

## SHORT COMMUNICATION

### Detection of conspecifics through olfaction in the Neotropical harvestman *Mischonyx cuspidatus* (Arachnida: Opiliones)

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**Abstract.** Chemoreception is a main sensory modality in harvestmen but there has been no experimental evidence of attraction to conspecifics by olfaction excluding contact chemoreception as an alternative explanation. Here, using a Y-maze olfactometer we tested whether the harvestman *Mischonyx cuspidatus* (Roewer, 1913) is attracted to volatiles of conspecifics. We found that males were attracted to volatiles of other males, but not females. Females were not attracted to female's volatiles, but were marginally more often attracted to volatiles of males. Our results help understanding mechanisms in mating and social behavior in the order Opiliones.

**Keywords:** Chemical communication, chemoreception, Laniatores, olfactometer, pheromone

The detection of chemical stimuli in the environment plays an important role in the natural history of many animal taxa (Wyatt 2014). Animals use chemical information to find food (Hölldobler & Wilson 1978), discriminate between colonies (Rottler et al. 2013), recognize parenthood (Waldman & Bishop 2004), find sexual partners (Thomas 2011) for homing, among other contexts (Hölldobler & Wilson 1978). Although the assessment of chemical information is widespread among animals, the ability to detect chemicals is probably more important for species less known to use other sensory modalities.

In harvestmen (order Opiliones), vision has recently been recognized as important at least for homing (Silva et al. 2018), but small air displacement, substrate-borne and air-borne vibrations, used in many arachnids, do not seem to be very important in this group (Willemart et al. 2009a). Contact chemoreception (chemical assessment through touching the stimulus, i.e., gustatory sense) is considered a main sensory modality in harvestmen (Willemart et al. 2009a) and empirical studies have shown that short-range olfaction (< 2 cm) may play a role in finding food, in detecting alarm chemicals and in associative learning (Machado et al. 2002; Willemart & Chelini 2007; Costa & Willemart 2013; Santos et al. 2013). Numerous types of olfactory sensilla on the first and second pairs of legs have now been described for species in the suborder Laniatores (Gainett et al. 2017), raising the possibility that olfaction is used in several contexts. It is unknown, for example, how male and females find each other. In species with territorial males (Munguía-Steyer et al. 2012), we do not know if guarding males can detect conspecifics moving into their territory at a distance. Harvestmen may form aggregations during the day (e.g., Machado & Macías-Ordóñez 2007a; Chelini et al. 2012; Grether et al. 2014), with both sexes being present, but it is unknown if conspecifics can detect aggregations at a distance. Therefore, attraction to conspecifics at a distance by olfaction may have important implications for understanding gregarious behavior and mating systems. Previous studies on attraction/recognition of conspecific chemicals in harvestmen have not provided evidence of olfaction while controlling for contact chemoreception (Donaldson & Grether 2007; Willemart & Hebets 2012; Fernandes et al. 2017; Harvey et al. 2017). Here we tested whether harvestmen are attracted to volatiles of conspecifics at long range. We expected that harvestman could show preference to (i) odor of conspecifics of the

opposite sex (an evidence of olfaction in the context of mating), (ii) odor of conspecific of either sex or same sex (an evidence of olfaction in the context of social behavior, possibly linked to aggregation).

We used the harvestman *Mischonyx cuspidatus* (Roewer, 1913) (Gonyleptidae). This species is included in Laniatores, a suborder with more than 4000 species in the order Opiliones (Giribet & Sharma 2015; Kury pers. webpage). It is synanthropic, active mostly at night (Pereira et al. 2004) and can be found aggregated with conspecifics under tree trunks during the day (Mestre & Pinto-da-Rocha 2004). Laniatorids typically feed on live animals that they capture, dead animals and plant matter (Acosta & Machado 2007; Costa et al. 2016). When active, they usually alternate between wandering around and remaining stationary on litter, trunks or leaves (Acosta & Machado 2007; Willemart et al. 2007). Males in Gonyleptidae may fight each other (Willemart et al. 2009b; García-Hernández & Machado 2018) and mating occurs by means of a penis (Machado & Macías-Ordóñez 2007b). Because it is difficult to rear laniatorid harvestmen to adulthood (Willemart 2007), we collected adult males and females of *M. cuspidatus* in November of 2016, at the “Parque Ecológico do Tietê” (23° 29' 46" S, 46° 31' 10" W), São Paulo city, São Paulo State, Brazil. We maintained the harvestmen in individual plastic boxes (12 cm x 8 cm x height 4 cm) with moistened soil and a piece of damp cotton. We fed the animals once a week with bananas, crickets or cooked pasta.

To test if *M. cuspidatus* is attracted by conspecific volatiles, we used a Y-maze glass cylindrical olfactometer (25 cm each arm, 6 cm diameter; Fig. 1). The tips of each arm of the olfactometer were closed with a glass lid (Fig. 1, L1, L2, and L3) that allowed the air to flow in and out of the system through silicon hoses. The stimuli were placed in glass chambers (9 cm diameter x 17 cm height; Fig. 1, SC1, SC2) connected to the system through the silicon hoses. A second set of silicone hoses connected the glass chambers to the air flowmeters (Fig. 1, FL1, FL2; Ki key Instrumentos). The air of the room was introduced into the system by an air pump (Prismatec). Before entering the system, the air passed through an activated carbon filter (Trigon Technologies). The measured airflow that entered the system (Fig. 1, near lids “L1” and “L2”) was 1 L/m. To prevent the influence of visual cues, we covered the glass chambers holding the stimuli with a sheet of black ethyl-vinyl acetate (EVA) and the whole system with a set of black pieces of Styrofoam that allowed us to see from the base

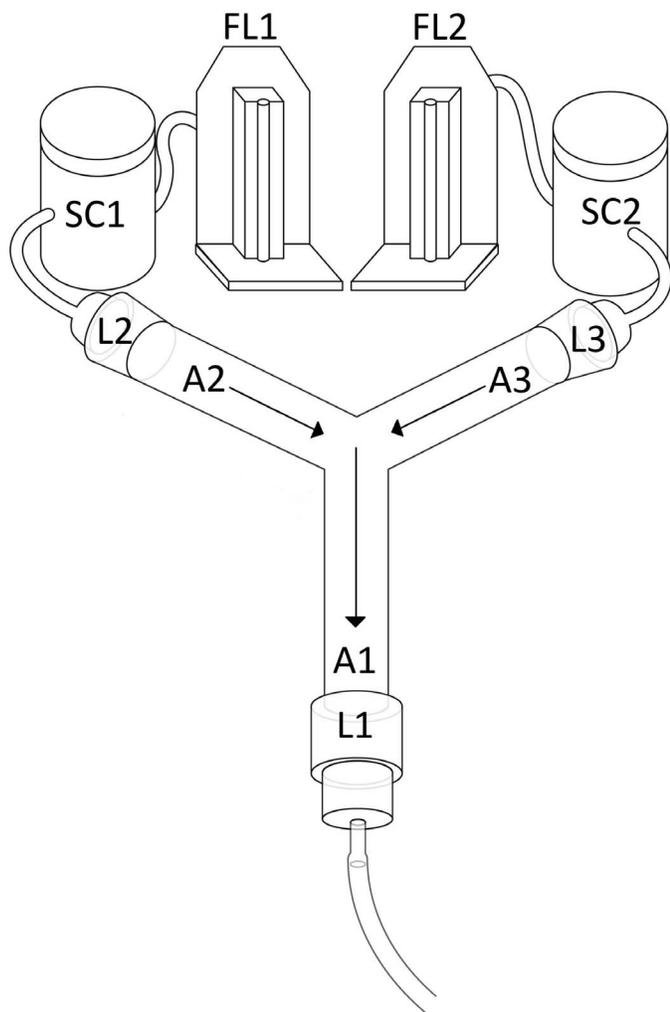


Figure 1.—Schematic representation of the olfactometer. The parts of the apparatus are illustrated with initials. Arms: A1, A2, A3. Lids: L1, L2, L3. Stimuli chambers: SC1 and SC2. Flowmeters: FL1 and FL2. The arrows indicate the direction of the airflow.

of the olfactometer. To minimize any effect of the vibrations produced by the air pump, we placed the olfactometer on a piece of EVA of 0.5 cm on a granite table, which attenuates vibrations (see Elias et al. 2004; Hebets 2004), and the air pump on the floor on a granite piece (2 cm thick) on a Styrofoam plate (4.5 cm thick).

Before each trial, we introduced a 5x3 cm piece of filter paper previously submerged for 5 seconds in water in each of the two glass chambers where the stimulus would be placed. This procedure was to provide humidity in the experimental environment. We introduced the experimental subject in the basal arm (Fig. 1, A1), and acclimatized for 3 minutes (with activated airflow). A black nylon mesh prevented the animal from entering the maze. After the acclimatization time, we carefully removed the nylon mesh and started the trial. We considered that the harvestman had chosen an arm when it entered one of the choice arms (Fig. 1, A2 or A3) with its entire body. The maximum time for each test was 20 minutes. We ran the trials between 8:00 pm and midnight, between November and December of 2016. After each test, we cleaned the olfactometer, glass lids, glass chambers and silicon hoses with 70% ethanol, allowing 15 min for drying with the help of a fan before starting the following trial. The mean temperature during the trials was 20°C (min: 18°C; max: 24.4°C; SD = 1.9) and the relative humidity was 84% (min: 80%;

max: 90.6%; SD = 3.7). We compared the frequency of choices for each arm of the olfactometer with a Chi<sup>2</sup> test.

We tested if males ( $n = 20$ ) and females ( $n = 20$ ) are attracted to odors of conspecifics of the same and opposite sexes. We used three different combinations of stimuli: (i) female vs. blank; (ii) male vs. blank and (iii) female vs. male. To avoid biases, we pseudorandomized the sides of the stimuli in the chambers. We used all animals, both experimental subjects and those used as stimuli only once and alternated the sexes of the experimental subjects and stimuli while running the trials.

Males of *M. cuspidatus* more frequently chose the arm with males when paired with the blank treatment. Nevertheless, we did not find a preference in any other combination (see Fig. 2). With regard to females, when given a choice between the pair of stimuli of “males” vs “blank”, females more often chose the arm with males (marginally significant; Fig. 2).

Previous studies also suggest differential attraction to chemicals of conspecifics in harvestmen depending on their sex. For example, some aggregations of one species of *Prionostemma* sp. (suborder Eupnoi) were male biased, while others were female biased (Grether et al. 2014). Since the authors did not find sexual differences in habitat preference, it was suggested that this pattern might be a result of sexual differences in chemical marking, with individuals preferring chemicals of conspecifics of the same sex (Grether et al. 2014). In translocation experiments with *Prionostemma* sp., individuals were collected in source populations and released in different spots in same sex groups of conspecifics (of males- or females-only). An analysis of the new recruits showed that females were mostly attracted to colonies founded by females. Males were attracted to colonies founded by females and males. Thus, colonies founded by females tended to attract more recruits in general (Harvey et al. 2017). The authors proposed that these patterns could be explained by: (i) differences in chemical compounds between males and females; and (ii) the larger sex (females in *Prionostemma* sp.) could deposit a larger amount of chemicals (Harvey et al. 2017). In our study, we found that males were attracted to conspecific volatiles of other males when paired with the blank treatment, but not with volatiles of females. We also found that females marginally more often chose volatiles of males, but not females, when the stimuli were paired with the blank treatment. Therefore, the hypothesis ii of Harvey et al. (2017) better explains our results. Males, the larger sex in *M. cuspidatus* (see Segovia et al. 2019), may release more volatiles, being easier to detect, but when paired with females, the chemical information of both sexes could overlap. Future studies could investigate if the amount of chemicals is an important factor in male attraction by trying to isolate the chemicals and testing for different quantities. The fact that the response to male volatiles in males was stronger than that of females may be the result of higher olfactory sensitivity. Although sexual dimorphism in olfactory sensilla has not been reported in laniatorids (Gainett et al. 2017, 2018), no studies have been specifically designed for that purpose. Another possibility is that only males produce aggregation pheromones. Our results now call for studies looking at sexual dimorphism in olfactory sensilla as well as the presence of the chemicals possibly involved in aggregation, a behavior present in many species of the order Opiliones (see Coddington et al. 1990; Machado et al. 2002; Chelini et al. 2012).

An additional hypothesis that may explain why female volatiles did not attract males is that females may release sexual pheromones only during specific windows at the reproductive season (Wyatt 2014). Unfortunately, we have no information on the age of the females, their mating history, or precise information on the natural history and seasonality of *M. cuspidatus*. Laniatorids typically reproduce throughout the year and peaks are common between November and March, the warm and wet season (Machado & Macías-Ordóñez 2007b). Eggs of this species have been found in January and

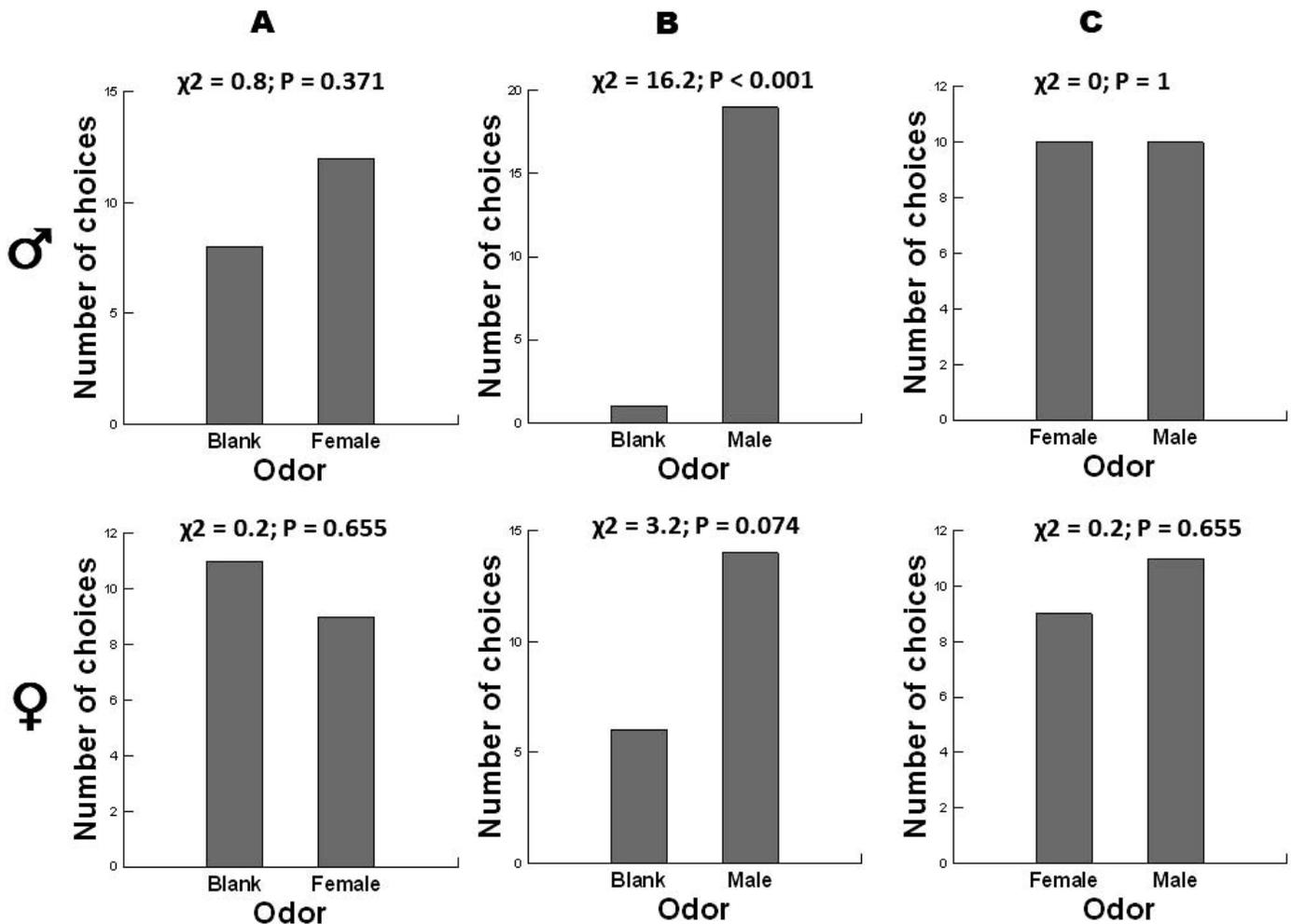


Figure 2.—Choices of the harvestman *Mischnonyx cuspidatus* of both sexes ( $n = 20$  for each experiment) tested in a Y-maze olfactometer with two stimuli paired. Upper row, focal males; lower row, focal females. (A) blank (control) vs. odor of females; (B) blank (control) vs. odor of males; and (C) odor of females vs. odor of males.

February, the warm and humid season (Mestre & Pinto-da-Rocha 2004). However, in captivity females of *M. cuspidatus* lay eggs even during cold and dry months (G.F. Pagoti, pers. obs.). But since we conducted our experiments between November and December, we were most probably within the reproductive period of the species, but it is possible that not all females were releasing sexual pheromones, during our experiments.

Gainett et al. (2017) reported olfactory receptors widespread in all 17 families of Laniatores studied. These authors also studied one species, *Heteromitobates discolor* (Soerensen, 1884), in more details, revealing several types of olfactory receptors, with distinct morphologies externally and internally. It is therefore possible that different receptors have distinct functions (see discussion in Gainett et al. 2017). Previously, laniatorids have been reported to use close-range olfaction for finding food, detecting and fleeing from alarm pheromones and in learning to find shelter (Machado et al. 2002; Willemart & Chelini 2007; Costa & Willemart 2013; Santos et al. 2013). Although we still need more studies to explain our results ecologically, now this study provides empiric evidence that finding conspecifics may be another role played by olfaction. This is a new step in understanding not only the sensory biology of harvestmen, but also in understanding how these animals get together in aggregations and find each other for sexual purposes.

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