

## Sexually dimorphic legs in a neotropical harvestman (Arachnida, Opiliones): Ornament or weapon?

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### ABSTRACT

The evolution of sexually dimorphic traits has been the focus of much theoretical work, but empirical approaches to this topic have not been equally prolific. Males of the neotropical family Gonyleptidae usually present a strong fourth pair of legs armed with spines, but their functional significance is unknown. We investigated the putative functions of the leg armature in the harvestman *Neosadocus maximus*. Being a non-visual species, the spines on male legs can only be perceived by females through physical contact. Thus, we could expect females to touch the armature on the legs of their mates if they were to evaluate it. However, we found no support for this hypothesis. We did show that (1) leg armature is used as a weapon in contests between males and (2) spines and associated sensilla are sexually dimorphic structures involved in “nipping behavior”, during which a winner emerged in most fights. Finally, we demonstrate that five body structures directly involved in male–male fights show positive allometry in males, presenting slopes higher than 1, whereas the same structures show either no or negative allometry in the case of females. In conclusion, leg armature in male harvestmen is clearly used as a device in intrasexual contests.

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### 1. Introduction

In several animal species males present sexually dimorphic structures that are used as weapons in male–male contests, as displays for females or as devices to grip on the female during copulation (see examples in Andersson, 1994). Among arthropods, for instance, beetle horns are certainly the best studied sexually dimorphic weapons (e.g., Eberhard, 1982; Eberhard et al., 2000; Brown and Rockwood, 1986; Conner, 1988, 1989a,b). They are used in male fights either to pry up and push a rival off his site or to grasp, lift and drop him to the ground (Eberhard, 1979, 1980). Displays involving the exhibition of enlarged chela in fiddler crabs (e.g., Murai and Backwell, 2006), tufts of setae in some lycosid spiders (Hebets, 2004), and color patterns in some insects, and vertebrates such as fish, salamanders, lizards, and birds (see examples in Andersson, 1994), are only a few examples of signaling male characteristics used by females to choose a mate among visually oriented species. Because the role of sexually dimorphic structures varies among

species and also because sexually dimorphic traits may be shaped simultaneously by intra- and intersexual selection (Johnstone and Norris, 1993), detailed observations of male–male interactions as well as of copulatory behavior are necessary to achieve an appropriate explanation of their functional meaning in different taxa.

Positive allometry, meaning that larger individuals have proportionately larger traits than smaller individuals, has been described in many sexually selected traits used as weapons or displays for females (e.g., Alatalo et al., 1988; Eberhard and Gutierrez, 1991; Green, 1992; Baker and Wilkinson, 2001; Simmons and Tomkins, 1996, but see Emlen and Nijhout, 2000 and Bonduriansky and Day, 2003 for examples of isometry, negative, and non-linear allometries in such traits). This pattern emerges probably due to the increasing benefits and decreasing costs of bearing an enlarged secondary sexual character as overall body size increases (Petrie, 1988, 1992). In the case of traits used as weapons in intrasexual contests, larger individuals may benefit from displaying a relatively large structure given its body size if it reduces the number and intensity of fights in which they are involved (e.g., Clutton-Brock, 1982). Additionally, small individuals that are of a lower quality may be prevented from producing large traits, since these traits are assumed to be costly and condition dependent (Zahavi, 1975). Small individuals may also

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invest fewer resources because there are few benefits to a structure that advertises low competitive ability (Petrie, 1988). Similar explanations have been proposed for positively allometric traits used as displays for females, i.e., small individuals would not benefit from, and may not be able to invest in, secondary sexual characteristics (Green, 1992). The empirical data on this subject, however, is scarce and heavily biased towards vertebrates and insects. Thus, further data with different taxonomic groups would be useful to shed more light on this issue.

The Opiliones is the third largest order within class Arachnida with nearly 6000 species (Machado et al., 2007). Sexual dimorphism in the group is incredibly diverse, including: (a) the presence of glands on the pedipalps, chelicerae, legs, sternal or anal region of the males; (b) differences in the size and armature of the pedipalps, chelicerae, legs, and ocularium; and (c) differences in the shape, size, and coloration of the body (see examples in Pinto-da-Rocha et al., 2007). Although sexual dimorphism may be the consequence of different selective forces (Anderson and Vitt, 1990; Forsman and Appelqvist, 1998), the great variety of forms of sexual dimorphism in Opiliones suggests that sexual selection may have played an important role in the evolution of the order. Among the neotropical species of the family Gonyleptidae, for instance, males usually present a strong fourth pair of legs, with several tubercles and spines on the coxa, trochanter, femur, and sometimes on the patella and tibia as well (see examples in Gonyleptidae, Cosmetidae, and Cranidae in Kury and Pinto-da-Rocha, 2007a,b and Pinto-da-Rocha and Kury, 2007). Although the leg armature is known in detail by the taxonomists of the group, and has been used as one of the main sources of information to identify harvestman species for more than two centuries, the functional meaning of the tubercles and spines is poorly understood. The only function so far demonstrated for the male armature in gonyleptids is self-defense because individuals use their fourth pair of legs to deliver a sharp pinch to the offending agent's hand between the armature of both coxae and femora (review in Gnaschini and Hara, 2007).

The primary goal of this paper was to describe the putative functions of the leg armature in the large-bodied neotropical harvestman *Neosadocus maximus* (Gonyleptidae). If the armature on legs IV of males is under intersexual selection, we predict that females would try to obtain information about this male trait before, during or after copulation. On the other hand, if the armature on legs IV of males is under intrasexual selection, we predict that spines would be somehow used in male–male contests. These scenarios, however, are not mutually exclusive. We tested these predictions by observing copulatory behavior and male–male interactions of captive individuals of *N. maximus*, and assessed the role of male specific morphological traits on such interactions, as well as their static allometries.

## 2. Material and methods

### 2.1. Behavioral observations in the laboratory

We collected adult males and females of *N. maximus* in the Parque Estadual Turístico do Alto do Ribeira (PETAR; 24°26′S; 48°34′W) and Parque Estadual Intervales (PEI; 24°14′S; 48°04′W), both in São Paulo state, southeastern Brazil. These two areas form a continuum located at the Ribeira Valley, a large Atlantic Forest remnant. The annual rainfall in the region ranges from 2000 to 3000 mm and the mean annual temperature ranges from 12 to 20 °C. The climate is seasonal with two well-defined seasons. The dry and cold season lasts from April to September and has a mean monthly rainfall of 139 mm and a mean monthly air temperature of 17.4 °C. The rainy and warm season lasts from October to March and has a mean

monthly rainfall of 306 mm and a mean monthly air temperature of 20.9 °C.

We reared individuals collected in the PETAR in individual (13 males) and collective (10 females) terraria containing humid soil. They were fed *ad libitum* on pieces of *Tenebrio* larvae, banana, and a variety of industrial food such as cream cheese, ham, and cat food. Observations on male–male interactions ( $n = 10$ ) were conducted in “fighting arenas” where two males and one female were introduced simultaneously. The individuals were chosen randomly and each individual was used only once a day. Dimensions of the fighting arenas were: (a) 45 cm × 20 cm base, 25 cm height, (b) 20 cm × 15 cm base, 15 cm height, both with humid towel paper on the bottom, and (c) 20 cm × 11 cm × height 9 cm with humid soil on the bottom. Although the arenas had different sizes, even the smallest one seemed to provide enough space for normal development of the fights. Individuals collected in the PEI (nine males and nine females) were individually marked with enamel color paint and reared in a collective terrarium, thereafter called “rearing arena”. The dimensions of this terrarium was 40 cm × 90 cm base, 20 cm height, containing humid soil, pieces of dead tree trunks, and two clumps of aroid plants. They were fed on pieces of dead cockroaches three times a week. Seventeen fights were observed and recorded inside this rearing arena.

We either video-recorded ( $n = 11$ ) or described the fights taking note of the entire sequence of events ( $n = 16$ ) (see Section 3 for an operational definition of the start and the end of the fights). Video recordings were made with a JVC GR-AX1010 video camera or a Sony DCR-TRV 740 digital video camera. Descriptions of the behavioral sequences as well as the drawings are based on the video images. To analyze the video-recorded fights we identified 16 behavioral acts using the software for behavioral description Etholog 2.25 (software by EB Ottoni, 1999). In order to explore predictions from fight theory (see Section 4), we carried out Pearson correlation analyses between size (dorsal scute length) of the smaller male and fight duration, and between size difference between contenders and fight duration in those cases for which there was a winner (see Table 1), fight duration and morphological data for both contenders ( $n = 12$ ). In both correlation analyses, fight duration was ranked transformed (Conover and Iman, 1981).

Observations on copulatory behavior ( $n = 2$ ) were conducted on individuals collected in the PETAR. We placed a male and a female randomly chosen in a terrarium (45 cm × 20 cm base, 25 cm height) with humid paper towel on the bottom and observed them for 30 min ( $n > 20$ ). If no sexual interaction occurred after this period, we placed a new pair of individuals in the terrarium. We video recorded the male–female interactions that included copulation with a JVC GR-AX1010 video camera. Descriptions of the behavioral sequences are based on the video images ( $n = 2$ ), as well as on copulations observed during fieldwork in PEI ( $n = 2$ ), which were not video-recorded, but described in detail.

Voucher specimens of the studied specimens are deposited in the Museu de Zoologia da Universidade de São Paulo (MZSP), Brazil.

### 2.2. Morphological features

Because our observations showed that nippings with legs IV could be triggered by introducing an object under the external apophysis of male coxa (see Section 3), we searched for sensilla that could trigger this behavior at this region using SEM micrographs. The body part to be investigated was submersed in a 10:1 (water vs. neutral detergent) solution for 3 min, then submersed in water, and finally in acetone for 3 min. The material was then dried in a stove at 40 °C for 24 h, mounted on an aluminum stub using double stick adhesive tape, sputter coated with gold (Sputter Coater Balzer

**Table 1**Operational definition of the behavioral acts accomplished by males of the harvestman *Neosodocus maximus* during intrasexual fights.

Behavioral act	Definition
1. Standing still	To remain in the same place supported by legs III and IV (sometimes also leg I)
2. Standing still with intense leg-tapping (ILT)	As described in (1), but displaying alternating or simultaneous dorso-ventral movements with one or both legs II in the air, tapping the substrate or the opponent. Legs I and III might also be moved in the same way (but not simultaneously)
3. Walking	To change location along the substrate supported by legs III and IV (sometimes also leg I). The movement may be forward, lateral or backwards
4. Walking with ILT	As described in (3), but displaying ILT
5. Legs IV wide opened	The posterior end of the body is raised, kept at 30–70° in relation to the ground, with the anterior end remaining less than 2 mm from the ground. The body stands on legs I, III, and IV, the latter wide opened; right tibia forming an angle between 80° and 180° with left tibia (Fig. 2A). The individual is either standing still or walking backwards
6. Legs IV wide opened with ILT	As described in (5), but displaying ILT
7. Leg IV positioning	The individual moves one leg IV dorso-ventrally in the air, sometimes resting it on the leg IV of the contender, backwards or laterally to him
8. Leg IV positioning with ILT	As described in (7), but displaying ILT (Fig. 2A)
9. Pushing	In “legs IV wide opened” posture, one of the contenders quickly moves backwards by a sudden extension of legs III and/or I
10. Nipping 1	With a sudden upward movement, one of the contenders brings a femur IV close to the body, pinching an object between the apophysis and either the tubercles of the trochanter or the spines of the femur on the same leg. Leg IV is kept upwards so that the object under the apophysis remains pinched. In our observations, the tarsus of the opponent leg was generally pinched (Fig. 2B)
11. Nipping 2	This action is similar to “nipping 1”, but occurs specifically when both contenders are backwards, close to each other (posterior end of one contender's abdomen less than 1 cm from the same region of the body of the other individual). Leg III might be entangled with contender's leg III, and usually the contender's femur IV is pinched. One of the individuals remains supported by legs III and IV while the other one is lifted, its body forming an angle between 90° (abdomen pointing downwards) and 180° (dorsum facing the substrate) with the substrate/contender (Fig. 2C)
12. Bite	Pinching an object between the chelae of chelicerae. In our observations only the legs of the contenders were bitten
13. Coxa-trochanter IV rubbing	The individual brings down one of the sides of the body, presses the coxa/trochanter region against the floor, and walks <5 cm forward, rubbing this region on the floor. The animal stands on legs IV, III and sometimes I
14. Tarsus IV rubbing	The individual drags the proximal part of the tarsus of a leg IV. Coxa/trochanter is not rubbed
15. Leg-threading	The individual stands still and slides one of the legs between the chelae of the chelicerae
16. Fleeing	Clearly walking away from the contender, usually moving fast and trying to climb the walls of the terrarium. This behavior defined the end of the fight, winner and loser

SCD 50), and photographed in scanning electron microscopes (Zeiss DSM 940 and LEO 440 Laika & Zeiss).

Additionally, we measured the following structures on preserved individuals (50 males and 30 females) deposited at MZSP: (A) dorsal scute length; (B) dorsal scute width; (C) distance between the tip of the external apophysis of male coxa IV; (D) right femur III length; (E) diameter at the base of right femur IV; (F) length of dorso-basal spine of male right femur IV, and (G) curvature of right femur IV. Except from dorsal scute length, which is a measure of body size and right femur III length, all the other structures measured are directly involved in male–male fights (see Section 3). Fig. 1 provides details on how these measures were taken under a dissecting microscope with an ocular micrometer. We estimated the slopes of bivariate lines between the  $\log_{10}$  of the dorsal scute length (an indicator of body size) and the  $\log_{10}$  of all other measured traits, and compared them to a slope value of 1 using a Standardized Major Axis Estimation (Warton et al., 2006). Analyses were carried out using the “smatr” module version 2.1 (Warton, 2005) of the R statistical package version 2.6.1.

### 3. Results

#### 3.1. Fighting behavior

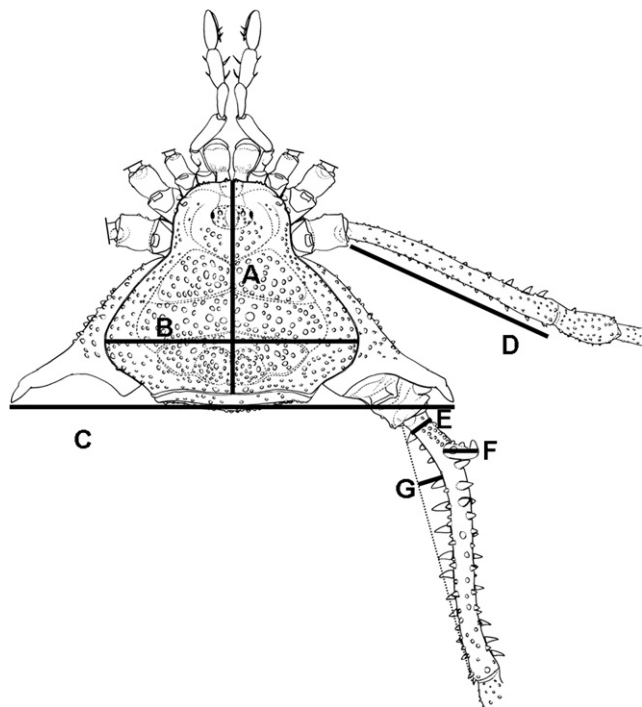
The ethogram of male–male fights is given in Table 1, in which we describe each behavioral act that may be exhibited by the contenders. We provide a video showing a complete fight between two males of *N. maximus* in Supplementary material. When fights were staged ( $n = 10$ ), the two males always touched the females before

fighting (fighting arenas). In the remaining 17 cases, females were present in the terrarium (rearing arena), but they were not touched by males immediately before the fights. We considered that a fight started when both contenders displayed “intense leg tapping” (ILT), which only occurred after they touched each other ( $n = 27$ ). Following initial contact, both males started to rotate, either backwards with “legs IV wide opened” (Fig. 2a) or forward. In some occasions ( $n = 11$ ), ILT was the only behavior observed before a winner could be defined (see definition of winner in Table 1). When the fights continued, they also involved “pushing”, “nippings” (Fig. 2b and c), and “bites”, but there is not a clear sequence of events (Fig. 3).

The median duration of the fights was 209 s (range = 60–1800 s,  $n = 20$ )—we could not record the duration of some non-staged fights). In three fights it was not possible to define a winner, since no individual clearly fled from the fighting place. The remaining 13 fights finished after “pushing” ( $n = 1$ ), “nipping 1” ( $n = 3$ ), and “nipping 2” ( $n = 9$ ). In the latter case, either the individual that stood above ( $n = 3$ ) or below ( $n = 2$ ) won the fight (in three cases we do not know and in one case there was no lifting from the substrate). There was no significant relationship between body size difference and duration of the fights ( $r = -0.304$ ;  $P = 0.912$ ), nor between the size of the smaller male in each contest and duration of the fights ( $r = -0.036$ ;  $P = 0.327$ ).

#### 3.2. Copulatory behavior

In one of the staged copulations, the male approached the female frontally and grasped female's dorsum with legs I and pedipalps for 10 s. Male's pedipalps then remained motionless along the ante-

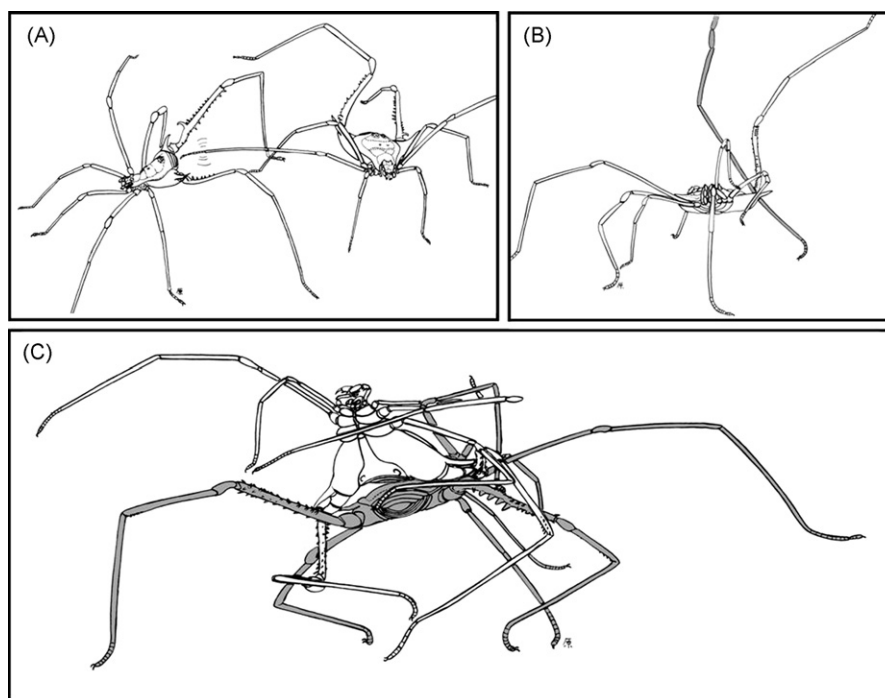


**Fig. 1.** Dorsal view of a male of the harvestman *Neosadocus maximus* showing the landmarks used to take the measures for allometric scaling: (A) dorsal scute length; (B) dorsal scute width; (C) distance between the tip of the external apophysis of male coxa IV; (D) right femur III length; (E) diameter at the base of right femur IV; (F) length of dorso-basal spine of male right femur IV; and (G) curvature of the femur IV, measured as the longest distance between the femur and an imaginary straight line from the upper extremity of this leg segment to its insertion on trochanter IV.

rior margin of the female's dorsal scute and the male alternated between keeping legs I motionless and gently rubbing tarsus I on the dorsum of the female up to the end of copulation. After 31 s of the initial contact, both individuals raised the frontal region of the body and the male introduced the penis into the female's genital opening. Throughout pre-copula and copulation the male displayed intense movement of chelicerae. The female remained motionless throughout copulation and resumed it by pulling back and intensively tapping the male with legs II 2 min after initial contact. The male immediately retracted the penis. Female then remained quiet and the male wandered in the arena. The second copulation, with a different pair from the one described above, was similar (minimal duration due to incomplete observation was 4 min 30 s) except that the male resumed copulation, walked away from the female and remained motionless. In none of the observed copulations did females touch any male spine on legs IV before, during or after copulation. Furthermore, in none of the remaining staged pairs, in which there was no copulation, female interaction with males involved touching legs IV. Two additional copulations were observed in the field and followed a behavioral pattern similar to that describe before. In both cases, copulations were apparently resumed by the females, which clearly did not touch male leg armature during or after intromission.

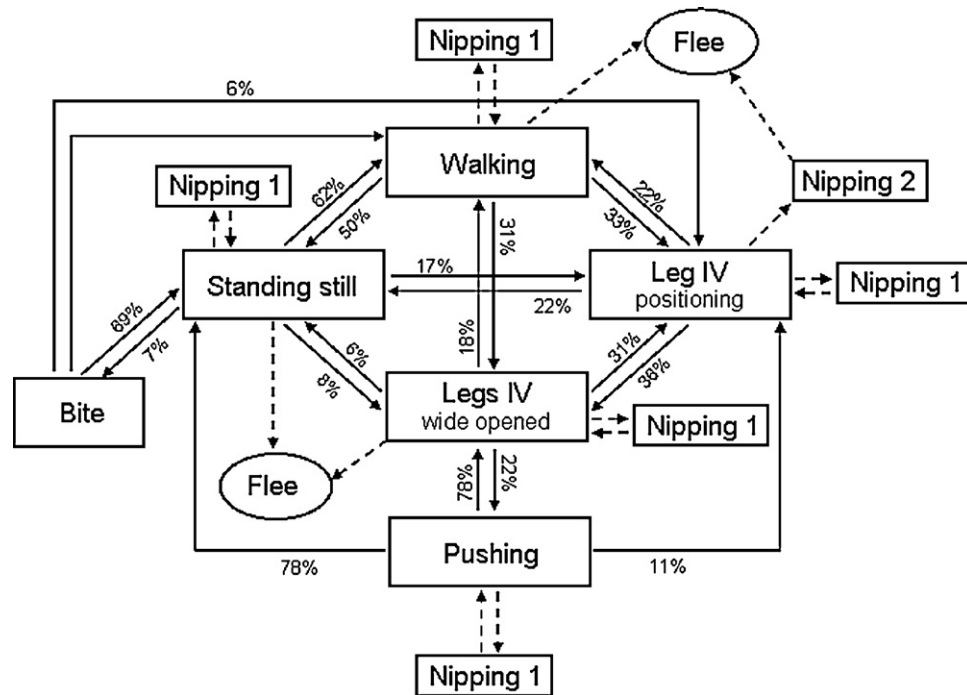
### 3.3. Morphological features

Sexual dimorphism in *N. maximus* is shown in Fig. 4. The external apophysis of male coxa IV (Fig. 4a–c) is a large spine under which the contenders leg is caught when nipping is delivered (Fig. 2b and c). Either the dorso-basal apophysis of femur IV (Fig. 4b) or the external apophysis of trochanter IV (Fig. 4b and c) are responsible for the fitting of the ventral region of the contenders leg. When the mechanoreceptor hair sensilla (*Sensilla chaetica*) on the male apophysis and trochanter (Fig. 4c and d) were stimulated by placing a dry ethanol preserved leg of a male harvestman *Goniosoma*

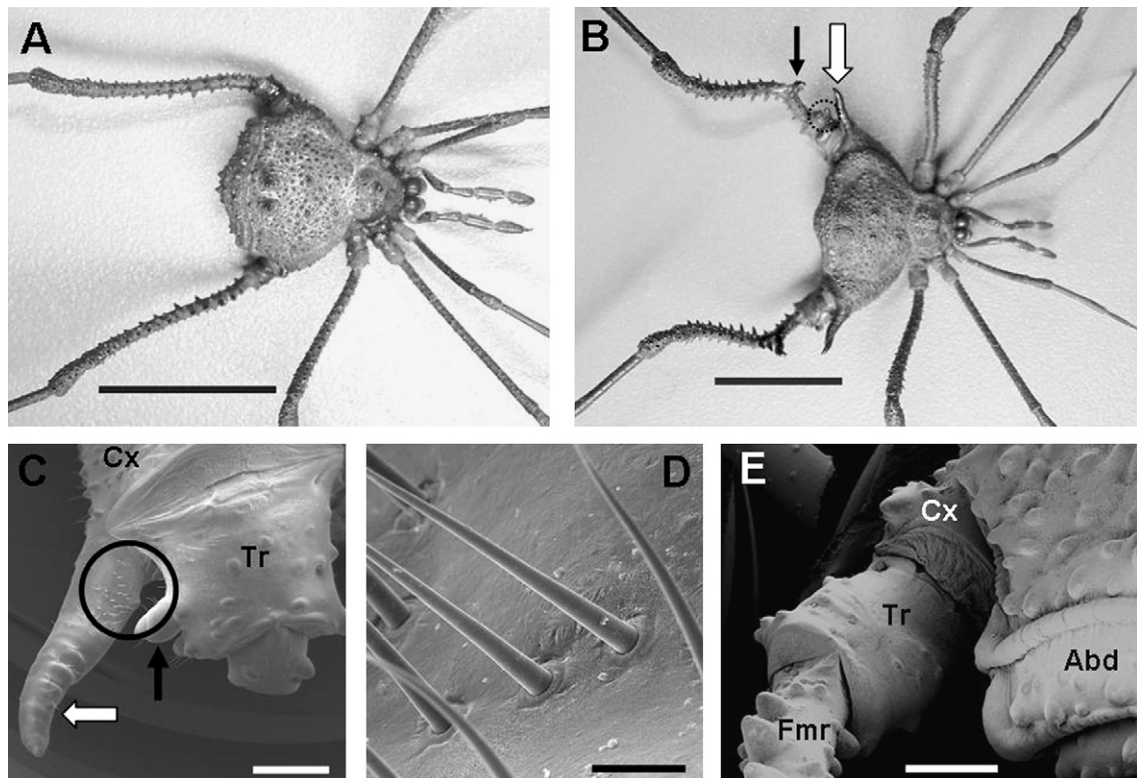


**Fig. 2.** Schemes of some behavioral acts accomplished by the harvestman *Neosadocus maximus* during intrasexual fights. (a) Male on the right displaying "leg IV positioning", and male on the left displaying "legs IV wide opened". (b) "Nipping 1": a male in frontal view flexes the femur IV towards the body and pinches the leg of a dead heterospecific harvestman (grey) experimentally introduced below the male's apophysis. (c) Two males in "nipping 2". Drawings by Marcos R. Hara.





**Fig. 3.** Fluxogram presenting the transitions between behavioral acts observed in 11 video recorded male–male fights of the harvestman *Neosadocus maximus*. “Standing still”, “Walking”, “Legs IV wide opened” and “Leg IV positioning” include acts with and without “Intense leg tapping”. We ignored the behavioral act “leg-threading” since it seemed to be unrelated to the fights and could be accomplished by both males and females in different situations. Solid lines represent transitions with frequencies higher than 5% and dashed lines represent transitions with frequencies below 5%. The numbers besides the arrows indicate the frequency of each transition. In [Supplementary material](#) we provide a video showing a complete fight between two males of *N. maximus*.



**Fig. 4.** Dorsal view of an adult (a) female and (b) an adult male of the harvestman *Neosadocus maximus*. Scale bars: 1 cm. The black arrow shows the subbasal dorsal apophysis of the femur, the white arrow shows the external apophysis of the coxa, and the ellipse shows the external apophysis of the trochanter. (c) Ventral view of external apophysis of trochanter and external apophysis of coxa on the right leg IV of a *N. maximus* male. Note a higher concentration of *Sensilla chaetica* (circle) below the external apophysis of the coxa (white arrow) and next to the external apophysis of the trochanter (black arrow). Tr: Trochanter; Cx: coxa. Scale bar: 10  $\mu$ m. (d) Magnified view of region shown in “(c)”, showing *Sensilla chaetica*, contact mechanoreceptors. Scale bar: 50  $\mu$ m. (e) Dorsal view of left trochanter IV of a female, showing no sensilla specialization. Abd: Abdomen; Cx: coxa; Fmr: femur; Tr: trochanter. Scale bar: 1 mm.

**Table 2**  
Slope values of bivariate lines between the  $\log_{10}$  of the length of the dorsal scute and the  $\log_{10}$  of all other measured traits, assessed using a Standardized Major Axis Estimation (Warton et al., 2006) in males and females of the harvestman *Neosadocus maximus*. The numbers in parenthesis represent the inferior and the superior limits of the 95% confidence interval. The “ $r$ ” value is the test statistic for a comparison against a slope value of 1 and represents the sample correlation between residuals and fitted values. The “ $p$ ” values are taken from the  $F$ -distribution. Numbers in bold represent significant differences ( $<0.05$ ) from a slope value of 1.

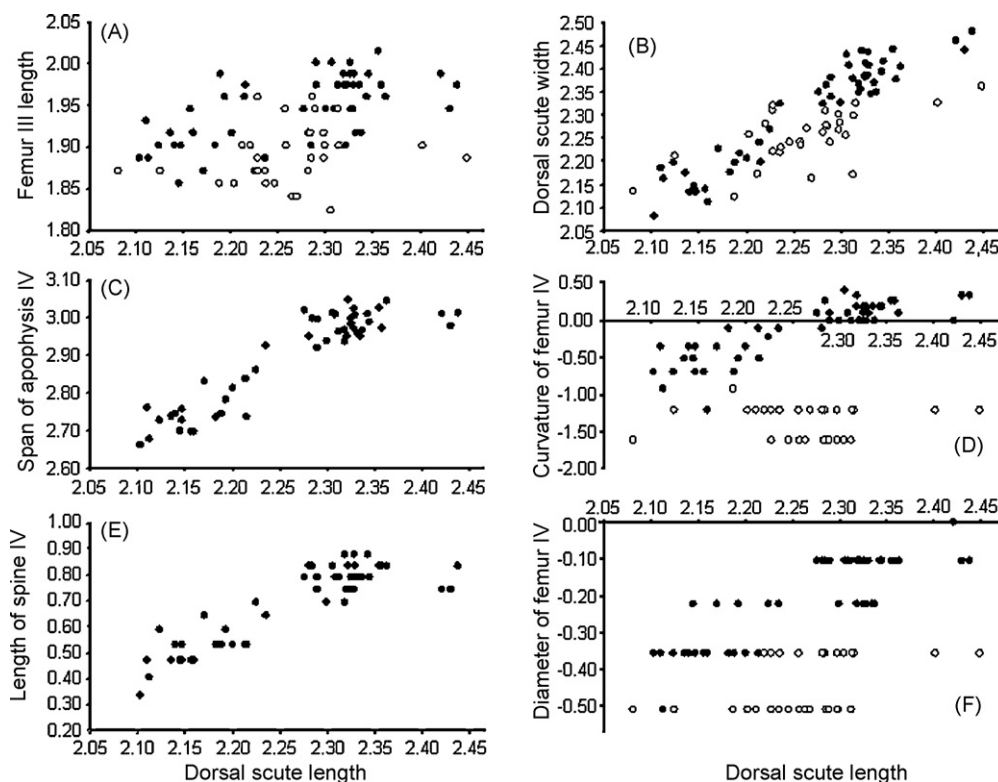
Structure	Males ( $n = 50$ )			Females ( $n = 30$ )		
	Slope	$r$	$p$	Slope	$r$	$p$
Length of femur III	0.443 (0.354–0.555)	–0.757	<b>&lt;0.001</b>	0.513 (0.354–0.742)	–0.593	<b>&lt;0.001</b>
Dorsal scute width	1.233 (1.125–1.352)	0.553	<b>&lt;0.001</b>	0.835 (0.623–1.118)	–0.231	0.219
Distance between the tips of the external apophyses of male coxa IV	1.336 (1.189–1.501)	0.589	<b>&lt;0.001</b>	–		
Diameter of the femur IV	1.299 (1.116–1.511)	0.451	<b>&lt;0.001</b>	1.122 (0.794–1.587)	0.126	0.44
Length of dorso-basal spine on femur IV	1.64 (1.430–1.880)	0.736	<b>&lt;0.001</b>	–		
Curvature of femur IV	4.048 (3.480–4.708)	0.964	<b>&lt;0.001</b>	–3.144 (–2.154 to 4.588)	0.816	<b>&lt;0.001</b>

*albiscryptum* below the apophysis, the males of *N. maximus* always displayed nipping behavior ( $n = 13/13$  trials). While wandering in the terraria, males also nipped the legs of females that happened to place their legs under the apophysis of males ( $n = 7$  observations). During fights, the contenders leg was also always nipped if placed under the apophysis ( $n = 5$ ). Females do not have these sensilla (Fig. 4e), and even when experimentally stimulated as described above, they did not display nipping behavior ( $n = 6/6$ ).

#### 3.4. Static allometry

Body size (measured as dorsal scute length) did not differ between males ( $9.67 \pm 0.87$  mm) and females ( $9.63 \pm 0.72$  mm) ( $t = 0.210$ ,  $DF = 74$ ,  $P = 0.417$ ). However, allometric scaling showed

sex-specific patterns (Fig. 5 and Table 2). The slope values of the bivariate line between dorsal scute length and all other body parts were either significantly lower than 1, or not significantly different from 1, for females (Table 2). For males, the slope value of the bivariate line between dorsal scute length and length of femur III (a structure that is not involved in the fights) was also significantly lower than 1 (Table 2). This result contrasts with the slope values of the bivariate lines between dorsal scute length and the other structures directly related to male fights (Table 2). The curvature of femur IV, for instance, presented a slope of around 4 (Table 2). Other three structures, namely, width of dorsal scute, distance between the tips of the external apophyses of coxa IV, and the length of dorso-basal spine on femur IV, also showed slope values significantly higher than 1 (Table 2).



**Fig. 5.** Relationship between an indicator of body size ( $\log_{10}$  dorsal scute length) and the following body parts of the harvestman *Neosadocus maximus* (all log-transformed): (A) right femur III length; (B) dorsal scute width; (C) span of apophyses IV, i.e., the distance between the tip of the external apophysis of male coxa IV; (D) curvature of the femur IV; (E) length of dorso-basal spine of male right femur IV; (F) diameter at the base of right femur IV. Data for males are presented as black circles and for females as open circles.

## 4. Discussion

### 4.1. Male–male fights

Male–male fights have been reported for several harvestman species, including representatives of the families Sclerosomatidae, Trogulidae, and Gonyleptidae (reviewed in Machado and Macías-Ordóñez, 2007; see also Willemart et al., 2006). In most cases described so far, the fights only involved biting with chelicerae, attacks with pedipalps, and pulling with legs and body. In only one case, namely *Phalangium opilio*, sexually dimorphic structures (the horned chelicerae and long pedipalps) have been recorded as being used by the males to fight conspecific males (Willemart et al., 2006). To the best of our knowledge, our study is the first to provide a functional explanation for the leg armature in male harvestmen. We clearly show that the spines and tubercles on coxa and femur IV work as weapons during fights between males. Although our data are all based on a single species, we hypothesize that the use of the leg armature as a weapon in intrasexual fights may be a widespread behavior in the family Gonyleptidae (and perhaps also in species that have similar structures in the families Cosmetidae and Cranidae) because many species show similar spines and tubercles on legs IV. In fact, in at least three other species not closely related to *N. maximus*, males also fight using their armed legs IV (G. Machado, unpublished data).

Most species of harvestmen are known to be non-visual, gathering information from their surroundings mainly with legs I, II and/or pedipalps (see Willemart et al., 2008). Contact has been shown to be necessary to detect live prey as well as conspecifics in some species (Macías-Ordóñez, 1997, 2000; Willemart et al., 2006; Acosta and Machado, 2007; Willemart and Chelini, 2007). In accordance with those sensory constraints, all fights we have observed only started after physical contact was established between males of *N. maximus*. Nipping behavior, in particular, required contact to occur, but was not chemically nor mechanically specific, since it was triggered by a female leg and by a leg of a different harvestman species. *S. chaetica*, which are contact mechanoreceptors of harvestmen (see Willemart et al., 2008), are present on the trochanter and on the ventral region of the male apophysis, but are absent on females. We suggest that these hairs are a non-specific sensory specialization of the males related to nipping behavior.

It seems clear that “intense leg tapping” is the first level of escalation in the fights, possibly giving the first information to the contenders about the strength or size of the opponent. When fights escalated further, males of *N. maximus* used their legs IV to grab the opponent and eventually deliver “nipping 1 or 2”, at which point 12 out of 13 fights ended with a winner. The “sequential assessment model” (SAM *sensu* Enquist and Leimar, 1983, 1987) states that when differences in fighting ability (e.g. due to size) are small, further escalation may be necessary for contenders to gather enough information to make a decision on whether to escalate further or retreat, thus predicting that fight duration will be inversely correlated to the size difference between contenders. Alternatively, the “war of attrition model” (WAM *sensu* Maynard-Smith, 1974) states that fights end when the weaker contestant gives up, thus predicting that fight duration will be directly correlated with the size of the smallest contestant. It is important to notice that both models predict that the larger individual will win the fight, although through different mechanisms (reviewed in Taylor and Elwood, 2003). Nevertheless, the correlations between time duration and contestant size difference and size of the smallest contestant in *N. maximus* did not provide support for either the SAM or the WAM models. This may be due to a low sample size, since this was not a question considered when designing this study.

### 4.2. Evolution of sexual dimorphism in leg armature

Although courtship often involves multimodal signals, male courtship displays depend on the sensory capabilities of the receiver, i.e., the females (Hebets and Papaj, 2005). Being a non-visual species, morphological features of *N. maximus* males such as the spines on their legs IV can only be perceived by females through contact, with the use of mechanoreceptors present on their legs I and II (Willemart et al., 2007). Therefore, we could expect females to touch the armature on the legs of their mates if they were to evaluate it. However, we did not observe female assessment of these secondary sexual characters either before, during or after copulation. In other gonyleptid species with similar sexual dimorphism in leg armature, copulatory behavior has already been described in more detail and in no case the authors have reported that females touch the spines on male's legs IV before, during or after copulation as well (review in Machado and Macías-Ordóñez, 2007). Although leg armature seems to play little or no role in female choice, it may be premature to rule out female assessment completely given the low number of sexual interactions in this study and the scarce available information in other studies.

There are two non-mutually excluding possibilities for the role of leg armature in male harvestmen besides female choice. First, males could be subjected to distinct selective pressures than females, such as higher predation, and this would have led to the development of different kinds of defenses (Shine, 1989), such as leg armature. Although *N. maximus* and other gonyleptids indeed use the spines for defense (review in Gnaspini and Hara, 2007), we reject this hypothesis by empirical evidence. Males and females of *N. maximus* occur in the same microhabitats, are active at the same period of the day, and are attacked by the same predators (Machado and Pizo, 2000; Willemart et al., 2007; Osses et al., in press). Thus we think it is unlikely that leg armature has evolved under natural selection as a defensive device against predation only in males. Moreover, spines of gonyleptids are only fully developed in adults (Muñoz-Cuevas, 1971; Gnaspini, 1995), which suggests a sexual role.

The second possibility is that leg armature has evolved as a weapon used in contests between males, as a typical case of intrasexual selection. Sexually dimorphic spines are widespread among insects, such as beetles and earwigs, and it is known that males use them in contests against other males (see examples in Andersson, 1994). In this study we have shown that in *N. maximus* spines and associated sensilla are sexually dimorphic and that both are directly related to the delivering of “nippings 1 and 2”. These observations suggest that these morphological traits are under intrasexual selection in harvestmen. Moreover, under sexual selection, male structures used as signaling devices and as weapons are frequently larger in larger individuals of a species, showing a positive allometry (see references in Pomfret and Knell, 2006). In our study, leg armature of *N. maximus* males also showed positive allometry: all five structures directly involved with male–male fights presented slopes significantly higher than 1, whereas these structures were either absent or showed values non-significantly different or lower than 1 in females (Table 2). In contrast, the length of femur III, which is not directly involved in male–male fights, presented a slope significantly lower than 1 in both sexes (Table 2). Therefore, our morphological and behavioral data combined are consistent with the idea of evolution, at least in part, through intrasexual selection.

An unanswered but highly relevant question is what do males fight for? In closely related species of the subfamily Gonyleptinae, such as *Gonyleptes saprophilus* and *Neosadocus* sp., males defend very specific sites (holes in roadside banks and trunks, respectively) as nesting sites, and the leg armature seems to be involved in the defense of this scarce resource against other males (Machado et al.,



2004). Field studies are necessary in order to understand the mating system of *N. maximus* and the form of parental care exhibited by the species. Males could be defending nest sites, like other closely related species, and thus leg armature may be involved in contests for the ownership of these sites, as described above. Males could also be defending territories with exclusive access to preferred oviposition sites on host plants, and thus to ovigerous females, like the gonyleptid *Acutisoma proximum* (Buzatto and Machado, 2008). In this case, leg armature may be directly involved in territorial fights among males. Finally, males could also be defending individual females, as in a typical female polygyny mating system. However, 17 fights begun without the presence of a female in the close vicinity (<10 cm) of the males, and in no case the winning male engaged in courtship or copulations with a nearby female after the fight.

The evolution of sexually dimorphic traits in animals has been, and still is, the source of much research and debate (review in Shine, 1989). In beetles for instance, sexually dimorphic horns have had their function ignored for decades until researchers discovered their importance in male–male contests (e.g., Eberhard, 1979). Currently, these beetles have been used as model organisms in studies of great general interest, such as the costs and benefits of bearing huge structures (Emlen, 2001) and on alternative mating tactics, for example (Moczek and Emlen, 2000). In this paper we demonstrate that leg armature in male harvestmen is used as a device in intrasexual contests, thus the group offers an additional opportunity to explore the evolution of sexually selected dimorphic traits such as sexual fighting behavior and weapons in a poorly known and diverse clade.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.beproc.2008.09.006.

## References

- Acosta, L.E., Machado, G., 2007. Diet and foraging. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), *Harvestmen: The Biology of Opiliones*. Harvard University Press, Massachusetts, pp. 309–338.
- Alatalo, R.V., Höglund, J., Lundberg, A., 1988. Patterns of variation in tail ornament size in birds. *Biol. J. Linn. Soc.* 34, 363–374.
- Anderson, R.A., Vitt, J., 1990. Sexual selection versus alternative causes of sexual dimorphism in teiid lizard. *Oecologia* 84, 145–157.
- Andersson, M., 1994. *Sexual Selection*. Princeton University Press, Princeton, NJ, 599 pp.
- Baker, R.H., Wilkinson, G.S., 2001. Phylogenetic analysis of sexual dimorphism and eye stalk allometry in stalk-eyed flies (Diopsidae). *Evolution* 55, 1373–1385.
- Bonduriansky, R., Day, T., 2003. The evolution of static allometry in sexually selected traits. *Evolution*, 2450–2458.
- Brown, L., Rockwood, L.L., 1986. On the dilemma of horns. *Nat. Hist.* 7, 54–61.
- Buzatto, B.A., Machado, G., 2008. Resource defense polygyny shifts to female defense polygyny over the course of the reproductive season of a neotropical harvestman. *Behav. Ecol. Sociobiol.* 63, 85–94.
- Clutton-Brock, T.H., 1982. The function of antlers. *Behaviour* 79, 108–121.
- Conner, J., 1988. Field measurements of natural and sexual selection in the fungus beetle, *Bolitotherus cornutus*. *Evolution* 42, 735–749.
- Conner, J., 1989a. Older males have higher insemination success in a beetle. *Anim. Behav.* 38, 503–509.
- Conner, J., 1989b. Density-dependent sexual selection in the fungus beetle *Bolitotherus cornutus*. *Evolution* 43, 1378–1386.
- Conover, W.J., Iman, R.L., 1981. Rank transformations as a bridge between parametric and nonparametric statistics. *Am. Stat.* 35, 124–129.
- Eberhard, W.G., 1979. The function of horns in *Podischinus agenor* (Dynastinae) and other beetles. In: Blum, M.S., Blum, N.A. (Eds.), *Sexual Selection and Reproductive Competition in Insects*. Academic Press, New York, pp. 231–258.
- Eberhard, W.G., 1980. Horned beetles. *Sci. Am.* 242, 166–182.
- Eberhard, W.G., 1982. Beetle horn dimorphism: making the best of a bad lot. *Am. Nat.* 119, 420v426.
- Eberhard, W.G., Gutierrez, E., 1991. Male dimorphism in beetles and earwigs and question of developmental constraints. *Evolution* 45, 18–28.
- Eberhard, W.G., García-C, J.M., Lobo, J., 2000. Size-specific defensive structures in a horned weevil confirm a classic battle plan: avoid fights with larger opponents. *Proc. R. Soc. B* 267, 1129–1134.
- Emlen, D.J., 2001. Costs and the diversification of exaggerated animal structures. *Science* 291, 1534–1536.
- Emlen, D.J., Nijhout, H.F., 2000. The development and evolution of exaggerated morphologies in insects. *Ann. Rev. Entomol.* 45, 661–708.
- Enquist, M., Leimar, O., 1983. Evolution of fighting behaviour: decision rules and assessment of relative strength. *J. Theor. Biol.* 102, 387–410.
- Enquist, M., Leimar, O., 1987. Evolution of fighting behaviour: the effect of variation in resource value. *J. Theor. Biol.* 127, 187–205.
- Forsman, A., Appelqvist, S., 1998. Visual predators impose correlational selection on prey color pattern and behavior. *Behav. Ecol.* 9, 409–413.
- Gnaspini, P., 1995. Reproduction and postembryonic development of *Goniosoma spelaeum*, a cavernicolous harvestman from southeastern Brazil (Arachnida: Opiliones: Gonyleptidae). *Inver. Reprod. Develop.* 28, 137–151.
- Gnaspini, P., Hara, M.R., 2007. Defense mechanisms. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), *Harvestmen: The Biology of Opiliones*. Harvard University Press, Cambridge, MA, pp. 374–399.
- Green, A.J., 1992. Positive allometry is likely with mate choice, competitive display and other functions. *Anim. Behav.* 43, 170–172.
- Hebets, E.A., 2004. Attention-altering signal interactions in the multimodal courtship display of the wolf spider *Schizocosa uetzi*. *Behav. Ecol.* 16, 75–82.
- Hebets, E.A., Papaj, D.R., 2005. Complex signal evolution: developing a framework of testable hypotheses. *Behav. Ecol. Sociobiol.* 57, 197v214.
- Johnstone, R.A., Norris, K., 1993. Badges of status and the cost of aggression. *Behav. Ecol. Sociobiol.* 32, 127–134.
- Kury, A.B., Pinto-da-Rocha, R., 2007a. Taxonomy: Cosmetidae. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), *Harvestmen: The Biology of Opiliones*. Harvard University Press, Cambridge, MA, pp. 182–185.
- Kury, A.B., Pinto-da-Rocha, R., 2007b. Taxonomy: Gonyleptidae. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), *Harvestmen: The Biology of Opiliones*. Harvard University Press, Cambridge, MA, pp. 196–203.
- Machado, G., Macías-Ordóñez, R., 2007. Reproduction. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), *Harvestmen: The Biology of Opiliones*. Harvard University Press, Cambridge, MA, pp. 414–454.
- Machado, G., Pizo, M.A., 2000. The use of fruits by the neotropical harvestman *Neosadocus variabilis* (Opiliones, Laniatores, Gonyleptidae). *J. Arachnol.* 28, 357–360.
- Machado, G., Requena, G.S., Buzatto, B.A., Osses, F., Rossetto, L.M., 2004. Five new cases of paternal care in harvestmen (Arachnida: Opiliones): implications for the evolution of male guarding in the Neotropical family Gonyleptidae. *Sociobiology* 44, 577–598.
- Machado, G., Pinto-da-Rocha, R., Giribet, G., 2007. What are harvestmen? In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), *Harvestmen: The Biology of Opiliones*. Harvard University Press, Massachusetts, pp. 1–13.
- Macías-Ordóñez, R., 1997. The mating system of *Leiobunum vittatum* Say 1821 (Arachnida: Opiliones: Palpatores): resource defense polygyny in the striped harvestman. Ph.D. Thesis. Lehigh University, Bethlehem, USA.
- Macías-Ordóñez, R., 2000. Touchy harvestmen. *Nat. Hist.* 109, 58–61.
- Maynard-Smith, J., 1974. Theory of games and the evolution of animal contests. *J. Theor. Biol.* 47, 209–221.
- Moczek, A.P., Emlen, D.J., 2000. Male horn dimorphism in the scarab beetle, *Ontophagus taurus*: do alternative reproductive tactics favour alternative phenotypes? *Anim. Behav.* 59, 459–466.
- Muñoz-Cuevas, A., 1971. Contribution à l'étude du développement postembryonnaire de *Pachylus quinamavidensis* Muñoz-Cuevas (Arachnides, Opiliones, Laniatores). *Bull. Mus. Hist. Nat. Paris* 12, 629–641.
- Murai, M., Backwell, P., 2006. A conspicuous courtship signal in the fiddler crab *Uca perplexa*: female choice based on display structure. *Behav. Ecol. Sociobiol.* 60, 736–741.
- Osses, F., Nazareth, T.M., Rossetto, L.M., Machado, G. Activity pattern of the neotropical harvestman *Neosadocus maximus* (Opiliones, Gonyleptidae): sexual and temporal variations. *J. Arachnol.*, in press.
- Petrie, M., 1988. Intraspecific variation in structures that display competitive ability: large animals invest relatively more. *Anim. Behav.* 43, 173–175.
- Petrie, M., 1992. Are all secondary sexual display structures positively allometric and, if so, why? *Anim. Behav.* 43, 173–175.
- Pinto-da-Rocha, R., Kury, A.B., 2007. Taxonomy: Cranidae. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), *Harvestmen: The Biology of Opiliones*. Harvard University Press, Cambridge, MA, pp. 185–188.



- Pinto-da-Rocha, R., Machado, G., Giribet, G., 2007. Harvestmen: The Biology of Opiliones. Harvard University Press, Cambridge, MA, 597 pp.
- Pomfret, J.C., Knell, R.J., 2006. Sexual selection and horn allometry in the dung beetle *Euoniticellus intermedius*. Anim. Behav. 71, 567–576.
- Shine, R., 1989. Ecological causes for the evolution of sexual dimorphism: a review of the evidence. Quart. Rev. Biol. 64, 419–461.
- Simmons, L.W., Tomkins, J.L., 1996. Sexual selection and the allometry of earwig forceps. Evol. Ecol. 10, 97–104.
- Taylor, P.W., Elwood, R.W., 2003. The mismeasure of animal contests. Anim. Behav. 65, 1195–1202.
- Warton, D.I., 2005. (Standardised) major axis estimation and testing routines (translated to R “smatr” module version 2.1 by J. Ormerod). In: R Development Core Team 2007 (Ed.), R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Warton, D.I., Wright, I.J., Falster, D.F., Westoby, M., 2006. Bivariate line-fitting methods for allometry. Biol. Rev. 81, 259–291.
- Willemart, R.H., Chelini, M.C., 2007. Experimental demonstration of close-range olfaction and contact chemoreception in the Brazilian harvestman *Iporangaia pustulosa*. Ent. Exp. App. 123, 73–79.
- Willemart, R.H., Farine, J.-P., Peretti, A.V., Gnaspini, P., 2006. Behavioral roles of the sexually dimorphic structures in the male harvestman, *Phalangium opilio* (Opiliones, Phalangidae). Can. J. Zool. 84, 1763–1774.
- Willemart, R.H., Chelini, M.C., Andrade, R., Gnaspini, P., 2007. An ethological approach to a SEM survey on sensory structures and tegumental gland openings of two neotropical harvestmen (Arachnida, Opiliones, Gonyleptidae). Ital. J. Zool. 74, 39–54.
- Willemart, R.H., Farine, J.-P., Gnaspini, P., 2008. Sensory biology of Phalangida harvestmen (Arachnida, Opiliones): a review, with new morphological data on 18 species. Acta Zool. 89, doi:10.1111/j.1463-6395.2008.0341.x.
- Zahavi, A., 1975. Mate selection: selection for a handicap. J. Theor. Biol. 53, 205–214.