

Acoustic communication of the red-haired bark beetle (*Hylurgus ligniperda*)

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Abstract:

Bark beetles (Coleoptera: Curculionidae: Scolytinae) are a speciose subfamily of weevils that primarily live in bark and consequently largely communicate using sound. Having colonised multiple countries outside its native range, *Hylurgus ligniperda* (Fabricius) is considered a successful invader, yet little is known about its acoustic communication. Here, we studied individual sound production and dyadic interactions among males and females of *H. ligniperda*. Two temporal parameters (duration and inter-note interval) and three spectral parameters (minimum, maximum, and centroid frequencies) were used as descriptors to quantify call variations depending on behavioural context. We also present a method for automatically extracting and analysing these calls, which allows acoustically discriminating amongst individuals. *Hylurgus ligniperda* exhibits sexual dimorphism in its stridulatory organ. Females do not produce stridulatory sounds, but males produce single-noted calls and modify their spectro-temporal parameters in accordance with context. Acoustic stimulation from nearby males does not appear to be a causative factor in such modification. Instead, hierarchical clustering analysis showed that physical interactions play a more important role in affecting call parameters than acoustic signals. Centroid and maximum frequencies were the largest contributors to the variability of the data, suggesting that call variations in *H. ligniperda* mainly occur in the spectral domain.

Keywords: acoustic detection; forest pest; insect behaviour; invasive species; similitude analysis; principal component analysis.

Introduction

Bark beetles (Coleoptera: Curculionidae: Scolytinae) are influential agents in forest ecosystems. They contribute to deadwood decomposition processes, ecological succession, and improve forest function via canopy thinning (Schowalter and Filip, 1993; Oliver, 1995; Raffa et al., 2015). Scolytines are also key players in nutrient cycling, water quality, and the diversification of stand structure and composition (Mikkelsen et al., 2013; Schowalter, 2012). However, some are also significant forest pests and can attack live and recently-felled trees in their natural or invaded geographic ranges (e.g., Schroeder, 2001; Brockerhoff et al., 2006). The two main groups of Scolytinae are the true bark beetles and ambrosia beetles. True bark beetles feed and reproduce in the inner bark of their hosts, colonizing and constructing galleries inside the tree (Kirkendall, 1983). Some of them are also carriers of specific symbiotic fungi (Harrington, 2005; McCarthy et al., 2010; Six and Wingfield, 2011; McCarthy et al., 2013) that can reduce the economic value of timber, or negatively impact tree health (Fraedrich et al., 2008; Lindgren and Raffa, 2013; Rouco and Muñoz, 2014). However, most species cause little or no economic damage (e.g., Brockerhoff et al., 2003; Sopow et al., 2015).

Bark beetles spend most of their life cycle in confined environments under bark or in wood, where visual and olfactory signals are difficult to transmit and detect (Fleming et al., 2013). Consequently, they use acoustic communication for significant aspects of their life history, including defensive behaviour, mate location, species recognition, aggression, and courtship (Barr, 1969; Lindeman and Yack, 2015; Ryker and Rudinsky, 1976). In this study, we use *Hylurgus ligniperda* (Fabricius), the red-haired bark beetle (also known as the golden-haired bark beetle), as a model organism to study the variability in sound production in Scolytinae in different behavioural contexts. *Hylurgus ligniperda* is a successful invader that is already established in many countries around the world (Wood and Bright, 1992, Brockerhoff et al. 2006). This bark beetle produces sounds using a stridulatory organ with a two-part elytro-abdominal mechanism (Liu et al., 2008). The mechanism (Figure 1) consists of a plectrum (i.e. a sclerotisation in one of the last abdominal tergites) that scrapes a file of parallel teeth (or *pars stridens*) on the ventral surface of the elytra (Hopkins, 1909; Wilkinson et al., 1967; Barr, 1969; Fleming et al., 2013).

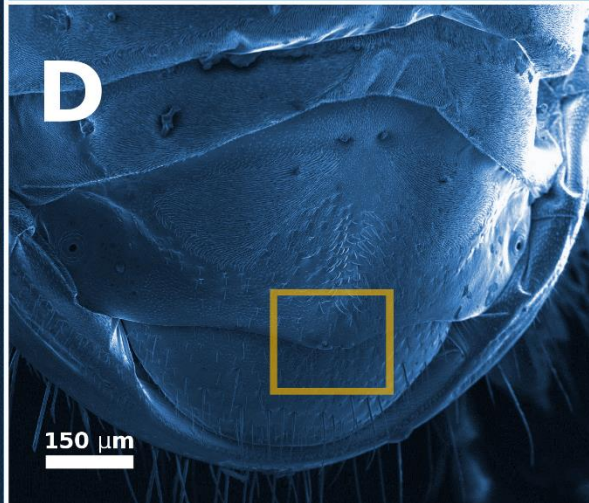
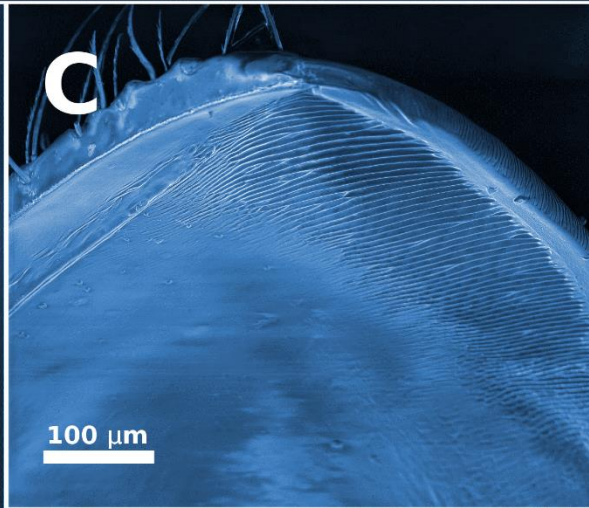
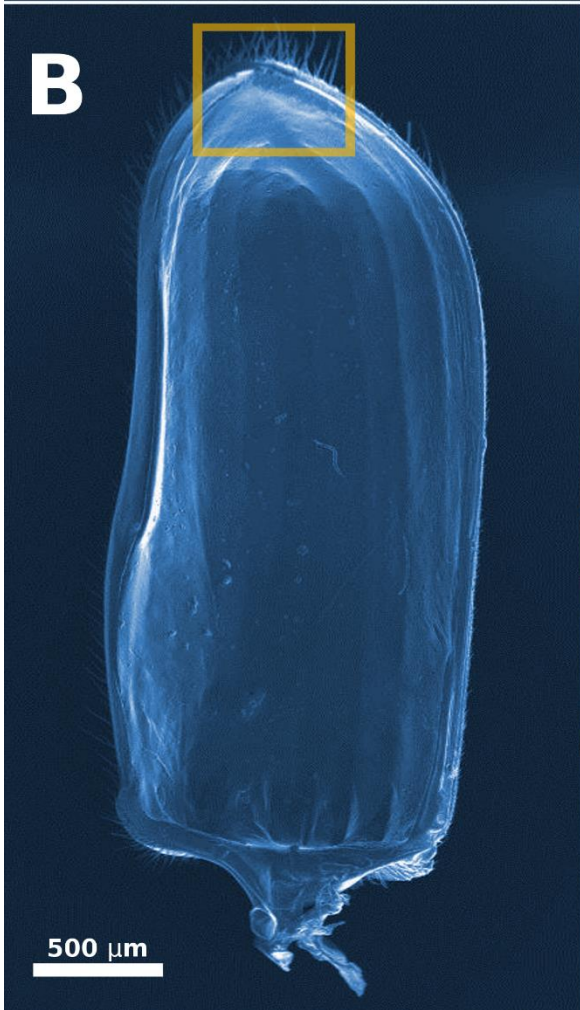


Figure 1. *Hylurgus ligniperda* sound production mechanism. The stridulatory organ consists of a two-part elytro-abdominal structure. (A) Male specimen of *Hylurgus ligniperda*. (B) Ventral view of the left elytron with the file of teeth, or *pars stridens*, highlighted in yellow. (C) Close-up showing detail of the parallel teeth of the *pars stridens*. (D) Dorsal view of the posterior four tergites showing the position of the plectrum (highlighted). Sounds are produced when the plectrum, located on the sixth abdominal tergite, scrapes the *pars stridens* located on the ventral surface of the elytra.

Bark beetle sounds typically consist of quasiperiodic strings of relatively uniform calls. These calls are either simple (i.e., a single note) or interrupted (i.e., multiple notes), and both types may be present during the same behaviour (Lindeman and Yack, 2015). Initially, it was demonstrated that behavioural context influences the type of call and its temporal parameters (Barr, 1969; Yandell, 1984; Lyal and King, 1996), and later Fleming et al. (2013) showed that call spectral parameters can also be influenced by behavioural context. Fleming et al. (2013) used the mountain pine beetle *Dendroctonus ponderosae* Hopkins as a model organism to measure variations in calls produced during different behaviours (namely distress, male-male, and male-female interactions) and found differences between male-male and male-female interactions. In another behavioural study, Lindeman and Yack (2015) evaluated calls during courtship in the red turpentine beetle *Dendroctonus valens* LeConte, finding a strong relationship between the parameters of the calls emitted by males and the conditions for mate choice in females. These findings illustrate the importance of acoustic communication for bark beetles, with calls varying depending on behavioural context. Nonetheless, calls of most scolytines are largely unknown, and the range and origin of these variations still need to be understood.

As many species of bark beetles are successful invaders globally, there is interest in using acoustic methods for the detection of invasive species in import pathways and for post-border detection (Mankin et al., 2011). With the advent of new technologies, data acquisition and automatic analysis of bark beetle calls have become feasible, leading to the potential option of using sound as a method for detecting or deterring beetles using acoustic devices (Aflitto and Hofstetter, 2014; Hofstetter et al., 2014). In this study, we quantified the variations of the spectro-temporal parameters of *H. ligniperda* calls in six different contexts, including the set-up behaviour (i.e., the sounds produced when the beetle is released into the recording

arena), distress, and both close and distant male-male and male-female interactions (i.e., four contexts). We conducted statistical, similitude, and principal component analyses to estimate differences amongst the calls and assess the contribution of the spectro-temporal parameters to the variability in each behaviour. Our objective was to determine the range of variation of the acoustic parameters of *H. ligniperda* calls, and the effect of the physical presence/absence of other individuals, or other calls in the variability of such parameters. Males of this species are known to initiate sound production when hearing stridulations of a conspecific or when in physical contact with another individual. Consequently, we wanted to evaluate if the calls elicited by an acoustic stimulus differ from the calls produced when in direct contact with another individual. We also wanted to verify if females had sound production capabilities, and if the calls produced under direct contact were dependent of the sex of the other individual. This research contributes to the understanding of the behavioural aspects of sound production in Scolytinae and guides future research on the development of acoustic detection tools. A broad range of applications could derive from this, including automatic detection mechanisms for border biosecurity and pest control.

Definitions

The terminology in bark beetle acoustics is somewhat unique and differs from the one used in other animal acoustics, which can lead to confusion. Therefore, we propose the use of a more standard bioacoustics terminology (following the definitions of Köhler et al., 2017) in order to make our results accessible for straightforward comparisons with other taxa (see definitions in Supplementary Material 1). In this study, we use a call-centred approach (Köhler et al., 2017) in which ‘call’ is the principal sound unit. A call can be further subdivided in other subunits called notes (Figure 2) and might consist of either one or several notes. The term ‘chirp’, previously used in other works (Yandell, 1984; Ryker and Rudinsky, 1976; Yturralde and Hofstetter, 2015; Lindeman and Yack, 2015), is re-defined as call; thus, simple chirps are single-noted calls and interrupted chirps multiple-noted calls. *Hylurgus ligniperda* calls only consist of a single note, hence, call and note, and their subsequent properties, are equivalent in this species (see Figure 2), but not in all Scolytinae; thus the use of a call-centred terminology will facilitate future comparisons with other bark beetle species.

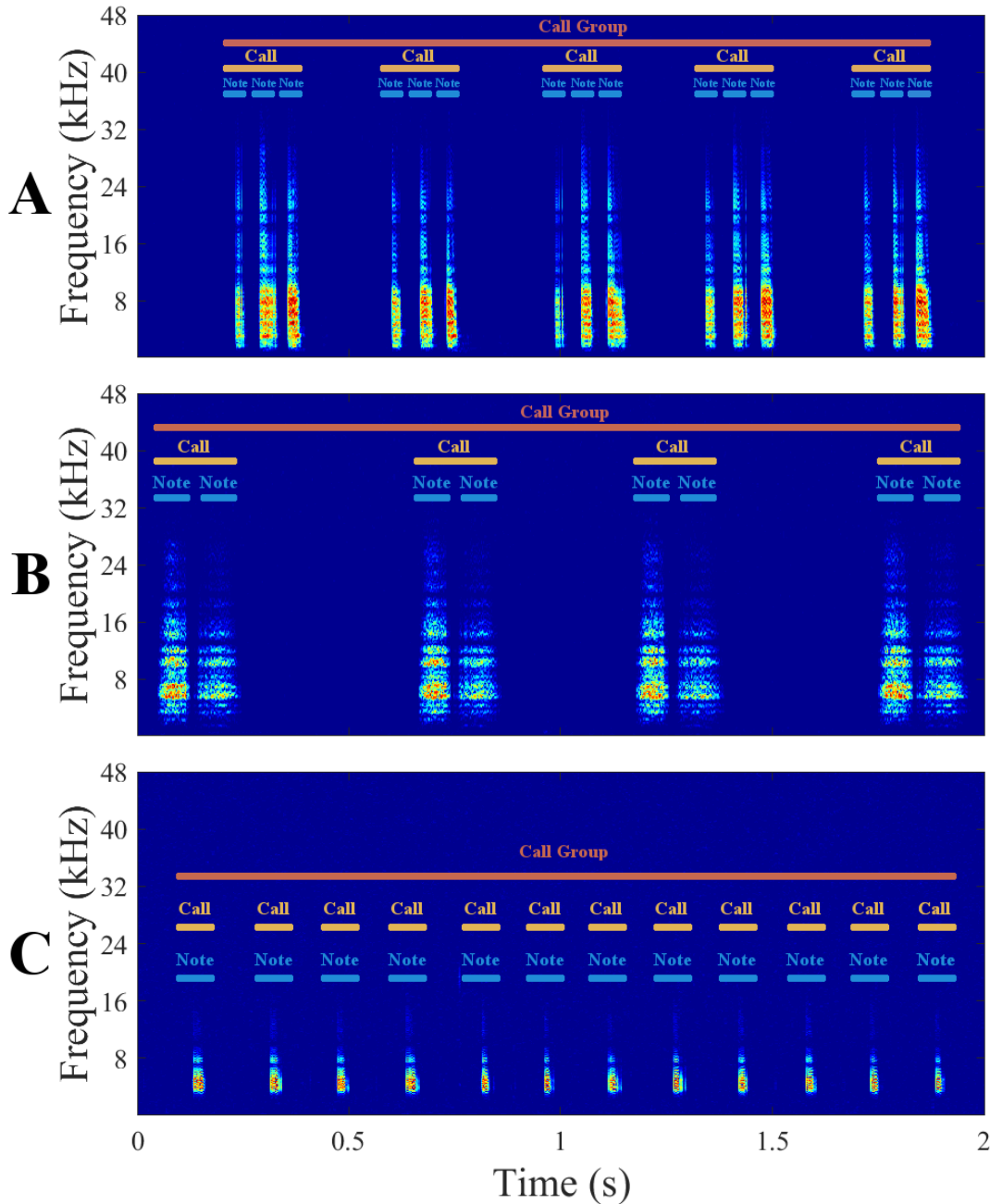


Figure 2. Stridulatory signals of three male individuals of (A) *Dendroctonus adjunctus*, (B) *Phloeosinus cupressi*, and (C) *Hylurgus ligniperda*. Here, we exemplify the use of the proposed notation on multiple-noted (A-B) and single-noted (C) calls. In some scolytines such as *Hylurgus ligniperda*, the term call and note are synonymous, but this is not a general characteristic of the group.

Materials and methods

Beetles

We used 15 males and 15 females of *H. ligniperda* in this study. Beetles were collected in the field from flight intercept panel traps located in Bottle Lake forest park, Christchurch, New Zealand (43°28'S, 172°41'E). Test subjects were randomly selected from the group of beetles, sexually differentiated using sound (only males are known to have a distress call) (Liu et al., 2008), and placed individually in 5 × 6 × 11 cm (w,h,d) containers in order to avoid potential habituation effects among males. For more information on the acoustic sexing see Supplementary Material 4. Males of *Hylurgus ligniperda* usually start singing when they are either in direct contact with another individual or within acoustic reach of another male call; therefore individuals had to be separated in different containers in order to avoid an excess of energy expenditure that could potentially bias the results of the experiment. Containers were kept in a dark environment at 21°C. Water was injected every 5 days via an atomizer to keep the containers humid. Each beetle was provided a piece of pine phloem of ca. 50 mm² that was replaced every 10 days. Each individual was recorded once in each of the six behavioural contexts (see below) in a randomised sequence.

Equipment

An FEI Quanta 250 scanning electron microscope (FEI Company, Hillsboro, OR, USA) was used to obtain the SEM images of the beetle's sound-producing organs. Specimens were mounted on stubs with adhesive carbon tabs and imaged uncoated, using an accelerating voltage of 5 kV and a spot size of 3.5 nm.

Acoustic data were collected in a temperature-controlled room at a constant temperature of 20°C under red light conditions. The room was located in a sealed physical containment facility and had no electronic equipment other than the recorder and the microphones (i.e., no noise but a faint ventilation sound was present during data acquisition; this was subsequently high-pass filtered out, see Supplementary Material 7). Signals were recorded with a two-channel SD 702 audio recorder (Sound Devices LLC, Reedsburg, WI, USA) and two sensors, an M50 ultrasonic omnidirectional microphone (3 Hz to 50 kHz frequency range – flat frequency response throughout the entire spectrum) (Earthworks Inc., Milford, NH,

USA) and an ME 66 super-cardioid microphone (Sennheiser KG, Wedemark, Germany). All spectro-temporal parameters in this study were computed using solely the data provided by the M50 microphone. The purpose of the ME 66 microphone was exclusively to generate redundancy to correctly isolate the sounds of the individual of interest in the dyadic interactions during signal processing. A sampling frequency of 96 kHz, a 48 dB gain, and 24 PCM bit depth were the recording parameters. After data acquisition, the collected recordings were moved from the recorder to a computer using a CompactFlash™ card. The subsequent automatic call detection, parameter estimation, and statistical analysis were performed in Matlab 2015a.

Two different set-ups were used to record the *H. ligniperda* calls (Figure 3). Both were ‘phloem sandwiches’ (Kinn and Miller, 1981; Taylor et al., 1992) which consisted of a flat piece of phloem embedded in two layers of 2 mm thick Perspex™ screwed into place. These were constructed to simulate the simplest possible galleries and chambers. The phloem layer had either one or two 1 cm² chambers, depending on the set-up, to ‘house’ individual beetles. The top Perspex layer had a 2 mm diameter hole above each chamber to introduce the beetles and to allow the microphones to record the stridulations without physical interference. For more information on the Perspex effects on the results of the spectral feature analysis, see Supplementary Material 6.

In the set-up for male-male and male-female interactions (Figure 3A), we simulated a single-tunnel gallery with two chambers. Close ($d < 1$ cm, where d is the distance between beetles) and distant ($d = 10$ cm) interactions were staged. To record calls, two microphones were placed at a 45° angle above each chamber with the edge of the microphone in contact with the uppermost Perspex layer (Figure 3). The ultrasonic microphone (M50) was aimed at the chamber containing the individual of interest (i.e., the individual whose sounds are going to be used for feature extraction), while the second microphone (ME 66) was aimed at the other individual. The information obtained with the ME 66 was later used to distinguish the origin of sounds of both individuals during signal processing.

For the study of individual sound production, a different set-up resembling a small confined space was used (Figure 3B). In this layout, calls produced during two specific behaviours were studied: distress calls and set-up calls (i.e., sounds made by the beetles when introduced

into the recording arena). In these tests, we used a single microphone (M50) located at a 45° angle above the chamber with the inferior edge of the microphone in contact with the top layer of Perspex (Figure 3B).

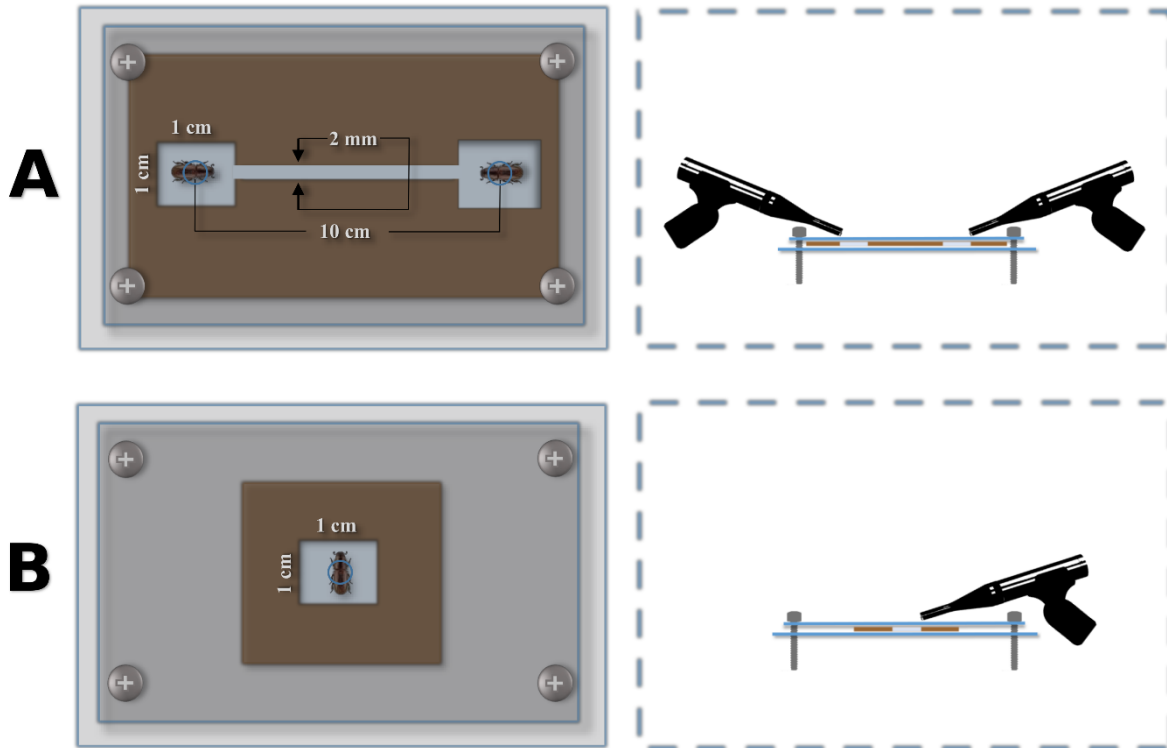


Figure 3. Experimental set-ups used for the study of the behavioural dependence in the sound production of *H. ligniperda*. Each set-up was composed of a flat piece of phloem embedded in two layers of acrylic screwed into place (superior and frontal views shown). **(A)** Set-up for dyadic interactions, simulating a single-tunnel gallery with two chambers. In this layout, male-male and male-female interactions were studied. **(B)** Set-up for individual sound production in a confined space. Set-up and distress calls were acquired using this configuration.

Database acquisition

For dyadic interactions at a distance, the path interconnecting the two chambers was blocked. Data acquisition started immediately after releasing the beetles in both chambers. A randomly selected individual, either male or female (depending on the context), was placed in the other

chamber. In close interactions, the path was unblocked, and data acquisition started once the beetles reached a distance < 1 cm.

Sounds emitted by individuals during the ‘set-up behaviour’ were obtained by releasing a beetle into the single-chamber phloem sandwich. For the distress behaviour, we used the same set-up, but compressed the two Perspex layers (without the beetle) in such a way that the beetle could not completely access the chamber using the hole on the top layer of the sandwich (Figure 3B), thereby exposing the posterior part of its body outside the set-up. Stridulations were elicited by stimulating the posterior part of the individual every 30 s with a small soft paint brush.

Sounds produced in each behavioural context were acquired over six minutes, which were shortened to five in pre-processing (by removing the first and last 30 s of the recording) to suppress audio tags, synchronization marks, and noises produced by the experimenter. After acquiring the calls of all individuals in all behaviours, the recordings were analysed automatically with Matlab. The algorithms for automatic call detection, parameter estimation, and source separation are presented in the following sections.

Automatic note detection and parameter estimation

The large amount of information contained in the dataset prohibited manual analysis. Therefore, we developed a method to automatically detect each note and estimate its spectro-temporal parameters (Figure 4). This approach removes any subjectivity in the analysis of the bark beetle sounds and considerably reduces the time needed for the estimation of their parameters. The method is a threshold and power-based approach that identifies and analyses each note based on the average value of the power distribution in the time domain of the spectrogram. Its goal is to automatically extract two temporal (note duration and inter-note interval, or INI) and three spectral (centroid, minimum, and maximum frequencies) parameters from each note for subsequent use in the behavioural analysis. An extended mathematical explanation of the method and the features extracted is presented in Supplementary Material 2.

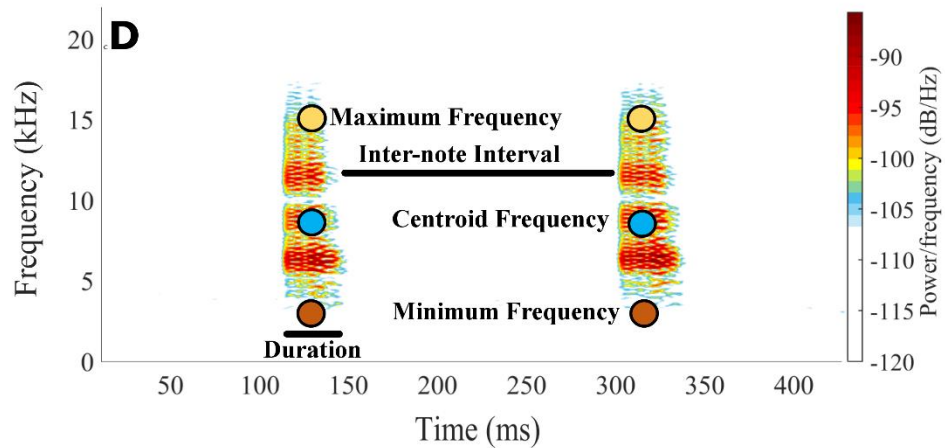
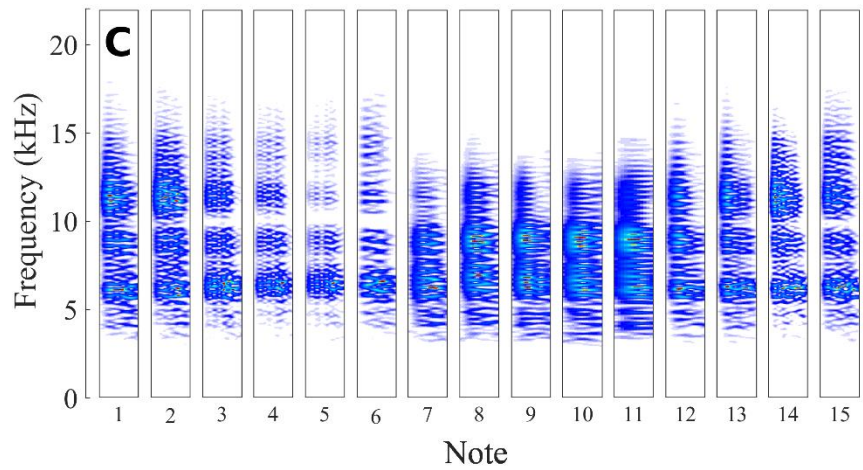
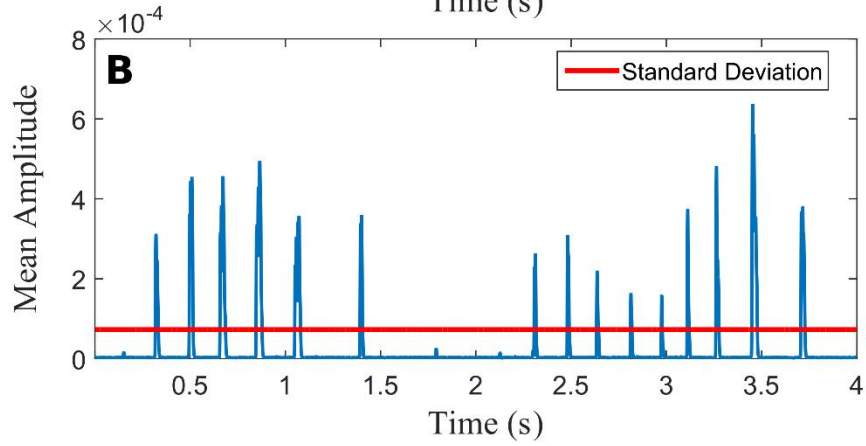
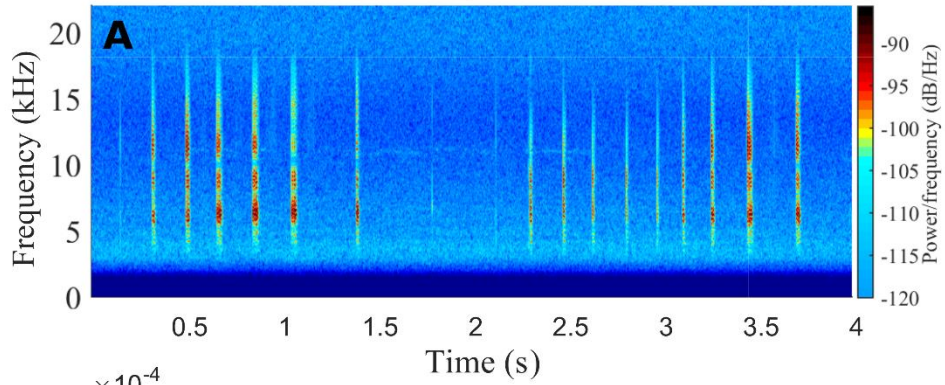


Figure 4. Method used for the extraction and analysis of bark beetle calls. **(A)** Filtering and estimation of the spectrogram. Each recording was high-pass filtered at 3 kHz to suppress the effects of ventilation noises during data acquisition. Then, the time-frequency representation (spectrogram) of the signal was computed. **(B)** Segmentation procedure. After obtaining the spectrogram, the average value of the power distribution in the time domain was estimated. This new vector contains information related to the temporal locations of each note in the recording. Subsequently, the standard deviation was used as a threshold criterion to estimate the beginning and ending of each note. **(C)** Note segmentation. Notes in each recording were individually extracted by using the information obtained in the previous step. In this panel, notes were evenly distributed across 4 s to enhance the visualisation; they were also independently feature-scaled in the frequency domain to highlight the most relevant components of each note. **(D)** Feature extraction. Two temporal (note duration and inter-note interval) and three spectral parameters (centroid, minimum, and maximum frequencies) were estimated from the extracted notes for subsequent analysis.

Source separation

In experiments involving more than one individual, discerning the source of the calls of the beetle that is being studied is complex. When individuals are close to each other, the sounds acquired by the sensor (i.e., microphone) cannot be clearly differentiated in the output signal (Figure 5A). An additional procedure was developed to solve this issue. This algorithm uses Principal Component Analysis (PCA) and redundant information provided by an extra microphone to extract and separate the calls of both beetles in dyadic interactions (Figure 5). The method assumes that the source with the largest power contribution to the signal acquired by a sensor is the source of interest for that sensor and that the number of sources is equal to the number of sensors. For a more detailed explanation of the method see Supplementary Material 3.

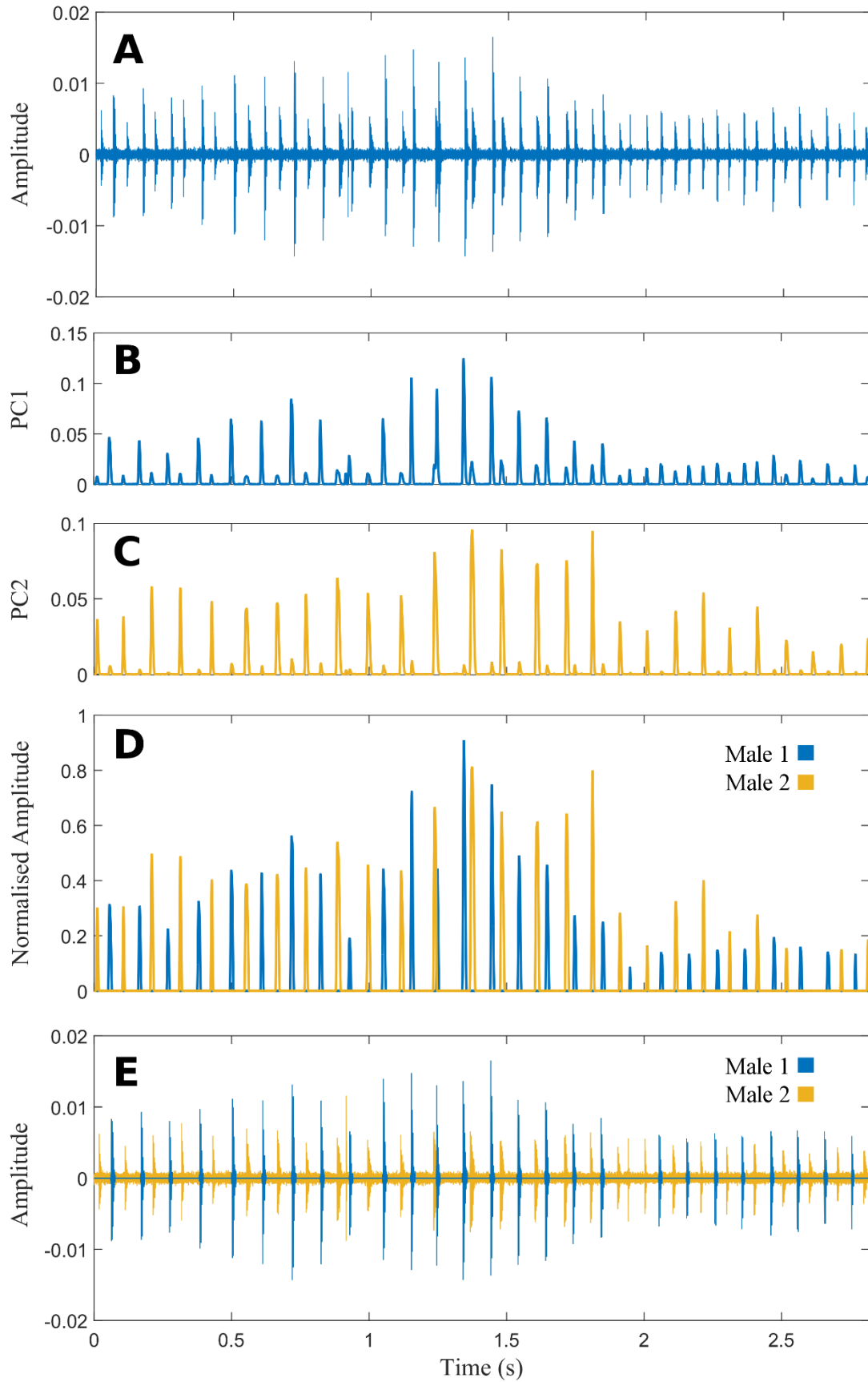


Figure 5. Method used to separate notes emitted by two different bark beetle (*Hylurgus ligniperda*) males in the same recording. A PCA-based approach was used for analysis of the calls. In these cases, the airborne signals emitted by the two beetles were in the range of acquisition of the microphone. An additional microphone generated redundant information to discern between the two sources. **(A)** Temporal representation of an example signal of 2.8 s with calls from two males. **(B)** Main component of variance estimated from the spectrogram of the recording of interest. A PCA was computed on the spectrogram of the recording obtained with the microphone aimed toward the individual of interest. Then, the modulus of the main component of variance was estimated, and information from the other components was disregarded. **(C)** Main component of variance estimated from the spectrogram of the redundant recording using the secondary microphone. In a similar procedure, the modulus of the main component of variance was estimated, but from the spectrogram of the recording obtained to add redundancy. **(D)** Temporal location of the notes of each male. The two previously obtained components (**B** and **C**) were thresholded, feature-scaled, and mutually subtracted in order to obtain a vector with the temporal locations of the notes of each male. **(E)** Visualization of the results of the method on the original signal. Notes emitted by each male are represented with different colours, with male 1 being the beetle of interest.

Algorithm set-up

Spectral content of *H. ligniperda* calls usually begins about three kHz (Figure 6); therefore, an IIR Butterworth high-pass filter of order eight (800 Hz stop-band frequency and 3000 Hz passband frequency) (Schubert and Kim, 2016) was used to filter the data (see Supplementary Material 7). For the estimation of the spectrogram, a flat top weighted window (Reljin et. al, 2007) of size $w = 1024$ and overlap $R = 512$ was selected. A Nyquist frequency $N_f = 512$ was chosen as the number of frequency bins (i.e., a FFT size of 1024).

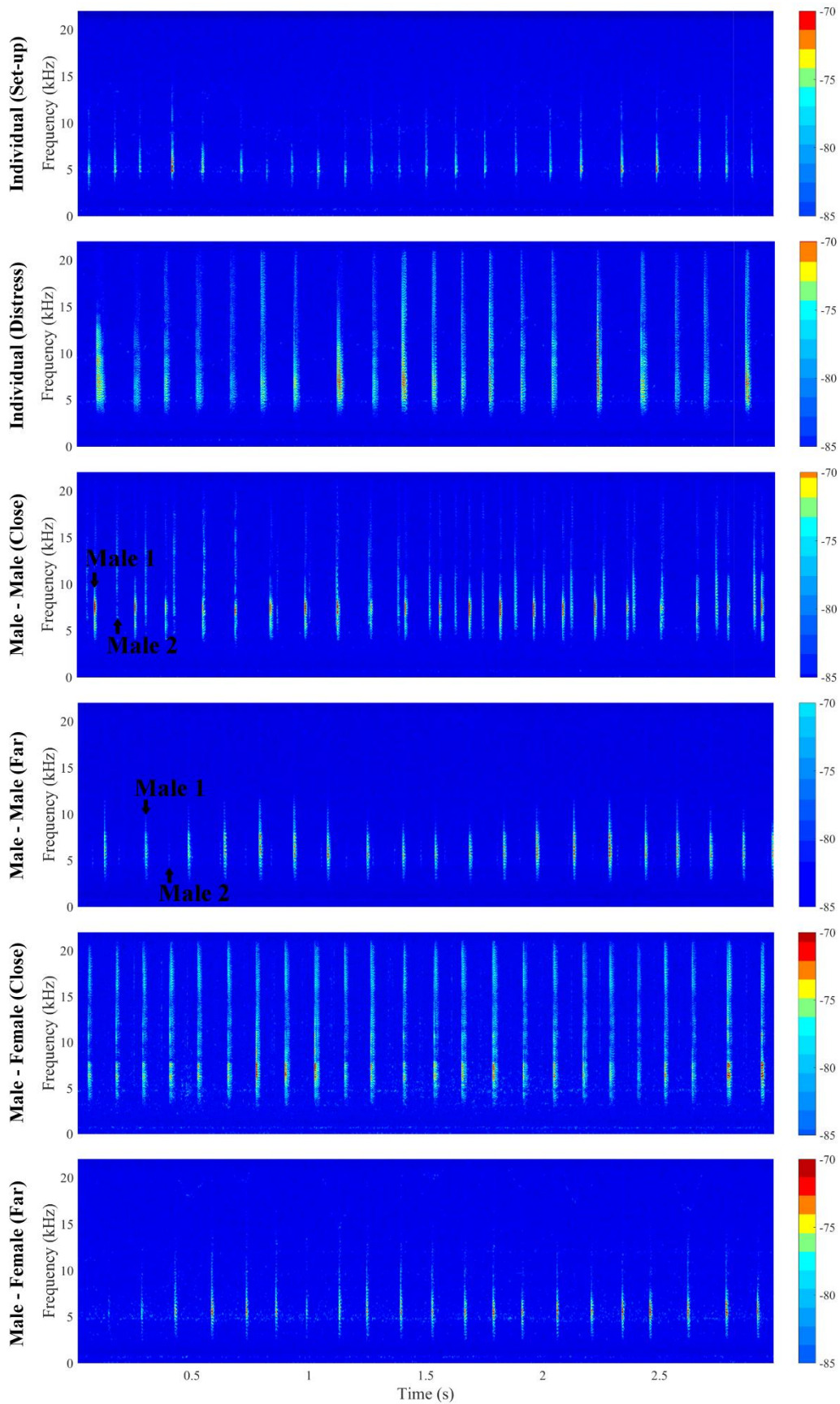


Figure 6. Spectrograms of call types emitted by the same male of *Hylurgus ligniperda* in different behavioural contexts. Colorbars in dB. The top two panels are calls emitted during individual sound production, i.e., distress and set-up calls. The set-up call is produced with no stimulus applied. The bottom four panels exemplify calls emitted during dyadic interactions. In male-male interactions, male 1 is the individual of interest. These plots do not have the filtering for data analysis, only a high pass filter at 500 Hz to reduce the DC offset and improve visualization.

Data analysis

In order to avoid pseudo-replication issues, all tests and analyses were conducted on the median values of the spectro-temporal parameters computed from the extracted notes of each individual. Initially, Friedman tests were performed to estimate differences in the calls emitted in each behavioural context. Bonferroni tests were then conducted for pairwise comparisons. A hierarchical clustering analysis was also performed to estimate similarities among the centres of the parameters of each type of call in the six behavioural contexts. In this case, an average linkage method (UPGMA) (Sokal and Michener, 1958) was used for the computation of the hierarchies of the calls in a dendrogram. An additional distance matrix (Gower, 1985) was estimated to show pairwise similarities in all behavioural contexts. Both the hierarchical analysis and the distance matrix were based on the estimation of the Euclidean distance between the cluster centres of the five call parameters in a five-dimensional space. Finally, the contribution of each spectro-temporal parameter to the variability of the calls in each behavioural context was estimated using Principal Component Analysis (PCA). The five spectro-temporal parameters were independently feature scaled (0-1), per behaviour, in order to reduce scale effects and make them comparable. The PCA was computed using single value decomposition, and five principal components ordered by the magnitude of their singular values were obtained. To measure contribution, the weights of each parameter in each component were assessed, but only the component that explained the largest amount of variance was taken into account.

Results

In total, 90 recordings were obtained from the 15 males in the six tested behavioural contexts (i.e. one recording per individual per behaviour). Although absence of a distress call in

females was previously known (Liu et al., 2008), we decided to perform the same experiments with females as with males in order to verify that female stridulations are also absent in other behavioural contexts. In our experiment, no stridulatory sounds were identified in any of the 90 recordings acquired from the 15 female individuals in the six behaviours. Additionally, we used morphological observations on SEM images of males and females (Figure 7) to verify the findings of Liu et al. (2008) regarding the existence of sexual dimorphism in the posterior two abdominal tergites of *H. ligniperda*. Males and females have a *pars stridens*, but males have a pronounced and arciform sclerotisation in the penultimate abdominal tergite, used as a plectrum, and that is barely present in females (Figure 7C). Additionally, the seventh (posteriormost) abdominal tergite in females is covered by the sixth (penultimate) tergite (Figure 7D), the size of which is almost the same as the last two abdominal tergites in males (Liu et al., 2008). Since females do not have distress calls (Liu et al., 2008), sound was used to sex the individuals. Consequently, there is a small chance that the obtained results were due to mute males wrongly classified as females. We estimated the probabilities of this error and they were negligible (see Supplementary Material 4). We also performed an additional experiment to determine the correlation between sexually dimorphic characteristics of the stridulatory organ and sound production capabilities. We obtained a 100% accuracy in separating males and females using the stridulatory call as sexing criterion. All mute individuals were classified as females, and all individuals that stridulated were classified as males (see Supplementary Material 4). Therefore, we are confident our sexing procedure did not bias the conclusions of our study.

In total, we extracted and analysed 181,389 notes from the 15 males across the six behaviours. For all data, the values of the maximum frequency oscillated around $10,273 \pm 3,314$ Hz (Mean \pm SD), $2,882 \pm 389$ Hz for the minimum frequency, $6,085 \pm 811$ Hz for the centroid frequency, 0.1157 ± 0.0658 s for the INI (inter-note interval), and 0.0321 ± 0.0062 s for duration. INIs with more than 3 s of separation were considered different call groups and not inter-note intervals. A call group is a sequence of calls separated by periods of silence longer than inter-call intervals. These periods of silence are stable and occur in a predictable manner.

Figure 6 exemplifies the types of notes emitted by the same male in each behavioural context. All notes in the six behaviours possessed a similar INI and duration, but relatively different spectral distributions, although male-male interactions (close and far) had the shortest note duration, and close interactions had the shortest INI (Table 1). Male-Female (far), Male-Male (far), and the set-up calls had a lower power concentration than the other three calls (distress, and Male-Female and Male-Male (close); Table 1). Male-Female (far) and Male-Male (far) interactions had similar spectral distributions, with a closer separation between the minimum and maximum frequencies than the other calls, and the lowest mean values for the maximum and centroid frequencies of all behaviours, followed by the set-up calls (Table 1).

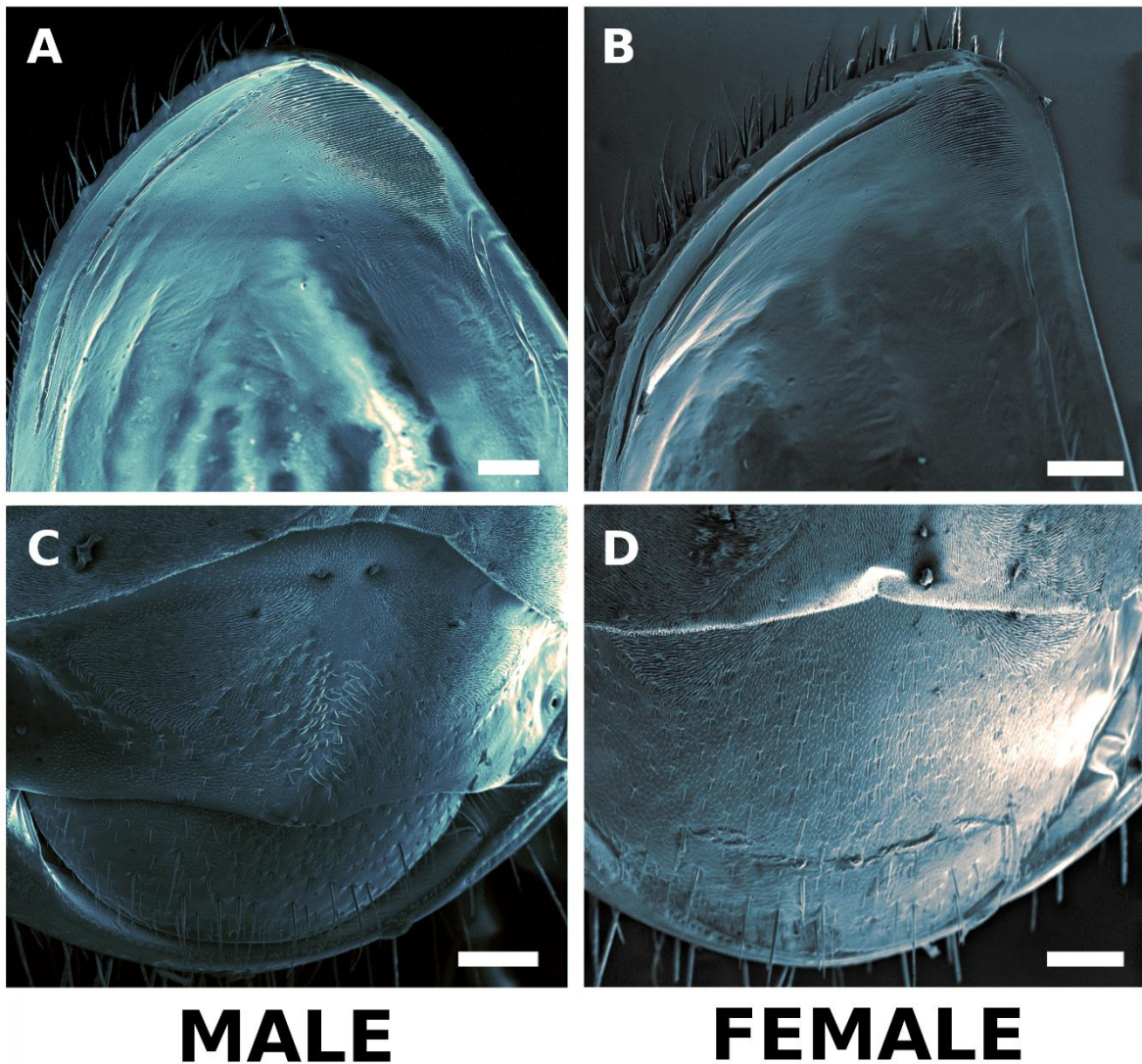


Figure 7. Sexual dimorphism in the stridulatory organ of *Hylurgus ligniperda*. (A-B) ventral view of the left elytron in a male and a female. (C-D) Dorsal views of the last three posterior tergites with elytra and wings removed. Both sexes possess a *pars stridens*, although males have a higher number of teeth distributed over a larger area. The seventh abdominal tergite in females is veiled by the sixth tergite, whose size is comparable with the combined size of the sixth and seventh tergites in males. The arciform sclerotisation in the posterior margin of the penultimate tergite (sixth) is the plectrum, which is responsible for stridulation in males. Scale bars are 100 μm .

Table 1. Spectro-temporal parameters (mean \pm SD) of *Hylurgus ligniperda* (N=15) calls in six behavioural contexts.

Behaviour	Spectral (kHz)			Temporal (ms)	
	Centroid	Minimum	Maximum	Duration	INI
<i>Set-up</i>	6.32 \pm 0.86	2.69 \pm 0.30	12.15 \pm 4.23	30.7 \pm 5.2	109.1 \pm 0.07
<i>Distress</i>	8.95 \pm 2.02	2.93 \pm 0.26	16.65 \pm 4.45	30.5 \pm 5.3	126.0 \pm 0.07
<i>Male-Male (close)</i>	7.59 \pm 1.45	2.79 \pm 0.46	15.31 \pm 4.33	28.2 \pm 3.9	96.1 \pm 0.07
<i>Male-Male (far)</i>	6.04 \pm 0.80	2.96 \pm 0.38	9.93 \pm 2.96	29.6 \pm 3.6	135.8 \pm 0.06
<i>Male-Female (close)</i>	7.32 \pm 1.12	2.53 \pm 0.29	16.43 \pm 4.59	31.6 \pm 6.9	62.2 \pm 0.06
<i>Male-Female (far)</i>	6.09 \pm 0.81	2.88 \pm 0.39	10.27 \pm 3.31	32.1 \pm 6.2	115.7 \pm 0.07

Friedman tests computed in each spectro-temporal parameter showed significant differences between behaviours ($p < 0.005$; $n = 15$) in the centroid frequency ($\chi^2_5 = 36.33$), maximum frequency ($\chi^2_5 = 41.34$), and duration ($\chi^2_5 = 18.06$). No statistically significant differences were found for minimum frequency ($\chi^2_5 = 13.02$) or INI ($\chi^2_5 = 8.72$). Pairwise comparisons (Table 2) showed significant differences in the same pairs of behaviours for the centroid and maximum frequencies (i.e., Set-up–Distress, Set-up–MF_{Close}, Distress–MM_{Far}, Distress–MF_{Far}, MM_{Far}–MF_{Close}, and MF_{Far}–MF_{Close}). The maximum frequency also presented differences for MM_{Far}–MM_{Close} (Table 2). For duration, statistically significant differences were found in the MM_{Close}–MF_{Close} and MM_{Close}–MF_{Far} pairs of behaviours (Table 2).

Table 2. Differences among the estimated group mean ranks for spectro-temporal parameters of *Hylurgus ligniperda* calls. * p<0.05, ** p<0.005, ***p<0.001.

Centroid						
	<i>Set-up</i>	<i>Distress</i>	<i>MM(close)</i>	<i>MM(far)</i>	<i>MF(close)</i>	<i>MF(far)</i>
<i>Set-up</i>	-	-2.667**	-1.600	0.267	-2.267*	0.066
<i>Distress</i>	-	-	1.067	2.933***	0.400	2.733***
<i>MM(close)</i>	-	-	-	1.867	-0.667	1.667
<i>MM(far)</i>	-	-	-	-	-2.533**	-0.200
<i>MF(close)</i>	-	-	-	-	-	2.333*

Minimum						
	<i>Set-up</i>	<i>Distress</i>	<i>MM(close)</i>	<i>MM(far)</i>	<i>MF(close)</i>	<i>MF(far)</i>
<i>Set-up</i>	-	-1.267	-1.100	-1.733	0.167	-1.267
<i>Distress</i>	-	-	0.167	-0.467	1.433	0.000
<i>MM(close)</i>	-	-	-	-0.633	1.266	-0.167
<i>MM(far)</i>	-	-	-	-	1.900	0.467
<i>MF(close)</i>	-	-	-	-	-	-1.433

Maximum						
	<i>Set-up</i>	<i>Distress</i>	<i>MM(close)</i>	<i>MM(far)</i>	<i>MF(close)</i>	<i>MF(far)</i>
<i>Set-up</i>	-	-2.633**	-1.500	0.567	-2.100*	0.467
<i>Distress</i>	-	-	1.133	3.200***	0.533	3.100***
<i>MM(close)</i>	-	-	-	2.067*	-0.600	1.967
<i>MM(far)</i>	-	-	-	-	-2.667**	-0.100
<i>MF(close)</i>	-	-	-	-	-	2.567**

Duration						
	<i>Set-up</i>	<i>Distress</i>	<i>MM(close)</i>	<i>MM(far)</i>	<i>MF(close)</i>	<i>MF(far)</i>
<i>Set-up</i>	-	0.367	1.833	0.967	-0.367	-0.600
<i>Distress</i>	-	-	1.467	0.600	-0.733	-0.967
<i>MM(close)</i>	-	-	-	-0.867	-2.200*	-2.433**
<i>MM(far)</i>	-	-	-	-	-1.333	-1.567
<i>MF(close)</i>	-	-	-	-	-	-0.233

INI						
	<i>Set-up</i>	<i>Distress</i>	<i>MM(close)</i>	<i>MM(far)</i>	<i>MF(close)</i>	<i>MF(far)</i>
<i>Set-up</i>	-	-0.300	1.133	-0.467	0.600	-0.367
<i>Distress</i>	-	-	1.433	-0.167	0.900	-0.067
<i>MM(close)</i>	-	-	-	-1.600	-0.533	-1.500
<i>MM(far)</i>	-	-	-	-	1.066	0.100
<i>MF(close)</i>	-	-	-	-	-	-0.966

In this study, calls of *H. ligniperda* were characterized by five spectro-temporal parameters extracted directly from the spectrogram. As each note can be mapped by its parameters as a point in a five-dimensional space, a distance matrix was estimated from these parameters in order to find similarities among the notes emitted in each behaviour. The matrix in Figure

8A was obtained by estimating pairwise distances among the centres of the clusters of the notes, using the Euclidean distance as a similarity measure. The distance values were then rescaled between zero and one for qualitative interpretations, where zero represents complete similarity and one is the largest obtained distance between two behavioural contexts. An additional hierarchical clustering analysis (average linkage) was performed on the centres of the clusters with the purpose of grouping types of calls by similarity (Figure 8B).

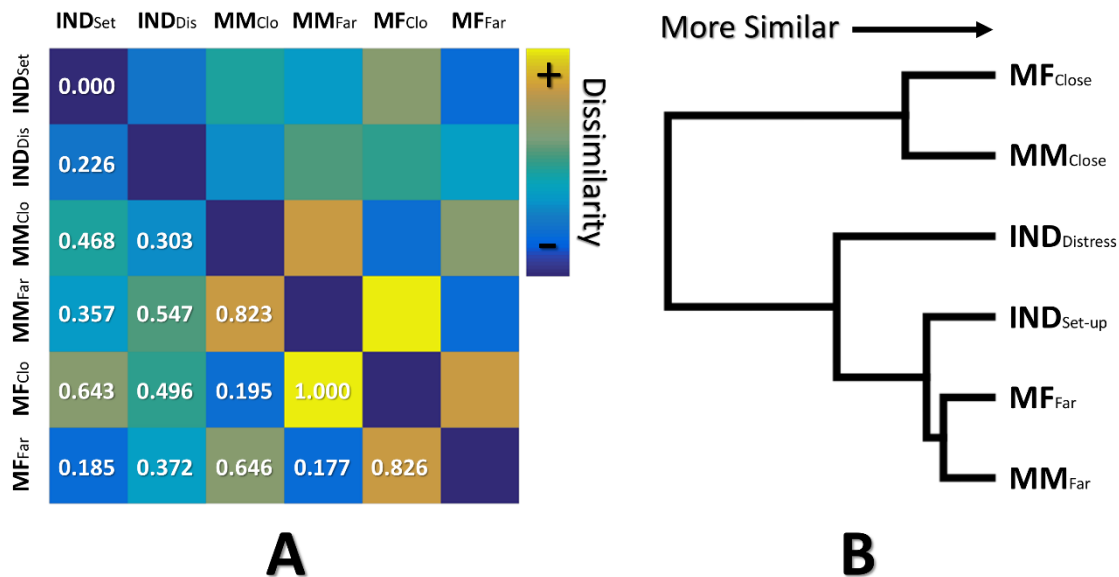


Figure 8. Similarity measurements among calls emitted by *Hylurgus ligniperda* in six behavioural contexts. **(A)** Scaled distance matrix (Euclidean distance) of calls between contexts. The minimum value represents complete similarity and the maximum value is the largest distance obtained between two behavioural contexts. **(B)** Dendrogram obtained by hierarchical clustering analysis (Median linkage - Euclidean distance) of the centre values of the spectro-temporal parameters in each context.

In dyadic interactions, Male-Male (far) and Male-Female (close) were the most dissimilar calls (Figure 8) and therefore have a rescaled Euclidean distance of one, with the second most dissimilar pair of calls being Male-Male (far) and Male-Male (close). Distant (far) interactions showed the most similarity. Calls produced during close interactions were similar between contexts, but were simultaneously dissimilar from calls produced in other

behavioural contexts (Figure 8). In individual sound production, the set-up call was more similar to the call produced in the Male-Female (far) interaction than to the distress call; however, the distress call was more similar to the set-up call than to any other type of call (Figure 8B). The distances between the set-up call and the calls produced in distant interactions were closer than the distances between the set-up call and calls produced in close interactions.

From 70.5% to 92.8% of the variance in each behavioural context was explained by a single principal component (PC1), whose main contributors were the centroid and maximum frequencies (Table 3 and Supplementary Material 5). Spectral parameters were more important than temporal ones in the explanation of the variability of the data; however, minimum frequency played a less significant role than the other two (Table 3). In temporal terms, the contribution of the INI to PC1 was inconsequential, and duration only had an effect in one behavioural context (Male-Male far) (Table 3).

Table 3. Coefficients of the first principal component (PC1) and percentage of variance explained by PC1 in all six behavioural contexts for *Hylurgus ligniperda* tests on acoustic communication. Centroid and maximum frequencies were the largest contributors to PC1.

Behaviour	PC1 coefficients					Explained variance (%)
	Centroid	Minimum	Maximum	Duration	INI	
<i>Set-up</i>	0.2287	-0.1022	0.9681	0.0003	0.0002	90.5
<i>Distress</i>	0.5645	-0.0165	0.8253	0.0030	0.0017	92.9
<i>Male-Male (close)</i>	0.2693	0.0412	0.9621	0.0059	0.0001	75.2
<i>Male-Male (far)</i>	0.3108	0.0173	0.9374	-0.1562	-0.0029	70.5
<i>Male-Female (close)</i>	0.2170	-0.0620	0.9742	-0.0010	-0.0010	85.0
<i>Male-Female (far)</i>	0.2778	-0.0600	0.9587	0.0089	-0.0035	84.6

Discussion

We studied the variations of the call parameters of *Hylurgus ligniperda* in individual sound production and dyadic interactions in 90 recordings of 15 males. We repeated the same experiments using 15 females, but did not find any stridulatory sound in any of the six studied behavioural contexts. We found that males of *H. ligniperda* solely produce single-noted calls, whose spectro-temporal parameters vary under stimulation by external sources (e.g. a brush) or direct contact with other individuals. Calls produced by other males (i.e. acoustic stimuli)

were irrelevant in the modification of such parameters. Maximum and centroid frequencies were the main contributors to the variance of *H. ligniperda* calls, with temporal parameters playing almost no role in the variability of the data. Our results suggest that *H. ligniperda* stridulates close to the limits of its capacity in temporal terms, and that most of the call modifications occur in the spectral domain.

Females did not produce sounds for acoustic communication at all. Additional morphological observations based on SEM images of *H. ligniperda* suggest that the sexual dimorphism in the stridulatory organ area (i.e., lack of plectrum in females) (Liu et al., 2008) makes acoustic communication in females highly unlikely. Some click-like sounds were initially found in females during data acquisition, but were discarded in further audio-visual inspections as either feeding or body-bark friction sounds. These ‘clicks’ can be confusing for the experimenter since some similar sounds have been reported for females of the Tomicini tribe (Rudinsky and Michael, 1973). However, the clicks we found in our experiment had an extremely low amplitude, lacked a structured pattern, and had a shorter duration than the click-like sounds we have heard in several females of *Dendroctonus* species (pers. obs.).

In nature, bark beetles are found in tunnels under several millimetres of tree bark, and recordings from ‘naked’ beetles extracted from tunnels would lead to unnatural behaviour. The set-ups using phloem sandwiches were designed to simulate simple galleries and to allow control and verification of beetle location (which is not possible when beetles are hidden in actual galleries). Nonetheless, the chambers housing beetles were made in the phloem of its main host tree species and in the presence of the monoterpenes and other volatiles associated with this, and they were readily ‘colonised’ by *H. ligniperda* placed at the 2-mm entrance hole. *Hylurgus ligniperda* is a saprophytic, secondary (non-aggressive) species and does not produce pheromones unlike primary (aggressive) bark beetle species; instead, it uses host volatiles (monoterpenes) as cues for finding its host material in which it establishes its galleries (e.g., Kerr et al., 2017). The set-ups were constructed to study short-distance and short-term acoustic interactions in terms of the acoustic responses once an individual of *H. ligniperda* encounters or hears another one of the same or different sex. This is a realistic scenario based on two observations of *H. ligniperda* behaviour: (i) Multiple individuals commonly attack the same tree and create galleries in close proximity, and (ii) males of *H.*

ligniperda almost unequivocally respond to the calls of another male. The set-up for ‘far’ interactions was specifically designed to evaluate changes in the acoustic behaviour when two individuals, in separate chambers, do not have direct contact, but can hear each other. The configuration for ‘close’ interactions was designed to estimate changes in the acoustic behaviour upon direct contact with another conspecific, and the set-up for individual sound production was designed to study communicatory interactions under distress conditions.

The set-up behaviour can be interpreted as a form of low-intensity distress call produced by moving the beetle from its container to the data acquisition set-up (phloem sandwich). This type of ‘distress’, and its associated stridulation, are significantly different from the ones found in the ‘distress behaviour’ obtained by direct contact with a brush which elicited louder and slightly faster sounds with a broader spectral distribution. For this reason, it was important to separate set-up and distress behaviours into two categories (or contexts). Additionally, some species can have distress calls but no set-up calls. For example, we know (pers. obs.) that females of the Southern pine beetle, *Dendroctonus frontalis* (Zimmerman), and the Western pine beetle, *Dendroctonus brevicomis* (LeConte), do not produce sounds when moved, pressed, or even under life-threatening situations, but stridulate when softly touched with a brush on the ventral surface of the abdomen. As we did not know if females of *H. ligniperda* could behave similarly to the previously mentioned species, it was important to evaluate the possible scenarios in which stridulations could be found.

Male calls of *H. ligniperda* are composed of a single note (i.e., simple), which differs from the mixture of simple and multiple-noted groups found in other scolytines with elytral-abdominal stridulatory organs (Fleming et al., 2013; Lindeman and Yack, 2015; Yturralde and Hofstetter, 2015). Some spectral components of *H. ligniperda* calls are distributed through the ultrasonic part of the spectrum, but most of the power is allocated between 3 and 10 kHz. Similar ultrasonic components have been reported for *D. ponderosae* (Fleming et al., 2013) and the Mexican pine beetle *Dendroctonus approximatus* Dietz (Yturralde and Hofstetter, 2015). Likewise, note duration (between 20 and 40 ms) is comparable with the single-noted calls of *D. valens* (Lindeman and Yack, 2015), *D. ponderosae* (Fleming et al., 2013), and *D. approximatus* (Yturralde and Hofstetter, 2015).

Hylurgus ligniperda is able to modify the spectro-temporal parameters of its calls in accordance with the behavioural context, as found in the genus *Dendroctonus* (Yandell, 1984; Fleming et al., 2013; Lindeman and Yack, 2015; Yturralde and Hofstetter, 2015). Such modification is perceivable to the human ear and could be described as an increase in pitch and amplitude. Temporal parameters, on the other hand, tended to be more stable regardless of the behavioural context and had minimal influence in the variability of the data. The maximum and centroid frequencies always covaried, suggesting that the modification of both spectral parameters was linked to the same action - possibly ventral movements to increase amplitude, as in other coleopterans (Alexander et al., 1963; Gibson, 1967; Hyder and Oseto, 1989; Wilson et al., 1993). This also explains the smaller range of variation of the minimum frequency, since it is more robust to changes in pressure. Attributable to the properties of the estimated parameters, centroid and maximum frequencies were the main contributors to the principal component, explaining over 70% of call variability. The centroid frequency is an accurate descriptor of the power distribution of a sound and, since the minimum frequency remained relatively stable, the maximum frequency played a substitute role (i.e., it was a proxy variable) for the bandwidth of the call ($f_{\max} - f_{\min}$). In temporal terms, *H. ligniperda* barely modified the INI and duration, and consequently, the number of notes per unit of time. Temporal parameters had minimal influence in the variability of the data and calling rate remained constant for the six studied behavioural contexts. The stridulatory organ of *H. ligniperda* consists of a two-part elytro-tergal mechanism, in which a movable part (plectrum), on the abdomen, scrapes a static part (pars stridens) on the ventral surface of the elytra. From this, it can be inferred that just one part of the dual organ, i.e. the plectrum, possesses the actuators to control the variations of the call. In general, there are two physical parameters that can be modified solely by using the plectrum, the speed of the ventral movement and the pressure on the pars stridens. As the inter-note intervals are stable amongst all the contexts and the call duration varied only slightly, it could be implied that *H. ligniperda* has little control of the speed of its ventral movements. Also, both maximum and centroid features covaried, which means their variation is probably linked to the same action (i.e., increasing the pressure from the plectrum on the pars stridens). We hypothesise that this increment of pressure is the physical parameter the beetle regulates to control the variability of the call frequency. Regarding the function, our results indicated that the biggest changes

in frequency mostly occurred under distress conditions, or when beetles were in direct contact with another individual, which are behaviours associated with aggressive interactions. These behavioural contexts have in common a direct line of sight between the individuals, which minimises the damping effects of the environment and makes calls spectrally distinguishable in the natural habitat. Furthermore, spectral content is intrinsically related to the loudness of the sound, which conveys information on the position of the other individual. To date we do not exactly know the location of these beetles' receptor organs, nor the internal mechanism they use to process the received signal. In 2019, Hofstetter et al, using playback experiments, tested and confirmed spectral discriminatory capabilities in the round-headed bark beetle *Dendroctonus adjunctus*, but the underlying mechanism for such discrimination is still unknown.

Dyadic interactions were used to evaluate how the physical presence of conspecifics could modify the calls of *H. ligniperda*, and the sex-dependence of such modification in case it existed. These interactions were also useful to estimate the effect of presence/absence of conspecific calls in the modifications of the spectro-temporal parameters. Calls emitted in distant interactions (male-male and male-female) were the most similar to each other, even though in just one of these scenarios (male-male) did both individuals produce sounds; a result mirrored in the close dyadic interactions. From this, we deduce that either visual or tactile interactions between individuals are more important than acoustic signals in the modification of the parameters of calls, and that the sex of the other individual is irrelevant for such modification in the studied dyadic interactions. Therefore, there is evidence to support the hypothesis that calls emitted by other males do not play a major role in the modification of the spectro-temporal parameters of the calls in the studied behavioural contexts. This does not mean that acoustic signals from other males are not important in mutual acoustic communicatory interactions. For instance, during data acquisition, we observed a phase coupling between the calls of males singing simultaneously. Additionally, we observed that males tended to reply acoustically to sounds emitted by other males.

While our laboratory set-up does not precisely mimic natural conditions, to date there is no tractable approach for such studies in the field. A priori knowledge of the species inside a tree is rather difficult or impossible to obtain, and there are no methods for acoustic detection

and identification of bark beetle sounds inside tree logs in such conditions (i.e., when the presence of species and the number and exact location of individuals are unknown). Nonetheless, our findings provide insight into strategies for automatic acoustic detection of bark beetles. Since acoustic signals produced by one beetle were irrelevant as modifiers of the parameters of the calls produced by the other beetle, improving the signal-to-noise ratio of these insects' calls using auditory stimuli would be challenging. However, playback procedures can be used to elicit an *ad libitum* acoustic response and to reduce the time of assessment in acoustic detection protocols. Future efforts in biosecurity aspects should focus on determining how to elicit acoustic responses in these beetles, and optimizing procedures for their automatic detection in several transmission media.

Our proposed approach for the analysis of *H. ligniperda* calls establishes a first step towards automatic detection of bark beetle sounds. This method considerably reduced the time needed for data analysis in comparison with manual procedures, permitted the analysis of large datasets in dyadic communicatory interactions, and removed observer bias. To discriminate between individuals, we applied a PCA-based method which relies on redundancy and requires the number of microphones to be equal to the number of sources (which is a common requirement in blind source separation algorithms; Cao and Liu, 1996; Naik and Wang, 2014), yet this method still needs to be refined for noisy and uncontrolled environments. Only a few approaches have been proposed previously for discriminating among calls of individuals in other taxa, such as canids and birds (Hartwig, 2004; Fox, 2008; Cheng et al., 2012; Ptacek et al., 2016), and to the best of our knowledge, this is the first time that acoustic identification of stridulatory sounds of simultaneously-stridulating conspecifics (i.e., telling apart calls of individuals of the same species that are stridulating at the same time) has been addressed in insects.

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References

- Aflitto NC, Hofstetter RW. (2014) Use of acoustics to deter bark beetles from entering tree material. *Pest Management Science*, **70**, 1808–1814.
- Alexander RD, Moore TE, Woodruff RE. (1963) The evolutionary differentiation of stridulatory signals in beetles (Insecta: Coleoptera). *Animal Behaviour*, **11**, 111–112.
- Barr BA. (1969) Sound production in Scolytidae with emphasis on the genus *Ips*. *Canadian Entomologist*, **101**, 636–672.
- Brockhoff EG, Knížek M, Bain J. (2003) Checklist of indigenous and adventive bark and ambrosia beetles (Curculionidae: Scolytinae and Platypodinae) of New Zealand and interceptions of exotic species (1952-2000). *New Zealand Entomologist*, **26**, 29–44.
- Brockhoff EG, Bain J, Kimberley M, Knížek M. (2006) Interception frequency of exotic bark and ambrosia beetles (Coleoptera: Scolytinae) and relationship with establishment in New Zealand and worldwide. *Canadian Journal of Forest Research*, **36**, 289–298.
- Brockhoff EG, Kimberley M, Liebhold AM, Haack RA, Cavey JF. (2014) Predicting how altering propagule pressure changes establishment rates of biological invaders across species pools. *Ecology*, **95**, 594–601.
- Cheng J, Xie B, Lin C, Ji L. (2012) A comparative study in birds: call-type-independent species and individual recognition using four machine-learning methods and two acoustic features. *Bioacoustics*, **21**, 157–171.
- Cao XR, Liu RW. (1996) General approach to blind source separation. *IEEE Transactions on Signal Processing*, **44**, 562–571.
- Fleming AJ, Lindeman AA, Carroll AL, Yack JE. (2013) Acoustics of the mountain pine beetle (*Dendroctonus ponderosae*) (Curculionidae, Scolytinae): sonic, ultrasonic, and vibration characteristics. *Canadian Journal of Zoology*, **91**, 235–244.

- Fox ES. (2008) A new perspective on acoustic individual recognition in animals with limited call sharing or changing repertoires. *Animal Behaviour* **75**, 1187–1194.
- Fraedrich SW, Harrington TC, Rabaglia RJ, Ulyshen MD, Mayfield III AE, Hanula JL, Eickwort JM, Miller DR. (2008) A fungal symbiont of the redbay ambrosia beetle causes a lethal wilt in redbay and other Lauraceae in the southeastern United States. *Plant Disease*, **92**, 215–224.
- Gibson LP. (1967) Stridulatory mechanisms and sound production in *Conotrachelus* (Coleoptera: Curculionidae). *Annals of the Entomological Society of America*, **60**, 43–54.
- Gower JC. (1985) Properties of Euclidean and non-Euclidean distance matrices. *Linear Algebra and its Applications*, **67**, 81–97.
- Harrington TC. (2005) Ecology and evolution of mycetophagous beetles and their fungal partners. In: Vega FE, Blackwell M, eds. *Ecological and Evolutionary Advances in Insect-Fungal Associations*. Oxford: Oxford University Press, 257–291.
- Hartwig S. (2005) Individual acoustic identification as a non-invasive conservation tool: an approach to the conservation of the African wild dog *Lycaon pictus* (Temminck, 1820). *Bioacoustics*, **15**, 35–50.
- Hofstetter RW, Dunn DD, McGuire R, Potter KA. (2014) Using acoustic technology to reduce bark beetle reproduction. *Pest Management Science*, **70**, 24–27.
- Hofstetter RW, Aflitto N, Bedoya CL, Yturralde K, Dunn DD. (2019) Vibrational Behavior in Bark Beetles – Applied Aspects. In: Hill et al., eds. *Biotremology – Studying Vibrational Behavior*. Animal Signals and Communication Series. Springer (In press).
- Hopkins AD. (1909) Contributions towards a monograph of the Scolytid beetles Part 1. The Genus *Dendroctonus*. *Technical series of the U.S. Department of Agriculture - Bureau of Entomology*, **17**, 35–51.
- Hyder DE, Oseto CY. (1989) Structure of the stridulatory apparatus and analysis of the sound produced by *Smicronyx fulvus* and *Smicronyx sordidus* (coleoptera, curculionidae, erirrhinae, smicronychini). *Journal of Morphology*, **201**, 69–84.
- Kerr JL, Kelly D, Bader MK, Brockerhoff EG. (2017) Olfactory cues, visual cues, and semiochemical diversity interact during host location by invasive forest beetles. *Journal of Chemical Ecology*, **43**, 17–25.
- Kinn DN, Miller MC. (1981) A phloem sandwich unit for observing bark beetles, associated predators, and parasites. USDA Forest Service Research Note, SO-269, 1–3.
- Kirkendall LR. (1983) The evolution of mating systems in bark and ambrosia beetles (Coleoptera: Scolytidae and Platypodidae). *Zoological Journal of the Linnean Society*, **77**, 293–352.
- Köhler J, Jansen M, Rodríguez A, Kok PJR, Toledo LF, Emmrich M, Glaw F, Haddad CFB, Rödel MO, Vences M. (2017) The use of bioacoustics in anuran taxonomy: theory, terminology, methods and recommendations for best practice. *Zootaxa*, **4251**, 1–124.

- Lindeman AA, Yack JE. (2015) What is the password? Female bark beetles (Scolytinae) grant males access to their galleries based on courtship song. *Behavioural Processes*, **11**, 123–131.
- Lindgren BS, Raffa KF. (2013) Evolution of tree killing in bark beetles (Coleoptera: Curculionidae): trade-offs between the maddening crowds and a sticky situation. *The Canadian Entomologist*, **145**, 471–495.
- Liu D, Flint ML, Seybold SJ. (2008) A secondary sexual character in the red-haired pine bark beetle, *Hylurgus ligniperda* Fabricius (Coleoptera: Scolytidae). *Pacific Entomologist*, **84**, 26–28.
- Lyal CHC, King T. (1996) Elytro-tergal stridulation in weevils (Insecta: Coleoptera: Curculionoidea). *Journal of Natural History*, **30**, 703–773.
- Mankin RW, Hagstrum DW, Smith MT, Roda AL, Kairo MTK. (2011) Perspective and promise: a century of insect acoustic detection and monitoring. *American Entomologist*, **57**, 30–44.
- McCarthy JK, Hood IA, Brockerhoff EG, Carlson CA, Pawson SM, Forward M, Walbert K, Gardner JF. (2010) Predicting sapstain and degrade in fallen trees following storm damage in a *Pinus radiata* forest. *Forest Ecology and Management*, **260**, 1456–1466.
- McCarthy JK, Brockerhoff EG, Didham RK. (2013) An experimental test of insect-mediated colonisation of damaged *Pinus radiata* trees by sapstain fungi. *PLOS ONE*, **8**, e55692.
- Mikkelsen KM, Bearup LA, Maxwell RM, Stednick JD, McCray JE, Sharp JO. (2013) Bark beetle infestation impacts on nutrient cycling, water quality and interdependent hydrological effects. *Biogeochemistry*, **115**, 1–21.
- Naik GR, Wang W. (2014) Blind Source Separation: Advances in Theory, Algorithms and Applications. New York, NY: Springer Publishing Company.
- Oliver WW. (1995) Is self-thinning in ponderosa pine ruled by *Dendroctonus* bark beetles? In: Eskew LG, ed. Forest health through silviculture: Proceedings of the 1995 National Silviculture Workshop. Mescalero, NM: USDA, 213–218.
- Ptacek L, Machlica L, Linhart P, Jaska P, Muller L. (2015) Automatic recognition of bird individuals on an open set using as-is recordings. *Bioacoustics*, **25**, 55–73.
- Raffa KF, Grégoire JC, Lindgren BS. (2015) Chapter 1 - Natural history and ecology of bark beetles. In: Vega FE, Hofstetter RW, eds. Bark Beetles. San Diego, CA: Academic Press, 1–40.
- Reljin IS, Reljin BD, Papic VD. (2007) Extremely flat-top windows for harmonic analysis. *IEEE Transactions on Instrumentation and Measurement*, **56**, 1025–1041.
- Rouco M, Muñoz G. (2014) Influence of blue stain on density and dimensional stability of *Pinus radiata* timber from northern Galicia (Spain). *Holzforschung*, **69**, 97–102.
- Ryker LC, Rudinsky JA. (1976) Sound production in Scolytidae: acoustic signals of male and female *Dendroctonus valens* LeConte. *Journal of Applied Entomology*, **80**, 113–118.
- Rudinsky JA, Michael RR. (1973) Sound production in Scolytidae: Stridulation by female *Dendroctonus* beetles. *Journal of Insect Physiology*, **19**, 689–705.

- Schowalter TD, Filip GM. (1993) Beetle-pathogen interactions in conifer forests. San Diego, CA: Academic Press, 5–7.
- Schowalter TD. (2012) Ecology and management of bark beetles (Coleoptera: Curculionidae: Scolytinae) in southern pine forests. *Journal of Integrated Pest Management*, **3**, 1–7.
- Schroeder LM. (2001) Tree mortality by the bark beetle *Ips typographus* (L.) in storm-disturbed stands. *Integrated Pest Management Reviews*, **6**, 169–175.
- Schubert TF, Kim EM. (2016) Fundamentals of Electronics: Book 3: Active Filters and Amplifier Frequency Response. San Rafael, CA: Morgan and Claypool Publishers, 670–671.
- Six DL, Wingfield MJ. (2011) The role of phytopathogenicity in bark beetle–fungus symbioses: a challenge to the classic paradigm. *Annual Review of Entomology*, **56**, 255–272.
- Sokal R, Michener C. (1958) A statistical method for evaluating systematic relationships. *University of Kansas Science Bulletin*, **38**, 1409–1438.
- Sopow SL, Bader MKF, Brockerhoff EG. (2015) Bark beetles attacking conifer seedlings: picking on the weakest or feasting upon the fittest? *Journal of Applied Ecology*, **52**, 220–227.
- Taylor AD, Hayes JL, Moser JC. (1992) A phloem sandwich allowing attack and colonization by bark beetles (Coleoptera: Scolytidae) and associates. *Journal of Entomological Science*, **27**, 311–316.
- Wilkinson RC, McClelland WT, Murillo RM, Ostmark EO. (1967) Stridulation and behavior in two southeastern *Ips* bark beetles (Coleoptera: Scolytidae). *Florida Entomologist*, **50**, 185–195.
- Wilson LM, Charles SH, James BJ, Joseph PM. (1993) Sound production in *Phrydiuchus tau* (Coleoptera: Curculionidae). *Annals of the Entomological Society of America*, **86**, 621–630.
- Wood SL, Bright DE. (1992) A catalog of Scolytidae and Platypodidae (Coleoptera), Part 2: taxonomic index. *Great Basin Naturalist Memoirs*, **13**, 1–1553.
- Yandell KL. (1984) Sound production of *Dendroctonus ponderosae* Hopkins (Coleoptera, Scolytidae): a comparison of populations from three host pines in Oregon. *Journal of Applied Entomology*, **97**, 180–187.
- Yturralde KM, Hofstetter RW. (2015) Characterization of stridulatory structures and sounds of the larger Mexican pine beetle, *Dendroctonus approximatus* (Coleoptera: Curculionidae: Scolytinae). *Florida Entomologist*, **98**, 516–527.

Supplementary Material 1 - *Definitions*

Due to the similarities among anuran and bark beetle calls, we have based our definitions and terminology on the work of Köhler et al. (2017). This terminology is standard to any type of communication that uses quasiperiodic pulse trains. Because of this, it could be applied, with just a few exceptions, to most taxa on earth. It is also unambiguously defined and it is not tied to the sound production mechanism or the function of the sound. Additionally, the term ‘chirp’ used in bark beetle acoustics, is not a chirp *sensu stricto*. In acoustics and signal processing, the term ‘chirp’ is a waveform whose instantaneous frequency increases, or decreases, with time. Due to the nature of bark beetle sounds, i.e. broadband and uniform, the instantaneous frequency tends to remain relatively stable, which differs from the most accepted definition of ‘chirp’. The term is also ambiguous and misleading, as it does not follow a hierarchical order, and thus, cannot serve as integral element in the description of the temporal parameters of the sound (see Broughton (1976) for a deeper discussion on this specific matter).

Call: The main acoustic unit of the stridulatory process (Figure S1 and Figure 2). These are separated from other calls by silent inter-call intervals. Calls with a single note are named single-noted calls, and calls with several notes are multiple-noted calls. A call might be composed by notes of different types.

Call duration: Length of the call; measured from the beginning of the first note to the end of the last note. In calls that consist of a single note, call duration is the same as note duration.

Call group: Sequence of calls separated by periods of silence longer than inter-call intervals (Figure S1 and Figure 2). These periods of silence must be stable and occur in a predictable manner.

Calling rate: Number of calls in a unit of time.

Centroid frequency: Also known as spectral centroid. This frequency is analogous to the centre of mass in mechanical systems. In general terms, it represents the frequency in which the centroid of the power spectral distribution is located (see supplementary material 2 for its mathematical definition).

Cut-off frequency: Frequency at which the energy of the note starts decreasing (see supplementary material 2 for its mathematical definition).

Inter-call-interval: Silent period between two consecutive calls, measured from the end of the last note of the call to the beginning of the first note of the next call. In calls with a single note, the inter-note-interval is the same as the inter-call-interval.

Inter-note-interval: Interval between two consecutive notes within the same call, measured from the end of one note to the beginning of the consecutive note.

Minimum frequency: Lower cut-off frequency of the mean spectrum of the note (see supplementary material 2 for its mathematical definition).

Maximum frequency: Upper cut-off frequency of the mean spectrum of the note (see supplementary material 2 for its mathematical definition).

Note: Main subunit of a call (Figure S1 and Figure 2). This subunit might have several types, and can only be further divided into pulses. A call might consist of a single note.

Note Duration: Length of a note within a call.

Pulse: Sound bursts within notes. This is the smallest acoustic unit. We did not perform any analysis at this level of hierarchy during this study.

Recording: Audio signal recorded with a data acquisition device.

Signal: Sequence of data points that convey information about the studied phenomenon. In our context, each datum contains acoustic information taken at equally-spaced points in time (i.e. chronologically).

Stridulation: Sound generated using stridulatory mechanisms. In our context it is also a synonym for call.

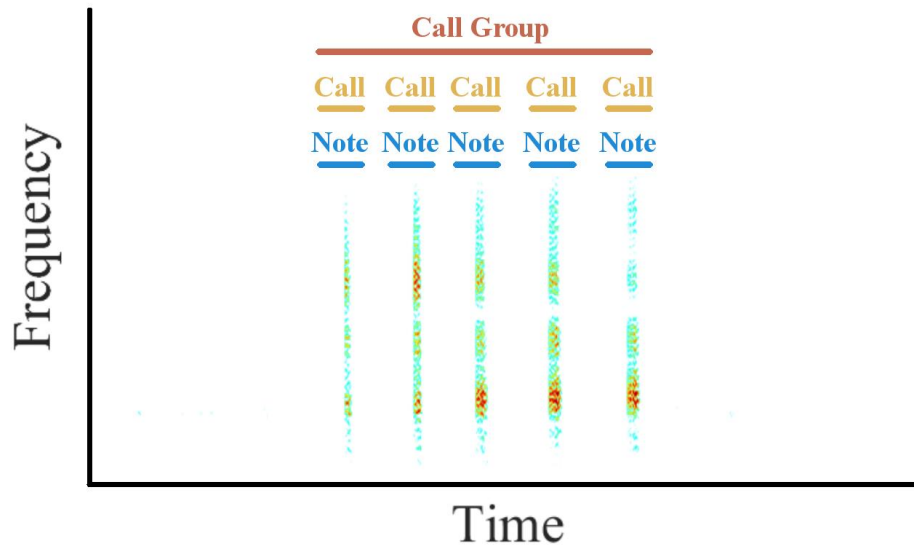


Figure S1. Hierarchy of acoustic units and subunits of the call-centred approach used to describe *Hylurgus ligniperda* stridulations. Since *H. ligniperda* only produce calls of a single note, the term note and call are synonymous.

Broughton WB. (1976) Proposal for a new term 'echeme' to replace 'chirp' in animal acoustics. *Physiological Entomology* 1:103–106.

Supplementary Material 2 - Automatic note detection and parameter estimation

The first step of the method consists in applying a high-pass filter to a signal $x \in \mathbb{R}^{N_x}$ that contains the information of interest (i.e., bark beetle stridulations), where N_x is the length of the signal in number of samples. Consequently, a signal $x_{\text{fil}} \in \mathbb{R}^{N_x}$ with the information filtered below the frequency f_0 was obtained. Here, we assume a bijective filter in which the filtered signal will have the same number of samples as the original signal. Afterwards, the spectrogram $S \in \mathbb{R}^{N_f \times N_t}$ is estimated from the signal x (Figure 4A), where N_f is the number of frequency bins and N_t is the number of points in the time domain after the time-frequency transformation. In this case, $S \equiv |X|^2$, where $X \in \mathbb{R}^{N_f \times N_t}$ is the magnitude of the discrete short-time Fourier transform (STFT) of x_{fil} , and the operator $|\cdot|$ represents the absolute value estimated individually from each element of the matrix. After this, the rows of the matrix S are averaged to obtain a vector $s \in \mathbb{R}^{N_t}$ with the mean value of all frequency bins for any specific point in the temporal domain (Figure 4B). This vector contains information related to the temporal location of each note in the recording. Subsequently, the standard deviation of s is used as a threshold criterion to estimate the beginning and ending of each note (red line in Figure 4B). Specifically, the new thresholded vector is defined as s_{th} , where each i -th element of s_{th} is obtained by the operation $s_{\text{th}i} = s_i \tau_i$; where $\tau \in \mathbb{R}^{N_t}$ is a $[0,1]$ bivalued vector, such that $\tau_i = 0$ if $s_i < \sigma_s$ and $\tau_i = 1$ otherwise, $\forall i = 1, \dots, N_t$. Here $\sigma_s \in \mathbb{R}$ is the standard deviation of s .

In the next step, the positions of all nonzero values of s_{th} are extracted, creating a new vector $p \in \mathbb{R}^{N_p}$ whose consecutive ordered pairs correspond with the beginning and ending of each note, i.e., $p = \{(p_1, p_2), (p_3, p_4), \dots, (p_{N_p-1}, p_{N_p})\}$. Here, $N_p = 2N_s$, and N_s is the number of notes. Each ordered pair is used to extract the notes directly from the spectrogram S . Consequently, a set of matrices $\mathbb{C} = \{C_1, C_2, \dots, C_{N_s}\}$ was obtained, where each element of \mathbb{C} is a matrix with the spectrogram of each note extracted directly from \mathbf{S} (Figure 4C).

Finally, the spectro-temporal parameters of each note are individually estimated (Figure 4D). This procedure is the same for all notes; therefore, for the sake of simplicity, the following part of the method will be solely demonstrated with an example note \mathbf{C} . Let $\mathbf{C} \in \mathbb{R}^{N_f \times N_c}$ be

a segment (note) directly extracted from the spectrogram, where N_c is the length of the note. Then, the mean spectrum $\mathbf{c} \in \mathbb{R}^{N_f}$ is defined as the arithmetic mean of all columns of the matrix \mathbf{C} . This vector contains the average values of \mathbf{C} in the frequency domain, which are used to estimate all the spectral parameters of the note.

Maximum and minimum frequencies were defined as the upper and lower cut-off frequencies of the mean spectrum of the note. Defining the cut-off frequencies as the maximum and minimum frequencies corresponding to the values of \mathbf{c} that exceed $\sigma_c/2$, where σ_c is the standard deviation of \mathbf{c} .

The centroid frequency (also known as spectral centroid) (Quan and Harris, 1997) f_c is analogous to the centre of mass in mechanical systems (Le et al., 2011). In general terms, it represents the frequency in which the centroid of the power spectral distribution is located (Eq. 1).

$$f_c = \frac{\sum_{i=1}^{N_f} f_i c_i}{\sum_{i=1}^{N_f} c_i} \quad (1)$$

Here, $\mathbf{f} \in \mathbb{R}^{N_f}$ is a vector with the frequency values of each bin in the spectrogram. f_c is obtained from a weighted mean and it is not based on the estimation of maxima points, which makes it robust to background noise and a more accurate predictor of the timbre of the sound than the dominant frequency (Schubert and Wolfe, 2006).

Temporal parameters were directly estimated from \mathbf{p} . Duration and INI were defined as $t_{\text{dur}} = p_{m-1} - p_{m-2}$ and $t_{\text{isi}} = p_m - p_{m-1}$, respectively; with $m = 3, 5, 7, \dots, N_p - 1$. The duration of each note is the difference between the two points in each ordered pair of \mathbf{p} , whereas the INI is the time between the ending and the beginning of two consecutive notes. These parameters are expressed in number of samples, but can be mapped to time using the following operation: $t = (t_{\text{samples}}(w - R) + R)/Fs$, where t is the temporal parameter in seconds, t_{samples} is the temporal parameter to be re-escalated, Fs is the sampling frequency of the original signal \mathbf{x} , w is the size of the window used to estimate the spectrogram, and R is the number of overlapping samples among windows.

Le PN, Ambikairajah E, Epps J, Sethu V, Choi EHC. 2011. Investigation of spectral centroid features for cognitive load classification, *Speech Communication* 53: 540-551.

Quan Y, Harris JM. 1997. Seismic attenuation tomography using the frequency shift method. *Geophysics* 62: 895-905.

Schubert E, Wolfe J. 2006. Does Timbral Brightness Scale with Frequency and Spectral Centroid? *Acta Acustica united with Acustica* 92: 820-825.

Supplementary Material 3 – Source separation

This method is used to separate notes emitted by two different bark beetle males acquired in the same recording. It consists of two main stages: (1) Estimation of the main component of variance from the spectrograms of recordings obtained with both main and secondary microphones. (2) A sequence of mathematical operations to find temporal locations of the notes of each male.

Let $\mathbf{S}_a \in \mathbb{R}^{N_f \times N_t}$ be the spectrogram of the signal acquired with the sensor pointing towards the individual of interest. Here we assume both spectrograms were estimated from synchronous signals of the same length. Then, a PCA is estimated from the spectrogram \mathbf{S}_a . This factors the spectrogram into two matrices $\mathbf{S}_a = \mathbf{Y}_a \mathbf{Z}_a$, where $\mathbf{Y}_a \in \mathbb{R}^{N_f \times N_z}$ are known as the principal component scores, $\mathbf{Z}_a \in \mathbb{R}^{N_z \times N_t}$ are the principal component coefficients (i.e., loadings), and N_z is the number of components. Afterwards, the transformation is reversed, but only using the component with the largest variance contribution, thus obtaining a matrix $\mathbf{S}'_a = |\mathbf{Y}_{a(i,1)} \mathbf{Z}_{a(1,j)}|$, $\forall i = 1, \dots, N_f$ and $\forall j = 1, \dots, N_t$; where the operator $|\cdot|$ represents the absolute value of each element of the matrix individually. Then, each column of $\mathbf{S}'_a \in \mathbb{R}^{N_f \times N_t}$ is thresholded around the median to clean the spectrum. This operation is performed by finding the values of each column of \mathbf{S}'_a less than \tilde{a}_j , if $S'_{a(i,j)} < \tilde{a}_j$, then $S'_{a(i,j)} = 0$; $\forall i = 1, \dots, N_f$ and $\forall j = 1, \dots, N_t$, where $\tilde{\mathbf{a}} \in \mathbb{R}^{N_t}$ is a vector that contains the median values of each column of \mathbf{S}'_a . In the next step, the arithmetic mean of all columns of the new spectrogram \mathbf{S}'_a is estimated, obtaining a vector $\mathbf{a} \in \mathbb{R}^{N_t}$ with the mean value of all frequency bins for any specific point in the temporal domain (Figure 5B). Then \mathbf{a} is feature-scaled in order to make it comparable with other vectors. The feature-scaling operation is defined as:

$$a_i' = \frac{a_i - \min(a)}{\max(a) - \min(a)} \quad (2)$$

where $\mathbf{a}' \in [0,1]$ is the rescaled representation of \mathbf{a} , $\forall i = 1, \dots, N_t$.

Now, let $\mathbf{S}_b \in \mathbb{R}^{N_f \times N_t}$ be the spectrogram of the signal acquired with the sensor pointing towards the other individual during the experiment (i.e., noise recording). In an equivalent

procedure to the previously shown with \mathbf{S}_a , the spectrogram \mathbf{S}_b is processed to obtain a vector $\mathbf{b}' \in \mathbb{R}^{N_t}$ (Figure 5C), analogous to the vector \mathbf{a}' estimated from \mathbf{S}_a . Finally, both vectors are subtracted, obtaining a vector $\mathbf{d} = \mathbf{a}' - \mathbf{b}'$ (blue line in Figure 5D), with $\mathbf{d} \in \mathbb{R}^{N_t}$. This vector is equivalent to the vector \mathbf{p} previously obtained (Figure 4B), and can be processed using the same method, as if it were a single-individual recording (see Automatic note detection and parameter estimation). The order in which the subtraction operation is performed gives the vector with the temporal locations of the individual of interest, hence, $\mathbf{d} = \mathbf{b}' - \mathbf{a}'$ gives information about the calls contained in \mathbf{S}_b (yellow line in Figure 5D). As the last step of the method is a subtraction operation, any external noise that affects both microphones in the same conditions is suppressed. Additionally, since the main component of variance contains essentially information from the source of interest, other sounds present in the recording are eliminated when the other components are neglected. However, this also makes the transformation irreversible, and a complete recovery of the original signal is not possible since this is an intended lossy compression technique (Goyal et al., 2008). In our case, this method is used to find the beginning and ending of the notes produced by the beetle of interest, but the spectro-temporal parameters are extracted directly from the original spectrogram. Therefore, there is no need to inverse the transformation to obtain the raw data. If total recovery of the original signal is desired, other methods such as Independent Component Analysis (ICA) (Hyvarinen et al., 2001) that guarantee independence of the sources are preferable.

Goyal VK, Fletcher AK, Rangan S. 2008. Compressive Sampling and Lossy Compression. IEEE Signal Processing Magazine 25: 48-56.

Hyvarinen A, Karhunen J, Oja E. 2001. Independent component analysis (1st ed.). New York, NY: Wiley.

Supplementary Material 4 – Probabilities of selecting a mute male

In this study, we used the distress call as sexing strategy to separate males from females. Since we found no female stridulatory sounds in any of the six studied behaviours, we decided to estimate the probabilities of obtaining these results due to the selected individuals all being mute males.

Let's assume an extremely unfeasible situation in which 20% of the males of the whole population were mute. In a set of n individuals with a 50/50 proportion of males and females, the probability of randomly selecting a mute male is given by the equation:

$$P(A \cap B) = P(A) * P(B/A)$$

Where $P(A) = 0.5$ is the probability of selecting a male, and $P(B/A) = 0.2$ is the probability of being mute given male.

$$\text{Thus: } P(A \cap B) = P(A) * P(B/A) = 0.5 * 0.2 = 0.1$$

Now, the probability of selecting an individual who does not stridulate (i.e. females or mute males) is given by:

$$P(\sim A \cup (A \cap B)) = P(\sim A) + P(A \cap B) = 0.5 + 0.1 = 0.6$$

Where $P(\sim A) = 1 - P(A)$ is the probability of not being male. Consequently, the probability of randomly selecting an individual who does not stridulate, and that is a mute male is given by:

$$P(C) = P((\sim A \cup (A \cap B)) \cap (A \cap B)) = P(\sim A \cup (A \cap B)) * P(A \cap B / (\sim A \cup (A \cap B))) = 0.6 * (1/6) = 0.1$$

Here we can see that in the scenario of 20% mute males in the collected population, if we use distress calls to differentiate males from females there is only a 10% chance of selecting a mute male from the pool of individuals and classifying him as a female during the sexing procedure. Now, let's estimate the probability of selecting 15 mute males from a population of size n . This can be done using the chain rule without replacement:

$$P(C_1 \cap C_2 \dots \cap C_{k=15}) = \prod_{k=1}^{15} \left(\frac{P(C) * n}{n - k - 1} \right)$$

Where $k=1, \dots, 15$ are the to-be-selected mute males and n is the number of total individuals. Let's suppose a set of $n=1000$ individuals, then:

$$P(C_1 \cap C_2 \dots \cap C_{k=15}) = \prod_{k=1}^{15} \left(\frac{0.1 \cdot 1000}{1000 - k - 1} \right) = 1.14 * 10^{-15}$$

In conclusion, a probability of $1.14 \cdot 10^{-15}$ is extremely small, which means that randomly selecting a subset of 15 mute males from a pool of 1000 individuals is technically impossible. Due to the previously shown calculations, we are confident that at least 13 of the 15 females we used in our study were not mute males.

We also performed an additional experiment to corroborate the results shown in our statistical analysis. First, we randomly selected 25 mute individuals and 25 individuals with sound production capabilities from one of our colonies. Then, we sexed them by inspecting the sexually dimorphic characteristics in the last two abdominal tergites, a method reported by Liu et al. (2008), which was verified by dissection of genitalia, with 100% accuracy separating males and females. Our results are reported in the following contingency table:

	Sound Production Capabilities		
Sex	<i>Sound</i>	<i>No Sound</i>	<i>Total</i>
<i>Male</i>	25	0	25
<i>Female</i>	0	25	25
<i>Total</i>	25	25	50

Using the method of inspecting abdominal tergites, all mute individuals were classified correctly as female, and all individuals that stridulated were classified correctly as males. This result unequivocally associates the lack or presence of distress calls in *Hylurgus ligniperda* with the sex of the individuals. This result, in conjunction with our statistical analysis, give us enough confidence to state that the sexing method we used in our experimental procedures did not have any influence in the acoustic data we are currently reporting in the manuscript.

Liu D, Flint ML, Seybold SJ. (2008) A secondary sexual character in the red-haired pine bark beetle, *Hylurgus ligniperda* Fabricius (Coleoptera: Scolytidae). *Pacific Entomologist*, 84, 26–28.

Supplementary Material 5 – *Principal Component Analysis*

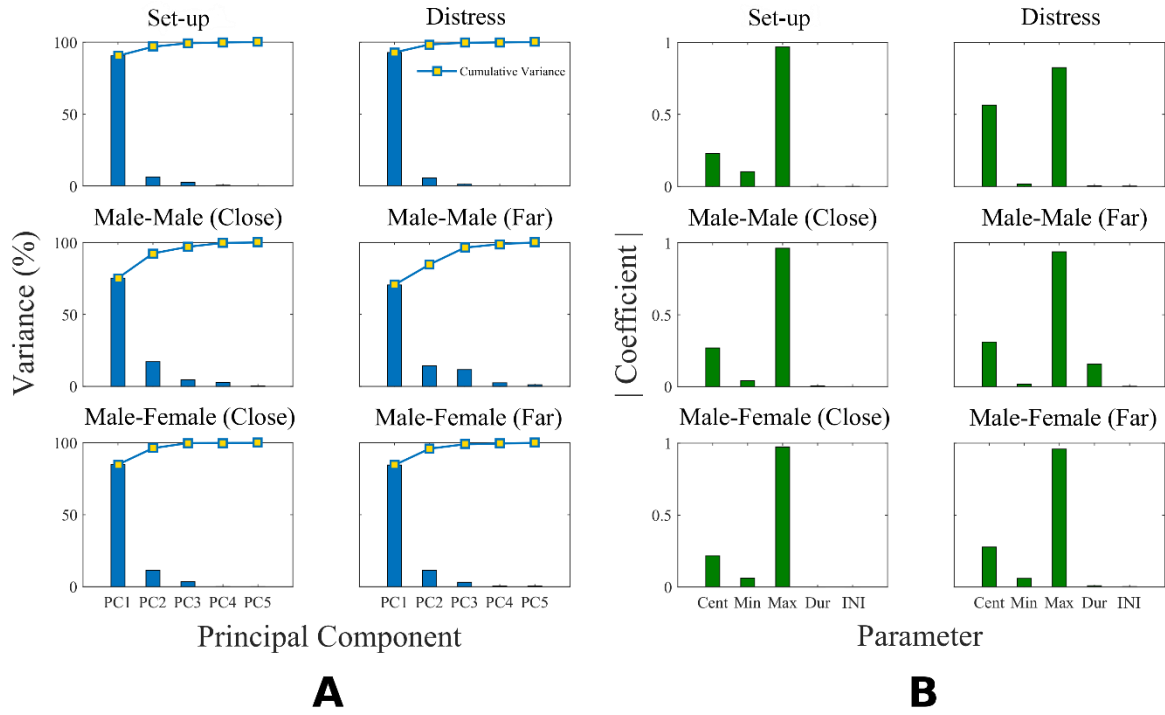


Figure S2. Principal Component Analysis of the five spectro-temporal parameters extracted from the notes of *Hylurgus ligniperda* calls emitted in six behavioural contexts. **(A)** Percentage of variance explained by each principal component. **(B)** Absolute value of the coefficients of each parameter for the first principal component (PC1).

Supplementary Material 6 – *Effects of the uppermost Perspex layer on data collection*

An additional experiment was performed to evaluate the effect of the Perspex layer on the data acquisition. Phloem sandwiches are commonly used in bark and ambrosia beetle studies, yet the effects of these on acoustic data collection have not been numerically determined. The experiment consisted of creating a sinusoidal signal with a linearly increasing frequency (also known as linear chirp) and constant amplitude, and then, recording that signal in our set-ups with and without the uppermost Perspex layer. This is an objective method to estimate the effects of the Perspex layer (with a 2 mm hole) on the spectral values. To accomplish this, a 60s sinusoid with an upward linear variation of its instant frequency from 400 Hz to 20000 Hz was created (Figure S3). Then, a circular (4mm radius) transducer generated the signal, which was instantly acquired using the same setup we used for data collection.

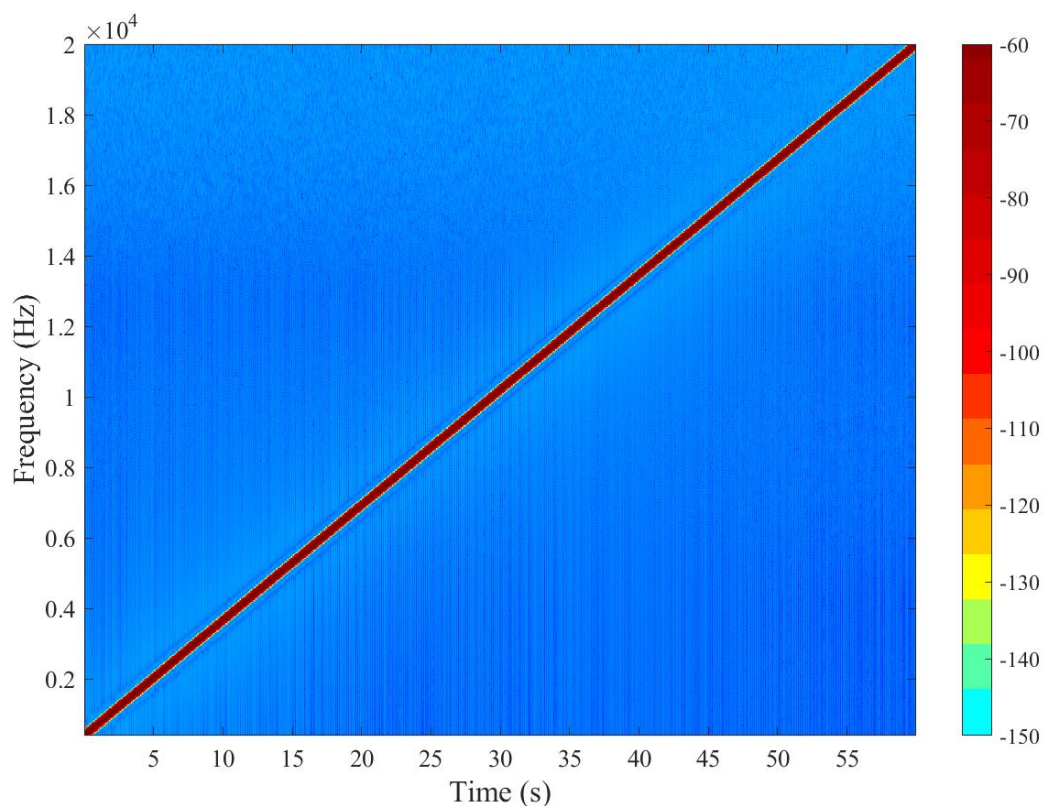


Figure S3. Linear Chirp. Sinusoid of 60s duration with an upward linear variation of its instant frequency from 400 Hz to 20000 Hz.

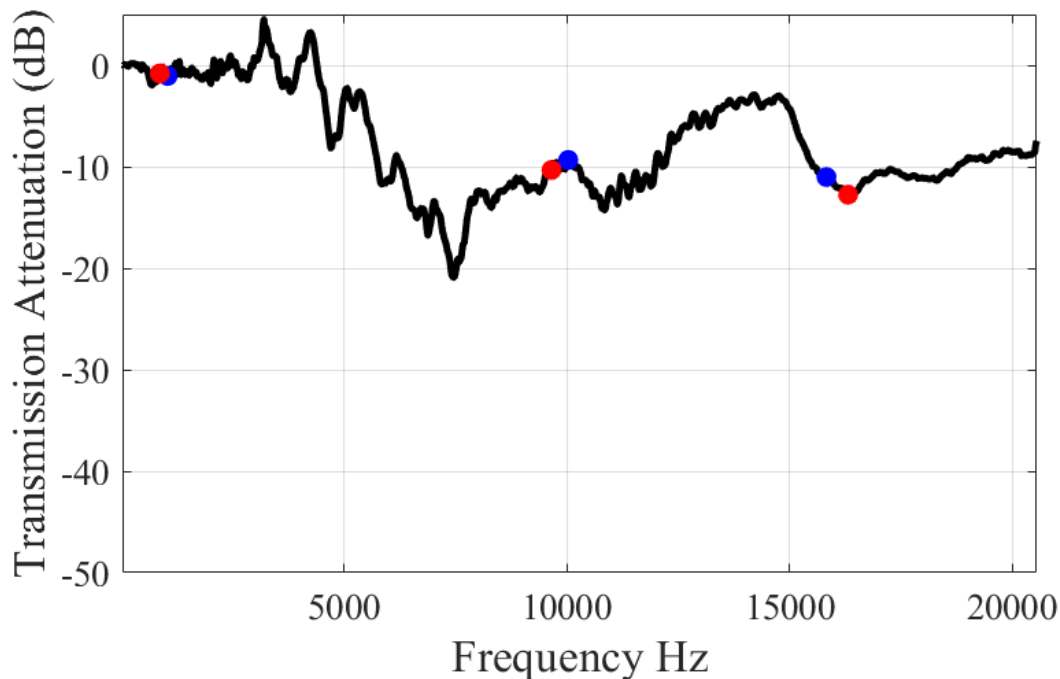


Figure S4. Transmission attenuation of the uppermost Perspex layer of the set-up. Points indicate the estimated spectral features: minimum, centroid, and maximum frequencies with (blue) and without (red) Perspex. Note that the values of the spectral features estimated from the signal recorded with Perspex closely match the values of the signal recorded without Perspex.

Since the features we extracted in our study are not amplitude-based, the changes produced by the Perspex have a very little effect on the currently reported data. To show this, we analysed the power spectral distribution of both signals acquired with and without Perspex exactly as the beetle calls in the manuscript were analysed (see Supplementary Material 2). The minimum, maximum, and centroid frequencies only differed by 171, 472, 364 Hz, (i.e., by approximately 0.9, 2.4, and 1.9 % of the signal bandwidth) respectively, when comparing set-ups with and without Perspex (Figure S4). Additionally, we also modelled the transfer function of the Perspex (Figure S4, black line) and inverse-filtered the measured signals by the transmission response of the Perspex; we found little difference in the acoustic features. We acknowledge that the Perspex layer in the set-up slightly changes the estimation of the spectral features; nonetheless, the variation is small (< 500 Hz or 2.5 % of the bandwidth) and the signals were all recorded under the same conditions. Therefore, this small variation

does not change the general conclusions of our study. In general, the Perspex layer attenuates the amplitude of the spectrum ~ 7 dB in average, but do not remove spectral content. This is the reason why the values of the estimated spectral features are comparable in both situations. In addition, the placement of the microphone on the uppermost Perspex layer had little to no effect on the data collection. In our experiment, the only part of the microphone that touched the Perspex was a very small area of its external case. The goal was to create a mechanical contact for stability; thus, all the recordings could be made from the same exact point. In Figure S5, we show the spectrograms of the same signal from Figure S3 recorded with and without touching the uppermost Perspex layer. There are barely perceptible variations of amplitude and no frequency shift, confirming that our microphone placement did not alter the recordings in any significant way.

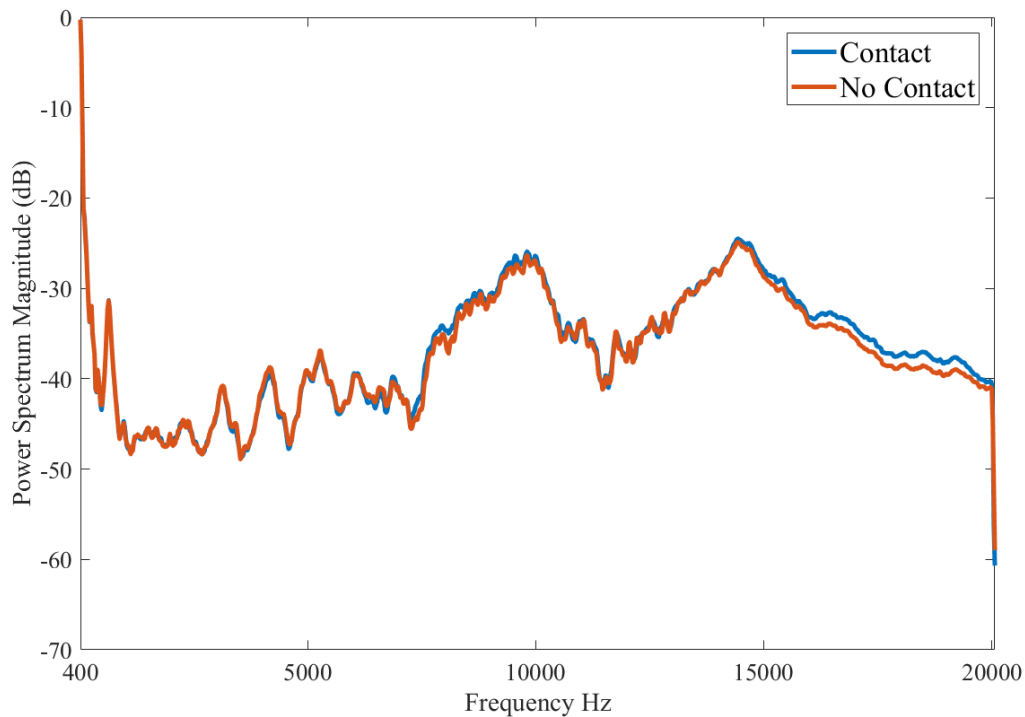


Figure S5. Power spectrum of signals recorded with the microphone in contact/ no contact with the uppermost Perspex layer.

Supplementary Material 7 – *Hylurgus ligniperda* recording

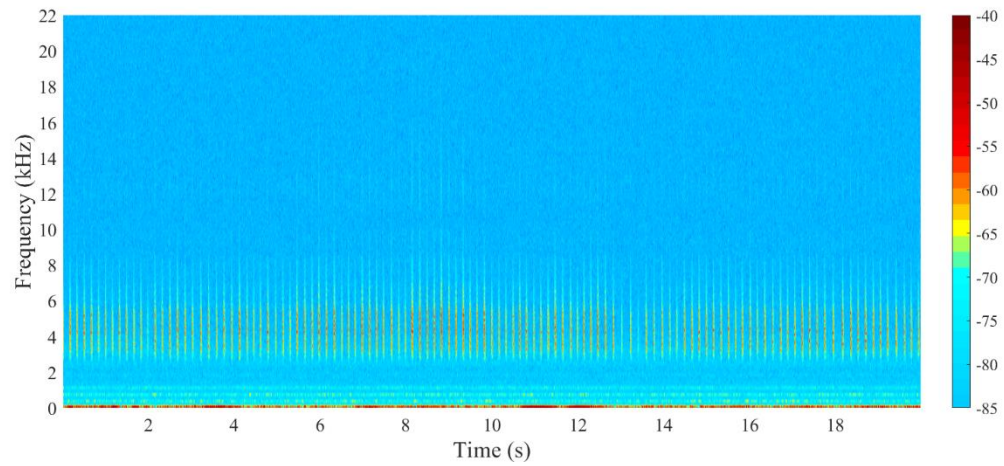


Figure S6. Unfiltered (raw) recording of a male individual of *Hylurgus ligniperda*. This plot shows the global structure of the signal and the spectral location of background noise (< 1.5 kHz). A corresponding sound file is accessible in the supplementary material.