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Characterization of stridulatory structures and sounds of the larger Mexican pine beetle, *Dendroctonus approximatus* (Coleoptera: Curculionidae: Scolytinae)

*Kasey M. Yturralde** *and Richard W. Hofstetter*

Abstract

We report the first description and analysis of acoustic signals and structures in the larger Mexican pine beetle, *Dendroctonus approximatus* (Coleoptera: Curculionidae: Scolytinae).We recorded acoustic signals during two behavioral contexts, disturbance and male-female interactions. The relationship between size and sound production was assessed by comparing temporal and spectral chirp characteristics with body size and sound-producing organs. During male-female interactions, males first produce the interrupted chirp, a relatively long chirp (x = 214.36 ± 5 ms) interspersed with periods of silence. Once inside female galleries, males switch to a simple chirp. Females produce chirps in response to male acoustic signals and disturbances. For male chirps produced in response to disturbance, frequency was positively correlated with the density of ridges on stridulatory structures and inversely correlated with beetle size characteristics. Small males were more likely to produce high frequency chirps when disturbed, compared with larger males. These results indicate that acoustic signals may be limited by morphology and size in bark beetles.

Key Words: acoustic communication, body size, stridulatory organ, bark beetle

Resumen

Presentamos la primera descripción y análisis de señales acústicas y estructuras en del scarabajo gigante mexicano del pino, *Dendroctonus approximatus* (Coleoptera: Curculionidae: Scolytinae). Se registraron las señales acústicas durante 2 contextos de comportamiento, la perturbación y el apareamiento. Se evaluó la relación entre el tamaño y la producción de sonido mediante la comparación de las características temporales y espectrales de los chirridos con el tamaño del cuerpo y de los órganos que producen sonidos. Durante la interacciones macho-hembra, los machos primero producen chirridos relativamente largos y complejos (x = 214.36 ± 5 ms) intercalados con periodos de silencio, el canto interrumpido. Dentro de las galerías de las hembras, los machos pronto cambian a un chirrido sencillo. Las hembras producen chirridos en respuesta a señales acústicas de los machos, así como a las perturbaciones. Para los chirridos de los machos producidos en respuesta a la perturbación, la frecuencia se correlacionó positivamente con la densidad de las rugosidades en las estructuras estridulatorias e inversamente correlacionada con las características de tamaño del escarabajo. Los machos pequeños tenían más probabilidades de producir chirridos de alta frecuencia cuando se les molesta, en comparación con los machos más grandes. Estos resultados indican que las señales acústicas pueden estar limitadas por la morfología y el tamaño de los escarabajos de corteza.

Palabras Clave: comunicación acústica, tamaño corporal, órgano estridulador, escarabajo de la corteza

Bark beetles (Coleoptera: Scolytinae) produce acoustic signals via stridulation that function in pair formation (Barr 1969; Ryker & Rudinsky 1976b), male-male competition (Rudinsky & Michael 1974; Rudinsky & Ryker 1976), release from predators (Lewis & Cane 1990) and the potential mediation of intraspecific competition among females (Rudinsky & Michael 1973). The acoustic signals and structures of many *Dendroctonus* bark beetle species were described in a series of papers by Rudinsky and colleagues in the early 1970s (Rudinsky 1969; Rudinsky & Michael 1972, 1973; Rudinsky et al. 1973). Yet, none of these studies investigated the relationship between acoustic signals and the underlying morphology in bark beetle species. Understanding how acoustic signals vary with acoustic structures can reveal the potential for sexual and natural selection pressures on morphology. Indeed, acoustic signals in bark beetles have likely been shaped by selection pressures through predator avoidance (Lewis & Cane 1990) and mate acceptance (Ryker & Rudinsky 1976b).

Bark beetles, like many other insects, are subject to limitations of sound production due to their small size (Bennet-Clark 1975, 1998). Among cicadas and crickets, small sized species produce higher frequencies compared to larger species (Bennet-Clark 1998). Insects often have difficulty producing sound that propagates over long distances efficiently, due to very small sound-producing structures (Bennet-Clark 1998). Typically when the diameter of a sound-producing structure is smaller than the signal wavelength, efficient sound production is highly challenging (Bradbury & Vehrencamp 1998). The relationship between insect body size and sound production has been demonstrated across species and taxa, as reviewed by Bennet-Clark (1998), but less so within species.

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We investigated variation in acoustic structures and signals in the larger Mexican pine beetle, *Dendroctonus approximatus* Dietz in order to better understand how acoustic signals may be constrained by size in bark beetles. This is the first description of stridulatory structures, acoustic signals and associated behaviors in *D. approximatus*, as it was excluded from earlier studies of bark beetle acoustics (Fleming et al. 2013). As one of the largest bark beetle species, *D. approximatus* is ideally suited for investigation of body size and acoustic signals as the majority of *Dendroctonus* spp. studied to date have focused on small to medium sized species (Wood 1982). In addition, *D. approximatus* exhibits considerable variation in body size (Wood 1982) and stridulates in response to disturbance. Chirps produced in a disturbance context are common across bark beetle species (Barr 1969; Michael & Rudinsky 1972) and easily recorded under controlled conditions.

Dendroctonus approximatus is distributed in Central and North America, ranging from Honduras and north into Utah and Colorado (Wood 1982). Like most other bark beetles, *D. approximatus* does not actively kill trees, but rather infests down trees or weakened trees already experiencing attacks initiated by primary bark beetle species (Wood 1982; Cibrián Tovar et al. 1995). Their flight period spans June into October and beetles emerge gradually from trees such that beetle abundance is relatively low (Furniss & Carolin 1977; Wood 1982). Acoustic structures and signals of *D. approximatus* were not investigated in previous reviews of sound production in bark beetles (Ryker 1988).

Here we provide a description of stridulatory structures and acoustic signals recorded during two different behavioral contexts, disturbance and encounters between male and female *D. approximatus*. We characterized the relationship between acoustic structures, overall body size, and acoustic signal characteristics. Temporal and spectral characteristics such as chirp duration, pulse rate, and peak frequency are reported. Ultimately, this analysis will contribute to a broader knowledge of how bark beetles use acoustic communication and allow comprehensive comparisons across bark beetle species.

Materials and Methods

SPECIMEN COLLECTION AND STORAGE

Specimens were collected between Jun and Sep in 2011 and 2012 from pheromone-baited traps located 15 km west of Flagstaff, Arizona, USA (35° 10′ N, 111° 45′ E; 2,080 m asl) in the Northern Arizona University/Arizona State Lands Centennial Forest and additionally from tornado-damaged trees in Coconino National Forest (34° 50′ N, 111° 23′ W; 2,300 m asl). Lindgren funnel traps were baited with western pine beetle lures (Synergy Semiochemicals Corp.) releasing frontalin (~5.2 mg/d), *exo*-brevicomin (~1.7 mg/d) and α-pinene (~150 mg/d). Species and sexual identification of *D. approximatus* specimens was conducted according to Wood (1982). Voucher specimens are stored at the Forest Entomology laboratory of R. Hofstetter in School of Forestry, Northern Arizona University.

We collected phloem tissue from recently felled Ponderosa pine (*Pinus ponderosae*) in Centennial Forest approximately every 3 weeks between Jun and Sep in 2011 and 2012. In the field, phloem was immediately placed in vacuum-sealed plastic bags and later stored in growth chambers at 4 °C. Phloem was necessary for construction of phloem slides [shown in Fig. 1, (Aflitto et al. 2014)] used to house bark beetles for acoustic recordings and behavioral observations.

ACOUSTIC TERMINOLOGY

Throughout the study we refer to acoustic signals produced by *D. approximatus* as chirps and defined based on temporal characteristics and associated context. Similar to Fleming et al. (2013), we follow the basic terminology of Michael & Rudinsky (1972) and refer to chirps as interrupted or simple. Simple chirps consist of a series of continuously produced sound pulses, whereas interrupted chirps include periods of silence. In addition to chirp type, we refer to the context within which chirps were recorded.

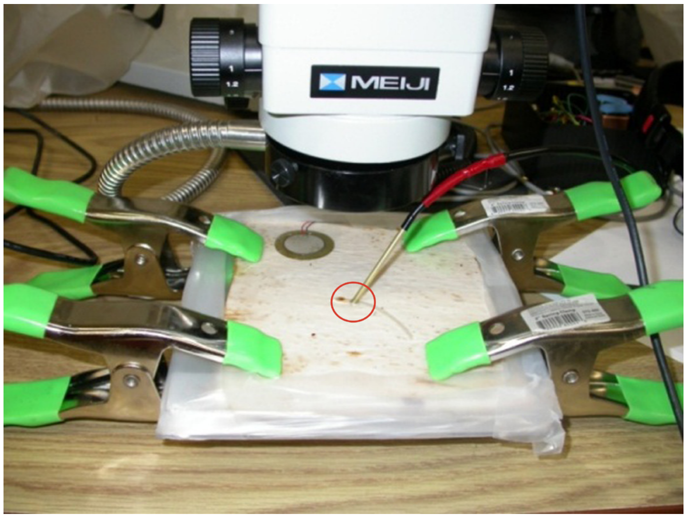

Fig. 1. Phloem slide positioned under dissecting scope. The ultrasonic electronic insertion microphone is shown positioned in the center of the slide (see circle), inserted into the tunnel entrance. A small piezo transducer, shown at the far end of the phloem slide, was not used in recordings for this study.

ACOUSTIC RECORDING AND SETUP

Chirps produced by male and female *D. approximatus* were recorded in similar behavioral contexts in which previous studies have reported acoustic communication for *Dendroctonus*, disturbance and male-female interactions. Chirps produced by male beetles in a disturbance context were recorded within 24-48 h of collection and these beetles were then maintained in growth chambers until a female was collected. The disturbance context was elicited by gently holding the male (or female) by the head and pronotum to allow for full range of movement of the elytral-abdominal structures. Out of eleven collected females, seven females produced chirps in a disturbance context that were of sufficient quality for acoustic analyses. A smaller subset of these females produced chirps in response to males. Thirty-one males produced chirps in a disturbance context, and 10 were successfully paired with females and produced chirps in a male-female interaction context. Of these 10 pairs, 5 females and 6 males produced a sufficient number of chirps for acoustic analysis.

Acoustic recordings of male-female interactions were conducted using beetle pairs in phloem slides (Fig. 1), which simulate male-female interactions that occur naturally within host trees, see Hofstetter et al. (2013) and Aflitto et al. (2014) for a description of the phloem slide. All male-female interactions were observed during recording, allowing for detailed annotations and identification of male and female chirps. Within 24-48 h of collection, a female was introduced to a phloem slide and upon signs of successful tunneling, a male was introduced. Male chirps were recorded from within 1-2 mm of the male elytral declivity. As the male entered the tunnel leading to the female gallery, he was continually recorded with the microphone placed into the tunnel entrance. From this point on for a beetle pair, recordings were made from the tunnel entrance, regardless of the proximity of either beetle. Thirty-second recordings were made continually for approximately 30 min from introduction of the male beetle. Recordings were annotated during observations such that chirps were identified as belonging to either the male or female beetle. During the male-female context females were paired with only one male. Individual beetles were used only once during recordings made in the male-female context.

Because the distance from the microphone to sound source could not be controlled during male-female interactions, spectral and temporal characteristics of chirps made in the male-female interaction context were not included in statistical tests. It is important to note that most recordings were made within 3 cm of chirping beetles and so recordings would have occurred within the near field. Subsequently, the frequencies we report might not accurately reflect the true frequency of each chirp, but do represent realistic distances within which bark beetles communicate with each other.

All recordings were made with an ultrasonic electronic insertion microphone developed by Dunn (2004). The ultrasonic insertion microphone was constructed using a Knowles Acoustics FG-3329 electret condenser microphone (Knowles Electronics, Itasca, Illinois), which is sensitive to frequencies between 10 Hz and 10 kHz at -53 dBV/0.1Pa and linearly declines above 10 kHz such that at 48 kHz sensitivity is approximately -70 dBV/0.1Pa (Knowles Electronics 2005, 2013). However, this microphone is reported to detect frequencies in excess of 100 kHz (Fujioka et al. 2011). Acoustic signals were recorded using an HD-P2 TASCAM digital audio recorder at 96 kHz and 24 bit sampling rate.

MORPHOLOGICAL MEASUREMENTS

Beetles were prepared and cleaned for the scanning electron microscope using increasing serial dilutions of ethanol. Specimens were sputter coated with gold for 120 s using a DESK II Dentron Vacuum unit. Specimens were examined using a LEO 435VP scanning electron microscope (SEM) and associated LEO software. Elytra length and thorax width were measured as an estimate of body length.

Stridulatory structures from SEM images were measured digitally using ImageJ 1.45s image processing software (Rasband 2012). Females exhibited poorly developed stridulatory structures, such that some females possessed little to no file making repeatable measurements impractical. Therefore, all stridulatory structure measurements refer only to male structures. The file was measured from the midpoint of the most posterior ridge (lower arrow, Fig. 2) to the midpoint of the final anterior ridge (upper arrow, Fig. 2). The final anterior ridge was defined as the most anterior ridge after which the ridges were significantly reduced in width (less than 2/3 of the previous ridge) and/or beginning to angle away from the previous ridges (see Fig. 2). The plectrum width was measured from the midpoint of each lobe. The total number of ridges on the file was counted for each file and also considered for the upper and lower half of each file, per Michael & Rudinsky (1972).

ANALYSIS OF ACOUSTIC OF ACOUSTIC REPERTOIRE AND CHIRPS

Recordings were analyzed using spectrograms and waveforms produced in Raven Pro (Version 1.4) Interactive Sound Analysis Software (www.birds.cornell.edu/raven). Spectrograms were produced using the following parameters: Hann window with a 698 sample size, 140 Hop size and 80 % overlap. All recordings were bandpass filtered between 1 and 48 kHz to exclude white noise at frequencies below 1 kHz that could not be controlled during recordings.

Individual chirps were identified using the band limited energy detector in Raven Pro. Detectors were specialized for each chirp type, simple and interrupted, produced by males and females. Preset band limited energy detectors were configured following the guidelines in the Raven Pro 1.4 User's Manual (Charif et al. 2010) and were based on the duration and frequency range of each chirp, as well as the time intervals between chirps (Table 1). Chirps were then sampled randomly using randomly generated numbers in Excel. In the disturbance context, a subset of twenty chirps was selected for males. For all other chirps, a subset of ten chirps was chosen.

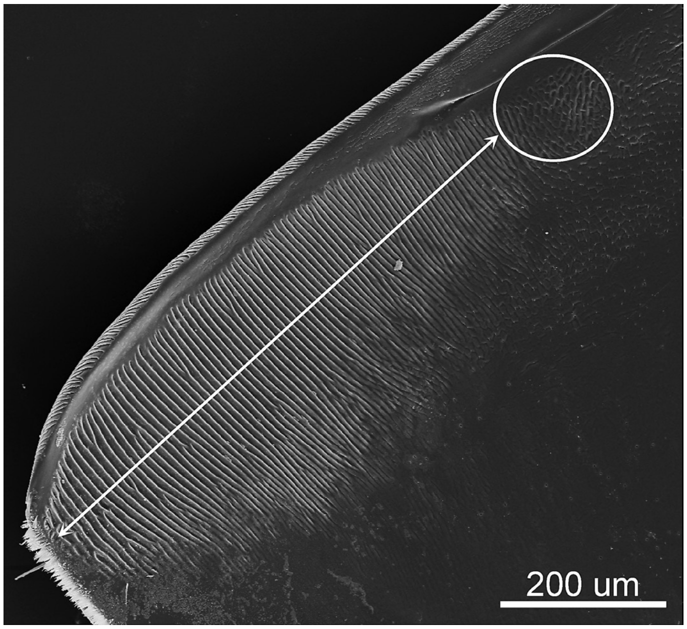

Fig. 2. File length was measured posteriorly from the most posterior ridge (see lower arrow) up anteriorly to the last well-developed ridge (top arrow). Ridges at the anterior edge become poorly developed (circle) and these were not measured. See Methods for further description.

Measurements reported here are peak frequency, median frequency, chirp duration, number of pulses within a chirp or syllable, pulse rate (pulses/s), and number of syllables per chirp (Fig. 3). Pulse refers to an individual sound pulse, while a train of sound pulses is referred to as a chirp. Interrupted chirps consist of several repeated short trains of sound pulses (Ryker & Rudinsky 1976b), hereafter referred to as syllables. Syllables are approximately 20-30% of the duration of simple chirps produced by males. Reported spectral characteristics, such as peak and median frequency, were calculated in Raven Pro (Version 1.4). Peak frequency is the frequency within a pulse train with the maximum power. Median frequency is the frequency about which equal amounts of energy lie above and below. Spectral profiles were constructed using the spectrogram slice view at the center of each pulse train, except for the female chirps. The center time of each pulse train is the time at which energy is divided equally within the pulse train.

DATA ANALYSIS

All statistical analyses were performed in JMP® 9.0.2 (SAS Institute Inc., Cary, NC). Summary statistics were utilized to characterize the range of calls produced by males and females. Analysis of the relationship between body size, stridulatory structures, and chirp characteristics was conducted using multivariate pairwise correlations function. Multiple regressions using stepwise backward elimination were utilized to identify predictor variables that accounted for the variation in response variables, such as chirp characteristics.

Table 1. Target signal and signal-to-noise (SNR) ratio parameters of energy detectors used to identify chirps.

		Target signal parameters					Signal-to-noise ratio parameters				
Sex	Chirp type	Min. freq. (kHz)	Max. freq. (kHz)	Min. duration (ms)	Max. duration (ms)	Min. separation (ms)	Min. occupancy[a]	SNR threshold (dB)	Block size (ms)	Hop size (ms)	Percentile
Male	simple	0.5	48.0	39.4	199.8	20.4	80.0	10.0	1,000.4	500.2	20.0
	interrupted	0.4	43.0	10.2	49.6	8.8	40.0	5.0	350.0	199.8	20.0
Female	Simple	0.4	43.0	70.0	109.4	49.6	30.0	5.0	399.6	199.8	20.0

[a]Minimum occupancy refers to the proportion of a sample in a selection that is required to exceed the background noise, as defined by the SNR threshold.

Results

ACCOUSTIC REPERTOIRE OF *D. APPROXIMATUS*

Dendroctonus approximatus males produced simple and interrupted chirps, while females produced only simple chirps (Table 2). Both male and female *D. approximatus* produce chirps when handled, simulating disturbance (Figs. 4 and 5). Males employ both simple and interrupted chirps during interactions with females (Figs. 6 and 7). As males enter a female gallery, they produce interrupted chirps (Fig. 6) and soon switch to simple chirps inside the gallery (Fig. 7), though some may alternate between the two different chirps. Females responded

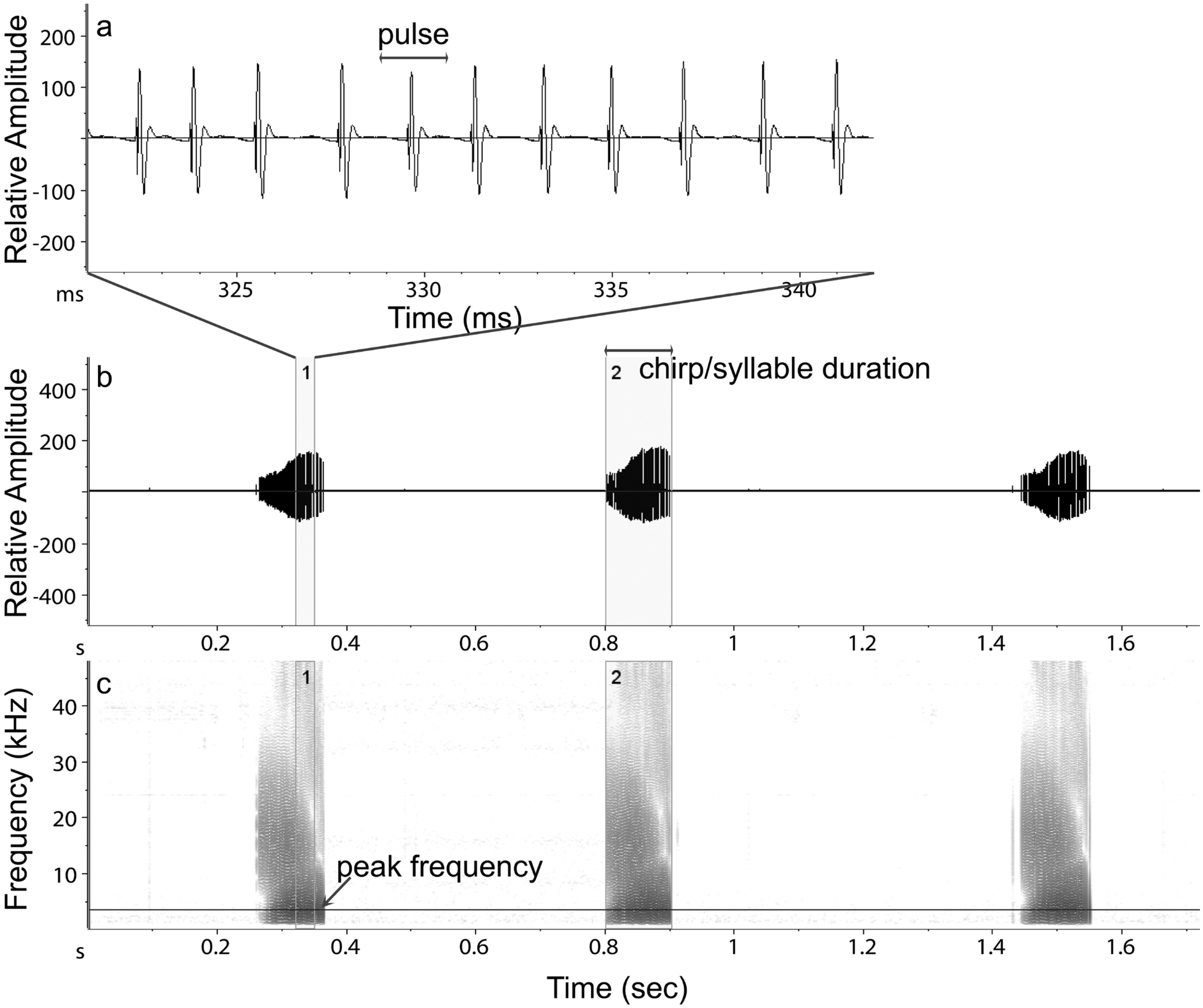

Fig. 3. Temporal and spectral characteristics assessed for simple chirps made in a disturbance context. Train of individual pulses a), waveform b), and spectrogram c) from sample chirp produced by male in a disturbance context. The peak frequency (3.468 kHz) is noted for the first recorded chirp (occurring at 0.3 s).

Table 2. Spectral and temporal characteristics of male and female simple chirps (mean ± SE).

Sex	Context	N	n	Duration (ms)	Peak frequency (Hz)	Median frequency (Hz)	Mean number of pulses	Pulse rate (pulses/s)
male	disturbance	31	20[a]	108.6 ± 1.2	5293.2 ± 110.6	6972.2 ± 117.9	55.1 ± 0.6	517.6 ± 23.0
	range			29.7 - 235.0	1218.8 - 17812.5	1968.8 - 16500.0	11 - 87	171.1 - 757.4
male	male-female	6	10[b]	124.5 ± 2.7	5685.9 ± 123.9	6116.8 ± 85.5	61.7 ± 1.2	505.7 ± 12.0
	range			86.0 - 168.0	3843.8 - 7593.8	5250.0 - 7968.8	44.0 - 84.0	320.8 - 678.3
female	disturbance	7	10	81.4 ± 3.6	11347.8 ± 352.4	13023.2 ± 414.9	12.6 ± 0.3	167.8 ± 6.0
	range			30.6 -172.1	3000.0 - 15843.8	6562.5 - 22031.2	5.0 - 18.0	64.5 - 359.1
female	male-female	5	10	88.2 ± 1.6	9335.6 ± 835.1	11613.8 ± 605.9	12.6 ± 0.3	143.1 ± 3.5
	range			70.0 - 0116.6	2062.5 - 18187.5	3468.8 - 17437.5	8.0 - 17.0	90.81 - 201.7

[a] A subset of males had a limited number of chirps for analysis, for these males n = 10, 13, 14, 18 and subsequently the total sample size was 595 chirps.

[b] One male only had 7 chirps, subsequently the total sample size was 57 chirps.

acoustically to male interrupted chirps with a simple chirp (see second and fourth chirps in Fig. 7). Female chirps produced in response to males were made at regular intervals of every 0.5 to 0.6 s. Many recordings included both the male interrupted chirp and the female simple chirp, making analysis challenging as chirps often overlapped temporally (Fig 8).

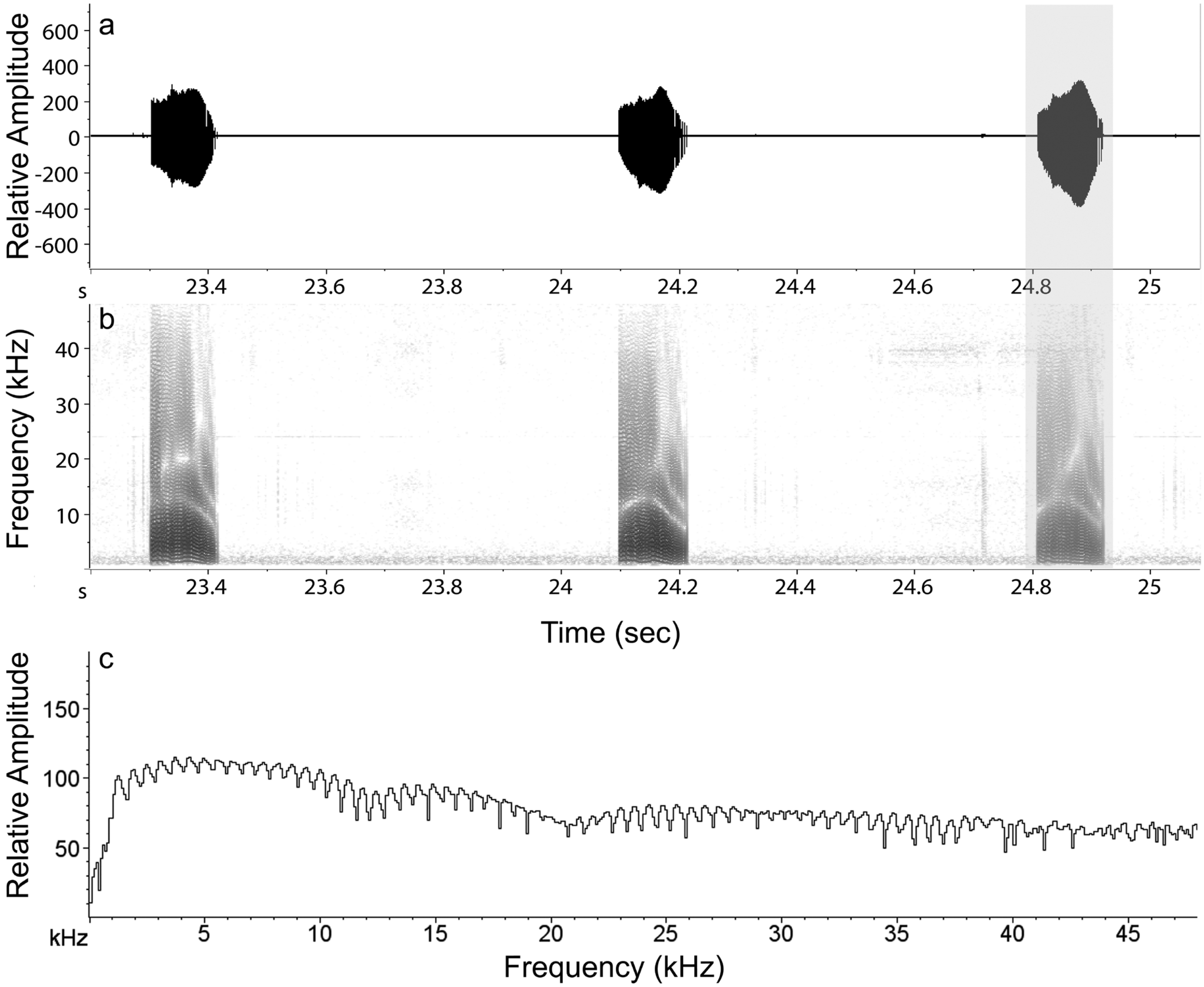

Fig. 4. Waveform a), spectrogram b), and spectral profile c) of male simple chirp in a disturbance context. The waveform shows the relative amplitude in generic units and the spectrogram shows frequency over time with darker shades indicating higher relative energy. The spectral profile was taken at the midpoint of the first chirp, highlighted in a and b.

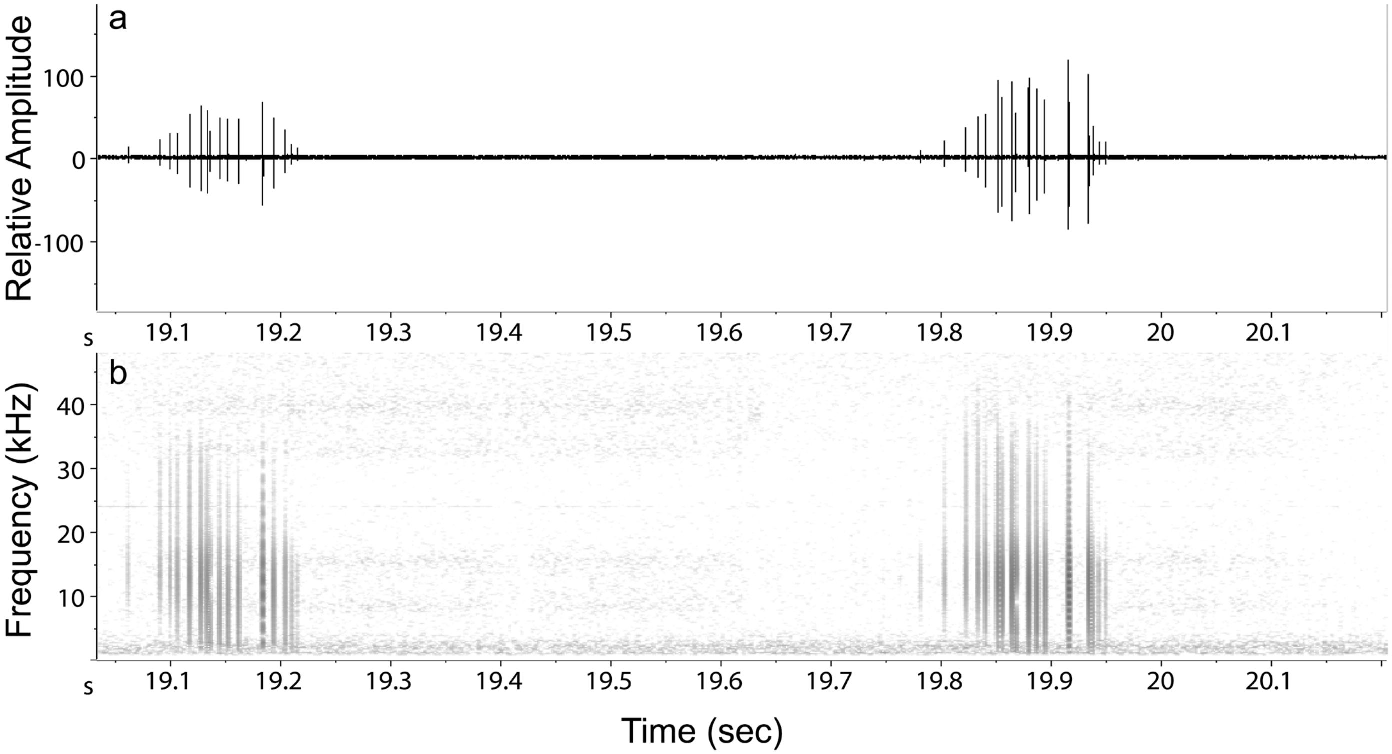

Fig. 5. Waveform a), and spectrogram b) of female simple chirp in a disturbance context. The waveform shows the relative amplitude in generic units and the spectrogram shows frequency over time with darker shades indicating higher relative energy.

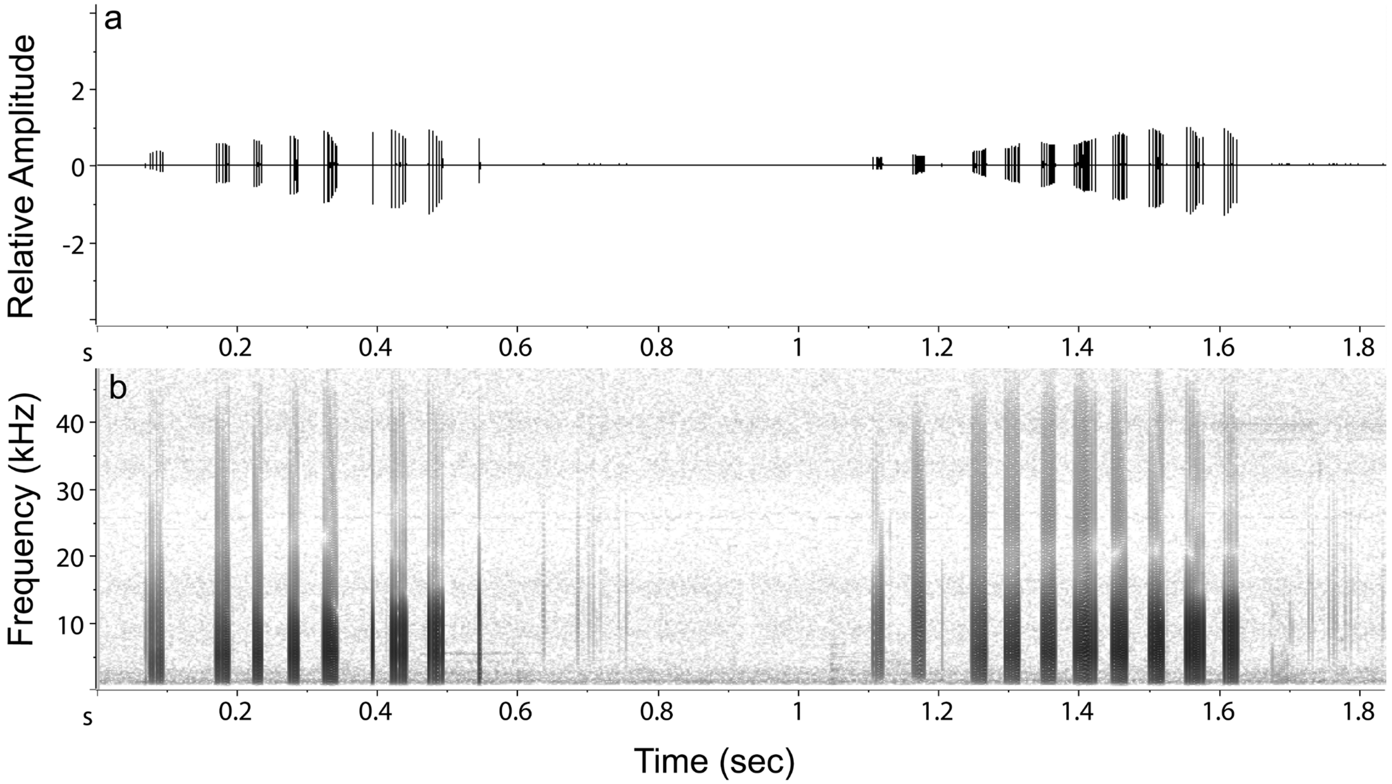

Fig. 6. Waveform a), and spectrogram b) of a male interrupted chirp showing individual syllables within each chirp.

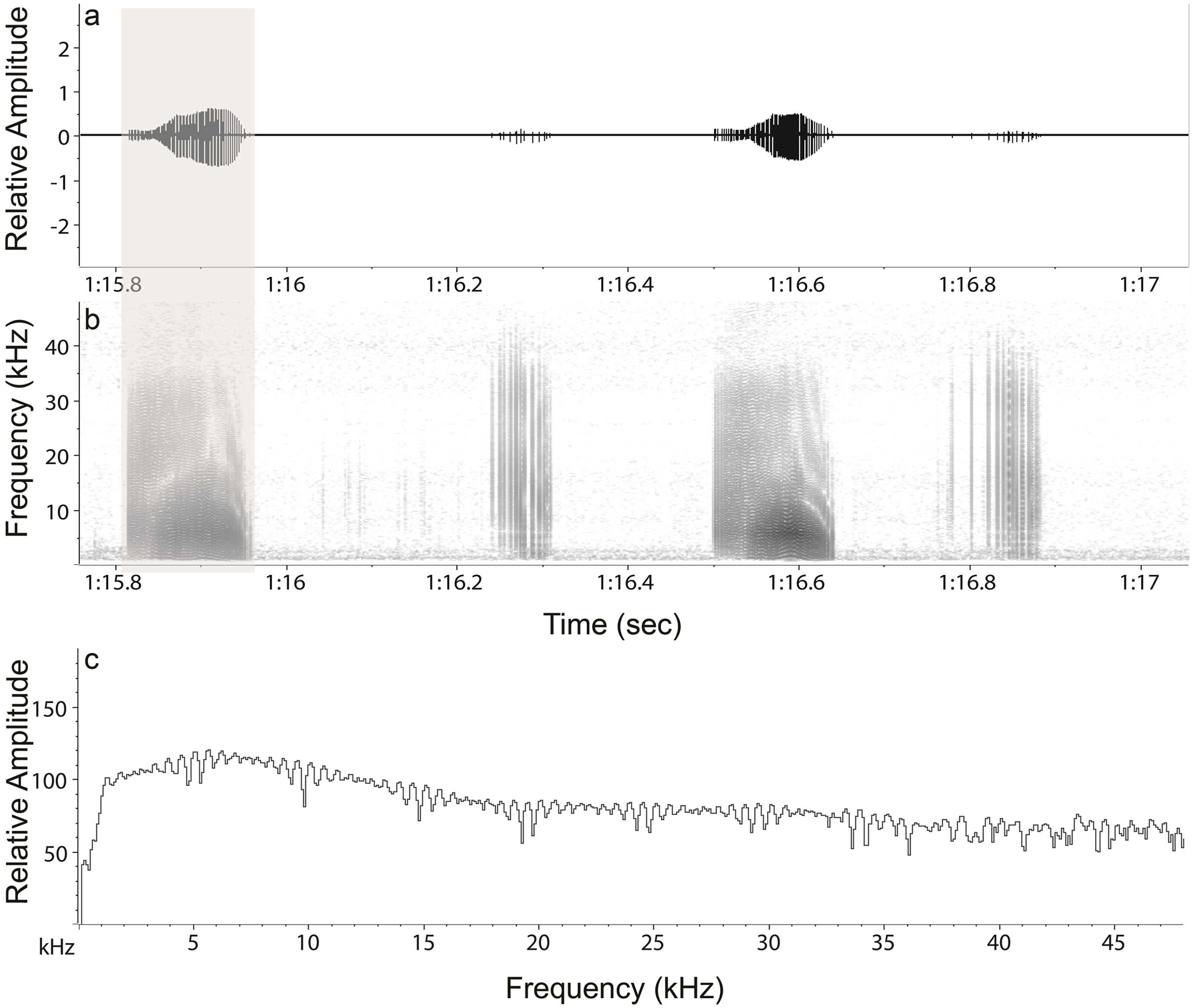

Fig. 7. Waveform a) and spectrogram b) of simple chirps produced by a male (first and third chirps) and female (second and fourth chirps) when paired together in gallery. The spectral profile c) was taken at the center time of the first chirp, highlighted in a and b. Center time is the point during a sample about which energy is divided equally.

Simple Chirps

Male and female *D. approximatus* produced simple chirps in response to disturbance. The duration of male simple chirps produced in response to disturbance ranged from 30-235 ms with a mean duration of 108.6 ms (Table 2). Male chirps had an average peak frequency of 5293 Hz (Table 2). Female chirps differed from males in their duration, having fewer individual pulses per chirp with a mean duration of 81.4 ms (Table 2). Females produced chirps with higher median and peak frequencies (11348 Hz) compared to male chirps.

Males produced simple chirps in response to females within galleries, often subsequent to interrupted chirps and females responded acoustically as well (Table 2). Male simple chirps had a mean duration of 124.5 ms with a mean peak and median frequency of 5686 and 6117 Hz, respectively. Females responded to males acoustically with simple chirps with a mean duration of 88.2 ms. The mean peak and median frequencies of female simple chirps were quite variable, possibly due to the distance between females and the microphone or distortion of sound traveling through tunnels in the gallery (Table 2).

Interrupted Chirps

When males initially began tunneling into a female gallery, they gave long chirps made up of multiple syllables. The mean duration of interrupted chirps was 214.36 ± 5.03 ms with a mean number of syllables and pulses per chirp of 7.93 ± 0.15 and 59.34 ± 1.49, respectively. We summarized spectral and temporal characteristics for the first 3 syllables (first syllables), syllables 4-7 (intermediate syllables) and syllables 8-11 (last syllables). Peak and median frequencies decreased from the first to last syllables (Table 3). Syllable duration, number of pulses per syllable, and pulse rate were greatest for the intermediate syllables and least for the last syllables (Table 3).

STRIDULATORY STRUCTURES, BODY SIZE AND CHIRP CHARACTERISTICS

The shape of stridulatory structures in male *D. approximatus* is consistent with other *Dendroctonus* spp. (Michael & Rudinsky 1972; Ryker & Rudinsky 1976a), consisting of a file on the underside of the

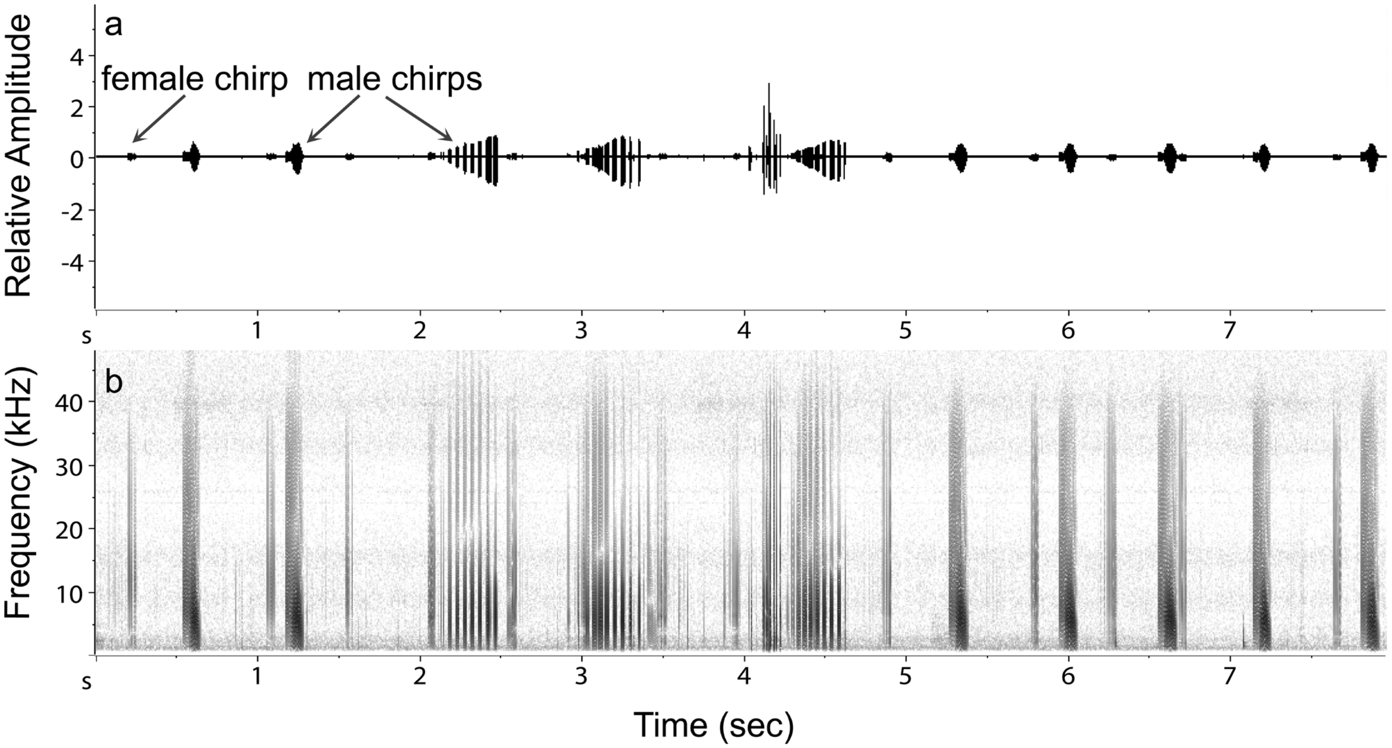

Fig. 8. Waveform a) and spectrogram b) of overlapping chirps produced by female and male *D. approximatus*. The first chirp in the sequence was produced by the female and is repeated approximately every 0.5 s. The female chirp overlaps with male chirps between 2 and 5 s in the recording. Sound occurring just past 4 s was not produced by either beetle, but rather was accidental noise produced by the recorder.

posterior elytra (located at the winglock) and the plectrum located along the posterior margin of the seventh tergite (Fig. 9 a). The plectrum is a paired structure consisting of 2 lobes approximately 50 *u*m wide (Table 4). The file is highly developed on the left elytron and only marginally developed on the right elytron (Fig. 10 a and b). One male whose right elytron had been damaged and partially lost was still capable of producing sound, as observed in other species (Michael & Rudinsky 1972).

Female *D. approximatus* exhibit an underdeveloped file on the left elytron and have very few ridges along the posterior portion of the right elytron (Fig. 10 c and d). A plectrum analogous to that found in males does not appear to exist in female *D. approximatus* (Fig. 9 b). Because of the high variability in the development of ridges in female files and lack of visible plectrum, only overall female body size measurements were included in subsequent analyses.

Overall, there were many significant correlations among body size characteristics and stridulatory structures. File length was highly positively correlated with all body size characteristics, as well as the total number of ridges (Table 5). Larger size males, estimated by elytra length and thorax width, tended to have longer files compared to smaller males. Ridge density, the total number of ridges divided by file length, was significantly negatively correlated with body size characteristics as well as file length (Table 5). Larger males with correspondingly large files had lower ridge densities compared to smaller males.

For male chirps produced in response to disturbance, peak frequency was significantly negatively correlated with body size characteristics and file length (Table 6), such that small males tended to produce higher frequency chirps in response to disturbance. In addition, the peak frequency of male chirps (disturbance context) was positively correlated with ridge density. Indeed, a multiple linear regression using

Table 3. Spectral and temporal characteristics of syllables in male interrupted chirps (mean ± SE).

		Median freq. (Hz)		Peak freq. (Hz)		Pulses		Duration (ms)		Pulse rate (pulses/s)	
First syllables[a]	Mean ± SE	7590.0	145.4	6717.7	163.4	7.0	0.2	24.2	0.4	292.7	5.5
	Min and Max	2625.0	14531.2	1312.5	15000.0	1.0	26.0	7.0	46.0	27.8	619.0
	N = 273										
Intermediate syllables	Mean ± SE	6580.3	73.5	5994.9	78.4	8.3	0.1	29.0	0.4	298.1	5.4
	Min and Max	3093.8	12375.0	2625.0	12281.2	1.0	15.0	6.0	84.0	114.8	1333.3
	N = 352										
Last syllables	Mean ± SE	6563.4	119.9	5957.6	135.4	6.3	0.2	27.0	1.1	249.2	8.2
	Min and Max	3093.8	10781.2	3093.8	12468.8	1.0	13.0	9.0	77.0	26.0	583.3
	N =104										

[a] First syllables consisted of syllables 1-3, while intermediate syllables consisted of syllables 4-7, and any syllables produced after the seventh syllable were evaluated at last syllables. Only interrupted chirps with 6 or more syllables were included in analyses.

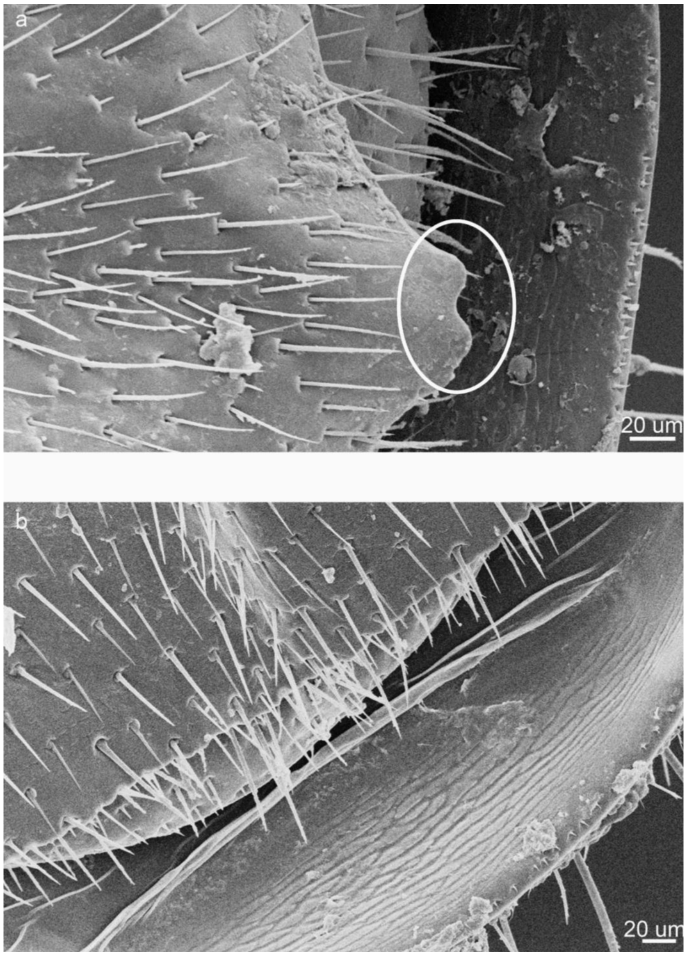

Fig. 9. Abdominal segments of male a) and female b) *D. approximatus*. The male plectrum is shown circled in a).

stepwise backward elimination identified one significant predictor of peak frequency, ridge density ($R^2 = 0.25$, $F_{(2,24)} = 4.076$, $p < 0.03$). Males with high ridge density produce relatively higher frequency disturbance chirps compared to males with lower ridge density.

As for temporal characteristics of male chirps produced in a disturbance context, the total number of pulses was significantly positively correlated with chirp duration (Pearson's $r = 0.62$, $p < 0.0001$). The total number of pulses per chirp was also positively correlated with the number of ridges on the lower file and total ridges ($p < 0.05$ for both correlations), suggesting that individual pulses result from activation of a single ridge. Chirp duration was significantly negatively correlated with pulse rate such that males producing longer chirps did so with fewer pulses per s (Pearson's $r = -0.60$, $p < 0.0001$). As for male simple chirps produced in response to females, there was a strong positive correlation between the total pulses per chirp and number of ridges on male files (Pearson's $r = 0.92$, $p < 0.001$).

Discussion

Both *D. approximatus* males and females produce chirps in similar contexts to those observed in other *Dendroctonus* spp. such as *D. ponderosae* (Ryker & Rudinsky 1976b; Fleming et al. 2013). Males produce an interrupted chirp, characterized by a series of short syllables upon entering a gallery of an attractive female, and soon after switch to a simple chirp. This is slightly different from that found in other species such as *D. frontalis* and *D. brevicomis*, whose males also produce interrupted chirps when first encountering an attractive female gallery, yet do not appear to produce a simple chirp to females (Rudinsky & Michael 1974; Ryker 1988). Male *D. approximatus* simple chirps in a disturbance context are more than twice as long as those reported for other *Dendroctonus* spp., except for *D. pseudotsugae* males with a mean duration of 68 ms (Michael & Rudinsky 1972; Rudinsky & Michael 1974; Ryker & Rudinsky 1976a; Fleming et al. 2013). The simple chirps produced by male *D. approximatus* are generally longer than those reported for other beetle families, such as Scarabaeidae and Cerambycidae, reported by Masters (1980). Female *D. approximatus* are unique in producing chirps in multiple contexts, both in response to disturbance and to prospective mates. Female simple chirps observed in a disturbance context in *Dendroctonus* spp. have previously only been reported for *D. valens* (Ryker & Rudinsky 1976a).

The functional importance of acoustic signaling during handling or disturbance has not been demonstrated in *Dendroctonus* spp., though Lewis & Cane (1990) showed a survival advantage against insect predators in another bark beetle, *Ips calligraphus* (Germar). Yet, Sivalinghem (2011) failed to demonstrate an anti-predator advantage to acoustic signaling in *I. pini* (Say). Insects that stridulate during encounters with arthropod and mammal predators have been shown to gain an advantage by decreasing predator handling time and in some cases avoiding predation (Masters 1979). Passalid beetles produce stridulations ranging between 2 and 9 kHz and increases the handling of time in crows following multiple encounters with stridulating passalid beetles (Buchler et al. 1981). Reduviid bugs also produce defensive stridulations, though at low frequencies, ~2 kHz, and are transmitted as substrate-borne vibrations (Schilman et al. 2001).

The broadband stridulations produced by insects in response to encounters with predators are similar to the chirps given by *D. approx-*

Table 4. Male and female body size and stridulatory structure size.

Sex	Body part or structure	N	Mean	SE	Min	Max
Male	Elytra length (mm)	30	3.59	0.05	2.96	4.28
	Thorax width (mm)	30	2.32	0.03	1.98	2.70
	File length (um)	28	622.14	15.31	452.57	754.06
	Plectrum width (um)[a]	27	49.79	1.86	31.15	72.32
	L-File[b]	28	37.71	0.77	30	46
	U-File[b]	28	47.54	0.85	40	56
	Total ridges	28	85.25	1.52	70	99
Female	Elytra length (mm)	11	3.54	0.10	2.98	4.01
	Thorax width (mm)	11	2.27	0.07	1.92	2.62

[a] The plectrum of one male was folded onto itself and could not be measured.
[b] This denotes the number of ridges on lower (L) and upper (U) halves of file.

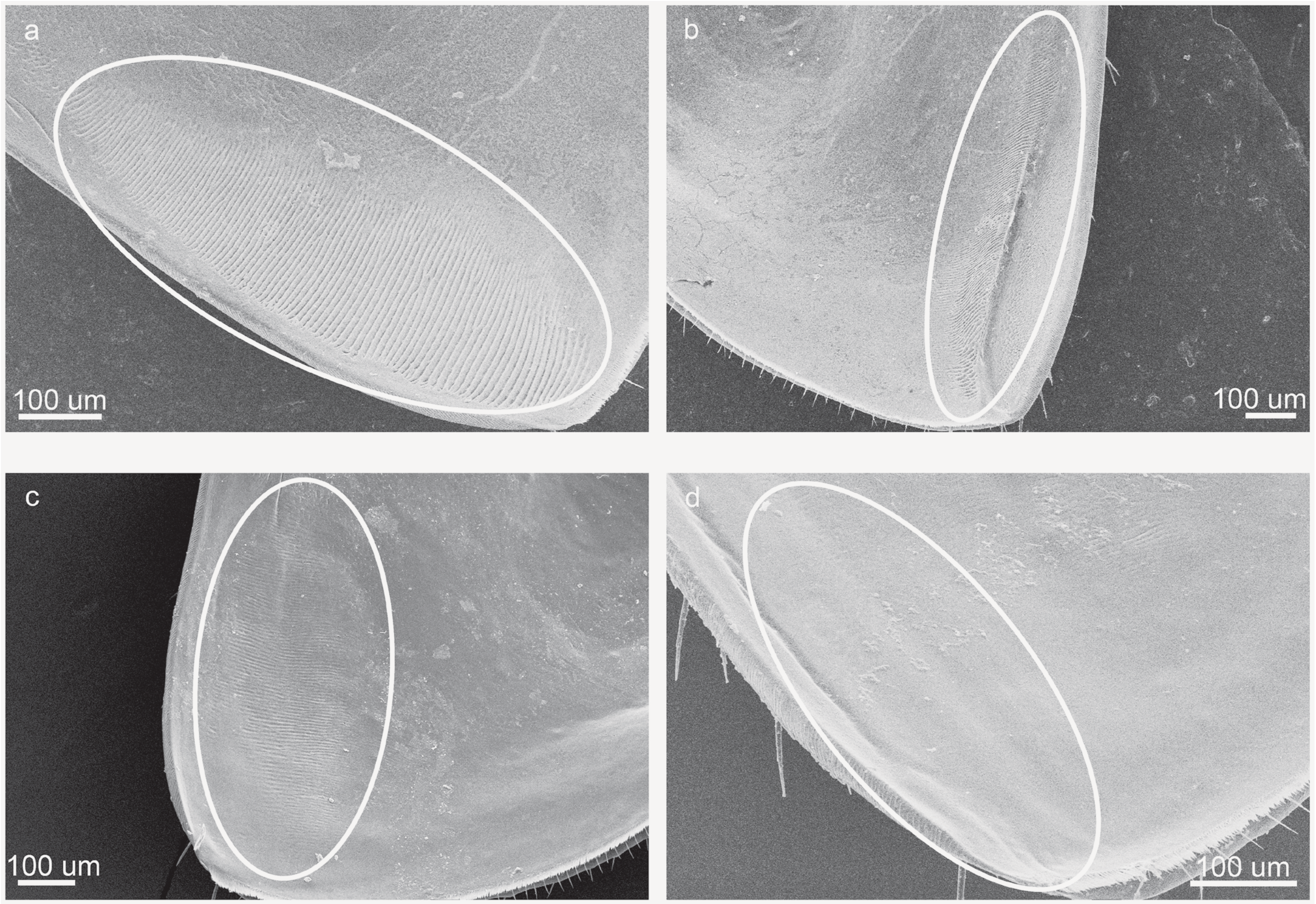

Fig. 10. Males possess a well-developed file on the left elytron a), with a relatively underdeveloped file on the right elytron b). The development of a file in female *D. approximatus* was highly variable and often almost non-existent. Left elytron of female with a well-developed file, relative to most other observed female files c), and of female with poorly-developed file d).

imatus during handling. Considering that most encounters between bark beetle adults and predators occur on the bark surface, decreasing the handling time and likelihood of being dropped may result in the successful escape of bark beetles. Experimental studies with manipulated bark beetles and their natural predators could test the importance of these acoustic signals as a means to avoid predation.

The method used by male *Dendroctonus* to produce interrupted chirps is unknown (Michael & Rudinsky 1972), though Rudinsky & Ryker (1976) note that *D. pseudotsugae* males primarily engage the upper half of the file while producing simple chirps and the lower half of the file while producing the interrupted chirp. Michael & Rudinsky (1972) hypothesized that males either lift the plectrum from the file or it remains engaged with the file but pauses briefly to produce the intervals between syllables. Other beetles produce syllables within a chirp by producing sound as the plectrum scrapes the file in both directions, with a pause between syllables (Mankin et al. 2009). This is unlikely to be the mechanism used by male *D. approximatus* as interrupted chirps contained both even and odd numbers of syllables and also because behavioral observations confirmed that males produced sound only in the direction of the downward movement made by the plectrum.

Table 5. Correlations between body size and stridulatory structures in male *D. approximatus.*

	Elytra length	Thorax width	L-file	U-file	Ridges	Ridge density	File length (um)	Plectrum width (um)
Elytra length	1	0.96 †	0.27	0.31	0.31	-0.44 *	0.59 **	0.38
Thorax width		1	0.21	0.31	0.28	-0.53 **	0.64 †	0.40
L-file[a]			1	0.75 †	0.93 †	0.31	0.40 *	0.00
U-file[a]				1	0.94 †	-0.12	0.73 †	0.03
Ridges					1	0.09	0.61 **	0.01
Ridge density						1	-0.73 †	-0.18
File length(um)							1	0.13
Plectrum width (um)								1

[a] This denotes the number of ridges on lower (L) and upper (U) halves of file.
*$p < 0.05$, **$p < 0.01$, †$p < 0.001$

Table 6. Correlations between chirp characteristics (produced in disturbance context), body size and stridulatory structures in male *D. approximatus*.

	Total pulses per chirp	Pulse rate	Duration (ms)	Peak freq (Hz)	Med freq (Hz)	Elytra length (mm)	Thorax width (mm)	Ridges on lower file	Ridges on upper file	Total ridges	Ridge density	File length (um)	Plectrum width (um)
Total pulses per chirp	1	0.25	0.62 †	-0.01	0.11	0.28	0.30	0.43 *	0.35	0.42 *	0.20	0.13	0.32
Pulse rate		1	-0.60 †	-0.08	-0.21	-0.03	0.02	-0.02	0.13	0.06	0.14	-0.08	0.33
Duration (ms)			1	0.07	0.27	0.22	0.20	0.34	0.16	0.26	0.09	0.12	0.01
Peak freq (Hz)				1	0.85 †	-0.38 *	-0.46 *	-0.04	-0.31	-0.20	0.44 *	-0.49 **	-0.04
Med freq (Hz)					1	-0.26	-0.36	-0.02	-0.15	-0.09	0.16	-0.20	-0.08

$*p < 0.05$, $**p < 0.01$, $†p < 0.001$

For chirps produced in a disturbance context, small males were more likely to have higher frequencies than chirps produced by larger males. However, this is complicated by the fact that ridge density was a significant predictor of frequency where males with high ridge density produced higher frequency chirps than males with files having lower ridge densities. Ultimately, small males had relatively small files and they had relatively high ridge density. Ridge density was a significant predictor of frequency, such that these small males produced higher frequency chirps compared to their larger counterparts.

The proximate explanation for the relationship between size and frequency in *D. approximatus* males warrants further discussion. Though peak frequency of simple chirps in the disturbance context was inversely related to file and elytra length, the most significant predictor of frequency was ridge density, measured as the number of ridges per unit length of the file. Males with higher ridge density produced significantly higher frequency chirps than males with low ridge densities. This would be expected assuming that the file acts as a frequency multiplier. However, the number of pulses per s was not correlated with peak frequency in chirps. Other insects, such as ants do exhibit a positive relationship between pulse rate and frequency (Hickling & Brown 2000).

The physical basis for frequency in orthopteran pure-tone songs has been well-documented and relies on a combination of a frequency multiplier and specialized resonating structure (Bennet-Clark 1999; Bennet-Clark 2003). For example, in a neotropical katydid the resonant frequency of the wings, 15 kHz, corresponds to that of the number of toothstrikes per s, 15,000/s, resulting in an acoustic signal with dominant frequency near 15 kHz (Montealegre-Z 2012). This one-to-one ratio is not apparent in *D. approximatus*, whose average peak frequency of 5.3 kHz in corresponds to approximately 518 pulses per s in simple chirps.

The inverse relationship between male size and frequency in *D. approximatus* is consistent with other stridulating insects. However, comparisons with other insect species are often only available for somewhat larger species. Male *D. approximatus* produce chirps in response to disturbance at similar frequencies to other beetles such as scarab beetles (Panneton et al. 2005) which stridulate at a peak frequency of approximately 5 kHz with approximate minimum and maximum frequencies of 2 and 14 kHz. Studies that address size-frequency relationships in small stridulating insects have found similar patterns. The velvet ant, *Myrmilla capitata*, varying in size from 5-10 mm exhibit a negative relationship between the maximum frequency of disturbance signals and the size of their file (Polidori et al. 2013). Small leaf beetles ranging from 5-9 mm in length produce frequencies between approximately 1- 10 kHz, though size relationships were not explicitly studied (Schmitt & Traue 1990).

The implications of a size-frequency relationship in male *D. approximatus* chirps are complex. This trend was observed in chirps produced in response to disturbance, which presumably includes predators and possibly male rivals. In this study, multiple males were not recorded together, but other studies have demonstrated that male *Dendroctonus* produce chirps when competing with other males (Rudinsky & Ryker 1976; Ryker & Rudinsky 1976b; Fleming et al. 2013). In fact, *D. rufipennis* produce disturbance chirps that are not significantly different (temporally) from chirps produced in a rivalry context (Rudinsky & Michael 1974). Likewise, male *D. ponderosae* produce chirps in response to disturbance and rival males with relatively similar dominant frequencies of 15.6 and 17.4 kHz, respectively (Fleming et al. 2013). Therefore, it is possible that male *D. approximatus* males could use rivalry chirps to discern the size of a competitor.

In summary, we examined stridulatory structures and acoustic signals in male and female *D. approximatus*. We determined that male

D. approximatus produces 2 chirp types, interrupted and simple, and most closely resembles the acoustic repertoire of *D. ponderosae*. We report female simple chirps in response to male acoustic signals and disturbance. Sound production in the latter context has only been reported for female *D. valens*. Sound production in *D. approximatus* may be size-limited as smaller males produced higher frequency calls (in a disturbance context) than did their larger counterparts.

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References Cited

Aflitto NC, Hofstetter RW, McGuire R, Dunn DD, Potter KA. 2014. Technique for Studying Arthropod and Microbial Communities within Tree Tissues. Journal of Visualized Experiments 93: e50793.

Barr BA. 1969. Sound production in Scolytidae (Coleoptera) with emphasis on Genus *Ips*. Canadian Entomologist 101(6): 636-672.

Bennet-Clark HC. 1975. Sound production in insects. Science Progress 62(246): 263-283.

Bennet-Clark HC. 1998. Size and scale effects as constraints in insect sound communication. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 353(1367): 407-419.

Bennet-Clark HC. 1999. Resonators in insect sound production: how insects produce loud pure-tone songs. Journal of Experimental Biology 202: 3347-3357.

Bennet-Clark HC. 2003. Wing resonances in the Australian field cricket *Teleogryllus oceanicus*. Journal of Experimental Biology 206(9): 1479-1496.

Bradbury JW, Vehrencamp SL. 1998. Principles of Animal Communication. Sinauer Associates, Inc., Sunderland, Massachusetts.

Buchler ER, Wright TB, Brown ED. 1981. On the functions of stridulation by the Passalid beetle *Odontotaenius disjunctus* (Coleoptera: Passalidae). Animal Behaviour 29(2): 483-486.

Charif RA, Waack AM, Strickman LM. 2010. Raven Pro 1.4 User's Manual. Cornell Laboratory of Ornithology, Ithaca, NY.

Cibrián Tovar D, Méndez Montiel JT, Campos Bolaños R, Yates HO, Flores Lara J. 1995. Forest Insects of Mexico. Universidad Autónoma Chapingo, Chapingo, Estado de México, México.

Dunn D. 2004. Microphones, Hydrophones, Vibration Transducers: Rolling Your Own. traktoria.org.

Fleming AJ, Lindeman AA, Carroll A, Yack JE. 2013. Acoustics of the Mountain Pine Beetle *Dendroctonus ponderosae* (Curculionidae, Scolytinae): Sonic, ultrasonic and vibration characteristics. Canadian Journal of Zoology-Revue Canadienne De Zoologie 91(4): 235-244.

Fujioka E, Mantani S, Hiryu S, Riquimaroux H, Watanabe Y. 2011. Echolocation and flight strategy of Japanese house bats during natural foraging, revealed by a microphone array system. Journal of the Acoustical Society of America 129(2): 1081-1088.

Furniss RL, Carolin VM. 1977. Western Forest Insects. USDA Forest Service, Washington, DC.

Hickling R, Brown RL. 2000. Analysis of acoustic communication by ants. Journal of the Acoustical Society of America 108(4): 1920-1929.

Hofstetter RW, Dunn DD, McGuire R, Potter KA. 2013. Using acoustic technology to reduce bark beetle reproduction. Pest Management Science 70: 24-27.

Knowles Electronics. 2005. FG-23329 Specifications. Knowles Electronics, Itasca, IL.

Knowles Electronics. 2013. Application Note 17. Knowles Electronics, Itasca, IL.

Lewis EE, Cane JH. 1990. Stridulation as a primary antipredator defense of a beetle. Animal Behaviour 40(5): 1003-1004.

Mankin RW, Moore A, Samson PR, Chandler KJ. 2009. Acoustic characteristics of Dynastid beetle stridulations. Florida Entomologist 92(1): 123-133.

Masters WM. 1979. Insect disturbance stridulation - its defensive role. Behavioral Ecology and Sociobiology 5(2): 187-200.

Masters WM. 1980. Insect disturbance stridulation: characterization of airborne and vibrational components of the sound. Journal of Comparative Physiology 135(3): 259-268.

Michael RR, Rudinsky JA. 1972. Sound production in Scolytidae - specificity in male *Dendroctonus* beetles. Journal of Insect Physiology 18(11): 2189-2201.

Montealegre-Z F. 2012. Reverse stridulatory wing motion produces highly resonant calls in a neotropical katydid (Orthoptera: Tettigoniidae: Pseudophyllinae). Journal of Insect Physiology 58(1): 116-124.

Panneton BE, Sprague MW, Kalmus GW, Charles TM, Daniel HJ. 2005. Stridulatory organs in the scarab beetle *Polyphylla occidentalis* (Scarabaeidae: Coleoptera): An SEM and acoustic study. Journal of North Carolina Academy of of Science 121(3): 135-144.

Polidori C, Ruffato G, Borruso L, Settani C, Payan G. 2013. Stridulatory organ and distress calls in males and females of a small velvet ant (Hymenoptera: Mutillidae). Bioacoustics 22(2): 121-135.

Rasband W. 2012. ImageJ. U.S. National Institutes of Health, Bethesda, MD.

Rudinsky JA. 1969. Masking of aggregation pheromone in *Dendroctonus pseudotsugae* Hopk. Science 166(3907): 884-885.

Rudinsky JA, Michael RR. 1972. Sound production in Scolytidae: Chemostimulus of sonic signal by Douglas-fir beetle. Science 175(4028): 1386-1390.

Rudinsky JA, Michael RR. 1973. Sound production in Scolytidae: Stridulation by female *Dendroctonus* beetles. Journal of Insect Physiology 19(3): 689-705.

Rudinsky JA, Michael RR. 1974. Sound production in Scolytidae: Rivalry behavior of male *Dendroctonus* beetles. Journal of Insect Physiology 20(7): 1219-1230.

Rudinsky JA, Morgan M, Libbey LM, Michael RR. 1973. Sound production in Scolytidae: 3-methyl-2-cyclohexen-1-one released by female Douglas fir beetle in response to male sonic signal. Environmental Entomology 2: 505-509.

Rudinsky JA, Ryker LC. 1976. Sound production in Scolytidae: Rivalry and premating stridulation of male douglas-fir beetle. Journal of Insect Physiology 22(7): 997-1003.

Ryker LC. 1988. Acoustic studies of *Dendroctonus* bark beetles. Florida Entomologist 71(4): 447-461.

Ryker LC, Rudinsky JA. 1976a. Sound production in Scolytidae: Acoustic signals of male and female *Dendroctonus valens* Leconte. Journal of Applied Entomology 80(2): 113-118.

Ryker LC, Rudinsky JA. 1976b. Sound production in Scolytidae: Aggressive and mating-behavior of mountain pine beetle. Annals of the Entomological Society of America 69(4): 677-680.

Schilman PE, Lazzari CR, Manrique G. 2001. Comparison of disturbance stridulations in five species of triatominae bugs. Acta Tropica 79(2): 171-178.

Schmitt M, Traue D. 1990. Morphological and bioacoustic aspects of stridulation in Criocerinae (Coleoptera: Chrysomelidae). Zoologischer Anzeiger 225(5-6): 225-240.

Sivalinghem S. 2011. Acoustic communication in the pine engraver bark beetle, *Ips pini* (Coleoptera: Scolytinae). Carleton University, Ottawa, Ontario.

Wood SL. 1982. The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae), a taxonomic monograph. Great Basin Naturalist Memoirs 6: 1-1356