standardized start point and chose an end point at the opposite side of the container. We released the individual at the start point after it touched the substrate with more than two legs, to prevent it from dropping to the ground. We interpreted the movement of the harvestmen as escape velocity, considering that grabbing it from the legs and releasing it on the soil could be a potential predator stimulus. We measured the distance from the start to the end point and the time period the individual used for getting from the start to the end point, or until the individual stood still for more than 10 s. We followed the walking trajectory of each individual by placing colored nails every time the harvestman changed the direction of its route from the start to the end points. We always placed the nails after the individual had changed the route and while it was moving away from the turning point. We did not observe that placing the nails affected the walking direction or speed of the individuals. We used a CASIO digital stopwatch (±0.1 s) to record the time and a measuring tape (±0.5 cm) to note the total distance the individual walked. We calculated the walking speed as a ratio of distance over time. We also registered the number of diversions from a straight-line path.

In group Ib, or observations of climbing speed, we used the same individuals as used in experiment group Ia. We placed one by one, each of the 99 individuals at the base of a cylindrical log of the genus *Eucalyptus* L'Hér. with a 10.0 cm diameter and a 130 cm length, which was leaned against a wall to make a 45° angle. We arbitrarily determined a start point at the base of the log and an end point near the top of the log. We followed a similar procedure to that described for group Ia and recorded the distance climbed and the time period required until the animal reached the end or remained still for more than 10 s.

In group II, or observations of food detection, we recorded the distance walked and time period of the individuals to touch a food source to report the food source detection speed. The setup was similar to group I and we placed each harvestman on the side of the container opposite to the Petri dish. We used as a test arena a one gallon plastic container of the same dimensions as that used for the group Ia and placed horizontally on a table, with soil as substrate and a 3.7 cm diameter and 0.41 cm high Petri dish with approximately 4 g of processed vegetable food (Gerber), following Guffey (1999). We considered that the harvestman had detected the food source when it touched the processed food with legs II. Only half of individuals tested started eating the food after 5 min, therefore we registered the time until touching the food source, not necessarily eating it.

After the experiments, we made sporadic video recordings (with a Nikon Coolpix P100 digital camera at 30 frames/s) of approximately two dozen individuals walking on a wooden table, including harvestmen with eight legs and others lacking both sensory and (or) locomotive legs. This allowed us to have a preliminary insight into the locomotion process and the qualitative differences in walking when the harvestmen lack certain legs. Afterwards, we examined each individual and recorded if they had any missing legs, specifying whether they were locomotive legs (first, third, or fourth pair) or sensory legs (second pair), and if the missing leg was left or right. To correct our locomotion data for body size, we photographed each harvestman three times with the digital camera mentioned above and measured their dorsal carapace area using ImageJ program (Abramoff et al. 2004). We reported the mean carapace area as an estimation of the harvestmen size. For six individuals it was not possible to photograph them because of their active movements, which resulted in pictures with inappropriate focus. Therefore, sample size in certain statistical analyses varied (see below). We deposited voucher specimens in the entomological collection of Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay.

Statistical analyses

We compared the walking, climbing, and food detection speeds between individuals categorized into three groups according to the number and type of legs that were missing: (1) complete (eight legs), (2) individuals lacking at least one sensory leg, and (3) individuals lacking at least one locomotive leg. We found that only 5% of the experimental individuals lacked more than one leg (see Results), so we assigned those individuals to group 2 or 3 depending to the type of missing leg. Four individuals included in our locomotion observations were lacking one sensory and one locomotive leg; therefore, we randomly assigned them to either group 2 or 3. We compared body size between individuals with eight legs versus individuals with less than eight legs (seven and six legs) with a Student's *t* test. To investigate the effect of the missing legs and body size on walking speed, we performed an analysis of covariance (ANCOVA). We used type of missing legs (complete, sensory leg missing, and locomotive leg missing) as a categorical predictor, individual size as a continuous predictor, and walking speed as response variable. We applied other ANCOVA tests using the number of turns in the trajectory during the walking trials, climbing speed, or food detection speed as response variables. We performed simple linear correlations to investigate a posteriori if body sizes of each harvestmen group (complete legs, missing one

Table 1. Frequency of the harvestman *Holmbergiana weyenbergi* (Scleresomatidae) found per substrate and activity.

Substrate	Activity		
	Bobbing	Walking	Standing still
Tree branches	3	0	7
Vertical tree log	0	10	1
Base tree log	1	2	6
Foliage	1	10	4
Leaf litter	2	59	4
Tree roots	1	0	3
Rocks	8	51	10
Soil	1	13	2
Fallen log	1	1	6

or more locomotive leg, or missing sensory legs) were associated with walking speed, and an additional set of correlations for climbing speed, for a total of six comparisons. Finally, we compared the walking and climbing speeds of the individuals tested with a paired *t* test. We present data as means \pm 1 SD. We performed the statistical analyses with Past Paleontological Statistics version 1.18 (Hammer et al. 2001) and NCSS 2001 (Hintze 2000).

Results

Field observations

We observed 209 individuals of the harvestman *H. weyenbergi* and 93% of them were found alone. Regarding the grouped individuals, we found six pairs and a group of three. Grouped individuals were mostly standing still on the ground, or they were walking slowly very close (<10 cm) between each other. We found 69% of the individuals walking or bobbing, mostly above rocks, leaf litter, or soil, and 21% were standing still (Table 1).

Overall, 65.5% of the total number of individuals had their eight legs. One third of the individuals lacked at least one leg: 13% lacked only one walking leg and 16% lacked only one sensory leg (from the second pair). Only 6% of individuals lacked more than one leg. Two individuals lacked both sensory legs, two individuals lacked two walking legs, seven individuals had one sensory leg and one walking leg missing, and only one harvestman had five legs, in which one sensory and two walking legs were missing. The individuals measured 6.05 ± 1.68 mm² of carapace area (range = 3.08–12.01 mm², $n = 99$). The body size did not differ between individuals with eight legs (6.03 ± 1.59 mm², $n = 59$) and individuals with less than eight legs (6.09 ± 1.87 mm², $n = 30$ individuals with seven legs and 5 individuals with six legs) (Student's *t* test: $t_{[93]} = 0.18$, $p = 0.86$).

Indoor observations

Harvestmen with eight legs, and the ones with at least one locomotive leg missing, walked faster than the group in which at least one sensory leg was missing (ANCOVA: $F_{[2,90]} = 5.53$, $p = 0.005$; Fig. 1A). The size of the individuals was positively associated with their walking speed ($F_{[1,90]} = 3.90$, $p = 0.05$). Larger harvestmen with all their legs walked faster than smaller ones ($r = 0.26$, $p = 0.04$, $n = 63$). That trend was not present with individuals lacking one or more locomotive legs ($r = 0.06$, $p = 0.83$, $n = 14$) or sensory legs ($r = 0.16$, $p = 0.47$, $n = 22$). The number of turns in the trajectory was not different from individuals with eight legs (2.1 ± 0.8 , range 1–6), individuals lacking at least one locomotive leg (1.9 ± 0.5 , range 1–3), or lacking at least one sensory leg (2.3 ± 1.5 , range 1–7) (ANCOVA: $F_{[2,90]} = 1.26$, $p = 0.29$). The number of turns was not associated with body size ($F_{[1,90]} = 1.79$, $p = 0.18$).

As for climbing speed, harvestmen with all eight legs walked faster than individuals lacking at least one sensory leg ($F_{[2,90]} = 7.52$, $p < 0.001$; Fig. 1B). Climbing speed was positively correlated with body size of the individuals ($F_{[1,90]} = 8.16$, $p = 0.02$; Fig. 2). Larger harvestmen with all their legs climbed faster than smaller

Fig. 1. Walking (A) and climbing (B) speeds (mean + SE) of individual harvestman *Holmbergiana weyenberghi* (Scleresomatidae) with all its legs, lacking at least one locomotive leg, and lacking at least one sensory leg. Sample sizes are shown in parentheses. The letter inside bars represents statistically different groups, derived from ANCOVA comparisons.

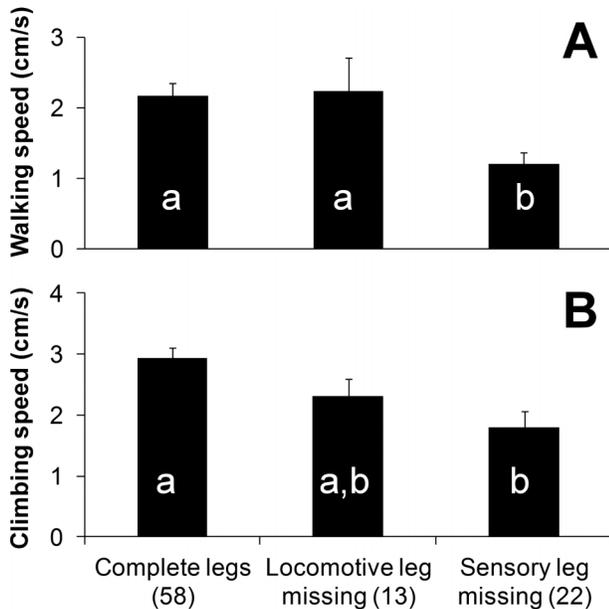
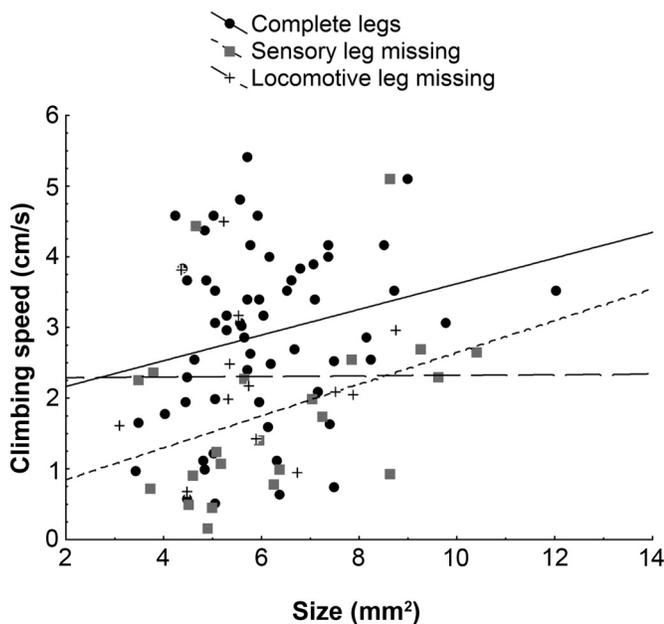
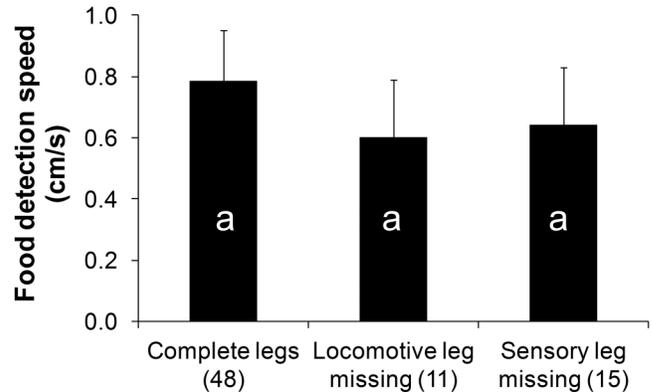


Fig. 2. Climbing speed of individual harvestman *Holmbergiana weyenberghi* (Scleresomatidae), with all its legs, lacking at least one locomotive leg, and lacking at least one sensory leg, relative to its size (carapace area). Linear fit of each group is shown to account for the pattern found in the ANCOVA.



individuals ($r = 0.23$, $p = 0.07$, $n = 63$; Fig. 2). However, size was not associated with climbing speed in individuals lacking a locomotive leg ($r = 0.36$, $p = 0.09$, $n = 14$; Fig. 2) and those lacking a sensory leg ($r = 0.01$, $p = 0.98$, $n = 22$; Fig. 2). On the other hand, the food source detection speed did not differ between harvestmen with or without leg autotomy ($F_{[2,71]} = 0.23$, $p = 0.79$; Fig. 3) or according to body size ($F_{[1,71]} = 0.01$, $p = 0.93$). Of the individuals tested, 23% did

Fig. 3. Food source detection speed (mean + SE) in an enclosure with harvestman *Holmbergiana weyenberghi* (Scleresomatidae) relative to its leg condition. Sample sizes are shown in parentheses. The letter inside bars represents statistically different groups, derived from ANCOVA comparisons.



not touch the food source in the 5 min interval: 13 individuals with eight legs, 7 individuals missing one sensory leg, and 3 individuals missing a locomotive leg. The characteristics of the individuals that did not touch the food source did not differ from the lacking legs pattern, hence no more individuals missing a sensory leg did not touch the food source than expected by chance ($\chi^2_{[2]} = 1.17$, $p = 0.56$). Fifty-six individuals began feeding 25.55 ± 54.24 s (range = 0–276 s) after detecting the food source.

The walking and climbing speeds were positively correlated ($r = 0.29$, $p = 0.01$, $n = 100$). However, the food source detection speed did not correlate with walking or climbing speed ($r = -0.11$, $p = 0.35$ and $r = -0.01$, $p = 0.95$, respectively, $n = 76$ for both tests). The climbing speed of all harvestmen tested (2.60 ± 0.76 cm/s) was 24% higher than their walking speed (1.97 ± 1.32) (paired t test: $t_{[98]} = -4.13$, $p < 0.001$). During the observations of climbing speed, only 13 individuals moved downwards from the start point, the rest walked upwards. Most harvestmen changed their direction only once while climbing and two individuals changed it twice. While walking, 64% of individuals changed their body orientation two times, 18% changed it once, 13% three times, and 5% between four and seven times.

In the video recordings, we anecdotally observed that individuals with eight legs walked with apparently shorter steps (proportionally to the anteroposterior reach of each leg) than individuals lacking at least one locomotive leg. The leg next to the where one leg is missing seemed to be extended farther while walking, perhaps to reach the position where the missing leg would have made contact with the substrate. Also, individuals lacking at least one leg walked with an apparent greater dorsoventral oscillation (both in height and frequency) of the prosoma than individuals with eight legs. One individual with five legs (two locomotive and one sensory leg missing) made more steps (5 in 10 s) than individuals with eight legs (1–2 in 10 s). Also, individuals lacking legs seemed to be more cautious while walking than individuals with eight legs. Two individuals lacking one sensory leg seemed to be more prone to orient their trajectory in the direction where the remaining sensory leg was (walked towards the left if left sensory leg was present).

Discussion

Our results confirm a negative effect of lacking one or more legs in the walking and climbing speeds of *H. weyenberghi*, but leg autotomy does not seem to affect food detection speed in this species. Although missing legs was frequent in nature (35% of individuals), only 6% of individuals lacked more than one leg. Similar frequencies of individuals missing at least one leg were found for the harvestmen *L. nigripes* and *L. vittatum* (Guffey 1998) and *Leiobunum formosum* (Wood, 1870) (Houghton et al. 2011). In

Leiobunum politum Weed, 1890, the frequencies of occurrence of leg loss are still higher (up to 64%) (Houghton et al. 2011). Therefore, leg autospasy would be a widely distributed phenomenon and frequent strategy in Sclerosomatidae harvestmen.

Lacking one or more legs affected locomotive activities in *H. weyenberghi*, so our results agree with the findings of Guffey (1999) and Houghton et al. (2011) on three species of *Leiobunum*. Individuals of *H. weyenberghi* that lacked one or more locomotive legs (leg I, III, or IV) did not differ from individuals with eight legs in walking or climbing speed. Guffey (1999) found that in *L. vittatum* only, individuals with five legs had slower initial escape velocity and mean velocity compared with individuals with eight legs. In *L. politum*, Houghton et al. (2011) found a gradual decrease in walking speed with the number of missing legs. Probably, in *H. weyenberghi* and as days go by after losing a leg, they can adjust locomotive behavior and compensate for the missing appendages, changing the walking pattern to reach the typical walking or climbing speeds, as has been cited for arachnids (Foelix 2011). The flexibility of behavior in this direction would be adaptive in organisms such as arachnids in which leg loss is common. Houghton et al. (2011) induced harvestmen to release one or more legs 24 h prior to the walking and climbing measurements. However, in this study, we used individuals captured at the field with missing legs; hence, we did not know the number of days that individuals had been lacking legs. However, the frequency of missing legs (35%) suggests that perhaps individuals had been lacking legs for several days. Although feasible, it is not possible to test this hypothesis with our data, and future studies controlling for the time that an individual has been lacking leg(s), as well as the number, type, and side of the missing leg(s), should lead to this approach.

The walking speed of *H. weyenberghi* was similar to that reported for *L. vittatum* (Guffey 1999), but lower than that of *Prionostemma* species (Smith et al. 2012) and *L. politum* (Houghton et al. 2011). The climbing speed was higher in *H. weyenberghi* than in *Prionostemma* species (Smith et al. 2012), but similar to *L. politum* (Houghton et al. 2011). The differences in speed could be due to body size, leg size, or the methods employed. Individuals of *Prionostemma* species show similar body size to *H. weyenberghi*, but climbing speed was tested with palm leaves in *Prionostemma* (Smith et al. 2012), which could explain the differences in walking speed with *H. weyenberghi*. For *L. politum*, the individuals were tested walking and climbing on a flat tabletop trackway (Houghton et al. 2011). Therefore, the differences in the type of substrate, its roughness, and traction for each species of harvestmen could account for the differences between our pattern and the other species. Another factor that could have affected walking and climbing speeds is the fact that individuals were freed at the start point without chasing or inducing them to walk or climb. However, previous studies (Guffey 1999; Houghton et al. 2011; Smith et al. 2012) used a different method while testing the effects of autotomy on walking speed on several harvestmen species, following the protocol described by Guffey (1999) who grasped the test animals by the posteriormost pair of legs, held them at the start point until movements ceased, and released them to test walking speed. In addition, we tested walking and climbing speeds in a dark room but during daytime hours and individuals are primary nocturnal, which could have affected their walking and climbing responses and speeds. All these hypotheses require further testing.

We found that *H. weyenberghi* had faster climbing than walking speeds. This is the opposite pattern to that found in *L. politum* (Houghton et al. 2011) and *Prionostemma* species (Smith et al. 2012). Differences in the natural history of the harvestmen species could account for this contrast. We found most individuals walking, standing, or perching on rocks, and in some sporadic observations during daytime, we saw individuals below rocks. Therefore, rocks could be the preferred substrate, suggesting that tree logs are not their most common substrate where they have better traction; therefore, they aim to walk (and escape) faster. In the genera

Leiobunum and *Prionostemma*, it is probably more common for individuals to climb trees to perch and forage (Houghton et al. 2011; Wade et al. 2011).

Finding that lacking a sensory leg, instead of a locomotive leg, affected walking and climbing speeds was an unexpected pattern and disagrees with results by Guffey (1999). Additionally, lacking a sensory leg did not affect food detection speed, contrary to our expectations based on the fact that the second leg pair is involved in chemical detection in other harvestmen species (Willemart and Chelini 2007). One possibility is that the sensory processes that occur in the second leg pair that are related to locomotion are more crucial than those occurring in locomotive legs. Certain tasks involving the sensory structures in the second leg pair that could be affected when *H. weyenberghi* lose this leg include the general body and space orientation, climbing balance, substrate recognition, and prehensile flexion, as has been suggested for other harvestmen species (Guffey et al. 2000). This could be true if most sensory functions of the harvestmen legs are performed in the second leg pair; however, there is no evidence currently to support this idea.

The lack of difference in food detection speed between individuals lacking sensory or locomotive legs does not support the exclusiveness of sensory tasks in the second pair of legs. This is congruent with Guffey (1999) who found that *Leiobunum* harvestmen with seven legs did not differ in their foraging skills and fly consumption from individuals with eight legs; the effect was present only in individuals with five legs. In our case, we did not find *H. weyenberghi* with five legs in the field, and individuals with six legs were seldom found, and therefore the comparison with Guffey (1999) is not possible. Additionally, other behaviors of *H. weyenberghi* could be affected because of the lack of a sensory leg, which are worth addressing in further studies, including the rapid detection of suitable microhabitats. Houghton et al. (2011) found that *Leiobunum* individuals missing one leg perched at lower height in vegetation. Sexual behavior could also be negatively affected by autospasy, particularly when the sensory leg pair is lost. The sensory leg pair in harvestmen is involved in mate detection, courtship, and copulation (Macías-Ordoñez 1997; Willemart et al. 2006; Pinto-da-Rocha et al. 2007). Hence, our results suggest the importance of the second leg pair in Eupnoi harvestmen, and that these legs could perform particular and perhaps unique functions in the behaviors of these arachnids.

The body size of *H. weyenberghi* correlated with walking and climbing speed only in individuals with eight legs, suggesting that there is a positive allometric relationship between body size and leg length in this species. A positive effect of body size on walking speed has been reported for various harvestmen species (Pinto-da-Rocha et al. 2007; Smith et al. 2012). However, we report for the first time the effect of body size on climbing speed in Eupnoi harvestmen, as well as the absence of a correlation between body size and food detection speed. Longer legs apparently favor greater locomotive speeds, perhaps due to the mechanical benefits of having more balance support or grabbing point with the legs, and the possibility of making larger steps (Guffey et al. 2000). What it is noticeable is the fact that this effect was only present when the individual had eight legs. Lacking at least one leg probably caused a negative effect that counteracted the allometric advantage of possessing a larger body size for locomotion. Additionally, the number of missing legs did not correlate with body size, suggesting that both juvenile and adult individuals lose legs.

In this study, we found no difference in the number of turns in the trajectory while walking between individuals with eight legs and individuals lacking legs. Therefore, we can discard this phenomenon as a potential mechanism affecting the slower escaping velocities of harvestmen lacking sensory legs. Some other factors that arose from our video-recording observations might contribute to the loss of speed and should be included in future research, with appropriated video recordings and fine-scale analysis of the

performance of the harvestmen. The frequency of stops during walking, the stride length, step frequency, sensory leg movements, walking direction and trajectory, dorsoventral oscillations while walking, the reach of sensory bouts of locomotive legs, and other qualitative details of the biomechanics of harvestmen remain to be tested as potential mechanisms for the costs of losing legs in locomotion.

Autospasy in *H. weyenberghi* could occur as a defense mechanism against predators such as lizards, frogs, birds, and spiders that are considered their most likely predators (Pinto-da-Rocha et al. 2007). Also, male–male aggressive encounters in a sexual context and competence for territories or refuges could account for leg loss in this species. In the study site and period, *H. weyenberghi* was abundant (209 individuals in a 3 h survey). We found approximately 10% of total individuals performing bobbing in the field, which could suggest they noted our presence and identified us as potential predators, therefore corroborating this behavior as a defense mechanism (Gnaspini and Hara 2007).

Summarizing, our results agree with Guffey (1999) in the genus *Leiobunum* and support the spare-leg hypothesis regarding autospasy on walking legs, but this would not apply to the loss of sensory legs. The spare-leg hypothesis states that losing one or more legs does not have a significant effect on the survival of individuals (Macías-Ordoñez 1997; Guffey 1999). Our study represents the first approach to understand the general locomotive behavior and the negative effect of leg autospasy on the Neotropical harvestmen *H. weyenberghi*. Considering that harvestmen do not regenerate legs even when they are lost in early instars (Guffey 1998; Pinto-da-Rocha et al. 2007), contrary to spiders (Foelix 2011), the question would be “how can leg autospasy be an adaptive defensive strategy for this group”? Further studies will focus on describing in detail how individuals of this species compensate for leg loss, perhaps by altering the typical walking pattern, and testing the effect of leg autospasy on reproductive activities. Finally, the locomotion performance of *H. weyenberghi* based on the number and type of legs lost was not compared between sexes, because there is no conspicuous sexual dimorphism in this species. However, future studies should address these topics, especially in species where behavioral patterns and foraging activities differ between sexes.

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