Lines of defense in the harvestman Hoplobunus mexicanus (Arachnida: Opiliones): Aposematism, stridulation, thanatosis, and irritant chemicals

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ABSTRACT

The lines of defense in the harvestman Hoplobunus mexicanus are reported including the complete chemical characterization of defensive secretion, a morphological description of the ozopore, and a spectrogram of stridulating sounds. Aposematic coloration may be regarded as the first line of defense, warning diurnal predators about the unpleasant attributes of the chemical secretion. At night, the aposematic coloration is probably not efficient and, if an individual is seized by a predator, thanatosis or stridulation can be used as second lines of defense. The last line of defense is the use of chemical irritants. The defensive secretion is a mixture of two compounds: 2-methyl-5-ethylphenol and 2,5-dimethylphenol. Whereas the anterior half of the ozopore is ornamented with many sharp projections, the posterior half is smooth and membranous, probably causing the secretion to flow only to the lateroposterior area of the body. The biological meaning of each defensive mechanism and the type of stimuli that triggers each of them is discussed.

1. Introduction

Most prey animals have several lines of defense against predation, including both morphological and behavioral adaptations that decrease their chance of encounter with the predator and/or increase the likelihood of surviving an attack (Edmunds, 1974). The first line of defense is generally to avoid detection by the predator, minimizing locomotion, noise production, and any visual cues that might denounce prey location (e.g., Edmunds, 1974; Lima and Dill, 1990; Caro, 2005). Among arthropods, common defensive strategies associated to this first line of defense are crypsis and anachoresis (Cloudsley-Thompson, 1995; Edmunds and Brunner, 1999; Field and Glasgow, 2001; Gnaspini and Hara, 2007; Witz, 1989). When the prey is spotted, it usually relies on other lines of defense, such as evasive responses that include fleeing, thanatosis, and deimatic behavior (Cloudsley-Thompson, 1995; Edmunds and Brunner, 1999; Field and Glasgow, 2001; Gnaspini and Hara, 2007). Even when the prey is cornered or effectively caught by a predator, the prey may exhibit aggressive responses that might hinder the attack and still avoid predation. In this last line of defense, some animals rely on chemical irritants and/or physical structures that retaliate and prevent a deadly strike by the predator (Witz, 1989; Edmunds and Brunner, 1999; Field and Glasgow, 2001; Eisner et al., 2005; Gnaspini and Hara, 2007).

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Representatives of the order Opiliones have a great variety of defensive strategies to cope with their predators (review in Gnaspini and Hara, 2007). Crypsis is widespread in the group, and some species even camouflage themselves with debris glued on the body and legs by a secretion from the integument (Pabst, 1953; Martens, 1993; Firmo and Pinto-da-Rocha, 2002). When detected by predators, many species of the suborders Laniatores and Dyspnoi respond to predator attacks with thanatosis behavior (Pabst, 1953; Gruber, 1993; Eisner et al., 1978; Cokendolpher, 1987; Pereira et al., 2004; Machado and Pomini, 2008). Stridulation has long been shown to be an effective mechanism deterring arthropod predators (Masters, 1979) and its occurrence has also been suggested in some species of Laniatores and Dyspnoi (Šilhavý, 1978; Hillard and Sankey, 1989; Gnaspini and Hara, 2007; Schönhofer, 2008), although as far as we know the actual stridulating sounds have not been recorded or formally described in any species. In species of the suborder Eupnoi, on the other hand, fleeing, bobbing, and autotomy of legs are the most common strategies employed by individuals that have already been spotted or attacked (Edgar, 1971; Anuradha and Parthasarathy, 1976; Holmberg et al., 1984). The last line of defense in harvestmen includes aggressive responses such as pedipalpal and cheliceral attacks, pinching, and releasing of chemical irritants (review in Gnaspini and Hara, 2007). Although chemical defense is considered the most effective defense mechanism in harvestmen of the suborder Laniatores (Eisner et al., 1978), it seems to be employed only when all other evasive measures were unsuccessful in preventing the predator attack (Duffield et al., 1981).

Hoplobunus mexicanus (Roewer, 1915) (Stygnopsidae) is a large and robust harvestman (body ca. 10 mm) that belongs to the suborder Laniatores and occurs in northeastern Mexico (Kury, 2003; Fig. 1a). The species seems to be aposematic since, in contrast to a dark brown body, the pedipalps and chelicerae are orange and the trochanter of all legs are light yellow (Fig. 1a). Individuals are active only at night, when they are found walking on the ground, on the bark of trees, or on the understory vegetation. During daylight, individuals take shelter under stones and rotten logs or inside natural cavities on roadside banks. In this study we report the lines of defense in *H. mexicanus*, including complete chemical characterization of defensive secretions with advanced spectroscopic experiments, detailed morphological description of the gland opening, and spectrographic analysis of stridulation under disturbance, the first of such description in Opiliones.
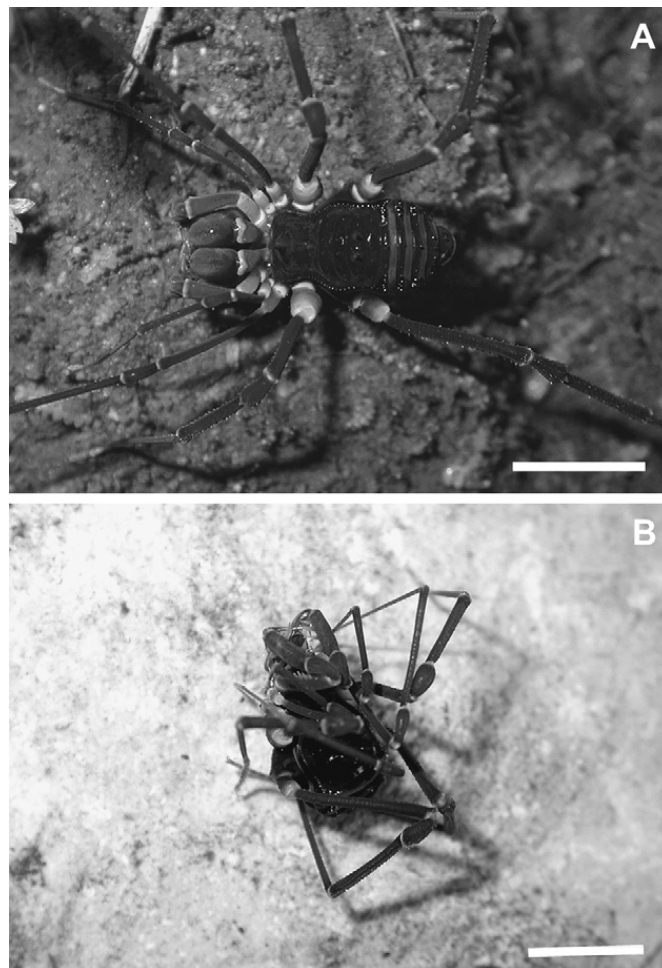

Fig. 1. (A) Male individual of the harvestman *Hoplobunus mexicanus* walking on a roadside bank at El Riscal area, Mexico. Note that the chelicerae, pedipalps, and trocanthers of all legs are lighter than the body. (B) Motionless individual of *H. mexicanus* exhibiting thanatosis behavior, with legs retracted over the body. Scale bars = 1 cm.

2. Materials and methods

2.1. Harvestmen collection and rearing

Individuals of *H. mexicanus* used in the chemical analyses and behavioral observations were collected in November 2006 at the El Riscal area (19° 28′ 51″ N, 96° 59′ 47″ W, 1550 m alt.), on the Eastern slope of the central Mexican plateau in the municipality of Coatepec, Veracruz, Mexico. The vegetation in the area is a cloud forest, especially well-preserved along small basins, with some secondary forest on the top of the crests. In the laboratory, 10 individuals (four males and six females) were reared in the same terrarium (60 × 40 cm base, 35 cm high) containing soil, pieces of rocks and trunks that could be used as shelters, and a dish with water to maintain the humidity. The harvestmen were fed twice a week with dead insects. Temperature and moisture conditions were controlled and ranged from 23 to 25 °C and from 80 to 90%, respectively, and a light schedule of 13L:11D was maintained in the laboratory. For the morphological description of the ozopore, another four individuals (two males and two females) of *H. mexicanus* were collected in the same place in July 2006. Voucher specimens were deposited at the Museu de Zoologia of the University of São Paulo (MZSP), Brazil.

2.2. Behavioral responses

To investigate the lines of defense of *H. mexicanus*, each individual was seized by the observer's hands in the distal region of right femur IV for 10 s ($n = 10$). During this period, it was recorded if the individuals stridulated or released chemical secretions. Afterward, the individuals were dropped on a plastic tray, from a height of 25 cm, a procedure that simulated a sudden release from the mouth or hand of a vertebrate predator, such as birds or opossums. Next, it was recorded if the individuals fled away or exhibited thanatosis behavior — in this case, the time individuals took to start moving again was registered. One week later, the same individuals were stimulated in another way: their dorsum (mesotergal area) and venter were pressed at the same time for 10 s ($n = 10$). In this case, the stimulus was applied using forceps which had the points covered with rubber tubes to avoid damaging the cuticle of the animals. Individuals were tested under a stereomicroscope in order to observe in detail the discharge of both the enteric fluid and the chemical secretions. They were scored as respondent if they released chemical secretions, which are easily recognized by the strong phenolic smell. The trials were conducted at room temperature (nearly 25 °C) from 14:00 to 18:00 h.

2.3. Chemical study of defensive secretions

Chemical defense exudates were collected by pressing cotton wool (50–70 mg) onto the gland openings of the harvestmen. The exudates from one male individual (5.0 mg) were then extracted with ethyl acetate (about 5 mL) and analyzed by GC–MS or with CDCl$_3$/TMS (600 μL) for the NMR experiments. All solvents were of high analytical grade, bi-distilled before use. The cotton wool was cleaned by successive extraction with bi-distilled ethyl acetate under refluxing conditions, followed by solvent removal under reduced pressure.

Analyses were carried out using an HP 6890/5973 system equipped with HP5 or MDN-5S fused silica capillary columns (30 m × 0.25 mm × 0.25 mm). Column temperatures were programmed from 50 to 200 °C at 10 °C min^{-1} and subsequently from 200 to 290 °C at 16 °C min^{-1}. The injector temperature was maintained at 250 °C. Helium was used as carrier gas, at a flow rate of 1 mL min^{-1}. The mass spectra were taken at 70 eV and the scanning speed was 3.54 scans s^{-1} from m/z 40 to 450. NMR spectral data were acquired with a Varian Inova spectrometer, operating at 499.88 MHz for ^{1}H NMR. CDCl$_3$ was used as solvent and TMS as an internal reference (δ 0.0). Chemical shifts were recorded in ppm and coupling constants J in hertz (Hz).

2.4. Morphology of the ozopore

Specimens preserved in 70% ethanol were maintained in KOH (0.1 M) overnight, and then cleaned with a solution of a commercial detergent in water (1:3) for 15–20 s in ultrasound equipment (cf. Pinto-da-Rocha, 2002). The detergent was removed with water and ultrasound equipment, in four sessions of 15 min each. Finally, the material was dehydrated in acetone for 15 min and left air-dry. After dehydration, the material was mounted on a stub and gold coated in a Balzer SCD 50 Sputter Coater. The material was examined with a Zeiss Leo 440 scanning electron microscope at the Electronic Microscopy Laboratory of the Museu de Zoologia of the University of São Paulo, Brazil. The terminology of the structures used here follows Hara and Gnaspini (2003).

2.5. Stridulation recording and spectrogram

Two males and two females were collected at night from the same field site in October 2009 and immediately placed in a small glass terrarium (25 × 15 cm base, 15 cm high) with pieces of rotten logs and branches. The tip of a syringe needle was inserted on the soft rotten wood within 15 cm of the site where individuals were standing, and a Vibrametrics 9002A accelerometer with a P5000 signal conditioner connected to a Marantz PMD Digital recorder was attached to the other end of the needle. The individuals were disturbed touching their bodies and legs with a dissecting needle until several stridulating sounds could be heard through earphones connected to the recorder. The largest series of continuous syllables was isolated

and noise was filtered using Cool Edit Pro 2.0 (Syntrillium Software Corporation) sound manipulating software with a noise profile taken from intervals between syllables of the same recording segment. The same software was used to produce a color spectrogram that was later turned into a black and white figure with enhanced contrast.

3. Results

3.1. Behavioral and chemical defenses

Three out of the 10 individuals stridulated when seized by hands in the distal region of their right femur IV. These same individuals (two males and one female) also released chemical secretions a few seconds after stridulating. The stridulation could be perceived as a sudden vibration of the harvestman's body, but no noise could be heard. The other seven individuals remained motionless during the 10 s of manipulation. After they were dropped, all the individuals that had not stridulated exhibited thanatosis behavior, and all individuals that had stridulated fled away as soon as they touched the tray. Individuals in thanatosis hold their legs either tucked in close to the body (Fig. 1b) or partially extended laterally, and became motionless for a period from 21 s to nearly 31 min (mean ± SD = 771 ± 623 s; $n = 7$). After the period of immobility, the individuals started moving slowly and none of them fled away like the individuals that stridulated.

When pressed on the dorsum and venter at the same time, nine out of the 10 individuals released chemical secretions. The chemical secretion is colorless, has an irritant smell, and is released in conspicuous amounts (ca. 5 mg per adult individual). Two types of secretion emission were recorded: (1) mixing the secretion with enteric fluid and discharging the resulting liquid along the lateral margins of the dorsal scute with accumulation of fluid at the lateroposterior area of the body forming a droplet ($n = 7$), and (2) a jet sprayed directly toward the aggressor without mixing the secretion with enteric fluid ($n = 2$). The mixture of chemical secretions with enteric fluid was recorded in the six cases in which there was liquid displacement along the body of the animals. In these cases, the enteric fluid was released before the chemical secretion ($n = 3$) or nearly at the same time as the chemical secretion, which was released directly into the flowing enteric fluid ($n = 2$). On one occasion, the chemical secretion was intermittently discharged into the enteric fluid.
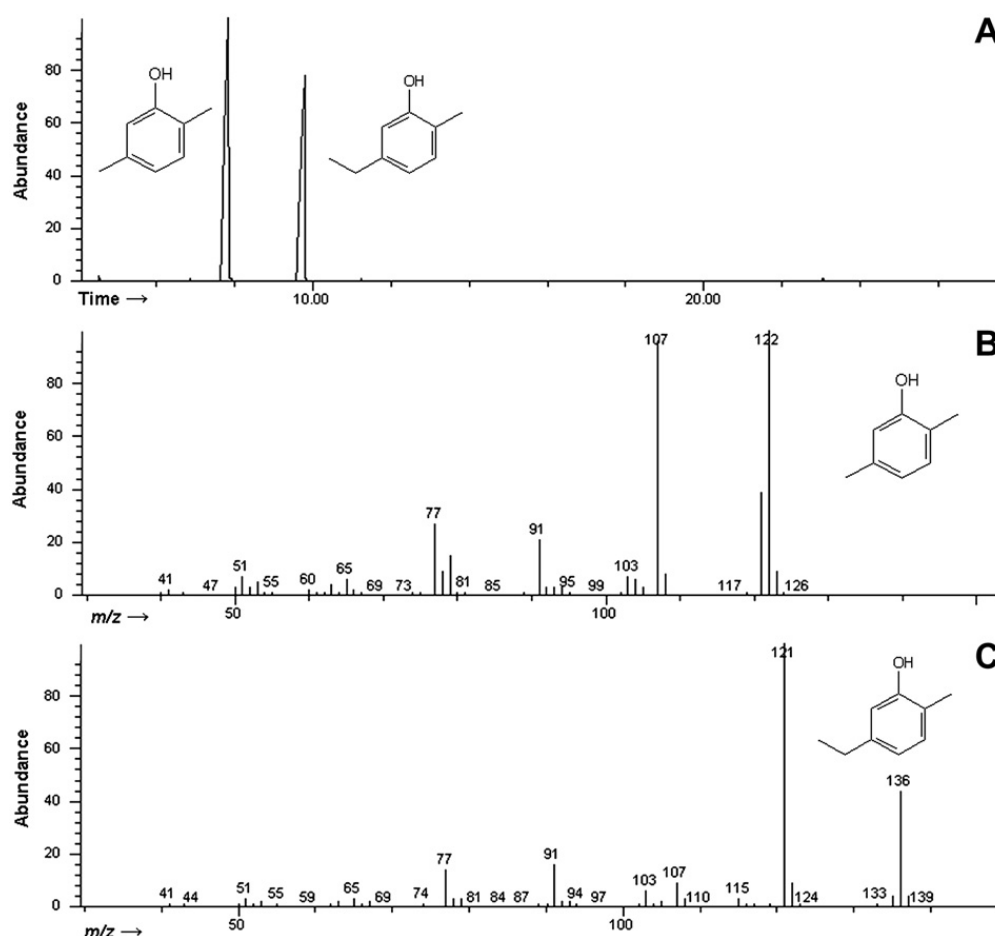

Fig. 2. (A) Total ion chromatogram (GC–MS, EI, 70 eV) of the harvestman *Hoplobunus mexicanus* defensive secretion. (B) 2,5-dimethylphenol (1) mass spectrum. (C) 2-methyl-5-ethylphenol (2) mass spectrum.

3.2. Chemical characterization of the defensive secretion

A GC–MS analysis revealed the presence of two volatile components in the chemical secretion, which were tentatively identified as (**1**) 2,5-dimethylphenol and (**2**) 2-methyl-5-ethylphenol, with relative abundances of 56% and 44%, respectively. Compound **1** displayed the molecular ion at m/z 122 as the mass spectrum base peak. The second most intense peak was m/z 107 (95%), corresponding to methyl loss from the parent molecular ion. Characteristic aromatic signals were observed at m/z 91, 21% (tropilium ion) and at m/z 77, 26% (phenyl ion). The mass spectrum of substance **2** displayed an intense molecular ion peak at m/z 136 (44%). The spectrum base peak at m/z 121 corresponds to a methyl loss ($M^+ - CH_3$). Characteristic aromatic ions at m/z 91 and 77 were also observed (Fig. 2).

Notwithstanding the GC–MS data relevance in the elucidation of harvestman defensive secretions, they do not provide accurate information on the differentiation of the many alternatives in the aromatic substitution. A traditional chromatographic purification of the defensive secretion was difficult due to the small amount available and considerable mass loss by volatilization. To overcome these problems, the crude defensive secretion, previously characterized by GC–MS as a 56:44 mixture of **1** and **2**, respectively, was analyzed by ^{1}H NMR and two sets of signals were distinguished based on the suggested structures and their concentration in the mixture. The aromatic region of the spectrum showed a 1,2,5 substitution pattern for substance **2** with a singlet at 6.63 ppm, and doublets with characteristic ortho-coupling at 7.03 ppm (J 7.6 Hz) and 6.69 ppm (J 7.6 Hz). The CH$_2$ group appeared as a quartet at 2.56 ppm (J 7. 6 Hz) coupled to the methyl group at 1.20 ppm (triplet, J 7.6 Hz), characterizing the ethyl group. A singlet corresponding to a methyl group ortho to a hydroxy group appeared at 2.21 ppm (Fig. 3a).

As previously observed from the GC–MS results (Fig. 2), substance **1**, major constituent in the mixture, displayed signals of higher amplitude in the ^{1}H NMR spectrum, with a singlet at 6.60 ppm, and two ortho-coupling doublets at 7.00 ppm (J 7.6 Hz) and 6.66 ppm (J 7.6 Hz). The methyl group on carbon 2 resonated at 2.20 ppm and the more deshielded signal at 2.27 ppm was assigned to methyl group on carbon 5 (Fig. 3a). Moreover the aromatic ring substitution patterns were further confirmed through 2D ^{1}H NMR (NOESY) correlations. The main correlations are shown in Fig. 3b. Finally, the NOE difference spectrum of substance **2** with pulse-selective excitation of methylene protons at 2.56 ppm produced signal enhancement of the protons signals at 6.63 (0.1%), 6.69 (0.12%) and 1.2 ppm (0.15%), confirming the presence of the ethyl substituent on C-5 of the aromatic ring (Fig. 3c).

3.3. Morphology of the ozopore

The anterior opening of the ozopore of *H. mexicanus* is covered by an integumentary dome that faces the laterodorsal area of the dorsal scute (Fig. 4). There is no sign of a sensorial peg, a "posterior opening" (sensu Hara and Gnaspini, 2003) of the ozopore, or of a V-shaped cut in the posterior rim of the opening. The anterior half of the ozopore is ornamented with microscopic and densely distributed sharp projections and the posterior half is smooth and membranous (Fig. 4). The lateral channel is well-defined and ornamented with small sharp projections near the posterior region of the ozopore (over the membranous margin of the lateral scutum). This lateral channel extends from the gland opening to the posterior region of the lateral margin (Fig. 4).
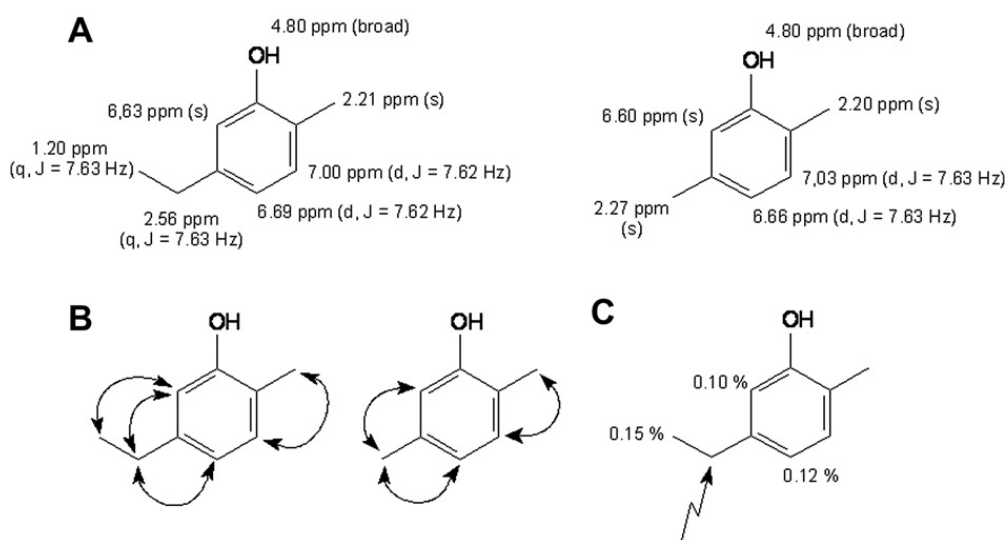

Fig. 3. (A) ^{1}H NMR chemical shifts of substances 1 and 2 produced by the harvestman *Hoplobunus mexicanus*. (B) Main correlations observed in ^{1}H NMR NOESY 2D experiment. (C) Signal nuclear Overhauser enhancements (^{1}H NMR NOESY 1D) observed for substance 2 after selective excitation of the methylene signal at 2.56 ppm.
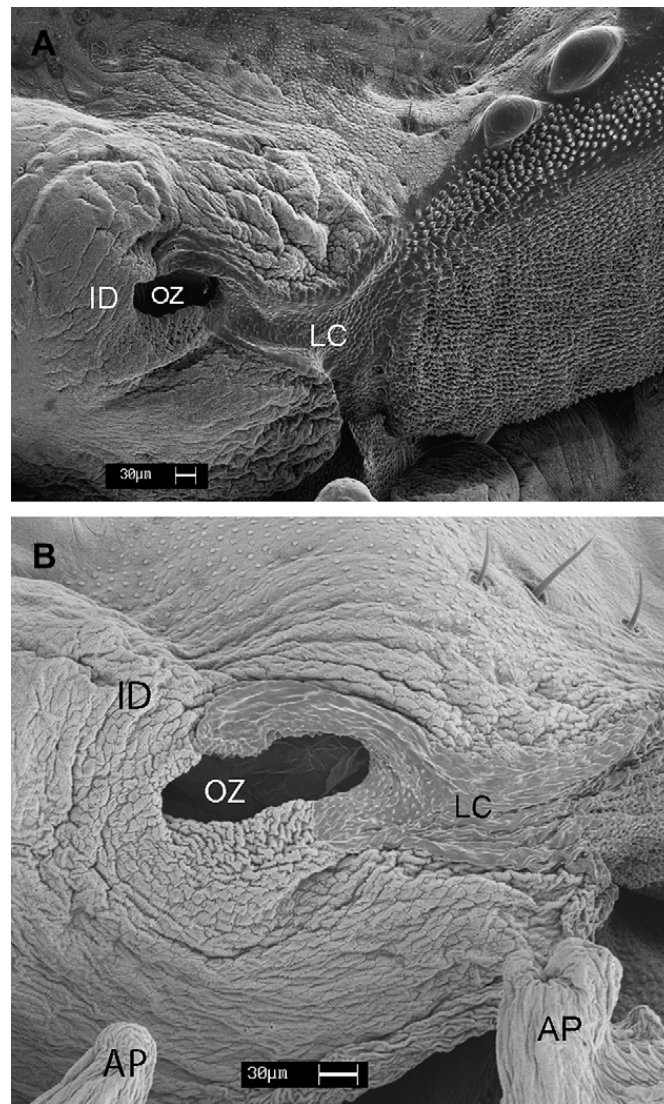
Fig. 4. (A) External morphology of the ozopore and lateral margin of dorsal scutum of a male harvestman *Hoplobunus mexicanus*. (B) Detail of the ozopore. Abbreviations: AP = apophyses of the front legs; OZ = ozopore; ID = integumentary dome; LC = lateral channel.

3.4. Stridulation

Although isolated syllables could be heard throughout the recording, the largest series of stridulating syllables was composed of five syllables produced in about 1.15 s, each lasting around 70 ms, and the interval between syllables varied between 150 and 500 ms (Fig. 5). All five syllables consisted of a frequency range below 5000 Hz, with a fundamental tone around 3000 Hz. Syllables 2, 3 and 5 had a slightly lower pitch since their frequencies were actually below 4000 Hz. The first syllable had a higher pitch since it also included an isolated frequency range around 7000 Hz.

4. Discussion

The lines of defense of the harvestman *H. mexicanus* include at least four mechanisms: aposematism, stridulation, thanatosis, and chemical irritants. Although each of these defensive mechanisms had already been reported or suggested for different harvestman species, as far as we know, this is the first study that reports that they can all be exhibited by the same species. As shown in the following paragraphs, the use of each defensive mechanism depends on the type of stimuli applied to the individuals. If the first line of defense (e.g., aposematism) is breached by the predator, then a second line of defense may come into operation (e.g., stridulation or thanatosis). The final, and perhaps most effective, line of defense is the use of chemical irritants, which are known to repel both vertebrate and invertebrate predators (Eisner et al., 2004; Machado et al., 2005).

Although thanatosis in harvestmen seems to be more common among small and cryptically colored species (Cokendolpher, 1987; Pereira et al., 2004; Machado and Pomini, 2008), individuals of *H. mexicanus* may become motionless for up to 31 min after being handled. Similar long-lasting thanatosis has been observed in some species of the families
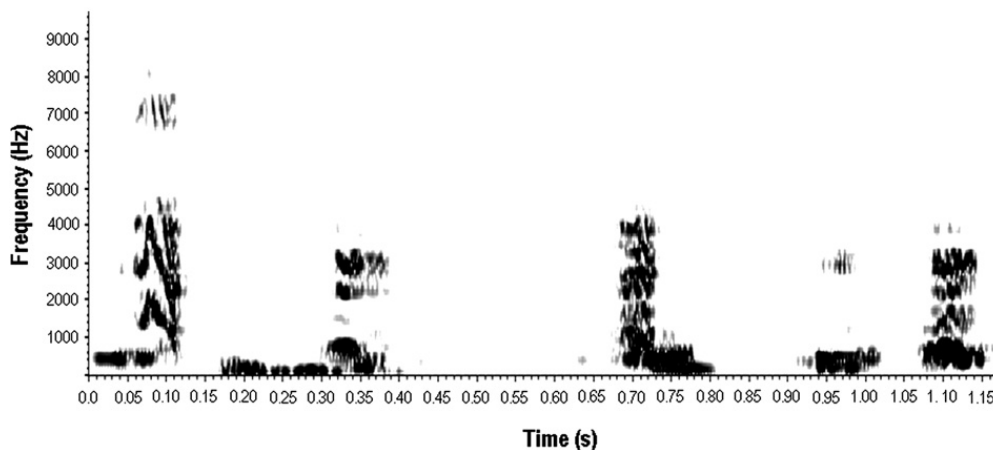
Fig. 5. Spectrogram of a series of five stridulation syllables by the harvestman *Hoplobunus mexicanus* as a result of disturbance by the observer poking the fourth leg and body with a dissecting needle.

Trogulidae, Escadabiidae, and Gonyleptidae (Pabst, 1953; Gnaspini and Hara, 2007). Contrary to these species, however, *H. mexicanus* is a large-bodied harvestman whose color pattern seem to be aposematic and not cryptic. The contrast between the dark body and the light parts of the appendages (trochanters of the legs, pedipalps, and chelicerae — see Fig. 1a) would suggest a function as a first line of defense warning diurnal predators about the unpleasant attributes of the chemical secretion. Since the species is active only at night, the aposematic value of its coloration remains to be investigated as its lighter parts shine under UV light.

Stridulation in harvestmen has been considered a means of sexual communication (Hillyard and Sankey, 1989), but it is also possible that this behavior functions as a defense mechanism (Gruber, 1969). Indeed, noises and vibration produced by stridulatory organs can be considered a special case of deimatic behavior, since they may frighten potential predators and hence promote the release of the seized individual (Dumortier, 1963; Masters, 1979). In this study, we showed that both males and females of *H. mexicanus* stridulate when one of their fourth legs is seized. Stridulation, which may be regarded as a second line of defense, was generally followed by the release of chemical secretions, but not by thanatosis behavior. In this way, it is possible that stridulation works as an acoustic and/or vibratory warning to nocturnal predators: if the harvestman is not set free, irritant chemicals will be released in sequence. The recording method was aimed to detect substrate-borne signals, and this substrate may be the actual body of the prey when handled by the predator, which could have a more effective startling effect than an airborne stimulus. Contrary to other harvestmen species, in which the putative stridulatory organs are present on the pedipalps and chelicerae (see references in Gnaspini and Hara, 2007), an examination of these appendages applying scanning electron microscopy did not reveal any stridulatory organ in individuals of *H. mexicanus*. We also searched for structures that would suggest a stridulatory function on internal and external areas of coxae, trochanters, femora, and patellae, but we did not find any structure that could be related to stridulation (unpublished data). Future morphological studies should be conducted to investigate where the stridulatory organs of *H. mexicanus* are located and how the vibration is produced. Likewise, functional studies on structures suggested to have a stridulatory role in Opiliones are lacking, thus their actual role remains to be shown.

The temporal pattern of stridulation recorded for *H. mexicanus* is remarkably similar to that of many insects that use stridulation as a defense mechanism. Masters (1980) sampled a broad range of such insect species and found that syllables were usually around 80 ms long (around 70 ms in our study), separated usually by about 90 ms (150–500 ms in our study), and with a rate between five and 10 syllables per second (five in our study). This author also reports a great variance in such temporal pattern probably due to the fact that the prey stridulates while struggling with the threat, as it was the case in our recording in which isolated syllables were also recorded. Although more extensive sampling of stridulation in *H. mexicanus* is required to confirm these patterns, they may suggest a potentially convergent temporal pattern of defensive stridulation among arthropods that might maximize antipredator effectiveness. The frequency of stridulatory sound in insects in the cited study averages 25,000 Hz, but varies widely and the range (5000–45,000 Hz) does barely cover the 4000–7000 Hz recorded for *H. mexicanus*.

The last line of defense in *H. mexicanus* is the use of chemical irritants. Here, we have successfully characterized the defensive secretion produced by the species, which is the first representative of the family Stygnopsidae chemically studied to now. Substance **2** (2-methyl-5-ethylphenol) is relatively common in the defensive secretions of many harvestmen of the suborder Laniatores, such as the gonyleptid *Camarana flavipalpi* (Machado and Pomini, 2008), the cosmetids *Cynorta astora* (Eisner et al., 1977) and *Eucynortula albipunctata* (Roach et al., 1980), and the stygnommatid *Stygnomma spiniferum* (Duffield et al., 1981). On the other hand, substance **1** (2,5-dimethylphenol) is a relatively rare defensive compound among harvestmen, being tentatively described only for the gonyleptids *Daguerreia inermis* and *Progonyleptoidellus striatus* (Hara et al., 2005). However, it is important to point out that many reports in this field employed only GC–MS or IR spectroscopy for the characterization of the secretions, and these authors indicate that the structural identification did not fully characterize the aromatic isomers (Hara

et al., 2005). In the present work, we have unequivocally attested the substitution patterns directly from the natural products on the basis of ^{1}H NMR, NOESY 1D and NOESY 2D experiments, thus confirming the identity of the compounds.

The types of secretion emission exhibited by *H. mexicanus* are similar to many species of the suborder Laniatores, in which there are both liquid displacement along the body margins of the individuals and direct jet sprays (Gnaspini and Hara, 2007). These two types of emission are supposed to increase the evaporating surface and to create a chemical shield around the body of the harvestman, protecting it after the emission of the secretion (Acosta et al., 1993; Gnaspini and Cavalheiro, 1998; Hara and Gnaspini, 2003; Machado et al., 2005). The morphological analysis of the ozopore indicated that, whereas the anterior half is ornamented with many sharp projections, the posterior half is smooth and membranous, probably causing the fluid to flow only to the lateroposterior area of the body. Like other harvestmen, enteric fluid is generally mixed with chemical fluids, which may increase the duration of the chemical shield, prolonging the time of evaporation of the chemical secretion (Machado and Pomini, 2008).

Although harvestman species that present thanatosis behavior are generally reluctant to release chemical secretions (Machado and Pomini, 2008), 30% of the individuals of *H. mexicanus* promptly released chemical secretions when grabbed by the fourth pair of legs. When their body was gently pressed, 90% of the individuals released chemical secretions. Experiments with *Stygnomma spinifera* have shown that 2-methyl-5-ethylphenol, the major component of the secretion in this species, is extremely effective against ants and lizards (Duffield et al., 1981). Therefore, it is possible that individuals of *H. mexicanus*, which release large amounts of chemical secretion containing 2-methyl-5-ethylphenol, are also well-defended against predators. An experimental approach like those used by Eisner et al. (2004) and Machado et al. (2005) could be used to test the effectiveness of the phenolic secretion released by *H. mexicanus* against different types of predators.

Acknowledgements

References

Acosta, L.E., Poretti, T.I., Mascarelli, P.E., 1993. The defensive secretions of *Pachyloidellus goliath* (Opiliones, Laniatores, Gonyleptidae). Bon. Zool. Beit. 44, 19–31.

Anuradha, K., Parthasarathy, M.D., 1976. Field studies on the ecology of *Gagrellula saddlana* Roewer (Palpatores, Opiliones, Arachnida) and its behaviour in the laboratory conditions. Bull. Ethol. Soc. India 1, 68–71.

Caro, T.M., 2005. Antipredator Defenses in Birds and Mammals. The University of Chicago Press, Chicago.

Cloudsley-Thompson, J.L., 1995. A review of the anti-predator devices of spiders. Bull. Br. Arachnol. Soc. 10, 81–96.

Cokendolpher, J.C., 1987. Observation on the defensive behavior of a neotropical Gonyleptidae (Arachnida, Opiliones). Rev. Arachnol. 7, 59–63.

Duffield, R.M., Olubajo, O., Wheeler, J.W., Shear, W.A., 1981. Alkylphenols in the defensive secretion of the nearctic opilionid, *Stygnomma spinifera* (Arachnida: Opiliones). J. Chem. Ecol. 7, 445–452.

Dumortier, B., 1963. Ethological and Physiological Study of Sound Emissions of Arthropoda. In: Busnel, R.-G. (Ed.), Acoustic Behaviour of Animals. Elsevier, Amsterdam, pp. 583–654.

Edgar, A.L., 1971. Studies on the biology and ecology of Michigan Phalangida (Opiliones). Misc. Pub. Mus. Zool., Univ.Mich. 144, 1–64.

Edmunds, M., 1974. Defence in Animals: a Survey of Antipredator Defences. Longman, Harlow.

Edmunds, M., Brunner, D., 1999. Ethology of Defenses against Predators. In: Prête, F.R., Wells, H., Wells, P.H., Hurd, L.E. (Eds.), The Praying Mantids. The Johns Hopkins University Press, London, pp. 276–299.

Eisner, T., Alsop, D., Meinwald, J., 1978. Secretions of opilionids, whip scorpions and pseudoscorpions. In: Bettini, S. (Ed.), Handbook of Experimental Pharmacology (Arthropod Venoms), vol. 48. Springer-Verlag, Berlin, pp. 87–99.

Eisner, T., Eisner, M., Seigler, M., 2005. Defenses of Insects, Spiders, Scorpions, and Other Many-Legged Creatures. Belknap Press (of Harvard University Press), Cambridge, MA.

Eisner, T., Jones, T.H., Hicks, H., Silberglied, R.E., Meinwald, J., 1977. Quinones and phenols in the defensive secretions of neotropical opilionids. J. Chem. Ecol. 3, 321–329.

Eisner, T., Rossini, C., González, A., Eisner, M., 2004. Chemical defense of an opilionid (*Acanthopachylus aculeatus*). J. Exp. Biol. 207, 1313–1321.

Field, L.H., Glasgow, S., 2001. Defense behaviour. In: Field, L.H. (Ed.), The Biology of Wetas, King Crickets and Their Allies. CAB International, Wallingford, pp. 297–316.

Firmo, C.L., Pinto-da-Rocha, R., 2002. A new species of *Pseudotrogulus* Roewer and assignment of the genus to the Hernandariinae (Opiliones, Gonyleptidae). J. Arachnol. 30, 173–176.

Gnaspini, P., Cavalheiro, A.J., 1998. Chemical and behavioral defenses of a Neotropical cavernicolous harvestman: *Goniosoma spelaeum* (Opiliones: Laniatores: Gonyleptidae). J. Arachnol. 26, 81–90.

Gnaspini, P., Hara, M.R., 2007. Defense mechanisms. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), Harvestmen: The Biology of Opiliones. Harvard University Press, Cambridge, pp. 374–399.

Gruber, J., 1969. Über Stridulationsorgane bei einem Ischyropsalididen: *Ceratolasma tricantha* Goodnight & Goodnight (Opiliones, Arachnida). Anz. Osterr. Akad. Wiss., Math.-Naturwiss. 11, 1–7.

Gruber, J., 1993. Beobachtungen zur Ökologie und Biologie von *Dicranolasma scabrum* (Herbst) (Arachnida: Opiliones). Teil I. Annln naturh. Mus. Wien 94/95B, 393–426.

Hara, M.R., Cavalheiro, A.J., Gnaspini, P., Santos, D.Y.A.C., 2005. A comparative analysis of the chemical nature of defensive secretions of Gonyleptidae (Arachnida: Opiliones: Laniatores). Biochem. Syst. Ecol. 33, 1210–1225.

Hara, M.R., Gnaspini, P., 2003. Comparative study of the defensive behavior and morphology of the gland opening area among harvestmen (Arachnida, Opiliones, Gonyleptidae) under a phylogenetic perspective. Arthrop. Struct. Develop. 32, 257–275.

Hillyard, P.D., Sankey, J.H.P., 1989. Harvestmen: keys and notes for the identification of the species. In: Kermack, D.M., Barnes, R.S.K. (Eds.), Synopses of the British Fauna, No. 4. E.J. Brill, Leiden, pp. 1–119.

Holmberg, R.G., Angerilli, N., Lacase, L., 1984. Overwintering aggregations of *Leiobunum paessleri* in caves and mines (Arachnida, Opiliones). J. Arachnol. 12, 195–204.

Kury, A.B., 2003. Annotated catalogue of the Laniatores of the New World (Arachnida, Opiliones) (vol. especial monográfico). Rev. Ibér. Aracnol. 1, 1–337.

Lima, S.L., Dill, L.M., 1990. Behavioural decisions made under the risk of predation. Can. J. Zool 68, 19–640.

Machado, G., Pomini, A.M., 2008. Chemical and behavioral defenses of the neotropical harvestman *Camarana flavipalpi* (Arachnida: Opiliones). Biochem. Syst. Ecol. 36, 369–376.

Machado, G., Carrera, P.C., Pomini, A.M., Marsaioli, A.J., 2005. Chemical defense in harvestmen (Arachnida: Opiliones): do benzoquinone secretions deter invertebrate and vertebrate predators? J. Chem. Ecol. 31, 2519–2539.

Martens, J., 1993. Further cases of paternal care in Opiliones (Arachnida). Trop. Zool. 6, 97–107.

Masters, W.M., 1979. Insect disturbance stridulation: its defensive role. Behav. Ecol. Sociobiol. 5, 187–200.

Masters, W.M., 1980. Insect disturbance stridulation: characterization of airborne and vibrational components of the sound. J. Comp. Physiol. 135, 259–268.

Pabst, W., 1953. Zur Biologie der mitteleuropäischen Troguliden. Zool. Jb., Abt. Syst., Ökol. u. Geog. Tiere 82, 1–156.

Pereira, W., Elpino-Campos, A., Del-Claro, K., Machado, G., 2004. Behavioral repertory of the neotropical harvestman *Ilhaia cuspidata* (Opiliones, Gonyleptidae). J. Arachnol. 32, 22–30.

Pinto-da-Rocha, R., 2002. Systematic review and cladistic analysis of the Caelopyginae (Opiliones, Gonyleptidae). Arq. Zool. 36, 357–464.

Roach, B., Eisner, T., Meinwald, J., 1980. Defensive substance of opilionids. J. Chem. Ecol. 6, 511–516.

Schönhofer, A.L., 2008. On harvestmen from the Soutpansberg, South Africa, with description of a new species of *Monomontia* (Arachnida: Opiliones). Afr. Invert. 49, 109–126.

Šilhavý, V., 1978. *Minuides milleri* sp. n., an opilionid with an unusual manner of stridulation (Phalangodidae, Phalangodinae). Acta Entomol. Bohemoslov. 75, 58–63.

Witz, B.W., 1989. Antipredator mechanisms in arthropods: a twenty year literature survey. Fla. Entomol. 73, 71–99.