Predatory bug *Picromerus bidens* communicates at different frequency levels

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**Abstract:** The Asopinae (Heteroptera: Pentatomidae) are a subfamily of stinkbugs with predaceous feeding habits and poorly understood communication systems. In this study we recorded vibratory signals emitted by *Picromerus bidens* L. on a non-resonant substrate and investigated their frequency characteristics. Males and females produced signals by vibration of the abdomen and tremulation. The female and male songs produced by abdominal vibrations showed gender-specific time structure. There were no differences in the temporal patterns of male or female tremulatory signals. The signals produced by abdominal vibrations were emitted below 600 Hz whereas tremulatory signals had frequency ranges extending up to 4 kHz. Spectra of male vibratory signals produced by abdominal vibrations contained different peaks, each of which may be dominant within the same song sequence. Males alternated with each other during production of rivalry signals, using different dominant frequency levels. We show that the vibratory song repertoire of *P. bidens* is broader than those of other predatory stinkbugs that have been investigated. The emission of vibrational signals with different dominant frequencies but the same production mechanism has not yet been described in heteropteran insects, and may facilitate location of individual sources of vibration within a group.

**Keywords:** Substrate-borne vibrational communication • *Picromerus bidens* • Signal production • Signal frequency variation

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1. Introduction

The subfamily Asopinae, with about 300 species, differs from other pentatomid subfamilies by having mainly predaceous feeding habits [1] which demand well-developed sensory and communication abilities. One species, the spined soldier bug *Podisus maculiventris* (Say), has received considerable attention with regard to its distribution, life history, biology, and food preferences. Its rearing, predator-prey relationships, and potential as a biological control agent were summarized by De Clercq [1]. The first attractant pheromone for any true bugs was identified for *P. maculiventris* [2]. This species also exploits the substrate as a medium for detection of vibrational cues produced by chewing prey [3], and for transmission of signals involved in intraspecific communication [4,5].

Less attention has been paid to the Palaearctic asopine species *Picromerus bidens* L. which is found predominantly in damp, shrubby areas and forests on vegetation up to 2 m above the ground [1]. As a generalised predator it feeds on the larvae of many insect species [6-9]. *P. bidens* disperses mainly by walking or probably by flight to avoid overpopulation of specific sites in summer [6,10,11]. Abdominal or sternal glands have not been found in *P. bidens* [12] and to our knowledge nothing is known about chemical communication between conspecifics. Thus, *P. bidens* represents an interesting model for behavioural studies because chemical communication has been shown to accompany substrate-borne sound communication in many of the stinkbug species investigated to date [13].

Gogala [14] observed stereotyped movements in male *P. bidens* which accompanied the emission of vibratory signals during mating behaviour. Recently Shestakov [15] described the vibratory signals of four asopine stinkbug species from European Russia. In *P. bidens* the author [15] identified only a male rival song and hypothesized that different signal spectra are the result
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2. Experimental Procedures

2.1 Material

For all the experiments we used 4-10 d old adult *P. bidens* males and females, reared in a laboratory culture at the All-Russian Centre for Plant Quarantine in Bykovo, Moscow Region, Russia. Bugs were separated by gender within the first day after the final moult.

2.2 Signal recording and analysis

All experiments were conducted at the All-Russian Centre for Plant Quarantine in Bykovo. Signals were recorded in December 2009 between 0900 and 1600 h under laboratory and daylight conditions at room temperature (23±1°C) and 40-50% relative humidity. For comparative reasons, and to eliminate the influence of substrate on characteristics of vibratory signals, we used the same recording technique as described for the spined soldier bug *P. maculiventris* [4]. Signals were recorded from bugs placed on a 10-cm-diameter low-midrange loudspeaker (40-6000 Hz frequency response, 8-Ω impedance, Radio Shack, Taipei, Taiwan). Loudspeaker membrane vibrations induced by singing bugs were amplified by a microphone amplifier (Sonifex, Redbox amplifier, tape RB-MA, Sonifex Ltd., Irthlingborough, Northamptonshire, United Kingdom), then digitized and stored via a sound card (24-bit, 96-kHz, 100-dB signal-to-noise ratio; Sound Blaster Extigy, Creative Laboratories Inc., Milpitas, Ca) on a laptop computer using Cool Edit Pro version 2.0 software (Adobe Systems Inc., San Jose, Ca). We analyzed recorded signals with Sound Forge version 6.0 software (Sonic Foundry Inc., Madison, WI). Frequency characteristics were described by frequency spectra (FFT size 32768, FFT overlap 75%, smoothing window Blackman-Harris, display range 60 dB, normalized to the dominant frequency peak amplitude value as 0 dB) and sonograms (FFT size 8192, FFT overlap 99%, smoothing window Blackman-Harris, display range 20 dB). Frequency values of spectral peaks were determined by the program with 1 Hz precision.

2.3 Data presentation

Songs were characterized as sequences of signals in the form of pulses and/or pulse trains. Pulses are defined as unitary homogenous parcels of vibrations of finite duration [17] and pulse trains as pulses arranged into repeatable and temporally distinct groups. We determined gender origin of emitted signals by observing the animals being tested. Song types were differentiated by temporal (duration and repetition time) and spectral properties of signals as well as by their behavioural context. In each spectrum we measured the frequency value of the dominant spectral peak (DFP further in the text), and of the peak with the second highest amplitude (SdFP1 further in the text) below the DFP. Data are represented as means of values obtained for different bugs when the difference between individual means was not significantly different (ANOVA, P>0.05). In the case of significant individual differences the minimal and maximal individual mean values are shown.

3. Results

When pairs of *P. bidens* adults were placed on the loudspeaker membrane, male and female substrate-borne signals were produced by abdomen vibrations (vibratory signals) and tremulations (tremulatory signals) visible to the observer. According to differences in their temporal and spectral characteristics (Table 1) we describe them as one female (FS) and four different male (MS1-4) song types. Male and female tremulatory signals showed no gender specificity.

3.1 Female song (FS)

The FS was emitted as a continuous sequence of regularly repeated pulse trains (Figure 1A, left) whose duration varied according to the pulse number per