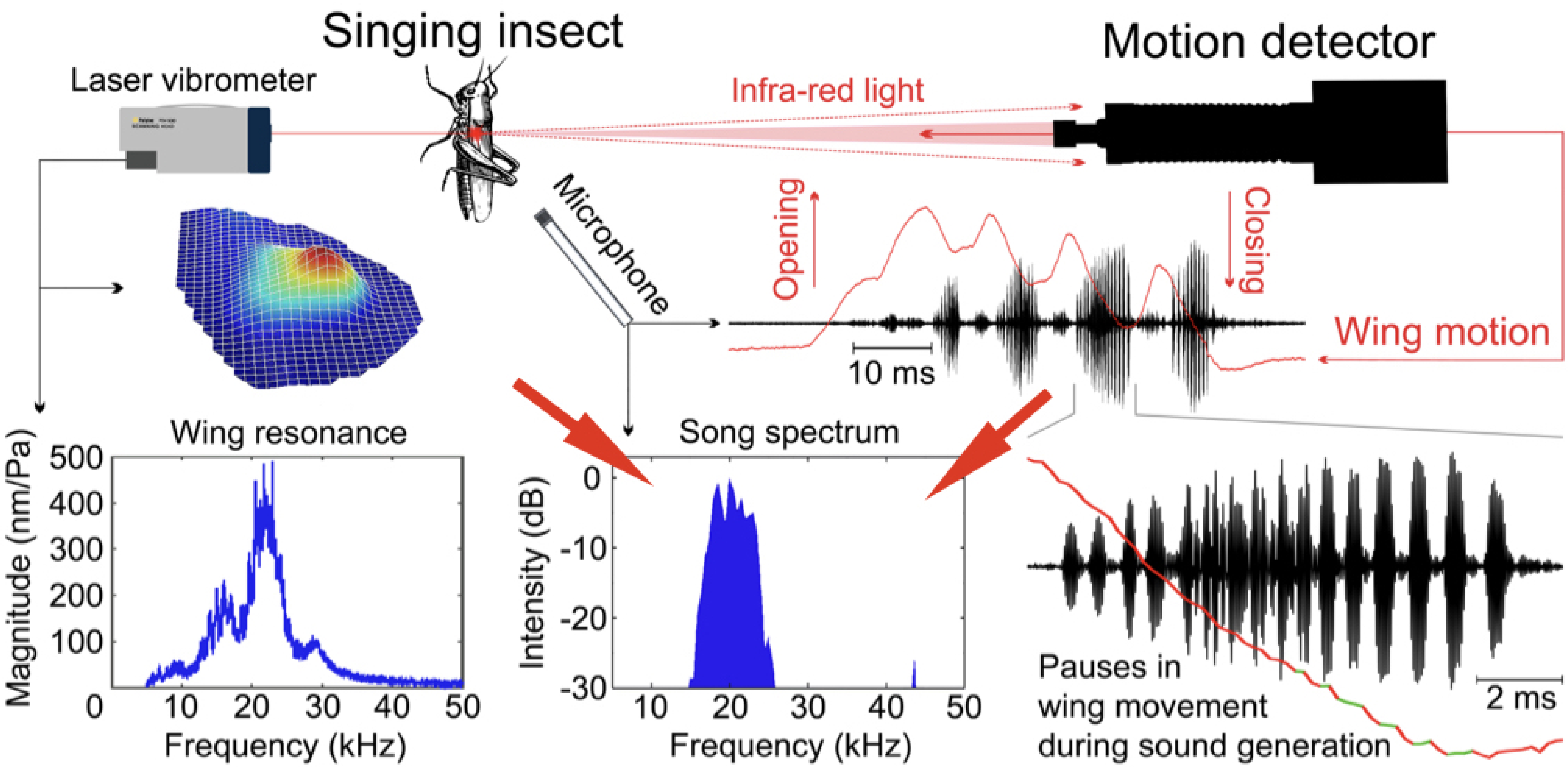

Singing insect
Motion detector
Laser vibrometer
Infra-red light
Microphone
Opening
Closing
Wing motion
10 ms
Wing resonance
Magnitude (nm/Pa)
500
400
300
200
100
0
10
20
30
40
50
Frequency (kHz)
Song spectrum
Intensity (dB)
0
-10
-20
-30
10
20
30
40
50
Frequency (kHz)
Pauses in
wing movement
during sound generation
2 ms

Complex wing motion during stridulation in *Nastonotus foreli* (Orthoptera: Tettigoniidae: Pseudophyllinae)

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Abstract

Male Katydids (Orthoptera: Tettigoniidae) rub together their specialised forewings to produce sound, a process known as stridulation. During wing closure, a lobe on the anal margin of the right forewing (a scraper), engages with a teeth-covered file on the left forewing. The movement of the scraper across the file produces vibrations which are amplified by a large wing cell adjacent to the scraper, the mirror. Katydids are known to stridulate with either sustained or interrupted sweeps of the file, generating resonant pure-tone (narrowband frequency) or non-resonant (broadband frequencies) calls. However, some species can conserve some purity in their calls despite incorporating discrete pulses and silent intervals. This mechanism is exhibited by many Cocconotini such as *Nastonotus* spp., *Cocconotus* spp., and *Eubliastes* spp. This study aims to measure and quantify the mechanics of wing stridulation in *Nastonotus foreli*, a Neotropical katydid that can produce complex, relatively narrowband calls at ≈20 kHz. It was predicted that this species will use a stridulatory mechanism involving elastic energy whereby the scraper bends and flicks along the file in periodic bursts. The calling behaviour and wing mechanics of seven males were studied using a combination of technologies (e.g. laser Doppler vibrometry, advanced microscopy, ultrasound-sensitive equipment and motion detectors) to quantify wing mechanics and structure. Analysis of recordings revealed no clear relationship between wing velocity and carrier frequency, and a pronounced distinction between wing velocity and scraper velocity during wing closure, suggesting that the scraper experiences considerable deformation. This is characteristic of the elastic scraper mechanism of stridulation. Curiously, *N. foreli* might have evolved to employ elastic energy to double the duration of the call, despite possessing muscles that can reach velocities high enough to produce the same frequency with a coupled scraper.

Keywords: Bush-cricket; ultrasound; bioacoustics; laser vibrometry; insect song; Neotropics

1. Introduction

Many arthropods are known to produce sound via a process known as stridulation where specialised body parts are rubbed together. Stridulation has been observed in crickets, beetles, flies, ants, cicadas, moths, spiders, scorpions and lobsters (Dumortier, 1963; Dupérré and Tapia, 2015; Golden and Hill, 2016; Kikuchi et.al., 2014; Lees, 1992; Luo and Wei, 2015; Ochoa and Ojanguren Affilastro, 2007; Puniamoorthy et al., 2009; Smith and Wirth, 2016). Katydids (also known as bush-crickets; Orthoptera: Tettigoniidae) use tegminal stridulation, the rubbing together of their specialised forewings (tegmina) (Heller and Hemp, 2014; Montealegre-Z, 2005; Robillard et al., 2015).

Male katydids generally produce mating songs by repeatedly opening and closing their wings, but the predominant sound components are usually generated during the closing phase (Montealegre-Z et al 2006; Montealegre-Z, 2012; Morris 1999; Walker and Dew, 1972; Walker 1975). During each wing closure, a structure on the anal margin of the right forewing, known as the scraper, engages with the anal end of a specialised, teeth-covered vein on the ventral surface of the left forewing called the stridulatory file, or pars stridens (Dumortier, 1963). The continuous movement of the scraper across the file produces a pulse (a continuous sound wave train isolated in time by considerable amplitude changes) (Montealegre-Z, 2005; Walker and Carlysle, 1975). These vibrations are further amplified and radiated by a membrane adjacent to the scraper on the right forewing called the mirror (Chivers et al., 2013; Chivers et al., 2017; Heller and Hemp, 2014; Montealegre-Z, 2009; Montealegre-Z, 2012; Xiao et al., 2013).

There are three common stridulatory mechanisms used by katydids. The first reaches resonance with pure tone sustained pulses associated with uninterrupted sweeps of the file using a coupled scraper (wing and scraper move at an almost identical velocity during closure). An oscillation of the mirror occurs every time a tooth is struck and is sustained in time by each subsequent tooth strike (Montealegre-Z and Postles, 2010). This produces a single continuous pulse of sinusoidal sound during a single closing stroke of the wing whereby, in theory, the number of teeth used should match the number of sine waves produced (Montealegre-Z and Mason, 2005; Walker and Carlysle, 1975). The scraper is a somewhat flexible structure so its velocity changes, relative to the rest of the wing, as it catches, bends and releases from each tooth (Montealegre-Z et al., 2006). As the scraper in this mechanism is only slightly flexible, the velocity of the scraper is close to the velocity of the wing closure. Using this basic mechanism, the muscles must move the wing at the velocity required to strike the teeth at a rate matching the resonant frequency of the mirror, thus efficiently producing the desired carrier frequency f_c (Prestwich and O'Sullivan, 2005). This is common in katydids that call under 40 kHz but, to reach a higher f_c, another, more advanced process must be used (Montealegre-Z and Mason, 2005; Montealegre-Z et al 2006; Morris et al., 1994).

Deformed elastic tissue can release energy much faster than a working muscle (Bennet-Clark and Lucey, 1967; Burrows, 2003; Knight 2009; Krasnov et al., 2004). The second mechanism of stridulation involves the use of such elastic energy to reach resonance by creating a train of discrete pulses during each wing closing stroke with a decoupled scraper (wing and scraper velocity are significantly different during wing closure), leaving little or no relationship between f_c and wing velocity and tooth density. Wing velocity in this mechanism is generally relatively low. During wing closure, the scraper is caught behind a tooth, stops and bends, gathering elastic energy. This energy is then suddenly released and the scraper moves at a greater velocity relative to the wing. Therefore, the scraper impacts several teeth at a much higher frequency, compared to a coupled scraper with the same wing velocity, and this cycle of catch and release is continued. Due to the release of elastic energy, scraper velocity is generally much greater than wing velocity (Montealegre-Z et al., 2006). Wing velocity can moderately decrease or even drop to zero when the scraper bends (Montealegre-Z, 2005). The extent to which the scraper bends is down to its shape and flexibility (Montealegre-Z et al., 2006). This elastic mechanism of stridulation is generally used by katydids with small body sizes to achieve particularly high f_c. This is usually because their small muscles lack the strength to close the wing fast enough (Montealegre-Z, 2009; Wallschläger, 1980).

Finally, the third mechanism of stridulation (roll-trigger) also produces resonant groups of discrete pulses during wing closure. Wing velocity gradually increases along the file so, to maintain a constant tooth contact rate, and a constant f_c, the scraper bends to its maximum then jumps over some of the file teeth, without stopping the wing movement, resulting in silent intervals of no scraper contact where the wing accelerates. This mechanism is used by several species of Cocconotini and Platyphyllini (Pseudophyllinae) katydids singing below ≈30 kHz (Montealegre-Z, 2005; Montealegre-Z et al 2006).

This study aimed to measure and describe the mechanics of wing stridulation in *Nastonotus foreli*, a Neotropical katydid (Pseudophyllinae: Cocconotini). The call of this species contains sequences of relatively narrowband discrete pulses with a proportional number of silent intervals, grouped in what seems to be various types of syllables. Using a combination of techniques (laser Doppler vibrometry, advanced microscopy, motion detectors, and ultrasound sensitive equipment) this paper reveals which mechanism of stridulation is utilised by *N. foreli* males.

2. Materials and methods

2.1 Study species

N. foreli is a Neotropical katydid found in Colombia (Chamorro-Rengifo et al., 2011; Orthoptera.speciesfile.org, 2017). Specimens were collected in Palmar de la Vizcaina (Magdalena medio) under official permits of the Colombian Ministry of Environment (National Parks section) and bred in captivity at the animal facility of the University of Lincoln, UK. Males in the final nymphal stage before adulthood were separated and kept in individual containers. The seven individuals studied in this project were kept in an incubator (Panasonic MIR-154-PE Cooled Incubator; PHC Europe B.V., Den Haag, Netherlands) on a 12-hour night (25 °C, ≈60% rel. humidity) day (30 °C, ≈60% rel. humidity) cycle with *ad libitum* access to fish flakes, corn, apple and water.

2.2 Wing resonance and laser vibrometry experiments

Wing resonance and vibration velocities were measured in five (of the seven males) specimens using a microscanning laser Doppler vibrometer (Polytec PSV-500-F, Waldbronn, Germany) with an OFV-056 scanning head, fitted with a close-up attachment, in response to sympathetic vibration. The laser spot location on the tegmen membrane was monitored by live video feed to the vibrometer's controlling computer. This system allows accurate measurement of the wing surface motion, without requiring the use of a reflective medium.

For the experiments, the whole stridulatory field in both tegmina was measured using 250-280 measurement points. Tegminal vibrations were examined in the frequency domain in response to acoustic stimulation with periodic chirp signals in the range 1-60 kHz. The spectrum of the stimulus was corrected to be flat at 60 dB SPL (re 20 µPa). The acoustic signals were generated by the PSV 500 internal data acquisition board (National Instruments PCI-4451, Austin, TX, USA), amplified (A-400, Pioneer, Tokyo, Japan) and passed to a loudspeaker (Ultrasonic Dynamic Speaker Vifa, Avisoft Bioacoustics, Glienicke, Germany) positioned 30 cm from the specimen. For recordings, an intact specimen was mounted on a Blu-Tack holder using metallic clamps to fix the body and legs. The wings were laterally extended by fixing the axillary sclerites with bee's wax. A Brüel & Kjær 1/8-inch condenser microphone was placed at the position of the wings to monitor and record the acoustic stimulus as a reference. Within the frequency domain setting of the vibrometer, a frequency spectrum was calculated for each point using a fast Fourier transform (FFT) with a rectangular window, at a sampling rate of 128 kHz, 128 ms sampling time, and with a frequency resolution of 7.8125 Hz. A high-pass filter of 1 kHz was applied to both the vibrometer and reference microphone signals during the scanning process, with an average of five samples taken at each recording point.

2.3 Acoustic recordings

At least two days after moulting to adult, the pronota of seven other katydids were labelled with a number using Canada balsam (Queen bee marking kit, EH Thorne (Beehives) Ltd, Market Rasen, UK) (Fig. S1). Then, after a minimum of two days of rest from the label attachment, acoustic recordings were taken. Shortly, before the night cycle began, the katydids were moved to a sound attenuating booth, placed in fine metal mesh cages (6 x 10 cm) and suspended from the middle of the ceiling to avoid sound reflection from the walls. The singing males were recorded with a wide-bandwidth response 1/8-inch microphone (Brüel & Kjær, 4138-A-015 with preamplifier model 2670, connected to a NEXUS Microphone Conditioner amplifier type 2690-A; Brüel & Kjær, Nærum, Denmark) and the PSV-500 acquisition board. Polytec software was used to acquire and save the files for later analysis (512 k-samples/s), (PSV 9.0.2, Polytec GmbH, Waldbronn, Germany). The microphone was placed 10 cm from the dorsum of the singing insect. In the booth, the temperature was 25.4 ± 0.9°C and the humidity was 33.0 ± 1.3%. After 10 control recordings were recorded, a 2x2 mm square of reflective tape (Scotchlite 7610 retro-reflective tape, 3M, Maplewood, MN, USA) was attached to the left forewing using canada balsam, adjacent to the stridulatory area in the distal position (Fig. S1) in preparation for wing motion experiments.

2.4 Wing motion and sound recordings

After application of the reflective tape, the seven specimens were left in their home containers for 2-3 days. Stridulatory movements of the reflective tape on the left forewing were recorded in the sound attenuating booth using a motion detector (von Helversen and Elsner, 1977; Hedwig, 2000, Heller, 1988), as described by Montealegre-Z and Mason (2005). The movements of the wing in the coronal plane of the specimen resulted in changes in the current of the diode which were recorded alongside sound signals on separate channels of a computer data acquisition board (Polytec hardware) at 512 kHz sampling rate. The motion signal was low-pass filtered at 1 kHz, and the acoustic signal was high-pass filtered at 1 kHz to remove high- and low-frequency noise, respectively. The light used for the photodiode had a red filter as not to disturb the katydids. During recordings, the temperature was 24.0 ± 0.7°C and the humidity was 35.7 ± 0.7%.

2.5 Morphological measurements

Various body parts of the seven males were measured using a digital calliper (Fowler High Precision, Newton, MA, USA). Body length was measured as the midline from the fastigium to the last abdominal tergite. The pronotal length was measured along the sagittal plane. Specimens were cooled to immobility, and the left forewing was carefully dissected from each male, the stridulatory areas gold sputter coated and viewed on a scanning electron microscope (Inspect S50, FEI, Eindhoven, Holland) using the manufacturer's software (xTm software, FEI). An Alicona Infinite Focus G5 (Alicona Imaging GmbH, Graz, Austria) was used to generate a 3D image of the stridulatory area.

2.6 Data analysis

Motion and acoustic signals were analysed using custom-written scripts in MATLAB (R2017a, The Mathworks Inc., Natick, MA, USA). The amplitude of each isolated syllable was calculated as the root-mean-square of the waveform. The f_c was calculated from the isolated syllables using a fast Fourier transform with a rectangular window and 1024 lines.

The quality factor Q is a dimensionless index that indicates the sharpness of the resonance: the higher the Q, the sharper the resonance (Bennet-Clark 1999, 2003). A system with low-quality factor ($Q < 1/2$) is said to be overdamped, while those with $Q > 1/2$ are said to be underdamped (Fletcher, 1992). In musical instruments, Q is usually high (>100), but in insects, Q usually varies between 1 and 50 (Baker et al., 2017; Bennet-Clark, 2003; Chivers et al., 2017; Montealegre-Z & Postles, 2010; Montealegre et al., 2006, 2011; Prestwich 2000, 2005). Q can be defined in several ways (Bennet-Clark 1999) and is here defined as the ratio of the frequency of the peak response divided by the spectral width at the two values above and below resonant frequency with amplitudes 0.707 times the peak value (Fletcher 1992). This is equivalent to measuring the bandwidth at -3 dB below resonance in a logarithmic scale, which is the Q reported here as Q_{-3}.

3D images of the stridulatory files of seven males were analysed with ImageJ (Rasband, 1.50i) to measure the morphology of the file. The inter-tooth distance (ITD) was measured as the middle of the edge of one tooth to the next (see Montealegre-Z and Mason 2005) and the tooth density was calculated as the number of teeth divided by the sum length of the ITDs. The ITDs were taken from the whole file but the tooth density and mean ITD was only calculated from the middle 50% of the file ITD as the anal and basal quarters were highly irregular (see Fig. 1 and S2).

Due to variation in the animal's position while singing, the motion provided unreliable wing displacement values during the recording process. To calculate accurate displacement values, the relative displacement of one closing stroke was compared with the total displacement of the verse to find the proportion of file used. This was under the assumption that the male used the entirety of its file during the verse. The total estimated displacement = length of whole file × proportion of file used. Therefore, the observed closing wing velocity = total estimated displacement ÷ duration of the closing stroke. To calculate the predicted scraper velocity, the tooth strike rate was calculated from the recorded f_c and time of a single closing stroke. For example, to reach a f_c of 21 kHz, a tooth must have been struck every 21000th of a second. If the mean ITD was 100 nm, predicted scraper velocity = 0.1 ÷ (1/21000) = 210 mm/s.

Statistical analysis was carried out using R (R Core Team, 2017). A one-way ANOVA was used to compare the mean f_c between males. The post hoc Tukey test was used to find which males had a different mean f_c. An ANCOVA was used to compare the mean f_c of the loudest syllables with and without reflective tape while taking into account the effects of temperature, humidity and age. Regression analyses were used to compare f_c of the loudest syllables with body, pronotal, tegmen, and stridulatory file length, mean ITD and tooth density. Mean estimated closing wing velocity and mean predicted scraper velocity were compared using a paired T-test to find an overall difference, then regression analyses were used to find a difference within recordings of the same individuals.

3. Results

3.1 Intraspecific f_c variation and call purity

Acoustic and wing motion recordings were successfully achieved in seven males. Katydids sang at a mean f_c ± SE (standard error) of 20.008 ± 0.27 kHz. The complex call of *N. foreli* exhibits a spectrum with features of both narrow and broadband sound, with a mean Q_{-3} ± SD of 5.593 ± 0.66 and bandwidth of 3.815 ± 0.25 kHz (Table 1; Fig. 2 and S3-9). The mean f_c of the different males was significantly different (ANOVA $F_{6,61}$ = 63.65, p < 0.001). M6 had the highest f_c, followed by M2. M7 and M1 had significantly higher f_c than M3, M4 and M5 (Post hoc Tukey test: p < 0.05; table 1). Among all specimens recorded, M3 shows an unusual broadband call associated with erratic, short and rapid decay syllables (Fig. 2 and S5).

3.2 Variation in f_c before and after tape attachment

Katydids sang at a mean ± SE of 19.646 ± 0.47 kHz without tape attached but sang at 20.312 ± 0.52 kHz with tape attached which comprises a mean increase in f_c of 0.666 ± 0.22 kHz. Despite this, there was no significant effect of tape attachment on f_c of the loudest syllables after controlling for the effect of temperature, humidity and the age (ANCOVA: $F_{1,9}$ = 0.00, p = 0.982).

3.3 Allometric relationships with f_c

Body length was negatively related to the f_c of the loudest syllable. M1 was partially cannibalised so it could not be included in this test. Pronotum, tegmen and stridulatory file length were also negatively related to the f_c of the loudest syllable (Table 2).

3.4 The morphology of the stridulatory file

The mean ± SE number of file teeth was 195 ± 3, the ITD was 9.946 ± 0.25µm and tooth density was 123 ± 3 teeth/mm between all males (n=7). Mean ITD was not related to the f_c of the loudest syllable ($F_{1,68}$ = 1.40, p = 0.241, R^2 (adj) = 0.0058; f_c = 23.63 – 0.334 mean ITD). However, tooth density had a very weak positive relationship with the f_c of the loudest syllable ($F_{1,68}$ = 4.18, p = 0.045, R^2 (adj) = 0.044; f_c = 15.17 – 0.0419 tooth density). There was a slight overall increase in ITD towards the basal end of the file in all males. The middle 50% of the file generally had a very uniform structure, unlike the anal and basal ends where the tooth spacing became increasingly irregular (Fig. 1).

3.5 Wing motion and velocity

N. foreli generally opened and closed their wings three to five times during one verse (Fig. 4A). Notice how almost all the maximum amplitude components of the sound are produced during the closing strokes. The opening stroke produces an irregular pulse train of low amplitude. The wing generally accelerates to about 200 mm/s during a silent interval, then decelerates just before and during a pulse and comes to a stop. This cycle repeats (Fig. 4B).

The wing velocity of M7 was positively related to syllable f_c, and while statistically significant, this relationship was not strong. All other males did not show a significant relationship between wing closure velocity and syllable f_c (Table 3; Fig. 3b). M4 was not included as many of its syllables were lacking in discrete pulses, thus leading to false estimated values of wing velocity (Fig. 2 and S6). Fig. 3A shows the calculated scraper velocities for the males, depending on f_c of the syllable.

There was a significant difference between mean estimated wing velocity and mean predicted scraper velocity values of closing stroke recordings (Paired t-test; $t = -18.02$, $df = 6$, $p < 0.001$). Predicted scraper velocity gave a mean of 203.38 ± 5.46 mm/s while closing wing velocity gave a mean ± SE of 91.57 ± 3.18 mm/s, differing by 111.81 ± 6.20 mm/s.

3.6 Wing vibrations measured with LDV

Vibrations in response to sound stimuli are limited to the mirror of both wings (Fig. 5), however, the mirror of the right wing dominated in amplitude (Fig. 6). The main vibrations of the right mirror occur in a basic mode corresponding to the dominant resonant frequency (at 21.2 ± 2.1 kHz). This vibration pattern was observed in all right wings of all specimens scanned (n=5). This frequency of vibration is close to the calling song frequency (20.0 ± 0.3 kHz). The resonant frequency of the left mirror, on the other hand, is lower (16.2 ± 2.1 kHz, n=5) than that of the right mirror. At resonance, peak vibration amplitudes of the right mirror are usually 4-5 times higher than those of the left mirror (Fig. 6), this corresponds to a difference of 13 dB. This difference could also be observed in the deflection maps shown in Fig. 5. In summary, the right mirror exhibits a natural tuning or resonance at a frequency close to the calling song and this matching suggests the natural frequency of the wings is functionally tuned to the specific sound frequency. Fig. 6B also shows the wing resonance of M3, a specimen with an unusual broadband call. This shows that the right mirror also influences the quality of the call emitted.

4. Discussion

Like other members of Ensifera (katydids and crickets), *N. foreli* exhibited infraspecific variation in f_c (Table 1). This variation is commonly explained by factors such as temperature, body size, natural wing resonance and tooth impact rate (Desutter-Grandcolas and Robillard, 2003; Montealegre-Z, 2005; Morris, 2008; Walker, 1962; Walker and Carlysle, 1975). The attachment of reflective tape (Fig. S1) to the forewing had no significant effect on f_c, despite some evidence to suggest that adding weight to the forewing would lower f_c (Almbro and Kullberg, 2011; Bailey and Broughton, 1970; Bailey, 1969; Montealegre-Z and Mason, 2005).

Body, pronotum, tegmen and stridulatory file length all had an inverse relationship with f_c (Table 2). This has previously been documented in other Ensiferans (Brown et al., 1996; Drosopoulos and Claridge, 2006; Howard and Hill, 2006) and is commonly explained by the correlation between larger males and lower tooth density, resulting in a lower f_c (Chivers et al., 2017; Montealegre-Z, 2005). However, this study only found a very weak relationship between f_c and tooth density and mean inter-tooth distance, so it seems likely that another factor may also cause the f_c variation. The stridulatory apparatus of the larger males may have had a lower natural resonance, as larger radiators tend to vibrate at lower frequencies (Montealegre-Z, 2009; Morris, 2008) due to increased mass and the more pronounced effect of inertia. The relatively gradually increasing inter-tooth distance between file teeth (Fig. 1B) and the right mirror resonances (Fig. 6) likely contributed to the control of the carrier frequency observed in the call. The observed variability in spectral breadth seems to result from mutable resonances on the right mirror, and from the combination of low and high resonances between both left and right mirrors (Fig. 6AB).

The estimated wing velocity from start to finish of one closing stroke is over 200 mm/s slower than the predicted scraper velocity during pulse generation. This is evidence for a stridulatory mechanism using a decoupled scraper (Montealegre-Z, 2005; Montealegre-Z et al. 2006). Fig. 3B demonstrates this disparity while also illustrating the lack of correlation between wing velocity and f_c (Table 3). This suggests elastic energy was stored and released in a relatively consistent fashion. The wing velocity would only affect the time taken to store the energy by bending the scraper, while the energy released would remain nearly constant resulting in the same f_c, regardless of wing velocity. This mechanism would also explain why f_c was not affected by the addition of tape even if this was reducing wing-closing velocity. It would also explain why temperature had no significant effect on f_c, even if it did increase wing velocity.

Fig. 4 also gives more evidence to suggest elastic energy was used. During the generation of discrete pulses, the wing stopped moving. This can only be explained by a decoupled scraper (Montealegre-Z, 2005). The wing velocity (shown in blue) increased during the silent interval, then, the scraper would have caught on a tooth, began to bend and brought the wing to a stop. At this point the elastic energy produced would be released, propelling the scraper across a small portion of the file at a velocity of about 200 mm/s. Interestingly, the wing itself reaches a velocity of 300 mm/s during many silent intervals, but its velocity during actual sound production is near zero. This can be seen in Fig. 4B as flat lines of displacement (shown in orange) indicating no wing movement. This clearly shows that the scraper must be moving independently of the wing.

The fact that this species can move its wings at a velocity equal to and beyond the required scraper velocity to produce calls at the same f_c begs the question, why do they not have a coupled scraper? The use of discrete pulses may be an adaption to lengthen the duration of the call (Robillard et al., 2013), without lowering the f_c to increase the chance of the female responding with phototaxis towards the male (Montealegre-Z et al. 2006). For example, if the scraper was coupled with the wing and both moved at 270 mm/s during one closing stroke covering 1.35 mm of file, the syllable produced would last about 5 ms, about 50% shorter than the current mechanism of calling (Fig. S10).

The stridulatory mechanism of *N. foreli* resembles that of *Metrioptera bicolor* [Tettigoniinae] (Heller, 1988), and similar mechanisms seemed to have evolved in other Neotropical species for example in Triecentrus spp. [Pseudophyllinae] (Montealegre-Z, 2005). The large phylogenetic distances between Tettigoniinae and Pseudophyllinae (Mugleston et al., 2018), suggest that this mechanism has evolved independently several times in the family Tettigoniidae. The advantages of this complex motion are not fully understood but deserve to be studied in the light of repertoire enrichment and optimization of wing motion.

Variation in closing wing velocity, commonly affected by temperature and body size, did not affect f_c because this species likely used an elastic scraper mechanism. This also could explain why the attachment of tape to the wing failed to alter the f_c, even if it did marginally lower wing velocity.

Curiously, *N. foreli* might have evolved to employ elastic energy during stridulation purely to lengthen the duration of the call, despite possessing muscles that can reach velocities high enough to produce the same f_c with a coupled scraper.

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Appendix

A range of terms have been used to describe acoustic signals produced by katydids in various publications. This study uses the following vocabulary. The term “discrete pulse” refers to a pulse that is separated from other pulses by a significant silent period. The sound produced during a single closing stroke is known as a “syllable”. The syllable can be made up of a group of discrete pulses. This study will focus on the syllables of the verse as these comprise almost all the sound produced. The “phonatome” is a term to describe all the sound produced in one tegminal cycle (wing opening and closing). A group of phonatomes, commonly produced in a sequence, is known as a “verse” (Stumpner et al., 2013; Walker and Dew, 1972). A “resonant” call refers to when the frequency of the sound produced matches the natural resonant frequencies of the driven system (the mirror, for example) resulting in the sound becoming accentuated and musical. A “non-resonant” call fails to reach this frequency and becomes transient. “Pure tone” is a term used to describe a sound wave made up of a single frequency, however, “broadband” characterises a sound wave consisting of a wide range of frequencies (Montealegre-Z, 2005). The Carrier frequency (f_c) is the frequency that holds the most energy (Montealegre-Z, 2012; Robillard et al., 2015). Depending on the species, katydids generally produce either pure tone continuous pulses associated with sustained sweeps of the file (Montealegre-Z et al 2006; Morris et al., 1994), or broadband discontinuous pulses resulting from non-resonance, unsustained sweeps of the file (Montealegre-Z and Morris, 2003). However, some katydids can produce resonant, pure tone calls with unsustained sweeps of the file (Montealegre-Z, 2005).

Appendix A. Supplementary data
The following are the Supplementary data to this article:
(https://www.elsevier.com/journals/journal-of-insect-physiology/xxxxxxxxxxxxxxxxxxxxxx

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Table 1. Tukey and Q_{-3} analysis output for each specimen (n=68). Means that do not share a letter are significantly different. [a]Only eight recordings were analysed due to sample rate inconsistencies.

Male	Mean ± SE [kHz]	Tukey grouping	Q_{-3} ± SD [kHz]	Q_{-3} bandwidth ± SD [kHz]
6	22.180 ± 0.13	A	5.148 ± 0.50	4337.761 ± 405.89
2	21.260 ± 0.15	B	5.764 ± 0.55	3721.158 ± 410.17
7	20.500 ± 0.23	C	3.781 ± 0.46	5482.616 ± 641.54
1[a]	20.163 ± 0.07	C	7.773 ± 2.22	2845.608 ± 1032.35
4	18.960 ± 0.30	D	5.319 ± 0.70	3624.614 ± 596.25
5	18.700 ± 0.07	D	5.133 ± 0.30	3650.295 ± 238.67
3	18.440 ± 0.14	D	6.234 ± 1.10	3040.372 ± 541.44

Table 2. Descriptive statistics and regression analysis output for each length against f_c of loudest syllable (n=7).

Length	Mean ± SE (mm)	R^2 (adj)	Test statistic	Regression equation	P-value
Body	40.220 ± 1.07	0.17	$F_{1,58} = 13.00$	$f_c = 30.91 - 0.2683$ length	0.001
Pronotum	5.947 ± 0.08	0.17	$F_{1,68} = 14.94$	$f_c = 34.65 - 2.3880$ length	<0.001
Tegmen	27.191 ± 0.63	0.28	$F_{1,68} = 27.92$	$f_c = 34.46 - 0.5203$ length	<0.001
File	2.045 ± 0.04	0.30	$F_{1,68} = 30.88$	$f_c = 37.39 - 8.3500$ length	<0.001

Table 3. Regression analysis output of wing velocity against f_c of loudest syllable for each male (n=10 verses per male, 3-4 syllables per verse). Unusual verses from male four were not included. Significant results are highlighted in bold.

Male	Mean ± SE (mm/s)	R^2 (adj)	Test statistic	Regression equation	P-value
1	84.412 ± 2.15	0.00	$F_{1,38} = 0.12$	$f_c = 21.656 - 0.00390$ velocity	0.729
2	95.658 ± 5.50	0.00	$F_{1,28} = 0.62$	$f_c = 21.677 - 0.00443$ velocity	0.438
3	92.516 ± 2.35	0.00	$F_{1,38} = 0.04$	$f_c = 19.580 + 0.00270$ velocity	0.841
5	94.705 ± 6.45	0.00	$F_{1,40} = 0.71$	$f_c = 18.471 + 0.00184$ velocity	0.404
6	80.457 ± 5.00	0.39	$F_{1,28} = 0.74$	$f_c = 21.653 + 0.00377$ velocity	0.397
7	101.677 ± 3.52	0.09	$F_{1,32} = 4.26$	$f_c = 17.220 + 0.02100$ velocity	**0.047**

Figure captions

Fig. 1. Stridulatory file morphology. A) 3D images of the stridulatory file of a male *N. foreli* from the anal (left) to basal (right) end. Tooth densities and mean inter-tooth distances (ITD) were calculated from the centre 50% of the files (see Fig.S2). B) ITDs over the full length of the stridulatory file for all seven males. The direction of the scraper was left to right (anal to basal). Faded points represent the measurements not used in calculating the tooth density and mean ITDs. Smooth local regression lines with 95% confidence intervals are shown for individual files, and in black for all files.

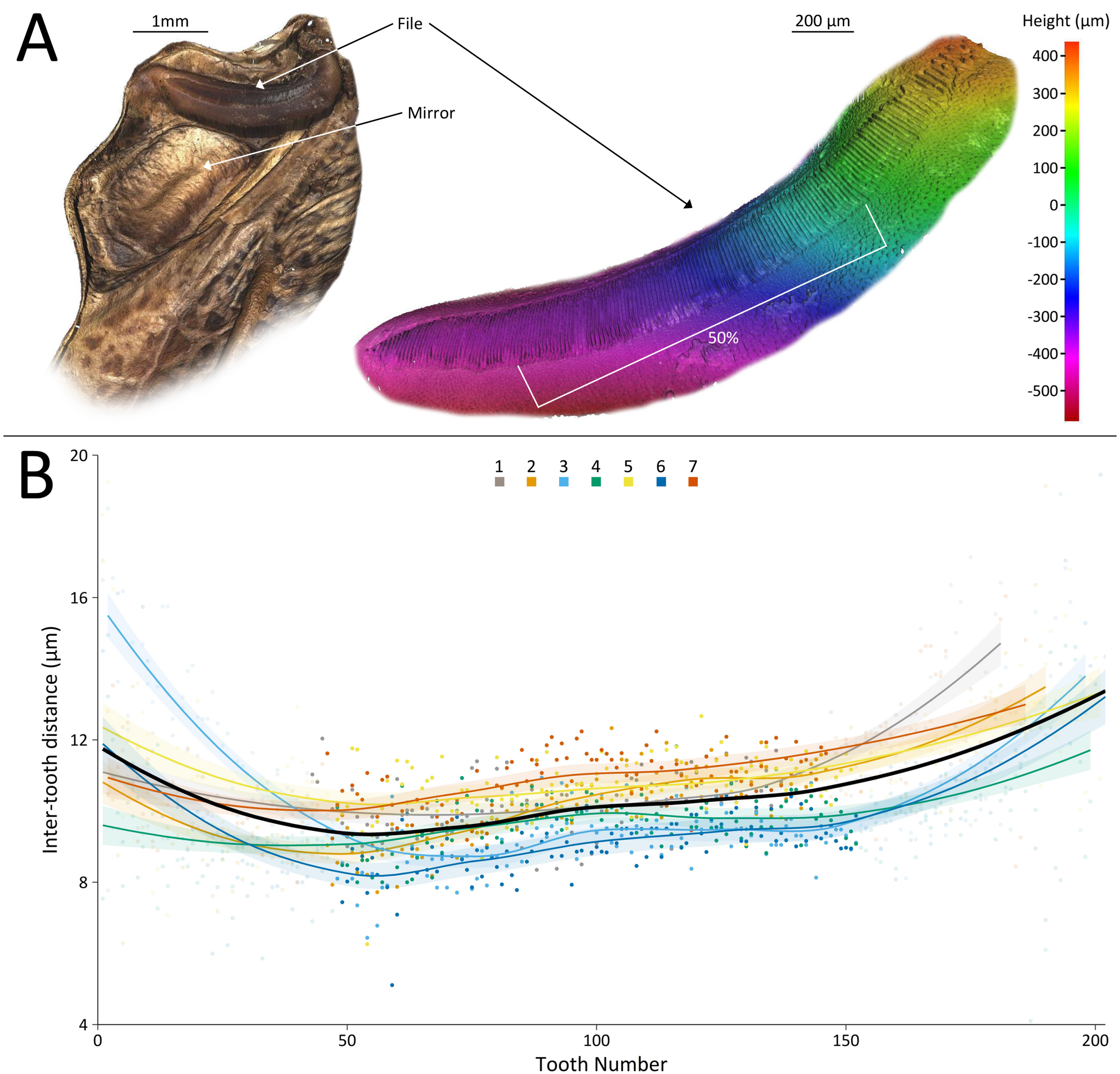
Fig. 2. Oscillograms and respective FFT analysis of each male's typical call. Note the strange broadband call of male 3 and lack of discrete pulses in male 4.

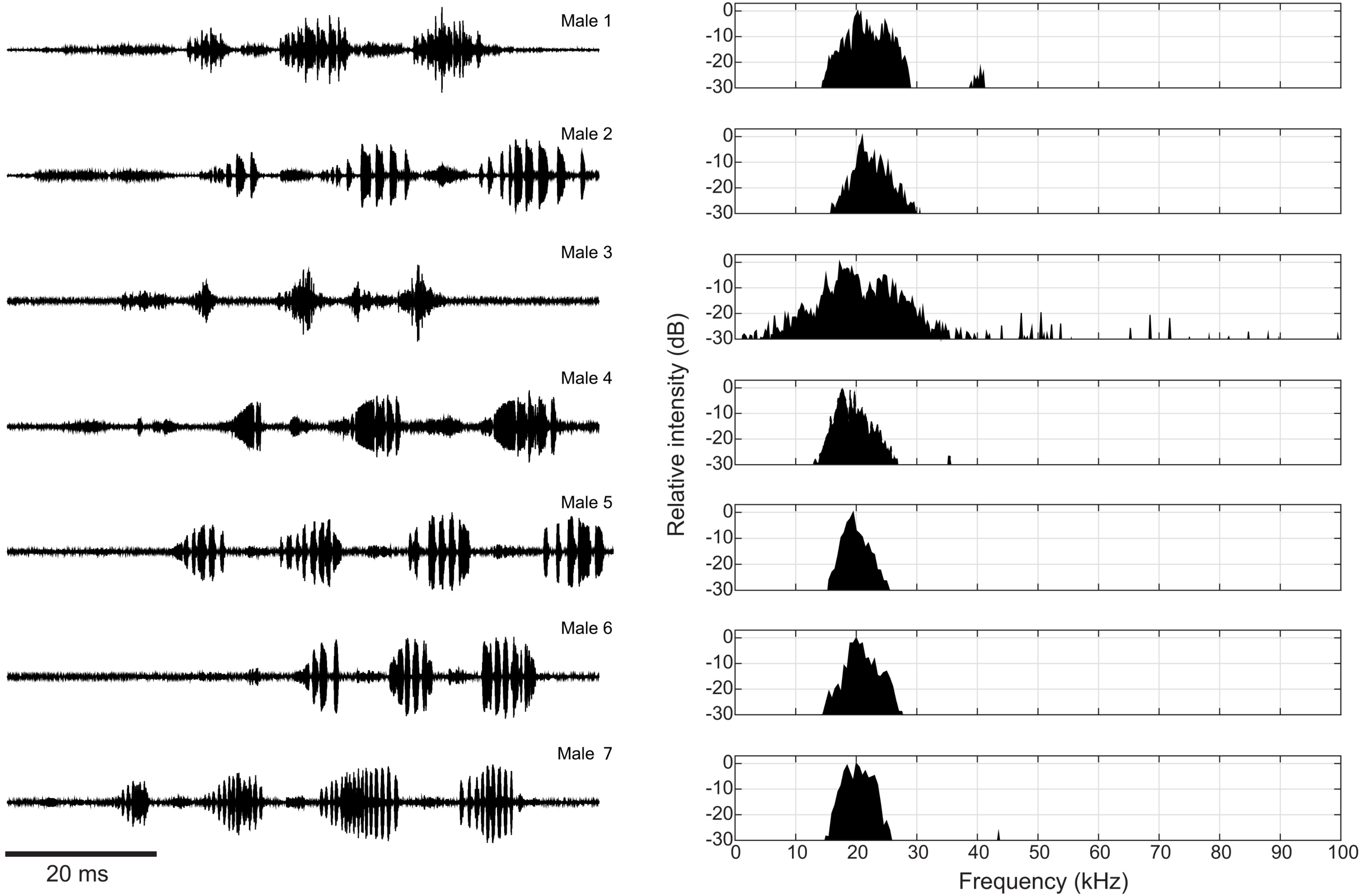
Fig. 3. Predicted scraper velocity (A) and measured closing wing velocity (B) against f_c of all syllables from all males. Unusual syllables from male four were not included. Linear regression lines for wing velocity have 95% confidence intervals.

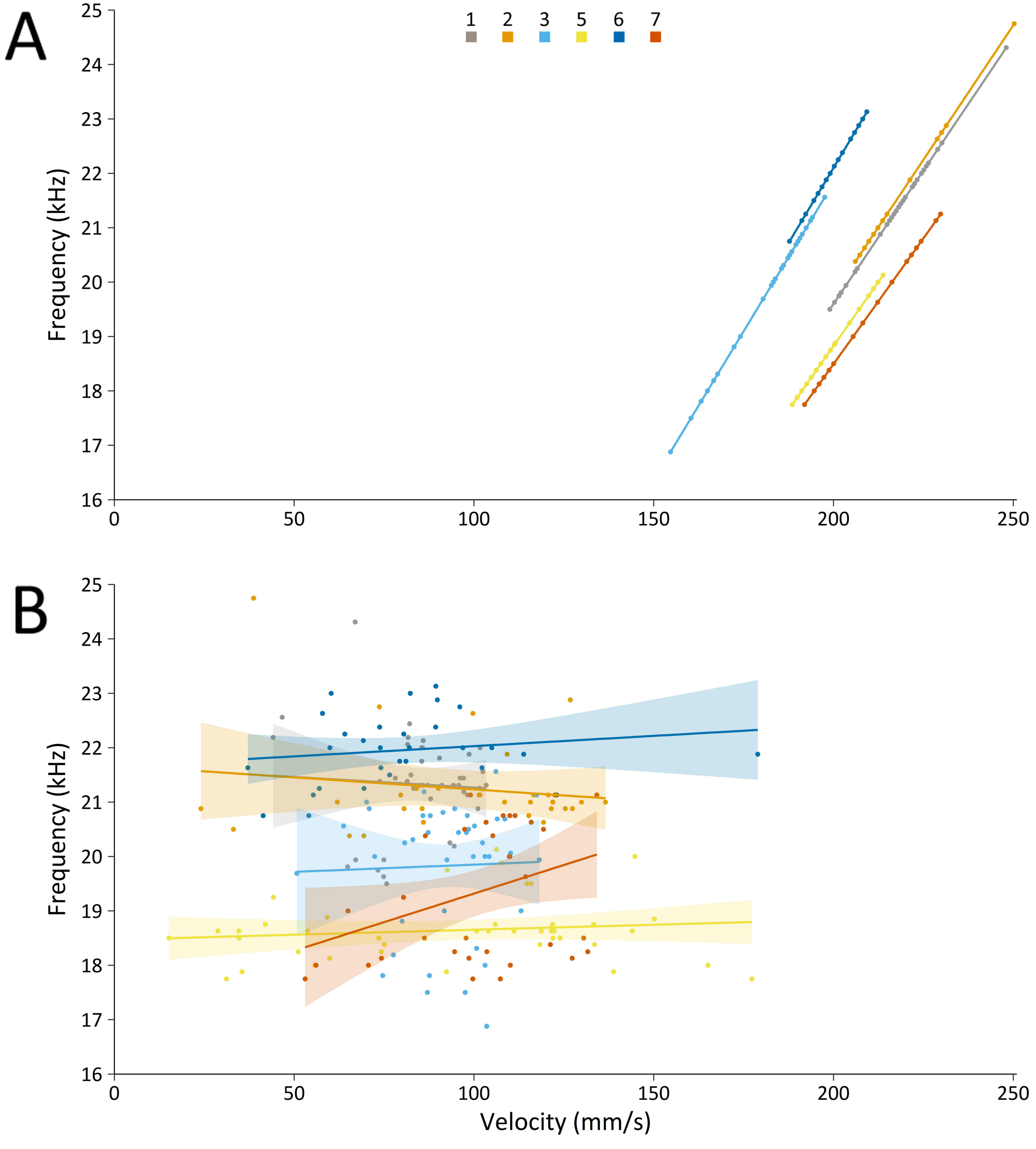
Fig. 4. Sound waveforms, wing displacement and velocity during the verse (A) and phonatome (B) from male seven. This is a typical call, representative of all males studied. The velocity is shown as positive for closing wing movement. Unlike the previous wing velocity calculations, the velocity line (blue) was estimated from the wing displacement trace (orange) as the distance change divided by time, at 5 k-samples/s.

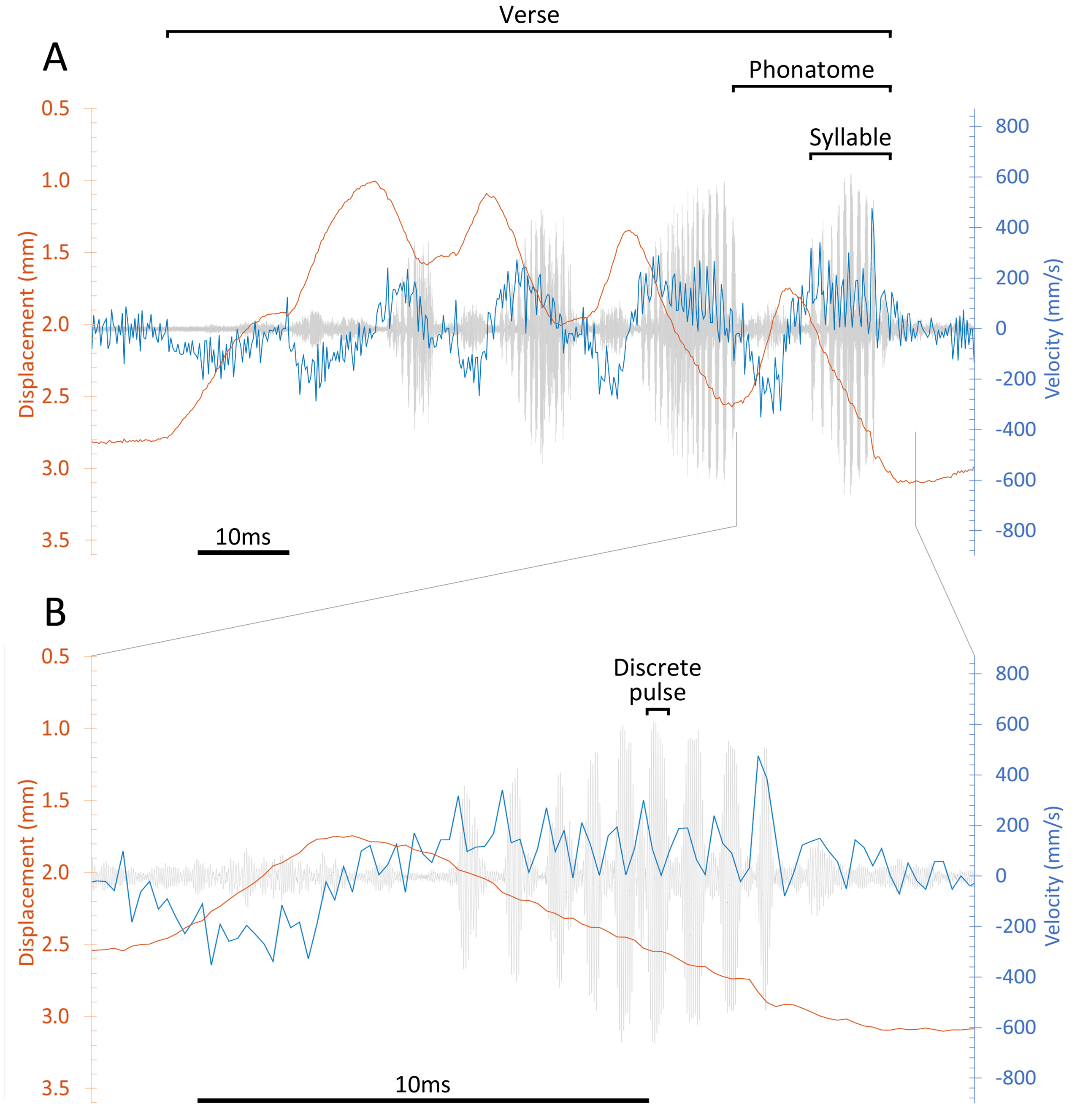
Fig. 5. Wing deflection map at the best resonance frequency of the right mirror (22 kHz in this case). A) The two wings extended for acoustic stimulation, note 1/8" microphone in the top middle. B) Stridulatory areas of the wings scanned. C) Scanning lattice of B showing the deflection pattern.

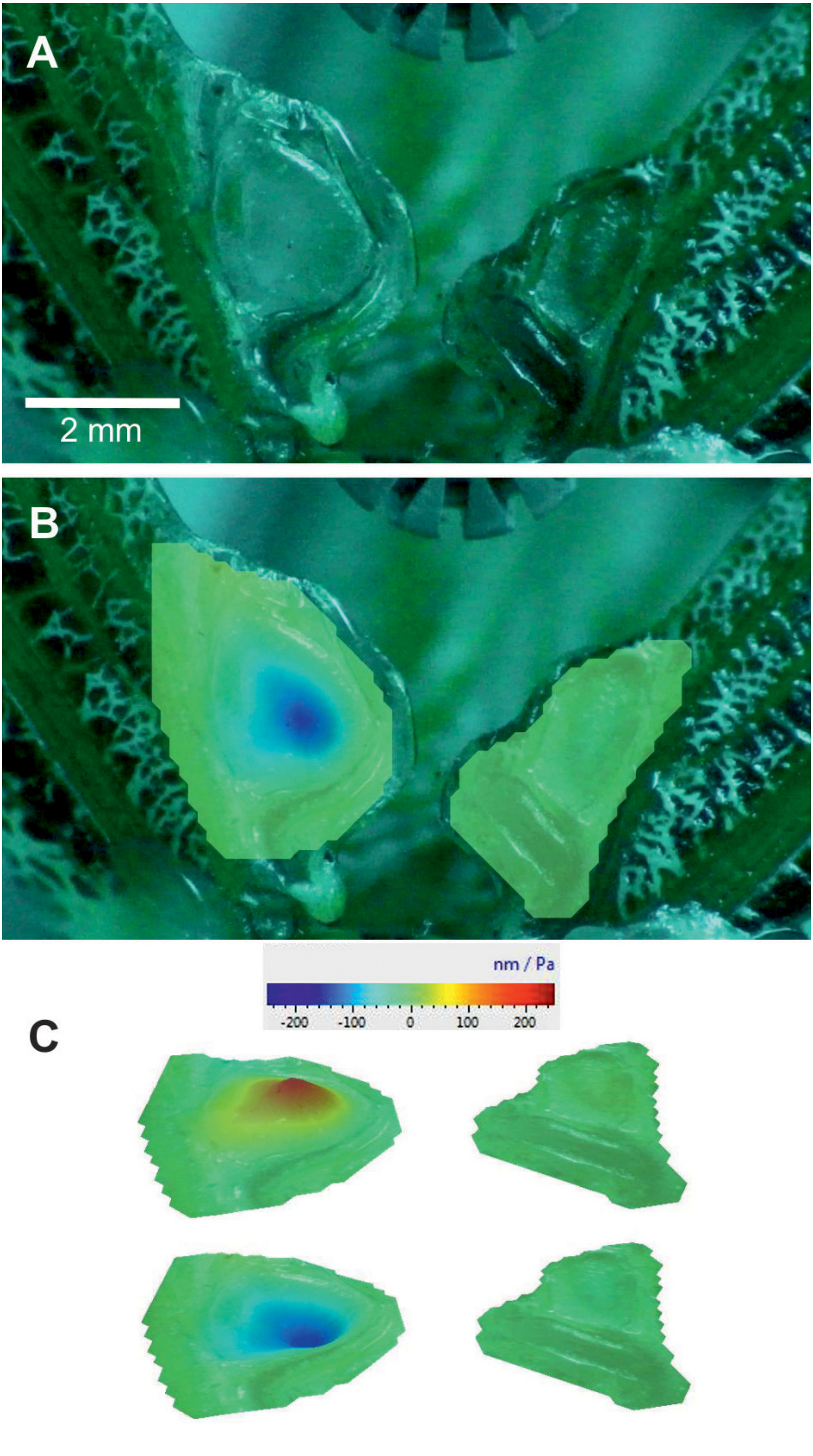
Fig. 6. Wing resonances of two specimens. A) A male with normal call peaking at around 21 kHz. Resonance of the right wing occurs at 22 kHz. B) A male with an unusual broadband, raspy call. The right wing of this male shows a broadband patter with best resonance at around 18 kHz, which is the main peak observed in the calling song of this male (male 3, see Fig. 2). Note that in both recordings the right wing mirror dominates in amplitude by a factor of 4-5x.


A
1mm
File
Mirror
200 µm
Height (µm)
400
300
200
100
0
-100
-200
-300
-400
-500
50%
B
1
2
3
4
5
6
7
Inter-tooth distance (µm)
20
16
12
8
4
0
50
100
150
200
Tooth Number


Male 1
Male 2
Male 3
Male 4
Male 5
Male 6
Male 7
20 ms
Relative intensity (dB)
0
-10
-20
-30
0 10 20 30 40 50 60 70 80 90 100
Frequency (kHz)


A
1
2
3
5
6
7
Frequency (kHz)
25
24
23
22
21
20
19
18
17
16
0
50
100
150
200
250
B
Frequency (kHz)
Velocity (mm/s)


Verse
A
Phonatome
Syllable
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Displacement (mm)
800
600
400
200
0
-200
-400
-600
-800
Velocity (mm/s)
10ms
B
Discrete
pulse
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Displacement (mm)
800
600
400
200
0
-200
-400
-600
-800
Velocity (mm/s)
10ms


A
2 mm
B
nm / Pa
-200
-100
0
100
200
C

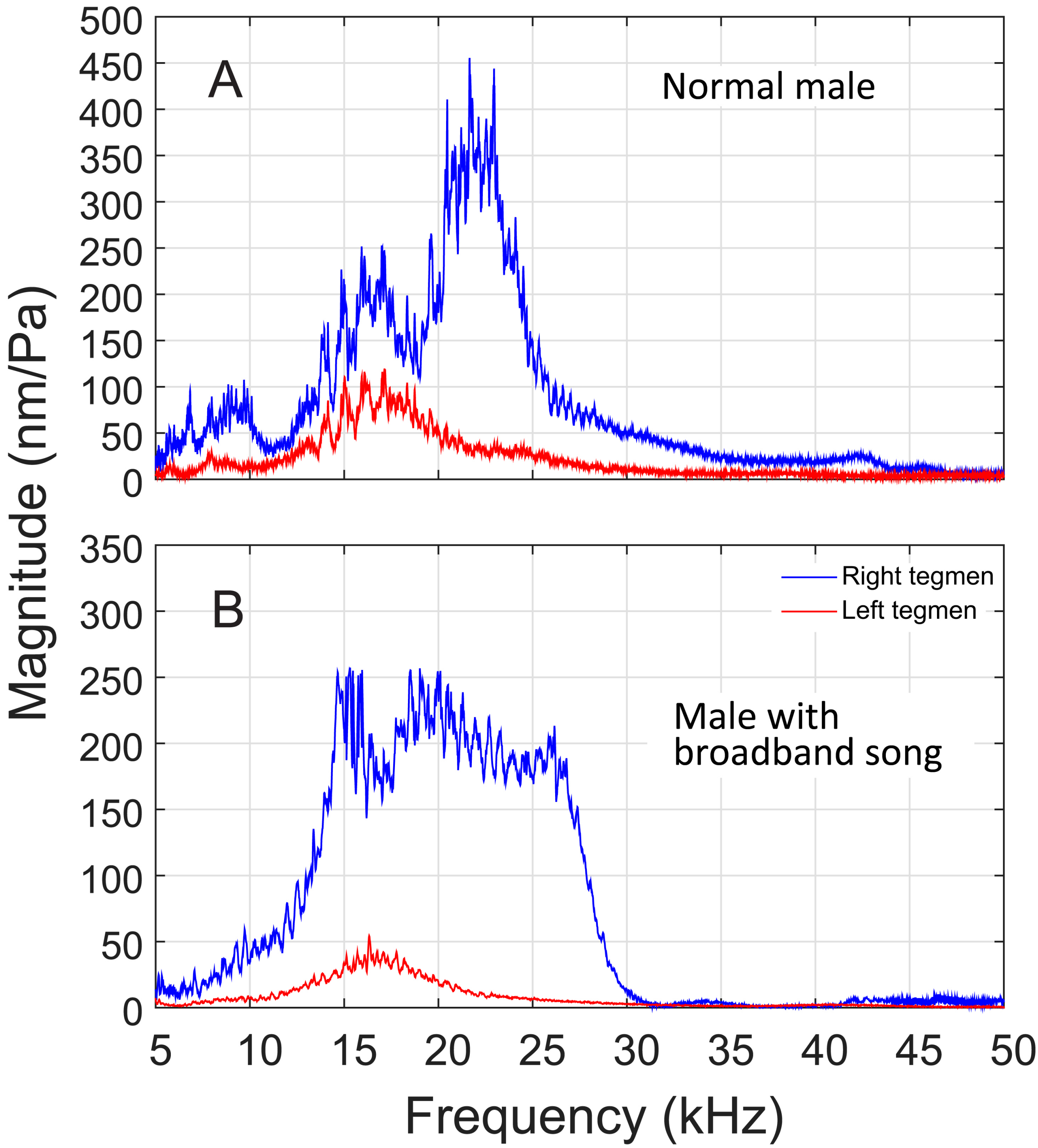

A
Normal male
B
Right tegmen
Left tegmen
Male with
broadband song
Magnitude (nm/Pa)
Frequency (kHz)
500
450
400
350
300
250
200
150
100
50
0
350
300
250
200
150
100
50
0
5
10
15
20
25
30
35
40
45
50

Fig. S1. Positions of the number label and square reflective tape on a live katydid. The individuals were cooled for ≈3 minutes to lower the chance of injury during attachment.

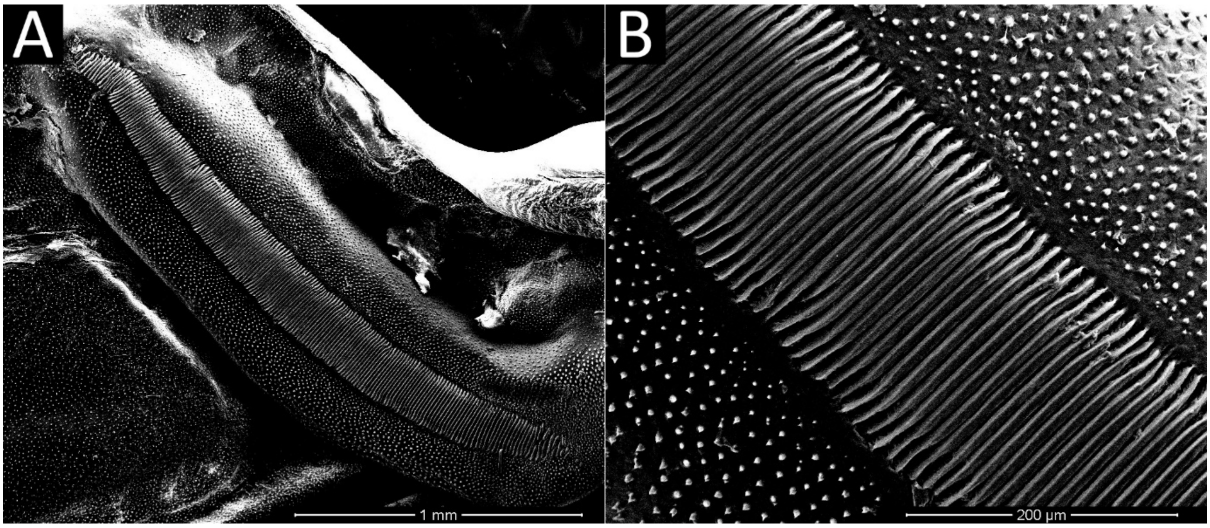


Fig. S2. Original SEM images used to measure stridulatory file morphology (example from male 5). A) Whole file from anal (left) to basal (right) end at lower magnification (61x). B) Middle of file at higher magnification (285x). Measurements were taken from a series of these images.

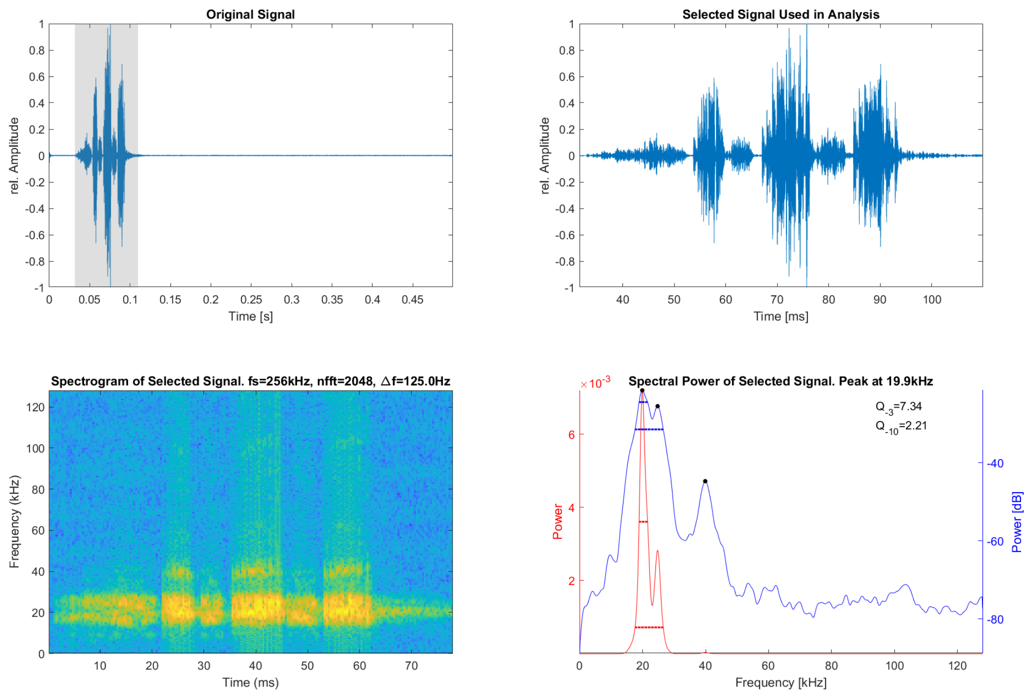


Fig. S3. Sound analysis and Q calculation of male 1. Original recording (top left), Signal selection (top right), Spectrogram (bottom left), and Q calculation (bottom right) using power spectrum (red), and relative intensity spectrum (blue).

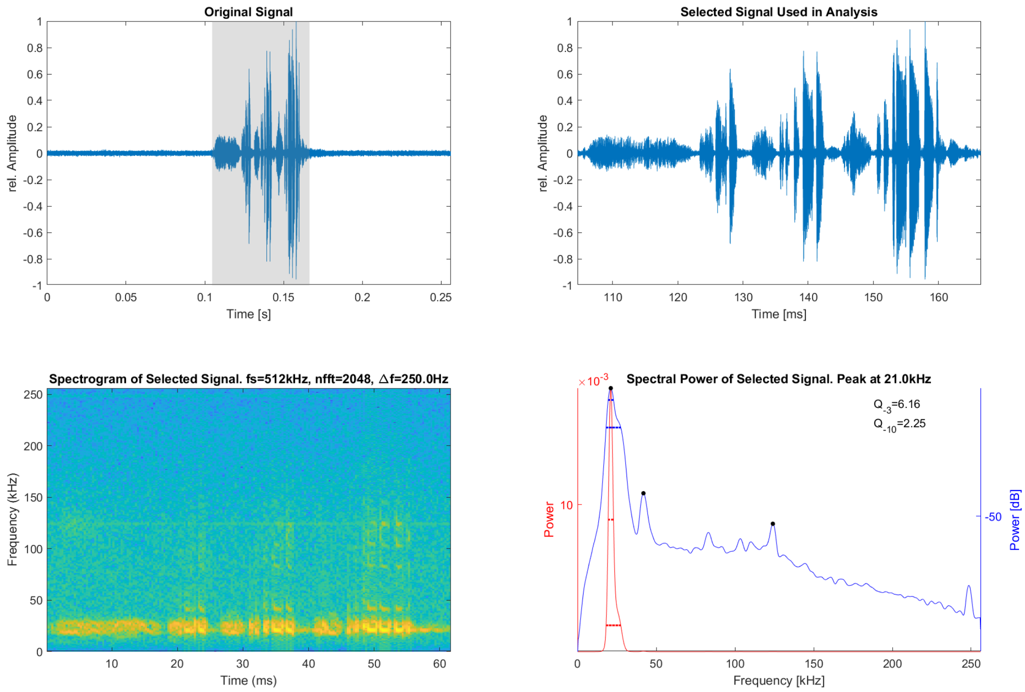


Fig. S4. Sound analysis and Q calculation of male 2. Original recording (top left), Signal selection (top right), Spectrogram (bottom left), and Q calculation (bottom right) using power spectrum (red), and relative intensity spectrum (blue).

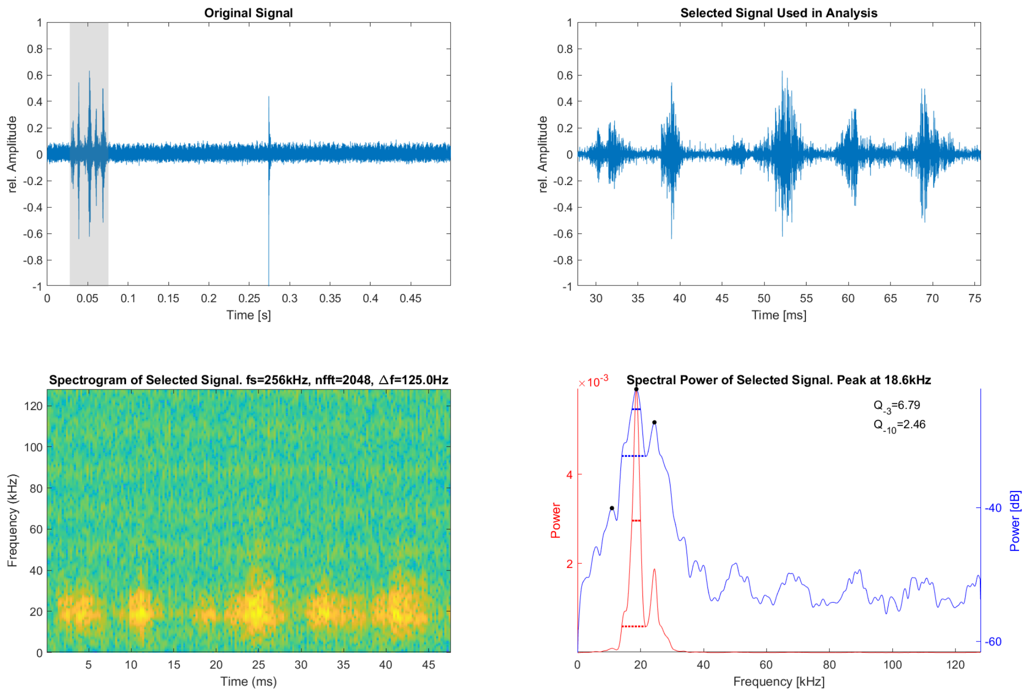


Fig. S5. Sound analysis and Q calculation of male 3. Original recording (top left), Signal selection (top right), Spectrogram (bottom left), and Q calculation (bottom right) using power spectrum (red), and relative intensity spectrum (blue).

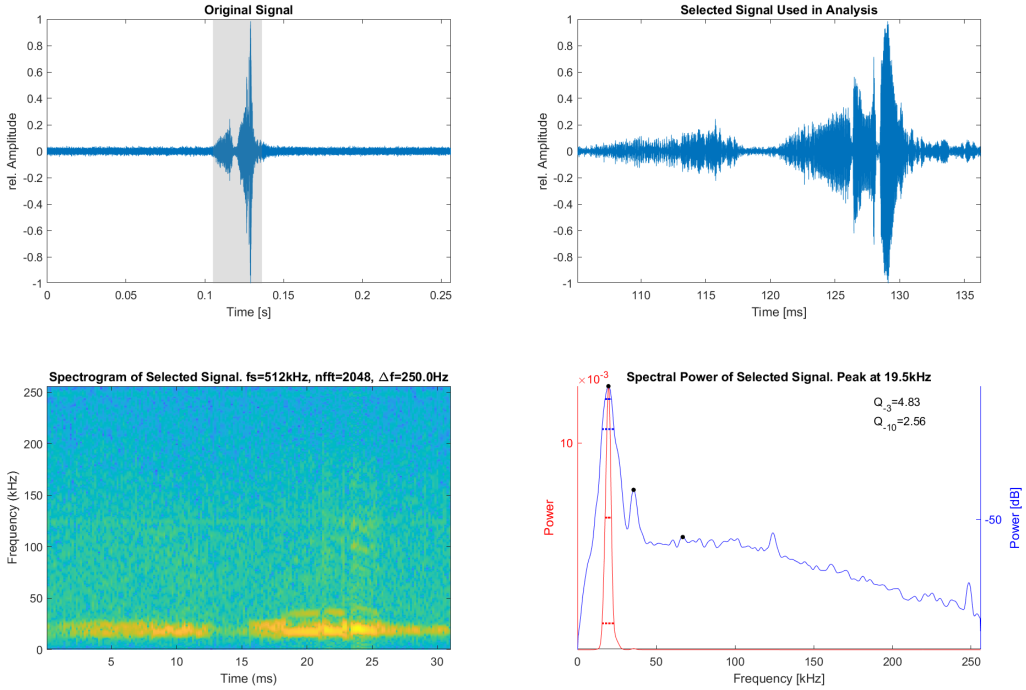


Fig. S6. Sound analysis and Q calculation of male 4. Original recording (top left), Signal selection (top right), Spectrogram (bottom left), and Q calculation (bottom right) using power spectrum (red), and relative intensity spectrum (blue).

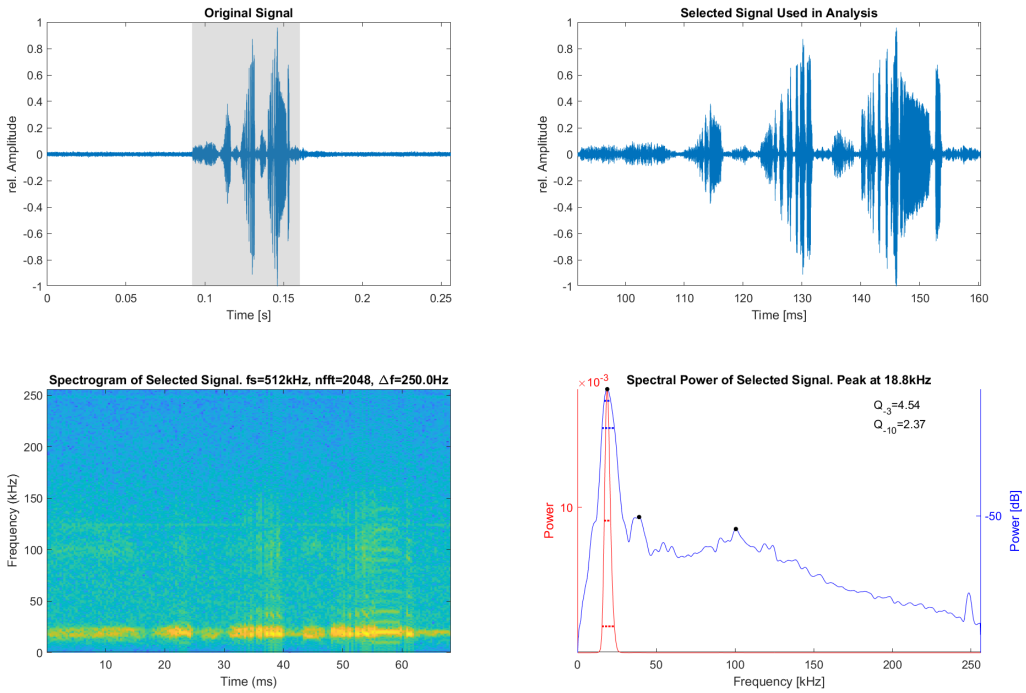


Fig. S7. Sound analysis and Q calculation of male 5. Original recording (top left), Signal selection (top right), Spectrogram (bottom left), and Q calculation (bottom right) using power spectrum (red), and relative intensity spectrum (blue).

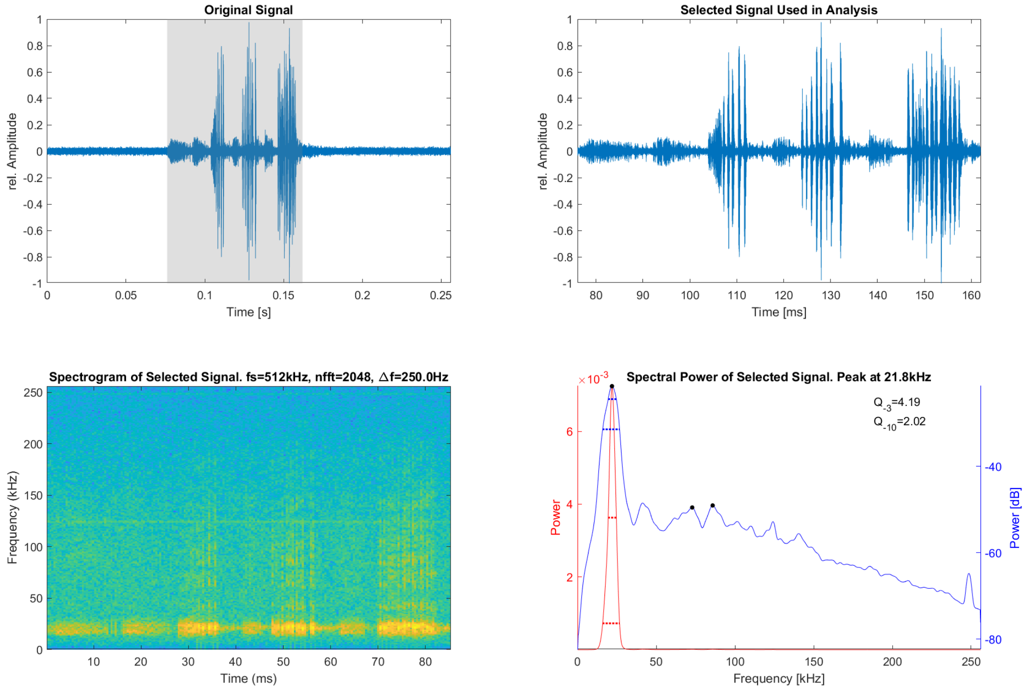


Fig. S8. Sound analysis and Q calculation of male 6. Original recording (top left), Signal selection (top right), Spectrogram (bottom left), and Q calculation (bottom right) using power spectrum (red), and relative intensity spectrum (blue).

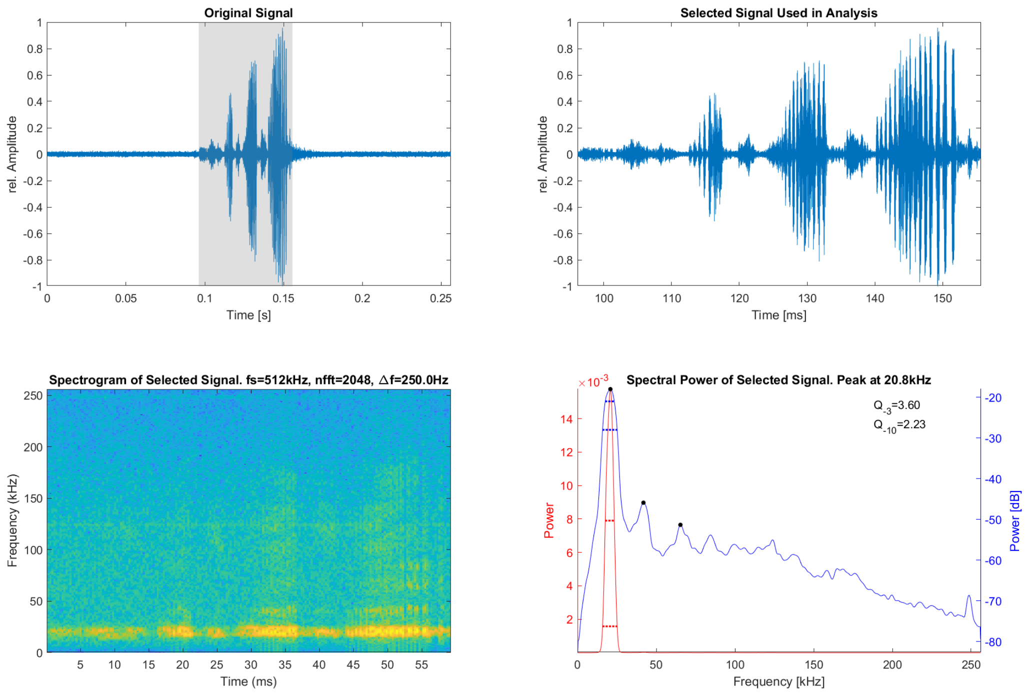


Fig. S9. Sound analysis and Q calculation of male 7. Original recording (top left), Signal selection (top right), Spectrogram (bottom left), and Q calculation (bottom right) using power spectrum (red), and relative intensity spectrum (blue).

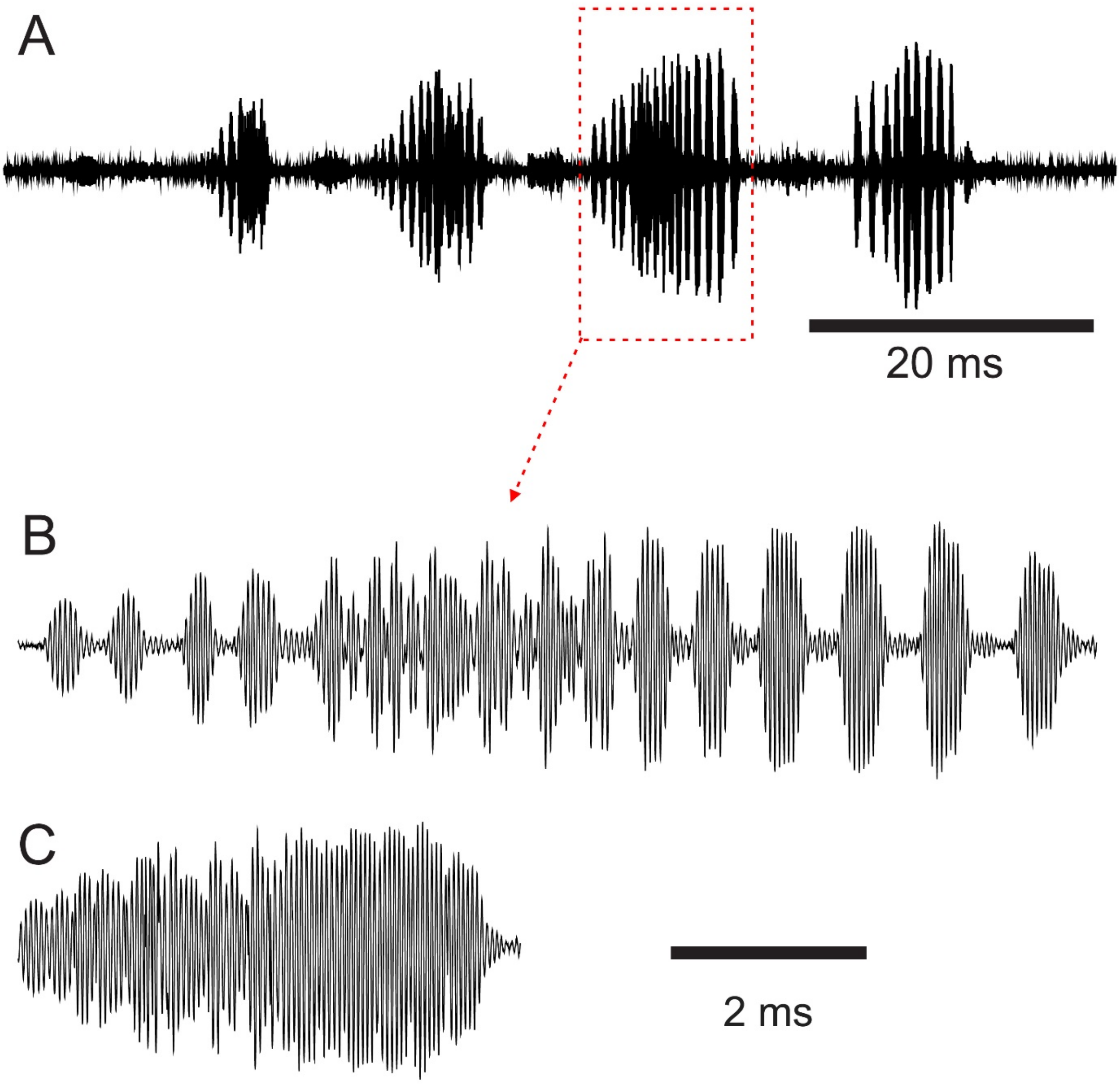


Fig. S10. Oscillograms of syllables with and without discrete pulses. A) A typical verse. B) The original syllable made up of discrete pulses separated by silent intervals. C) A theoretical syllable without silent intervals.