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COMPORTAMENTO ACUSTICO DI *PENTATOMA RUFIPES* (HETROPTERA, PENTATOMIDAE) DURANTE ACCOPPIAMENTO O INTERAZIONE DI RIVALITÀ UNO-A-UNO

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Abstract - Cesare Brizio, Filippo Maria Buzzetti & Lorenzo Lolli - Acoustic behaviour of *Pentatoma rufipes* (Heteroptera, Pentatomidae) during one-to-one mating or rivalry interactions.

During a night, wide-band field recording session in the Bolognese Apennine at 1200 meters asl, aimed at orthopteran songs, a regular pattern of buzzing sounds of non-orthopteran origin was heard, and visually traced to a group of Forest Bugs *Pentatoma rufipes* (Linnaeus, 1758) apparently engaged in mating. Even though the investigating team didn't have any photographical equipment available during the encounter, the peculiar sounds were recorded and are here described in detail, with a narrative report about the associated behaviour.

Keywords: bioacoustic, ethology, sound analysis.

Riassunto - Cesare Brizio, Filippo Maria Buzzetti & Lorenzo Lolli - Comportamento acustico di *Pentatoma rufipes* (Hetroptera, Pentatomidae) durante accoppiamento o interazione di rivalità uno-a-uno.

Durante una sessione di registrazione notturna rivolta agli ortotteri nell'Appennino Bolognese a 1200 m di quota, è stato udito una emissione sonora ronzante di origine non ortotterica, visualmente identificata in un gruppo di *Pentatoma rufipes* (Linnaeus, 1758) apparentemente impegnati in accoppiamento. Sebbene il gruppo di ricerca non avesse equipaggiamento fotografico disponibile durante l'incontro, il suono peculiare è stato registrato e viene qui descritto in dettaglio, con un resoconto del comportamento ad esso associato.

Parole chiave: bioacustica, etologia, analisi del suono.

Introduction

Sounds are emitted among many orders of insects, typically in Orthoptera and Homoptera, and by mean of different structures. These sounds are mostly species-specific and related to male-female pairing. Within Heteroptera, many families are known to produce sounds (e.g. Reduviidae, Cydnidae, Pentatomidae). A distinction has to be made between "vibrational signals", i.e. the sounds mediated by a solid substrate, and "acoustic signals", that are transmitted as sound waves in the atmosphere/hydrosphere and are emitted by interaction between body parts. While vibrational signals are the subject of the recent field of research named "biotremology" and are usually studied by vibration detectors such as piezoelectric cells, aerial sound waves are the subject of traditional "bioacoustics". Nevertheless, the two disciplines can effectively blend together to provide a more complete understanding of animal ethology. Furthermore, vibrating substrates may transmit acoustic waves in the surrounding medium, thus blurring the distinction among the two fields of research. In the case of this paper, our findings partially overlap with the observations by Shestakov (2015), who analysed the vibrational signals of some Pentatomidae, including *Pentatoma rufipes*, but include novel data about sound generation as well as the description of a behavior that, to our best knowledge, wasn't reported previously in scientific literature.

Materials and methods

The recording of *P. rufipes* was obtained by a low-cost, highly portable USB microphone (Ultramic 250 by Dodotronic) with a sampling rate of 250 kHz, and thus capable to provide monophonic recordings in the 1- 125 kHz range, a wide bandwidth extending well into the inaudible ultrasonic range. Previous papers (Brizio & Buzzetti, 2014; Brizio, 2018; Buzzetti *et al.*, 2019), illustrated some technical limitations of Ultramic 250 (including the emergence of characteristic, extremely narrow intrinsic noise bands for USB cable lengths higher than 50 cm), and defined a protocol for the comparison of digital audio recordings taken at 250 kHz and digital recordings obtained at any lower sampling frequency, down to 44.1 kHz.
The Ultramic 250 was connected via USB cable to an Asus Eee PC 1225B notebook pc, and recording was performed by the SeaWave software by CIBRA, under Windows 7 64-bit.
Envelopes, spectrograms and frequency analysis plots were generated by Adobe Audition 1.0 software. Sound level is on an arbitrary scale, expressed in dB ref Full Scale Level. No real sound pressure level can be extrapolated. Spectrograms (normalized spectral energy) were generated with the following parameters: FFT and Window size 8192 samples, Windowing function Welch (Gaussian), logarithmic energy plot, sound level expressed in dB ref Full Scale Level.
Frequency analyses were made with the same parameters and Blackman-Harris windowing function.
The illustrations of frequency analyses were generated with a scan of the whole audio sample as described in the figure captions. The screenshots obtained from Adobe Audition were then post-produced with Adobe Photoshop Elements, by converting them in black and white and removing the background grid, and finally horizontal / vertical reference rulers were manually added with MS-Paint. Those intervention did not alter the data nor the analysis results.
Scatter plots were generated by Excel 2010, based on the intervals and durations appearing in Table 1, showing values measured by the sound pressure envelope view available in Adobe Audition 1.0.
For a better understanding of the terminology adopted here, Fig. 1 shows the criteria adopted to plot the duration of echemes and intervals: considering that the duration of any echeme was variable and that echemes may contain one or more subequal volume peaks, there is no univocal definition of "peak to peak interval", while the beginning of each echeme can unambiguously be observed. When measuring echeme duration, small tails occurring after echeme ending (marked by decrease of volume under the bottom noise at any frequency) are not considered, as shown in Fig. 1. Similarly, aborted echemes or isolated syllables occurring during some intervals were ignored, to privilege the analysis of the repetitive, rhythmical pattern constituting the major feature of the observed acoustic behavior.
For what concerns audible sound description we use an array of terms from sources including Buzzetti & Barrientos (2011), Moore (1989) and Ragge & Reynolds (1998):

- Syllable (*or phonatome*): a short, clearly definable sound, produced by a complete opening and closing movement of the forwings (elytrae or tegmina), or by an upward and downward movement of the hind legs. In the song of some species, two subunits (hemisyllables) are clearly recognizable, often characterized by pulses with opposite phases. In some cases

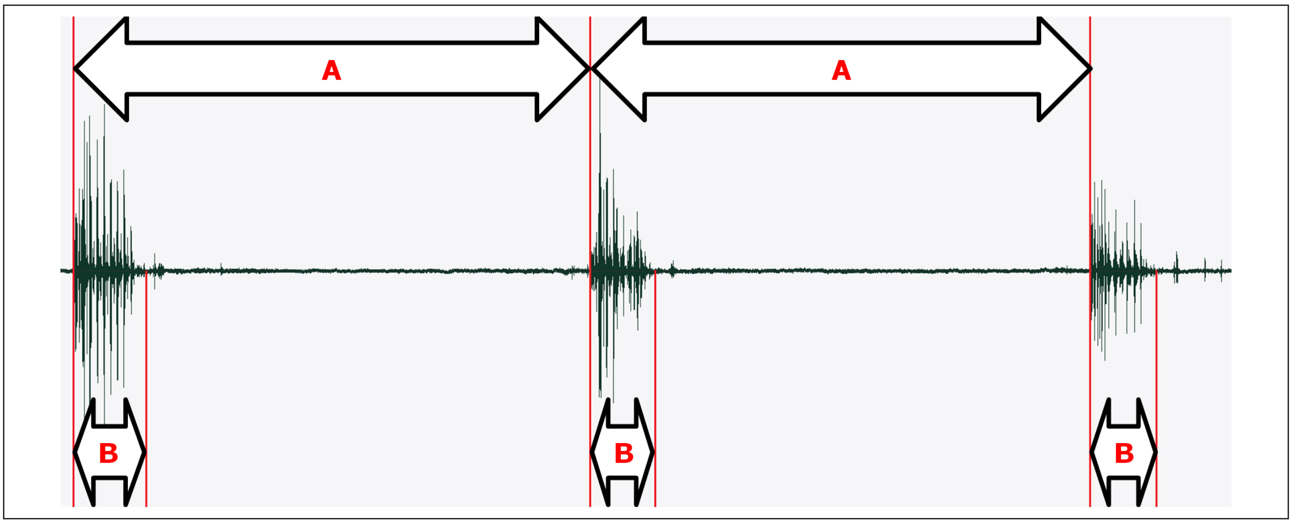

Fig. 1 - Terminology applied to the scatter plots: A = Interval to subsequent echeme initiation; B = Echeme duration.

those subunits are symmetrical, in other cases one of the subunits can be clearly different or barely visible, depending on the contact of the moving parts;

- Echeme: most basic and simple assemble of syllables.

Encounter

At around 23:10 on 16 August 2022, in Madonna dell'Acero (Bolognese Apennines), at an elevation slightly above 1200 meters above sea level, CB and LL equipped with headlights were engaged in an Ultramic 250 recording session aimed at the orthopteran *Pholidoptera griseoaptera* (De Geer, 1773) in the immediate vicinities of their family house. Other orthopterans, including *Leptophyes laticauda* (Frivaldsky, 1867), *Pholidoptera aptera goidanichi* Baccetti, 1963 and *Tettigonia viridissima* (Linnaeus, 1758) could be seen or heard in the immediate vicinities. An abrupt, loud rhythmic buzz was heard over the background noises; with a long experience in recording local orthopterans, CB was immediately aware that such a sound didn't match any orthopteran song previously heard in the area. LL quickly located the source of the sound: a group of around 10 *P. rufipes* (a common species in this forested area, easily encountered by day and frequently attracted by lights at night) had gathered near an external light and one individual was buzzing loudly while apparently engaged in mating.

Not equipped to take pictures, and not knowing how long the opportunity to capture the unusual sound would have lasted, rather than abandoning the station to collect a camera the team opted for an immediate recording. It should be clarified that Ultramic recordings

Tab. 1 - Echeme interval and duration in a bout of 30 echemes.

Echeme n°	Interval to subsequent pulse train initiation (msec)	Duration of main pulse train (msec)
1	884	101
2	853	99
3	989	98
4	856	111
5	610	75
6	1071	104
7	780	95
8	1077	130
9	977	99
10	901	102
11	845	72
12	899	79
13	785	75
14	953	87
15	872	72
16	826	92
17	850	100
18	863	76
19	803	90
20	860	96
21	826	99
22	823	76
23	857	87
24	754	93
25	686	94
26	836	111
27	848	77
28	836	91
29	828	75
30	--	88

Fig. 2 - *Pentatoma rufipes* (L.) photographed at the recording station shortly after the acoustic behavior here described.

Fig. 3 - Another specimen of *Pentatoma rufipes* (L.) photographed at the recording station shortly after the acoustic behavior here described.

are performed engaging both hands, one holding the personal computer or tablet, the other holding the microphone attached by a short (30 cm) cable (see Brizio & Buzzetti, 2014 for the shortcomings of longer USB cables): while one team member recorded the sound monitoring the display, the other kept his headlamp pointed on the sound source, thus allowing an accurate aiming of the microphone to the exact direction of the buzzing insect.

The buzzing *P. rufipes* was apparently mating, overlying another individual and orientated in the same direction, with abdominal tips touching.

Several other individuals (no less than four at a time) kept sight of the interaction, at a range of five to fifteen centimetres, while other individuals could be observed in the immediate vicinities. This kind of gathering is reminiscent of the nocturnal mating aggregations reported by Ramsay (2016) for the same species.

This notwithstanding, among the factors recommending a good degree of caution in hypothesizing the sexual nature of the one-to-one interaction that caused the acoustic behaviour here described, we cite:

- the lack of high-quality photographic evidence that could have helped ascertaining its details,
- the impossibility to grasp by the unaided eye both the sex of the involved specimens and the details of the interaction, despite the help of the headlight,
- the availability via simple Google searches of pictures of *P. rufipes* mating with both sexes facing opposite directions.

The sound was very clearly emitted by vibrating the open wings: we have no audio records of flying *P. rufipes* but subjectively, based on previous encounters with the same species, the buzz closely matched the noise of the flying insect. In fact, even though the buzzing specimen never got off the ground, the observed wing motion was comparable with that observed in daytime at take off.

A continuous song bout, described in detail herein under, with a total duration of around 35 seconds was recorded. Two song bouts of similar duration and structure were heard before the recording could begin. After the end of the recording, the couple split and no other sound was heard.

A few minutes later, once completed the planned recordings of *P. griseoaptera*, CB returned to the *P. rufipes* recording station with a photocamera, and observed four remaining individuals, taking pictures of two specimens (Fig. 2 and Fig. 3).

Sound analysis

The recording covers 38 seconds of sound emission (Fig. 4), that can roughly be divided as follows:

- a 10 sec song bout, including 9 subequal echemes (short buzzes) and a long buzz lasting 2300 msec,
- a 3 sec pause,
- a 25 sec song bout, including 30 subequal echemes.

Scatter plots were derived from the longest song bout including only standard echemes. Rhythm of emission (intervals between echemes as described in Fig. 1) averaged around 850 msec, corresponding to around 12 echemes every 10 seconds, while echeme duration averaged around 90 msec. The scatter plots of intervals (Fig. 5) and durations (Fig. 6) confirm that the echemes are emitted at a fairly regular rate, and with consistent durations, a fact supporting the non-casual, behavioural nature of the sound emissions.

As mentioned above, before the recording took place, two similar song bouts were heard, reinforcing our impression that the recorded sounds constitute a behavioural trait.

Echemes, or "short buzzes", can be composed of 10 – 20 syllables that, pending further investigation, most probably correspond to as many opening/closing motions of the wings, with the down stroke generating the highest volume peaks. The sound pressure envelope of a typical echeme with a duration of around 100 msec is shown in Fig. 9. Syllable duration, for the loudest and better defined syllables, is around 5 msec.

The time-frequency spectrogram of a single echeme (Fig. 8) shows a typical buzzing sound with components up to 85 kHz arranged in three inconspicuous bands, whose existence is more clearly revealed by the frequency / sound pressure analysis performed by scanning the whole bout of 30 echemes (Fig. 9). Low-frequency components concentrate under 2 kHz then - immediately before and after 10 kHz - two peaks, respectively with two and three spikes, can be observed. The two most relevant but scarcely defined bands, separated at around 40 kHz by a marked pressure decrease, respectively peak at 24990 Hz (-68,47 dBfs) and 47720 Hz (-69,5 dBfs). With a bottom noise at any frequency slightly below -100 dBfs, components up to 85 kHz can be safely attributed to the buzzing insect.

The same analyses were performed on the other relevant feature, the long buzz (2300 msec). Its first distinguishing feature are the uninterrupted syllables of subequal duration and different volume, around 80 per second (around 13 msec per syllable, unequally divided

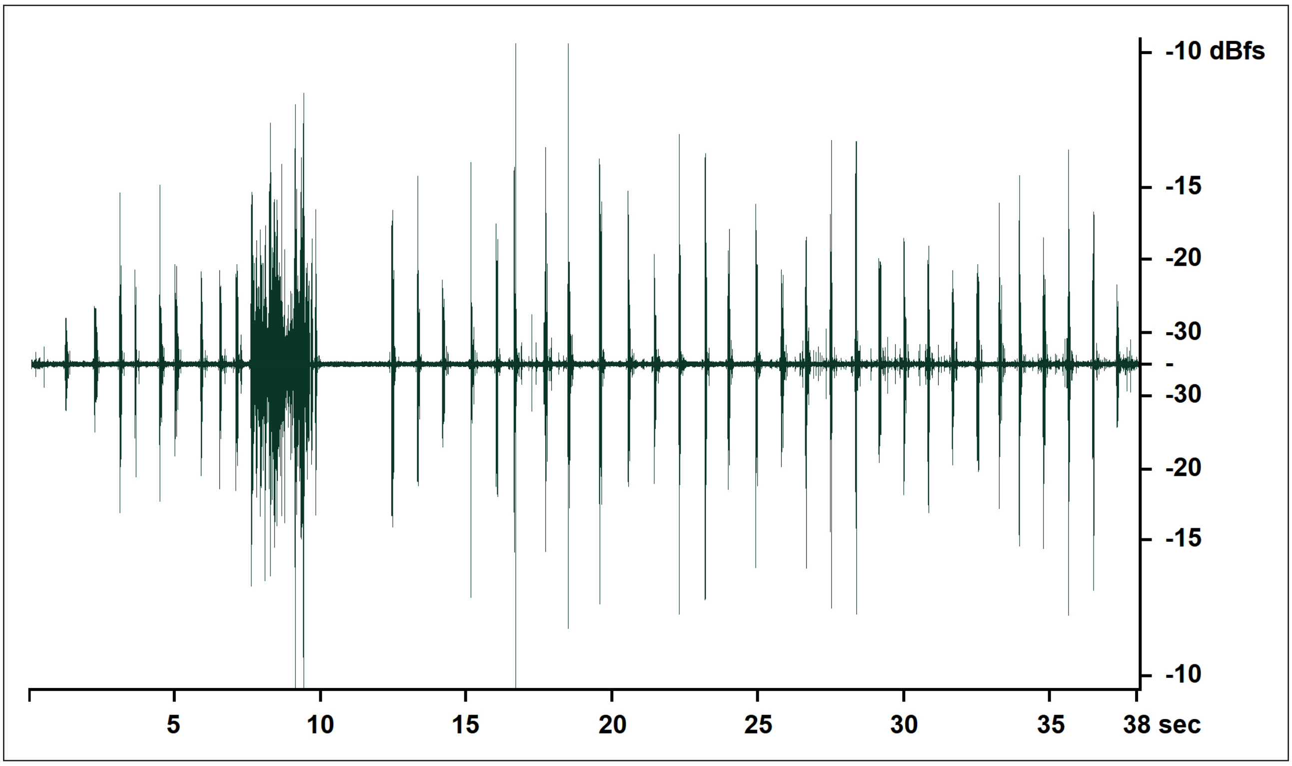

Fig. 4 - Full recording, sound pressure envelope.

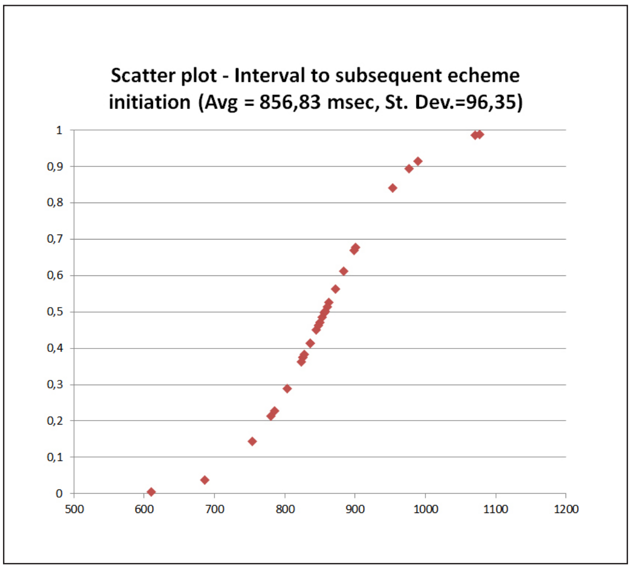

Fig. 5 - Echeme intervals, scatter plot - See Fig. 1 and Table 1 for details.

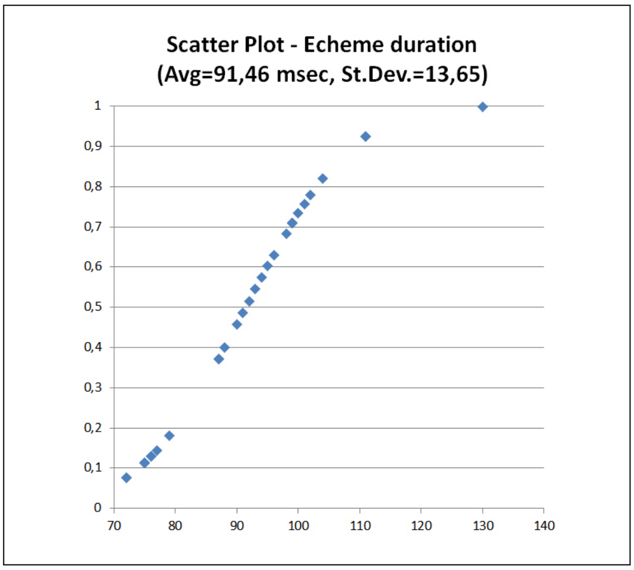

Fig. 6 - Echeme durations, scatter plot – See Fig. 1 and Table 1 for details.

between a short and loud initial pulse and a lower volume continuous buzz often containing a final momentary spike). Figures 10 and 11 respectively illustrate the whole long buzz and a detail of 300 msec. In many parts of the long buzz the volume is decidedly higher than in the echemes.

The time-frequency spectrogram of the long buzz (Fig. 12) is similar but not identical to the corresponding illustration of the echemes. As clarified by the frequency/sound pressure analysis performed by scanning the whole buzz (Fig. 13) a slight shift towards higher frequencies can be observed, and overall the shape of the

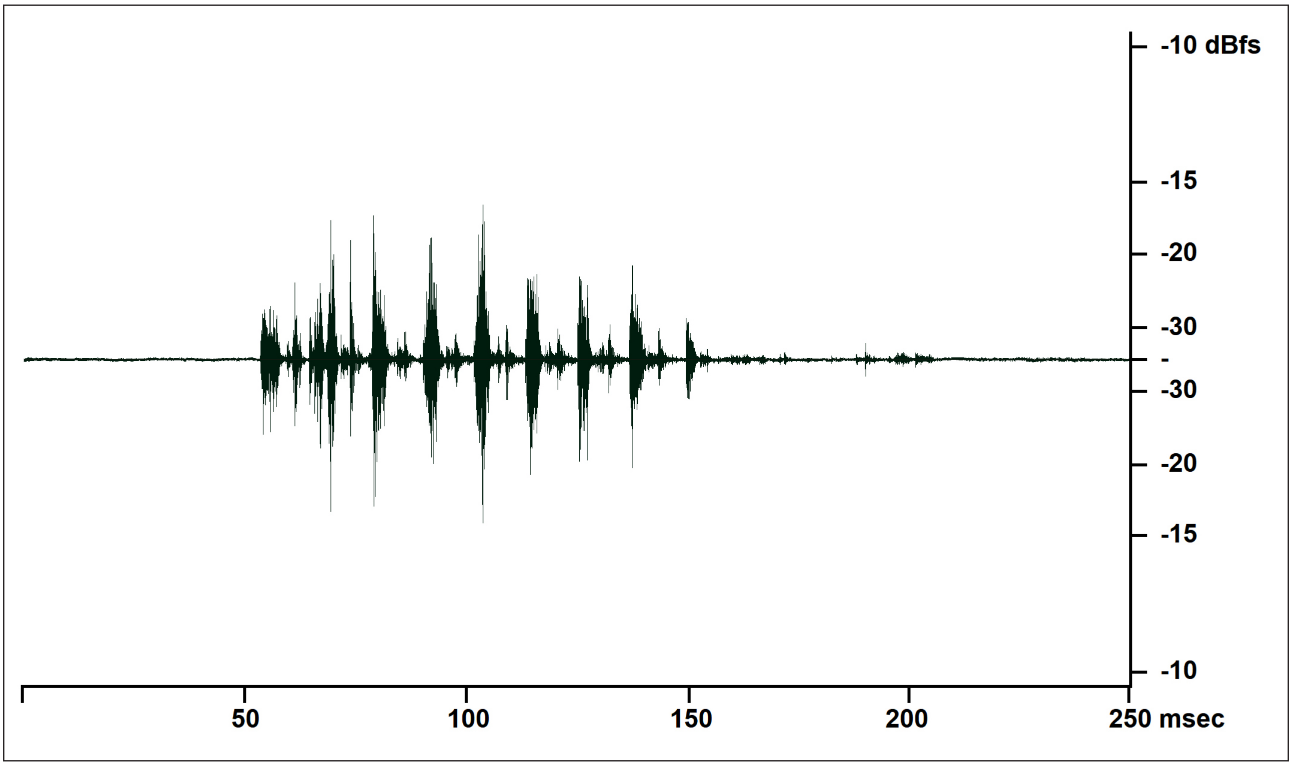

Fig. 7 - Echeme, sound pressure envelope.

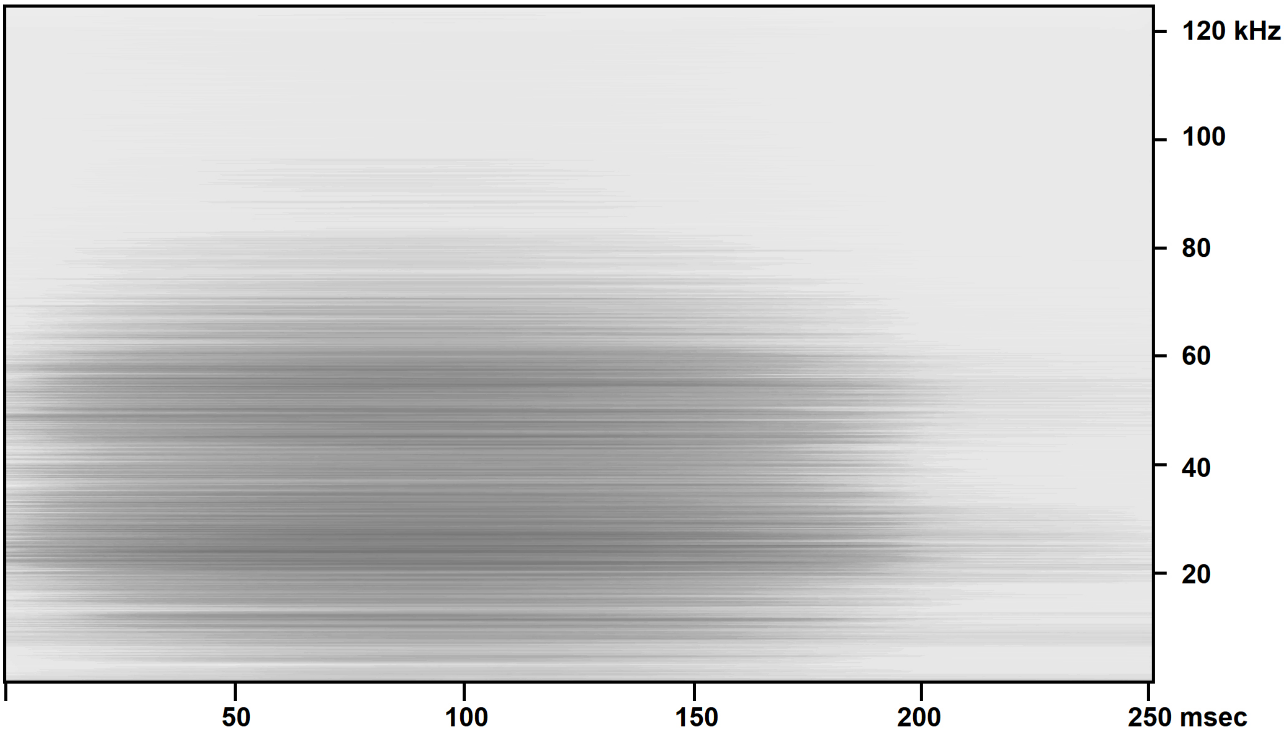

Fig. 8 - Echeme, time/frequency spectrogram.

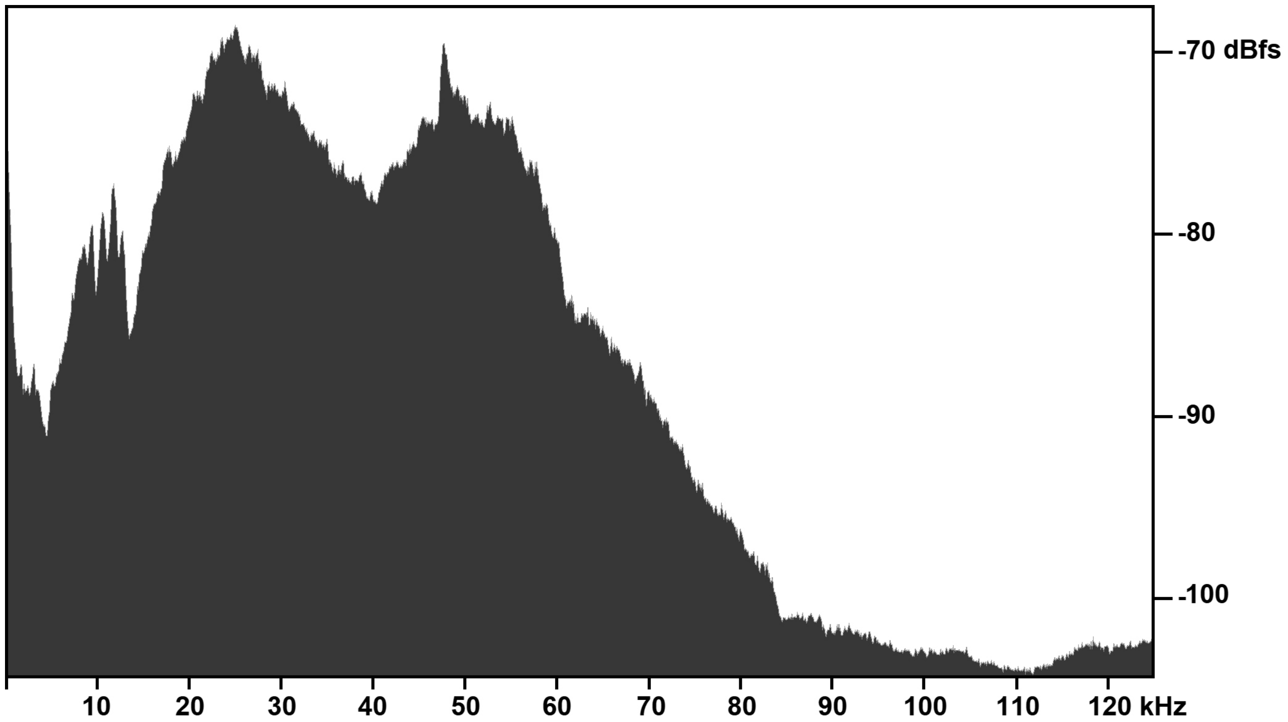

Fig. 9 - Song bout of 30 echemes, frequency/sound pressure analysis.

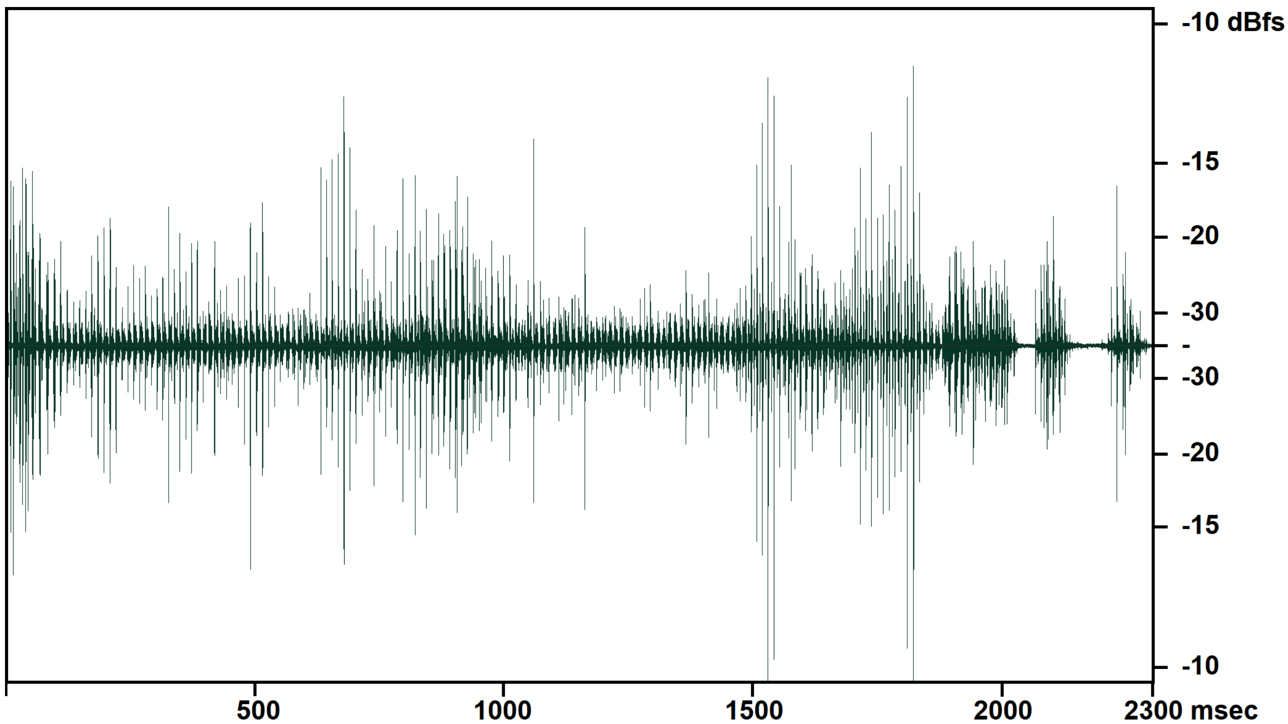

Fig. 10 - Long buzz, sound pressure envelope.

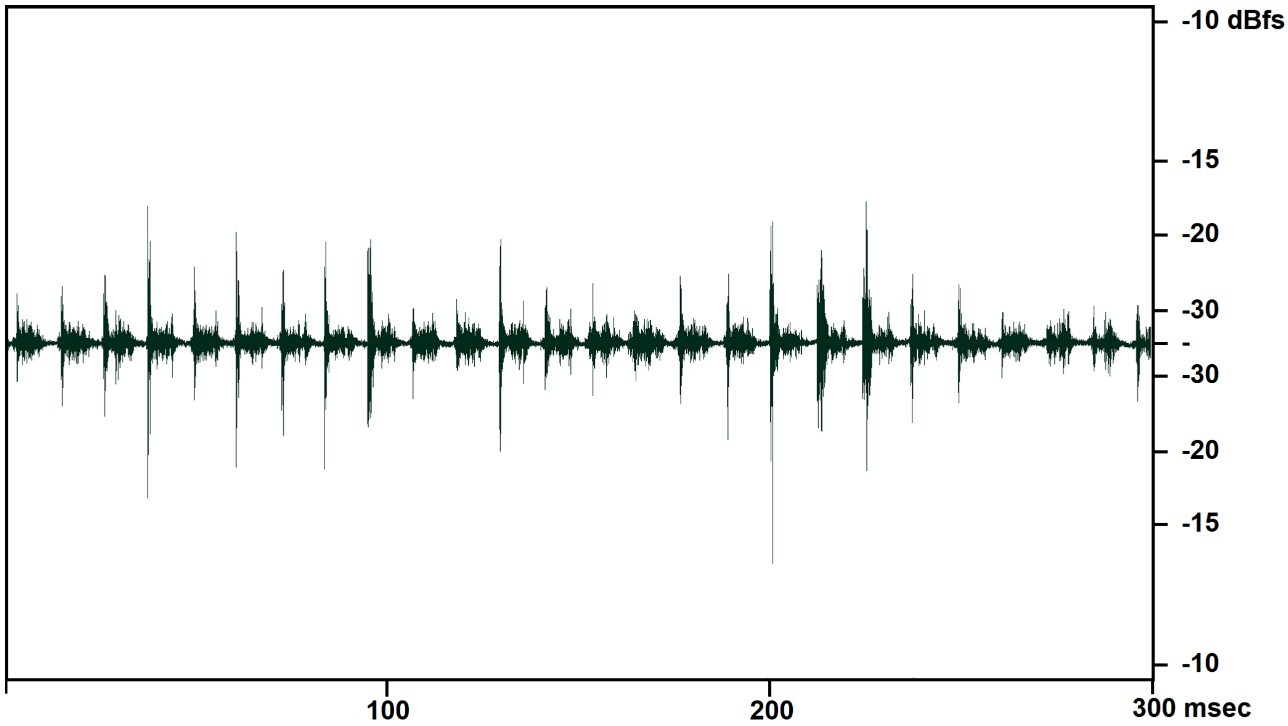

Fig. 11 - Long buzz, sound pressure envelope of 300 msec.

frequency distribution is blunter. Up to 20 kHz the structure is very similar, including the three-spiked peak immediately above 10 kHz and a band separation at around 40 kHz, but peak pressures are at higher frequencies, respectively at 26550 Hz (-58,91 dBfs) and 51840 Hz (-64,6 dBfs). Also in this case the sound of the buzzing insect seems to reach around 85 kHz.
To test compatibility with a time/frequency spectrogram covering *P. rufipes* in the paper by Shestakov, 2015, who used an entirely different vibration recording equipment (GZK-661 or GZP-311 monophonic piezoelectric cartridges), Figure 16 was generated by considering just the lowest (0-1000 Hz) band of the long buzz. At this level of detail, the thin lines appearing in the full-band spectrogram are displayed as regularly spaced bands: the illustrations of *P. rufipes* vibrational signals by Shestakov, including the spectrogram and the sound pressure envelopes, are different in many respects, lending support to the idea that Shestakov's investigations did not include the wingbeat-generated buzz here reported.

Conclusions

For a purely descriptive report like this, and for a novel behavior apparently unreported in scientific literature, there is not much room for discussion.
It's not demonstrated that such a serendipitous encounter could be reproduced an better studied in controlled conditions: we decided that the mating/rivalry buzz of *P. rufipes* deserved description regardless some degree of uncertainty - the lack of specimen collection and of video evidence may cast some doubts on the exact context (rivalry or actual mating) in which the bioacoustic phenomena described here take place.
On the other side, the acoustic evidence collected by digital recordings, the well-established presence of the species in the area, and the photographs taken in the place of the encounter cast no doubt on the involvement of *P. rufipes* as the sound-emitting species, and allow the accurate description contained in this paper.
Considering that, despite our efforts, we could not locate any previous description of the acoustic behavior reported here, we are convinced that this is in fact the first report of its kind. Despite the modest number of scientific works covering the species, *P. rufipes* is widespread and encounters with naturalists should abound:

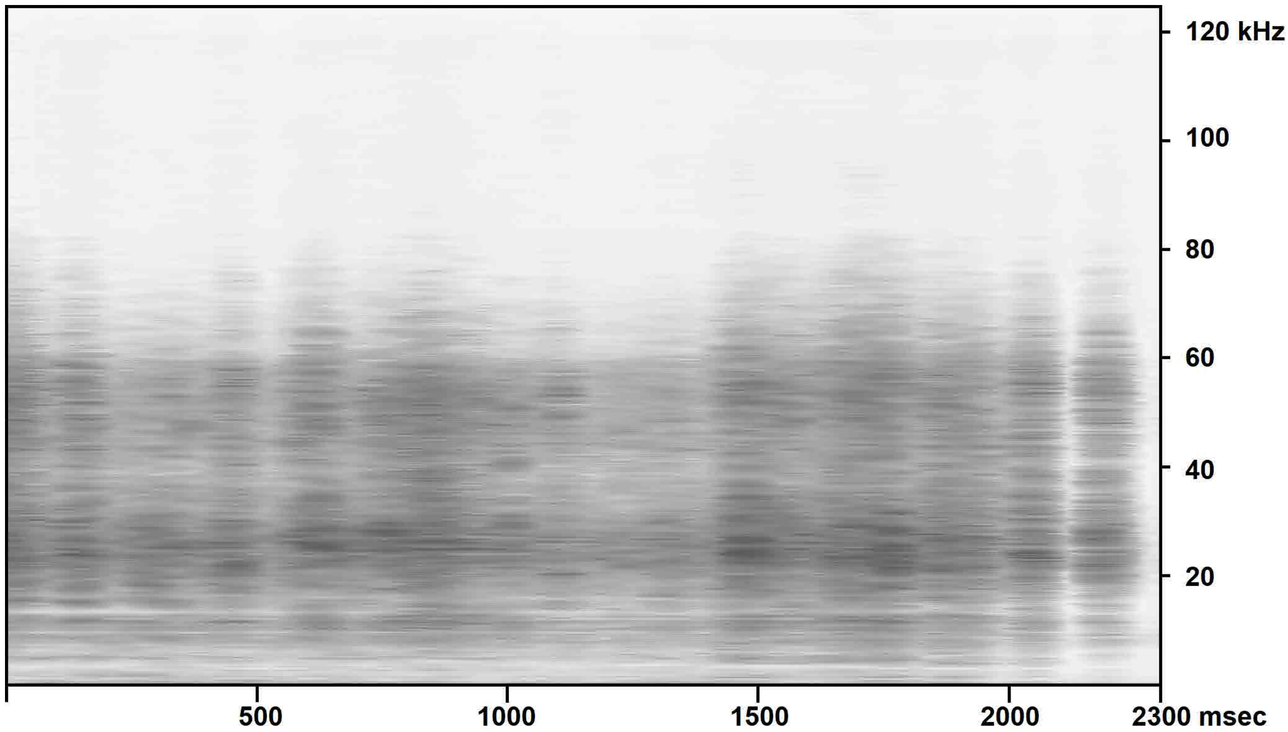

Fig. 12 - Long buzz, time/frequency spectrogram.

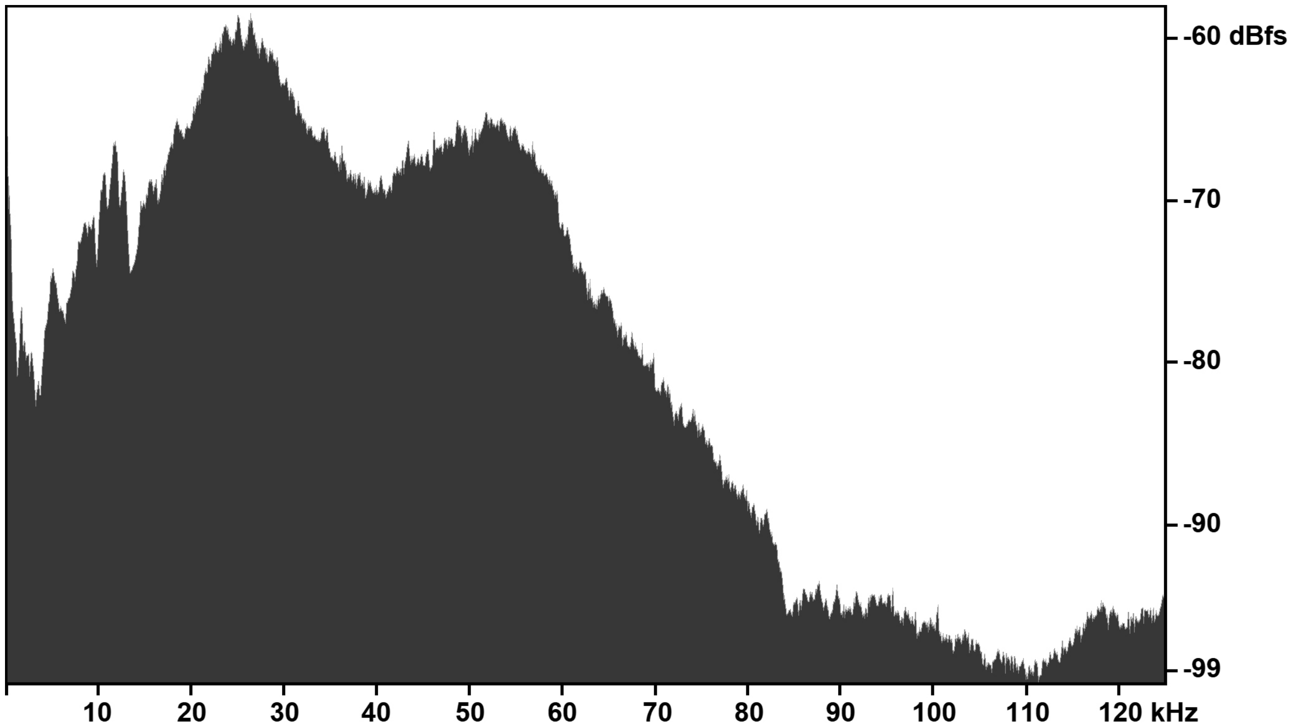

Fig. 13 - Long buzz, frequency/sound pressure analysis.

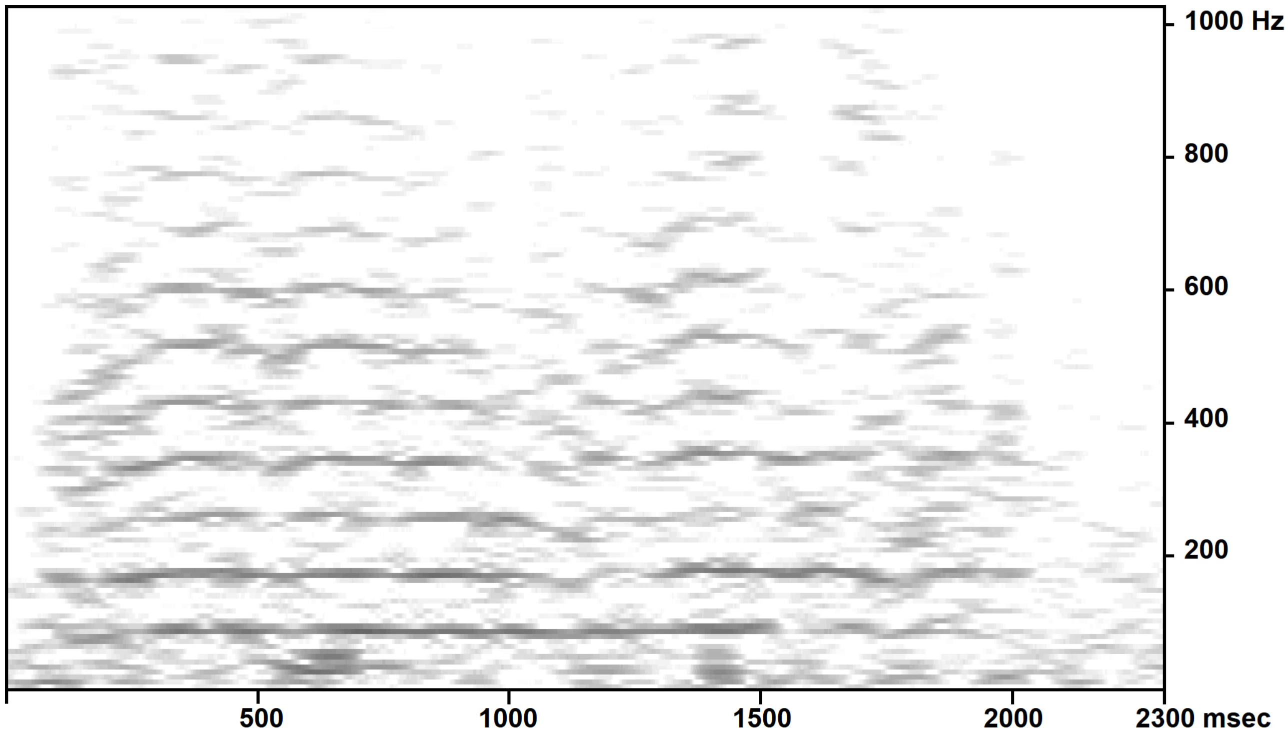

Fig. 14 - Long buzz, time/frequency spectrogram limited to 1000 Hz.

the lack of previous reports of the buzzing sounds associated with mating may hint an occasional, not obligatory occurrence. Its possible rarity is one more reason why this uneasily observed behavior deserves being described.

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