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Research Article

High-frequency components in the distress stridulation of Chilean endemic velvet ants (Hymenoptera: Mutillidae)

Componentes de alta frecuencia en la estridulación de angustia en especies endémicas chilenas de hormigas terciopelo (Hymenoptera: Mutillidae)

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Abstract. The emission of distress stridulation is a common defense mechanism among insects, including species from the order Hymenoptera (ants, bees and wasps). Wasps from the family Mutillidae (also known as velvet ants) emit audible distress stridulations when attacked. While species-specific variation in the acoustic properties of velvet ants stridulation has been described, one common theme across species is the absence of ultrasonic frequency components (> 20 kHz). We recorded and analyzed the distress stridulation emitted by females of three species of Chilean endemic velvet ants, popularly known as panda ants: *Euspinolia chilensis (*Spinola, 1851), *E. militaris* (Mickel, 1938) and *Reedomutilla gayi (*Spinola, 1851). In general, the stridulation emitted by *R. gayi* is similar to the distress signal of other velvet ant species described to date. In contrast, the distress signals of both *Euspinolia* species differ from previously reported Mutillidae stridulations, including the presence of high-frequency spectral components, reaching the ultrasonic range. Due to the scarce information on the natural predators of South American velvet ant species, the defensive significance of distress stridulation having ultrasonic components remains unknown, however, its potential role against reptile and rodent predators is discussed and its potential role as intra-specific communication is suggested.

Key words: Distress signals, panda ant, stridulation, wasp.

Resumen. La emisión de estridulación de angustia es un mecanismo común de defensa entre los insectos, incluidas las especies del orden Hymenoptera (hormigas, abejas y avispas). Las avispas de la familia Mutillidae (también conocidas como hormigas terciopelo) emiten estridulaciones de angustia audibles cuando son atacadas. Si bien se ha descrito variación especie-específica en las propiedades acústicas en la estridulación de las hormigas terciopelo, un tema común es la ausencia de componentes de frecuencia ultrasónica (> 20 kHz). Registramos y analizamos la estridulación de angustia emitida por hembras de tres especies de hormigas terciopelo endémicas chilenas, conocidas popularmente como hormigas panda: *Euspinolia chilensis (*Spinola, 1851), *E. militaris* (Mickel, 1938) y *Reedomutilla gayi (*Spinola, 1851). En general, la estridulación emitida por *R. gayi* es similar a la señal de angustia de otras especies de hormigas terciopelo descritas hasta la fecha. Por el contrario, las señales de angustia de ambas especies de *Euspinolia* difieren de las estridulaciones de Mutillidae reportadas anteriormente, incluida la presencia de componentes espectrales de alta frecuencia que alcanzan el

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rango ultrasónico. Debido a la escasa información sobre los depredadores naturales de las especies de hormigas terciopelo sudamericanas, la importancia defensiva de la estridulación de angustia con componentes ultrasónicos sigue siendo desconocida; sin embargo, se discute su posible rol contra los depredadores reptiles y roedores y se sugiere su rol potencial en comunicación intraespecífica.

Palabras clave: Avispas, estridulación, hormiga panda, señales de angustia.

Introduction

Despite their name, velvet ants are parasitoid wasps belonging to the Mutillidae family, a cosmopolitan taxon comprising over 4,200 described species (Lelej and Brothers 2008) characterized by sexual dimorphism: females are wingless and males usually have wings (Brothers 2006). Mutillid females are ectoparasites of mature larvae or pre-pupae of other insects, mostly of other aculeate Hymenoptera (Tormos *et al.* 2010).

Stridulation, a widespread form of acoustic behaviour in arthropods, is the production of sounds that results from rubbing two rigid parts of the body (Claridge 2006). This behaviour has been observed on both sexes in mutillids (Polidori *et al.* 2012, 2013a), where the stridulatory organ is located in the second and third tergite of the gaster and consists of a file (*pars stridens*) and a scraper (*plectrum*) (see Polidori *et al.* 2012 for a detailed description of these organs). Sound is produced when the file rubs the scraper, activated by dorsoventral movements (Polidori *et al.* 2012, 2013a). Although the ecological function of stridulation in mutillids is not clear, it has been proposed that these sounds could act as warning, defensive (Schmidt and Blum 1977; Masters 1979; Polidori *et al.* 2012) and/or mating signals (Manley 1977; Brothers 2006; Tormos *et al.* 2010; Polidori *et al.* 2012, 2013a, 2013b).

Few studies on mutillid biology, ecology or mating have been conducted, and these have been almost exclusively performed on European or North American species (Brothers 2006; Polidori *et al.* 2012, 2013a, 2013b), but little is known about South American mutillid species, particularly about their acoustic behaviour. In Chile, mutillids are known as panda ants due to its fuzzy appearance and white/black coloration, but nothing is known about their ecology. In the present article, we describe the distress stridulation produced by three endemic Chilean panda ant species: *Euspinolia militaris* (Mickel, 1938), *E. chilensis (*Spinola, 1851) and *Reedomutilla gayi (*Spinola, 1851) and compare these signals to those described for other Mutillidae species.

Materials and Methods

On February 2017, live females of *E. militaris*, *E. chilensis* and *R. gayi* (one of each species) were collected from the Likandes Educational Park (Cajón del Maipo, Región Metropolitana de Santiago, Chile, 33°64’S - 70°33’W; 1,124 m above sea level) and transported to the laboratory of Neuroethology in Santiago, Chile for testing. Wasps were kept in individual containers with soil and fed daily with a 10% honey solution. Recordings were conducted two days after the arrival of the individuals to the laboratory.

Stridulation recording

For each of the three panda ant species, the stridulation of one female individual was recorded. To facilitate the manipulation of experimental subjects, wasps were cooled by placing them inside a refrigerator at -20 °C for three minutes, and then rapidly mounted on a plastic rectangular slide (75 x 25 x 3 mm) attaching them with two thin (1-2 mm width) strips of masking tape. The head and thorax were carefully attached to the slide preventing their movement, while the abdomen protruded from the superior edge of the slide, allowing this segment to move freely. The slide with the mounted insect was then placed vertically, with the head of the experimental subjects pointing downwards and the abdomen upwards (Fig. 1). Wasps recovered from cooling within few minutes after mounting. A small tie-clip omnidirectional microphone (Sennheiser MKE2-60 with a K6 power module, flat frequency response between 20-20,000 Hz) was placed laterally at 1.5 cm from the abdomen of the mounted insect and spontaneous stridulations were recorded for at least six minutes with a digital recorder (16 bits and 44.1 kHz, Olympus LS-100). Between trials, the plastic slide was thoroughly cleaned with 99% ethanol to minimize the presence of chemical cues on this device. The experimental procedure did not harm the insects, as individuals moved and behaved normally after being dismounted. Recordings were conducted between 17:00-17:30 hours at 26 °C inside a sound-attenuated booth having walls and ceiling covered with 50 cm height foam wedges.

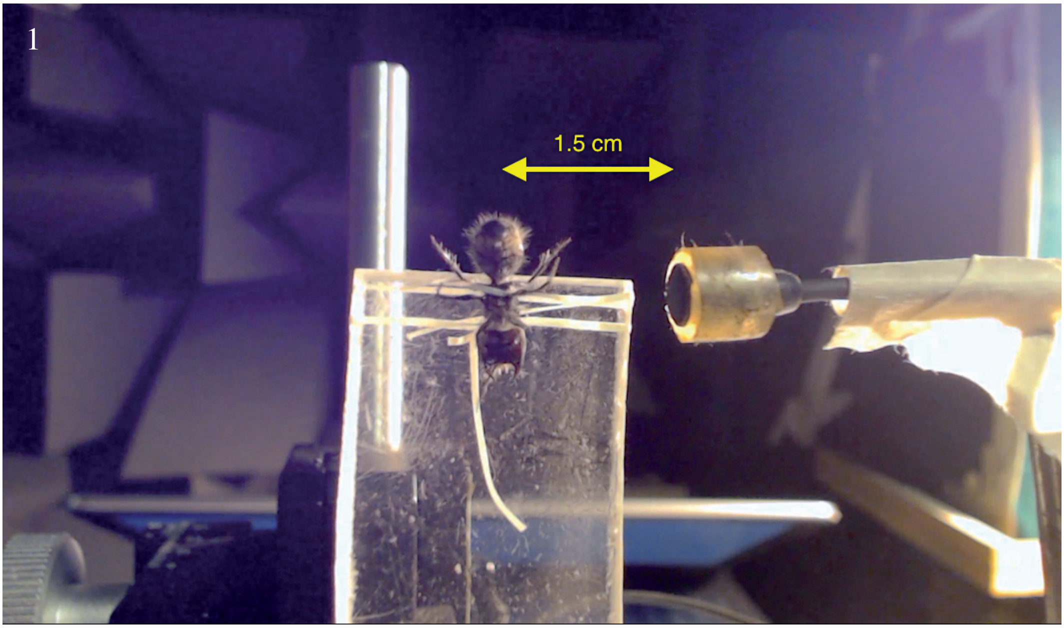

Figure 1. Experimental setup used to record the distress stridulation of velvet ants. The tip of the microphone (Sennheiser MKE2-60 with K6 power module) was placed laterally at 1.5 cm from the abdomen of the mounted individual.

Signal analysis

The stridulatory signal emitted by Mutillidae wasps corresponds to a chirp composed of two different notes generated by the forward and backward motion of the stridulatory organ (Polidori *et al.* 2013a) (Fig. 2). For each female, a sequence of five consecutive chirps was chosen for acoustic analyses. Recordings were band-stop filtered between 0 and 500 Hz to reduce the presence of low-frequency noise. To compare our measurements with other mutillids, we measured acoustic variables similar to those used by Polidori *et al.* (2013a) to describe the signal of six species of European velvet ants. Four acoustic variables were computed for forward and backward notes: 1) number of pulses in each note, 2) note duration (ms), 3) minimum frequency, the frequency (Hz) at which the spectrum amplitude was 10 dB below the peak amplitude on the low-frequency side (hereafter min.

freq.$_{-10dB}$) and 4) maximum frequency, the frequency (Hz) at which the spectrum amplitude was 10 dB below the peak amplitude on the high-frequency side (hereafter max. freq.$_{-10dB}$). In addition, the following chirp acoustic properties were measured: 1) inter-note interval (ms), 2) chirp duration (ms), 3) chirp repetition interval (ms) and 4) chirp rate (chirps/s). Spectral variables (i.e., min. freq.$_{-10dB}$ and max. freq.$_{-10dB}$) were obtained from mean power spectra (Hanning window, 512 points, 0% overlap) computed for each note (Fig. 3) and the repetition interval corresponded to time interval between the onsets of two consecutive chirps. All the acoustic analyses were conducted with RavenPro 1.4 (Cornell Lab of Ornithology, Ithaca, NY, USA) and the package 'seewave' (version 2.0.5, Sueur *et al.* 2008) in R (version 3.3.3, R Core Team 2017).

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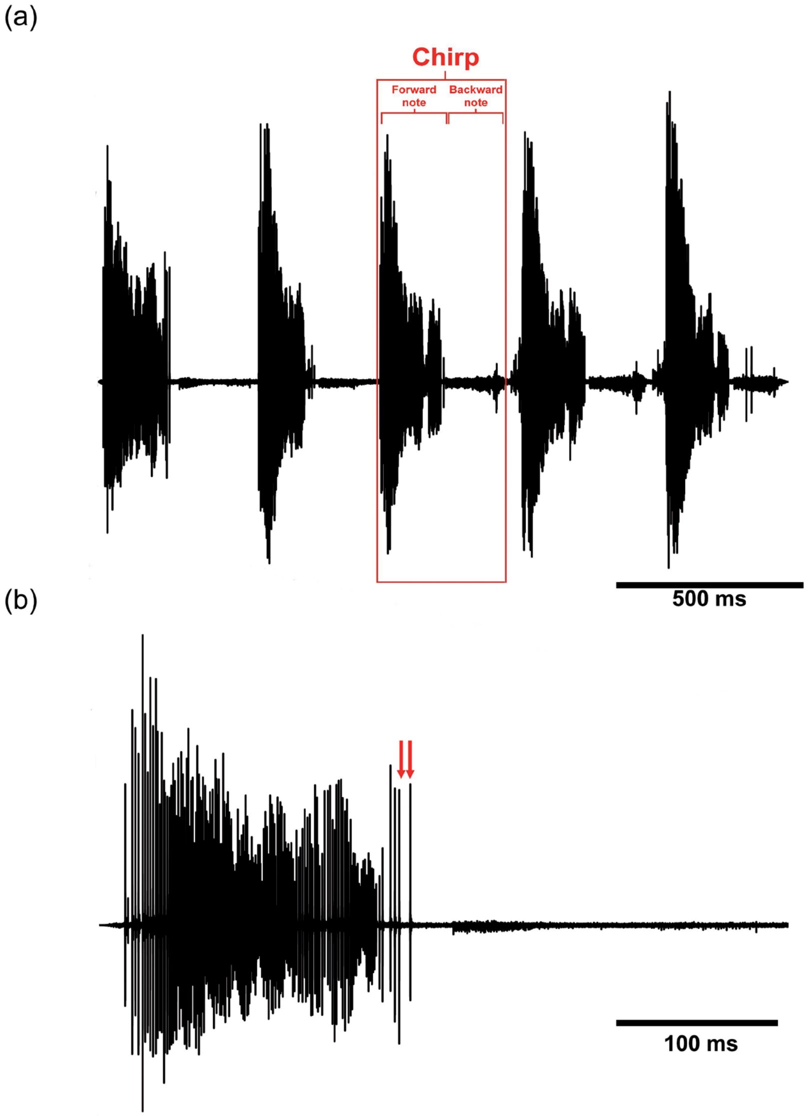

Figure 2. Distress stridulation of *Euspinolia chilensis*. (a) The forward and backward notes of the third chirp of the sequence are highlighted in red. (b) Expanded view of the first chirp of the sequence. The red vertical arrows show the last two pulses of the forward note of the chirp. Note the low amplitude of the backward note relative to the amplitude of the forward note.

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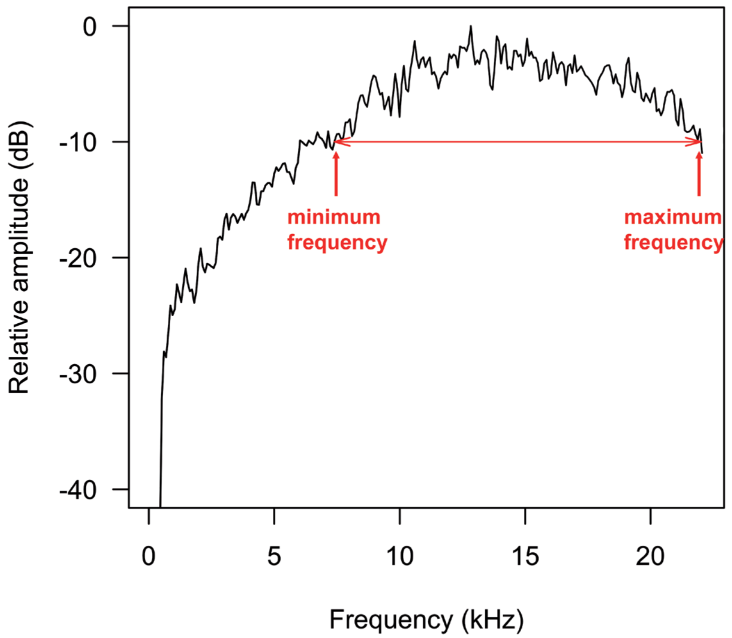

Figure 3. Mean power spectrum (Hanning window, 512 points, 0% overlap) obtained for the forward note of the chirp shown in Figure 2. The horizontal red arrow shows the frequency bandwidth 10 dB below the peak amplitude of the spectrum and the vertical red arrows show the minimum and maximum frequencies measured for this note.

Results

Similarly to the distress stridulation emitted by other Mutillidae wasps, the chirps of female *E. chilensis*, *E. militaris* and *R. gayi* comprise two notes. The backward note of *R. gayi* was visually identifiable in the waveform, however the backward notes emitted by *E. chilensis* and *E. militaris* had very low amplitude, and thus the spectral properties and number of pulses could not be measured (Figs. 4(a), (b), (c)). Table 1 summarizes

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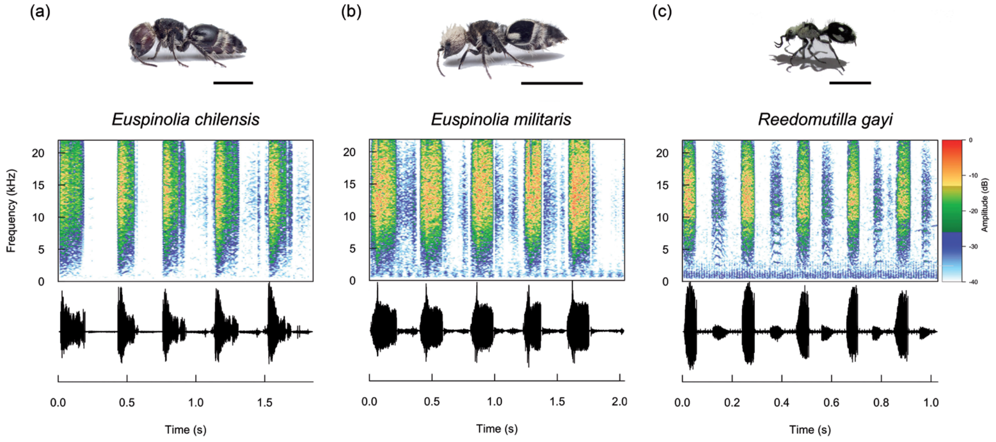

Figure 4. Spectrograms (Hanning window, 512 points, 0% overlap) and oscillograms of the distress stridulation of female (a) *Euspinolia chilensis*, (b) *Euspinolia militaris* and (c) *Reedomutilla gayi*. Temperature during recordings was 26 °C. Black scale bars represent 0.5 cm.

the acoustic variables measured for the three species and the values reported for other Mutillidae wasps (Polidori *et al.* 2013a). Overall, our acoustic analyses show that many of the variables measured for Chilean velvet ant species do not fall within the ranges reported for European species. This is the case for *E. chilensis* and *E. militaris*, as all the temporal and spectral properties measured for the distress stridulation emitted by these species are beyond the range of values previously reported for Mutillidae wasps (Table 1). Among the differences we found that the forward notes of *E. chilensis* and *E. militaris* have a larger number of pulses and longer duration. Also, chirp duration and chirp repetition interval are longer, and chirps are emitted at a lower rate in these two species. Most notably, we found that the max. freq. $_{\text{-10dB}}$ of the forward notes, which are the main chirp components, extends beyond the about 17 kHz previously reported for other Mutillidae wasps, reaching values of up to 22 kHz, which is near the limit of frequencies detectable with our recording setup. This result indicates the presence of ultrasonic components in the stridulation of *E. chilensis* and *E. militaris*.

In contrast to the stridulation of *E. militaris* and *E. chilensis*, our acoustic analyses show that several of the variables measured for *R. gayi* are within the values previously reported for European velvet ants, except for a higher min. freq. $_{\text{-10 dB,}}$ a lower max. freq. $_{\text{-10 dB}}$, a larger inter-note interval and a lower chirp repetition interval (table 1).

Table 1. Temporal and spectral properties of the distress stridulation of female *Euspinolia chilensis*, *Euspinolia militaris* and *Reedomutilla gayi*. The values in the table show the mean ± SD [min. – max.] of the five chirps analyzed for each subject of each species. The range [min. – max.] of average values reported by Polidori *et al.* (2013a) for six species of European Mutillidae wasps are also shown for comparison.

Signal component	Acoustic variable	*E. chilensis*	*E. militaris*	*R. gayi*	Other Mutillidae wasps
Forward note	Min. freq$_{\text{-10 dB}}$ (kHz)	8.4 ± 0.7 [7.8 – 9.5]	7.8 ± 0.3 [7.4 – 8.3]	10.5 ± 1.0 [9.5 – 11.8]	[4.1 – 6.0]
	Max. freq$_{\text{-10 dB}}$ (kHz)	21.6 ± 0.3 [21.2 – 21.8]	21.6 ± 0.3 [21.1 – 21.9]	13.1 ± 1.5 [11.2 – 14.8]	[15.3 – 17.4]
	Number of pulses	191.6 ± 30.1 [160 - 223]	250.8 ± 9.2 [235 - 259]	65.0 ± 9.1 [51.0 – 76.0]	[25.5 – 110.4]
	Duration (ms)	182.3 ± 21.0 [152.1 – 204.2]	183.5 ± 22.2 [145.9 – 216.7]	51.8 ± 4.6 [45.6 – 58.1]	[34.6 – 85.9]
Backward note	Min. freq$_{\text{-10 dB}}$ (kHz)	-	-	9.5 ± 1.1 [7.6 – 14.4]	[4.4 – 6.0]
	Max. freq$_{\text{-10 dB}}$ (kHz)	-	-	11.1 ± 2.2 [8.1 – 10.4]	[15.3 – 17.4]
	Number of pulses	-	-	62.8 ± 6.8 [55.0 – 72.0]	[24.2 – 112.8]
	Duration (ms)	155.2 ± 24.2 [128.1 – 193.1]	205.9 ± 42.3 [161.5 – 272.3]	50.5 ± 6.4 [38.7 – 58.1]	[31.9 – 91.5]
Chirp	Inter-note interval (ms)	11.8 ± 7.7 [2.0 – 23.2]	10.5 ± 3.3 [7.5 – 15.6]	55.5 ± 7.3 [46.9 – 64.0]	[12.7 – 37.0]
	Chirp duration (ms)	349.2 ± 34.8 [303.7 – 397.1]	395.3 ± 45.4 [339.2 – 463.8]	156.4 ± 12.7 [143.7 – 172.2]	[89.5 – 196.1]
	Repetition interval (ms)	368.1 ± 38.6 [326.4 – 415.7]	393.6 ± 27.9 [352.9 – 417.5]	51.3 ± 6.9 [43.4 – 60.1]	[109.8 – 233.2]
	Chirp rate (chirps/s)	2.7	2.5	5.1	[4.4 – 9.6]

Discussion

Although quantitative data on the stridulation of velvet ants is scarce, detailed acoustic analyses have been conducted for the distress stridulation emitted by females of six species of European mutillids (Polidori *et al.* 2013), which allowed our comparison with the three South American species. Our analyses show that the distress stridulation emitted by the females of three velvet ant species native to Chile greatly differ from those studied in other mutillid species, particularly in the number of pulses in each note, note duration minimum and maximum frequency of the forward note.

Although in the present study we did not examine the metasomal stridulatory organ, the differences found between the distress calls of European and South American velvet ant species could be explained by differences in the morphology of this structure. A previous study found that some morphological measures of the stridulatory organ, such as the width of the third tergite and the file, the thickness of the ridges, and the inter-ridge distance were strongly associated with the spectral properties of the distress signals of the velvet ant *Myrmilla capitata* (Lucas, 1849) (Polidori *et al.* 2012). In contrast, in this species, most of the temporal parameters of the stridulation (i.e. number of pulses of the forward note, duration of the forward syllable, and chirp duration, among others) were unrelated to the morphology (Polidori *et al.* 2012), suggesting that other mechanical processes could account for temporal patterns of stridulation in Mutillidae. Yet, further biomechanical studies are required to elucidate the interplay between morphology and sound production in velvet ants, especially in South American species.

As there is no available information on the natural predators of *E. chilensis* and *E. militaris*, the defensive significance of emitting distress stridulatory signals having ultrasonic components is unknown. Reptiles are known to predate velvet ants (Best and Pfaffenberger 1987; Manley and Sherbooke 2001) and the selective pressure exerted by lizard predation has been suggested to drive the evolution of Müllerian colour mimicry in several North American and African velvet ant species (Wilson *et al.* 2012, 2018; Pan *et al.* 2016). However, reptile hearing sensitivity is restricted to relatively low frequencies, typically below 8 kHz (Manley 2011), and thus they are an unlikely audience for the ultrasonic components of the distress stridulation of *E. chilensis* and *E. militaris*. Rodents have also been reported to predate velvet ants (Schmidt and Blum 1977; Masters 1979), and the auditory sensitivity of these mammals usually extends beyond 20 kHz (Heffner *et al.* 2001). Therefore, the ultrasonic stridulation of some South American velvet ants could be involved in deterring rodent predators or at least serve as warning signals for this type of potential predators (Tschuch and Brothers 1999; Polidori *et al.* 2012). This is a plausible idea, given that in an experimental setup, mice seemed to be more hesitant in attacking a stridulating velvet ant (Masters 1979). Also, the species studied in the present report are larger than 10 mm, and the sound power level of insect stridulation depends on body size (Polidori *et al.* 2012).

Besides its predator deterrence function, the distress stridulation of velvet ants could also have a role in intra-specific communication. Nearby individuals may benefit from detecting and responding to the distress stridulation emitted by conspecifics. For example, weeping lizards (*Liolaemus chiliensis* Lesson, 1830) remain immobile for a longer period of time after hearing conspecific distress calls as compared to individuals exposed to a control sound, suggesting that in the presence of this signal individuals adjust their behavior to reduce predation risk (Hoare and Labra 2013). Although this possibility has not been formally evaluated in Mutillidae wasps, further behavioral experiments combined with field observations will contribute to elucidate the adaptive significance of distress signaling in velvet ants, and its potential role in inter- and intra-specific communication.

In summary, we provide the first detailed acoustic description of the stridulatory distress signal of South American velvet ants. Both the spectral and temporal properties of the stridulation emitted by two of the studied species differ from the values reported for other Mutillidae wasps; however, the lack of repetitions (only one female per species was analyzed) limits the conclusions drawn in this study, thus there is a necessity to analyze more specimens, as well as more species, to allow any generalization. Additionally, more studies are needed to understand the defensive role of the broadband distress stridulation of South American velvet ants, including the identification of their natural predators or explore the potential role of stridulation as a mating communication signal, as has been observed in other Mutillidae such as *Nemka viduata* (Tormos *et al.* 2010).

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