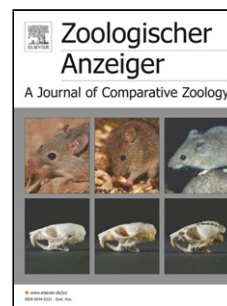


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Author: Fabio Sarria-S Kallum Buxton Thorin Jonsson
Fernando Montealegre-Z



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Wing mechanics, vibrational and acoustic communication in a new bush-cricket species of the genus *Copiphora* (Orthoptera: Tettigoniidae) from Colombia

Fabio Sarria-S¹

Kallum Buxton¹

Thorin Jonsson¹

Fernando Montealegre-Z^{1,*}

¹ *School of Life Sciences, University of Lincoln, Joseph Banks Laboratories
Green Lane, Lincoln, LN6 7DL, UK*

* Corresponding author: E-mail address: fmontealegrez@lincoln.ac.uk

Abstract

Male bush-crickets produce acoustic signals by wing stridulation to call females. Several species also alternate vibratory signals with acoustic calls for intraspecific communication, a way to reduce risk of detection by eavesdropping predators. Both modes of communication have been documented mostly in neotropical species, for example in the genus *Copiphora*. In this article, we studied vibratory and acoustic signals and the biophysics of wing resonance in *C. vigorosa*, a new species from the rainforest of Colombia. Different from other *Copiphora* species in which the acoustic signals have been properly documented as pure tones, *C. vigorosa* males produce a complex modulated broadband call peaking at ca. 30 kHz. Such a broadband spectrum results from several wing resonances activated simultaneously during stridulation. Since males of this species do rarely sing, we also report that substratum vibrations have been adopted in this species as a persistent communication channel. Wing resonances and substratum vibrations were measured using a μ -scanning Laser Doppler Vibrometry. We found that the stridulatory areas of both wings exhibit a relatively broad-frequency response and the combined vibration outputs fits with the calling song spectrum breadth. Under laboratory conditions the calling song duty cycle is very low and males spend more time tremulating than singing.

Keywords Resonance; Laser Doppler Vibrometer; Tremulation; ultrasound; bioacoustics; bush-cricket; Biotremology

1. Introduction

Males of several Ensifera (Gryllidae, Gryllotalpidae, Tettigoniidae and Prophalangopsidae), with some exceptions, generate sounds using wing stridulation (Morris, 1999). Typically, one wing bears a row of teeth, the file, and the other bears a scraper that is swept along this file to produce a series of stimuli (impacts) with the file teeth. The energy released by tooth impacts is transmitted to the surrounding cell membranes, usually the mirror and harp (Bethoux, 2012; Montealegre-Z and Postles, 2010). Many species of bush-crickets communicate using ultrasonic channels, with pure tone ultrasound

signals prevailing in neotropical species (Montealegre-Z, 2009). Pure tone signals seem to be common in species of the genus *Copiphora* (Montealegre-Z and Postles, 2010; Morris, 1980; Morris et al., 1994), with most of the energy occurring in the ultrasonic range (Montealegre-Z, 2009).

Such acoustic behaviour is commonly combined with body vibrations transmitted through the substrate as an alternative channel of communication (Morris et al., 1994; Stumpner et al., 2013). Vibrational communication, also known as seismic tremulation, is the communication between organisms by the vibration of a substrate. In bush-crickets it is speculated that tremulation functions to attract females whilst reducing the cost of acoustic eavesdropping by predators and also allowing for an alternative communication channel (Morris, 1980; Morris et al., 1994; Römer et al., 2010). Some species seem to have dramatically reduced their acoustic behaviour and adopted tremulation as a key modality for communication with females (Belwood and Morris, 1987).

Vibratory signals occur commonly in the environment of all living organisms and many species intentionally produce such signals for communication. It is therefore advantageous to detect these with appropriately receptive organs. The ability of animals to detect substrate borne vibrations through mechanoreception appears to precede the ability to hear audible airborne sounds and predates that of the vertebrate ear mechanism (Hill and Shadley, 2001; Lakes-Harlan and Strauß, 2014). An estimated 150,000 species of insects solely use substrate-borne vibrations for conspecific communication, with a further 45,000 species using vibration along with another method of communication (Cocroft and Rodríguez, 2005). Due to the prevalence and diversity of acoustic activity in certain groups (e.g. Orthoptera), vibrational communication has been relatively overlooked in many studies.

This paper reports on a new species of neotropical bush-cricket from Colombia, which has adopted persistent substrate vibrations as an alternative channel for communication, and different from its congeneric, exhibits relatively broadband signals. We establish that the broadband spectrum of the call results from the activation of different resonators on the sound generator

and that substrate vibration is an additional communication channel in this species.

2. Material and methods

2.1 Collection area

Palmar de la Vizcaina: El Palmar de la Vizcaina is an oil palm research centre surrounded by patches of tropical moist forest situated in the valley of the Magdalena river in Colombia, ~ 32 km from the municipality of Barrancabermeja, Santander, Colombia (lat. 6° 59' 02.3" N; long. 73° 42' 20.2" W). This locality has an elevation ranging between 85-95 m, an average annual temperature of 29.3 °C, an annual rainfall of 2693 mm and a relative humidity between 72-77%.

2.2. Capture and rearing

Specimens of *Copiphora vigorosa* were collected at night (18:00 – 24:00) along established trails in the sampling area, with a total of 54 hours of sampling activity during December 2014 and November 2015. Specimens were caught from understory vegetation and stored in 300 ml plastic pots. Upon return to the base camp, the collected insects were placed in a wire mesh cylindrical cage and provided with food (fresh apple) and water. Specimens were transported to the University of Lincoln, UK, under collection and exportation permit No COR 5494-14 (issued by the Administrative Unit of National Natural Parks of Colombia).

Specimens were maintained in captivity in rectangular terraria (Exo Terra, 60x45x30 cm) in a Fitotron plant growth chamber (25 °C, photoperiod 12:12 h, and 80% relative humidity). The terraria contained a mix of potted Bromeliads and small plastic plants with an imitation rock wall. The animals were fed *ad libitum* with fresh apple, baby corn, porridge oats and fish flakes; water was also provided.

2.3. Song recordings

Recordings were done in a sound-attenuated booth at the School of Life Sciences, University of Lincoln, at a temperature of 30 °C and relative

humidity of 40%. The specimens were placed on an artificial perch at 10 cm from a 1/8" condenser microphone (G.R.A.S. 40DP; G.R.A.S. Sound & Vibration, Denmark), connected to a 1/4" Preamplifier (G.R.A.S. 26TC). The microphone was calibrated at 94 dB SPL (re 20 μ Pa), using a Brüel & Kjaer sound level calibrator (Type 4231, Brüel & Kjaer, Nærum, Denmark). Data was obtained via an acquisition board (PCI-6110, National Instruments, Austin, TX, USA) and stored on a computer hard disk at a sampling rate of 512 k-samples/s using the Polytec acquisition software (PSV 9.0.2, Polytec GmbH, Waldbronn, Germany). Sound was analysed using Matlab (R2015a, The MathWorks, Inc., Natick, MA, USA).

2.4. Forewing resonance

Wing resonance was measured in four male specimens using micro-scanning laser Doppler vibrometry (LDV; PSV-500, Polytec GmbH, Waldbronn, Germany). For the experiments, the insect was mounted on a horizontal brass platform following the procedure described by Montealegre-Z et al. (2011a). Briefly, the wings were laterally extended by fixing the axillary sclerites with a mixture of beeswax (Fisher Scientific, Loughborough, UK) and colophonium (Sigma-Aldrich Company Ltd., Dorset, UK). The dorsal region of the tegmen corresponding to the mirror and contiguous cells were excited via synthetic sound stimulation, and scanned with the LDV using approximately 1500 grid points. Acoustic stimulation consisted of broadband periodic chirps in the range 2-100 kHz. The spectrum of the stimulus was corrected to be flat (± 1.5 dB) at 60 dB SPL at all frequencies. The acoustic signals were generated by the PSV-500 internal data acquisition board (PCI-4451; National Instruments, Austin, TX, USA), amplified (A-400, Pioneer, Kawasaki, Japan) and passed to a loudspeaker (Ultrasonic Dynamic Speaker Vifa, Avisoft Bioacoustics, Glienicke, Germany) positioned 30 cm from the specimen. The reference signal was recorded using a 1/8" condenser microphone positioned horizontally at a distance of 2-3 mm from the wings (Brüel & Kjaer, 4138-A-015 and preamplifier model 2670, Brüel & Kjaer, Nærum, Denmark). Laser and sound recordings were all obtained in a sound-attenuated booth (1.8 x 1.8 x 2 m) at approximately mid-day in the animals' light cycle.

2.5. Recording of vibratory signals

A sago palm (*Cycas* sp., Cycadaceae) planted in a plastic flower pot was used as the host plant for measurements. *Cycas* is an old world palm and not a natural substrate for *C. vigorosa* but was chosen due to its similarity to true palms (Arecaceae) present in South American forests. The plant was pruned, leaving two parallel touching leaves (approximately 400 mm x 10 mm) for ease of scanning. Reflective tape patches (approximately 5 mm x 20 mm) were placed at the base of the leaf stems (50 mm from the trunk) to increase the signal response of the laser vibrometer beam.

Vibrations produced by *C. vigorosa* were recorded at 128 kHz sampling rate with the LDV front end placed approximately 30 cm from the host plant. On the days of the recording, the bush-crickets were isolated and transferred from the incubator to a sound-attenuated booth. A microphone (1/8" 4138 with a 2670 preamplifier connected to a Nexus conditioning amplifier type 2692; Brüel & Kjaer, Nærum, Denmark) was mounted next to the plant using a magnetic table clamp to monitor the stridulatory activity. Stridulation calls often preceded tremulation events and indicated that the specimens had become habituated to the experimental conditions, and were therefore used to inform the observer of the best time to obtain vibration recordings. All experiments were performed on a vibration isolation table.

After the equipment set up, the chamber lights were switched off in line with the specimens' photoperiod, and any light sources such as the conditioning amplifier screen were covered with black fabric to reduce possible disturbance. Bush-crickets begin activity at night time by feeding, and therefore they were supplied with baby corn, slices of apple and water in their individual containers. The specimens were left for an hour to become acclimatised, feed and become active. After the allocated time, the insects were observed by one of the experimenters.

One male and one female were placed on separate leaves of the palm, and left again to habituate. The insects were monitored using the Laser Vibrometer camera video-feed and vibration recordings were triggered

manually by observing their behaviour in the camera. One of the experimenters was inside the acoustic booth, taking note of any behaviour and notifying the others on the position of the insect.

After copulation, a second male was placed on the host plant along with the male and female to record any possible male-male aggression behaviour.

Additionally, under the same experimental setup, six hours sessions of filming were done on six isolated males using a tripod mounted Sony HDR CX240 Handycam (Sony, Tokyo, Japan), and saved as MPEG-4 AVCHD 5M. Light for recording was provided by an LED red-light head torch.

2.6. Analysis of tremulations, sound, and laser measurements

Tremulations: After LDV recording, a low-pass, 300 Hz Hanning filter was applied (using the acquisition software's inbuilt digital filters; PSV 9.0.2, Polytec GmbH, Waldbronn, Germany) to all recordings to reduce noise and produce clear waveforms. FFT analysis was run on filtered recordings, all data were analysed in Matlab (R2015a, The Mathworks, Inc., Natick, MA, USA). Waveform analysis was carried out using the LDV acquisition software (PSV 9.0.2, Polytec GmbH, Waldbronn, Germany).

Video recordings: one hour of video from each male's filmed nocturnal activity was analysed. Stridulatory and tremulation events were counted and the time spent in each activity compared.

Wing resonances: Data of wing vibrations were analysed using the LDV presentation software (PSV 9.0.2, Polytec GmbH, Waldbronn, Germany). Data was also exported in ASCII format for further analysis in Matlab.

Sound recordings: For identification purposes, sound signals of *C. vigorosa* were compared with those of *C. brevirostris*, a morphologically similar species. Calls of *C. brevirostris* were obtained under laboratory conditions from specimens collected at Pericos, a site within the small community of El Salto, of the municipality of Buenaventura, Valle del Cauca, Colombia (lat. 3° 56' N, long 76° 47' W).

3. Results

3.1. Systematics

Family. Tettigoniidae Krauss, 1902

Genus. *Copiphora* Serville, 1831

Species. *Copiphora vigorosa* sp. nov.

Derivation of name. The specific epithet refers to the vigorous vibratory displacement of the male after mating.

Diagnosis. Insects of robust body (Fig. 1H). Head with fastigium of vertex forming a down-curved sharp cone of about 3.5 mm in females and 3.0 in males (more elongated than in *C. breviostris*). Ovipositor elongated (49-57 mm).

Body colouration. Frons with fumeus patch fading towards the edges of the rostrum. Clypeus with a flavus area bearing two ligneous dots equidistant of each other. Apical area of the clypeus testaceous, extending to the labrum. Mandible surface regularly aquamarine hue, tips brunneous (Fig. 1A). Femoral spines small, azure. Abdomen colourful in both sexes, with spiracle area ruber tarentinus (red-pinkish). Abdominal sternites aureus, with pullus marks in the lateral margins, and ruber tarentinus marks in the anterior corners (Fig. 1G).

Description. Head.— Fastigium relatively short ($\text{♂}3.09 \pm 0.29$ mm, $\text{♀}3.52 \pm 0.35$ mm), acuminate, with rufous coloration. Apex slightly downcurved and basal portion with a ventral projection. (Fig. 1A, B). Frontal ocellus white, circular, as broad as 0.5 times the eye diameter. Thorax— Dorsal surface of pronotum flat and smooth; anterior and posterior margin slightly curved. Lateral lobes with posterior angle acute. Thoracic auditory spiracle large and covered by the pronotum lateral lobes. Wings: Fully developed. Forewings showing a prasinous tone, irregularly distributed brunneous spots, and ostrinus outer margin. Apices rounded, extending well beyond the abdomen. Hind wings transparent. Right tegmen mirror subovoid, with an area of ca 6.0 mm², fusion of veins CuPa β +CuPb+AA1 massive, branch CuPa β surrounding the mirror

about 3 times thinner than the fusion (Fig. 2A). Stridulatory file holding 163 teeth, from which 125 appear to be functional (Fig. 2B); inter-tooth distance gradually increasing within the first 90 teeth, and then abruptly increases towards basal region of the file (Fig. 2C); tooth density = 63.2 teeth/mm. Abdomen.— Abdominal sternites display a flavus coloration and intersegmental membrane with two lateral ferruginous spots (Fig. 1D). Male genitalia: Subgenital plate basally broad, gradually narrowing, with medial round notch, bearing elongate styles (Fig. 1 C and D). Tenth tergite weakly bilobular at the apex, lobes separated from each other by a shallow depression at middle. Female genitalia: Subgenital plate distally narrowed, notched (Fig. 1 E and F). Ovipositor straight and 2.5 times longer than the hind femur; dorsal and ventral margins smooth. Measurements—Body measurements are given in Table 1. Males were an average weight of 0.79 ± 0.3 g and females were an average 1.51 ± 0.2 g.

General biology. Individuals of *C. vigorosa* are found on bushes at heights between 1-3 m from the ground. In the field, some specimens were observed feeding on small flowers or grass shoots. In captivity cannibalism was also observed.

Holotype. 1 ♂ MEUV (Museo de Entomología de la Universidad del Valle, Cali), November 17-19, 2015 (Fabio A. Sarria-S & Stephany Valdés-R).

Allotype. 1 ♀ MEUV, November 17-19, 2015 (Fabio A. Sarria-S & Stephany Valdés-R).

Paratypes. 5 ♂♂, 1 ♀ MEUV, November 17-19, 2015 (Fabio A. Sarria-S & Stephany Valdés-R). Type locality. COLOMBIA, Dept. of Santander, Barrancabermeja, Palmar de la Vizcaina, elevation 85-95 m.

3.2. Bioacoustics

The calling song of *C. vigorosa* consists of a series of syllable trains (7 to 9) and lasts on average 2.55 ± 0.65 s ($n=4$) at 30 °C. Males stridulate approximately four times in an hour, consequently the duty cycle (percentage of the total time spent singing during an hour) is extremely low (average 0.3%, $n=7$). Syllable trains last between 92 to 110 ms and contain 8 to 10 complex syllables (Fig. 3A). Individual syllables consist of a continuous set of

oscillations with mixed structure, and last 7.61 ± 1.03 ms, with a gradual rise in amplitude, which reaches its maximum value after 4.55 ± 0.88 ms. Mean syllable train period is 0.36 ± 0.08 s. Recordings of the stridulatory wing motion involved in the production of these syllables were not made. However, considering file morphology and tooth distribution, it is very likely that the major amplitude components of the call are produced during the closing phase of the wings, as in other congeners (Montealegre-Z & Postles 2010). The carrier frequency determined from the power spectrum of a fast Fourier transform calculated on a single chirp shows that the calling song exhibits a broadband nature with carrier frequencies in the high audio to the low ultrasonic range, extending between 10.19 and 37.21 kHz. Calls have a maximum peak at 32.68 ± 3.37 kHz and two more peaks, with energy at ca 10 kHz and 16 kHz (Fig. 3D). In spite of such broadband tendency suggested by the spectrum, spectrogram analysis shows that acoustic energy is harmonically related (Fig. 4A). Frequency analysis in the time domain shows frequency modulation (FM), with the syllables modulated from high to low frequencies and partitioned into two areas. The first FM occurs in the first 4.5 ms of the pulse from 18 to 13 kHz, then an abrupt change occurs between 4.5 and 9.0 ms in which frequency decreases between 12 and 6 kHz. The total FM of each syllable is from 18-6 kHz (Fig. 4A). Measured with a calibrated microphone at 10 cm dorsal to the specimen, the average intensity of the song was 102.81 ± 1.49 dB SPL ($n= 4$).

3.3. *Wing resonances*

Wings resonances were obtained using synthetic sound stimulation and recorded with a μ -scanning LDV. These experiments show that the right wing mirror dominated in amplitude response, while the deflection amplitude of the left mirror is lower (Fig. 5A and B). FFT analysis of the right stridulatory area shows a broadband spectrum between 5 and 45 kHz, with resonant peaks at around 8, 14, 21 and 32 kHz (Fig. 5B). At 32 kHz (a frequency similar to the average dominant of the calling song) the mirror frame does not vibrate like a piston up and down, but rather it appears to be clamped at the site of vein CuPa β +CuPb+AA1, and exhibits its maximum around the handle (h) and

CuPb join (Fig. 2A). This motion drags the adjacent cell where vein CuPa is located (Figs. 5A, 5H-G, see also supplementary video 1). Therefore in *C. vigorosa* the active wing area of sound production is not limited to the vibration of the mirror, as in other bush-cricket species [e.g., *Supersonus* spp., other *Copiphora* spp, (Montealegre-Z and Postles, 2010; Sarria-S et al., 2014)]. The 3D reconstruction of the wing deflection at 32 kHz suggests a rather complex mode of vibration with maximum displacement centred in the mirror (Fig. 5A, C-G, see video 1).

The amplitude of deflection of the left stridulatory area is lower than that of the right mirror, and shows resonances at ca. 8, 20, 30, and 40 kHz (Fig. 5A and B). The higher amplitude peak of the LW was measured at around 30 kHz, but this peak shows approximately half the energy of the maximum peak of the right mirror (Fig. 5B). Different from the right stridulatory area, the only area that deflects with higher amplitude in the left wing is centred in the mirror (Fig. 5A). The broadband form of the calling song spectrum appears to result, in part, from the activation of several resonating areas on both wings (Fig. 5B).

3.4. Seismic communication

Vibration signals were recorded using LDV from three males and one female *C. vigorosa*, but observed through video recordings in six males. Tremulating actions of both male and female are easily observable with the human eye and are faintly audible through movement of the host plant substrate. Both sexes begin tremulations by slightly elevating the abdomen, causing the anterior end to lower, possibly to allow for greater movement (and therefore a stronger signal) while avoiding striking the substrate (see Supplementary video 2). Tremulations consist of a series of continuous pulses (Fig. 6A-C), with pulse duration averaging 0.50 ± 0.18 s (males, $n=3$), and 0.39 ± 0.26 s (female, $n=1$). Pulses are discretely separated by silent intervals of 1.51 ± 0.21 s (males) and 1.55 ± 0.17 s (female), and are delivered at a rate of 24 pulses per minute. Both male and female exhibited similar tremulation peak frequencies (Fig. 6B-D): male 9.1-15.4 Hz (mean 12.1 Hz, $n=3$) and female 11.5-12.2 Hz (mean 11.7 Hz, $n=1$). Singing often preceded bouts of male tremulation and stopped altogether when the female began tremulating in

response. Pulse duration of mating tremulations does not significantly differ between male individuals (T test=2.14, df=6, p=0.076).

Three males performed a suspected aggressive tremulation when interacting with other males. These tremulations had an average pulse duration of 0.92 ± 0.13 s, with a 1.75 ± 1.4 s interval (n=18 pulses), and a frequency range of 7.8-8.6 Hz (mean 8.3 ± 0.4 Hz, n=3). Post-copulation tremulation was observed in one male, which performed tremulation pulses at a repetition rate of 9/min.

Tremulations in males and females exhibit a very similar envelope and temporal pattern (Fig. 6A-C). Singing and tremulation were both part of the calling behaviour, though they did not occur simultaneously. Several hours of observation and video recording under laboratory conditions suggests that tremulations are the primary communication channel used by males of *C. vigorosa*, while stridulation occurred sporadically. The percentage of time males spent tremulating is significantly higher than the time spent singing. In one hour, the total time of the males' tremulations represents $6.44\% \pm 3.58$, while singing events only $0.06 \pm 0.045\%$ (Wilcoxon, $Z=2.20$, $p=0.028$, n=6).

4. Discussion

We observed after comparing the acoustic calls duty cycle against the vibrational duty cycle that under experimental conditions, tremulation is the most commonly used communication channel in the test specimens. The broadband tendency of the call frequency spectrum results from different resonators in the wings being activated simultaneously. We found that the sound generator's mirror and surrounding areas, resonate at different frequencies and the breadth of these resonances encompasses the call spectrum range. Below we discuss these major findings.

4.2. Bioacoustics and wing mechanics

Most *Copiphora* species recorded up to now using appropriate ultrasound sensitive equipment produce pure-tone calls, e.g. *C. rhinoceros* (Morris, 1980), *C. brevis* (Morris et al., 1994), *C. gracilis* (Montealegre-Z, 2005, 2009), *C. gorgonensis* (Montealegre-Z and Postles, 2010). Males *C. vigorosa* produce

acoustic signals with spectral bandwidth of ca 30 kHz (Fig. 3D). The spectrum exhibits usually three energy peaks at ca 10 kHz, 16 kHz, and one dominant frequency at nearly 30 kHz. Nevertheless, frequency analysis in the time domain shows that the call preserves some tonal quality as suggested by some harmonic relationships (Fig. 4A). This pure-tone nature seems to be partially masked by the resonant properties of the wing (Fig. 5A and B). The right stridulatory area shows complex resonances, which are dominated by four resonant peaks of variable amplitude between 8 and 32 kHz (Fig. 5B). The left wing mirror on the other hand vibrates with lower amplitude, but is not strongly damped as in other species of bush-crickets (Montealegre-Z, 2012; Montealegre-Z and Postles, 2010; Sarria-S et al., 2014). Even though most of the acoustic energy seems to be radiated by the right stridulatory area, the left mirror exhibits resonant peaks of low amplitude between 8 and 20 kHz, and a stronger dominant peak at ca. 33 kHz (Fig. 5B). The broadband aspect of the calling song spectrum appears to result from simultaneous excitation of these resonators, which seem to vibrate with different phase, as observed in the laser recordings. However, amplitude gain at around 30 kHz might result from some of the left and right stridulatory areas vibrating in synchrony during stridulation, but this remains speculative until further evidence is collected. If careful attention is paid to Fig. 5B, it is apparent that some of the main peaks observed in the power spectrum of the calling song are represented in the wings resonances, but some 1.5-2.0 kHz down. When wings engage in stridulation different properties seem to change: membrane tension, stiffness, etc., thus affecting the wing resonance. These variables have been proposed as determinant conditions for sound production in other species of bush-crickets and crickets (Bennet-Clark, 2003; Montealegre-Z and Postles, 2010).

An increasing pattern of inter-tooth spacing is associated with regular tooth-scraper impacts, and in turn with a relative constant strike period in the resultant oscillations. Stridulation involving these features usually results in pure tones. FM in the first part is relatively low (Fig. 4A) but increases markedly once the exponential part of the file curve is reached (Fig. 2C). The relative stable instantaneous frequency in the first part of the syllable (Fig. 4B) could be associated to the file area of lower variance in inter-tooth distances

(the first 80 teeth), while the more modulated part of the pulse could be in association with the area of the file with rapidly increasing inter-tooth spacing (Fig 2B and C).

There are, however, other bush-cricket species that have sharp wing resonances, but exhibit calls with broadband spectra. For example, the sharp wing resonance in *Panacanthus pallicornis* has been associated with an erratic tooth distribution pattern of the teeth in the file. In spite of a broadband spectrum, spectral peaks preserve a harmonic relationship in this species (Montealegre-Z and Mason, 2005).

The LDV recordings also show that at its best resonance the mirror vibrates in a single mode (Fig. 5), and that the mirror frame is clamped on the massive vein CuPa β +CuPB+AA1. Similar to *C. gorgonensis*, the mirror is not clamped from the wing hinge at the base of vein CuPB as suggested in the past by Morris and Pipher (1967). This pattern is more similar to the vibrations inferred by Bailey (1970) for *Homorocoryphus nitidulus*.

4.2. Tremulation signals

Both males and females of *C. vigorosa* performed tremulation signals with a similar mean peak frequency (around 12.1 Hz). It is assumed that due to the random nature (changes in shape, size and density) of substrates for communication, any organism should broadcast vibrations in a wide spectrum, as differing transmission properties of substrates would make specialisation on a single frequency unlikely to function well (Michelsen et al., 1982).

Substrate transmission properties are known to differ between plant species and also between plant parts (Römer et al., 2010), with specialist species (such as treehoppers, Membracidae) suffering the least signal attenuation on their own host plant species (McNett and Coccoft, 2008). The ability of *C. vigorosa* to produce a lower frequency aggression signal informs us that they have some control of the frequency of the signals they generate, but whether *C. vigorosa* specifically compensates for the substrate to produce this range remains untested. Perhaps this lower frequency aggressions signal could be

associated with abrupt large body movements. Complex vibrational signals might be the result of the adoption of reduced singing behaviour as a defensive adaptation against bats (Morris et al. 1994), see below.

Morris et al. (1994) measured substrate vibrations of several bush-cricket species, including *C. brevirostris*, using an accelerometer attached to a leaf of the host plant (*Heliconia*). Substratum vibrations in these species show energy peaks at 35 - 45 Hz. Thus the tremulations of *C. brevirostris* and those of *C. vigorosa* (here reported) differ in frequency, but it is uncertain if this is due the different methods used for recording, host plant, and/or position of the recording area in relation to the position of the vibrating insect. Males of *C. brevirostris* sing with a very low duty cycle, and produce acoustic signals mostly with ultrasonic energy (Fig. 3, see also Morris et al. 1994). Similar to *C. brevirostris*, *C. vigorosa* tremulations dominate over acoustic signals for interspecific communication; males' calling songs, although rare, are loud and perhaps audible to eavesdropping predators.

Several authors have attributed the reduction of singing behaviour and the adoption of substrate vibration (as the main channels of intraspecific communication) to predation pressures, mostly by gleaning/eavesdropping bats (Belwood, 1988, 1990; Belwood and Morris, 1987; Morris et al., 1994; Römer et al., 2010). Eavesdropping seems to explain why some bush-crickets use such bewilderingly high principal carriers (Belwood and Morris, 1987; Falk et al., 2015; Montealegre-Z et al., 2006; Montealegre-Z et al., 2011b; Morris et al., 1994; Sarria-S et al., 2014). Several of these species using ultrasonic carriers and pure tones alternate their low-duty-cycle acoustic signals with substratum vibrations (Morris et al. 1994, F. Sarria-S personal observations). The calling song of *C. vigorosa* is not as pure as the song of related species, since a considerable amount of acoustic energy is delivered in a broadband signal (Fig. 3D, Fig. 5B), perhaps rendering them more conspicuous to eavesdropping predators. This might be one of the reasons why their singing duty cycle is extremely low in relation to tremulation signals. Morris et al. (1994) suggest that as the role of airborne sound in pairing is reduced, substratum vibration became increasingly the preferred channel for bringing mates together in a large number of neotropical tettigoniids. In fact some

species (e.g., *Schedocentrus differens*) seem to have completely lost their acoustic calling song (Morris et al. 1994). In this situation hearing might still be elaborated (Hartbauer et al., 2010; Hummel et al., 2014; Montealegre-Z et al., 2012; Palghat Udayashankar et al., 2012; Stölting and Stumpner, 1998) to detect the echolocation calls of gleaning bats.

Anecdotally, tremulation velocity in *C. vigorosa* seems to differ greatly between sexes, with the female consistently performing tremulation pulses with twice the amplitude of the males (Fig. 6A and C). Although such differences might be related to body size (body average weight: ♀ 1.51 ± 0.2 g, ♂ 0.79 ± 0.3 g), the amplitude response might also depend on the angle of incidence of the laser beam on the plant, but we did not control for this. Limitations of our work on seismic signalling in *C. vigorosa* arise by the fact that substrate vibrations were not recorded from the native host plants, but from palms we acquired on the UK market. We also recorded vibrations from a single point at the base of each palm branch, and did not try other locations. Römer et al. (2010) showed that the host plant of *Docidocercus gigliotosi* filters vibrations, depending on the location of the signaller on the plant, and that some parts of the plant enhance the transmission of the vibration. For instance, the leaves seem to act as resonators in the range of 10-15 Hz. We did not measure vibration from the leaves, but the frequency of the vibrations recorded from the base of the plant fall in the range reported by Römer et al. (2010).

4.3. Systematics

Twenty six species of *Copiphora* are currently listed in the Orthoptera Species File online data base (Eades et al. 2016), from which eight have been described/reported from Colombia (Chamorro-R et al., 2011). *Copiphora vigorosa* resembles *C. brevisrostris* in some morphological and behavioural aspects (e.g., face coloration, low call duty cycle, tremulations), but revealing the relatedness of these two species requires further phylogenetic analysis of the genus.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at <http://dx.doi.org/xxxxxxxxxxxxxx>

References

- Bailey, W.J., 1970. The mechanics of stridulation in bush crickets (Tettigoniidae, Orthoptera) I. Tegminal Generator. *J. Exp. Biol.* 52, 495-505.
- Belwood, J.J., 1988. Foraging behavior prey selection and echolocation in Phyllostominae bats (Phyllostomidae), in: Nachtigall, P.E., Moore, P.W.B. (Eds.), *Animal Sonar, Processes and Performances*. Plenum Press, New York, pp. 639-644.
- Belwood, J.J., 1990. Anti-predator defences and ecology of Neotropical forest bush-cricket, especially the Pseudophyllinae, in: Bailey, W.J., Rentz, D.C.F. (Eds.), *The Tettigoniidae: Biology, Systematics and Evolution*. Crawford House Press, Bathurst, pp. 8-26.
- Belwood, J.J., Morris, G.K., 1987. Bat Predation and Its Influence on Calling Behavior in Neotropical Bush-cricket. *Science* 238, 64-67.
- Bennet-Clark, H.C., 2003. Wing resonances in the Australian field cricket *Teleogryllus oceanicus*. *J. Exp. Biol.* 206, 1479-1496.
- Bethoux, O., 2012. Grylloptera - a unique origin of the stridulatory file in bush-cricket, crickets, and their kin (Archaeorthoptera). *Arthropod Systematics & Phylogeny* 70, 43-68.
- Chamorro-R, J., Cadena-Castañeda, O.J., Braun, H., Montealegre-Z, F., Romero, R.I., Serna-Marquez, F.H., Gonzales -O, R., 2011. Checklist and new distribution records of bush-cricket (Orthoptera: Tettigoniidae) from Colombia. *Zootaxa* 3023, 1-42.

- Cocroft, R.B., Rodríguez, R.L., 2005. The Behavioral Ecology of Insect Vibrational Communication. *Bioscience* 55, 323-334.
- Eades, D.C., Otte, D., M., C.M., Braun, H., Orthoptera Species File. Version 5.0/5.0. , 2015 ed. The Orthopterists' Society.
- Falk, J.J., ter Hofstede, H.M., Jones, P.L., Dixon, M.M., Faure, P.A., Kalko, E.K.V., Page, R.A., 2015. Sensory-based niche partitioning in a multiple predator–multiple prey community. *Proceedings of the Royal Society of London B: Biological Sciences* 282.
- Hartbauer, M., Radspieler, G., Römer, H., 2010. Reliable detection of predator cues in afferent spike trains of a bush-cricket under high background noise levels. *J. Exp. Biol.* 213, 3036-3046.
- Hill, P.S.M., Shadley, J.R., 2001. Talking back: Sending soil vibration signals to lekking prairie mole cricket males. *Am. Zool.* 41, 1200-1214.
- Hummel, J., Wolf, K., Kössl, M., Nowotny, M., 2014. Processing of simple and complex acoustic signals in a tonotopically organized ear. *Proc Biol Sci* 281.
- Lakes-Harlan, R., Strauß, J., 2014. Functional Morphology and Evolutionary Diversity of Vibration Receptors in Insects, in: Cocroft, R.B., Gogala, M., Hill, P.S.M., Wessel, A. (Eds.), *Studying Vibrational Communication*. Springer Berlin Heidelberg, pp. 277-302.
- McNett, G.D., Cocroft, R.B., 2008. Host shifts favor vibrational signal divergence in *Enchenopa binotata* treehoppers. *Behav. Ecol.* 19, 650-656.
- Michelsen, A., Fink, F., Gogala, M., 1982. Plants as transmission channels for insect vibrational songs. *Behav. Ecol. Sociobiol.* 11, 269-281.
- Montealegre-Z, F., 2005. Biomechanics of musical stridulation in bush-crickets (Orthoptera: Ensifera: Tettigoniidae): an evolutionary approach, Department of Zoology. University of Toronto, Toronto, p. 328.
- Montealegre-Z, F., 2009. Scale effects and constraints for sound production in bush-crickets (orthoptera: tettigoniidae): generator morphology constrains signal parameters. *J. Evol. Biol.* 22, 355–366.
- Montealegre-Z, F., 2012. Reverse stridulatory wing motion produces highly resonant calls in a neotropical bush-cricket (Orthoptera: Tettigoniidae: Pseudophyllinae). *J. Insect Physiol.* 58, 116-124.
- Montealegre-Z, F., Jonsson, T., Robert, D., 2011a. Sound radiation and wing mechanics in stridulating field crickets (Orthoptera: Gryllidae). *J. Exp. Biol.* 214, 2105-2117.
- Montealegre-Z, F., Jonsson, T., Robson-Brown, K.A., Postles, M., Robert, D., 2012. Convergent evolution between insect and mammalian audition. *Science* 338, 968-971.
- Montealegre-Z, F., Mason, A.C., 2005. The mechanics of sound production in *Panacanthus pallicornis* (Orthoptera : Tettigoniidae : Conocephalinae): the stridulatory motor patterns. *J. Exp. Biol.* 208, 1219-1237.
- Montealegre-Z, F., Morris, G.K., Mason, A.C., 2006. Generation of extreme ultrasonics in rainforest bush-crickets. *J. Exp. Biol.* 209, 4923-4937.

- Montealegre-Z, F., Morris, G.K., Sarria-S, F.A., Mason, A.C., 2011b. Quality calls: phylogeny and biogeography of a new genus of neotropical bush-cricket (Orthoptera: Tettigoniidae) with ultra pure-tone ultrasonics. *Syst. Biodivers.* 9, 77-94.
- Montealegre-Z, F., Postles, M., 2010. Resonant sound production in *Copiphora gorgonensis* (Tettigoniidae: Copiphorini), an endemic species from Parque Nacional Natural Gorgona, Colombia. *J. Orthoptera Res.* 19, 347-355.
- Morris, G.K., 1980. Calling display and mating behaviour of *Copiphora rhinoceros* Pictet (Orthoptera, Tettigoniidae). *Anim. Behav.* 28, 42-51.
- Morris, G.K., 1999. Song in arthropods, in: Davey, K.G. (Ed.), *Encyclopedia of Reproduction*. Academic Press, San Diego, pp. 508–517.
- Morris, G.K., Mason, A.C., Wall, P., Belwood, J.J., 1994. High ultrasonic and tremulation signals in neotropical bush-crickets (Orthoptera, Tettigoniidae). *J. Zool. (Lond.)* 233, 129-163.
- Morris, G.K., Pipher, R.E., 1967. Tegminal amplifiers and spectrum consistencies in *Conocephalus nigropleurum* (Bruner), Tettigoniidae. *J. Insect Physiol.* 13, 1075-1085.
- Palghat Udayashankar, A., Kössl, M., Nowotny, M., 2012. Tonotopically arranged traveling waves in the miniature hearing organ of bushcrickets. *Plos One* 7, e31008.
- Römer, H., Lang, A., Hartbauer, M., 2010. The Signaller's Dilemma: A Cost–Benefit Analysis of Public and Private Communication. *PLoS ONE* 5, e13325.
- Sarria-S, F.A., Morris, G.K., Windmill, J.F.C., Jackson, J., Montealegre-Z, F., 2014. Shrinking Wings for Ultrasonic Pitch Production: Hyperintense Ultra-Short-Wavelength Calls in a New Genus of Neotropical Bush-crickets (Orthoptera: Tettigoniidae). *Plos One* 9.
- Stölting, H., Stumpner, A., 1998. Tonotopic organization of auditory receptors of the bushcricket *Pholidoptera griseoptera* (Tettigoniidae, Decticinae). *Cell Tissue Res.* 294, 377-386.
- Stumpner, A., Dann, A., Schink, M., Gubert, S., Hugel, S., 2013. True Bush-crickets (Pseudophyllinae) from Guadeloupe: Acoustic Signals and Functional Considerations of Song Production. *Journal of Insect Science* 13, 157.

Table1. Body measurements (mm) of examined material of *Copiphora vigorosa*. R=right, L=left, (*)=Holotype and (**)=Allotype specimens.

	Female 1**	Female 2	Mean female	SD	Male 1	Male 2	Male 3	Male 4*	Male 5	Male 6	Male 7	Male 8	Mean male	SD
Body	45.8	45.69	45.75	0.05	41.9	40.69	41.16	31.63	39.48	34.36	31.84	31.84	36.61	4.61
Fastigium	3.17	3.87	3.52	0.35	3.03	3.34	2.84	3.34	3.44	3.27	2.63	2.83	3.09	0.29
Pronotum	9.73	10.45	10.09	0.36	10.44	10.29	9.91	10.29	10.82	11.06	8.97	10.29	10.26	0.63
Tegmen	39.58	41.18	40.38	0.80	35.19	35.47	32	35.47	36.99	31.83	29.5	34.73	33.89	2.51
F-Femur R	10.22	10.1	10.16	0.06	9.1	9.24	8.24	9.24	9.81	9	7.24	8.92	8.85	0.78
F-Femur L	10.11	10.03	10.07	0.04	8.2	9.25	8.3	9.25	9.29	9.05	7.09	8.72	8.64	0.76
M-Femur R	10.11	10.71	10.41	0.30	8.16	9.63	8.51	9.63	9.28	9.53	7.46	8.78	8.87	0.79
M-Femur L	10.14	11.11	10.63	0.48	8.94	9.11	8.21	9.11	9.17	9.41	7.58	8.73	8.78	0.60
H-Femur R	20	22.8	21.40	1.40	18.17	19	17.15	19	18.61	M	15.36	17.87	17.88	6.43
H- Femur L	19.98	22.3	21.14	1.16	18.11	18.7	20.07	18.7	18.69	18.31	15.1	17.86	18.19	1.41
F-Tibia R	12.49	13	12.75	0.26	11.18	12.03	11.1	12.03	11.54	10.51	0	9.61	9.75	4.02
F-Tibia L	11.16	12.1	11.63	0.47	12.03	11.6	10.37	11.6	11.18	10.99	8.53	9.58	10.73	1.18
M-Tibia R	11.33	12.59	11.96	0.63	9.9	10.86	10.05	10.86	10.44	9.84	8.74	9.43	10.01	0.72
M-Tibia L	11.08	12.62	11.85	0.77	10.11	11.22	9.66	11.22	10.86	10.05	8.51	9.47	10.14	0.94
H-Tibia R	20.86	23.36	22.11	1.25	18.32	20.36	17.53	20.36	19.16	19.58	15.59	18.4	18.66	1.59

H-Tibia L	21.22	22.92	22.07	0.85	18.23	19.97	20.13	19.97	19.25	0	15.29	18.49	16.42	6.82
S-plate	2.51	2.62	2.57	0.06	3.2	4.34	3.61	4.34	3.65	3.48	2.72	3.19	3.56	0.56
Cercus R	3.22	2.53	2.88	0.35	2.56	2.74	2.01	2.74	3.05	2.43	2	2.28	2.47	0.37
Cercus L	3.15	2.27	2.71	0.44	1.97	2.22	2.45	2.22	2.78	2.36	2	2.21	2.27	0.26
Ovipositor	57.61	49.08	53.35	4.27	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Eye Diameter R	1.98	1.93	1.96	0.03	1.59	1.43	1.56	1.43	1.84	1.62	1.67	1.54	1.58	0.13
Eye Diameter L	1.8	1.8	1.80	0.00	1.47	1.4	1.53	1.4	1.77	1.65	1.68	1.55	1.55	0.13

Table 2. Analysis of acoustic parameters. Values shown for each male represent an average of different recordings (see N). ST= Syllable train. LF= low frequency and HF= high frequency: spectral width measured at 10 dB below maximum peak. Syllable maximum amplitude refers to the time when its wave envelope reaches the highest value.

Individuals	Call duration (s)	Call/hour	No. ST/call	ST duration (ms)	ST Period (s)	ST duty cycle %	Syllables per ST	Syllable duration (ms)	Syllable Max. amplitude (ms)	dB (SPL)	Max. peak (kHz)	LF at 10dB down Max. (kHz)	HF at 10dB down Max. (kHz)
Male 1/Dec 2014 (N=4)	1.87	3	7-8	104.48	0.26	5.68	9-10	8.68	4.87	103.98	29.38	9.88	35.13
Male 2/Nov 2015 (N=4)	3.23	4	8-9	92.13	0.43	2.90	8	7.65	5.63	103.98	37.38	10.78	40.68
Male 3/Nov 2015 (N=2)	2.97	4	8	101.85	0.41	3.43	8-9	7.90	4.10	100.85	32.25	10.00	35.15
Male 4/Nov 2015 (N=2)	2.13	4	3-6	110.80	0.33	8.63	8-10	6.20	3.63	102.45	31.70	10.10	37.90
Averages	2.55	~4	~5	102.31	0.36	5.16	8	7.61	4.55	102.81	32.68	10.19	37.21
STDV	0.65	NA	NA	7.76	0.08	2.61	NA	1.03	0.88	1.49	3.37	0.40	2.65

Figure legends

Fig. 1. Morphological features of *C. vigorosa*. (A) Frontal view of the male rostrum showing the frons coloration. (B) Lateral view showing gena and pronotum. (C-D) Male and female terminalia, respectively, showing male cerci and female ovipositor base. (E-F) Male and female subgenital plates (respectively). (G) Abdomen (ventral view) of female displaying pigmentation pattern. (H) Male right cercus. (I) Ovipositor. (J) Female resting on a leaf.

Figure 2. The wing's structures involved in the sound production. (A) Scanning electron microscope of the mirror (dorsal surface) and adjacent cells in the right tegmen. (B) Stridulatory file. Image obtained using Alicona Infinite Focus Technology. (C) Inter-tooth distances measured in the direction of scraper movement during stridulation.

Figure 3. Song structures of *Copiphora* spp. (A-D). (A) *C. vigorosa*, four syllable trains recorded at 30°C. (B) Close up of one syllable train in (A) at higher resolution. (C) A single syllable. (D) Power spectrum of a single train of syllables. (E-H) Same data as in A-D for the potentially closest relative *C. brevirostris*, (E) Oscillogram of the calling song. (F) Three syllables in a single stridulatory event. (G) Last syllable of the calling song at higher resolution. (H) Power spectrum of the call: Fundamental frequency at 17.29 kHz and a more intense harmonically-related peak at 34.67 kHz.

Figure 4. Frequency analysis in the time domain. (A) Spectrogram showing the variation in frequency over time. Lateral ghost plot in transparent red depicts the spectrum of the syllable in B. (B) One syllable extracted for frequency analysis.

Figure 5. Scanned areas and deflection profiles of the right wing mirror. (A) Dorsal view of the extended wings and the scanned areas. (B) FFT analysis of left and right wing resonances (blue and red outlines), over plotted on the spectrum of the calling song (dashed spectrum in black). The light blue and pink shaded areas indicate one standard deviation from the mean of right and left mirror resonances, respectively. (C-G) Lateral view of the right wing indicating the mirror area deflections at 32 kHz. Each panel shows deflection taking in steps of 45° along the oscillation cycle. Red indicates positive displacements and blue negative displacements. The colour coded scale bar indicates the velocity range of the response.

Figure 6. Substratum vibrations recorded with a LDV. (A, C) Temporal pattern of female and male, respectively, showing a short sequence of pulses in each case. (B, D) FFT analysis of the temporal segments shown in A and C.

