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Neotropical harvestmen (Arachnida, Opiliones) use sexually dimorphic glands to spread chemicals in the environment



Deux opilions (Arachnida, Opiliones) néotropicaux utilisent des glandes sexuellement dimorphiques pour répandre des sécrétions dans l'environnement

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ABSTRACT

Sexually dimorphic glands have convergently appeared in animals and are often responsible for the production of pheromones. In the suborder Laniatores of the order Opiliones (Arachnida), glands of such type are widespread, but there is not a single paper on how they are used. Using Scanning Electron Microscopy and a behavioral approach, we describe glandular openings and how these glands are used, in the harvestmen *Gryne perlata* and *Gryne coccinelloides* (Cosmetidae). Males of these two species have glandular openings on the metatarsi of legs I and on the metatarsi IV. Males were shown rubbing the glands of the metatarsi I against their other legs, whereas glands on the metatarsi IV are gently touched on the substrate or rubbed either against other legs, or against the substrate. Not all behaviors were seen in both species.

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R É S U M É

Les glandes sexuellement dimorphiques ont évolué de manière convergente chez les animaux et sont souvent à l'origine de la production de phéromones. Dans le sous-ordre des Laniatores (Arachnida, Opiliones), ce type de glandes est très répandu, mais il n'existe aucune observation concernant la manière dont elles sont utilisées. À l'aide de la microscopie électronique à balayage et d'une approche comportementale, nous avons pu décrire l'existence d'ouvertures glandulaires et le mode d'utilisation de ces glandes chez deux opilions Cosmetidae : *Gryne perlata* et *Gryne coccinelloides*. Les mâles de ces deux espèces présentent des ouvertures glandulaires sur les métatarses des pattes I et IV. Différents types de comportements ont pu être observés selon l'espèce étudiée. Les glandes du métatarse I sont uniquement frottées contre les autres pattes, alors que celles situées sur le métatarse IV sont utilisées de différentes façons : elles peuvent être, soit frottées contre les autres pattes et contre le sol, soit mises uniquement en contact avec le sol.

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1. Introduction

Pheromones are chemicals produced by exocrine glands that mediate the communication between individuals of the same species [1]. According to their function, they are classified in alarm, aggregation, trail, sexual or territorial pheromones [2], the latter two being often produced by sexually dimorphic glands found both in vertebrates [3–5] and invertebrates [6,7]. Like other secondary sexual characters, sexually dimorphic glands are under sexual selection through mate choice, direct competition for sexual partners or resources used by the opposite sex [8,9].

In the class Arachnida, there are few studies on chemical communication, despite being the third group among arthropods in number of species [10]. Among spiders, most studies investigate the behavioral responses of males to pheromones released by females [11–15]. There are few studies on the elucidation of the chemicals of these pheromones [16,17] or the morphology of the glands [18]. Concerning other arachnids, sexually dimorphic glands are known in the metasoma (“tail”) of some scorpions (used in mating) [19], in the glandular sacs close to the legs IV in some pseudoscorpions (used in chemical marking) [20] and pedipalps of some amblypygids (mode of use unknown) [21].

Sexually dimorphic glands are known in the four suborders of Opiliones. In Cyphophthalmi, males of all species have glands that open in the adenostylus of legs IV, in addition to anal and sternal glands, all of unknown mode of use [22]. In Dyspnoi, males of some Ischyropsalididae and Nemastomatidae have glands in their chelicerae used in mating [23–25]. In Eupnoi, males of several species of Phalangioidea have glands on their penis also used in mating [26–28]. In Laniatores, sexually dimorphic glands have been described in only 18 species: glandular openings are present on the metatarsi IV of males of *Iporangaia pustulosa* [29] and on other 16 species [30], whereas the internal morphology of glands in the basitarsi of legs I has been described in males of *Rhopalocraneus albilineatus* (Manaobiidae) [31]. The mode of use of these glands of laniatorids is unknown. Males of *Gryne perlata* (Melo-Leitão, 1936) and *Gryne coccinelloides* (Melo-Leitão, 1935), belonging to the family Cosmetidae in the suborder Laniatores, have the metatarsi of legs I and IV swollen, suggesting the presence of sexually dimorphic glands [30]. These regions are similar to glandular areas of several other species in distinct clades within the suborder [30]. Though possibly not homologous, these glands may be analogous, meaning that findings on these two species may potentially be generalized. There is not a single paper describing either the morphology of the sexually dimorphic glands in these two species or how a sexually dimorphic gland is used in any species, in a suborder with over 4100 species. Such lack of behavioral data hampers studies on chemical communication in the group, since these often depend on this basic information of how the glands are used. We therefore studied the external morphology and conducted a behavioral study on the use of two sexually dimorphic glands in the group: the metatarsal gland in legs I and in legs IV.

2. Materials and methods

2.1. Collection and maintenance in the laboratory

Adult males and females of both species were collected during the day, manually. *G. coccinelloides* was collected in Campinas, State of São Paulo, in November 2011, under tiles in a synantropic area across the street from a humid forest. *Gryne perlata* was collected in a pasture in Irajuba (savannah area), southwest of the State of Bahia, in December 2011. The individuals were aggregated in termite nests and under logs. At night they were seen leaving the termite nests. Nothing is known about the natural history of either of the species.

In the laboratory, each specimen was maintained individually in plastic recipients (20 cm diameter × 8 cm height) with dry soil on the bottom. Water was provided with a moistened cotton ball in a cap. They were fed on humid dog food once a week. Temperature was ambient and photoperiod was 12/12 h.

2.2. Scanning Electron Microscopy

In populations of both species, there seem to be distinct male morphs (which may be a common feature in harvestmen: [32,33] that can be distinguished by the length of legs IV (Fig. 1 shows a large male). A large male and a female of each species were fixed in alcohol 70%, their metatarsi I and IV were dissected and cleaned in three steps: agitation in water for 1 min, agitation in a 10:1 water and detergent solution for 3 min and finally agitation in water for 1 min. The legs were then mounted on aluminum stubs with double stick adhesive tape and kept in a stove at 64 °C for 24 h. The stubs were sputter-coated with gold (Sputter Coater Balzer SCD 50) and photographed in a scanning electron microscope (SEM–Zeiss DSM 940).

2.3. Behavior

To understand how the glands of the metatarsi I and IV are used by *G. perlata* and *G. coccinelloides*, males and females were digitally recorded in the laboratory. A

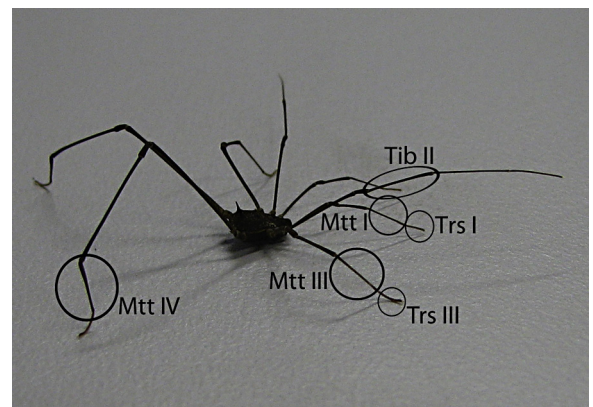


Fig. 1. *Gryne perlata* male. Body regions involved in rubbing of metatarsus I (legs I and II) and metatarsus IV (legs III and IV). Abbreviations: Mtt–metatarsus; Trs–tarsus; Tib–tibia.

minimum of five large males and five conspecific females were introduced in an arena ($40 \times 18.5 \times 22$ cm height), each species being tested separately. A total of 10 *G. perlata* males (all large individuals), seven females, eight *G. coccinelloides* males (all large individuals) and five females were used for the behavioral observations. Small males remain to be tested. The individually marked specimens were randomly recorded in every context, such as walking, climbing, staying still, feeding and touching conspecifics. Recording of each individual lasted a maximum of 10 min. Males of *G. perlata* have been recorded for 42 h and females for 14 h. Males of *G. coccinelloides* have been recorded for 19h30 min and females for 6 h 30 min. The arenas had dry soil on the bottom, sticks and live plants. Water was provided by means of a moistened cotton in a cap. Because preliminary observations showed that these species are nocturnal, recordings were carried out between 20 h and 3 h AM, in the dark, with a Sony HDXR550 V at Night Shot mode.

3. Results

The body regions involved in the observed behaviors are shown in Fig. 1. The metatarsus of both species is, as in most laniatorids, clearly divided between the astragalus and the calcaneus (Fig. 2). We first show the results for the metatarsus I and then for the metatarsus IV.

3.1. Metatarsus I

3.1.1. External morphology of the glandular area

The calcaneus I of males is slightly thicker than that of females. In both species, males have glandular orifices found only in the retrolateral region (Fig. 3A and B). The small orifices are grouped in several larger orifices, the morphology being similar in both species. In females, the correspondent region does not have such orifices (Fig. 3C).

3.1.2. Behavior

We have observed three ways of using the metatarsus I, all involving bringing a leg forward and rubbing it against the glandular opening on the retrolateral region of legs I. Rubbing lasted from 0.7 to 7.9 seconds and occurred in

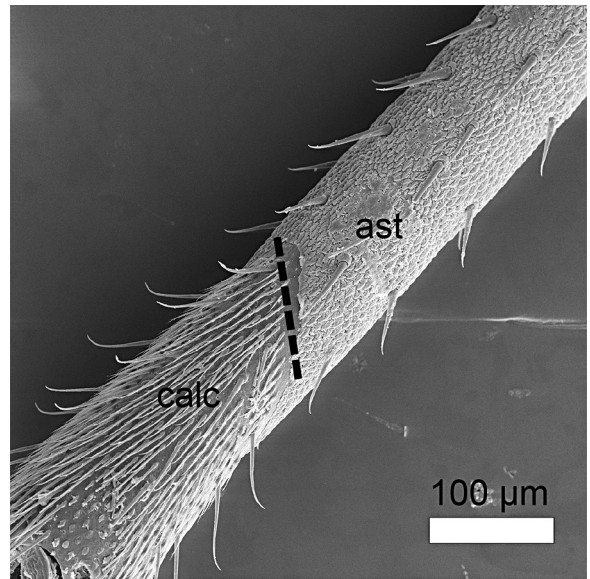


Fig. 2. Metatarsus IV of *Gryne perlata* male. Astragalus (ast) and calcaneus (calc) delimited by a dashed line.

both species (Table 1), except for “mtt I \times tibia I” in *G. coccinelloides* (Fig. 4).

3.2. Metatarsus IV

3.2.1. External morphology of the glandular area

In *G. perlata* and *G. coccinelloides* adult males, the metatarsus IV is swollen at the region of the astragalus, unlike in females (Fig. 5A–B). In both sexes there are small glandular orifices throughout the metatarsus, but in males they are more abundant in all regions, dorsal, lateral and ventral. The texture of the integument is also different between the sexes (Fig. 6A–H).

3.2.2. Behavior

We have observed four ways of using the metatarsus IV: rubbing it against either the substrate or another body part (“rub tIII \times mttIV” and “rub mttIV \times tibia III” – Table 2) or gently and repeatedly touching it against the substrate.

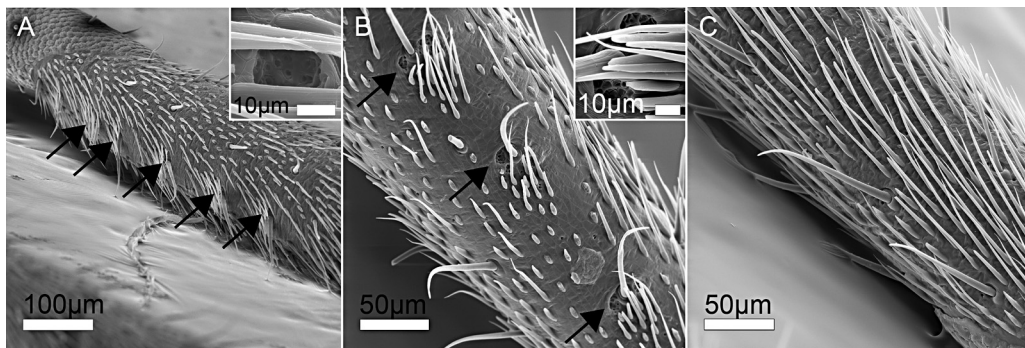


Fig. 3. Calcaneus of the metatarsus I, retrolateral region. A. Gland orifices grouped in larger orifices in males of *Gryne perlata* and (B) *Gryne coccinelloides*. C. Absence of orifices in a *Gryne perlata* female.

Table 1

Behavioral categories and definitions of how the glandular area of the metatarsus I is used in the harvestmen *Gryne perlata* and *Gryne coccinelloides* (Cosmetidae). In the three categories, the retrolateral region of leg I and legs on the same side of the body are involved in the described behaviors.

Behavioral categories	Definitions	Species	
		<i>G. perlata</i>	<i>G. coccinelloides</i>
Rub tarsus III against tarsus I and metatarsus I (trsIII × mtt I)	The tarsus III is directed forward and its ventral region rubbed only once against the metatarsus and tarsus I	<i>n</i> = 1; 1.7 s	<i>n</i> = 4 (one male, median = 1.4 s; min = 0.9 s; max = 1.9 s)
Rub tarsus III against metatarsus I (trsIII × mtt I)	The tarsus III is directed forward and its ventral region rubbed from one to nine times against the metatarsus I	<i>n</i> = 2; 1.3 s; 3.8 s	<i>n</i> = 9 (one male, median = 3.1 s; min = 0.7 s; max = 5.8 s)
Rub metatarsus I against tibia II (mtt I × tibII)	The metatarsus I is rubbed only once against the tibia II (lateral)	Not observed	<i>n</i> = 1; 7.9 s

Dorsal, lateral and ventral parts were used for gently touching or rubbing, in behaviors that lasted from 0.5 to 6.5 seconds and occurred in both species except for “rub mttIV × tibia III” and “rub tIII × mttIV” (Table 2).

After rubbing two legs together, the harvestmen only wandered (median time of observation after rubbing: 110 s; max: 240 s; min: 60 s) except in one case: a *G. perlata* male touched a conspecific female with a leg II, rubbed a metatarsus IV against a twig, moved quickly forward towards the female (as when attacking prey), rubbed tarsus III against

the metatarsus IV, then rubbed this tarsus III against the substrate and finally repeated these last two behaviors.

The category “rub mttIV × subst” was observed in both species. Males of *G. perlata* rubbed the dorsal (*n* = 2), ventral (*n* = 2), prolateral (*n* = 4) and retrolateral (*n* = 2) regions of the metatarsus IV on dry twigs (*n* = 8), leaves of live plants (*n* = 1) and soil (*n* = 1). One male was also observed waving the leg in the air (duration = 6 s) before rubbing it against a dry twig. Males of *G. coccinelloides* rubbed the ventral (*n* = 5), prolateral (*n* = 5) and retrolateral (*n* = 4) regions of the metatarsus IV on dry twigs (*n* = 12), leaves of live plants (*n* = 1) and soil (*n* = 1). Two males waived the leg IV in the air before rubbing it against the substrate (duration = 3.4 and 8.2 s).

Concerning the category “touch mttIV × subst”, males of *G. perlata* touched the dorsal (*n* = 5), ventral (*n* = 3), prolateral (*n* = 1) and retrolateral (*n* = 1) regions of the metatarsus IV on dry twigs (*n* = 1), leaves of living plants (*n* = 1) and soil (*n* = 8). *Gryne coccinelloides* touched the dorsum of the metatarsus IV on soil (*n* = 1) (Fig. 7).

4. Discussion

We hypothesized that the use of the sexually dimorphic glands on the metatarsi I and IV is related to spreading pheromones for two main reasons:

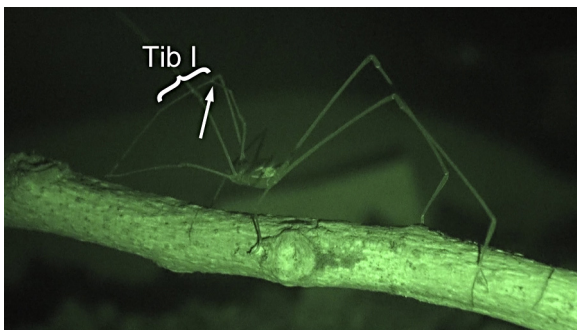


Fig. 4. *Gryne coccinelloides* rubbing the metatarsus I on the tibia of a leg II. Abbreviation: Tib -tibia. Arrow shows the region of contact between the two legs (color online).

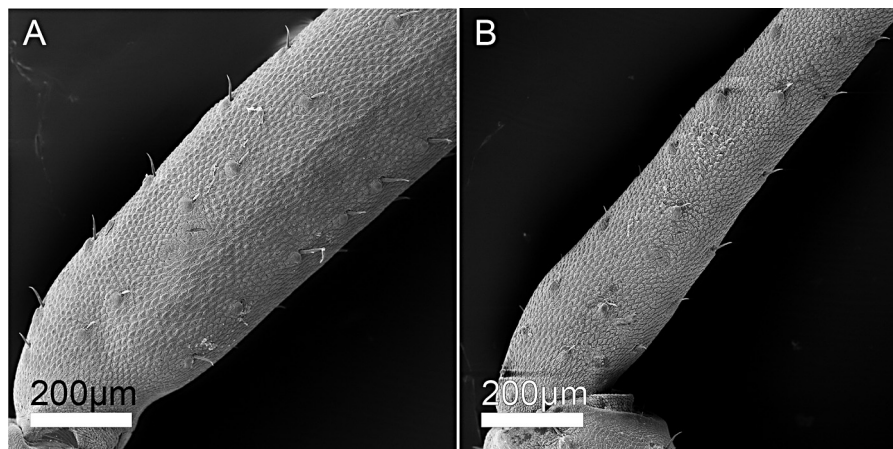


Fig. 5. Metatarsus IV swollen in *Gryne perlata* males (A) and not swollen in females (B).

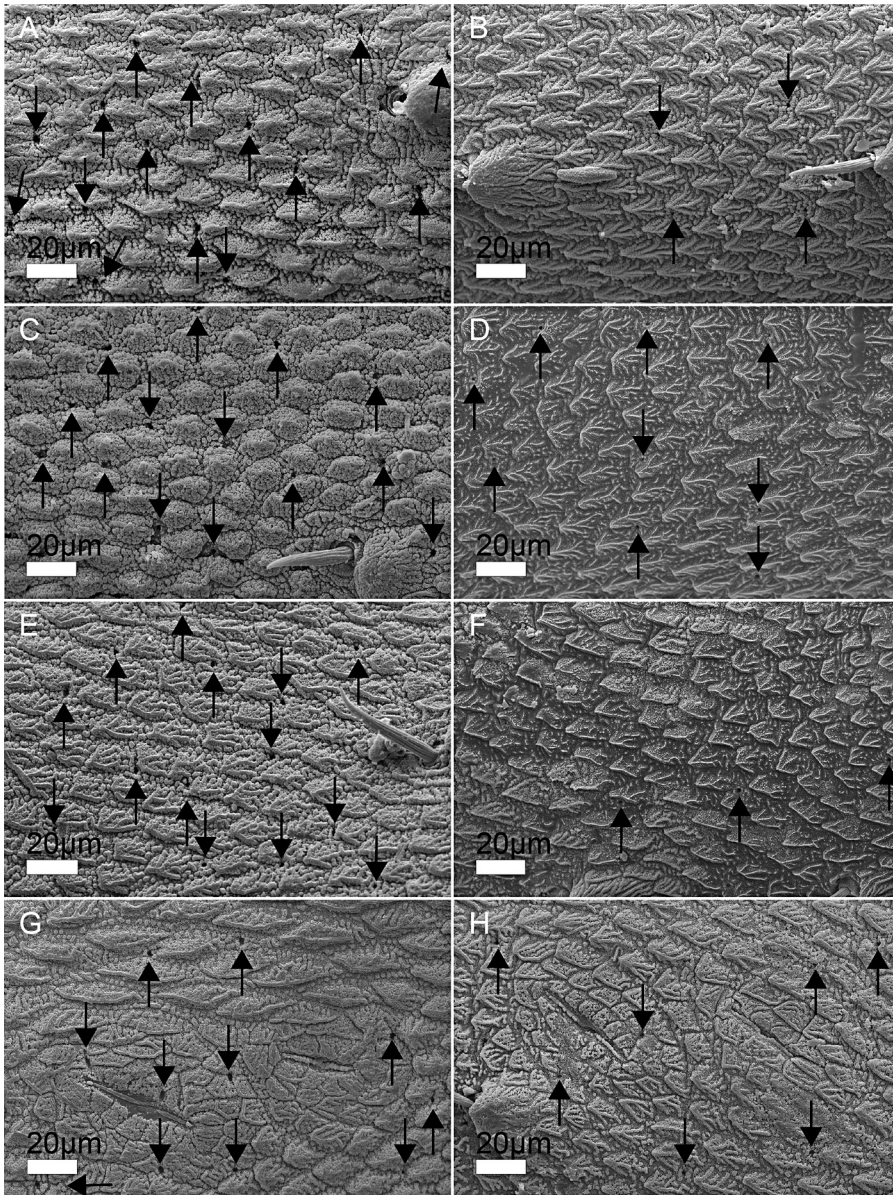


Fig. 6. Glandular orifices on the metatarsus IV of *Gryne perlata*, dorsal view: male (A) and female (B); ventral view: male (C) and female (D); Glandular orifices on the metatarsus IV of *Gryne coccinelloides*, prolateral: male (E) and female (F); retrolateral: male (G) and female (H). Setae indicate orifices. The metatarsi of males have more orifices in all regions.

- evidence from comparative data: rubbing body parts to leave pheromones is a widespread behavior in vertebrates and invertebrates [2];
- the specific body parts involved in the described behaviors: dorsal, lateral and ventral regions of metatarsi IV and only the retrolateral region of metatarsi I. These match the exact locations of the observed glandular openings.

Only males have been observed rubbing the metatarsi I and IV. Transferring the secretions directly to the substrate has also been described in other taxa [34–38] as well as rubbing the glands against other body parts. In this case, animals either subsequently rub this other part on the

substrate [39] or, by rubbing body parts together, simply mixture secretions of different glands [40,41]. We suggest that rubbing the glands against its own body can also be a way of expanding the body area containing chemicals and thus amplifying the signal to conspecifics. Unexpectedly, we have also observed males waving the metatarsus IV, which may be related to dispersing volatiles as observed in roaches [42] and salamanders [43,44]. Because harvestmen are not known for an accurate olfactory sensitivity, this could be important for communicating at short range (laniatorid harvestmen can detect odors at short range [45,46]). In fact, there were conspecifics in the arena when this behavior was observed.

Table 2

Behavioral categories and definitions of how the glandular area of the metatarsus IV is used in the harvestmen *Gryne perlata* and *Gryne coccinelloides* (Cosmetidae).

Behavioral categories	Definitions	Species	
		<i>G. perlata</i>	<i>G. coccinelloides</i>
Rub metatarsus IV against the substrate (rub mttIV × subst)	The metatarsus IV (dorsal, lateral and ventral) is rubbed against twigs, soil and leaves with dorso-ventral and latero-lateral movements	<i>n</i> = 10, 5 males; med = 3.4 s; min = 1.1 s; max = 6.5 s	<i>n</i> = 14, 6 males; med = 1.8 s; min = 0.3 s; max = 5.5 s
Touch metatarsus IV on the substrate (touch mttIV × subst)	The metatarsus IV (dorsal, lateral and ventral) is gently and repeatedly touched on twigs, soil and leaves	<i>n</i> = 10, 4 males; med = 1.2 s; min = 0.6 s; max = 3.2 s	<i>n</i> = 1, 5.9 s
Rub metatarsus IV against tibia III (rub mttIV × tibia III)	Leg III is kept motionless at its regular position Leg IV of the same side of the body is brought forward and its metatarsus is rubbed against the lateral region of tibia III	Not observed	<i>n</i> = 3, 2 males; med = 2.9 s; min = 1.9 s; max = 4.6 s
Rub tarsus III against metatarsus IV (rub tIII × mttIV)	Leg IV is kept motionless on the substrate. Tarsus III of the same side of the leg is moved backwards and its ventral part is rubbed on the ventral region of metatarsus IV	Not observed	<i>n</i> = 2, 1 male; 0.5 s; 1.9 s

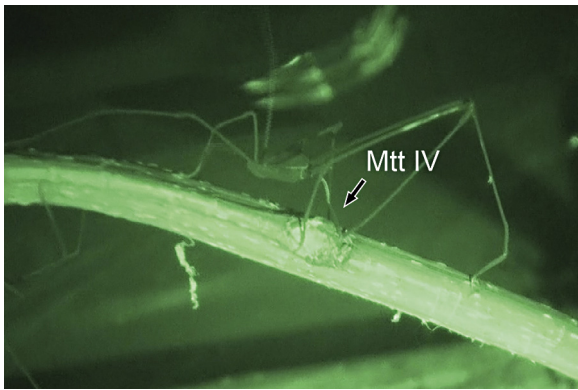


Fig. 7. Male of *Gryne perlata* using their metatarsal glands on leg IV: Touching the dorsum on a twig. Abbreviation: Mtt IV–Metatarsus IV (color online).

The function of rubbing is still unknown. It is probably related to sexual attraction and/or territory demarcation, maybe informing about the quality of the males that left the signal behind. Collecting information from chemicals left on the substrate is widely known in other animals including arachnids such as spiders. By touching silk lines left on the substrate, spiders can access information on gender, reproductive state, health, symmetry and age among others [47]. The same could occur in harvestmen but this remains to be investigated.

Glandular openings are important taxonomic characters in laniatorid harvestmen, helping to understand relationships among superfamilies [48]. Males of *G. perlata* and *G. coccinelloides* have sexually dimorphic glandular openings on the metatarsi of legs I and IV. Similar orifices to those of legs I have been described on legs I of six species in the subfamily Progonyleptoideliinae. However, those are on the tarsi and not the metatarsi [30]. In legs IV, these same six species appear to have the exact same dimorphism and orifices. The subfamily Progonyleptoidellinae is within Gonyleptidae, which is closely related to Cosmetidae [49], but such orifices are absent in most other

gonyleptids. Therefore, these are probably independent acquisitions.

We have described harvestmen rubbing sexually dimorphic glands on the substrate. Several questions can now be raised. What are the functions of rubbing? Why such variation in the time males spend rubbing or touching the metatarsi on the substrate (0.6 to 12 s in one of the cases)? Can females access the quality of males by touching their chemicals? If chemical marking is for territorial purposes, do small males [which may not be territorial - 33] also rub? This is just a sample of the number of questions that remain to be answered, but the first step is now made.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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