

# The Acoustic Behaviour as a Tool for Biodiversity and Phylogenetic Studies: Case of the *Rhammatocerus* Species Inhabiting Uruguay (Orthoptera, Acrididae, Gomphocerinae)

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## 1. Introduction

Species diversity does have a pivotal role in the study and perception of biodiversity (Boero, 2010). One of the goals of Zoology is the study of the animal diversity, that is, the animal species, and Taxonomy is one of the basic disciplines to achieve it. Currently, biodiversity research requires a multidisciplinary approach (Boero, 2009), many different disciplines being involved, such as morphology, molecular biology, ecology, ethology..., that provide new characteristics to be considered. The study of biodiversity should proceed with the contribution of integrative taxonomy (Boero, 2010), taking into account, in addition to the traditional taxonomy, other disciplines of great utility, such as the study of the behaviour.

In this context, behaviour and sounds are relevant characteristics to discover new taxa (Valdecasas, 2011). Sound production in insects is widespread and has been recorded in different orders. It is involved in different behaviours, the most important of which are defence against predators, aggression and mating or sexual behaviour. Four types of senses are used by insects in their sexual behaviour: tactile, visual, chemosensory and acoustics. The acoustic sense is that generally have received more attention, this stimulus is often heard by humans and their production involves the movement of specialized structures that can be seen, usually directly. The types of sounds produced and producing mechanisms can be framed primarily in three categories: stridulation, vibration and percussion. The sounds of insects have been classified in the context of behaviour in several types: call or proclamation, courtship, aggregation, aggression, mating and sounds of interaction (Lewis, 1984; Ragge & Reynolds, 1998).

The sound on Orthoptera serves to promote social relations in the broadest sense of the term. This type of acoustic behaviour cannot be studied in isolation and cannot be understood except within the framework of the general behaviour of the species that is not just the sound production. From this point of view, we show that an exact knowledge of the physical elements of the sounds, as well as the morphology of these sound-producing organs is essential (Busnel, 1954). The sounds produced by the Orthoptera are important from a taxonomic point of view and has an important role as a mechanism of identification. They are of great value to establish the real status of local populations that may have few morphological differences (Blondheim, 1990; García et al., 1995).

The process of acoustic communication in reproduction is amply documented in Ensiferan insects (crickets and katydids): males of singing Ensifera (Orthoptera) emit specific calling songs used for species recognition, while courtship songs are generally less specific and could be mostly under the influence of sexual selection. In contrast, singing Caelifera (Orthoptera) perform more diverse behaviour prior to mating. In the subfamily Gomphocerinae (Acrididae) more specifically, many species emit calling songs, which are sufficiently specific to be used for species identification in the field, but they also perform very complex, often multimodal, courtship behaviour involving sequences of acoustic, vibrational and/or visual signals (Nattier et al., 2011; Ragge & Reynolds, 1998).

Neotropical Gomphocerinae form a group of grasshoppers whose taxonomy, systematics and biology are still poorly known (Otte, 1979; Otte & Jago, 1979). Within this group, *Rhammatocerus* Saussure, 1861 is widely distributed, from southern USA to central Argentine. Its species have a great economic importance; many of them are important crop and grazing pests (Carbonell et al., 2006; Cigliano & Lange, 1998; Salto et al., 2003), especially in Brazil and Colombia (Lecoq & Assis-Pujol, 1998). The genus *Rhammatocerus* is related to other genera such as *Parapellopedon* Jago, 1971 and *Cauratettix* Roberts, 1937.

Since its description, the most important studies on this genus are due to Jago (1971) and Carbonell (1995), but no taxonomical revisions of its entire species have been performed. At present, the genus is composed of 13 species (Assis-Pujol, 1997a 1997b, 1998; Carbonell, 1995), some of them still not clearly defined (Carbonell pers. comm.). The group is characterized by a high intraspecific variation and certain heterogeneity of its external morphology. So, other characters than morphological have been searched to clearly separate the species. As regards the species identification, in tribe Scyllinini, like in other Gomphocerinae, the genitalia allow differentiation between close species in only rare cases (Carbonell, 1995). The phallic complex is an important morphological character among Acrididae but, in Gomphocerinae, it has not a practical value (Carbonell, 1995). The female genitalia, especially the spermatheca, have revealed its utility in identifying some species (Assis-Pujol & Lecoq, 2000). Furthermore, molecular studies have not provided information helping species identification (Loreto et al., 2008).

As pointed before, the sounds produced by Orthoptera are of great taxonomic value. They play a well-known role as identification system during mating, and for this reason they are of great value for establishing the real status of local populations that display small morphological differences (Blondheim, 1990; García et al., 1995). The study of sounds produced by Gomphocerinae has repeatedly demonstrated its utility to solve species identification problems (Ragge & Reynolds, 1998); however the acoustic behaviour of

Neotropical Gomphocerinae has been studied to a very limited extent (COPR, 1982; García et al., 2003; Lorier, 1996; Lorier et al., 2010; Riede, 1987).

*Rhammatocerus pictus* (Brunner, 1900) and *Rhammatocerus brunneri* (Giglio-Tos, 1895) are robust species inhabiting wet high pastures in northern and central South America, between about latitudes 15 and 40 South. *Rhammatocerus pictus* is distributed in central and southern Brazil (Rio Grande do Sul), Paraguay, Chile (Malleco), northern and central Argentina, Bolivia and Uruguay (Assis-Pujol, 1998; Carbonell et al., 2006) and *Rhammatocerus brunneri* in central-southern Brazil, Paraguay, Bolivia and Uruguay (Assis-Pujol, 1997a). In Uruguay, both species occupy similar habitats (low humid areas covered with tall and dense grass and low hill slopes). They are distributed in the country (Assis-Pujol, 1998) and are more frequently found north of Río Negro. Both species are sympatric in Artigas, Rivera and Lavalleja Departments (Assis-Pujol, 1997a, 1998).

These two species are systematically close to each other, having been separated by Assis-Pujol (1998) just on the basis of morphological criteria: colour of hind femora and tibiae and the spermatheca shape. Their acoustic behaviour is up to date unknown.

Our objective is to establish the real status of these two taxa at the light of their sound production and acoustic behaviour, describing the songs produced in different behavioural situations, the behavioural units identified and the sound-morphological structures.

## 2. Materials and methods

This study on the sound production and stridulatory structures of *Rhammatocerus pictus* and *Rhammatocerus brunneri* was conducted with males and females proceeding from the "Colección de Entomología de la Facultad de Ciencias de la Universidad de la República", Uruguay, and specimens captured in Uruguay.

### 2.1 Microscopy techniques and characters considered in stridulatory file study

To study the structure of the stridulatory apparatus, 10 specimens of each sex and species (Table 1) were observed under a binocular microscope (Olympus SZH provided with 10X ocular lenses, 0.66-4X zoom objective, 2X lens and graduated eye piece) as well as with a Jeol 6100 scanning microscope, equipped with SEI (secondary electron images), working at an acceleration voltage of 15 kV and at 21 mm (working distance). Images were captured with the LINK ISIS program. Because the samples are hard and have not risk of being dehydrated, they only had to be cleaned as proposed by Clemente et al. (1989) and, then, coated with pure gold.

Measurements were taken by means of a sliding stage mounted on the stereoscopic binocular microscope, the displacement of which was measured by an attached dial calliper in combination with a graduated eye piece. The accuracy of the dial calliper was 0.05 mm.

For this study, the shape of the file and pegs have been taken into account, as well as the number of pegs in the file, femur length (HFL), file length (FL), peg density all along the file (PD) and in its middle area (PDM) and file length / femur length ratio (FLx100/HFL). Measurements are expressed in mm (Table 2).

	Specimens	Locality	Date	Collector
<i>Rhammatocerus brunneri</i>	4 males (n° 61, 62, 63, 65)	Cerro Chato Dorado. Rivera. Uruguay	12-II-2000	Clemente, García, Lorier
	1 male (n° 64) 2 females (n° 93, 94)	Sierra de la Aurora. Rivera. Uruguay	14-III-1961	C.S.C, A. Mesa, P. San Martín
	2 males (n° 67, 68) 1 female (n° 91)	Cuchilla de Cuñapirú. Rivera. Uruguay	21-I-1956	C.S.C.
	1 males (n° 69) 3 female (n° 86, 90, 95)	Ronda Alta. Río Grande do Sul. Brasil	24-II-1964	A. Mesa, M.A. Monné
	1 males (n° 70)	Bom Jesús. Río Grande do Sul. Brasil	26-II-1964	A. Mesa, M.A. Monné
	1 male (n° 71) 4 females (n° 87, 88, 89, 92)	40 km. N de Caaguazú. Paraguay	13-III-1965	C.S.C, A. Mesa, M.A. Monné
<i>Rhammatocerus pictus</i>	2 males (n° 56, 59)	Cerro Chato Dorado. Rivera. Uruguay	17-18-III- 2001	E. Lorier
	2 males (n° 60, 66)	Lunarejo. Rivera. Uruguay	1-IV-1999	E. Lorier
	1 male (n° 57)	V.Lunarejo Rivera. Uruguay	13-II-2000	Clemente, García, Lorier
	1 male (n° 72) 1 female (n° 79)	S <sup>a</sup> de la Aurora. Rivera. Uruguay	14-III-1961 11-III -1961	C.S.C, A. Mesa, R. San Martín
	1 male (n° 73) 1 female (n° 76) 1 female (n° 77)	Las Piedras. Canelones. Uruguay	5-II-1966 5-XI-1966 20-III-1964	A. Cármenes
	1 male (n° 74)	Pto. Pepeají. Paysandú. Uruguay	IV-1954	C.S.C.
	1 male (n° 58)	Buena Vista Agraciada. Soriano. Uruguay	8-II-2001	García, Lorier, Presa
	1 male (n° 75) 1 female (n° 83)	Lagoa Vermelha. Rio Grande do Sul. Brasil	18-II-1964	C.S.C, A. Mesa, M.A. Monné
	1 female (n°78) 1 female (n° 80)	Ayo. Tres Cruces. Artigas. Uruguay	12-XI-1955 14-II-1955	Fac. Humanidades y Ciencias
	1 female (n° 81)	Tartagal. Salta. Argentina	29,31-I- 1965	A. Mesa, R. Sandulski
	2 females (n° 82, 84)	San Lorenzo. Salta. Argentina	3-II-1965	A. Mesa, R. Sandulski
	1 female (n° 85)	Nonoai. Río Grande do Sul. Brasil	20-II-1964	A. Mesa, M.A. Monné

Table 1. Summary of the information concerning origin, collection date and number of specimens used to study the sound producing organs of *Rhammatocerus*.

		HFL (mm)	FL (mm)	Pegs	PD (mm <sup>-1</sup> )	PDM (mm <sup>-1</sup> )	FL x 100/HFL
<i>R. brunneri</i>	Males n=10	16.96 ± 0.76 (15.80-17.90)	5.09 ± 0.76 (4.5-5.70)	74.4 ± 12.4 (58-99)	14.60 ± 1.98 (11.60-18.68)	19.5 ± 3.2 (13-24)	29.96 ± 0.97 (28.61-31.84)
	Females n=10	23.86 ± 0.86 (22.70-25.25)	11.61 ± 0.73 (10.70-12.90)	84 ± 7.1 (72-95)	7.26 ± 0.70 (5.81-8.12)	12.6 ± 1.9 (10-16)	49.02 ± 3 (44.95-53.69)
<i>R. pictus</i>	Males n=10	17.92 ± 0.94 (16.50-19.40)	5.21 ± 0.62 (4.40-6.00)	73 ± 10.70 (58-90)	14.18 ± 2.61 (11.05-18.88)	18.6 ± 2.8 (14-22)	29.04 ± 2.78 (24.71-33.33)
	Females n=10	22.78 ± 1.32 (21.10-24.65)	10.78 ± 1.05 (8.95-12.05)	74.2 ± 3.29 (69-79)	6.94 ± 0.69 (6.09-8.16)	9.8 ± 1.8 (8-12)	47.57 ± 6.10 (36.30-54.50)

Table 2. Summarized data related to stridulatory apparatus of *Rhammatocerus brunneri* and *R. pictus*. HFL: hind femur length; FL: whole stridulatory file length; Pegs: total number of pegs in the whole file; PD: density of pegs in the whole file; PDM: density of pegs in the middle of the file. Values are expressed as mean ± SD and range in parenthesis.

Two-ways multivariate analysis of variance (MANOVA) was used to assess whether there were overall differences between study species regarding the stridulatory file, considering as dependent variables the abovementioned morphological traits. In this case separated analyses were run for each sex. For these and all other statistical analyses, software SPSS (v. 15.0) was used. All variables were log-transformed. The signification value, in all the cases, was  $P \leq 0.05$ .

## 2.2 Study of behaviour and sound production

This study was conducted with 9 males (Table 3), 6 males of *R. pictus* and 3 males of *R. brunneri*, and 312 different songs (126 from *R. pictus* and 186 from *R. brunneri*), registered in 5 different tapes (Table 3). The specimens were held in cages in the laboratory and fed mainly with grasses that were changed daily. Humidity was provided by daily watering and by cotton imbibed in water. Two types of cages were used: a glass cage, with net top, 20 x 11 x 14 cm, and a wooden cage with metal mesh top and glass front, 35 x 35 x 55 cm, both exposed to natural light or artificial light provided by a 40W bulb 12 hours per day (Table 3).

The sounds produced were recorded under different conditions, such as isolated specimens, a male together a female of the same species, a species together other different species, both species together.

Sound recordings were made in the laboratory (Table 3) using a Uher 4000 and a Uher 6000 analogical tape recorders (Uher Werke München, Barmseestrasse 11, 8000 München 71, Germany), at a tape speed of 9.5 cm/s, with a frequency response (in Hz) of 20-25000 and signal-to-noise ratio better than 66 dB A remote-control Uher M655 and a Uher M518 dynamic microphones, were located 10-20 cm from the specimens.

		Locality of capture	Date and collector	Recording date	Recording conditions	Tape	Type of sound and recording number
<i>R. pictus</i>	1 male	Cerro Batoví. Tacuarembó. Uruguay	1/IV/1999 E.Lorier	18/IV/1999	Wood cage, (bulb 25W) 20°C	EL6/1999	1 calling 4 courtship Rec.3/6/99
	1 male	Valle del Lunarejo. Rivera. Uruguay	13/II/2000 Clemente, García, Lorier	14/II/2000	Wood cage, (bulb 40W) 33°C	EL3/2000	1 calling Rec. 2/3/2000 Rec.3/3/2000
	1 male	Cerro Chato Dorado. Rivera. Uruguay	11/II/2000 Clemente, García, Lorier	22/II/2000	Wood cage, (2 bulbs 40W) 31-32°C	EL4/2000	4 calling 14 courtship Rec. 2/4/2000
	3 males	Rivera. Uruguay	18/III/2001 Lorier	20/III/2001 23/III/2001	Glass cage (bulb 40W) 27-28 °C	EL3/2001	96 disturbance 6 courtship Rec. 1/3/2002 Rec. 2/3/2001
<i>R. brunneri</i>	3 males	Cerro Chato Dorado. Rivera. Uruguay	12/II/2000 Clemente, García, Lorier	14/II/2000	Wood cage, (bulb 40W) 33°C	EL2/2000	36 disturbance 2 calling Rec. 8/2/2000
					Wood cage, (bulb 40W) 32°C	EL3/2000	118 disturbance 10 calling 7 courtship Rec. 1/3/2000 Rec. 3/3/2000
				22/II/2000	Wood cage, (2 bulbs 40W) 32°C	EL4/2000	1 courtship 12 disturbance Rec. 1/4/2000

Table 3. Summary of the information concerning specimens used to study the acoustic behaviour of *Rhammatocerus* and studied recordings.

Observations of communicative and interactive behaviour and of the general activity of individuals were made in the laboratory and recorded with a JVC GR-AXM23 video-camera for subsequent analysis. Specimens were observed throughout the recordings, including mute periods, and the behaviour of the specimens in each situation was noted.

Sound recordings were analysed using a Mingograph 420 System attached to a digital oscilloscope (Tektronix 2211) and to a Krohn-Hite 3550 filter. To study the physical

characteristics of the sound, the analogical signal was digitized with a Sound Blaster® AWE64 Gold at 8 bits and at a 44 kHz sampling frequency, and then studied using the Avisoft® SAS Lab Pro 3.8. PC software for MS-Windows. Oscillograms were obtained using the option One-dimensional functions, selecting the function Time-signal. The spectral characteristics were obtained using the same option than before, selecting, in the function Amplitude spectrum (linear), the Hamming evaluation window, bandwidth 0.526 Hz and resolution 0.336 Hz.

Males of both species produce different types of sound in different behavioural situations fitting with the categories from the literature (García et al., 2003; García et al., 2005; Ragge & Reynolds, 1998). The terminology used to describe songs follows that of Ragge & Reynolds (1998). The variables used for the study of the sounds are: echeme length, number of syllables, syllables length, rate of emission of syllables, peak of maximum amplitude, low quartile, middle quartile, upper quartile, minimum frequency, maximum frequency and band width. The following types of sounds were identified: (1) the calling song, produced spontaneously by a male; (2) the courtship song, produced by males when close to a female and (3) the disturbance song, produced by males when interacting with other individuals.

To explore the relationship among different types of sounds produced by the studied species, a principal component analysis (PCA) was performed considering all the variables used to study the sounds (see before). Since for disturbance songs not all response variables could be recorded, they were not included in the PCA. Two-ways MANOVA was used to formally assess the differences among type of sound (calling song and courtship song) and species (*R. pictus* and *R. brunneri*), considering the variables used for the study of sounds as dependent variables. When cases corresponding to different groups overlapped after running the PCA, a separate MANOVA was performed for these groups considering the same dependent variables and factors as for the overall MANOVA. Variables were log-transformed for both PCA and MANOVA and a significance level of 5% was selected.

Specimens and recordings are kept in the “Colección de Entomología de la Facultad de Ciencias de la Universidad de la República”, Uruguay, and in the “Colección del Área de Zoología de la Universidad de Murcia”, Spain. Recordings can be reviewed using the recording number (Table 3). Sample songs are also available at OSF (<http://osf2.orthoptera.org>) (Eades 2001).

### 3. Results

The sounds recorded, emitted by males in different behavioral situations, were all produced by femoro-tegmina mechanism, rubbing the pegs of the stridulatory file of hind femora against some specialized tegmina veins.

No differences between songs have been observed in relation to the recording conditions. No sound produced by females has been registered in any case, although they produce movements with their hind legs. These movements are of moderate amplitude, starting with the leg folded at about 45° respect the vertical and displacing about 45° from the starting position. This movement can be isolated, from resting position to the vertical, or be repeated several times, when close to other individuals, males or females.

### 3.1 Stridulatory file

In *Rhammatocerus pictus* the hind femora of both sexes have, along their inner surface, a stridulatory file that is almost linear and long in relation to femur length. The stridulatory pegs are well developed and regularly spread except at ends, where they are more irregularly and more separately distributed (Fig. 1A-D).

Male file, although shorter than that of the female, contains more pegs than that of the female. The peg density on the whole file is greater in males than in females (Table 2, Figs. 1A and C). The male pegs are conic shaped. They are inserted in the alveolus, which have a raised margin, and have a short peduncle (Fig. 1B). The female pegs, well developed, are also conic shaped with rounded apex, and have a short peduncle. They are also inserted in alveolus with raised margins (Fig. 1D).

In both sexes of *Rhammatocerus brunneri* the stridulatory file is linear and long in relation to femur length. Pegs are well developed and regularly spread except at ends, where they are more separated and irregularly disposed (Fig. 1 E-H).

Male file is half the length than that of female, but it has almost the same number of pegs and, so, the peg density is greater (Table 2, Fig. 1E and G). The male pegs are conic shaped and have a short peduncle. They are inserted by in the alveolus, which have a raised margin (Fig. 1F). The female pegs are also conic shaped, slightly irregularly spread and more separated than in males. Pegs are also inserted by a short peduncle in alveolus with raised margins (Fig. 1H).

The MANOVA revealed the lack of overall differences among species regarding the stridulatory file in the case of males ( $F_{5, 14} = 1.563$ ;  $P = 0.234$ ), although significant differences were detected for femur length ( $F_{1, 18} = 6.205$ ;  $P = 0.023$ ). Females of different species showed significant overall differences ( $F_{5, 14} = 3.724$ ;  $P = 0.024$ ), as well as for the responses variables number of pegs in the file ( $F_{1, 18} = 15.947$ ;  $P = 0.001$ ) and peg density all along the file ( $F_{1, 18} = 11.920$ ;  $P = 0.003$ ).

### 3.2 Calling and courtship songs

The PCA performed extracted two components explaining most of the variance of the original data (79.5%). Component 1 was positively correlated with those variables dealing with frequency and negatively with duration of the echeme. Component 2 was positively correlated with both the number of syllables and the rate of emission of syllables and negatively with the duration of syllables (Fig. 2).

When scores for each case were plotted against component 1 and 2 three major groups were identified: 1) courtship and calling songs of *R. brunneri* (closely overlapped); 2) calling song of *R. pictus* and one of the types of courtship song of this species (*pictus* 1); 3) other type of courtship song of *R. pictus* (*pictus* 2) (Fig. 3). The MANOVA performed for both the courtship and calling songs of the studied species indicated the existence of overall significant differences among species ( $F_{11, 23} = 18.042$ ;  $P < 0.0001$ ) and type of song ( $F_{33, 68.47} = 6.318$ ;  $P < 0.0001$ ).

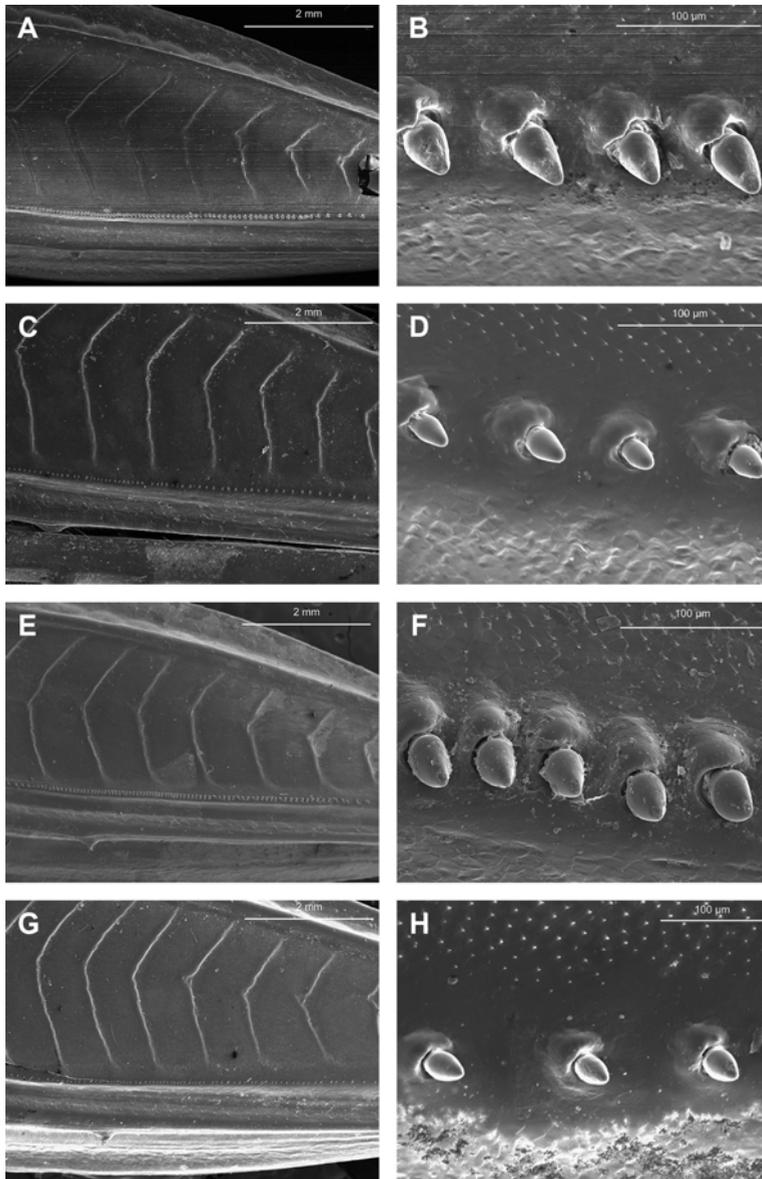


Fig. 1. Stridulatory files. A-D: *Rhammatocerus pictus*. A: male file general appearance, B: detail of male pegs of middle zone, C: female file general appearance, D: detail of female pegs of middle zone. E-H: *Rhammatocerus brunneri*. E: male file general appearance, F: detail of male pegs of middle zone, G: female file general appearance, H: detail of female pegs of middle zone.

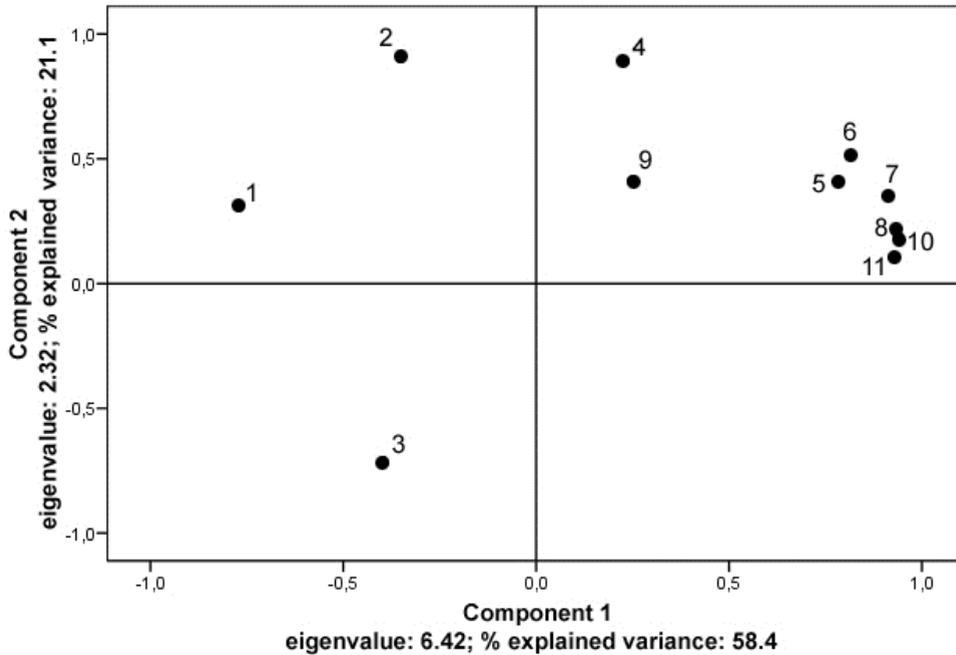


Fig. 2. Results of PCA performed to explore relationships among types of sound considering all the variables used: 1 echeme length; 2 number of syllables; 3 syllables length; 4 rate of emission of syllables; 5 peak of maximum amplitude; 6 low quartile; 7 middle quartile; 8 maximum frequency; 9 minimum frequency; 10 upper quartile; 11 bandwidth

### 3.2.1 *Rhammatocerus pictus*

Calling song was a spontaneous song, consisting of echemes composed of a variable number of syllables (Table 4).

Its frequency spectrum occupied a broad band, with the peak of maximum amplitude at around 7 kHz (Table 4, Fig. 4C).

The echeme started with short and almost inaudible syllables. The sound increased in intensity after 1/3 of the echeme had been emitted. Syllables were clearly separated one from other by evident gaps (Figs. 4A and B).

It was produced by synchronously rubbing the two hind femora against the tegminae, both hind legs moving rapidly. At the beginning of the echeme the legs movement was hardly visible.

Courtship songs were composed of echemes of a variable number of syllables (Table 4). Two different types of song have been observed, the here called pictus 1 (Fig. 4D-F) emitted at a

broad band, with the maximum amplitude at around 5 kHz, and the called pictus 2 (Fig. 4G-I) which occupied a much narrower band, with the maximum amplitude at around 2 kHz; both are significantly different (Fig. 3).

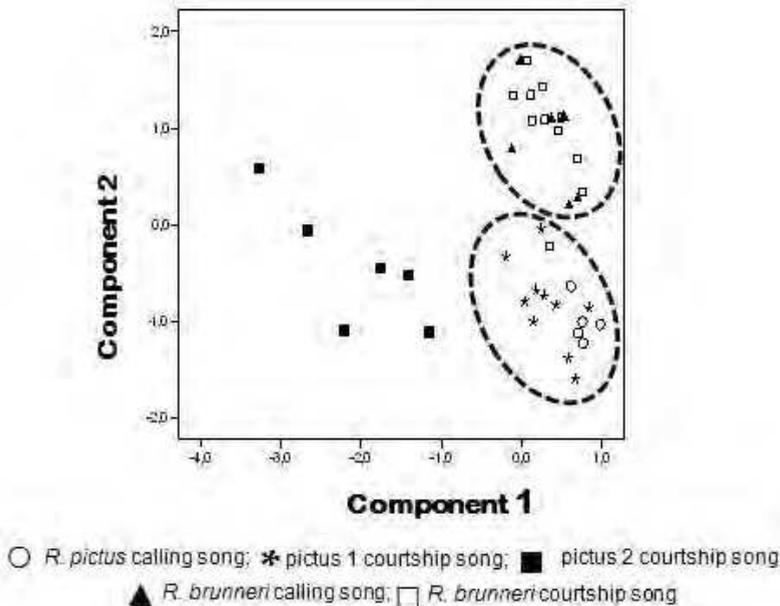


Fig. 3. Graphic showing the results of plotting scores for each cases against components 1 and 2 of PCA.

When the courtship started, the male moved walking towards the female; stopping when close and perpendicular to her. Then he moved his antennae up and down, synchronously at the beginning and, then, alternately, and started to sing. While singing, he directed his antennae towards the female, forming an around 150° angle. After the song, the male usually jumped suddenly on the female, trying to mate while touching her with his antennae. In most cases, the mating was not effective, the male being rudely rejected by the female by kicking the male, raising the hind legs or simply going away him.

While the courtship song, males performed two kinds of movement: 1) Mute movement: one to three slow and wide up and down movements of hind legs. They rose synchronously from the rest position (around 30° in relation to the main corporal axis) to around 75°, and then bent slightly asynchronously. 2). Stridulatory movement: a series of synchronous, quick and small up and down movements, of little amplitude (30-55°) respect to the corporal axis. In some cases, the movement 1 lacked, the courtship starting directly with the quick movement.

The songs started as imperceptible, almost inaudible, and sound increased in intensity until the end. At the final section, syllables were intense, similar in structure, with gaps between them (Fig. 4 D-E and G-H).

The MANOVA run separately for the group 2 identified by the PCA revealed that for this species, overall, calling song and pictus 1 courtship song were not significantly different ( $F_{11, 3} = 0.702$ ;  $P = 0.712$ ), existing differences only in the case of some of the response variables dealing with frequency ( $P \leq 0.037$ ): peak maximum amplitude ( $F_{1, 13} = 5.401$ ;  $P = 0.037$ ), low quartile ( $F_{1, 13} = 11.494$ ;  $P = 0.005$ ), upper quartile ( $F_{1, 13} = 6.026$ ;  $P = 0.029$ ), middle quartile ( $F_{1, 13} = 7.279$ ;  $P = 0.018$ ) and minimum frequency ( $F_{1, 13} = 11.527$ ;  $P = 0.005$ ).

### 3.2.2 *Rhammatocerus brunneri*

The calling song consisted of an echeme composed of syllables (Table 4, Fig. 5A and B), short and almost inaudible at the beginning and increasing in intensity further on. At the end, the echeme had a high intensity and the syllables are very close with almost no gap between them. The frequency spectrum of the sound occupies a broad band, the main peak being at around 8500 Hz (Table 4, Fig. 5C).

The leg movements to produce the sound are almost imperceptible at the beginning of the echeme. They became wider as the echeme went on and at the end legs seemed to move much quickly than before.

The courtship song was composed of echemes of a variable number of syllables (Table 4, Fig. 5D and E). As regards the spectral characteristics (Fig. 5F) they were similar to that of calling song there having not been found any statistically significant difference between this song and calling song.

The leg movements to produce the sound were also similar to that to produce calling song. When a male was performing a courtship, could be interrupted by other males, who started to perform the disturbance song.

During courtship the male follows the female, standing behind very close to her.

The MANOVA run separately for the group 1 identified by the PCA revealed that for *R. brunneri* there were not significant overall differences between courtship and calling songs ( $F_{11, 5} = 0.607$ ;  $P = 0.773$ ), although the duration of the echeme (Table 4) was significantly ( $F_{1, 15} = 4.786$ ;  $P = 0.045$ ) different.

## 3.3 Disturbance song

### 3.3.1 *Rhammatocerus pictus*

Songs were composed of isolated syllables of variable duration (Table 4) irregularly emitted (Fig. 6A and B). Its frequency spectrum showed a quite broad band of emission; the maximum amplitude peak being at below 3 kHz (Fig. 6C). To produce song, the males moved rapidly and almost synchronously either hind legs or only one hind leg.

Males produced these sounds when the specimens were at near or in contact with each other. The signals were sometimes emitted in alternation. Some individuals were observed singing while walking. When a male was courting a female, other males near the couple started to sing the disturbance song that, in this case, played the role of rivalry song.

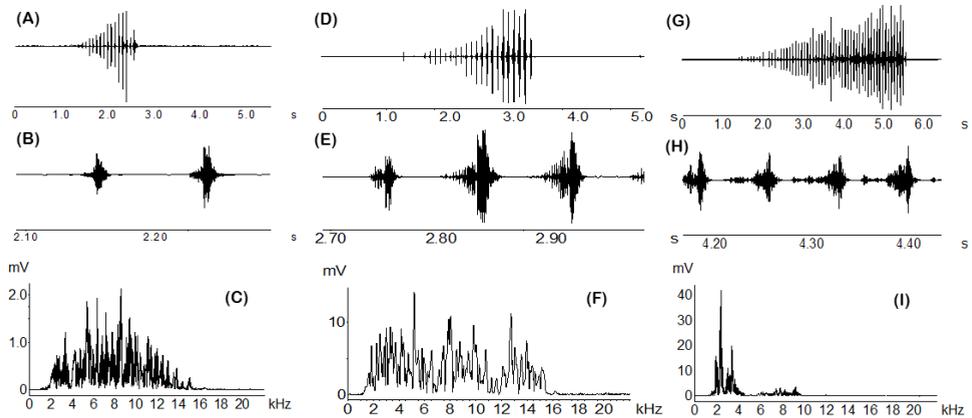


Fig. 4. *Rhammatocerus pictus*. Calling (A-C) and courtship (*pictus* 1 D-E and *pictus* 2 G-I) songs. Echeme (A, D and G); syllable detail (B, E and H), and frequency spectra (C, F and I).

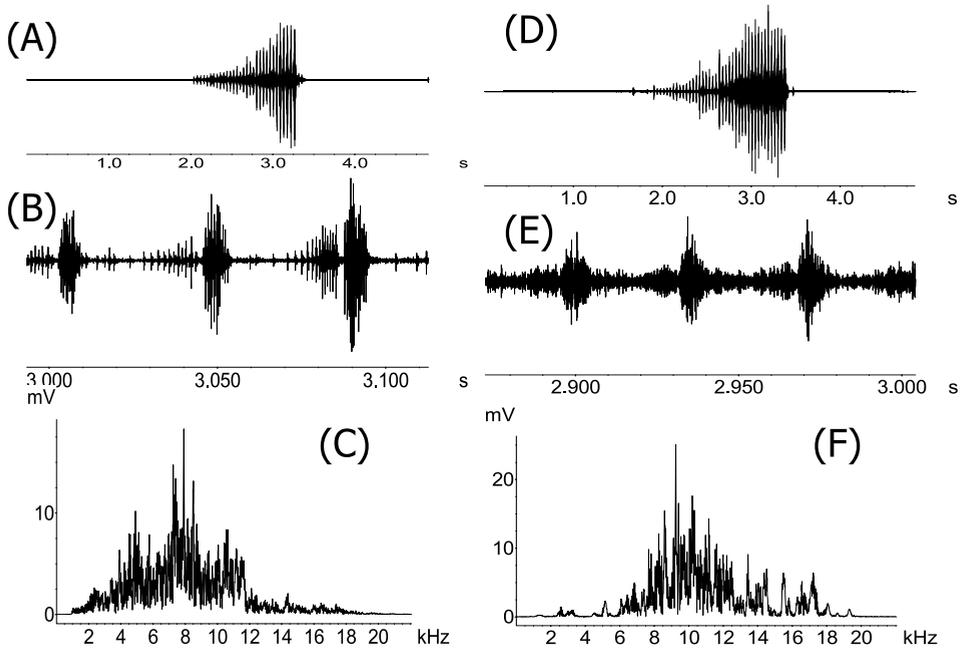


Fig. 5. *Rhammatocerus brunneri*. Calling song (A-C) and courtship song (D-F). Echeme (A and D); syllable detail (B and E) and frequency spectra (C and F).

### 3.3.2 *Rhammatocerus brunneri*

Songs were composed of isolated syllables (Table 4, Fig. 6D and E) emitted alternately by different individuals. The frequency spectrum occupied a broad band, with the peak of maximum amplitude at around 10 kHz (Fig. 6F). The leg movements to produce this song were similar to that performed by *R. pictus*, usually one or two quick asynchronous up and down movements.

This song was emitted in different circumstances, such as coinciding with or at the end of a calling song of other male or as rivalry song.

For disturbance songs, indicated the existence of overall significant differences among species (MANOVA:  $F_{8, 15} = 53.073$ ;  $P < 0.001$ ).

No disturbance songs produced by females have been recorded. One or two mute up and down movements of their hind legs were observed after a male interaction or courtship song or when interacting with other female. These movements have been also observed performed by males when close to other individuals or interacting with them.

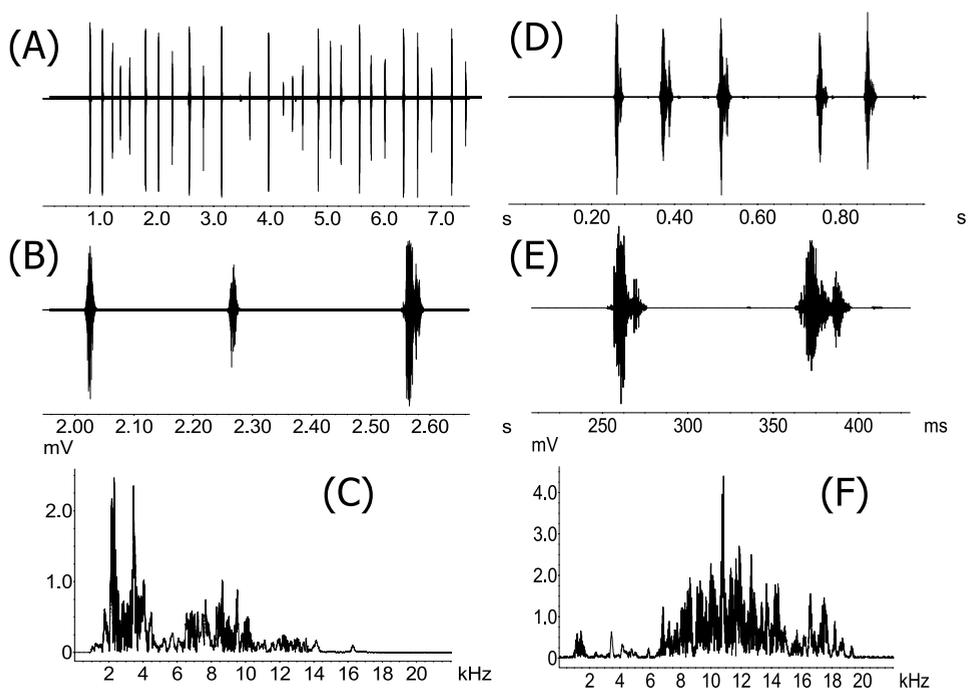


Fig. 6. Disturbance song. *Rhammatocerus pictus* (A-C) and *Rhammatocerus brunneri* (D-F). Sequence of syllables (A and D); syllable detail (B and E) and frequency spectra (C and F).

#### 4. Discussion

The stridulatory file of *R. pictus* and *R. brunneri* are well developed in both males and females. The peg spread, regular all along the file except at the ends, fits with that of other Gomphocerinae (Clemente et al., 1989; Pitkin, 1976). It can be pointed that the metrical characteristics referred to the stridulatory file are not species specific for males of *R. pictus* and *R. brunneri* but can serve to differentiate the females of both species. Nevertheless, there seem to be some morphological differences between stridulatory pegs of males. In both cases pegs are conic shaped, but *R. pictus* have pegs clearly more elongated, with a more acute apex, and the alveolus have the raised margin more irregular than *R. brunneri*.

The acoustic repertoire of *R. pictus* and *R. brunneri* is similar to that of other neotropical Gomphocerinae species, such as *Parapelopedon instabilis* (Rehn, 1906), *Euplectrotettix ferrugineus* Bruner, 1900 and *Fenestra bohlsi* Giglio-Tos, 1895 (García et al., 2003; Lorier, 1996; Riede, 1987), being made up of:

1. Calling song, as defined by Bailey (1991), Dumortier (1963), Ewing (1989), García et al. (1995), Green (1995), Helversen & Helversen (1983), Lorier et al. (2010), Ragge (1987), Ragge & Reynolds (1998), Reynolds (1986), Riede (1987), among others, since the songs were produced by males being apart from other specimens.
2. Courtship song, as defined by Ragge & Reynolds (1998) among others, that is, the special song produced by a male when close to a female.
3. Disturbance song, characterized by the physical structure and context in which it is emitted, that is the sound produced when the specimens were near or in contact with each other (García et al., 2003, among others). In the literature, reference exists to a "contact cry" (Kontaktlaut). All these signals are brief and sometimes emitted in alternation (Dumortier, 1963; Faber, 1953; Jacobs, 1953) and it is said are of little or no importance in taxonomy or identification (Ragge & Reynolds, 1998). These sounds can be interpreted in an aggressive context as rivalry song when produced by several males as an answer to a courting male, as described for *Fenestra bolshi* (Lorier et al., 2010). From females, despite having a stridulatory file, no sound has been registered.

The function of these different types of signals linked to the reproductive behaviour in Orthoptera and other insects has been analysed and interpreted in the context of the sexual selection theory. Sound can evolve, among other possibilities, through sexual selection. Evolutionary pressures have to act towards a minor energetic cost and a minimum risk of predation in the mating (Bailey, 1991). The significance of the male calling songs of Orthoptera lies in the fact that they are believed to provide the main means of mate recognition and hence of reproductive isolation of sympatric species (Ragge, 1987; Ragge & Reynolds, 1998). Acoustic signals provide enough relevant information on species identification and sexual selection. So, sound is considered essential to solve taxonomical problems at the specific level. Closely related, sympatric species often use strikingly different acoustic criteria to discriminate the same set of conspecific and heterospecific signals (Gehardt & Huber, 2002). A marked difference between songs is a strong indicative of different species (Ragge & Reynolds, 1998). When two morphologically similar populations of Orthoptera have consistently different calling song, it is likely that they belong to different species. Conversely, when two populations show small morphological differences but have exactly the same calling song, they are probably forms of a single species (Ragge, 1987).

In most Gomphocerinae some acoustic signals identify the species and can avoid the crossing between sympatric close species. This is the case of *Rhammatocerus* species here studied, which show differences in physical characteristics of all types of song. Both species have overall different songs, as shown in Table 4 and Figures 4, 5 and 6. Differences are more or less evident depending on the type of song.

The calling song of *R. brunneri* is very similar to the courtship song (Fig. 5). Thus, this species uses a similar song, with little differences (Table 4), in two different behavioural situations. In *R. pictus* a similar situation exists between the calling song and one of the courtship songs recorded (*pictus* 1) (Fig. 4A-F). At the light of the recorded songs, this species may start courting with a song similar to the calling song (*pictus* 1) changing afterwards to a particular courtship song (*pictus* 2), accompanied by legs and antennae movements. Although the whole sequence has not been registered, it would be not surprising since the use of a courtship song similar to calling song has been observed in other Gomphocerinae species, such as *Chorthippus binotatus binotatus* (Charpentier, 1924) (García et al., 1995) and *Omocestus antigai* (Bolívar, 1987) (Clemente et al., 1999). In the last case, it could be observed that the courtship started with the calling song and, later, the courtship song was emitted.

This kind of behaviour fits with that observed, for example, in genus *Stenobothrus* Fischer, 1853, the ancestor of which should have had produced simple and likely identical calling and courtship songs. The complex courtship songs observed in many species resulted from the addition of new acoustic traits derived from the common calling song pattern and from the addition of visual signals produced by the movement of legs and antennae (Berger, 2008 as cited in Nattier et al., 2011). In *R. pictus*, during the courtship new traits related to the spectral characteristics of the sound and, on the other hand, visual signals by legs and antennae movements would have been added.

These similarity of calling and courtship songs in *R. brunneri* and, partly, in *R. pictus* could be explained by the signals flexibility observed in acridids by which the same signal can be used in different contexts, as here occurred. This would imply following the opinion of Otte (1977), a further stage in an evolutionary process not ended still, probably accompanying a sympatric speciation process. Probably, sounds should not be the unique barrier to avoid hybridizing but there could also take part other kind of signals, such as those visual or chemical (Otte, 1977), although behavioural traits are often evolutionary labile and hence communication systems often diverge more rapidly than do morphological and molecular characteristics (Gehardt & Huber, 2002). Nevertheless, the question of which of the two song types calling or courtship song is the phylogenetic older one remains open (Berger & Gottsberger, 2010) but a simplification of courtship repertoires in some species suggests that the evolution of courtship song, and that of mating behaviour as a whole, could also result from a dynamic process (Berger, 2008 as cited in Nattier et al., 2011).

As regards the disturbance song, in some species phonotaxis is reduced when other males interfere, diminishing the efficiency of calling or courtship song. It could be pointed, for male disturbance songs - either spacing or answering other male courtship -, that the answering male, when alternating its song, uses its interference potential of acoustic channel

	Calling song		Courtship song		
	<i>R. pictus</i>	<i>R. brunneri</i>	<i>R. pictus 1</i>	<i>R. pictus 2</i>	<i>R. brunneri</i>
<b>Echeme length (sec)</b>	1.509 ± 0.164 (1.241-1.658)	2.130 ± 0.480 (1.618-3.22)	2.022 ± 0.588 (1.21-3.23)	3.02 ± 0.968 (2.151-4.807)	1.651 ± 0.380 (1.233-2.291)
<b>Number of syllables</b>	20.166 ± 1.602 (18-23)	49.416 ± 12.101 (34-67)	24.38 ± 7.65 (14-40)	38.85 ± 12.33 (28-61)	38 ± 12.23 (25-56)
<b>Syllables length (sec)</b>	0.049 s ± 0.012 (0.028-0.064)	0.028 ± 0.002 (0.024-0.032)	0.041 ± 0.008 (0.031-0.070)	0.052 ± 0.005 (0.043-0.059)	0.027 ± 0.003 (0.023-0.032)
<b>Rate of emission (syll/sec)</b>	13.471 ± 1.772 (12.06-16.61)	22.684 ± 3.542 (13.63-27.47)	12.07 ± 1.682 (9.14-15.39)	13 ± 0.328 (12.68-13.56)	22.9 ± 4.67 (13.63-29.29)
<b>N</b>	6	12	18	7	8
<b>Peak of maximum amplitude</b>	7198.08 ± 2962.745 (2790-11040)	8570.63 ± 2201.574 (1571-11864)	5014 ± 2239.707 (1297-10680)	2196.71 ± 422.754 (1884-3474)	7772.93 ± 2307.929 (3466-11170)
<b>Low quartile</b>	5316.54 ± 796.125 (3760-6540)	6687.78 ± 1343.213 (3334-8645)	4340.39 ± 771.349 (2530-5351)	2236.79 ± 330.158 (1913-3213)	6129.47 ± 1524.788 (3639-9000)
<b>Middle quartile</b>	8419.23 ± 803.845 (6990-9880)	9081.74 ± 1357.565 (6193-11148)	6879.75 ± 1361.498 (3924-8893)	3416.43 ± 1426.617 (2330-7380)	8720.00 ± 1293.614 (6341-10675)
<b>Upper quartile</b>	11354.62 ± 801.969 (8910-12270)	12040.63 ± 1507.873 (9146-14499)	10159.11 ± 1635.277 (6346-12917)	7058.50 ± 3157.601 (2853-14136)	11790.47 ± 1228.933 (8694-13339)
<b>Minimum frequency</b>	1864.62 ± 281.769 (940-2340)	1865.52 ± 1207.976 (963-7068)	1349.04 ± 327.541 (931-1920)	1301.29 ± 292.857 (1014-1838)	2078.73 ± 1178.164 (807-5307)
<b>Maximum frequency</b>	15971.92 ± 1312.951 (13130-18820)	17174.26 ± 2293.155 (12220-20916)	14593.64 ± 2035.698 (9735-17520)	7315.57 ± 3018.844 (3441-12973)	16105.13 ± 2529.865 (10874-18809)
<b>Band width</b>	14102.69 ± 1365.625 (11140-17070)	15306.85 ± 2635.109 (10150-19902)	13243.75 ± 1932.220 (8780-15993)	6013.64 ± 3030.971 (1606-11929)	14025.87 ± 3022.831 (8640-17463)
<b>N</b>	26	27	28	14	15

Table 4. Data summary concerning the physical characteristics of the songs emitted by the males of *brunneri* on the time and frequency domains (data of which are expressed in Hz)

(Cade, 1985; Greenfield, 1997). Thus, the emitters compete for the receipt's attention, in this case the silent female. Thereby it could be understood, for *R. brunneri*, the courtship interruption provoked by rivalry songs (McGregor & Peake, 2000). Differences between disturbance songs of the two species, *R. pictus* and *R. brunneri*, support the specific communicative value even of this type of song.

Anyway, since the acoustic communication involves a particular signal recognisable by the conspecifics to avoid the interspecific mating, its characteristics have to be specific. In the studied cases, it has been proved that the sound characteristics, both in the time and in the frequency domains, allow clearly discriminating cryptic species, and offer useful elements for phylogenetic analysis. Up to now, the only characters to separate between *R. pictus* and *R. brunneri* was the colour of hind femora and tibiae and the spermatheca shape (Assis-Pujol, 1998), but the real status of both taxa remained obscure. So, our results can have consequences on taxonomy since they bring new elements to the genus revision, justifying the separation of the two species, *R. pictus* and *R. brunneri*, as valid species (Carbonell pers. comm.).

## 5. Conclusion

Exploration of biodiversity is imperative (Boero, 2009) but is needed of features helping to the specific identification and differentiation especially among cryptic species. Currently, in addition to the morphological characters, easily observed and absolutely useful when well defined, other characteristics are available, such as the genetic or the behavioural ones. The sound production in insects provides excellent traits allowing discriminating species on the basis of the sound characteristics, in the time and in the frequency domains, as well as in the behavioural traits of the context in which sound is produced.

*Rammathocerus brunneri* and *R. pictus* are sympatric Neotropical Gomphocerinae species with very similar morphological traits, even those related to the stridulatory file, that is, the sound producing organ. Nevertheless, they produce songs that result species specific, offering new features to be considered for their actual specific status. In addition to that, the sounds and its accompanying behaviour provide new evidences to be taken into account in the phylogenetic history of the genus.

Thus, the acoustic behaviour, when present, is worth of being considered in taxonomic revisions due to its demonstrated utility. The job of naming animals is far from having been carried out (Boero, 2009) and sound can help to complete that job.

## 6. Acknowledgment

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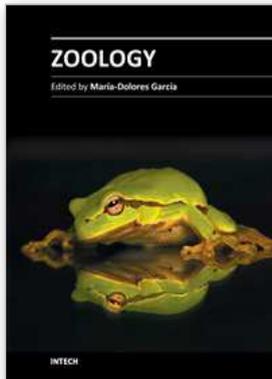
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The present book is not a classical manual on Zoology and the reader should not expect to find the usual treatment of animal groups. As a consequence, some people may feel disappointed when consulting the index, mainly if searching for something that is considered standard. But the reader, if interested in Zoology, should not be disappointed when trying to find novelties on different topics that will help to improve the knowledge on animals. This book is a compendium of contributions to some of the many different topics related to the knowledge of animals. Individual chapters represent recent contributions to Zoology illustrating the diversity of research conducted in this discipline and providing new data to be considered in future overall publications.

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