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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.

RÉSUMÉ

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 *Rhopalocranaus 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

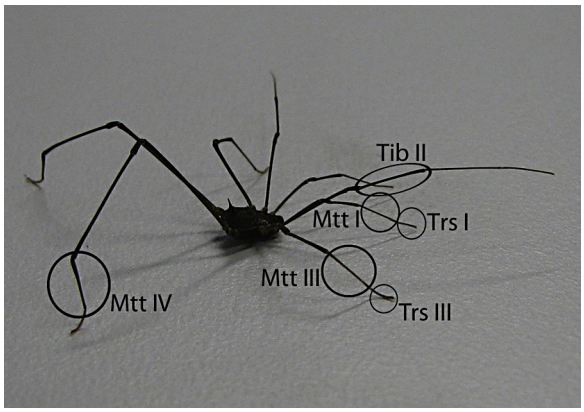

Fig. 1. *Gryne perlata* male. Body regions involved in rubbing of metatarsus I (legs I and II) and metatarsus IV (legs III and IV). Abbreviations: Mtt–metatarsus; Trs–tarsus; Tib–tibia.