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SHORT COMMUNICATION

# First experimental evidence that a harvestman (Arachnida: Opiliones) detects odors of non-rotten dead prey by olfaction

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ABSTRACT. Harvestmen feed on live, dead and fresh, or decomposing animals, fungi, and plant matter, being very dependent on chemoreception to find food. Herein we performed an experiment to test if individuals of *Discocyrtus pectinifemur* Mello-Leitão, 1937 (Gonyleptidae) (n = 23) behave differently when in contact with olfactory cues from different sources (rotten prey, non-rotten prey and a control). Using dead crickets in a box covered with a mesh, and recording the time the harvestmen spent in the vicinities of the box, we show that *D. pectinifemur* detects non-rotten prey and stays longer on it than on the other two treatments. Our results contrast with a previous study on another species, showing that we should not generalize results obtained for one species. Our data also suggest that olfactory receptors occur on the legs of these harvestmen and that *D. pectinifemur* might choose dietary items based on olfaction.

KEY WORDS. Chemoreception; Laniatores; sensilla; sensory ecology.

Harvestmen (Arachnida) are usually found on leaf litter, leaves, tree trunks and caves (Curtis & Machado 2007). They feed on live, dead and fresh or decomposing animals, fungi and plant matter such as flowers and fruits (Acosta & Machado 2007). Most of these arachnids are nocturnal and do not rely on mechanoreception at a distance or vision to find food. In the suborder Laniatores, individuals tap their surroundings with the first and second pair of legs when foraging. On the distal parts of the legs there are setae responsible for contact mechanoreception and chemoreception (Willemart *et al.* 2009). There is no electrophysiological or histological evidence of olfactory receptors in Laniatores (Willemart *et al.* 2009).

Among the 4,200 laniatorid species (Kury 2011), only *Iporangaia pustulosa* Mello-Leitão, 1935 (Gonyleptidae) has been subjected to experimental tests on food detection at close range by olfaction: the results suggest that they are attracted only to decomposing prey, a strong odored food (Willemart & Chelini 2007). Herein we provide experimental evidence that the harvestman *D. pectinifemur* can detect non-rotten dead prey by olfaction.

We collected individuals of *D. pectinifemur* in the city of Campinas, State of São Paulo, in January 2012, in a residential area close to a fragment of Atlantic forest. We brought the animals to the laboratory and housed them individually in plastic containers (12 x 8 x 4 cm) with soil on the bottom, screens on the walls and a shelter. We provided water in a wet cotton ball and fed them once a week with moistened dog food. We ran the trials in April and May 2012. The average temperature during the experiments was 18.6°C ± 5.6 (st dev) (n = 6 days). Voucher specimens were deposited at the Museum of Zoology of the University of São Paulo, Brazil (MZSP 42536 and 42537).

We ran an experiment to compare the reaction of the tested animal to non-rotten and rotten food and a control, always at a close distance. The test arena was circular, measuring 10 cm in diameter and 20 cm in height, with paper towel on the bottom. We introduced the stimulus used in each treatment in a 2 cm diameter round plastic box covered with a mesh on the top, which was placed adjacent to the arena wall, inside the arena. We used only one stimulus at a time, the distance between the cricket and the mesh being 1 cm. We introduced the tested animal (n = 23, 15 females and 8 males) in the arena 7 cm away from the box. We then left the animals in a vial for three minutes to acclimate. Next, we released them and recorded their behavior during 14 minutes. We used the following stimuli: (1) rotten odor: 3 mm *Gryllus* sp. killed by freezing, removed from the freezer 24 hours before the experiment and left at 100% humidity until the trial; non-rotten odor: 3 mm *Gryllus* sp. killed by freezing, removed from the freezer three minutes before the trial; humidity control: a square 0.5 cm piece of wet towel paper. Because the crickets were very small, we used two crickets per trial (adult crickets are 2.5 cm long).

Before running the trials, we starved the animals for eight days. We ran the trials at night, between 6 p.m. and 1 a.m. We recorded the animals using a Sony Handycam HDR-XR550/

night shot positioned above the arena forming a 90 degree angle with it. We cleaned the arena thoroughly with alcohol 70% and changed the paper towel after each trial.

In order to identify whether or not the animals would behave differently according to the stimulus provided, we counted the time that the tested animal had its pedipalps and at least one leg I on or touching the lateral of the box (cf. WILLEMART & CHELINI 2007). Individuals that did not display this behavior in any of the treatments were not included in the analyses. Because we did not have a sufficient number of individuals and did not want to split them in three treatments and therefore reduce the sample size, we used a repeated measures design, using the same individual in a random order in randomized hours of the night, and in such a manner that the number of individuals tested in each treatment, each day, was the same (MARTIN & BATESON 2011). We compared the three treatments using a non-parametric Friedman test followed by post hoc Student-Newman-Keuls (SNK) (ZAR 1999). We also quantified and compared among treatments (comparison by Friedman test followed by SNK when necessary): the number of times the animals extended their pedipalps and the time spent with the pedipalps extended, since this is their typical behavior when they attempt to capture prey.

Our results show that *D. pectinifemur* stays longer (in seconds) on the non-rotten odor than on the rotten odor or control ($\chi^2 = 6.32$, DF = 2, $p = 0.026$, SNK $p < 0.05$) (Fig. 1). There was no difference between the rotten odor and control (SNK $p > 0.05$) and no difference between males and females (Mann-Whitney between treatments: $p > 0.05$). The median number of times the harvestmen extended the pedipalps was 0 (max:10; min:0) for the rotten odor; 1 (max:20; min:1) for the non-rotten odor; 0 (max:14, min:0) for the control, the difference being non-significant ($\chi^2 = 4.5$, DF = 2, $p = 0.105$). The median time the animals spent with their pedipalps open was 0 (max: 128; min: 0) for the rotten odor; 4 (max: 391; min: 0) for the non-rotten odor; and 0 (max: 260, min: 0) for the control ($\chi^2 = 6.59$, DF = 2, $p = 0.037$; SNK test, however, did not detect differences between groups).

This is the first experimental evidence that a harvestman detects weak odors, namely a non-rotten dead prey, by olfaction. This result was somehow unexpected because WILLEMART & CHELINI (2007) had reported that the laniatorid *I. pustulosa* detects dead prey by olfaction only if it was rotten, and because olfactory receptors are still unknown in this suborder.

In the absence of olfactory receptors, the contact chemoreceptors sensilla chaetica could potentially detect strong odors in high concentrations (CHAPMAN 1998). For example, MACHADO *et al.* (2002) reported laniatorid harvestmen detecting the strong odor of defensive secretions from conspecifics at close distance. But this is unlikely to happen with prey that exudes a weak odor, such as non-rotten dead crickets, suggesting that olfactory receptors are present in laniatorid legs. Sensilla basiconica on the dorsal region of first tarsi and second pair of legs (absent in tarsi III and IV) (WILLEMART *et al.* 2007,

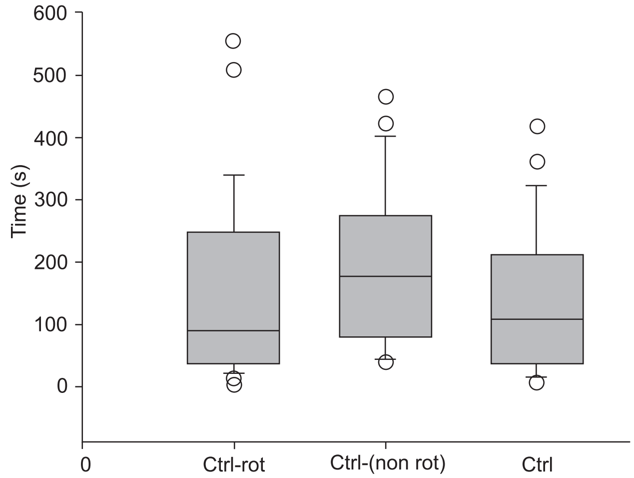


Figure 1. Results of the experiment where we compared the time that individuals of *Discocyrtus pectinifemur* spent with its pedipalps and at least one leg I on or touching the lateral of the box containing one of the three stimuli: rotten cricket, non-rotten cricket and humidity control. We used a repeated measures design and therefore the box-plot is showing the difference between the two treatments (rotten and non-rotten) and the humidity control, which is shown as "zero". Bottom and top of the box show the 25th and 75th percentiles and the horizontal line in the box shows the median. Whiskers show the 10th and 90th percentiles, individual points show outliers.

2009) are potential candidates. Their external morphology resembles olfactory receptors found in other taxa (STEINBRECHT 1984, ZHANG 2013), but only histological sections or electrophysiological studies could definitely answer the question.

Laniatorid harvestmen do not use their pedipalps to detect prey but to capture them. Pedipalps were extended for longer in the non-rotten treatment odor, again suggesting more interest in this type of prey ($p = 0.037$), even though the differences were non-significant in the post hoc tests ($p > 0.05$).

Showing more interest in non-rotten than in rotten crickets using olfactory cues may be related to a choice of food with better quality, which has been shown in other taxa (EGAS *et al.* 2003, SIMÕES *et al.* 2012). It could also suggest that this species has a tendency towards capturing live prey instead of rotten items. Unfortunately, we cannot further discuss these two hypotheses because there are no data on the feeding habits or the physiology of feeding in *D. pectinifemur*. Our results also suggest that we should not generalize results for the whole suborder: *I. pustulosa* was only attracted to rotten crickets and in *D. pectinifemur* there was no difference between the time spent on rotten prey and control. This may also reflect a difference on the diet or the microhabitat of these two species. Whereas at least adults of *I. pustulosa* inhabit the vegetation, *D. pectinifemur* leaves on the leaf litter, where decomposing items are abundant. It is possible that non-decomposing food items stand out in this environment just as decomposing items stand out on the vegetation, which would help explaining our results. If this hypothesis is correct, we could predict that species living on leaf litter would show more interest in non-rotten items and species living on vegetation would prefer rotten items.

In summary, our data suggest that olfactory receptors must occur on the legs of laniatorids, that *D. pectinifemur* may possibly choose what to eat using olfaction, and that we should be careful when generalizing results about the sensory capabilities of laniatorids.

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