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Caterpillar sonic defences: mechanisms and diversity of mandible stridulation in silk and hawk moth (Bombycoidea) larvae

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ABSTRACT

Silk and hawk moth caterpillars produce a variety of defence sounds described as clicking, chirping, whistling, and vocalising. Such diversity provides opportunity to test hypotheses on the functions and evolution of insect defence sounds using comparative analyses. A key step to conducting comparative analyses is to categorise different phenotypes. Here, we describe mandible sounds of 20 species of late instar Bombycoidea caterpillars and establish objective criteria for differentiating between sound-producing mechanisms. First, we assess how the two mandibular mechanisms – clicking and chirping – differ from one another using two reference species: *Antheraea polyphemus* (clicker) and *Saturnia pyri* (chirper). In these references, clicks are produced by ridged and serrated mandibles and have short duration units with few pulses, whereas chirps are produced by scalloped mandibles and have longer duration units with more pulses. Second, we characterise acoustic and morphological traits of 18 additional species. These are categorised as clickers (13 species), chirpers (4 species), or other (1 species) using diagnostic features identified from the reference species. Third, these categorisations are tested using a predictive logistic regression model. The results of this study contribute to our understanding of acoustically mediated defences in caterpillars and provide necessary criteria for conducting further comparative studies.

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Introduction

Many species of silk and hawk moth (Bombycoidea) caterpillars have been reported to produce defence sounds (see Bura et al. 2016). These sounds are widespread across the Bombycoidea phylogeny, occurring in a third of species tested to date (n = 20/61 species tested; Bura et al. 2016). Four different categories of defensive sound production have been documented: clicking (Brown et al. 2007; Bura et al. 2012), chirping (Bura et al. 2009), whistling (Bura et al. 2011), and vocalisation (Rosi-Denadai et al. 2018). Clicking and chirping are both produced by stridulation using the mandibles, whereas whistling and vocalisation are produced by forcing air out of the spiracles and the foregut, respectively. While defence sounds are common in insects, little is

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understood about their effects on predators or their evolutionary origins (Low et al. 2021). The Superfamily Bombycoidea is ideal for testing hypotheses on the functions and evolution of insect defensive sound production using a phylogenetic comparative approach due not only to the variability between sounds and the availability of genetic sequences, but also because the evolution of juvenile defence sounds is not confounded by sexual selection. However, prior to conducting comparative studies on these sounds, it is essential to be able to distinguish between the different phenotypes. For example, not all whistling species use the same pair of spiracles to produce sounds (see Bura et al. 2011; Sugiura et al. 2020), and these species should therefore be scored according to spiracle pair rather than simply as ‘whistling’ in a comparative analysis. Mandible sounds present a similar problem where two categories have been proposed (clicking and chirping), but whether these represent distinct phenotypes is unclear.

Over half of the sound-producing Bombycoidea caterpillar species reported to date produce one of the two categories of mandible sounds (Bura et al. 2016). Clicking has been experimentally confirmed in three species, *Antheraea polyphemus*, *Actias luna*, and *Manduca sexta* (Brown et al. 2007; Bura et al. 2012), and involves rubbing rows of teeth on the distal edge of one mandible against the teeth on the distal edge of the opposing mandible, resulting in short sound units (clicks). Chirping on the other hand is reported to be a different mechanism, as studied in *Saturnia pyri* (Bura et al. 2009), whereby the distal edge of one mandible slides along the smooth inner surface of the opposing mandible, producing longer sound units (chirps). However, whether clickers and chirpers represent distinct phenotypes has not been thoroughly investigated. Other species that were subsequently designated as clickers or chirpers were done so sometimes based on incomplete data, especially as many species were collected as single specimens in the wild, and therefore categorisation into the two sound types may have been subjective. In order to objectively score these sound-producing mechanisms in future comparative studies, it is necessary to clarify how clicking and chirping differ, or alternatively, determine if mandible sounds constitute a single mechanism that varies along a continuum.

This study reports on mandibular defence sounds in 20 species of Bombycoidea caterpillars. There are three goals. 1) We compare the acoustic, morphological, and behavioural characteristics of a previously designated clicker (*A. polyphemus*, Brown et al. 2007) and a previously designated chirper (*S. pyri*, Bura et al. 2009) to create references for the two proposed categories. We chose these species because the mechanisms of sound production had been experimentally investigated in previous studies (see Brown et al. 2007; Bura et al. 2009). We identify primary distinguishing features between clicking and chirping based on these reference species. 2) For 18 additional species, we describe the mandibular defence sounds and accompanying morphological and behavioural characteristics (where available). We use the distinguishing features identified in goal one to assess if these species fall into the established clicker or chirper categories. 3) We test the categorisations from goal two by developing a predictive logistic regression model. The results from this study provide a comparative overview of mandibular defence sounds in silk and hawk moth caterpillars and provide objective criteria for scoring phenotypes.

Materials and methods

Caterpillar sampling

A total of 20 species of Bombycoidea caterpillars proposed to produce clicks or chirps were included in this study: 6 from Sphingidae (Sphinginae: *Acherontia atropos*, *Manduca diffissa tropicalis*, *Manduca lefeburii*, *Manduca pellenia*, *Manduca sexta*; Macroglossinae: *Eumorpha satellitia*) and 14 from Saturniidae (Ceratocampinae: *Adeloneivaia jason*, *Citheronia lobesis*, *Schausiella santarosensis*; Saturniinae: *Actias luna*, *Actias selene*, *Antheraea oculea*, *Antheraea pernyi*, *Antheraea polyphemus*, *Callosamia promethea*, *Calosaturnia mendocino*, *Copaxa curvilinea*, *Copaxa rufinans*, *Saturnia pavonia*, *Saturnia pyri*). These caterpillars were collected and tested as part of an ongoing survey of the diversity of sound production within Bombycoidea caterpillars by the Yack bioacoustics lab at Carleton University. Specimens were obtained opportunistically from various sources worldwide, including Canada, USA, Europe, Costa Rica, and Ecuador, between the years 2005–2021 (NCC permit #3654, and CFIA permits *P*-2004–02683, *P*-2007–03105, *P*-2008–02614, and P-2016–02619). Caterpillars were collected either as larvae from their host plants in the wild or reared from eggs. Eggs were obtained both from wild-caught gravid females and online suppliers, and reared at Carleton University using local host plants suitable to each species. All caterpillars in this study were tested as late instars, usually from III to V. Wherever possible, specimens were preserved as late instars in 70–95% EtOH for use in morphological analyses (see *Morphology* below). As a variety of species were collected from numerous geographical regions and under different collecting conditions, the sample sizes, recording methods, and datasets on morphology, behaviour, and sound production varied between species. The numbers, sources, location of voucher specimens, and plants fed to each species included in this study are provided in Table S1.

Sound and video recordings

Three types of sound and video recording scenarios were used to collect the acoustic and behavioural data analysed in this study. In all instances, caterpillars were induced to produce sounds by placing them on a cutting of host plant, allowing them to rest for 15 minutes, and then pinching them with blunt forceps behind the head capsule or on the posterior end of their body. The three recording scenarios were as follows: 1) Sounds were recorded using one of two broadband, ¼ inch microphones: Earthworks or Bruel & Kjaer (B&K). Earthworks recordings were conducted using a QTC40 Earthworks microphone (Milford, NH, USA) and recorded to a Fostex FR-2 Field Recorder (Gardena, CA, USA) at a sampling rate of 192 kHz. B&K recordings used a B&K Type 4939 microphone (Naerum, Denmark) amplified with a B&K Type 2690 Nexus conditioning amplifier (Naerum, Denmark) and were recorded to the Fostex at a sampling rate of 192 kHz. The microphones were placed 2–10 cm from the caterpillar's head. Resulting sound files were saved as .wav files. These recordings were used in the current study to analyse acoustic traits in both temporal and spectral domains. 2) Video recordings of attack trials coupled with audio recordings were obtained using a SONY HDR XR-500 high-definition camcorder (Tokyo, Japan) and a SONY ECM-MS907 microphone (Tokyo, Japan) placed 5–10 cm from the caterpillar's head. Sounds were extracted from the video files, saved as .wav files, and used for selected temporal analyses. 3) Close-up video recordings of mandibular movements coupled with audio recordings were obtained using the SONY camcorder and microphone described above. Caterpillars were held on their dorsum beneath the camcorder and pinched on their posterior end to elicit sound production. A SONY MT4037 S magnifying lens (Tokyo, Japan) was added to the camera for a closer view of mandibular movements and the microphone was arranged 5–10 cm from the head of the animal. These video recordings were used to assess mandibular movements during sound production (see *Sound and Video Analyses of References* below). All recording scenarios were performed in an Eckel Industries Ltd. acoustic chamber (Cambridge, MA, USA) at Carleton University or in a portable chamber lined with acoustic foam in the field.

Sound and video analyses of references

To identify how the two clicking and chirping reference species differ in acoustic and behavioural traits, we analysed sound files of 10 specimens for each of the two references, and mandible videos of 13 *A. polyphemus* and 6 *S. pyri* individuals. Sound files were analysed using Avisoft SASlab Pro (Avisoft Bioacoustics, Berlin, Germany) for six temporal and three spectral characteristics (Figure 1). Temporal characteristics included unit duration, number of pulses per unit (PPU), pulse rate, pulse duration, duty cycle, and interpulse interval (IPI) within a unit. We define a unit as an individual sound as perceived by the human ear (Broughton 1963). A pulse is defined as a component of a unit that forms a transient waveform with a distinct rise and fall (Broughton 1963). Duty cycle was calculated as the sum of all pulse durations divided by the unit duration. Spectral characteristics included dominant frequency (defined as the peak with the most energy on the power spectrum) as well as bandwidths at −3 and −10 dB below dominant frequency. Spectra were produced and analysed in Avisoft SASlab Pro using 1024-point Fast Fourier Transform (Hann window). Single-pulse units were excluded from the frequency analyses. Sound amplitude comparisons were not included in this study due to the variability between species in recording distances and microphones used. All sound units for both temporal and spectral analyses were sampled randomly from the second train of units produced after pinching, where a train refers to a series of sound units following an attack. Three units each per individual were measured. Acoustic differences between references were identified using statistical analyses (see *Statistical Analyses* below). Video attack trials and close-up mandible videos were analysed to confirm that a single sound unit corresponds to a single mandibular movement, and we compared which parts of the mandibles were associated with sound production as well as laterality where possible.

Morphology of references

To identify how the two clicking and chirping reference species differ based on mandible morphology, and to assess how mandible morphological features relate to sound characteristics, mandibles of five (n = 5) preserved specimens per species were studied. Mandibles were photographed in situ, then dissected out and photographed again using a Leica M205C stereoscope (Leica Microsystems, Wetzlar, Germany) equipped

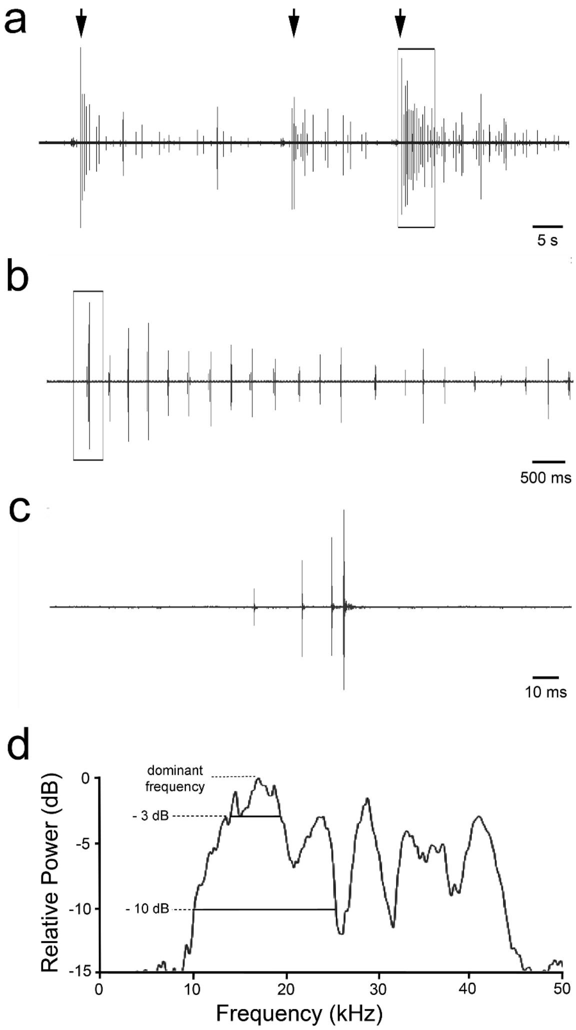

Figure 1. Acoustic parameters measured in this study using defence sounds produced by a late instar *Antheraea polyphemus*. (a) Three sound trains resulting from three simulated attacks (arrows). Box encloses the first 8 seconds of the third attack, expanded in part b. (b) Multiple click units are shown from the first part of the third train of part a. Box encloses the first sound unit, expanded in part c. (c) One click unit from part b expanded to show individual sound pulses. (d) A power spectrum showing the spectral parameters measured in this study.

with a Leica DMC4500 camera. Mandibles were prepared for scanning electron microscopy by mounting them on aluminium stubs, sputter-coating with gold-palladium, and examining them with a TESCAN VEGA II XMU scanning electron microscope (TESCAN USA Inc., Warrendale, PA).

To compare the mandibular morphological traits of the two reference species, but also for categorising the comparative species (see next section), we developed three categories of mandible morphology adapted from previous descriptions of caterpillar mandibles (Snodgrass 1935; Bernays and Janzen 1988; Bura 2010) (Figure 2): smooth (distal edge flat to almost block-like in form, while inner and outer surfaces are smooth and without

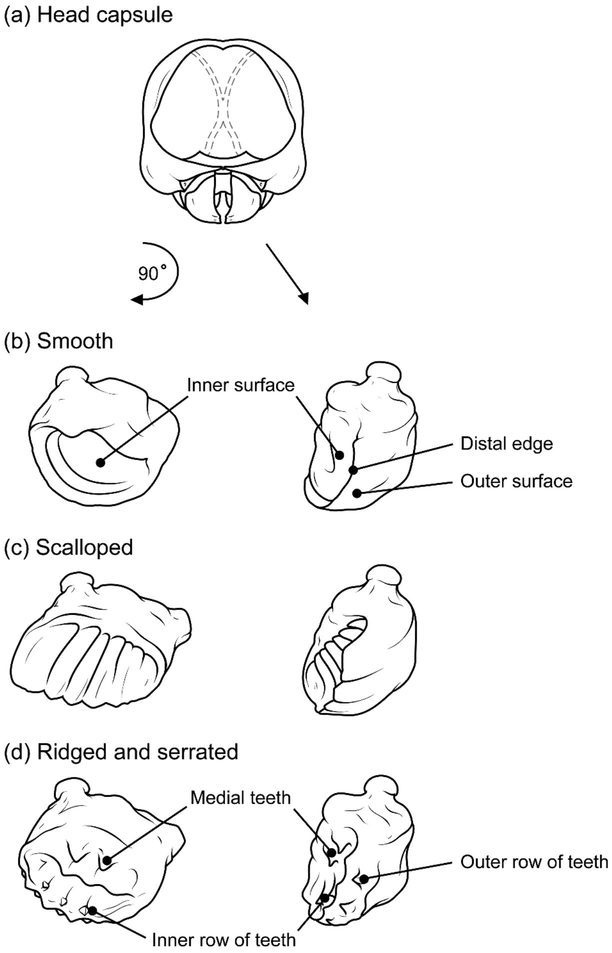

Figure 2. Line drawings of generalised mandibles representing the three main categories. The animals' left mandibles are drawn showing the inner surface, while the right mandibles are drawn showing a side profile. (a) Location of intact mandibles on a caterpillar head capsule (posterior view). (b) Smooth mandibles. (c) Scalloped mandibles. (d) Ridged and serrated mandibles representing Type 3 (see text for descriptions of the three types).

teeth); scalloped (distal edge scalloped or distinctly serrated, and inner surface with faint to distinct longitudinal grooves); and ridged and serrated mandibles with three types: ridged and serrated Type 1 (single row of teeth along distal edge, and one to two medial teeth on the inner surface), ridged and serrated Type 2 molar-like (one row of teeth along distal edge, and two additional rows of teeth just proximal to distal edge: one on the inner surface, and one on the outer surface), and ridged and serrated Type 3 (three rows of teeth similar to Type 2, and one to two medial teeth on the inner surface). It should be noted that mandibles within pairs were morphologically symmetrical as mirror images.

To compare the head capsule morphological traits of the two reference species, but also for categorising the comparative species (see next section), head capsules of six ($n = 6$) preserved specimens per species were studied. Head capsule width was measured as a proxy for mandible size to assess if unit duration is correlated to mandible size, or if size differs between clicking and chirping species (see *Statistical Analyses* below). We measured the head capsule width of final instars using digital calipers (Fisherbrand 9002449). Head capsule shape was also assessed as Bura (2010) had predicted a relationship between mandibular sound production and shape due to the required musculature. We determined the head capsule shape as one of the three categories as per Bura (2010): round, oval, or triangular, where oval is when the difference between width and height is less than −0.1 (i.e. the capsule is taller than it is wide).

Comparative species

Eighteen additional Bombycoidea species were described based on their sound features, morphology, and behaviour. Acoustic analyses of up to five individuals (15 sound units) per species were performed. Scanning electron micrographs (SEMs) of mandibles were obtained from one specimen per species, where possible. When preserved specimens were unavailable, head capsule images of late instars were obtained online and the relative head width-to-height ratio was measured to categorise head shape. See methods described above for further details of data collection and analysis of acoustic and morphological traits. Details on the number of specimens and data available for each species are reported in Table S1. We assessed if these additional Bombycoidea species could be categorised as clickers or chirpers using the criteria identified from the reference species (goal two) and we tested the categorisations using predictive logistic regression modelling (goal three) (see *Statistical Analyses* below).

Statistical analyses

Statistical analyses were implemented in R version 4.0.2 (R Core Team 2020). A standard alpha value of 0.05 was used for all analyses unless otherwise stated. When performing multiple comparisons, p-values were adjusted using the Bonferroni correction.

To determine how the reference species differ (goal one of this study), we compared each of the nine measured acoustic traits as well as the head capsule widths between the two references using Mann–Whitney U-tests with adjusted p-values. Additionally, a Spearman's rank-order correlation analysis was performed to determine first, if any of the acoustic traits were correlated within each species, and second, if these correlations differed between the two species. Correlations that differed between the species were analysed for significance by converting the correlation coefficients into z-scores and finding the observed z-test statistic. The results of these analyses provided key criteria that we used to distinguish between the references.

To categorise the additional 18 species as either clickers or chirpers for goal two, we determined whether the acoustic traits of each species differed significantly from either of the reference species using Mann–Whitney U-tests with adjusted p-values. We also ran a Spearman's rank-order correlation analysis on the acoustic traits within each species to determine if any traits were correlated, and if correlations coincided with those of either reference.

To test the categorisations of the 18 species obtained from comparisons with the reference species (from goal 2), a logistic regression was used to model the probability of a species being either a clicker or a chirper based on their acoustic characteristics. The data were divided into a training set and a test set. Rather than restricting the model to only the two references, the model was built on a training set containing sounds from five species with a total of 85 data points (40 chirps, 45 clicks), thereby providing a broader range of acoustic traits than from the reference species alone. The five training set species included the references as well as *Saturnia pavonia*, *Actias luna*, and *Manduca sexta*. We chose *S. pavonia* as an additional chirping representative due to its similarity to *S. pyri*, and *A. luna* and *M. sexta* as additional clicking representatives as they were both described at the same time as *A. polyphemus* (Brown et al. 2007). The dependent variable was the category of sound production, with a value of either 0 (click) or 1 (chirp). We evaluated the effects of all nine acoustic traits as predictor variables using univariate analyses, as well as forward stepwise regression where statistically significant predictor variables with the lowest AIC value were added one by one to the model. The fit of our final model was assessed using a likelihood ratio test, Hosmer–Lemeshow goodness of fit test, and McFadden's pseudo-R^2. The test set consisted of the average sounds from 18 species for which we had both temporal and spectral data. Using the *predict()* function in R, we estimated whether a species' sounds could be assigned to either clicking or chirping using a cut-off value of 0.5, and constructed confidence intervals for each probability using endpoint transformation.

To account for potential effects of body size on sound characteristics between species, we tested for a relationship between head capsule width (as a proxy for mandible size) and unit duration using linear regression and Spearman's rank-order correlation analysis.

Results

Clicker reference: *Antheraea polyphemus*

To establish the identifying characteristics of a clicker, we describe the acoustics, morphology, and behaviour of 10 individuals of *A. polyphemus* (Figure 3(a-c)). A summary of *A. polyphemus* acoustic (n = 30 units) and morphological (n = 6 individuals) characteristics is included in Table 1.

Acoustics

Click units range from 0.3 to 67.6 ms (mean 17.1 ± 16.5 ms) in duration. Units consist of 1 to 6 PPU (mean 2.2 ± 1.1) with an average rate of 167.3 ± 115.9 pulses/second. Pulses average 1.09 ± 0.78 ms in duration with a median IPI of 16.2 ± 16.0 ms. The unit dominant frequency averages 31.4 ± 7.3 kHz and ranges from 13.5 to 46.1 kHz.

Morphology and behaviour

Antheraea polyphemus mandibles are classified as ridged and serrated Type 2 molar-like (n = 5 pairs) (Figure 3(a,b)). The mandibles possess a serrated distal edge of four to five teeth with an additional row of three teeth on the outer surface, as well as a row of four teeth on the inner surface of the mandible just proximal to the distal edge. The teeth along the distal edge are molar-like in form with a dip in their middle, though this varied slightly with some specimens appearing more worn with reduced distal teeth. The head capsule of *A. polyphemus* is round with an average width of 6.22 mm (n = 6).

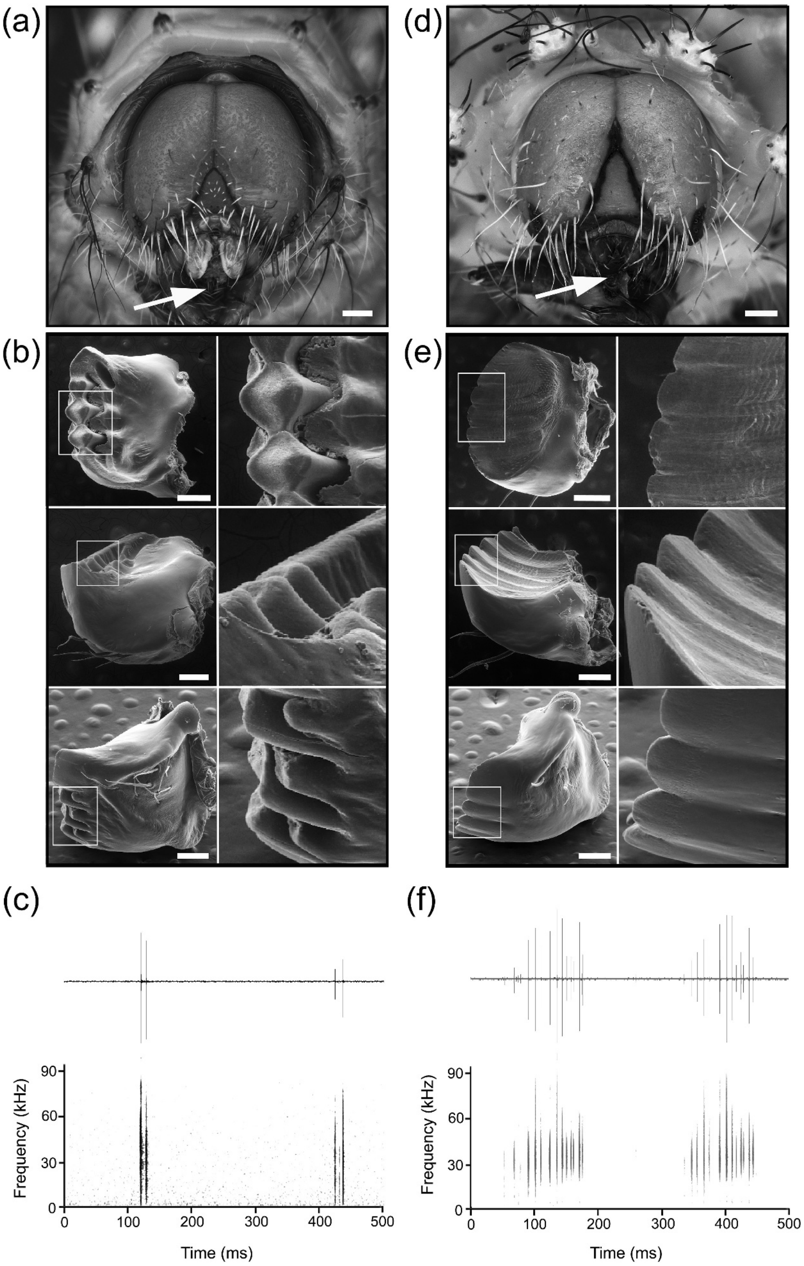

Figure 3. Morphological and acoustic characteristics of the reference clicker *Antheraea polyphemus* (a-c), and the reference chirper *Saturnia pyri* (d-f). (a) Head capsule of *A. polyphemus* with arrow pointing to location of mandibles. Scale bar: 1 mm. (b) Scanning electron micrographs showing different orientations of the mandibles. Left column shows the entire mandible; from top to bottom: inner (scale bar: 0.4 mm), lateral (scale bar: 0.3 mm), and outer (scale bar: 0.3 mm). Boxes outline enlarged regions shown on the right. (c) Sound waveform (top) and spectrogram (bottom) of two clicks (i.e. two sound units). (d) Head capsule of *S. pyri* with arrow pointing to location of mandibles. Scale bar: 1 mm. (e) Scanning electron micrographs showing different orientations of the mandibles. Left column shows the entire mandible; from top to bottom: inner (scale bar: 0.4 mm), lateral (scale bar: 0.3 mm), and outer (scale bar: 0.3 mm). Boxes outline enlarged regions shown on the right. (f) Sound waveform (top) and spectrogram (bottom) of two chirps (i.e. two sound units).

Table 1. Acoustic and morphological traits of all species analysed in this study. The two reference species, *A. polyphemus* and *S. pyri*, are in bold. Acoustic measurements were obtained from 3 sound units each for up to 5 different individuals per species. Morphological measurements were obtained from up to 6 specimens per species, and standard deviations are provided when n > 1. All values in this table are provided as 'Mean (SD)'. Sample sizes per species are provided in Supplementary Table S1.

	Acoustics									Morphology			
	Temporal Traits						Spectral Traits			Head Capsule		Mandibles	
Taxon	Unit Duration (ms)	PPU	Pulse Rate (#/s)^a	Pulse Duration (ms)	Duty Cycle (%)^a	IPI Median (ms)^b	Dominant Frequency (kHz)	-3dB (kHz)	-10dB (kHz)	Shape	Width (mm)	Category^c	Movements Observed
SATURNIIDAE: Ceratocampinae													
Adeloneivaia jason	27.3 (32.9)	1.6 (0.5)	72.3 (65.0)	0.54 (0.29)	3.4 (3.7)	44.2 (31.6)	26.4 (1.1)	7.0 2.6)	28.5 (11.0)	Oval	n/a	n/a	N
Citheronia lobesis	132.1 (27.6)	21.7 (6.4)	163.9 (30.8)	0.90 (0.20)	11.6 (2.7)	5.5 (1.3)	36.7 (3.8)	9.5 (5.1)	47.8 (14.2)	Round	n/a	Scalloped	N
Schausiella santarosensis	84.8 (29.1)	12.9 (4.4)	158.8 (57.0)	0.60 (0.41)	6.3 (3.9)	6.9 (2.8)	36.3 (3.9)	10.7 (4.3)	44.8 (16.7)	Round	6.3	Smooth	N
SATURNIIDAE: Saturniinae													
Actias luna	43.1 (39.2)	2.3 (1.0)	267.0 (400.7)	1.26 (1.16)	18.5 (27.0)	42.7 (35.3)	34.0 (6.7)	6.8 (3.0)	21.2 (7.0)	Round	5.6 (0.2)	R+S Type 2	Y
Actias selene	64.7 (44.8)	3.5 (2.4)	74.3 (49.7)	0.50 (0.47)	2.7 (2.0)	39.1 (36.6)	27.1 (7.2)	4.8 (2.3)	18.5 (10.3)	Round	6.6 (0.2)	R+S Type 2	Y
Antheraea oculea	29.1 (39.4)	2.1 (1.2)	259.9 (321.4)	1.41 (1.27)	18.5 (25.3)	35.0 (35.9)	24.2 (7.7)	5.2 (2.4)	18.5 (7.6)	Round	7.9	R+S Type 2	N
Antheraea pernyi	100.8 (65.2)	4.3 (1.5)	92.4 (100.1)	1.25 (0.75)	10.8 (11.4)	30.1 (35.0)	19.3 (7.8)	5.3 (3.1)	21.0 (10.0)	Round	8.8 (0.1)	R+S Type 2	Y
Antheraea polyphemus	**17.1 (16.5)**	**2.2 (1.1)**	**167.3 (115.9)**	**1.09 (0.78)**	**14.4 (9.3)**	**16.2 (16.0)**	**31.4 (7.3)**	**7.1 (3.1)**	**22.6 (7.3)**	**Round**	**6.2 (0.4)**	**R+S Type 2**	**Y**
Callosamia promethea	42.9 (26.2)	4.5 (2.2)	115.2 (38.6)	0.63 (0.17)	5.8 (2.1)	12.0 (7.2)	28.5 (2.6)	4.8 (2.4)	24.5 (4.7)	Round	3.9 (0.2)	Scalloped	Y
Calosaturnia mendocino	40.0 (35.2)	4.9 (2.8)	226.1 (186.8)	1.19 (0.83)	23.4 (18.8)	4.5 (2.6)	n/a	n/a	n/a	Round	3.8	Scalloped	N
Copaxa curvilinea	4.2 (3.2)	1.8 (0.8)	390.1 (60.2)	0.43 (0.21)	10.1 (2.0)	4.3 (0.7)	31.4 (6.1)	6.7 (2.1)	19.3 (7.0)	Round	4.4	n/a	Y
Copaxa rufinans	52.1 (51.8)	2.8 (1.4)	70.0 (41.8)	1.14 (0.52)	8.2 (5.6)	37.8 (58.1)	30.0 (2.5)	6.2 (1.8)	15.9 (3.0)	Round	5.2 (0.4)	n/a	Y
Saturnia pavonia	79.8 (40.6)	4.5 (2.1)	75.6 (59.0)	0.47 (0.37)	4.0 (4.4)	23.8 (16.3)	39.1 (5.5)	7.3 (1.9)	24.8 (7.0)	Round	4.3 (0.3)	Scalloped	Y
Saturnia pyri	**83.3 (40.4)**	**8.6 (5.1)**	**103.0 (37.9)**	**0.61 (0.34)**	**4.4 (1.5)**	**12.5 (7.2)**	**34.3 (12.6)**	**9.9 (4.3)**	**29.6 (9.0)**	**Round**	**6.0 (0.4)**	**Scalloped**	**Y**
SPHINGIDAE: Sphinginae													
Acherontia atropos	34.6 (17.0)	2.6 (1.1)	78.4 (38.6)	1.29 (0.63)	9.2 (3.2)	22.3 (11.5)	18.8 (9.9)	6.9 (4.9)	22.4 (10.6)	Round	n/a	R+S Type 3	N
Manduca diffissa tropicalis	19.2 (3.7)	4.6 (1.5)	240.3 (62.3)	0.96 (0.42)	20.5 (3.1)	4.4 (3.0)	4.3 (0.1)	1.1 (0.4)	3.9 (1.0)	Round	5.6	R+S Type 3	Y
Manduca lefeburii	27.4 (24.3)	3.2 (1.9)	147.3 (102.0)	0.56 (0.42)	3.8 (2.7)	12.9 (12.9)	8.0 (0.7)	1.7 (0.5)	10.0 (5.8)	Round	5.5	n/a	N
Manduca pellenia	26.4 (26.6)	1.8 (0.8)	58.5 (19.5)	0.81 (0.29)	4.1 (1.8)	31.7 (9.6)	27.4 (11.2)	5.9 (3.7)	22.0 (10.5)	Round	6.8	R+S Type 3	N
Manduca sexta	53.0 (34.6)	3.2 (1.0)	80.9 (52.6)	0.72 (0.32)	6.8 (4.0)	22.0 (15.2)	28.1 (8.7)	5.4 (3.4)	27.4 (14.3)	Round	5.2 (0.2)	R+S Type 3	Y
SPHINGIDAE: Macroglossinae													
Eumorpha satellitia	28.3 (20.7)	2.3 (1.0)	92.7 (47.8)	0.69 (0.31)	3.7 (1.5)	21.8 (15.4)	n/a	n/a	n/a	Round	5.8 (3)	R+S Type 1	Y

[a] Units composed of single pulses were not included in pulse rate or duty cycle calculations, or frequency analyses.

[b] Median (rather than mean) IPI values were included as IPI tends to be heavily skewed.

[c] R+S refers to ridged and serrated mandibles.

Clicking was confirmed to coincide with mandibular movements in 35 videos from 13 individuals (Movie S1). Each of the 150+ click units observed in these videos corresponded to one closing movement, while no sound was produced as the mandibles re-opened. Most individuals are 'ambidextrous' in that sound units can be generated by either mandible moving along the distal edge and inner surface of the other. Generally, individuals are consistent with the side used within trains, although some individuals will switch which mandible moves internally between units within a train. It was possible to assess which rows of teeth were involved in sound production in 14 videos. Sound production begins when the distal edge of one mandible snaps against the distal edge of the opposing mandible, and sound continues as the distal edge contacts the row of teeth on the inner surface of the opposing mandible. The outer row of teeth, if contacted at all, only serves as a stopping point.

Proposed mechanism

Based on our results of the number of pulses per unit, mandible morphology, and observed mandibular movements, we propose that *A. polyphemus* produces sounds in a manner similar to snapping fingernails together. As one row of teeth is struck by a row of teeth on the opposing mandible, an individual pulse is produced. *Antheraea polyphemus* mandibles possess three rows of teeth, though because the distal edge row has a molar-like form with a dip in the middle, this row could act as two separate rows of teeth rather than one. There could therefore conceivably be up to nine PPU in a single unit. However, the maximum number of PPU observed in our data was 6 PPU.

Chirper reference: *Saturnia pyri*

To establish the identifying characteristics of a chirper, we describe the acoustics, morphology, and behaviour of 10 individuals of *S. pyri* (Figure 3(d–f)). A summary of acoustic (n = 30 units) and morphological (n = 6 individuals) characteristics is included in Table 1.

Acoustics

Chirp units range from 1.3 to 162.1 ms (mean 83.3 ± 40.4 ms) in duration and consist of 1 to 22 PPU (mean 8.6 ± 5.1) with an average rate of 103.0 ± 37.9 pulses/second. Pulses average 0.61 ± 0.34 ms in duration with a median IPI of 12.5 ± 7.2 ms. The unit dominant frequency averages 34.3 ± 12.6 kHz and ranges from 9.5 to 54.1 kHz.

Morphology and behaviour

Saturnia pyri mandibles are classified as scalloped (n = 6 pairs). The mandibles have a lightly serrated distal edge with no teeth on the inner or outer surfaces, but with longitudinal grooves on the inner surface that may correspond to the serrations of the opposing mandible's distal edge (Figure 3(d,e)). The head capsule of *S. pyri* is round with an average width of 5.95 mm (n = 6).

Chirping was confirmed to coincide with mandibular movements in 48 videos (Movie S2). We observed 300+ chirp units from six individuals, and each unit corresponded to a single closing movement, while no sound was produced as the mandibles re-opened. Individuals are 'ambidextrous' in that they can alternate which mandible slides across the other. Individuals are consistent with which mandible moves internally within a train, but may switch mandibles between trains. Based on 18 videos, it was determined that the distal edge/outer surface of one mandible slides along the inner surface of the other mandible.

Proposed mechanism

Based on the mandible morphology, observed mandibular movements, and the number of pulses produced per unit, we propose that the sounds of *S. pyri* are produced by a stick and slip mechanism (cf. Patek 2001). The distal edge and/or outer surface (scraper) of one mandible runs along the inner surface (file) of the other, and as the scraper slides, static friction causes it to become stuck. A build-up of sliding friction occurs as the scraper is pulled, causing the mandible to slip and produce the individual pulses of the sound units. While the terms 'file' and 'scraper' are often restricted to morphologically distinct structures of a sound-producing mechanism in insects (Dumortier 1963), since in this case the caterpillar can alternate which mandible slides along the other to produce sounds, it could be argued that functionally, the roles of file and scraper can alternate between the two mandibles.

How do clicking and chirping differ between references?

Antheraea polyphemus and *S. pyri* differ acoustically and morphologically in several respects (Figures 3, 4). The sounds differed significantly in unit duration, PPU, and duty cycle (Mann–Whitney U-tests, $p < 0.00001$), and −10 dB bandwidth (Mann–Whitney U-tests, $p < 0.005$) (Figure 4). *Antheraea polyphemus* mandibles are categorised as ridged and serrated Type 2 molar-like, while *S. pyri* mandibles are categorised as scalloped. Both species' head capsules are round and did not differ significantly in width (Mann–Whitney U-test, $p > 0.05$).

Spearman's rank-order correlation analysis revealed that the relationships between different acoustic traits varied between the two species. Two differences were verified as significant using Fisher's *z*-transformation (Table S2). Unit duration was significantly correlated with pulse rate ($r_S = -0.8329$) and IPI median ($r_S = 0.7749$) at $p < 0.00001$ in *A. polyphemus*, but not in *S. pyri*. In *S. pyri*, PPU was significantly correlated to pulse rate ($r_S = 0.5776$) and duty cycle ($r_S = 0.4603$) at $p < 0.05$, but these correlations were not significantly different from those of *A. polyphemus* (Table S2). Some traits were found to correlate in both species with no significant differences (Table S2), including unit duration being significantly correlated to PPU (*A. polyphemus* $r_S = 0.6896$; *S. pyri* $r_S = 0.8225$; $p < 0.0001$).

Based on these findings, we predict that the most reliable features for distinguishing between *A. polyphemus* and *S. pyri* sound-producing mechanisms are unit duration, PPU, mandible morphology, and correlated acoustic traits. Clicking species should possess ridged and serrated mandibles of any of the three types, while chirping species should possess mandibles with a smooth inner surface such as smooth or scalloped mandibles. Additionally, of the criteria based on analysis of correlations between acoustic traits, we predict that clicking species will show a stronger correlation between unit duration and pulse rate as well as between unit duration and IPI median than chirping species (but see next section). Duty cycle, while significantly different between the two

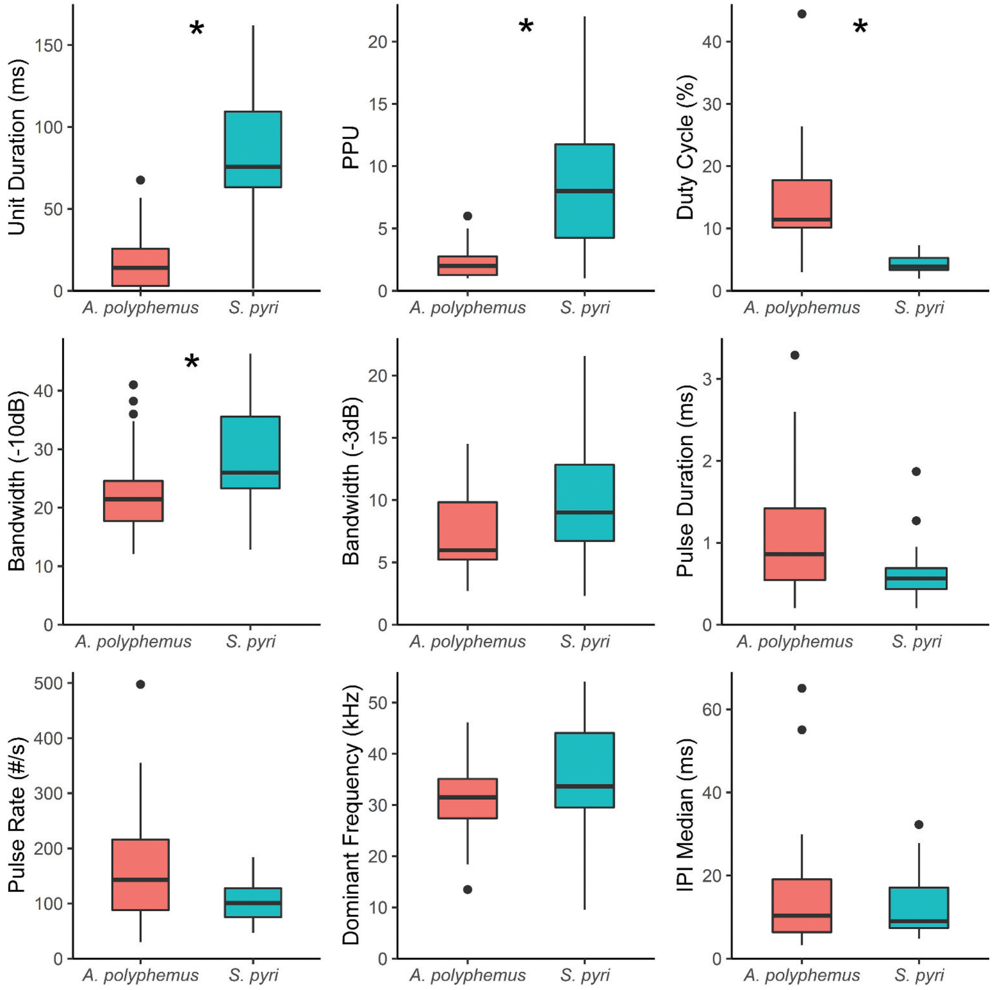

Figure 4. Boxplots of *Antheraea polyphemus* and *Saturnia pyri* acoustic traits. Traits that differed significantly between the two species are indicated with an asterisk, * (Mann–Whitney U-tests, $p < 0.005$).

species, is partially dependent on PPU and therefore any differences in duty cycle should be captured by differences in PPU. Bandwidth measurements may be less reliable as spectral data is not always available for all species. These results provide a guide for differentiating clicking and chirping mechanisms in other mandibular sound-producing species.

Classifying clickers and chirpers: comparisons with references

For each of the 18 additional species, we describe their acoustic, morphological, and behavioural characteristics (Table 1, Figure 5). We then propose for each species whether they are clickers or chirpers based on the main criteria established from the reference species (see previous section). These criteria include unit duration and PPU (assessed for significant differences from the references using Mann–Whitney U-tests), as well as mandible morphology and correlation analysis results (Table 2). However, correlation analysis results were highly variable among the species (Table 2). Therefore, our categorisations are based primarily on the Mann–Whitney results and mandible morphology. Below, we provide a brief summary of our categorisations and rationale.

Eight of the 18 species were categorised based on their similarities to one of the reference species with regards to unit duration and PPU. Mandible information was available for six of these eight species, and matched the morphology of the reference they were more similar to acoustically. These species are *A. jason*, *A. luna*, *A. oculea*, *C. curvilinea*, *M. lefeburii*, *M. pellenia*, and *E. satellitia* as clickers, and *S. santarosensis* as a chirper (Table 2). The remaining 10 species did not meet all predictions from the previous section, and therefore were assessed using a combination of criteria described below. Sound waveforms and morphological features (where available) of all species are shown in Figure 5, and Table 2 summarises our comparison of each species to the references. Note that Table 2 also includes the predicted category from the logistic regression model (see next section). An expanded version of Table 2 that includes *p*-values and correlation coefficients is available as supplementary material (Table S3).

Citheronia lobesis, Saturniidae: Ceratocampinae

Citheronia lobesis is an outlier because it produced sound units that were significantly longer in duration and with 14–33 PPU (n = 6 units), significantly more PPU compared to *S. pyri* and *A. polyphemus*. Though *C. lobesis* was designated as a chirper in a prior publication (Bura et al. 2016), without mandible videos to confirm that movements correspond with sound production, we are not confident in categorising this species as either a clicker or a chirper (see *Discussion* for alternative hypotheses explaining the proposed mechanism).

Actias selene, Saturniidae: Saturniinae

Actias selene sound units were significantly longer in duration than *A. polyphemus* and contained 1–6 PPU (n = 15 units), although there was one outlier unit that contained 11 PPU. Though PPU differed significantly from both *A. polyphemus* and *S. pyri*, the range of PPU is more similar to *A. polyphemus* (also 1–6 PPU) than to *S. pyri* (1–22 PPU). The lower values and narrow range of PPU in addition to ridged and serrated Type 2 mandibles lead us to classify *A. selene* as a clicker.

Antheraea pernyi, Saturniidae: Saturniinae

Antheraea pernyi sound units were significantly longer in duration than *A. polyphemus* and contained 2–7 PPU (n = 15 units). Though PPU differed significantly from both *A. polyphemus* and *S. pyri*, the range of PPU is more similar to *A. polyphemus* (1–6 PPU) than to *S. pyri* (1–22 PPU). The lower values and narrow range of PPU in addition to ridged and serrated Type 2 mandibles lead us to classify *A. pernyi* as a clicker.

Callosamia promethea, Saturniidae: Saturniinae

Callosamia promethea produced sound units significantly longer in duration than *A. polyphemus* and shorter than *S. pyri*. The units contained 2–9 PPU (n = 11 units), significantly more than *A. polyphemus* and less than *S. pyri*. Though *C. promethea* was designated as a clicker in a prior publication (Bura et al. 2016), the scalloped mandibles

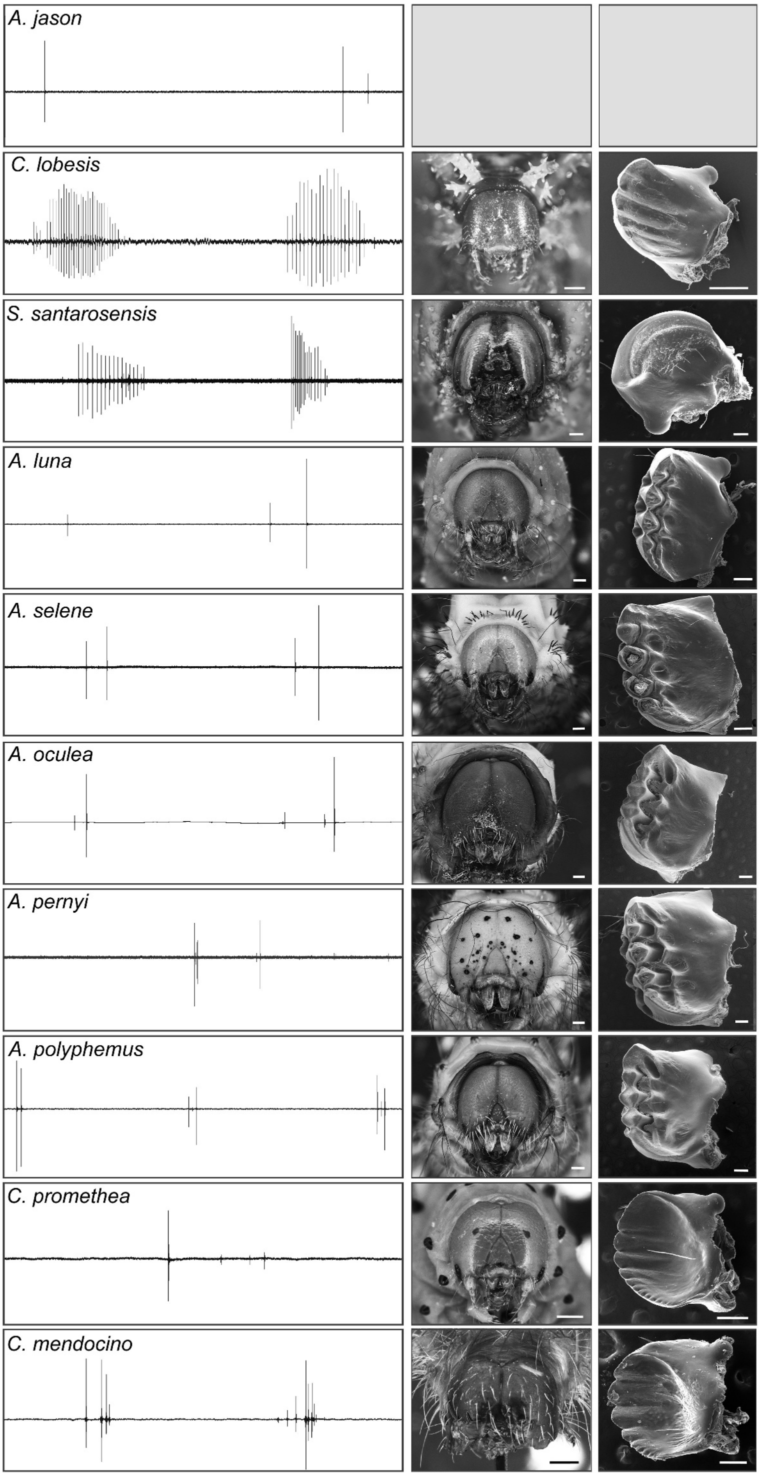

Figure 5a. Representative sound waveforms, head capsules, and a single mandible for species examined in this study. Species are presented in the same order as they are listed in Table 1. All sound traces are 700 ms in duration. Scale bars for head capsules are 1 mm and for mandibles are 250 µm. Gray boxes indicate that data were not available for that species.

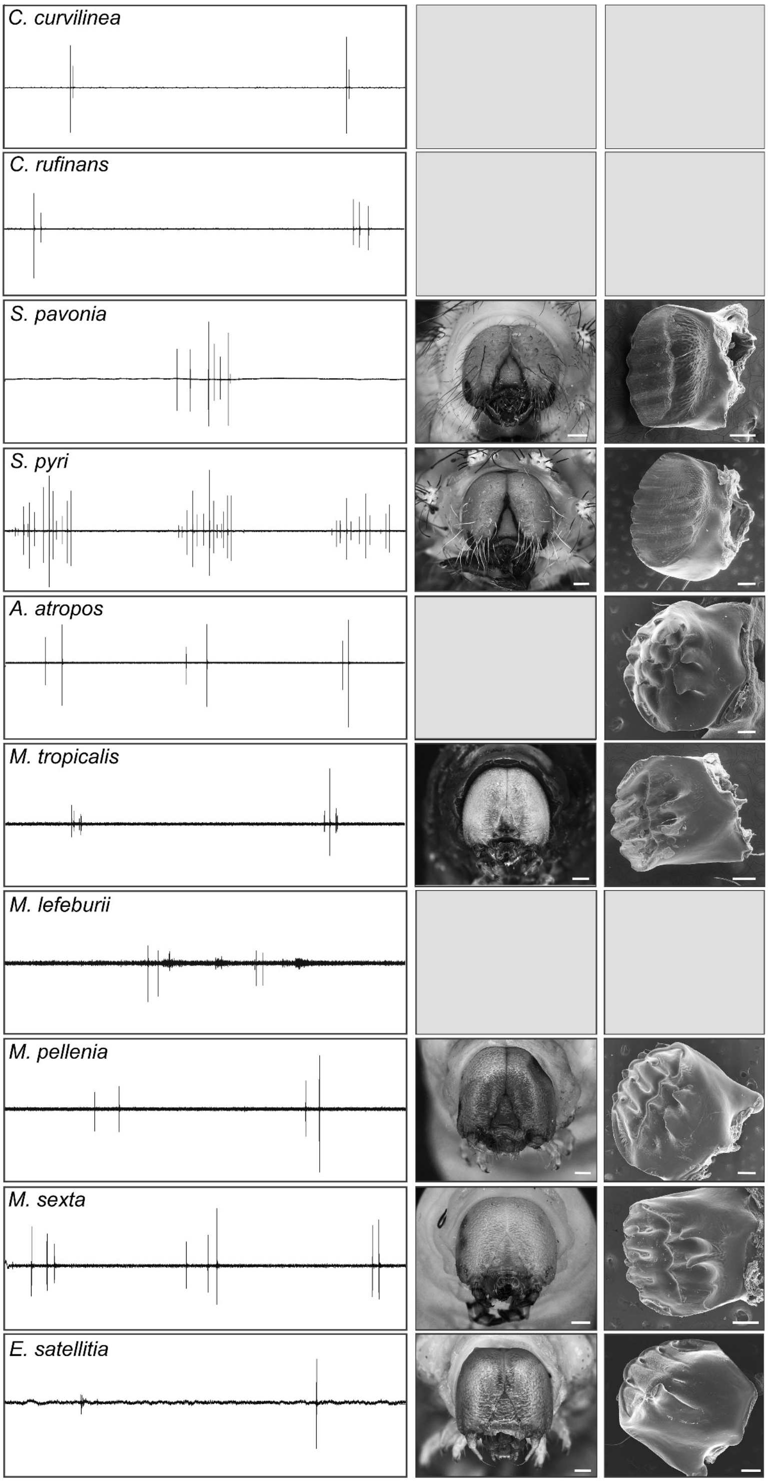

Figure 5b. (Continued).

Table 2. Comparative analysis of 20 species of Bombycoidea including the two reference species, *A. polyphemus* and *S. pyri* (in bold), and 18 additional species. Mann–Whitney U-tests were conducted for each species with the two reference species for unit duration and PPU. Significant differences are indicated with '*' ($p < 0.025$), while non-significant differences are indicated with 'ns'. Correlations between unit duration and pulse rate or between unit duration and IPI median are indicated by 'Present/Absent'. Mann–Whitney *p*-values and correlation coefficients are presented in Supplementary Table S3.

	Comparative Analyses								
	Mann–Whitney U-tests				Correlations				
	A. polyphemus		*S. pyri*		Unit Duration				
Species	Unit Duration	PPU	Unit Duration	PPU	Pulse Rate	IPI Median	Mandible Category[a]	Proposed Category	Logistic Regression Category
Adeloneivaia jason	ns	ns	*	*	n/a	n/a	n/a	Clicking	Clicking
Citheronia lobesis	*	*	*	*	Absent	Absent	Scalloped	Undetermined	Chirping
Schausiella santarosensis	*	*	ns	ns	Absent	Absent	Smooth	Chirping	Chirping
Actias luna	ns	ns	*	*	Present	Present	R+S Type 2	Clicking	Clicking
Actias selene	*	*	ns	*	Present	Present	R+S Type 2	Clicking	Clicking
Antheraea oculea	ns	ns	*	*	Present	Absent	R+S Type 2	Clicking	Clicking
Antheraea pernyi	*	*	ns	*	Present	Present	R+S Type 2	Clicking	Clicking
Antheraea polyphemus	**n/a**	**n/a**	**n/a**	**n/a**	**Present**	**Present**	**R+S Type 2**	**Clicking**	**Clicking**
Callosamia promethea	*	*	*	*	Absent	Absent	Scalloped	Chirping	Clicking
Calosaturnia mendocino	ns	*	*	ns	Present	Absent	Scalloped	Chirping	n/a
Copaxa curvilinea	ns	ns	*	*	n/a	n/a	n/a	Clicking	Clicking
Copaxa rufinans	ns	ns	ns	*	Present	Absent	n/a	Clicking	Clicking
Saturnia pavonia	*	*	ns	*	Absent	Absent	Scalloped	Chirping	Chirping
Saturnia pyri	**n/a**	**n/a**	**n/a**	**n/a**	**Absent**	**Absent**	**Scalloped**	**Chirping**	**Chirping**
Acherontia atropos	*	ns	*	*	Absent	Absent	R+S Type 3	Clicking	Clicking
Manduca diffissa tropicalis	ns	*	*	ns	Absent	Absent	R+S Type 3	Clicking	Clicking
Manduca lefeburii	ns	ns	*	*	n/a	n/a	n/a	Clicking	Clicking
Manduca pellenia	ns	ns	*	*	n/a	n/a	R+S Type 3	Clicking	Clicking
Manduca sexta	*	*	ns	*	Present	Absent	R+S Type 3	Clicking	Clicking
Eumorpha satellitia	ns	ns	*	*	Absent	Absent	R+S Type 1	Clicking	n/a

[a] R+S refers to ridged and serrated mandibles.

lead us to classify *C. promethea* as a chirper. However, this species may warrant further investigation.

Calosaturnia mendocino, Saturniidae: Saturniinae

Calosaturnia mendocino produced short sound units with 1–9 PPU ($n = 8$ units), traits which did not differ significantly from *S. pyri*. However, unit duration also did not differ significantly from *A. polyphemus*. Nevertheless, based on the higher number of pulses and the scalloped mandibles, we classify *C. mendocino* as a chirper.

Copaxa rufinans, Saturniidae: Saturniinae

Copaxa rufinans produced short sound units with 1–5 PPU (n = 8 units), traits which did not differ significantly from *A. polyphemus*. While unit duration did not differ significantly from *S. pyri* sounds either, the number of PPU did, and we therefore classify *C. rufinans* as a clicker.

Saturnia pavonia, Saturniidae: Saturniinae

Saturnia pavonia sound units were long in duration and contained 2–9 PPU (n = 15 units), both traits that were significantly different from *A. polyphemus*. While PPU also differed significantly from *S. pyri*, the length of the sound units, the higher number of pulses, and the scalloped mandibles lead us to classify *S. pavonia* as a chirper.

Acherontia atropos, Sphingidae: Sphinginae

Acherontia atropos produced short sound units with 1–5 PPU (n = 15 units), traits that differed significantly from *S. pyri*. While unit duration also differed significantly from *A. polyphemus*, due to the sounds differing significantly from *S. pyri* and the ridged and serrated Type 3 mandibles, we classify *A. atropos* as a clicker.

Manduca diffissa tropicalis, Sphingidae: Sphinginae

Manduca diffissa tropicalis produced short sound units not significantly different in duration from *A. polyphemus*, but with 3–7 PPU (n = 5 units) which was not significantly different from *S. pyri*. However, due to the very short sound units and the ridged and serrated Type 3 mandibles, we classify *M. diffissa tropicalis* as a clicker. It should be noted that the tested individual was heavily parasitised at the time of the recordings, and so the sounds produced may not be truly representative of this species.

Manduca sexta, Sphingidae: Sphinginae

Manduca sexta sound units were significantly longer in duration than *A. polyphemus* and contained 1–5 PPU (n = 12 units). While their PPU differed significantly from both *A. polyphemus* and *S. pyri*, the range of PPU is more similar to *A. polyphemus* (1–6 PPU) than to *S. pyri* (1–22 PPU). The lower values and narrow range of PPU in addition to ridged and serrated Type 3 mandibles lead us to classify *M. sexta* as a clicker.

Classifying clickers and chirpers: predictive model

A model using logistic regression analysis was designed to test our categorisations of clickers and chirpers from goal two. Univariate analysis on the training dataset revealed each acoustic trait to be a significant predictor variable at $p < 0.1$. The final model after stepwise regression included PPU, duty cycle, and dominant frequency, in order of most to least significant at classifying clicking versus chirping (Equation 1; Table 3). The final model was significantly more effective at predicting clicking versus chirping than the null model (likelihood ratio test, $p < 0.01$), fit the data well (Hosmer–Lemeshow test, $p > 0.05$), and had a pseudo-R^2 value of 0.49 indicating the included acoustic traits were important to predicting sound category (Table 3). When tested on the remaining species, the model supported all of our categorisations from the previous section except for *C. promethea* which the model classified as a clicker (Table 4, Figure 6). However, there was considerable

Table 3. Results of logistic regression analysis for predicting whether a sound is a click or a chirp.

Trait	Estimate	Standard Error	*z* value	*p*
(Intercept)	−4.8922	1.4640	−3.3420	0.0008*
PPU	0.7959	0.2316	3.4360	0.0006*
Duty Cycle	−0.1515	0.0812	−1.8650	0.0621*
Dominant Frequency	0.0612	0.0359	1.7040	0.0883*
Model Evaluation		**χ2**	**df**	***p***
Likelihood Ratio Test[a]		57.7910	3	1.74E-12*
Hosmer–Lemeshow Test		5.0885	8	0.7481

Significant values are indicated with asterisks, * ($p < 0.1$).
Pseudo-R^2 (McFadden) = 0.4917.
[a] Likelihood ratio test was conducted against the null model.

Table 4. Probability estimates and confidence intervals of each species producing either clicks or chirps based on the logistic regression model. Probabilities 0–0.5 are designated as clicks, and 0.5–1 are designated as chirps.

Species	Probability	95% Confidence Interval	Category
Adeloneivaia jason	0.1322	(0.0446, 0.3324)	Click
Citheronia lobesis	1.0000	(0.9693, 1.0)	Chirp
Schausiella santarosensis	0.9985	(0.8889, 1.0)	Chirp
Actias luna	0.0297	(0.0030, 0.2363)	Click
Actias selene	0.4239	(0.2363, 0.6362)	Click
Antheraea oculea	0.0126	(0.0010, 0.1356)	Click
Antheraea pernyi	0.1436	(0.0306, 0.4710)	Click
Antheraea polyphemus	0.0460	(0.0083, 0.2178)	Click
Callosamia promethea	0.4781	(0.2695, 0.6946)	Click
Copaxa curvilinea	0.0746	(0.0219, 0.2248)	Click
Copaxa rufinans	0.1650	(0.0728, 0.3321)	Click
Saturnia pavonia	0.7367	(0.5310, 0.8737)	Chirp
Saturnia pyri	0.9734	(0.7528, 0.9977)	Chirp
Acherontia atropos	0.0608	(0.0139, 0.2288)	Click
Manduca diffissa tropicalis	0.0128	(0.0004, 0.3130)	Click
Manduca lefeburii	0.1018	(0.0156, 0.4476)	Click
Manduca pellenia	0.1438	(0.0543, 0.3295)	Click
Manduca sexta	0.2303	(0.1158, 0.4061)	Click

overlap of the confidence interval of *C. promethea* with chirping species (Figure 6). A similar overlap with chirping species was observed in *A. selene*. *Calosaturnia mendocino* and *E. satellitia* were not included as we lack spectral data on their sounds.

$$Log(p/(1-p)) = -4.8922 + 0.7959(PPU) - 0.1515(duty\ cycle) + 0.0612(dominant\ frequency) \tag{1}$$

Size and unit duration

To account for differences between species' acoustic traits due to differences in size, we investigated if there is a relationship between head capsule width (as a proxy for mandible size) and unit duration. Unit duration was not dependent on head capsule size in either clickers or chirpers (clickers: adjusted $R^2 = 0.264$, $p = 0.0502$; chirpers: adjusted $R^2 = 0.577$, $p = 0.0845$), nor was a significant correlation observed (clickers: $r_S = 0.58$, $p = 0.0502$; chirpers: $r_S = 0.83$, $p = 0.0845$). Head capsule size therefore does not appear to be

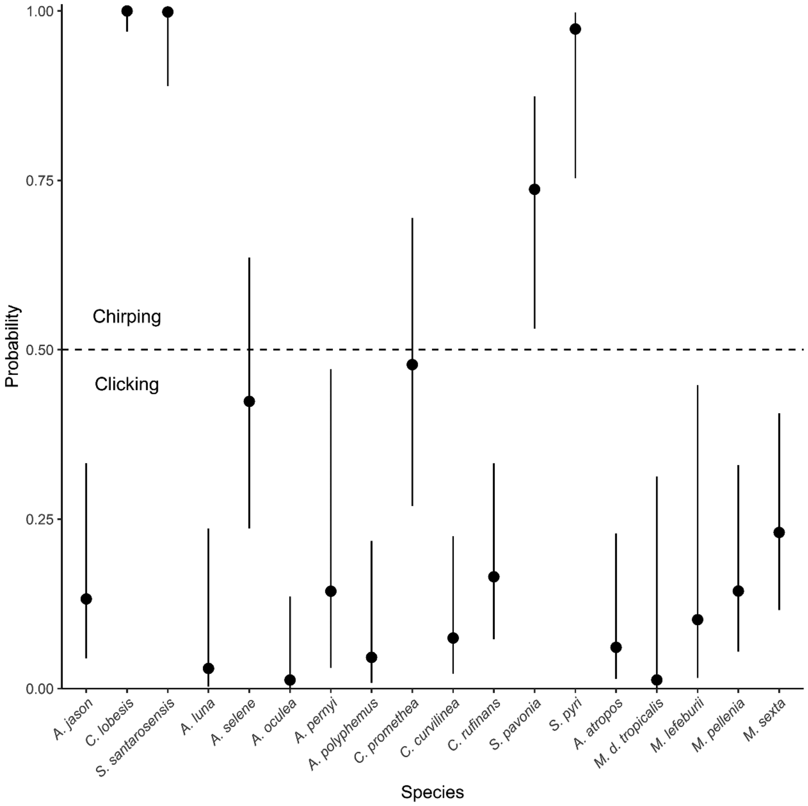

Figure 6. Predicted probabilities and confidence intervals of each species being either a clicker (lower half) or chirper (upper half) based on the logistic regression model.

important for distinguishing clicking versus chirping, nor is size likely to have a large effect on unit duration.

Classifying clickers and chirpers: an overview

We conclude that clicking and chirping are distinct mechanisms. Based on results from the two reference species, we identified unit duration, PPU, correlated acoustic traits, and mandible morphology as criteria to distinguish the mechanisms. However, correlated acoustic traits were not consistent in classifying all species and varied by family (Table 2). The logistic regression model also identified PPU as an important predictor, in addition to duty cycle and dominant frequency. Of the 18 species tested by the model, only one species (*C. promethea*) was categorised differently than from our comparisons to the references.

Following the categorisation of all species into clickers and chirpers, the range of characteristics per category can be described as follows. Clicks range from 0.29 to 222.90 ms in duration (mean 40.42 ± 42.78) with 1–11 PPU (mean 2.72 ± 1.54) (Figure 7). The dominant frequencies of clicks range from 4.10 to 46.62 kHz (mean 25.69 ± 10.12), while duty cycle ranges from 0.25 to 89.62% (mean 10.64 ± 12.90) (Figure 7). All clicking species possess ridged and serrated mandibles of one of the three types. Chirps range

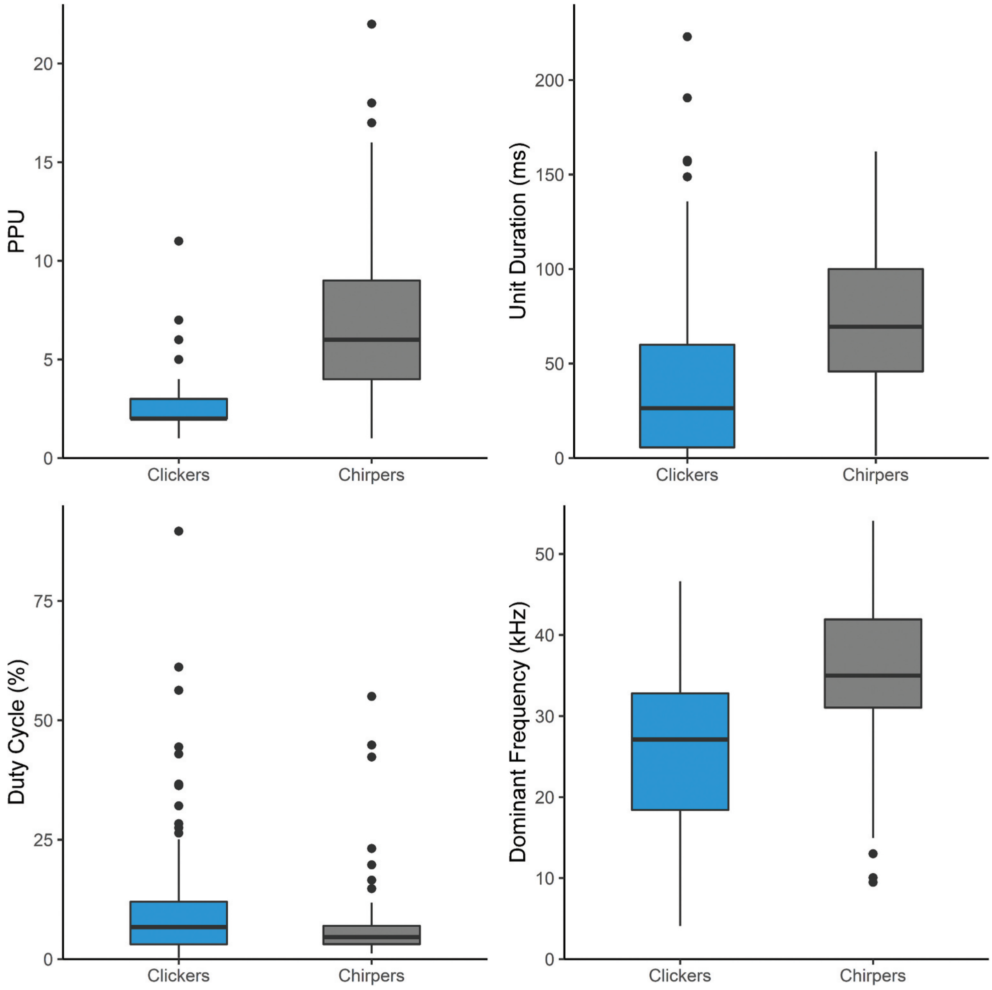

Figure 7. Overview of acoustic traits identified as important for distinguishing clickers and chirpers based on our analysis of the reference species and the logistic regression model. Boxplots include data from all categorised clickers and chirpers (with *Callosamia promethea* included as a chirper), except for *Citheronia lobesis.*

from 1.27 to 162.10 ms in duration (mean 71.96 ± 40.22) with 1–22 PPU (mean 7.25 ± 4.81) (Figure 7). While units with only single pulses do occur in chirping species, they are not common (2/79 single-pulse units among chirpers, compared to 32/157 single-pulse units among clickers). The dominant frequencies of chirps range from 9.49 to 54.07 kHz (mean 35.52 ± 10.26), while duty cycle ranges from 1.16 to 55.00% (mean 7.21 ± 9.47) (Figure 7). All chirping species possess smooth or scalloped mandibles.

When considering these results, we propose the following approach when evaluating new mandible sound producing species. We recommend assessing PPU, unit duration, and mandible morphology by comparing them to the reported acoustic ranges and mandible categories of clicks and chirps. PPU and mandible morphology may be more reliable than unit duration for categorising species since unit duration was not an important predictor in the logistic regression model, likely due to a strong correlation between unit duration and PPU in most species (results not shown). While the regression model was very reliable for categorising species, the model requires duty cycle and dominant frequency measures – traits that can be difficult to measure depending on the availability of spectral data or the quality of the sound files. Additionally, the model does not take mandible morphology into consideration, but we argue that mandible morphology should always be assessed. Therefore, we recommend that if spectral data is available, then the model is used in conjunction with mandible morphology.

Discussion

Clicking and chirping are distinct mechanisms

In this study, we report on the acoustic and morphological traits of silk and hawk moth caterpillars that use their mandibles to produce defence sounds. Differences in acoustic traits and mandible morphology allowed us to conclude that there are two separate categories of mandibular sound-producing mechanisms: clicking and chirping. Clicking sounds are produced when the distal edge of one mandible snaps against the rows of teeth of the other mandible, producing pulses as the rows of teeth encounter each other. Chirping sounds are produced as the distal edge of one mandible slides along the inner surface of the other, producing pulses as the mandible sticks and slips due to a build-up of friction. We found PPU and mandible morphology are the most reliable features for assigning a species to either clicking or chirping, though unit duration is also helpful (see *Overview* above). However, not all species were categorised the same by our two methods (i.e. comparison to reference species and the logistic regression model). *Actias selene* and *C. promethea* were classified as clickers according to the model, though their confidence intervals (Figure 6) overlapped with chirpers. We nevertheless assigned *A. selene* as a clicker and *C. promethea* as a chirper based on our conclusion that mandible morphology is very important for classifying these sound-producing mechanisms. Another species, *Citheronia lobesis*, had previously been categorised as a chirper (Bura et al. 2016), and this is in agreement with the model results. However, given the significant differences between this species' sounds and the two references, and the fact that mandibular movements were not observed in this species, we are not confident in this categorisation. It is more likely that *C. lobesis* is a vocaliser as two of its sister species, *C. bellavista* and *C. sepulcralis*, produce sounds similar to *C. lobesis*, but do so while holding their mouthparts open (Low, unpublished). We strongly recommend when assessing sound-producing mechanisms in a new species that researchers first confirm whether sounds coincide with mandibular movements.

Why is it important to distinguish between clickers and chirpers? As both involve sound production via mandibular movements, it could be argued that they be categorised as one mechanism in a comparative analysis with other sound producers. However, for any comparative analysis it is highly recommended that researchers account for phylogeny due to the non-independence of related species (Felsenstein 1985). Since mandible morphology, an important distinguishing trait between clickers and chirpers, can be tied to phylogeny (e.g. Bernays and Janzen 1988; Bernays 1991), categorising clicking and chirping separately in a comparative analysis allows one to account for some of the variation among species due to their phylogenetic non-independence. Additionally, because the two sound-producing mechanisms are strongly linked to mandible type, and mandible morphology has been linked to diet (see Bernays 1991), it is possible that diet type could be a predictor of mandibular sound-producing mechanisms in Bombycoidea.

Evolution of mandibular stridulation

Sound production using mandibles is not unique to Bombycoidea caterpillars, occurring in different insect orders and in different contexts. For example, in Orthoptera, mandibular stridulation has been reported in Acrididae grasshoppers in the contexts of both defence and courtship (Alexander 1960; Blondheim and Frankenberg 1983), and in Anostostomatidae tusked wetas in the context of defence (Field 1993). Mandibular scraping against the leaf surface (a form of stridulation) is used in other larval Lepidoptera for defence against intruders (e.g. Gracillariidae, Fletcher et al. 2006; Drepanidae, Guedes et al. 2012). Scarabaeidae chafers (Coleoptera) produce sounds using maxilla-mandibular stridulation, though the function of these sounds isn't fully understood (Görres and Chesmore 2019). Sound production using mandibles has presumably evolved convergently in these taxa, and which selection pressures or conditions favoured the evolution of mandible sounds is an interesting question. These conditions could be related to pre-existing morphological and/or behavioural traits, such as hard mandibles used for eating wood or tough leaves, or for biting during fighting. Strong mandibles may also require increased head capsule musculature or certain types of muscle fibres (Clissold 2007). In soft-bodied insects such as caterpillars, there may be few alternative mechanisms for sound production as mandibles are one of only a few sclerotised structures (Low et al. 2021).

In Bombycoidea caterpillars, we propose a few hypotheses relating to the evolution of mandibular defence sounds. Mandibular stridulation may have originated from non-signalling defensive behaviours such as biting or regurgitation. Several caterpillars attempt to bite attacking forceps (*M. sexta*, *M. pellenia*, *A. atropos*; see Bura 2010), and biting motions may have resulted in mandibles rubbing to produce incidental sounds. Alternatively, sounds may have evolved from movements associated with re-imbibing regurgitant. Many caterpillars that regurgitate defensively are capable of re-imbibing their regurgitant (see Grant 2006; Bura et al. 2016), and we have observed several species producing occasional clicking sounds incidentally while re-imbibing (*A. polyphemus*, *A. luna*, *C. rufinans*; personal observations). Thus, clicking and chirping defence sounds may have originated through the process of ritualisation of these non-signalling defences (cf. Scott et al. 2010). Other factors that may have influenced the evolution of sound production in these caterpillars include pre-existing defences, morphological or behavioural traits, or selection pressures imposed by predators. For example, Bura et al. (2016) provided evidence that mandibular sounds (clicking and chirping) were highly correlated with chemical defences, suggesting that these sounds function as warning signals. In contrast, non-mandibular defence sounds (vocalising or whistling) were poorly correlated with chemical defences and proposed to function in startling predators. Morphological factors such as head shape could also be a precursor to the type of sound production mechanism (Bura 2010). For instance, in this study, we found that the majority of species that produce mandibular sounds have a round head capsule (Table 1), which may allow for greater muscle attachment points to provide sufficient force to produce sounds. Future research should take a phylogenetic comparative approach to studying selection pressures that may have resulted in the evolution of different sound-producing mechanisms.

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Data availability statement

For access to acoustic data from this study, please contact the corresponding author at jayneyack@cunet.carleton.ca.

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