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An ethological approach to a SEM survey on sensory structures and tegumental gland openings of two neotropical harvestmen (Arachnida, Opiliones, Gonyleptidae)

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Abstract

We studied the sensory structures and tegumental glands of two harvestmen species, *Neosadocus* sp. and *Iporangaia pustulosa*. We also provide field data of dietary items and data on the foraging behavior of *Neosadocus* sp. in captivity. Food include mostly immobile items such as dead insects, fruits and feces; the mobility of foraging animals in starvation was greater than in satiation conditions; no trichobothria was found; metatarsal paired slit sensilla and three other sensilla are described for the first time in harvestmen. We discuss the possible relationship between sensory structures, diet and foraging mobility, comparing with other groups of Arachnida. We also describe five new tegumental glands, one sexually dimorphic in the metatarsus IV of *I. pustulosa* males and two that are rubbed against the substrate while walking, present in both species. This is the first morphological evidence that harvestmen might leave chemical marks on the substrate.

Keywords: *Sensory biology, sensilla, gland, foraging, Opiliones,* Neosadocus *sp.,* Iporangaia pustulosa

Introduction

Arachnids are plesiomorphically predatory animals that rely, among other characteristics, on effective prey detection that allow them to feed on mobile, sometimes fast-moving prey, such as crickets, cockroaches and other arthropods (Humphreys 1989; McCormick & Polis 1990; Foelix 1996; Punzo 1998, 2000; Hebets 2002; Andrade & Gnaspini 2002; Condé & Adis 2002; Platnick 2002; Woas 2002; Berndt et al. 2004). Prey detection in arachnids involves, besides contact, mainly mechanical clues such as substrate-borne vibrations and wind currents (Haupt 1996; Weygoldt 2000; Brownell 2001; Barth 2002a).

Two types of receptors are most important in this prey detection: slit sense organs and trichobothria. Slit sense organs are detectors of mechanical stresses in the cuticle, the latter being caused by muscular activity, hemolymph pressure, and by substrate-borne vibrations (Barth 2004). Trichobothria are fine and long hairs that detect slight air currents (Reissland & Görner 1985). In some arachnid groups, such as spiders (Araneae), whip-spiders (Amblypygi) and whip-scorpions (Thelyphonida), slit sensilla may group and form a functional unit called the lyriform organ (Barth & Stagl 1976). Trichobothria and slit sense organs are not only important for feeding, but also for predator detection and for intra-specific communication (e.g. Barth 1982).

Harvestmen are omnivorous arachnids that feed opportunistically on fruits, excrement, fungi, and a variety of invertebrates such as arthropods (often dead) and annelids (see references in Newton &Yeargan 2001; Willemart 2002). Exceptions do exist, such as specialists on gastropods (Nyffeler & Symondson 2001), and there are almost no data on the most basal suborder, Cyphophthalmi (the other suborders being Eupnoi, Dyspnoi and Laniatores—Giribet et al. 2002). Several of these items are not mobile, and therefore cannot be detected by mechanical clues at a distance (substrate-borne vibrations and wind currents). When motionless food is an important part of its diet, the animal is expected to wander while foraging in order to find this food (Riechert & Luczak 1982). Although no specific studies have been made, some harvestmen species have been reported to wander while foraging

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(Comstock 1940; Edgar 1971; Acosta et al. 1995; Macías-Ordóñez 2000; Elpino-Campos et al. 2001; Newton & Yeargan 2001; Santos & Gnaspini 2002; Willemart & Gnaspini 2004), appearing that they are not typical sit-and-wait predators. These two characteristics (omnivory and wandering while foraging) are not common in at least the most studied arachnids, such as most spiders and scorpions. These are mostly predatory, and forage by sit-and-wait mode (Riechert & Luczak 1982; Benton 1992). A notable exception to this general rule but that corroborates this idea is found in solpugids (Solifugae), which are also generalist predators (Punzo 1998). These do not have trichobothria and slit sensilla are in low numbers (Millot 1949; Punzo 1998), possibly hampering long-range detection of prey. However, they have a high density of hair sensilla and some species have been reported to wander while foraging (Punzo 1998), characteristics that probably help them make contact with their prey. Based on these data, we raised these hypotheses: that a harvestman would wander during foraging, and, since wandering would be related to feeding, that foraging harvestmen in starvation would wander more than satiated ones. These two hypotheses were tested herein.

Concerning the sensory structures related to prey capture, scorpions and spiders have trichobothria and slit-sense organs that allow detection of mobile prey (Brownell 2001; Barth 2002a). Among slit sensilla, the metatarsal organs appear to be the most important to detecting prey (Barth 2002a). Previous studies reported that Eupnoi harvestmen have no trichobothria and no metatarsal organs (Millot 1949; Barth & Stagl 1976; Luque 1993), which would limit their ability to detect live prey at a distance. This could be related to their omnivorous and wandering habits, leading us to another question of this study: do laniatorean harvestmen bear sensilla such as trichobothria and slit-sense organs (more specifically metatarsal organs) or analogous structures? As discussed above, this subject would provide important data to understand why omnivory, more specifically feeding on motionless food, has arisen as a putative synapomorphy of Opiliones, a group within a predatory clade and thus phylogenetically constrained to bear sensory structures related to predatory behavior. In order to help in our discussion, we also provide field data on the diet of *Neosadocus* sp.

The second part of the study concerns glandular structures. Spiders are known to leave draglines with pheromones (e.g. Tietjen & Rovner 1982), and scorpions leave pheromones on the substrate (Steinmetz et al. 2004 and references therein). Gaffin and Brownell (2001) suggested a 'chelicerate mode' of mate finding that would involve mostly clues left on the substrate (but see for example Searcy et al. 1999) rather than the 'mandibulate terrestrial mode' of mate finding, in which air borne clues would be more important. The second question of this study is therefore: are there glands that open in regions of the body that could be rubbed against the substrate? If this is the case, it would be the first necessary evidence to start investigating further a possible role of chemical trails in a harvestmen's life, including mate finding.

Finally, since there are few papers published on the external morphology of cuticular structures of harvestmen (see references in Willemart & Gnaspini 2003), we also provide general data on sensory structures and cuticular gland openings.

Materials and methods

Biological material

We used *Neosadocus* sp. (Gonyleptidae, Gonyleptinae) and *Iporangaia pustulosa* Mello-Leitão 1935 (Gonyleptidae, Progonyleptoidellinae) because they have been the subject of study of other research projects on behavioral biology, to which our data might relate to in the future. We collected the latter in Parque Estadual Intervales (Ribeira Valley, state of São Paulo, southeastern Brazil), in the margins of a river, on leaves in shrubs or trees, and the former in Parque Estadual Turístico do Alto do Ribeira (PETAR) (Ribeira Valley, state of São Paulo, southeastern Brazil). Voucher specimens are deposited at the Museu de Zoologia da Universidade de São Paulo (MZUSP).

Microscopical preparations

The material was submersed in a 10:1 (water:neutral detergent) solution for 3 min, then submersed in water, and finally either cleaned ultrasonically or submersed 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 SCD 50) and photographed in scanning electron microscopes (SEM—Zeiss DSM 940 and LEO 440 Laika and Zeiss).

Field data on diet

Dietary items were opportunistically collected during field trips to PETAR. In March 2003, we collected dietary items day and night for an approximately 45 h effort. In February 2004, we collected items found during a 2 h searching period.

Maintenance of the animals in captivity

We maintained 10 males and 6 females of *Neosadocus* sp. in the laboratory. They were individually housed in terraria (45 × 20 × 25 cm), of which two adjacent walls were of Styrofoam. Two Styrofoam stripes of 3 cm length crossed within the terrarium, each with one tip on the bottom and the other resting on the upper part of the terrarium, in the opposite side. This "X" formed by the intersection of the two stripes aimed at increasing the area where the animals could wander. A dark shelter (9 × 6 × 5 cm) was available for the animals in the corner where the two adjacent Styrofoam walls made contact. We fed the animals approximately twice a week with pieces of dead *Tenebrio* larvae and bread. Pieces of food left by the harvestmen after feeding were always removed.

Behavioral observations on mobility

We wanted to test whether the harvestmen remained mostly in the same place or wandered in the terrarium while foraging, to infer if their strategy could be classified as sit-and-wait. Since wandering may also reflect mate searching instead of food searching, we compared the same individuals, of both sexes, well fed and starving. An increase in mobility in starving conditions and the absence of difference between the sexes would suggest that wandering in our terraria was related to feeding instead of mate searching.

Our observations consisted of three blocks, all conducted in December 2003 and the beginning of January 2004. In the first block, we monitored the same individuals for three consecutive nights (16, 17 and 18 days of fasting period). We then fed them to satiation and began the second block of observations (11 days after the end of the first block), for another 3 consecutive nights. Since there could be a reduced mobility due to habituation with the terraria and not due to hunger (the observed animals were being monitored in the same terraria in which they were being maintained), we conducted a third block of observations in starvation conditions, 13 days after the end of the second block. This time, the fastening period was of 14, 15 and 16 days in each of the 3 nights.

During each night of each block, we conducted a scan-sampling (see Lehner 1996), monitoring the animals at every 30 min, starting at 21:15–0:15 and resuming observations at sunrise or soon after it, between 4:45 and 6:45, totaling between 13 and 16 observations per night. This period of the day was chosen because *Neosadocus* sp. has been reported to be nocturnal (Machado & Pizo 2000). Two adjacent walls of the terrarium were ruled into squares of 5 cm per side, so we could note the position of the animals in each observation, with the help of a headlamp covered with red filter. Whenever an individual was found in distinct squares in two consecutive observations, we considered it to have displaced.

Analysis of data on mobility

As the same individuals were being used, we used a repeated measures design to test for distinct mobility between the same individuals in each block. We averaged the percentage of "wander" observations of distinct days of the same block and this gave us a single data point for each individual, for each block (see Martin & Bateson 1996). We then compared the three blocks with a Friedman Repeated Measures Analysis of Variance on Ranks followed by a post-hoc Student Newman Keus test (SigmaStat software). Though we started the observations with 10 males and 6 females, some died in captivity. Therefore, only those individuals that were observed in the three blocks could be used for statistics, since repeated measures tests compare the same individual in each group.

Results

SEM survey, general features

The structures found are presented below. For a general view of the region of the leg in which they occur please check Figures 1 and 2. Except for the

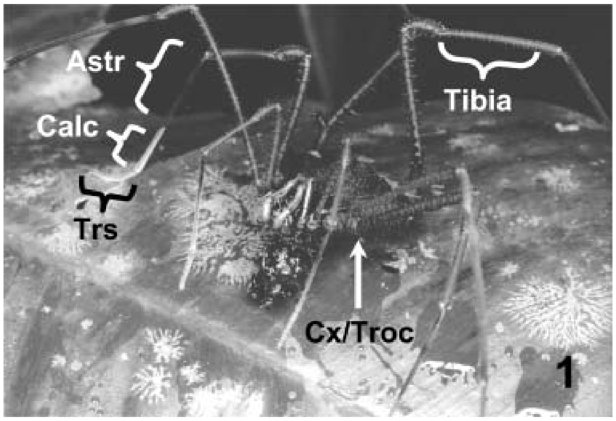

Figure 1. *Neosadocus* sp. male feeding on vertebrate feces. Ast=astragalus; Calc=calcaneus; Cx=coxa; Troc=trochanter. Body length of the individual=~1 cm.

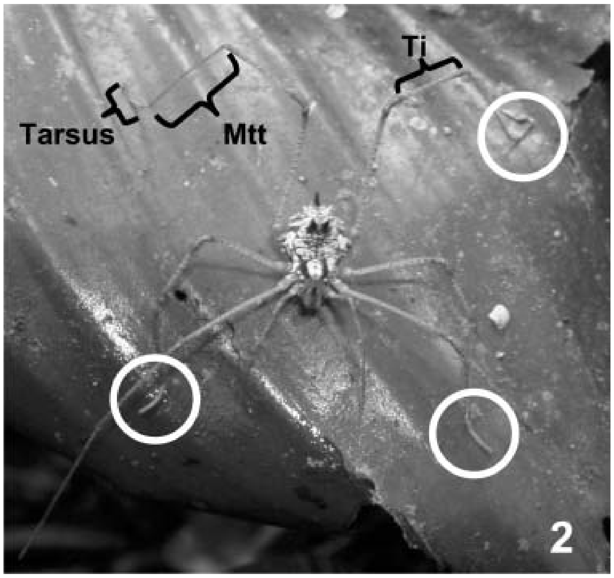

Figure 2. *Iporangaia pustulosa* female. Ti=tibia; Mtt=metatarsus. Circles show the tarsus and distal region of the metatarsus (calcaneus). Body length of the individual=~0.7 cm.

metatarsal gland of *I. pustulosa* (see below), all the structures described occur in both sexes of both species. Structures described for the first time are discriminated as (n. st.).

SEM survey, hairs

Trichomes (Figures 3–12): they occur from the tip of the tarsus to the most proximal part of the calcaneus. Absent in the rest of the body. The length of the shaft is between 50 and 80 μm, orienting towards the distal end of the leg, with an angle of insertion between ~15° and 30°. The shaft tapers in its distal portion, bearing longitudinal grooves and no wall pores. There is no basal membrane, and broken hairs revealed no internal lumen.

Sensilla chaetica (Figures 3–5, 8, 10, 11, 13 and 14): from the tip of the tarsus to the most proximal part of the calcaneus, they are usually less abundant than trichomes, with exceptions made to legs I and II, where in the distal part they are the most abundant sensilla. Also present on the whole body, in very low density and on the posterior side of the mouth (Figure 14). The length of the shaft is between 80 and 200 μm, orienting towards the distal end of the leg, with an angle of insertion between ~50° and 80°. The shaft tapers in its distal portion, bearing longitudinal grooves and no wall pores. A basal membrane surrounds the proximal part of the shaft, and an internal lumen occurs (Figure 13). Sensilla chaetica are readily distinguished from

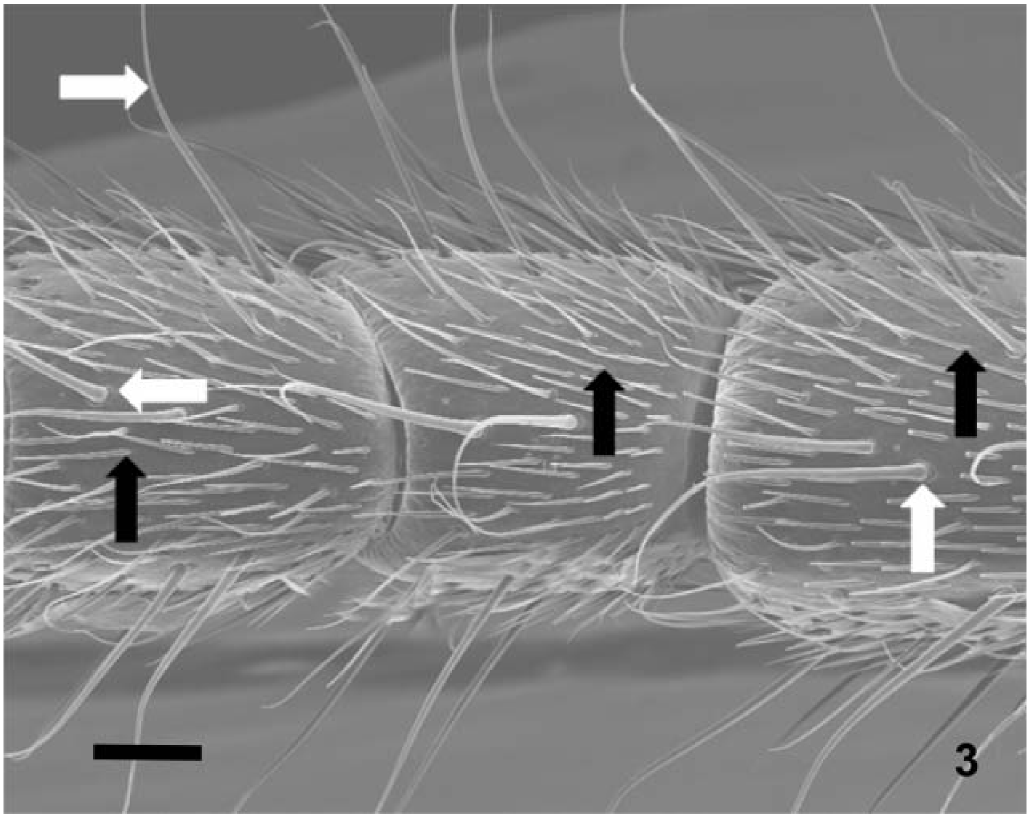

Figure 3. *Trichome* (black arrow) and *sensillum chaeticum* (white arrow), at the dorsal region of tarsus I, in *Neosadocus* sp. female. Scale bar=100 μm.

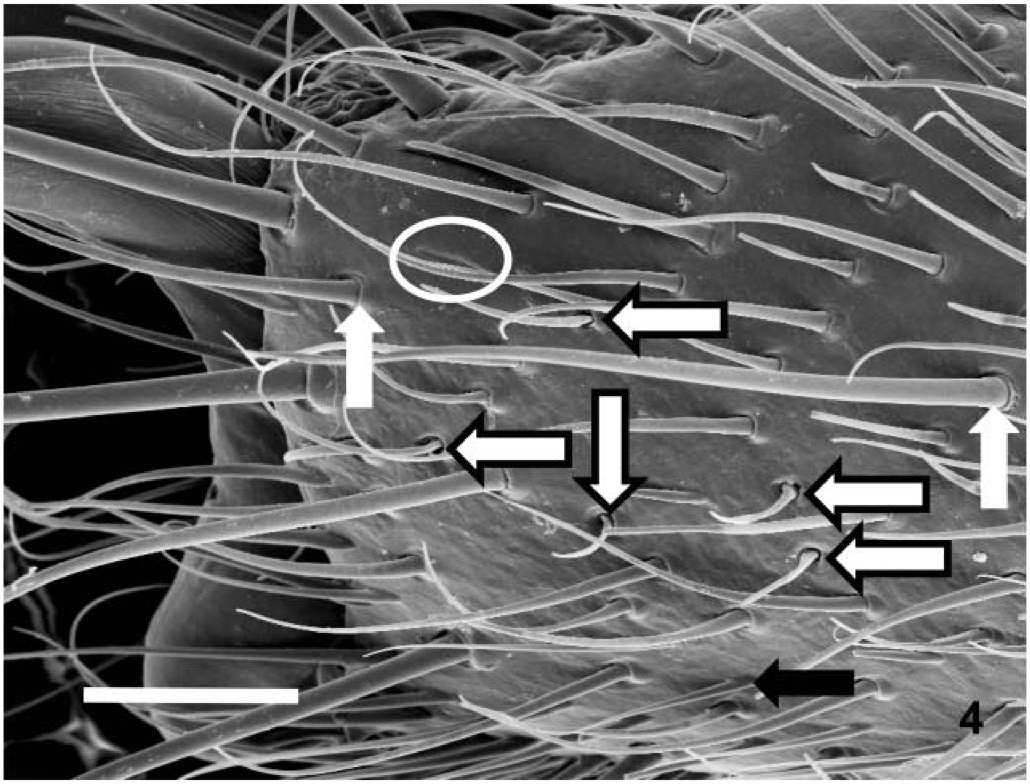

Figure 4. *Trichome* (black arrow), *sensillum chaeticum* (white arrow), falciform hair (black and white arrow) and single slit II (ellipse), at the most distal tarsomere of leg II, retrolateral region, in *Neosadocus* sp. female. Scale bar = 100 µm.

trichomes since, besides the morphological differences, they overtop the latter.

Falciform hairs (n. st.; Figures 4 and 15): they occur on the retrolateral and prolateral region of the most distal tarsomere of legs I and II. The length of the shaft, which is inserted in a cuticle depression, is between 25 and 50 µm. The format of the shaft varies, being more or less abruptly curved (Figure 4).

Sensilla basiconica (n. st.; Figures 5, 16 and 17): they occur in all four legs, on the dorsal region of the calcaneus (2–4), distally to the metatarsal paired slits (see below), and in legs I and II on the prolatero-dorsal region of the last tarsomere (2, on an irregular depression of the cuticle). We also found isolated ones in the distal portion of the last tarsomere, dorsally in legs III and IV. The length of the shaft is ~4.5 µm, it bears irregular slight grooves and no wall-pores. It opens in the apical region by means of a longitudinal aperture. The shaft is inserted in a cuticle depression surrounded by a basal membrane. In a morphological variation, the shaft is longer (~6.5 µm) and not inserted in a cuticle depression.

Spines (Figures 10 and 11): they occur in all four legs, in pairs, on the latero-ventral region of the distal part of the calcaneus. The length of the shaft is ~240 µm, orienting towards the distal end of the leg. The shaft tapers in its distal portion and bears longitudinal grooves. There are no wall pores or a pore at the tip. A basal membrane surrounds the proximal part of the shaft. They are easily distinguished by their large size and typical position.

SEM survey, slit sensilla

Metatarsal paired slits (n. st.; Figures 5 and 10): they occur in the dorso-lateral region of all four legs, on the most distal region of the calcaneus. They are positioned transversally to the longitudinal axis of the leg, always in pairs, and the more distal one is longer and bears a "labium". Being along the transversal plane, we did not measure it, but its length can be assessed in the figures.

Single slits I (Figure 15): they occur throughout the calcaneus and tarsus, on lateral and ventral regions. We failed to find them on the dorsal region. Measuring ~10 µm long, they are disposed longitudinally to the longer axis of the leg.

Single slits II (n. st.; Figures 4 and 6): they occur in the retrolateral region of the distal part of the legs. Measuring ~50 µm long, they are disposed sub-longitudinally to the longer axis of the leg.

Tibial slit (Figure 18). Found in the dorsal region, distal portion of the tibia III of *I. pustulosa* female. It measures ~120 µm long.

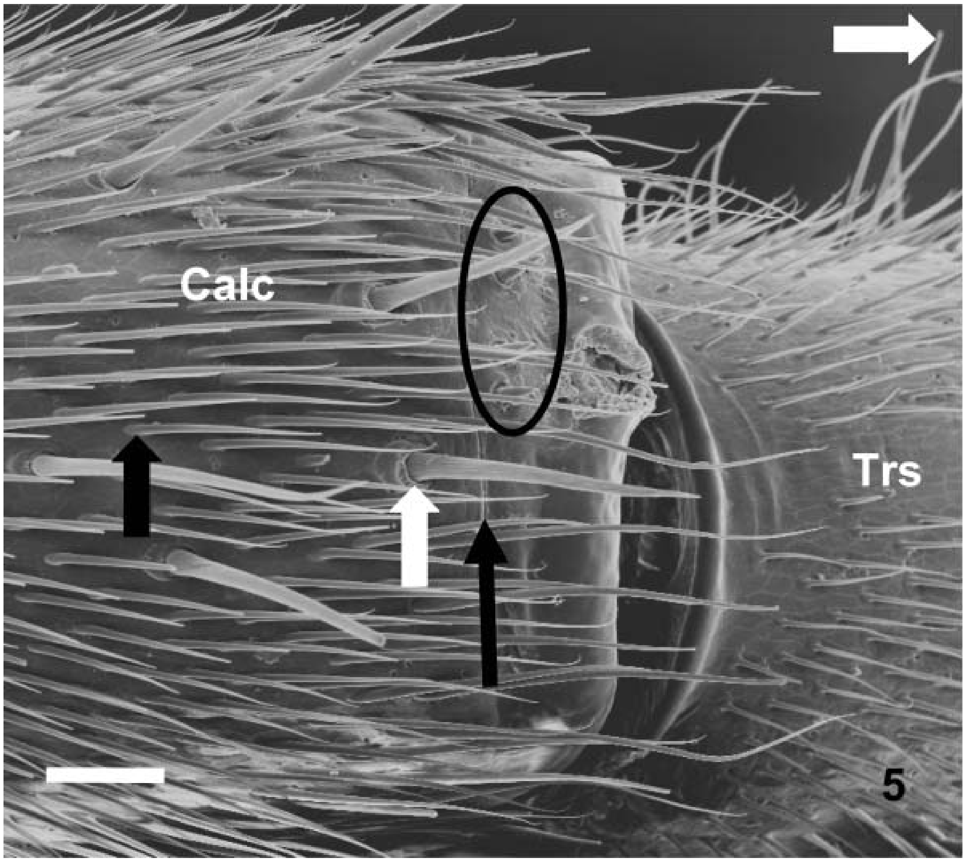

Figure 5. *Trichome* (short black arrow), *sensillum chaeticum* (white arrow), a slit of the paired metatarsal slits (long black arrow) and four sensilla basiconica (ellipse), at the dorsal region of calcaneus (calc) and tarsus (trs), leg II, in *Neosadocus* sp. female. Scale bar=50 µm.

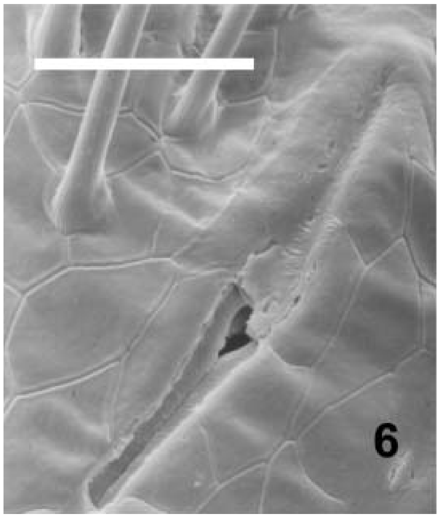

Figure 6. Single slit II with membrane partially broken, showing the interior region. In the upper left, three trichomes, at the most distal tarsomere of leg IV, prolateral region, in *I. pustulosa* male. Scale bar=10 µm.

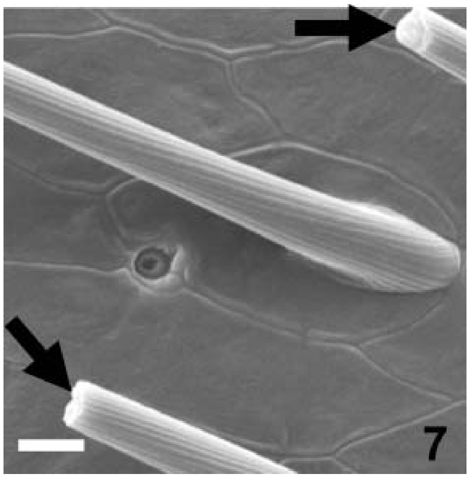

Figure 7. Single pore and trichomes (arrows point broken ones, showing no internal lumen), at the tarsomere of leg I, prolateral region, in *Neosadocus* sp. male. Scale bar=5 µm.

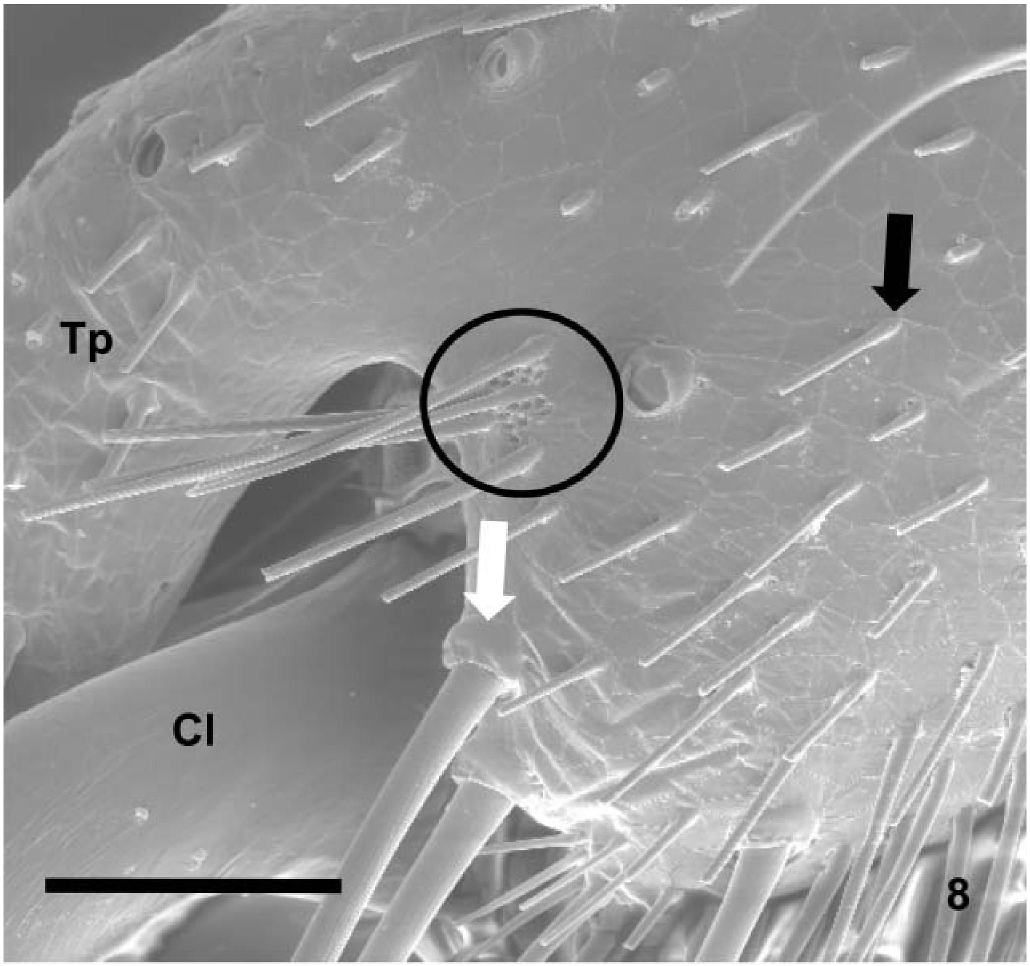

Figure 8. *Trichome* (black arrow), *sensillum chaeticum* (white arrow) and tarsal aggregated pores (circle), at the most distal tarsomere of leg III, retrolateral region, in *I. pustulosa* female. Cl=claw; TP=tarsal process. Scale bar=100 µm.

SEM survey, glandular openings

Articulation porous region (n. st.; Figures 19 and 20): these gland openings occur in the coxa-trochanter articulation of all legs. The articulation bears a subrectangular region with multiple pores, which number varies. The size of the articulation porous region varies according to the width of the leg, being therefore much larger in legs IV (width in medial region ~450 µm) than in legs I (width in medial region ~100 µm) for example.

Metatarsal gland (n. st.; Figures 21–24): a clearly differentiated region (Figure 24) that occurs in the metatarsus of legs IV of males. Without magnification, the gland can be seen as an enlarged area, absent in females (Figures 21A, B). This area bears several pores (Figure 22), through which secretions are exuded (Figures 22 and 23). Pores measure ~1.7–2 µm.

Rough pit glands (n. st.; Figures 19 and 25): We found these multi porous gland openings in the trochanter of all legs. Two general kinds of pits occur, though there are intermediaries: one is convex in relation to the integument and the other one is in the same plane of the integument. They form an incomplete ring distally to the articulation porous region. A pit measures between 30 and 40 µm.

Single pores (Figures 7 and 26): they occur all over the body, including dorsal, lateral and ventral regions of the legs. In the calcaneus and tarsus, they are always between polygons of the cuticle. The opening of the internal lumen measures ~0.6 µm.

Tarsal aggregated pores (n. st.; Figures 8 and 9): they occur in the retrolateral and prolateral regions of the distal part of legs III and IV, proximally to the tarsal process. It is an aggregation of glandular openings that is always close to the base of an aggregation of trichomes. The number of openings observed was ~15, each opening measuring ~1 µm.

Tarsal perforated organ (n. st.; Figures 11 and 12): they occur in all legs, on the ventral region of the most proximal tarsomere. There are two parallel rows, parallel to the longitudinal axis of the leg, each

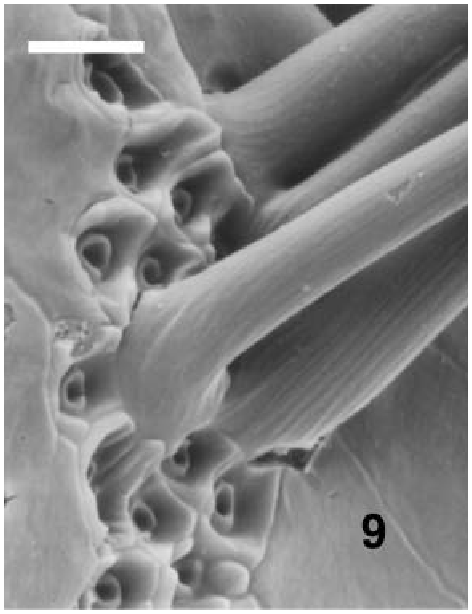

Figure 9. Tarsal aggregated pores and trichomes, at the most distal tarsomere of leg III, retrolateral region, in *I. pustulosa* female. Scale bar=5 µm.

with 10–20 perforated subrectangular parts. The rows are separated by a region identical to the rest of the legs. The length of all the adjacent subretangular parts varies from leg to leg, ranging from ~120 µm to ~350 µm.

Field observations on diet of Neosadocus *sp.*

Twice, distinct females of *Neosadocus* sp. have been observed feeding on pieces of earthworms, once a male was eating an isopod. We also observed individuals eating conspecifics eggs (*n*=2; one male and one female; Figure 27), feces (*n*=1 male; Figure 1), a bee cadaver (*n*=1 female), and remains of chewed fruits fallen from a tree (*n*=2, one male and one female). Once, a male and a female were sharing an earthworm. All these items were <50 cm from the ground. Apart from the bee cadaver, we do not know whether animal items were captured dead or alive.

Laboratory data on foraging of Neosadocus *sp.*

Individuals of *Neosadocus* sp. have displaced from the original square in 47.6% cases (median; range:

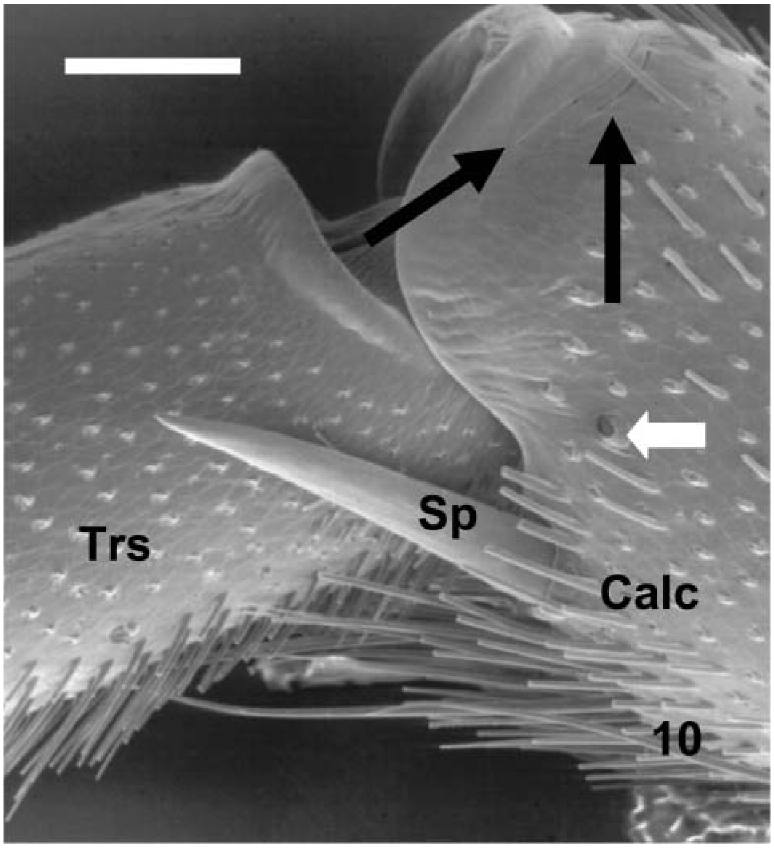

Figure 10. Spine (Sp) and paired metatarsal slits (black arrows). White arrow=*sensillum chaeticum* with the shaft missing. Lateral region of calcaneus (calc) and tarsus (trs) of leg IV, in *I. pustulosa* female. Scale bar=100 µm.

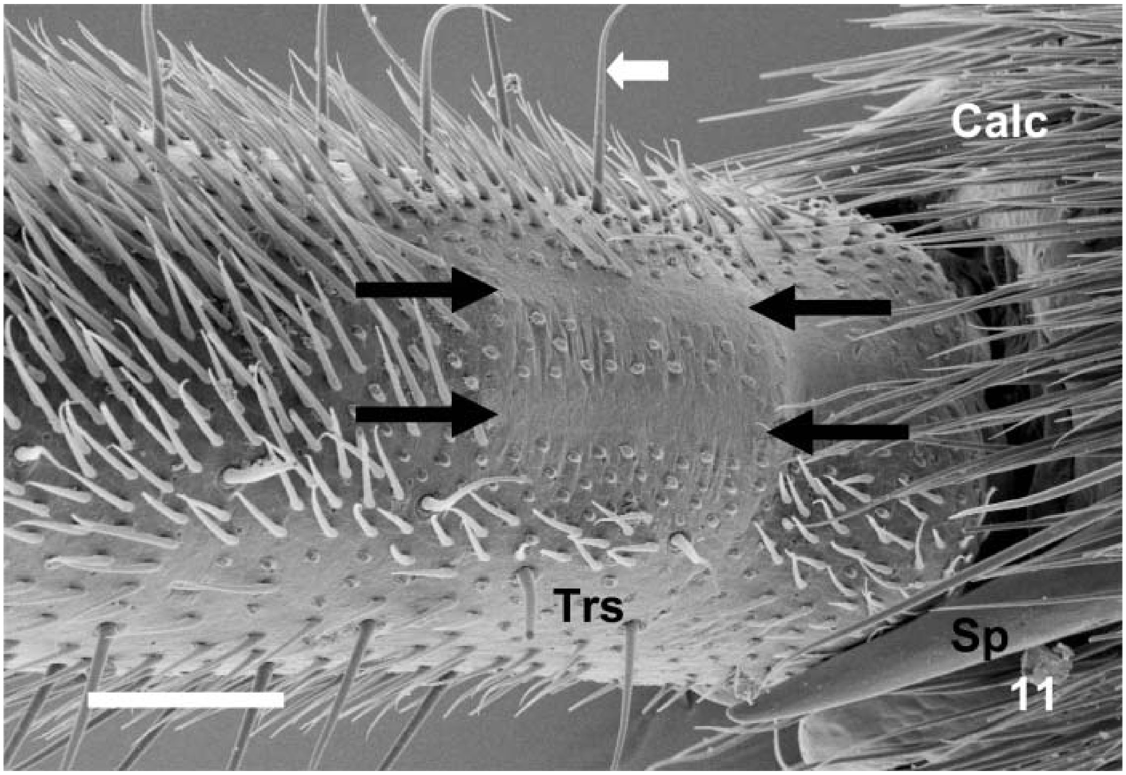

Figure 11. Spine (Sp), tarsal perforated organ (black arrows) and *sensillum chaeticum* (white arrow). The clear spots between the two lines of the tarsal perforated organ are broken trichomes. Ventral region of calcaneus (calc) and tarsus (trs) of leg I, in *Neosadocus* sp. female. Scale bar=100 µm.

6.7–84.0%; $n=15$) in the first block of observations (starvation condition). In the second block (satiation), they moved to different squares in 13.8% cases (median; range: 0.0–54.8%; $n=11$). In the third block (starvation condition), they moved to different squares in 25.0 % cases (median; range: 6.4–75.0%; $n=9$). There was a significant difference between the blocks ($\chi^2=1.556$, df=2, $P=0.001$, $n=9$). The second block differed both from the first block ($q=4.67$, $P<0.05$) and from the third block ($q=4.71$, $P<0.05$); but the first and the third blocks did not differ between each other ($q=1.88$, $P>0.05$).

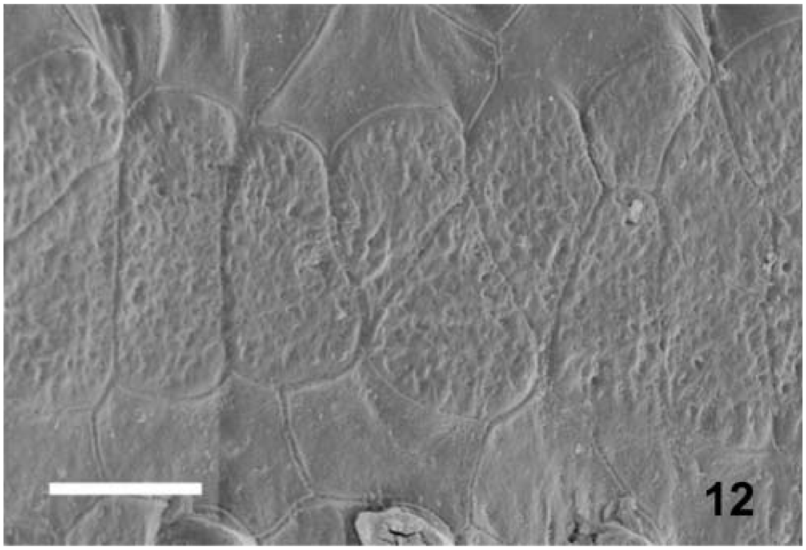

Figure 12. Detail of the tarsal perforated organ, at the ventral region of tarsus (trs) of leg I, proximal portion, in *Neosadocus* sp. female. Broken trichomes can be seen in the lower part of the picture. Scale bar=10 µm.

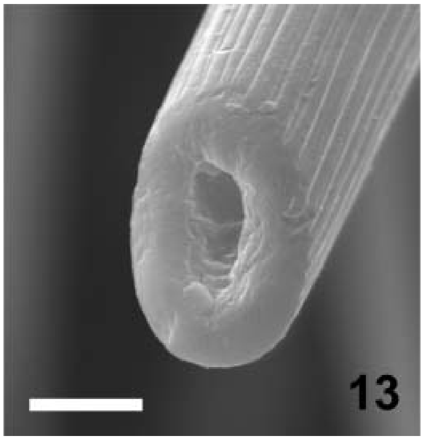

Figure 13. Broken *sensillum chaeticum*, showing internal lumen, at the most distal tarsomere of leg III, retrolateral region, in *Neosadocus* sp. male. Scale bar=5 µm.

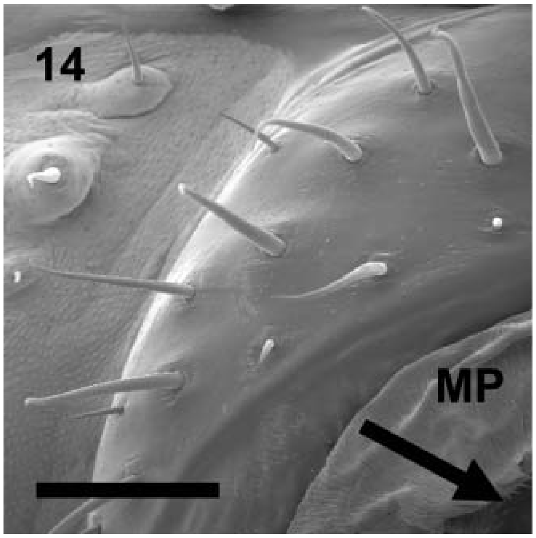

Figure 14. Lumen of the mouth (arrow), mouth parts (MP) and surrounding region, in *Neosadocus* sp. male. All hairs seen are sensilla chaetica. Scale bar=50 µm.

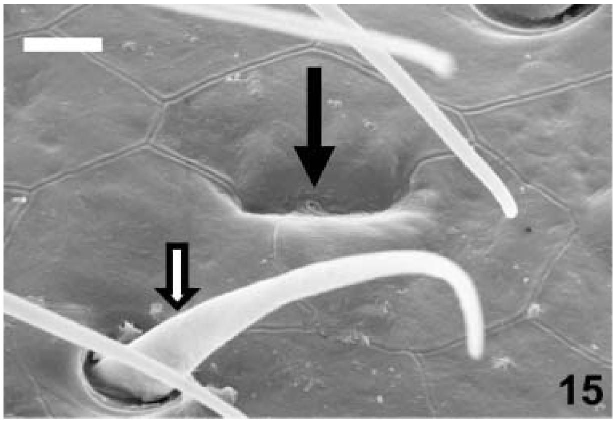

Figure 15. Single slit I (arrow points the possible insertion of the dendrite) and a falciform hair (black and white arrow). Tarsomere of leg II, lateral region, in *Neosadocus* sp. female Scale bar=5 µm.

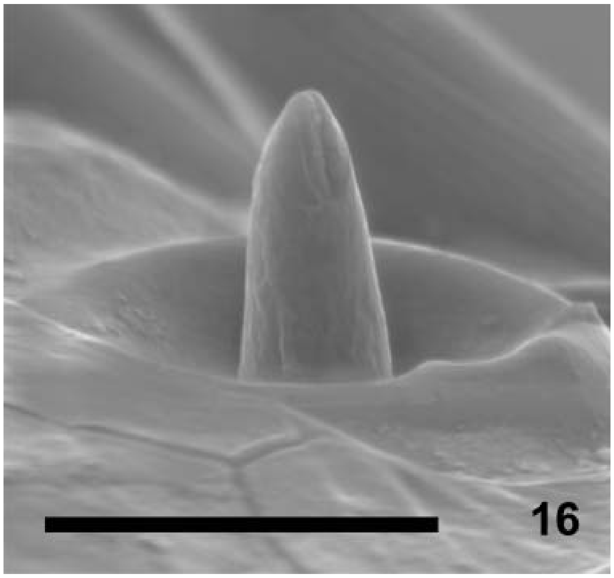

Figure 16. *Sensillum basiconicum*, at the most distal tarsomere of leg III, dorsal region, in *Neosadocus* sp. male. Scale bar=5 µm.

Although no quantitative observations were conducted, *I. pustulosa* also seems to alternate between wandering and standing in captivity (RHW, own observation).

Discussion

Detection of substrate-borne vibrations

The metatarsal paired slits, described here for the first time in harvestmen, are positioned perpendicular to

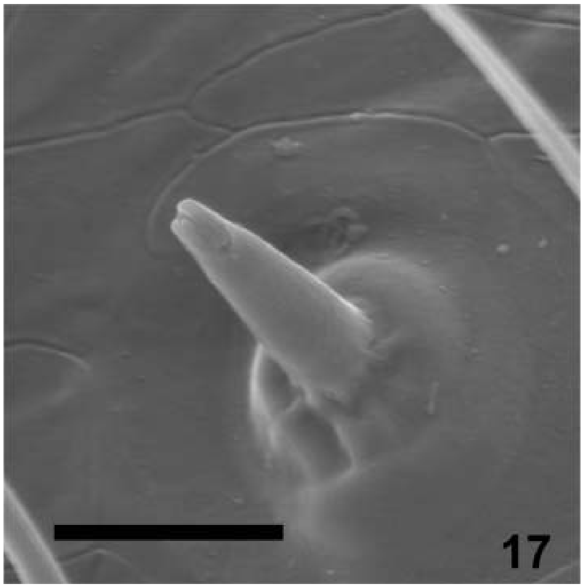

Figure 17. *Sensillum basiconicum*, morphological variation, at the most distal tarsomere of leg IV, dorsal region, in *Neosadocus* sp. male. Scale bar=5 µm.

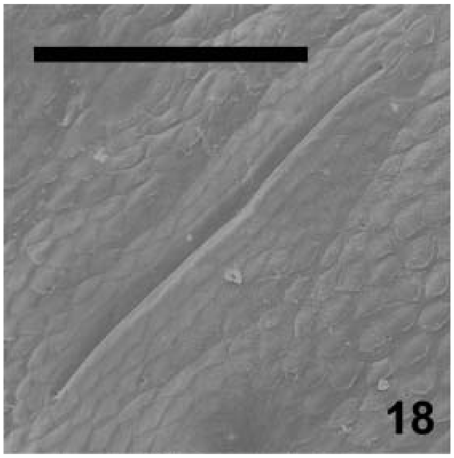

Figure 18. Tibial slit sensillum, at the dorsal region of the tibia, distal portion, in *I. pustulosa* female. Scale bar=100 µm.

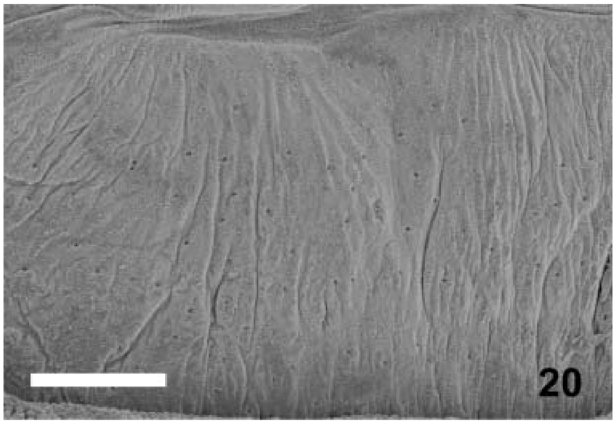

Figure 20. Detail of the pore plate gland, at the coxa/trochanter articulation of leg II, in *I. pustulosa* male. Note the multiporous surface. Scale bar=50 µm.

the longitudinal axis of the leg, in a region that is usually close to the substrate when the animal stands (see Figures 1 and 2). This characteristic makes them appropriate to detect substrate-borne vibrations, because slits are more sensitive when positioned in a way that it forms a 90° angle with the direction of the imposed force (Barth 2002b). Moreover, the fact that they are long is important since both directional and absolute sensitivity increase with length of the slit (Barth 2002a). The metatarsal organ of spiders is their most important detector of substrate-borne vibrations (Barth 2002a), and it is also located dorsally on the distal region of the metatarsus. These organs are also typical of other arachnids, such as scorpions and whip scorpions (Barth & Stagl 1976). Despite the similarity in position and orientation relative to the leg, the metatarsal paired slits appear not to be homologous to the metatarsal organs. They have very distinct morphologies and phylogeny does not suggest a single appearance within arachnid phylogeny (see Giribet et al. 2002). The single slits I that were found all over the legs are possibly proprioceptors. The functions of the single slits II are unknown.

Detection of wind currents

In accordance with what has been found in other harvestmen (Eupnoi), no trichobothria was found

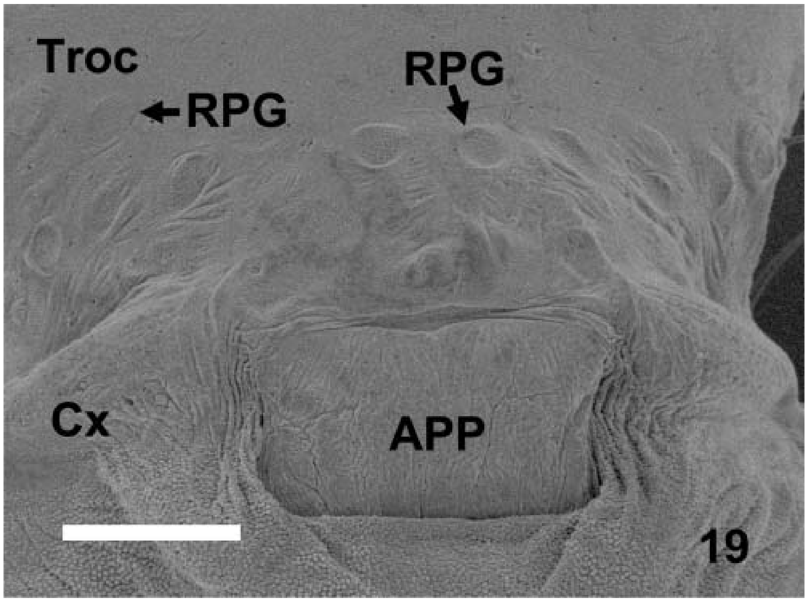

Figure 19. Rough pit gland (RPG) and articulation pore plate (APP), at the dorsum of the coxa (cx)/trochanter (troc) articulation of leg I, in *I. pustulosa* male. Scale bar=100 µm.

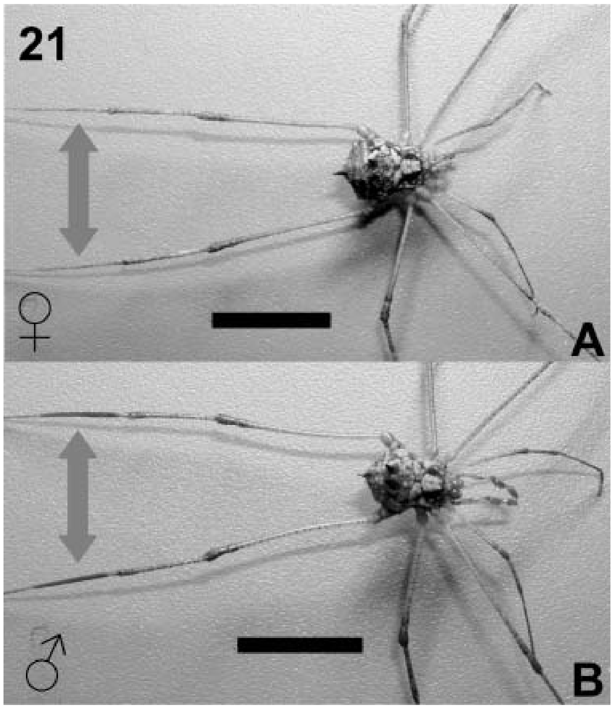

Figure 21. *I. pustulosa* female (A) and male (B). Arrows show metatarsus of legs IV, with a glandular area in males. Scale bar=1 cm.

either in *Neosadocus* sp. or in *I. pustulosa*. Nor have we found equivalent thin hairs such as the filiform hairs of insects (see Gnatzy 2001). It therefore seems reasonable to believe that the detection of air currents in these harvestmen is limited.

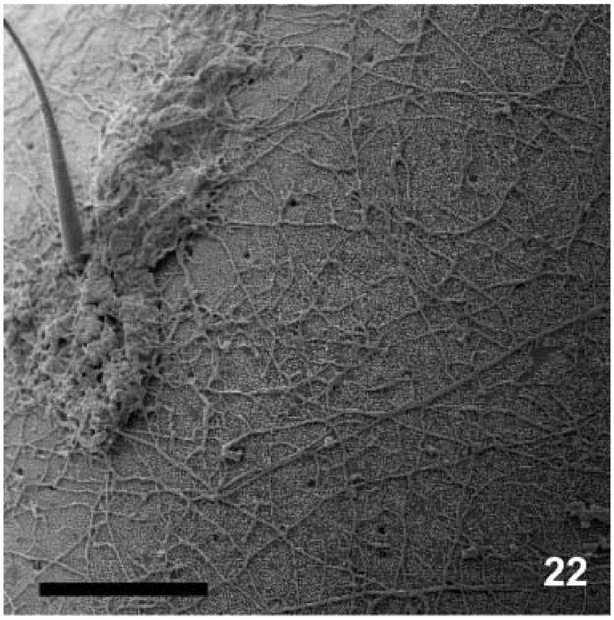

Figure 22. Dorsal region of the metatarsal gland of leg IV, with several threads of secretions, in *I. pustulosa* male. Scale bar=50 µm.

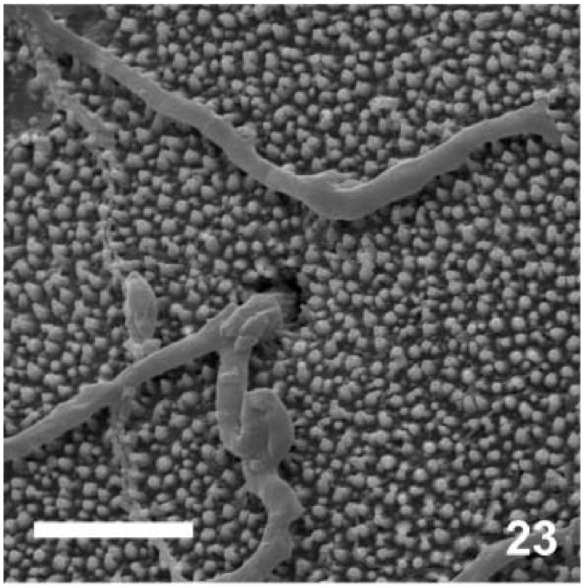

Figure 23. Detail of secretions expelled from a glandular opening of the metatarsal gland of leg IV, in *I. pustulosa* male. Scale bar=5 µm.

Remaining sensilla

Trichomes and sensilla chaetica have been observed in several harvestmen species (see references in Willemart & Gnaspini 2003). Although the former is long and thin, it has limited mobility in the base, thus its role role as a mechanoreceptor is improbable, although we cannot rule out this possibility until histological and electrophysiological studies are conducted. Having no pores, we discard a chemoreceptive role (Zacharuk 1985). If it is not a

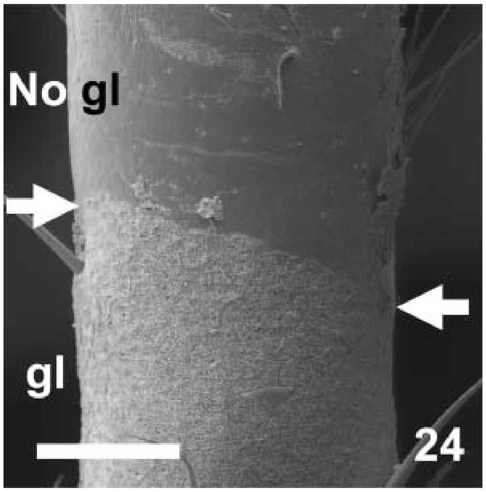

Figure 24. Distal limit of the glandular area of the metatarsal gland of leg IV, in *I. pustulosa* male. gl=glandular area; No gl=non-glandular area, close to the joint with the tarsus. Arrows show the exact limit of the gland. Scale bar=100 µm.

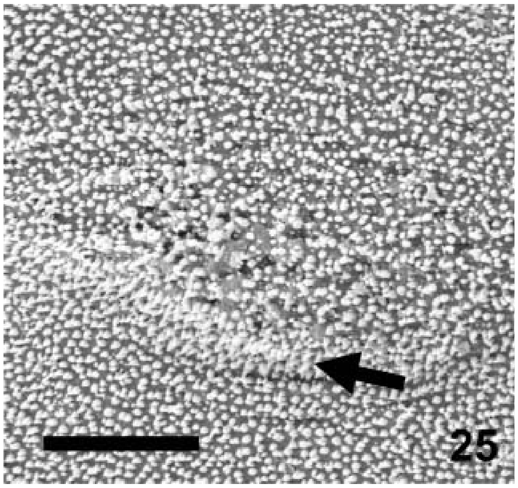

Figure 25. Detail of a rough pit gland, at the dorsal region of the trochanter of the leg III, in *Neosadocus* sp. male. Arrow points the inferior limit (white granulation) of the pit. Note the pores in the pit.

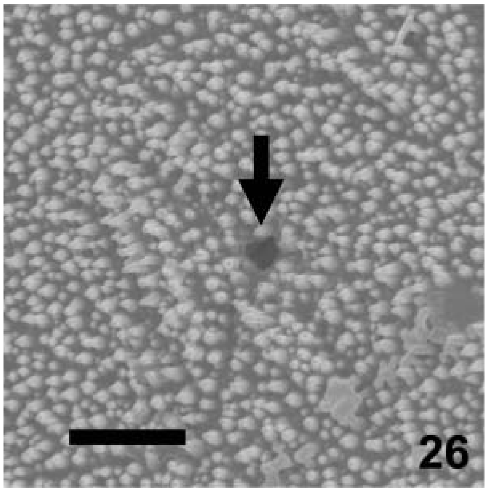

Figure 26. Single pore, at the coxa, ventral region, in *Neosadocus* sp. male. Scale bar=5 µm.

Figure 27. *Neosadocus* sp. female feeding on a conspecific egg of an unprotected clutch. Body length of the individual=~1 cm.

mechanoreceptor, it has been proposed that it may protect the integument and help in cleaning the appendages (Willemart & Gnaspini 2003). Sensilla chaetica has no wall pores, but has an internal lumen that may lead to a tip opening (see also Guffey et al. 2000; Willemart & Gnaspini 2003), thus probably functioning as a contact chemoreceptor (Steinbrecht 1984). Because it has a basal membrane and can be deflected in all directions, it may also function as a contact mechanoreceptor (Keil 1997). Their more abundant occurrence on the distal ends of legs I and II are probably related to the fact that these are mainly sensory legs, generally not used for supporting the body but for tapping the animal's surroundings (e.g. Anuradha & Parthasarathy 1976; Guffey 1999; Willemart 2002). The sensilla chaetica close to the mouth probably aids when deciding whether to accept/reject a food item, since mouth-contact with the food always occur prior to ingestion. Hairs with wall pores (e.g. in arachnids: Foelix 1985; Igelmund 1987; Talarico et al. 2006) were not found in these two species.

Sensilla basiconica seem to have limited mobility and they do not occur in any region in which they could function as a proprioceptor (see Chapman 1998). With the longitudinal opening that appears to open to the environment, and by exclusion, it may function as a chemo, hygro and/or thermoreceptor. The spines have a basal membrane (and are therefore mobile), and bear no pores. They are possibly contact mechanoreceptors that inform that the calcaneus is close to the substrate. The function of the falciform hairs is unknown.

Sensory capabilities, diet and foraging behavior

The sensory biology of an animal is obviously related to several factors, such as mate and predator detection and recognition and searching for microclimatic conditions. We herein restrict our discussion to feeding since our intention is specifically an attempt to understand why omnivory has arisen in harvestmen. It is known that harvestmen, particularly lanitorean, are omnivorous animals, and that several items of their diet are motionless, as mentioned in the introduction. Gnaspini (1996) and Machado and Pizo (2000) also reported *Neosadocus* sp. feeding on fruits. This was corroborated by our field observations, since eggs, feces, fruits and the bee cadaver are motionless items. Finally, it has been shown that live isopods were hardly caught by *I. pustulosa* in captivity (Willemart 2005).

We have also shown in the introduction that harvestmen wander while foraging, which was also corroborated by our observations in captivity. Both males and females of *Neosadocus* sp. do wander and increase their mobility when hungry, a similar behavior that was found for other harvestmen (Williams 1962) and some predatory beetles (Coleoptera, Carabidae) in areas with low prey density (Wallin & Ekbom 1994). Wandering when foraging seems to be an exception among arachnids, and this might be related to the consumption of motionless food items (Riechert & Luczak 1982). Concerning sensory structures, *I. pustulosa* and *Neosadocus* sp. seem not to be very sensitive to air currents, which would limit prey detection prior to contact (see Weygoldt 2000). They appear to be able to detect substrate-borne vibrations, although we cannot speculate about their sensitivity. Finally, they have hairs (sensilla chaetica) that allow them to perceive, by contact, the chemical properties of an item.

Dietary data (motionless items), foraging behavior (wandering behavior) and sensory structures (limited ability to detect moving prey) therefore seem to be related functionally. These factors might help to explain why, in spite of belonging to a group plesiomorphically predatory, Opiliones (but cyphophthalmids for which there is no detailed data) are omnivorous. Behavioral experiments on prey detection would now be required to test harvestmen's ability to perceive prey movement.

Gland openings

Four of the six glands reported herein are described for the first time in harvestmen. A complete list of the tegumental glands present in harvestmen will be provided elsewhere.

The function of the rough pit glands and the articulation porous region is unknown—we do not know their secretions, their histological characteristics, and apparently they are not sexually dimorphic. Neither do we have behavioral evidences of their use, although we know that males and females of *Neosadocus* sp. do not touch them while copulating or in male–male fights (see detailed description in Willemart 2005). Because it is sexually dimorphic, the metatarsal gland of *I. pustulosa* probably secretes a sexual pheromone. This might function at allowing sex recognition, to attract females or as an aphrodisiac before copula, similarly to the tibial gland in legs I of some spiders (Lycosidae—Kronestedt 1986).

We have observed, in captivity, males and females of both species rubbing legs III and IV on the substrate while walking, and males of *Neosadocus* sp. also do it during male–male fights (Willemart 2005). While walking, they extend one of the legs IV, in a way that the proximal part of the tarsus, and sometimes also the distal part, is dragged against the substrate. Leg III is gently twisted, allowing contact with the latero-dorsal region with the substrate. This behavior is probably related to the glands found on their tarsi, namely the tarsal aggregated pores and the tarsal perforated organ. In insects, substrate demarcation may relate to mate finding, territorial demarcation and general orientation (Wyatt 2004). Upon contact, male scorpions recognize substrate on which a female has walked (Steinmetz et al. 2004). In harvestmen, Gnaspini (1996) suggested that *Goniosoma spelaeum* (Mello-Leitão 1933) (Gonyleptidae) might leave chemical trails that would allow them to always use the same routes when leaving the cave. The function of leaving chemical trails in harvestmen is still an open question, but we have provided here the first morphological evidence that this might occur.

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