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Comparative Density of Hair Sensilla on the Legs of Cavernicolous and Epigean Harvestmen (Arachnida: Opiliones)

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Abstract. To allow an animal to behave appropriately, the location of sensorial structures is expected to be related to their function. As the different leg pairs of arachnids may have different functions (probing x supporting the body), one could expect them to have a different density of sensilla. Moreover, different regions of the same leg (dorsal, lateral, and ventral) would also be expected to have different densities of sensilla, according to the use of each region (e.g., the ventral part is often in contact with the substrate while the dorsal part is not). As cavernicolous animals are expected to be more sensitive than their epigean relatives, one could also expect a different density of sensilla when comparing cavernicolous and epigean animals. Using three epigean and three cavernicolous species of harvestmen (Arachnida, Opiliones), this study aimed at describing the morphology of hair sensilla on the legs and answering three questions: (1) Are there differences in the density of hair sensilla between the dorsal, lateral and ventral regions of each leg pair of the same individual? (2) Are there differences in the density of hair sensilla between the leg pairs of the same individual? (3) Are there differences in the density of hair sensilla when comparing the leg pairs of individuals of cavernicolous and non-cavernicolous species? The tarsi and metatarsi of all right legs of the six studied species were analyzed under a scanning electron microscope. The results (P < 0.05) showed that, in general: the ventral region of the tarsus was denser in *sensilla trichodea* than the lateral and dorsal regions, particularly on legs I and II; the density of *sensilla chaetica* did not differ on legs III and IV, but was greater on the dorsal region of legs I and II; the ventral part of legs I had the higher density of *sensilla trichodea* of the four pairs, whereas the second pair had the lower density; *Holcobunus citrinus* (Eupnoi) was the species with higher density of *sensilla trichodea*, on all legs; the cavernicolous species had a lower density of sensilla than the epigean species. The results are tentatively related to harvestmen behavior.

Key words. Opiliones, harvestman, *sensilla chaetica, sensilla trichodea*, morphology.

1. INTRODUCTION

Animals need up-to-date information about their environment to behave appropriately, and they are therefore highly dependent on their sense organs (YOUNG 1989). Sense organs may be related to several types of stimulus, such as chemical (olfaction and gustation), mechanical, radiant, and changes in temperature and humidity (CROWSON 1981). Arthropods have sensory structures located in specific sites on the body (FOELIX 1996; DALY et al. 1998), which are expected to be related to the function of the structure. HANSSON et al. (1986) showed that more exposed sensilla on a moth antennae are receptors of the less concentrated components of the pheromone released by females, and KLEIN (1981) suggested that the outward-facing side of olfactory sensilla on the palp of a cricket provides a better exposure to odorant stimulus.

In arachnids, although there are sensory structures in several parts of the body, they are concentrated at their extremities (PUNZO 1998). The first pair of legs of Thelyphonida, Schizomida, Palpigradi, Araneae, Solifugae, Amblypygi, and the pedipalps of Scorpiones and Pseudoscorpiones are used to probe the environment (SAVORY 1964; WEYGOLDT 1969, 2000; FOELIX 1996; PUNZO 1998; FARLEY 2001).

Harvestmen (Arachnida, Opiliones) have traditionally been divided in the suborders Cyphophthalmi, Palpatores and Laniatores (e.g., SHULTZ 1998), although sometimes the first two are grouped in the single suborder Cyphopalpatores (e.g., MARTENS et al. 1981) or Palpatores is divided into the suborders Eupnoi and Dyspnoi (e.g., GIRIBET et al. 2002). They are non-visual (HILLYARD & SANKEY 1989), omnivorous (revised by WILLEMART 2002) arachnids, which rely mostly on their legs to gather information from their surround-

ings (Phillipson 1960; Macías-Ordóñez 1997). The second pair of legs has been considered to be the most important sensorial appendage of harvestmen, and is generally not used for walking, which is accomplished mainly by the third and fourth pair of legs (Hillyard & Sankey 1989). Some studies showed that the first pair of legs is also important as a sensorial appendage (Anuradha & Parthasarathy 1976; Guffey 1999; Willemart 2002). One would therefore expect a different density of sensilla when comparing different legs (since they have different functions), and between different regions of the same leg, according to the use of each part of the leg (e.g. the ventral part is often in contact with the substrate while the dorsal part is not). Very little has been done in the field of sensorial structures of harvestmen. Some papers on general aspects of harvestmen report the presence of hair and slit sensilla and lyriform organs (Hansen & Soerensen 1904; Berland 1949; Cloudsley-Thompson 1958; Kaestner 1964; Edgar 1971; Eisenbeis & Wichard 1987; Hillyard & Sankey 1989). More specific papers described and discussed the function of the hair sensilla, tarsal and campaniform organs, and slit sensilla (Edgar 1963; Barth & Stagl 1976; Foelix 1976; Lopez et al. 1980; Spicer 1987; Kauri 1989; Luque 1993; Guffey et al. 1999). Finally, photographs of sensorial structures can be found in some taxonomic papers (e.g., Juberthie & Massoud 1976; Holmberg & Cokendolpher 1997; Giribet 2002). None of the works above dealt with Neotropical species.

Cave species are interesting models for the study of the evolution of sensorial organs. These species are subdivided into three categories: troglobites are restricted to the cave, troglophiles include populations that complete their life cycles outside caves and populations that complete their life cycles in caves, and trogloxenes inhabit caves but must leave it to forage and/or reproduce (Howarth 1983). "Old" troglobites may have troglomorphisms, i.e., characteristics that appeared in the cave and are related to this environment. One of the known troglomorphisms is the increase in the number and/or sensitivity and/or size of the sensorial structures (other than visual ones), which is related to the low food availability in caves (Christiansen 1992; Gnaspini & Hoenen 1999). As troglophiles may complete their life cycle in the hypogean or epigean environment, we have to consider the possibility that they may be more sensitive than strict epigean species, which would be a preaptation for the colonization of caves (Gnaspini & Hoenen 1999).

To detect whether the increase in sensorial structures is related to evolution in the cave environment or is due to plesiomorphy, the phylogeny of the studied group has to be well known and it must include epigean and cavernicolous species. Unfortunately, because the systematics of Opiliones are still unresolved, it is so far not possible to develop a complete phylogenetic study. However, a comparison between sensorial structures of cavernicolous and epigean harvestmen is a first step towards understanding how these structures have evolved. Herein we studied three epigean and three cavernicolous harvestmen species. In addition to describing the morphology of the most abundant hair sensilla on their tarsi, this study intended to answer three questions: (1) Are there differences in the density of hair sensilla between the dorsal, lateral and ventral regions of each leg pair of the same individual? (2) Are there differences in the density of hair sensilla between the leg pairs of the same individual? (3) Are there differences in the density of hair sensilla when comparing the leg pairs of individuals of cavernicolous and non-cavernicolous species?

2. MATERIALS AND METHODS

2.1. Species studied

We used the following laniatorean (all Gonyleptidae) species (between parentheses: subfamily, relationship between the species and the cave environment, and provenence – all in the state of São Paulo, Brazil): *Promitobates ornatus* (Mello-Leitão, 1922) (Mitobatinae, epigean, Paranapiacaba [= Alto da Serra], Santo André), *Neosadocus maximus* (Mello-Leitão, 1935) (Gonyleptinae, epigean, Iporanga), *Goniosoma albiscriptum* Mello-Leitão, 1932 (Goniosomatinae, trogloxene, Gruta da Quarta Divisão, Ribeirão Pires), *Daguerreia inermis* Soares & Soares, 1947 (Pachylinae, troglophile, Gruta dos Buenos, Iporanga), *Pachylospeleus strinatii* Silhavy, 1974 (Pachylospeleinae, troglobite, Gruta das Águas Quentes, Iporanga). One Eupnoi (Sclerosomatidae) was also used: *Holcobunus citrinus* Pocock, 1903 (Gagrellinae, epigean, Paranapiacaba, Santo André). Only females were used, except for *P. strinatii*. Because there was an available preserved male, and this species is rare and endangered, we decided not to collect females.

2.2. Microscopical preparations

Individuals preserved in alcohol had the legs cut at the level of the astragalus (Fig. 1). The cut portion of the leg (tarsus, calcaneus and part of the astragalus) was then submerged in a 5 : 1 (water : neutral detergent) solution and cleaned ultrasonically. Thereafter, the leg was transferred to a vessel containing only water and cleaned ultrasonically again. The leg was then dried in a stove at 40 °C for 24 h, mounted on an aluminum stub using double sided adhesive tape, sputter coated with gold (Sputter Coater Balzer SCD 50) and photographed with two different scanning electron microscopes (SEM – Zeiss DSM 940 and LEO 440 Laika & Zeiss).

2.3. Counting of hair sensilla

Only tarsus and calcaneus were used to quantify the hair sensilla because the remaining parts of the leg have a very small density of hair sensilla (see section 3; Figs. 1, 2, and 3). The dorsal, lateral and ventral parts of the second and third tarsomeres and the distal third of the calcaneus were photographed. Two magnifications were used, x and ~2x, the latter for a possible need of observing structures minutely, totaling at least 24 photographs per individual. Photographs were then transferred to the software Corel Draw, on which sample squares of 6.45 cm² of area were distributed side-to-side, without superposition, on the medial part of each region (dorsal, lateral, ventral) of the tarsus/calcaneus (Fig. 4). Positioning squares on the medial portion was to avoid for instance that a square sample placed on the upper lateral region put too high on the lateral area would reach the dorsal part of the leg. We also avoided putting sample squares in the region between two tarsomeres, since this region never contained hair sensilla. Except for these two criteria, sample squares were randomly distributed (Fig. 4). The average ± standard deviation of samples per tarsus/calcaneus was 8.96 ± 1.35 (range = 6–14). Each type of hair sensilla was counted separately. We considered that a hair sensillum was inside the sample square whenever at least a part of its insertion on the leg was inside the square sample. If a hair sensillum insertion was inside two sample squares, this hair sensillum was counted only once. As several different magnitudes were used when making the photographs, and the leg width varied between species, a conversion was made to allow comparison between species. The number of hair sensilla found on each sample square was multiplied by the magnification of the photograph.

This study was based on ~160 available SEM photographs, of six individuals of six species. Instead of making qualitative comparisons, we decided to use several sample squares from the same individual to allow a quantitative comparison, even if, strictly speaking, these comparisons concern the individuals used herein, and not a sample of the species.

The nomenclature of sensilla used herein followed that used by GUFFEY et al. (2000), which does not necessarily correspond to that used by some entomologists, due to the confusion in sensilla terminology (see ALTNER & PRILLINGER 1980; MERIVEE et al. 1999).

2.4. Leg-measurements

In order to determine the total area of the calcaneus and tarsus (the "high-density of hair sensilla region" – see Fig. 1), some measurements were taken with the help of digital calipers. The length was measured from the distal portion of the tarsus (base of claws) to the most proximal part in which there was still a high density of hair sensilla (the most proximal part of calcaneus), and width was measured at the same region in which the photographs were taken (see 2.3).

2.5. Statistical analysis

The values obtained from counting were organized in the Software Sigmastat, which tests for normality and homocedascity before using parametric tests. To compare groups, we used non-parametric (Kruskal-Wallis) and parametric ANOVA, which were followed, when necessary, by a posteriori tests (parametric: Tukey test; non-parametric: Dunn test). In cases in which only two groups were compared, we used a t or Mann-Whitney test. The P value to reject normality and homocedascity, and to conclude groups were different was 0.05. As mentioned above, the values of the numbers of hair sensilla were multiplied by the magnification of the photograph. This did not affect tests made in groups that did not have "zero" values, since all the numbers increased proportionally. However, since "zeros" do not change when multiplied, groups that contained "zero" hair sensilla in one or more sample squares could not be compared.

3. RESULTS

The two most abundant hair sensilla found were *sensilla chaetica* and *sensilla trichodea*. Except for some particular cases (the dorsal region of calcaneus II and tarsus I, lateral region of tarsus I, and the dorsal, lateral and ventral regions of tarsus II of *D. inermis,* and the dorsal region of tarsus II of *P. strinatii*), *sensilla trichodea* were much more abundant than *sensilla chaetica*. These two sensilla shared the following characteristics: their shafts were both oriented towards
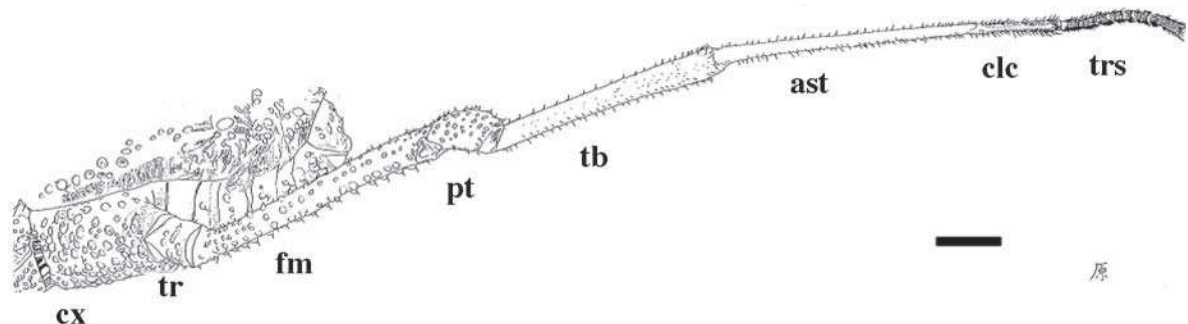

Fig. 1. The leg of a laniatorean harvestman (*Neosadocus maximus*). Scale bar = 3 mm. cx = coxae; tr = trochanter; fm = femur; pt = patella; tb = tibia; ast = astragalus; calc = calcaneus; trs = tarsus (drawing by M.R. Hara).
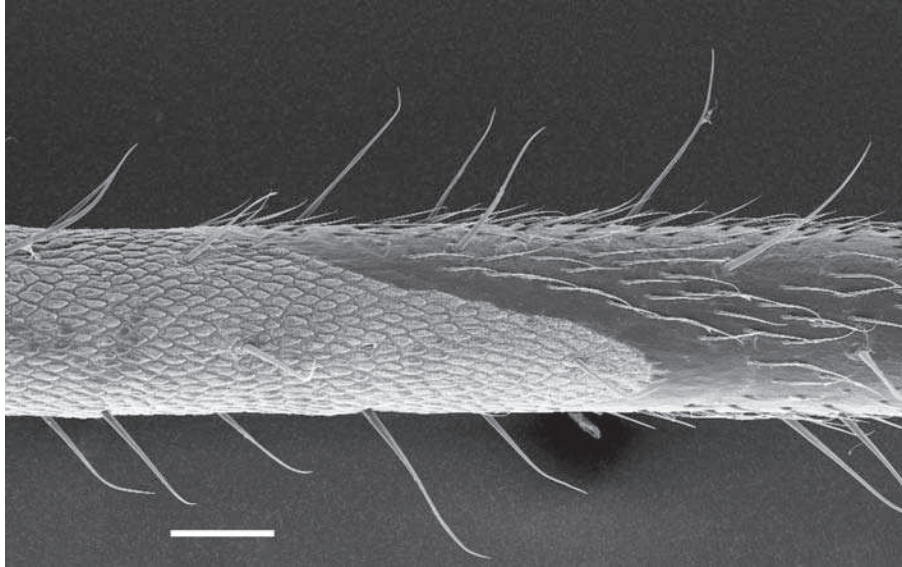
Fig. 2. Lateral view of the two parts of the metatarsus: astragalus (rough surface, few hair sensilla, proximal) and calcaneus, on the leg II of *Daguerreia inermis*. Scale bar = 100 μm.
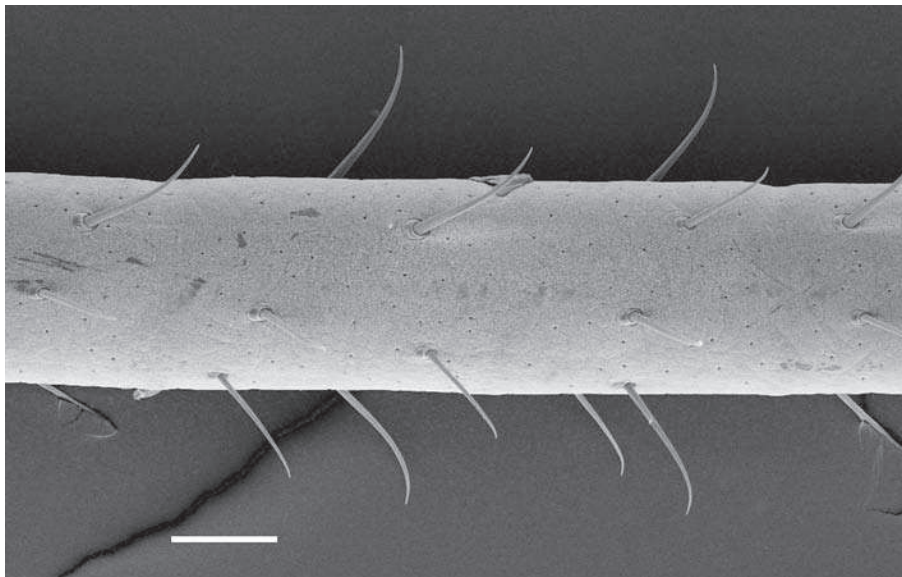
Fig. 3. Lateral view of the astragalus I of *Goniosoma albiscriptum*. Scale bar = 100 μm.
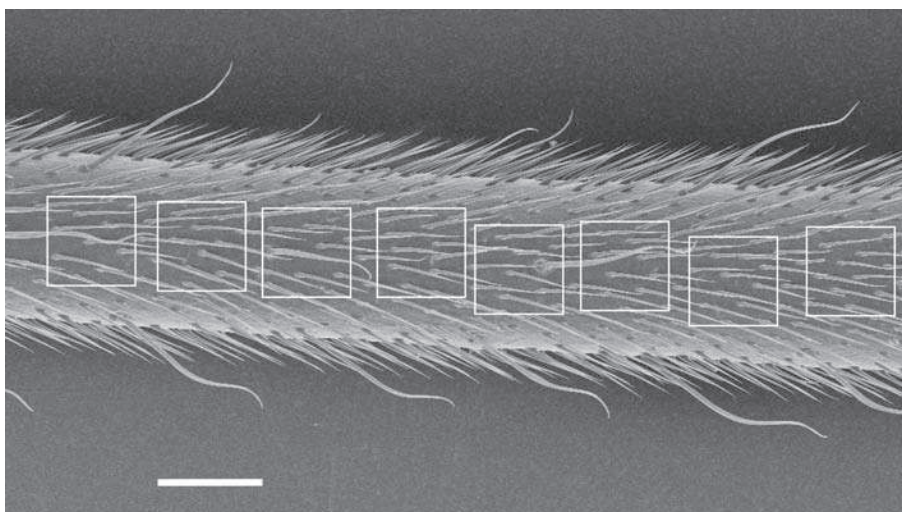
Fig. 4. Sampling method used to quantify the hair sensilla (see text for further information), on the calcaneus IV of *Promitobates ornatus*, lateral view. Scale bar = 100 μm.

the distal end of the leg (angle of insertion relative to the leg: *sensilla trichodea*: ~15–30°; *sensilla chaetica* ~50–80° – exceptions: on the tarsus I and II of *P. strinatii* and tarsus II of *D. inermis*, the angle of insertion of *sensilla chaetica* varied between ~15–80°); their distal regions were slightly or abruptly curved, generally towards the distal end of the leg; their shafts had deep or soft longitudinal grooves and no wall pores (Figs. 5 and 6). They differed in the following characteristics: *sensilla chaetica* had a basal membrane whereas *sensilla trichodea* did not, and the former generally exceeds the latter. Among *sensilla chaetica*, there were variations in the size of the shaft, and among *sensilla trichodea* there were slight variations

of form in *N. maximus* and *P. ornatus*. In these two species, some shafts had an enlargement at the base. Broken sensilla revealed the presence of a single, circular lumen in some *sensilla chaetica* and *sensilla trichodea*, but not in all of them (Figs. 5 and 6). A pore tip seemed to be present in at least some *sensilla chaetica* but not in *sensilla trichodea* (Figs. 7 and 8). All species studied herein had the proximal parts of the legs covered by a rough microgranulate surface. The five laniatoreans that we observed had a few hair sensilla from the coxae to the distal part of the astragalus (Figs. 1, 2, and 3), and the Eupnoi studied had a few hair sensilla from the coxae until the distal part of the tibia.

Tab. 1. Legs and species in which one region of the calcaneus or tarsus (dorsal, lateral, ventral of the same leg) was significantly denser in *sensilla trichodea* or *sensilla chaetica* than another region (P < 0.05). I = leg I; II = leg II etc.

dorsal > lateral	dorsal > ventral	lateral > dorsal	lateral > ventral	ventral > dorsal	ventral > lateral
sensilla trichodea in calcaneus					
–	–	II *P. ornatus*	–	I *D. inermis*	I *D. inermis*
–	–	III *N. maximus*	–	I *P. ornatus*	II *D. inermis*
–	–	–	–	II *D. inermis*	III *H. citrinus*
–	–	–	–	II *N. maximus*	III *P. ornatus*
–	–	–	–	III *N. maximus*	–
–	–	–	–	III *P. strinatii*	–
–	–	–	–	III *P. ornatus*	–
sensilla trichodea in tarsus					
–	III *H. citrinus*	I *P. strinatii*	–	I *D. inermis*	I *G. albiscriptum*
–	–	II *P. ornatus*	–	I *G. albiscriptum*	I *H. citrinus*
–	–	IV *D. inermis*	–	I *H. citrinus*	I *N. maximus*
–	–	–	–	I *N. maximus*	II *D. inermis*
–	–	–	–	I *P. strinatii*	II *G. albiscriptum*
–	–	–	–	II *D. inermis*	II *N. maximus*
–	–	–	–	II *G. albiscriptum*	II *P. ornatus*
–	–	–	–	II *N. maximus*	IV *G. albiscriptum*
–	–	–	–	II *P. ornatus*	IV *H. citrinus*
–	–	–	–	II *P. strinatii*	–
–	–	–	–	III *D. inermis*	–
–	–	–	–	IV *D. inermis*	–
–	–	–	–	IV *G. albiscriptum*	–
–	–	–	–	IV *H. citrinus*	–
sensilla chaetica in calcaneus					
–	II *D. inermis*	–	II *D. inermis*	–	–
sensilla chaetica in tarsus					
II *P. ornatus*	I *D. inermis*	–	I *G. albiscriptum*	III *D. inermis*	–
–	I *G. albiscriptum*	–	I *N. maximus*	–	–
–	I *N. maximus*	–	II *G. albiscriptum*	–	–
–	I *P. strinatii*	–	II *N. maximus*	–	–
–	II *N. maximus*	–	–	–	–
–	II *P. ornatus*	–	–	–	–

3.1. Comparisons between dorsal, lateral and ventral regions of the legs of the same species

The differences in the densities of hair sensilla between the dorsal, lateral, and ventral parts of the legs are summarized in Tab. 1. *Neosadocus maximus* had a very high density of hair sensilla on the ventral part of tarsi III and IV, thus obscuring their type and number (Fig. 9). Although they were not considered for the statistical tests, we believe that the great majority of these sensilla were probably *sensilla trichodea*, which were the most abundant on all regions of all legs in *N. maximus*.

Sensilla trichodea

The ventral region of the calcaneus was generally denser than lateral and dorsal regions, although on calcaneus IV there was no difference between the six species (Tab. 1). On the tarsus, the ventral region was also generally denser than lateral and dorsal regions, mostly among tarsi I and II, which include 17/23 (74%) of the significant differences between the ventral region vs. lateral/dorsal regions (Tab. 1). The ventral region of the legs IV was also generally denser than lateral and dorsal regions.

Sensilla chaetica

Except for legs II of *D. inermis*, there was no difference in the density on the calcaneus (Tab. 1). Generally, the ventral region of tarsi I and II was less dense than lateral and dorsal regions, and there was no difference between the regions on legs III and IV (Tab. 1).

3.2. Comparisons between the ventral part of the different leg pairs of the same species

Sensilla trichodea

None of the studied species had a greater density of *sensilla trichodea* on calcaneus II than on calcaneus I (Tab. 2). Tarsi I were denser than tarsi II in four of the six studied species.

Sensilla chaetica

This type of hair sensilla was homogeneously distributed among the calcaneus of distinct leg pairs in most species (Tab. 2). Except for legs IV of *H. citrinus*, tarsus I was not denser than the other tarsi. Except for *D. inermis*, tarsus II was not denser than the other tarsi (Tab. 2).

3.3. Comparisons among different species

A comparison among *sensilla chaetica* was not possible because the data were not comparable due to the lack of these sensilla in several samples (see Materials and Methods). *Holcobunus citrinus* had the greatest density of *sensilla trichodea* of all species. The cavernicolous *D. inermis* and *P. strinatii* had the lowest density of these hair sensilla (Tab. 3).

3.4. Measurements of the "high-density of hair sensilla" region

Neosadocus maximus had the larger "high-density of hair sensilla" region (calcaneus + tarsus – see Materials and Methods) (9.35 mm²), followed by *H. citrinus* (9.08 mm²), *G. albiscriptum* (7.81 mm²), *P. ornatus* (4.37 mm²), *D. inermis* (3.79 mm²) and *P. strinatii* (3.50 mm²).

4. DISCUSSION

4.1. General features

The microgranulate surface on the proximal parts of the leg was also observed on other harvestmen species (Juberthie & Massoud 1976; Holmberg & Cokendolpher 1997), and the extension of the "high-density of hair sensilla region" that we found in the laniatoreans studied was similar to that observed by Juberthie & Massoud (1976) in a cyphophthalmid; the extension of the "high-density of hair sensilla region" that we found in the Eupnoi studied was similar to that observed by Holmberg & Cokendolpher (1997) in another Eupnoi species. The general morphology of the hair sensilla was similar to that found by Juberthie & Massoud (1976), Spicer (1987), Holmberg & Cokendolpher (1997) and Guffey (1999), which would suggest similar functions.

4.2. Morphology of *sensilla chaetica*

Pores in hair-sensilla have been related to a chemoreceptive function (Foelix 1970, 1985; Shanbag et al. 1999 and references therein). There may be a single terminal pore (contact chemoreception – e.g. van Baaren et al. 1999) or several pores on the shaft wall (olfaction – e.g. Ochieng et al. 2000). Although pores were hardly seen and are known on some occasions to be plugged by extruding fluid (Foelix & Chu-Wang 1973; Kauri 1989), they seem to occur at least in some of the *sensilla chaetica* observed (Fig. 7). Because in the species studied herein, no wall-pores were found in *sensilla chaetica* (as also reported by Guffey et al. 2000), these sensilla are probably not related to olfaction. The steeper angle in the leg cuticle and larger length, allowing *sensilla chaetica* to extend beyond *sensilla trichodea*, suggests a contact chemoreceptive function (Foelix & Chu-Wang 1973; Altner &

PRILLINGER 1980). No terminal pores were found on *sensilla chaetica* of the harvestmen studied by GUFFEY et al. (2000), but they stressed that the histological characteristics suggested a chemoreceptive function. Broken *sensilla chaetica* revealed (1) a thick shaft wall, which is generally associated with contact chemoreceptors (SLIFER 1970), but (2) a single lumen in the shaft, and not a double lumen as recorded in chemosensitive hairs in spiders (FOELIX 1970). As con-tact chemoreceptive sensilla generally posses mechanoreceptive dendrites, assuming a bimodal function (FOELIX & CHU-WANG 1973; ALTNER & PRILLINGER 1980), and because of the presence of an articulating membrane, we suggest that *sensilla chaetica* are gustatory contact mechanoreceptors. It should be mentioned, however, that the *sensilla chaetica* that had no internal lumen (Fig. 5A) probably had no terminal pore, and would therefore function only as tactile hairs.

Tab. 2. Significant results in the comparisons of *sensilla trichodea* and *sensilla chaetica* of the ventral region of the calcaneus and tarsus among different legs (P < 0.05). For instance, I>II = higher density of hair sensilla on legs I than on legs II. *Dague = Daguerreia inermis*; *Goni = Goniosoma albiscriptum*; *Holco = Holcobunus citrinus*; *Neos = Neosadocus maximus*; *Pachy = Pachylospeleus strinatii*; *Promi = Promitobates ornatus*.

| I > II	I > III	I > IV	II > I	II > III	II > IV	III > I	III > II	III > IV	IV > I	IV > II	IV > III
sensilla trichodea in calcaneus											
Neos	*Dague*	*Neos*	–	*Goni*	*Goni*	*Pachy*	*Promi*	*Pachy*	–	–	–
Promi	*Goni*	*Promi*	–	–	*Pachy*	–	–	–	–	–	–
sensilla trichodea in tarsus											
Dague	–	*Dague*	–	–	–	–	*Dague*	*Dague*	–	*Dague*	*Holco*
Goni	–	*Goni*	–	–	–	–	–	–	–	*Holco*	–
Holco	–	–	–	–	–	–	–	–	–	–	–
Pachy	–	–	–	–	–	–	–	–	–	–	–
sensilla chaetica in calcaneus											
Promi	–	–	–	–	–	–	–	–	–	–	–
sensilla chaetica in tarsus											
–	–	*Holco*	*Dague*	*Dague*	*Dague*	–	–	–	–	–	–

Tab. 3. Comparison of the density of *sensilla trichodea* on the ventral and dorsal region of the legs of different species. The number of "x" represents the number of species that have significantly lower density of hair sensilla on the same part of the leg than the one represented at the first line of the column (P < 0.05).

|	*D. inermis*	*G. albiscriptum*	*H. citrinus*	*N. maximus*	*P. strinatii*	*P. ornatus*
VENTRAL						
calcaneus I			×××			×××
calcaneus II		×	××			
calcaneus III		×	×××××	××	×××	×××
calcaneus IV			×××			××
tarsus I		×	×	××		
tarsus II		××	×	××		
tarsus III			×××			
tarsus IV		××	×××			
DORSAL						
calcaneus I			×××			
calcaneus II			×××			
calcaneus III			×××			
calcaneus IV			×××			×
tarsus I			×××			
tarsus II			×××	×		
tarsus III		×	××			×
tarsus IV			××××	×		××××

4.3. Morphology of *sensilla trichodea*

Sensilla trichodea had no wall pores. They are therefore probably not olfactory detectors, as discussed above. There was no discernable apical pore (Fig. 8), which does not mean pores were not actually present, as also discussed above. However, as *sensilla chaetica* extend beyond *sensilla trichodea*, a contact chemoreception function is more likely attributable to the former, as it contacts the substrate first (ALTNER & PRILLINGER, 1980). We would like to propose three possible functions for these sensilla, which are not mutually exclusive. The first is that they may function as tactile hairs, although there was no articulating membrane, which are commonly found in tactile hairs in insects and other arachnids (FOELIX 1985; SNODGRASS 1993; CHAPMAN 1998). Unsocketed hair sensilla without wall pores being the most abundant sensilla were also found in other taxa, but their function was not discussed (e.g., Hymenoptera: AMORNSAK et al. 1998; Diptera: SHANBAG et al. 1999; Opiliones: GUFFEY et al. 1999). Because of their abundance and very acute angle of insertion, possibly reducing the contact between the substrate and the integument, a second possible role for *sensilla trichodea* is to protect the integument and/or other sensilla, as has been suggested for some sensilla found on beetles (MUSTAPARTA 1973;
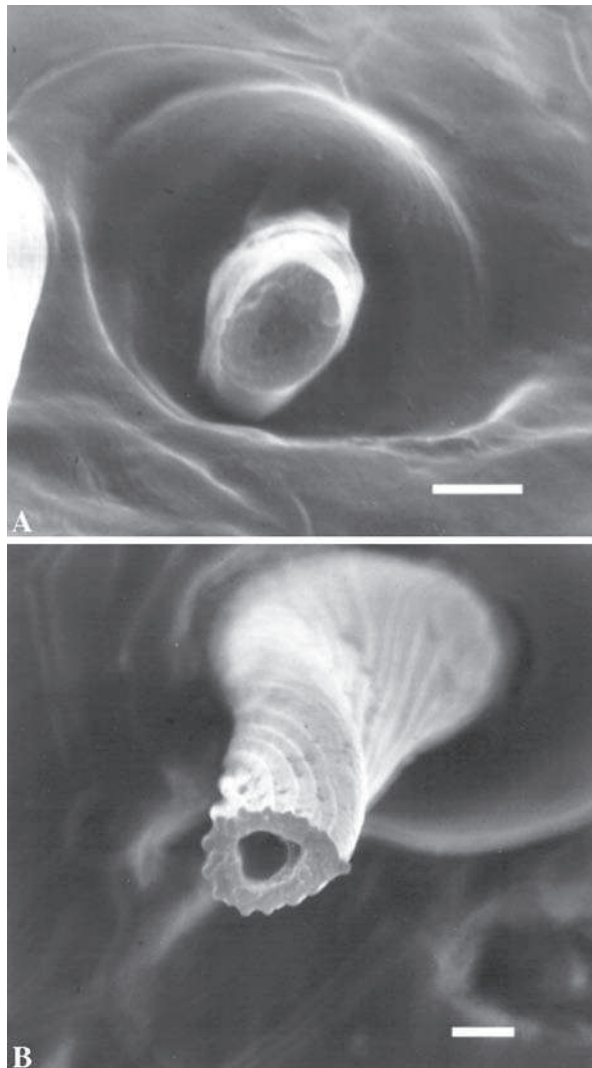
Fig. 5. A: Broken *sensillum chaeticum* without visible internal lumen, on tarsus I of *Daguerreia inermis*; **B:** Broken *sensillum chaeticum* with internal lumen, on calcaneus I of *Promitobates ornatus*. Scale bar = 2 μm.
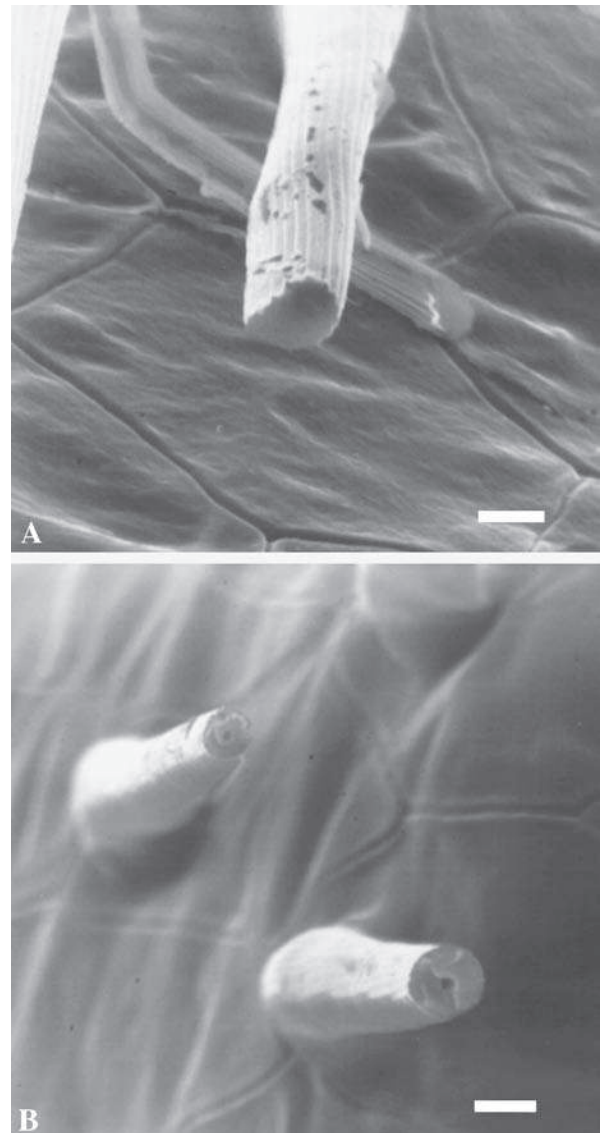
Fig. 6. A: Broken *sensilla trichodea* without visible internal lumen, on tarsus I of *Daguerreia inermis*; **B:** Broken *sensilla trichodea* with internal lumen, on tarsus I of *Pachylospeleus strinatii*. Scale bar = 2 μm.

FAUCHEUX 1989). Finally, they may serve as a brush when the harvestmen groom themselves. These animals are known to repeatedly intercalate between passing their legs I and II on their body (cephalothorax, abdomen and legs) and between their chelicerae, which might be related to the cleaning of sensorial structures (EDGAR 1971; see also HILLYARD & SANKEY 1989; SANTOS & GNASPINI 2002).

4.4. Distribution of *sensilla chaetica* and *trichodea*

The three main questions of this paper are discussed and we propose, as working hypotheses, possible relationships between the distribution of hair sensilla and harvestmen behavior.
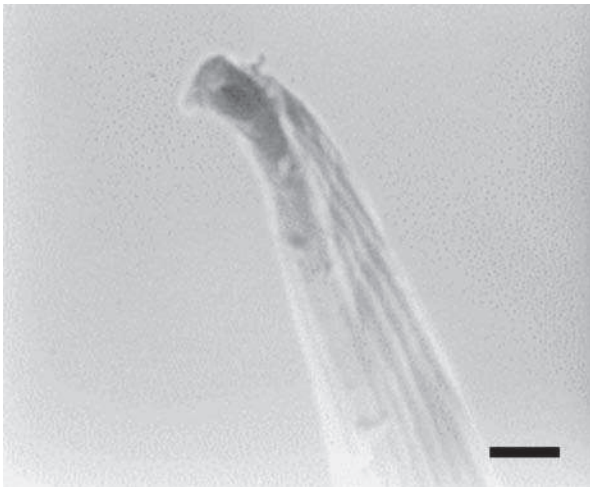
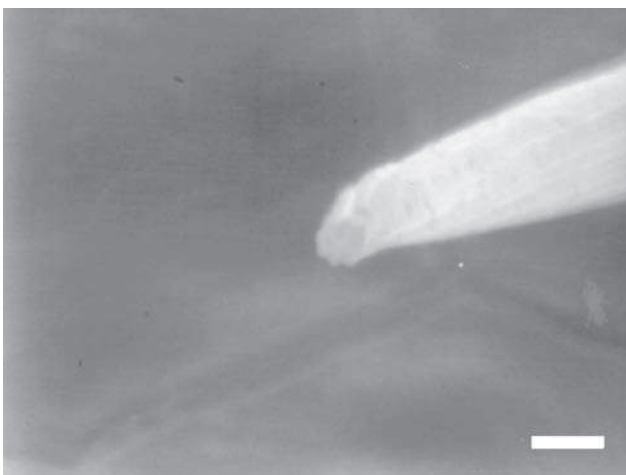**Fig. 7.** Tip of *sensillum chaeticum*. Scale bar = 500 nm.

Fig. 8. Tip of *sensillum trichodeum*. Scale bar = 1 µm.

4.4.1. Calcaneus

The distribution of sensilla on the calcaneus did not follow the pattern found in the tarsus. As there seem to be no functional difference between the regions (e.g. tapping the substrate or supporting the body by the ventral region, as known to occur with the tarsus), one might expect a similar distribution of sensilla throughout the calcaneus. However, some significant differences did occur. Further studies are needed to clarify this point.

4.4.2. Tarsus

The first question was whether the dorsal, lateral and ventral part of each leg differed in the density of hair sensilla. The results indicated that the ventral region of the tarsi were denser in *sensilla trichodea* than the lateral and dorsal regions, mainly on legs I and II. Harvestmen seem to use these legs mostly for probing the environment (see GUFFEY 1999; WILLEMART 2002). Since it is usually the ventral part that touches the substrate/food, large numbers of hair sensilla on this region may be associated with mechanical perception (size, form and texture), what would explain the greater density of hair sensilla on this region. The more sensilla are present, the more accurate is the mechanical perception of resources (see BROWNELL 2001), such as the physical characteristics of the environment, food, habitat and oviposition sites. The fact that not only legs II but also legs I have more *sensilla trichodea* on the ventral region than on the dorsal and lateral regions is interesting because legs II have historically received much attention when sensorial mechanisms are discussed (CANALS 1936; CLOUDSLEY-THOMPSON 1958; KAESTNER 1968; EDGAR 1971; GOODNIGHT & GOODNIGHT 1976; HILLYARD & SANKEY 1989; ACOSTA et al. 1995; MACHADO et al. 2000), and they were sometimes referred to as "sensorial legs" (HOENEN & GNASPINI 1999; ELPINO-CAMPOS et al. 2001). Although they are indeed important as sensorial organs, observations by ANURADHA & PARTHASARATHY (1976), ELPINO-CAMPOS et al. (2001), WILLEMART (2002), and WILLEMART & GNASPINI (in press) suggested that legs I are also important sensorial organs, even more important for identifying food than the second pair (GUFFEY 1999).

The second question was whether there were differences in the density of hair sensilla between the ventral region of the leg pairs of the same individual. It is noteworthy that the ventral region of legs II had less *sensilla trichodea* than the ventral region of legs I in four of the six studied species. As noted above, besides aiding in cleaning behavior and protecting the integument/other sensilla, *sensilla trichodea* may have a tactile function. Legs II seem to be used to determine the physical dimensions of the environment (GUFFEY

1999), like "general features", whereas legs I seem to be responsible for recognition of resources (such as food – Guffey 1999) – "detailed features". Therefore, it would be important to concentrate *sensilla trichodea* on the ventral region of legs I for increased tactile perception. Concerning the distribution of *sensilla chaetica*, the few distinct distributions found in *D. inermis* and *H. citrinus* remain to be explained.

Finally, the third question was whether the density of hair sensilla was different among the species. *H. citrinus* was the species with higher density of *sensilla trichodea*, on all legs. Roters (1944, apud Macías-Ordóñez 1997) and Phillipson (1960) reported that, in some Eupnoi, prey perception is only possible after physical contact. Macías-Ordóñez (1997) observed that the Eupnoi *Leiobunum vittatum* Say 1821 only detected conspecifics after touching them. The fact that *H. citrinus* has a high density of *sensilla trichodea* and that the Eupnoi studied by the authors above are not able to detect prey at a distance would be a behavioral indication that these hair sensilla are probably not responsible for long range perception (be it chemical or mechanical). However, why then would *H. citrinus* have such a high density of hair sensilla? In addition to the high density of *sensilla trichodea*, this species also has very long legs, and, unlike the laniatorean studied herein, hair sensilla are abundant all over the metatarsus, not only on its distal part (calcaneus). *Holcobunus citrinus* has the second highest density of sensilla among the studied species, just behind *N. maximus,* which has very thick legs. To tentatively explain these results, some behavioral data of palpatorean harvestmen have to be mentioned. Two typical defensive

behaviors of *H. citrinus* are fleeing and autospasy of the legs, which were also noted in some other species of Eupnoi (Berland 1949; Kaestner 1968; Edgar 1971; Roth & Roth 1984; Hillyard & Sankey 1989). The body at the center of the legs also keeps it relatively far from invertebrate predators. The fact that a predator reaches the body of a palpatorean could mean its death, since they do not have effective physical defense as laniatoreans (see Machado & Raimundo 2001). The great density of hair sensilla and the extended sensorial region of the metatarsus might be associated with the need of rapid detection of a predator, through contact, allowing fleeing or autospasy if a leg happens to be caught.

Concerning the Laniatores studied, the epigean species (*N. maximus* and *P. ornatus*) and the trogloxenic *G. albiscriptum* have a higher density of *sensilla chaetica* than the troglophilic *D. inermis* and the troglobitic *P. strinatii*. The latter two, as mentioned in the introduction, might be expected to have a greater density of hair sensilla than the epigean species (see Christiansen 1992; Gnaspini & Hoenen 1999; Hüppop 2000). Since the opposite occurred, this subject deserves further studies. Obviously, historical features cannot be forgotten, and phylogenetically closely related species should be investigated. Nevertheless, some considerations may be made. First, *D. inermis* and *P. strinatii* were considered by Trajano & Gnaspini (1991) to be omnivorous. Second, Pinto-da-Rocha (1996a, b) noticed that both species seemed concentrate near the cave streams, where there is a higher availability of food (detritus carried by the stream). Third, Hoenen & Gnaspini (1999) noticed
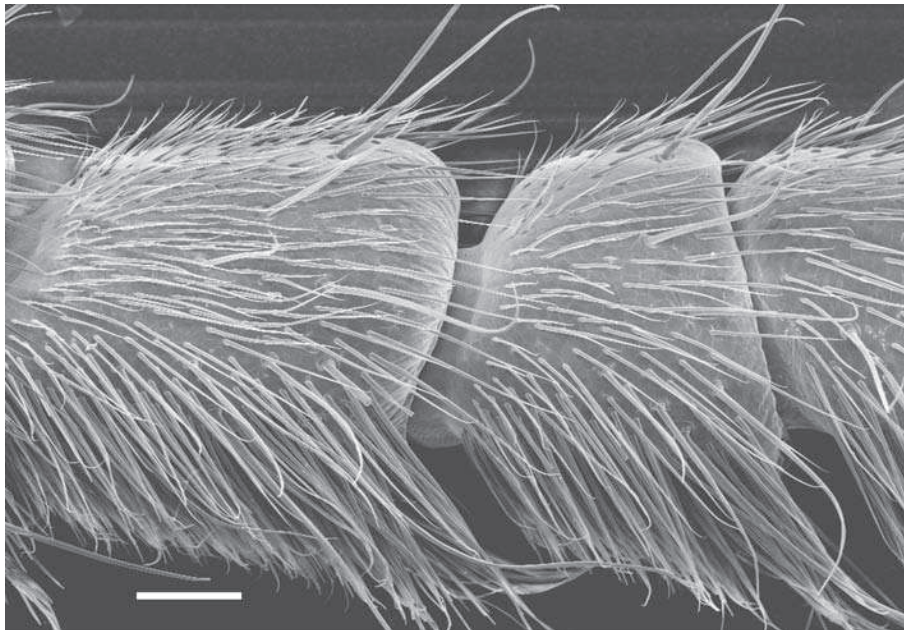
Fig. 9. Lateral view of tarsus IV of *Neosadocus maximus*, showing the high density of hair sensilla on the ventral region. Scale bar = 100 μm.

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that *P. strinatii* showed a large amount of activity, which they suggested may be related to the scarcity of food and/or mates typical of caves. Even considering that the availability of food is higher near streams, it is not available in a concentrated way. In other words, *P. strinatii*, as an omnivorous species, shows preference for places where its food is more available, and shows a large amount of activity (see POULSON 1963). In a low food environment (as in caves), the animals have several evolutionary routes to improve finding food: becoming more generalist, shifting the diet, enhancing sensorial detection and/or enhancing the chance of finding food by walking more (see HÜPPOP 2000). As harvestmen are generally omnivorous (revised by WILLEMART 2002), it is therefore not a feature acquired by *P. strinatii*. Thence, *P. strinatii* may followed the latter route. This may also explain our findings on *D. inermis*.

Another point to explore is the fact that the sensorial apparatus is also related to defensive behaviors. It is possible that the predation pressure on *P. strinatii* is lower inside the caves, where predators are in lower numbers than in the epigean environment. This seems to have occurred with other cave animals (e.g., cave fishes – TRAJANO 1989) and might explain why the sensorial apparatus of *P. strinatii* was not larger when compared to epigean species: because there was no need (= no selective pressure). Finally, the "shift" from *sensilla trichodea* to *sensilla chaetica* in some regions of legs I and II in *D. inermis* and *P. strinatii* may be related to a need of increased chemical perception. We should finally stress that our study is pioneer for harvestmen, and that we are already studying other behavioral aspects that will hopefully solve questions about the use of legs by harvestmen.

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