

1 **Title:**

2 The Ander's organ: A mechanism for Anti-predator Ultrasound in a Relict
3 Orthopteran

4

5 **Running title:**

6 Ultrasonics in a Relict Orthopteran

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24 **Summary statement**

25 Motion tracing, acoustic recording, and micro-CT imaging reveals a unique ultrasonic
26 organ in a relict insect. Statistical quantification of organ morphology reveals sexually
27 driven loss of the organ in males.

28 **Abstract**

29 The use of acoustics in predator evasion is a widely reported phenomenon amongst
30 invertebrate taxa, but the study of ultrasonic anti-predator acoustics is often limited to
31 the prey of bats. Here, we describe the acoustic function and morphology of a unique
32 stridulatory structure in the relict orthopteran *Cyphoderris monstrosa* (Ensifera,
33 Hagloidea): the Ander's organ. This species is one of just eight remaining members
34 of the family Prophalangopsidae, a group with a fossil record of over 90 extinct
35 species widespread during the Jurassic. We reveal that the sound produced by this
36 organ has the characteristics of a broadband ultrasonic anti-predator defence, with a
37 peak frequency of 58 ± 15.5 kHz and a bandwidth of 50 kHz (at 10 dB below peak).
38 Evidence from sexual dimorphism, knowledge on hearing capabilities and
39 assessment of local predators, suggest the signal likely targets ground-dwelling
40 predators. Additionally, we reveal a previously undescribed series of cavities
41 underneath the organ that likely function as a mechanism for ultrasound
42 amplification. Morphological structures homologous in both appearance and
43 anatomical location to the Ander's organ are observed to varying degrees in 4 of the
44 7 other extant members of this family, with the remaining 3 yet to be assessed.
45 Therefore, we suggest that such structures may either be more widely present in this
46 ancient family than previously assumed, or have evolved to serve a key function in
47 the long-term survival of these few species, allowing them to outlive their extinct
48 counterparts.

49

50 **Introduction**

51 Invertebrates have evolved a remarkable array of modes of communication, from
52 chemical markers and aposematic colours to acoustic, vibrational, and behavioural
53 cues. Of these, some of the best studied are the mechanisms for conspecific
54 communication; be it for kin recognition, competition, or mate attraction [1]. But of all
55 communication channels, the most crucial in the context of natural selection are the
56 signals for anti-predator defense. These are typically divided into two classes: 1)
57 primary defense mechanisms; the passive traits of an organism which operate
58 regardless of predator presence; and 2) secondary defense mechanisms; those
59 traits which function exclusively in direct or anticipated presence of a predator [2,3].

60 In animals as small as insects, passive physical defenses such as spines may serve
61 little resistance against vertebrate predators that are often much larger, faster, and
62 have more sophisticated sensory and cognitive abilities [4]. As a consequence,
63 insects have evolved a vast array of secondary defence mechanisms that fulfil an
64 important role in avoiding predation [3]. These defences include the more
65 characteristic traits of insects such as the hymenopteran sting, the spray of
66 bombardier beetles [5], and the saliva of assassin bugs [6], and often function as a
67 composite of behavioural, physical, and chemical elements. The mechanisms by
68 which these defences communicate to a predator differ greatly depending on both
69 the predator and the prey. While the examples above highlight physical deterrents,
70 many insects have evolved alternative ways to communicate to predators, such as
71 visual displays or acoustics. Acoustic secondary defences have driven studies of
72 predator-prey dynamics ever since early studies of invertebrate communication [7].
73 In this context, sounds have been found to facilitate predator startle responses [8],
74 aposematic (Batesian) mimicry [9], and even signal jamming of echolocating
75 predators [9].

76 In Ensifera (Insecta, Orthoptera; primarily bush-crickets or katydids, crickets, wētā
77 and grigs), a great variety of primary and secondary anti-predator defences exist
78 [10]. Their acoustic signals have evolved as a key mechanism in both conspecific
79 communication and secondary anti-predator defence [11]. These signals are typically
80 produced by wing stridulation, in which a series of teeth (the file) on one wing
81 engages with a scraper on the opposite wing to produce vibrations subsequently
82 amplified as sound by specialized wing cells [12–14]. However, other mechanisms,
83 including many which may have initially evolved as a by-product of a physical
84 defensive component, are also observed [15–17]. Most well studied are the
85 abdominal stridulatory mechanisms of Stenopelmatoidea (wētā, gryllacridids, and
86 allies; [17–20]). All known species of this superfamily exhibit femoro-abdominal
87 stridulation, in which pegs or scrapers on the base of the hindlegs strike a series of
88 abdominal teeth during defensive kicking behaviour [18]. Some also display tergo-
89 tergal stridulation, in which successive abdominal tergites which carry file and
90 scraper mechanisms strike one another during telescopic abdomen compression,
91 resulting in sound production [17]. The sounds produced by these organs vary
92 greatly in carrier frequency, from as low as 4 kHz, up to 32 kHz [18,21]. However,

93 despite the ample knowledge of conspecific acoustic communication in Ensifera, the
94 diversity and role of sound in the evolution of ensiferan anti-predator defense, and
95 descriptions of organ presence, remains limited [18,21].

96 In the great grig, *Cyphoderris monstrosa* Uhler, a relict ensiferan of the family
97 Prophalangopsidae whose members were widespread during the Jurassic [22],
98 secondary defence mechanisms appear to have taken on a variety of forms that
99 differ between the sexes. When disturbed, males produce an acoustic signal by wing
100 stridulation (the same mechanism as used for production of the conspecific song
101 [23]). However, all individuals of this species possess an additional stridulatory
102 mechanism on the abdomen, the Ander's organ. In 1939, Swedish entomologist Kjell
103 Ander hypothesised (reasoning from dead museum specimens) that this organ, a
104 small pair of stridulatory files located on the lateral surfaces of the first abdominal
105 tergite (Fig. 1), should function with an accompanying row of teeth along the
106 posterior edge of the metanotum to produce sound [24], however this hypothesis and
107 any acoustic components of the organ have not since been investigated, nor have
108 any investigated the organ function or significance in the evolution of anti-predator
109 defence. *Cyphoderris monstrosa* offers a unique opportunity to understand ancient
110 ensiferan biology; as one of only eight extant species (Table S1) of a family
111 containing over 90 species known only from fossils [22,25,26]. Therefore, 80 years
112 on, we test Ander's hypotheses; statistically quantifying the morphology of the organ
113 between sexes and life stages, investigating the acoustic signal, and describing the
114 mechanism and function of the Ander's organ. In addition, we comment on the
115 identification of similar morphological features across four of the other seven extant
116 prophalangopsids and discuss the broader implications of these findings in the
117 evolution of anti-predator acoustics.

118

119

120 **Materials and methods**

121 ***Live Specimens***

122 *C. monstrosa* were hand-captured from William A. Switzer Provincial Park, Alberta,
123 Canada (53°29'0.51"N, 117°49'32.55"W) after sunset at the Kelley's Bathtub day use

124 area (N = 57) as well as the area around the Blue Lake centre (N = 53) between July
125 6th and 13th, 2019. From this sample of individuals collected as part of a project on
126 temporal and geographic variation in selection, a subset (4 adult males, 4 adult
127 females and 2 juveniles [1 of each sex]) were sent to the University of Lincoln, UK for
128 bioacoustic experiments. Differences in sex were identified by sex-specific external
129 genitalia.

130 While at the University of Lincoln, specimens were maintained on an *ad libitum* diet
131 of bee pollen (Sevenhills, Wakefield, West Yorkshire, UK), fresh carrot, and cat
132 biscuits (James wellbeloved, Somerset, UK) and had access to water. Each animal
133 was kept in an individual container in a cooled 24-hour incubator (PHCbi MIR-154,
134 PHC Holdings Corporation, Tokyo, Japan) on a 4-step temperature cycle (14h:10h
135 light/dark cycle) around a mean of 8 °C.

136

137 **Collection Specimens**

138 In addition to 4 of the individuals used in the bioacoustic study, a variety of other
139 specimens were used for a study of the morphology of the Ander's organ in *C.*
140 *monstrosa* (N = 53) and homologous structures in congeners *C. buckelli* (N = 6) and
141 *C. strepitans* (N = 4) and a closely related prophalangopsid species, *Paracyphoderris*
142 *erebeus* (N = 1). Details on collection locations and dates for all specimens are given
143 in Table S2.

144

145 **Acoustic recordings**

146 For measurements of the frequency composition and intensity of the Ander's organ
147 acoustics, specimens were placed on a 30 cm² surface of sound absorbent foam in
148 an acoustic chamber. A B&K 1/8" Type 4138 omnidirectional microphone (Brüel &
149 Kjær, Nærum, Denmark), calibrated using a B&K Type 4237 sound pressure
150 calibrator (Brüel & Kjær, Nærum, Denmark) was positioned 10 cm above the animal.
151 The specimen was agitated using a soft paintbrush to test the best methods of
152 inducing the use of Ander's organ. Brushing across the face elicited the most
153 frequent response. The consequent acoustic signals were recorded using a PSV-500
154 internal data acquisition board (PCI-4451; National Instruments, Austin, TX, USA) via

155 a preamplifier (B&K 2670, Brüel & Kjær, Nærum, Denmark) at a sampling frequency
156 of 512 kHz. A high pass filter set at 2 kHz was used to remove any low frequency
157 background noise, and final recordings were saved as .txt files.

158

159 ***Motion capture of Ander's organ mechanism***

160 Specimens were immobilised prior to experiments by freezing at -2 °C for 2 mins.
161 This method was selected as a natural method of immobilisation, as the species has
162 a freeze tolerance down to ~-9 °C [27]. While immobile, specimens were restrained
163 with non-toxic Blu tack (Bostik Ltd, Stafford, UK) to a custom-made acrylic mount.
164 Thin foam strips were attached laterally along the abdomen to prevent leg kicking but
165 allow unrestricted abdomen movement. A 1 mm² piece of reflective, non-scattering
166 tape (Salzmann 3M Scotchlite Reflective Tape, 3M Minnesota, USA) was then
167 attached using non-toxic insect marking glue (E.H. Thorns Ltd, Wragby, Lincolnshire,
168 UK) to the centre of the first abdominal tergite, which possesses the Ander's organ
169 stridulatory files. Vertical motion of the abdomen could then be recorded from the
170 reflection of this tape using a custom made opto-electronic photodiode motion
171 detector [28,29]. The mounted animal was then left for 5 minutes to fully recover
172 from freezing prior to recording. Data acquisition followed the same setup as with
173 acoustic recordings, but with an additional recording channel for the motion detector
174 (Fig. S1). All recordings were carried out at 18-22 °C and 50% RH between the
175 hours of 09:00 and 16:00.

176

177 ***Signal analyses***

178 Analysis of Ander's organ signals and averaging of frequency spectra was carried
179 out using custom scripts written in MATLAB R2019a (MathWorks, Natick, USA). This
180 analysis used 4096 FFT lines with signals recorded at a sampling frequency of 512
181 kHz. No filters were used on the signals other than a 2 kHz high-pass used during
182 recording. Averaging consisted of 50 pulses from 6 bouts across 4 individuals. Signal
183 waveforms and frequency spectra were also plotted using MATLAB.

184

185 ***Morphological analysis***

186 In adult and juvenile males and females used in the bioacoustic study, following
187 death by senescence, the length and width of the Ander's organ stridulatory file, as
188 well as tooth distribution and density, was imaged using an Alicona InfiniteFocus
189 microscope (Bruker Alicona Imaging, Graz, Austria) at 10x objective magnification,
190 resulting in images with a resolution of ~100 nm. Length, distribution, and density of
191 teeth were defined and measured using standardized techniques (Fig. S2).

192 Specimens from collections prior to 2019 (Table S2) had their Ander's organs (left
193 and right) imaged using a Leica Si9 stereomicroscope (Leica Microsystems, Wetzlar,
194 Germany). Morphological dimensions of the Ander's organ were measured from
195 microscope photographs using ImageJ [30] by standard scale bar calibration,
196 following the same standardized measurement definitions. Microscope photographs
197 of head width and left femur length were bundled using tpsUtil v1.70, and tpsDig2
198 v2.26 (<http://life.bio.sunysb.edu/morph/>) was used to place landmarks from which
199 linear dimensions were calculated.

200

201 ***μCT imaging and measurement***

202 X-ray μ-CT of one adult of each sex of was performed using a SkyScan 1172 μ-CT
203 scanner (Bruker Corporation, Billerica, MA, USA) with a resolution of 3 μm (45 kV
204 source voltage, 185 μA source current, 400 ms exposure and 0.1-deg rotation
205 steps). Prior to the scan, specimens (already preserved in ethanol) were removed
206 from their preservation containers and positioned in a custom-built holder in the CT
207 scanner. μ-CT projection images were reconstructed to produce a series of
208 orthogonal slices with NRecon (v.1.6.9.18, Bruker Corporation, Billerica, MA, USA),
209 and the 3D image captured using CTvox (Bruker Corporation, Billerica, MA, USA).
210 3D segmentation and rendering of the organ, and measurements of sub-organ cavity
211 length, was carried out in Amira-Aviso 6.7 (Thermo Fisher Scientific, Waltham,
212 Massachusetts, USA). We used the length measurements of the cavities to calculate
213 an estimate of resonance. This was calculated by the assumption that the cavity acts
214 as a cylindrical tube. In such tubes, the fundamental frequency (f_0) corresponds to
215 the wavelength that is twice the length of the tube, calculated as:

$$216 \quad f_0 \text{ (Hz)} = \frac{c}{2L} \quad (1)$$

217 where c is the speed of sound in air, and L is the length of the tube in metres.

218

219 **Statistical analyses of *Ander's organ morphology***

220 We used seven measurements to describe *Ander's organ morphology* in *C.*
 221 *monstrosa*: 1) number of stridulatory file teeth, 2) average file tooth length, 3)
 222 standard deviation of file tooth length, 4) average inter-tooth distance, 5) standard
 223 deviation of inter-tooth distance, 6) metanotal spine number, and 7) file length. 3 and
 224 5 allowed for a quantification of variation in organ morphology within individuals. In
 225 all cases, we averaged the calculated values for both left and right *Ander's organs* of
 226 each individual prior to statistical analysis. We analysed variation in *Ander's organ*
 227 morphology using a Multivariate General Linear Model (GLM) with Sex (male or
 228 female), Stage (juvenile or adult) and their interaction as fixed factors, and the above
 229 seven measurements as dependent variables. All statistical analyses were
 230 completed in IBM SPSS Statistics Version 26 (IBM Corporation, 2019).

231

232 **Results**

233 ***Morphology of the *Ander's organ****

234 The *Ander's organ* of *C. monstrosa* is present in both sexes, and all assessed life
 235 stages (Fig. 1), with larger individuals possessing larger organs (Table 1, Fig. 2).
 236 Given that body size differs both between females and males and between adults
 237 and juveniles (Table 1, Fig. 2A), we included body size as a covariate in our analysis
 238 of sex differences and developmental changes in *Ander's organ morphology*. We ran
 239 a multivariate GLM with seven *Ander's organ* measurements as dependent variables
 240 (see methods), initially including all possible three- and two-way interaction terms
 241 between the fixed factors (Sex and Stage) and covariate (Size). All nonsignificant
 242 interactions were removed in a stepwise fashion starting with the three-way
 243 interaction (Sex * Stage * Size: $F = 1.164$, $df = 7, 35$, $p = 0.348$), then the Sex * Size
 244 interaction ($F = 0.800$, $df = 7, 36$, $p = 0.592$), and then the Stage * Size interaction (F
 245 $= 1.972$, $df = 7, 37$, $p = 0.086$). *Ander's organ morphology* is sexually dimorphic, but

246 this dimorphism differs when considering either adults or juveniles (Sex * Stage
247 interaction, $F = 2.567$, $df = 7, 38$, $p = 0.029$). We therefore ran separate multivariate
248 GLMs for adults and juveniles with Sex as the fixed factor, Size as the covariate, and
249 their two-way interaction as independent variables. In neither adults nor juveniles
250 was the Sex * Size interaction term statistically significant (adults: $F = 0.511$, $df = 7,$
251 22 , $p = 0.816$; juveniles: $F = 2.049$, $df = 7, 7$, $p = 0.182$) and so it was removed from
252 both models. After controlling for body size, female *C. monstrosa* had larger Ander's
253 organs than males, but that difference was only statistically significant for adults
254 (adults: Sex $F = 14.399$, $df = 7, 23$, $p < 0.001$; juveniles: Sex $F = 1.601$, $df = 7, 8$, $p =$
255 0.261). This multivariate pattern was replicated for each individual measurement
256 (Fig. 2), with females having more stridulatory teeth, longer teeth, greater tooth
257 length standard deviation, longer inter-tooth distance, greater inter-tooth distance
258 standard deviation, more spines and longer organs than males, but only statistically
259 significantly so as adults (Fig. 2).

260 An assessment of other extant prophalangopsid species (*C. buckelli*, *C. strepitans*,
261 *Paracyphoderris erebeus*) uncovered undescribed morphological structures with
262 varying levels of similarity to the Ander's organ, sharing the presence of trailing
263 spines or hairs along the posterior edge of the metanotum (Fig. S3). In
264 *Paracyphoderris erebeus*, an Ander's-like organ was found, with ridges acting as
265 stridulatory teeth, and 8 clear metanotal spines ($n=1$, Fig. S3A). Due to the low
266 number of accessible specimens for these additional species, a detailed
267 quantification and comparison of morphological parameters has not yet been
268 possible.

269

270 **Acoustic signal analysis**

271 We attempted to elicit use of the Ander's organ in 4 adult males, 4 adult females and
272 2 juveniles (1 of each sex). Adult males consistently failed to produce a sound using
273 the organ, instead producing an acoustic defence with tegmina when perturbed (Fig.
274 S4). All adult females and all juveniles successfully produced sound using the organ
275 on at least one occasion, and of these, all but 1 adult female provided acoustic
276 recordings suitable for temporal and spectral analysis.

277 The signal generated by the Ander's organ of female and juvenile *C. monstrosa* was
278 found to consist of temporally unstructured pulse sequences (or bouts; Fig. 3A-C),
279 each containing a series of broadband ultrasonic pulses, with a peak frequency of 58
280 ± 15.5 kHz (mean \pm SD, N=50 pulses from 4 animals; Fig. 3D). The waveform of the
281 signal is highly variable in amplitude, ranging from \sim 30-100 dB SPL (re 20 μ Pa @ 1
282 cm). Pulses also display a high, but inconsistent, repetition rate at 69.1 ± 22.3
283 pulses/second, with 4-12 pulses per bout (Fig. 3B). Each pulse is extremely rapid,
284 with an average duration of 0.30 ± 0.02 ms (Fig. 3C). Welch's power spectral density
285 (PSD) analysis revealed the signal is highly broadband, with a bandwidth ranging
286 from 40-90 kHz at 10 dB below the maximum energy peak (Fig. 3D).

287

288 ***Ander's organ mechanism and additional defensive components***

289 We attached a 1 mm² piece of reflective tape to the metanotum of partially restrained
290 specimens of *C. monstrosa* and tracked Ander's organ use by following the
291 movement of the tape with a custom made opto-electronic photodiode motion
292 detector [28,29]. This was coupled with a microphone to record the association
293 between motion and acoustic signal (for full details, see methods). Motion traces
294 confirmed the mechanism of Ander's organ is tergo-tergal stridulation, in which the
295 first abdominal tergite, containing the stridulatory files, moves by telescopic abdomen
296 compression underneath the posterior edge of the metanotum which possesses
297 several spines that act as scrapers (Fig. 4B). The acoustic signals are generated
298 during this abdomen compression, but low amplitude signals are also generated as
299 the file disengages the metanotum during abdomen expansion (Fig. 4D). Organ use
300 was exclusively exhibited by females and juveniles during physical contact, and was
301 accompanied by either leg kicking behaviour while the animal was on its back,
302 mandible opening, or both, forming a composite signal. This sound may also be
303 amplified by a trio of tracheal cavities that underlie the stridulatory file (Fig. 4C,5).
304 These cavities are independent from the respiratory trachea, found to be present in
305 all assessed individuals (1 adult male and 2 adult females, Fig. S5), and absent
306 under other abdominal tergites, suggesting they are a specialised structure for
307 amplification. We used the actual length measurements of the cavities to provide a
308 preliminary estimate of the resonant frequency of each cavity in an example adult

309 female, to discern whether these structures are likely to be involved in signal
310 amplification. The 3 cavities had lengths of 1850.6 μm , 2646.9 μm , and 1969.3 μm ,
311 which, using eq. (1), suggest a resonance of 92 kHz, 64 kHz, and 87 kHz,
312 respectively.

313

314 **Discussion**

315 In his initial observations of this ‘chirping organ’, Ander provided no comprehensive
316 explanation of the function of the stridulatory mechanism [24] and the organ as such.
317 Various hypotheses were made from his morphological description which we here
318 investigated; namely that sounds are generated during organ use and proving that
319 the contraction of the abdomen forms the more significant motion in acoustic signal
320 generation. The broad frequency range of the acoustic signal is almost certainly
321 facilitated by extreme irregularity in organ morphology and feature distribution,
322 highlighted by the variation in tooth length and inter-tooth distance within individuals
323 (Fig. 2D,F; Fig. 4C, E). In addition, the finding of a cavity underneath the organ
324 suggests a resonant mechanism for amplification of the sound. This broad frequency
325 range, the impulsive waveform of the sound, and organ use only during physical
326 contact are key indicators that the organ functions as an acoustic anti-predator
327 defence [15,16,31,32], and thus we support this hypothesised function of the signal.
328 This is further supported by the additional observed behaviours associated with
329 organ use; namely leg kicking and mandible opening, which could serve to physically
330 deter a predator. The amplitude and frequency spectrum of the signal leads to the
331 conclusion that *C. monstrosa* is targeting predators with an ultrasonic hearing range
332 that extends to at least 60 kHz, most likely to elicit an acoustic startle response to
333 increase chances of survival [33]. The assessment of sub-organ cavity resonance
334 suggests that the measured organ can, in theory, produce broadband resonant
335 sound amplification from ~ 64 kHz to 92 kHz. As individuals possess one organ per
336 body side which could display an increased morphological variation and consequent
337 resonance, we believe it is plausible that amplification is the role of these cavities,
338 however a more robust modelling, such as by finite element modelling, will prove
339 useful in confirming this theory.

340 Quantification of the morphology of the organ and an observed lack of Ander's organ
341 use in males also support the notion of functional loss in males posed by Ander [24],
342 with females displaying larger organs in every measured parameter. This may
343 initially suggest a difference in predation risk between groups, perhaps as a result of
344 behavioural ecology. During the mating season, males climb several meters into the
345 canopy, ascending higher as the evening progresses. Females are assumed to
346 spend their days in underground burrows, only ascending trees at night to locate
347 singing males [34]. However, both sexes have been observed on/under the ground,
348 and there are as of yet no full descriptions of the disparity in male and female daily
349 activities.

350 A more plausible explanation is that such a dimorphism represents an example of
351 sexual selection. The sex by stage interaction of morphological analysis indicated
352 that males have significantly smaller organs, but only when they are sexually mature,
353 suggesting that features exclusive to adult males are prioritized over those for
354 disrupting a predator, and the pre-existing antipredator signal (Ander's organ) has
355 lost its function as a result. We identify these sexually selected features as the
356 tegminal calling song for mate attraction, and the development of thick hindwings as
357 a nuptial gift for female consumption [35]. These likely render the Ander's organ
358 useless, as both pairs of wings overlie the stridulatory file and metanotum.

359 Additionally, while it could be argued that males may not require the functional organ
360 as they can produce a warning signal by tegminal stridulation [34], this sound does
361 not have the same broadband energy or ultrasonic components as the Ander's organ
362 signal (Fig. S4), so may be used against a different predator, or one whose hearing
363 range includes both the male tegminal aggression, and the Ander's organ
364 ultrasonics. Females may indeed be at higher predation risk than males during their
365 time spent following the male calls for mating, a process known as phonotaxis, which
366 would prolong predator exposure. In addition, juveniles are likely under strong
367 selection to acquire food to grow and mature, and females under fecundity selection
368 to acquire sufficient nutritional resources to produce embryos. Previous studies of
369 grigs have shown that nutritionally-deprived adult females are more likely to mate
370 and feed on male hind wings under close conditions in the lab [35,36], possibly
371 indicating that they may engage in more phonotaxis when hungry, further increasing
372 predator exposure. On the other hand, adult males feed little in captivity (KAJ, pers.

373 obs.) and may also not feed much in the wild if they acquire all the resources needed
374 to mate before moulting to adulthood. Overall, this difference supports the concept
375 that females and juveniles have an increased likelihood of predator exposure. A
376 female-bias in morphological disparity may suggest an element of maternal-offspring
377 communication, however the frequency composition of the signal far exceeds the
378 hearing range of this species [37].

379 There are several hypotheses of how an acoustic signal such as that of the Ander's
380 organ can be used to evade predation. The simplest of these is the predator startle
381 hypothesis, by which the impulsive stop/start waveform of the sound at high sound
382 pressure levels acts to frighten a predator, increasing the temporal window for
383 escaping death (release call) [16]. These signals may also have evolved as a form of
384 Batesian mimicry for nocturnal species [16,21], whereby insects without additional
385 secondary defences (unarmed) have evolved the same acoustic signal as insects
386 with additional defences. This allows unarmed species to take advantage of the pre-
387 programmed predator association between the sound and the true defence, and
388 deter the same predator with a false warning. Such unarmed insects tend to
389 represent the greatest presence of stridulatory warning signals [21], suggesting this
390 mode of mimicry [15,16,21]. Others have posed that due to similar spectral and
391 temporal characteristics, and the possession of additional defensive components in
392 almost all insects, these disturbance sounds exhibit a widespread Müllerian mimicry
393 [15], whereby the signals always represent a true warning to the predator, with no
394 unarmed species. This mimicry has been confirmed in the acoustic behaviour of deaf
395 moths [38] and for ultrasonic signals, may disrupt echolocating predators [39,40]. In
396 *C. monstrosa*, the signal in this context may serve as a warning of the potential bite
397 the predator would receive if it were to prolong its attack.

398 Although the obvious predators that come to mind when considering ultrasonic
399 capabilities are bats, the auditory processing of *C. monstrosa* provides evidence for
400 non-bat predation, as this species possesses one population of auditory receptors
401 tuned to low frequencies, and a second type of auditory receptor tuned to a broader
402 frequency range that includes the male song [35,41]. As frequency discrimination
403 beyond these two categories of receptor is not possible, *C. monstrosa* is unlikely to
404 use its high frequency receptors for predator detection [41,42], as these receptors
405 are preoccupied with the role of detection of the male song. Instead, it is the other

406 population of receptors, best tuned to low frequencies ~2 kHz, that are suspected to
407 be associated with detecting terrestrial predators moving through a substrate [41].
408 However, this does not mean that *C. monstrosa* is unable to detect surface gleaning
409 bats, as it is known from other orthopteran insects that cercal organs may also be
410 involved in the detection of aerial predators, via the motion of hairs in response to
411 wind produced by the wings of an approaching bat [43]. *C. monstrosa* possesses the
412 necessary cerci, and an investigation into their function could prove beneficial to
413 investigating this alternate mode of predator detection.

414 Several bats do indeed share a geographic overlap with *C. monstrosa*. These
415 species are the big brown bat (*Eptesicus fuscus*), the silver-haired bat (*Lasionycteris*
416 *noctivagans*), and the hoary bat (*Lasiurus cinereus*, [44]). However, these bats are
417 primarily aerial predators, hunting on the wing, and *C. monstrosa* is not capable of
418 flight. *E. fuscus* for example is a common species across the habitat range of *C.*
419 *monstrosa*, but is considered to be a “beetles-specialist”, with nearly 90% of its diet
420 consisting of small to mid-sized flying insects [44-46]. *Lasiurus cinereus* is also
421 adapted for fast, unmanoeuvrable flight, and so mainly feeds on small flying insects
422 [47,48]. *Lasionycteris noctivagans* flies more slowly and manoeuvrably than *L.*
423 *cinereus* [40], often feeding in clearings over relatively short distances [47,48], so is
424 a potential surface gleaning predator. Recorded diets of these predators also often
425 contain high levels of unidentified insect matter (up to 15 %; [47,48]). This could
426 include *C. monstrosa* in the category of generally large, unknown ground dwelling
427 insects, but due to the argument suggested above, and the heavy percentage of
428 their diet consisting again of small, flying insects [47,48], we believe this to be
429 unlikely. Due to this lack of evidence for bat predation, we suggest echolocating and
430 high frequency communicating shrews as a more likely ultrasonic predator. The two
431 species which coexist with *C. monstrosa* are the cinereus shrew (*Sorex cinereus*)
432 and dusky shrew (*S. monticolus*). *S. cinereus* has been reported to echolocate,
433 particularly in novel environments and while foraging [49]. While the echolocation
434 bandwidths of these particular two species are unknown, the known upper
435 frequencies of other shrew species vary from ~30 to 95 kHz [49-52], meaning they
436 very likely have the capability to hear the frequency range of the Ander’s organ
437 signal.

438 There are also certain non-echolocating species which are insectivorous and share a
439 geographic overlap. The northern flying squirrel (*Glaucomys sabrinus*), may be an
440 interesting candidate as one of the only species likely to encounter *C. monstrosa*
441 both in the trees and on the ground. However, invertebrates make up less than 1%
442 of the diet of this species [53]. Other species include the yellow-pine chipmunk
443 (*Tamias amoenus*), red squirrel (*Tamiasciurus hudsonicus*), western jumping mouse
444 (*Zapus princeps*), long-tailed vole (*Microtus longicaudus*) and deer mouse
445 (*Peromyscus maniculatus*; [44] Mice have generally been found to produce
446 communication signals up to ~110-120 kHz [54,55], so may also represent a key
447 predator; particularly *P. maniculatus*, who is likely abundant within the range of *C.*
448 *monstrosa* and lives a heavily arboreal lifestyle, allowing for predation of *C.*
449 *monstrosa* both on the ground, and in the trees. While this species is known to
450 commonly eat insects [56], there has not yet been a study of *P. maniculatus* diet
451 within this region.

452 We suspect the predation ecology of *C. monstrosa* to be similar to the New Zealand
453 wētā (Anostomatidae), which possess similar stridulatory organs, and are an
454 integral part of the diet of stoats and other ground-dwelling mammals [17,57].
455 However the only known native predator of wētā capable of hearing ultrasound is the
456 (ground hunting) lesser short-tailed bat (*Mystacina tuberculata*, [58]), and so
457 increased knowledge on the ultrasonic hearing capabilities of the other predators of
458 wētā could assist in refining this comparison.

459 In another closely related species to *C. monstrosa*, *Paracyphoderris erebeus*, the
460 morphology of a structure with extreme likeness to the Ander's organ
461 (Supplementary figure 3) suggests the ability to produce a similar signal. We
462 propose, based on the morphology of both the Ander's organ and the tergal
463 stridulatory mechanisms of certain weta (*Deinacrida* sp., [20]), that it has a similar
464 function. Remnants of these structures and others in 5 of the 8 known extant
465 prophalangopsid species suggest that abdominal stridulation could be a common
466 trait of the family (Fig. S3). Alternatively, it may be that this structure is a key
467 convergent adaptation that, along with other traits, has allowed these species to
468 persist where other members of the family have gone extinct.

469 We believe that the discovery of ultrasonic signals in *C. monstrosa*, and the
470 presence of these morphological traits across other extant prothelangsids,
471 provide a foundation of evidence that ultrasonic stridulatory organs could be present
472 earlier in the history of Ensifera than previously assumed. Observations of the
473 natural behaviour of *C. monstrosa* and its predators would provide more information
474 on the function of the Ander's organ. In addition, efforts should be made to discover
475 such organs in fossil species, as this may provide insight into the origin of ultrasonic
476 anti-predator defences, and the hearing ranges of ancient terrestrial predators.

477

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486

487 **Author contributions**

488 All authors were involved in writing and revision of the manuscript. CW collected
489 acoustic and motion data, organ measurements, micro-CT data, and wrote the initial
490 manuscript. KAJ provided live and stored specimens for data collection, collected
491 body size measurements, and completed final statistical analyses. CP assisted with
492 acoustic and motion data collection. TJ assisted with acoustic analyses and organ
493 imaging. FMZ led the lab, built the motion detector, assisted with idea development,
494 and provided equipment training.

495

496 **Competing interests**

497 No competing interests declared

498

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506

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675 **Figure legends**

676 **Figure 1.** Location of the Ander's organ of *Cyphoderris monstrosa* and examples of
677 the stridulatory file. A, adult female stridulatory file; B, adult male stridulatory file; C,
678 juvenile male stridulatory file.

679

680 **Figure 2.** Differences in body size (GMS; Geometric mean size) and estimated
681 marginal means (EMM) of Ander's organ morphology in *C. monstrosa* based on sex
682 and life stage. GMS is calculated as the square root of head width X left femur
683 length. Error bars represent 1 SEM. Asterisks indicate statistically significant
684 differences at $p < 0.05$. Statistical comparisons of Ander's organ measurements (b-h)
685 are between the sexes for adults and juveniles separately because of a Sex by
686 Stage interaction effect (see Results).

687

688 **Figure 3.** Acoustic analysis of Ander's organ signals. A, waveform of multiple
689 Ander's signals, with B highlighted in red; B, one bout, with C highlighted in red; C,
690 an individual pulse of one bout; D, relative magnitude mean frequency spectrum over
691 individual pulses (N=50, the solid red line signifies the mean, the shaded area ± 1
692 standard deviation); E, example signal waveforms with spectrograms from an adult
693 female, juvenile female, and juvenile male.

694

695 **Figure 4.** The mechanism for Ander's signal generation. A, metanotum edge with
696 spines that scrape the abdominal file; B, mechanism of Ander's signal generation by
697 telescopic abdomen compression; C, Motion of Ander's mechanism, showing the
698 relationship between the motion of the abdomen (red), and the consequent acoustic
699 signal (blue). Highlighted areas show full signals, which occur exclusively during
700 telescopic abdomen compression

701

702 **Figure 5.** 3D reconstructed morphology of the Ander's organ of an adult female; highlighting
703 the anatomy of the sub-organ cavities.

704

705 **Tables**706 **Table 1.** Descriptive statistics for morphological measurements of *C. monstrosa* (N = 53) broken down by sex and stage.

| Measurement | Juvenile Male | Juvenile Female | Adult Male | Adult Female |
|--------------------------------|---------------------------|----------------------------|----------------------------|----------------------------|
| Head Width (mm) | 5.15 ± 0.49 (4.03-5.69) | 5.88 ± 0.39 (5.31-6.27) | 6.12 ± 0.22 (5.79-6.64) | 6.83 ± 0.22 (6.29-7.09) |
| Left Femur Length (mm) | 8.46 ± 0.95 (6.22-9.37) | 9.17 ± 0.75 (8.04-9.90) | 11.47 ± 0.62 (10.53-12.60) | 10.82 ± 0.36 (10.19-11.39) |
| Geometric Mean Size (mm) | 6.60 ± 0.68 (5.01-7.23) | 7.34 ± 0.53 (6.63-7.84) | 8.38 ± 0.36 (7.90-9.15) | 8.59 ± 0.26 (8.01-8.97) |
| Tooth Number | 8.2 ± 1.7 (5-10.5) | 12.4 ± 3.1 (9-17) | 12.8 ± 2.3 (8-17) | 16.0 ± 3.6 (10.5-21.5) |
| Mean Tooth Length (µm) | 114.7 ± 43.3 (41.0-184.8) | 166.9 ± 51.8 (107.8-241.9) | 166.4 ± 42.2 (93.8-253.5) | 204 ± 45.4 (146.2-278.4) |
| Tooth Length SD (µm) | 35.2 ± 14.4 (10.1-52.8) | 50.0 ± 15.1 (28.3-77.7) | 51.9 ± 17.2 (25.2-89.5) | 66.0 ± 19.5 (30.6-99.8) |
| Mean Inter-tooth Distance (µm) | 14.4 ± 2.2 (11.2-18.8) | 15.4 ± 2.9 (11.9-20.3) | 13.8 ± 2.9 (9.3-20.7) | 18.3 ± 4.7 (13.1-28.1) |
| Inter-tooth Distance SD (µm) | 4.1 ± 1 (2.9-5.4) | 5.5 ± 1.2 (4.1-8.2) | 6.1 ± 1.7 (3.5-9.4) | 7.4 ± 5.1 (3.5-24) |
| Spine number | 7.6 ± 4.2 (0-15) | 10.0 ± 2.9 (6-14.5) | 7.2 ± 2.4 (4.5-11.5) | 11.1 ± 2.3 (7-14.5) |
| File Length (µm) | 242.2 ± 78.9 (100-340) | 338.1 ± 116.6 (210-570) | 286.3 ± 66.3 (170-417.5) | 485.4 ± 61.3 (365-570) |

707 Values are means ± s.e.m. with the range in parentheses. SD = standard deviation.

708 **Table 2.** Matrix of Pearson r correlations among body size and Ander's organ measurements for 49 *C. monstrosa* individuals included in the
 709 morphological analysis.

| Measurement | GMS | HW | LFL | TN | MTL | TLSD | MITD | ITDSD | SN |
|--|--------------|--------------|--------------|--------------|--------------|--------------|--------------|-------|--------------|
| Head Width (mm) | 0.918 | | | | | | | | |
| Left Femur Length (mm) | 0.947 | 0.742 | | | | | | | |
| Tooth Number | 0.488 | 0.575 | 0.359 | | | | | | |
| Mean Tooth Length (μm) | 0.467 | 0.540 | 0.351 | 0.618 | | | | | |
| Tooth Length SD (μm) | 0.407 | 0.457 | 0.317 | 0.661 | 0.854 | | | | |
| Mean Inter-Tooth Distance (μm) | 0.279 | 0.404 | 0.144 | 0.209 | 0.228 | 0.139 | | | |
| Inter-Tooth Distance SD (μm) | 0.350 | 0.361 | 0.298 | 0.299 | 0.227 | 0.134 | 0.645 | | |
| Spine Number | 0.252 | 0.402 | 0.101 | 0.027 | -0.040 | -0.055 | 0.375 | 0.042 | |
| File Length (μm) | 0.469 | 0.692 | 0.233 | 0.583 | 0.471 | 0.370 | 0.402 | 0.258 | 0.479 |

710 **GMS:** geometric mean size = $\sqrt{\text{HW} \times \text{LFL}}$; **HW:** head width (mm); **LFL:** left femur length (mm); **TN:** tooth number; **MTL:** mean tooth length
 711 (μm); **TLSD:** tooth length standard deviation (μm); **MITD:** mean inter-tooth distance (μm); **ITDSD:** inter-tooth distance standard deviation;
 712 **SN:** spine number; **FIL:** file length (μm). Bolded correlations are statistically significant at $p < 0.05$.

713

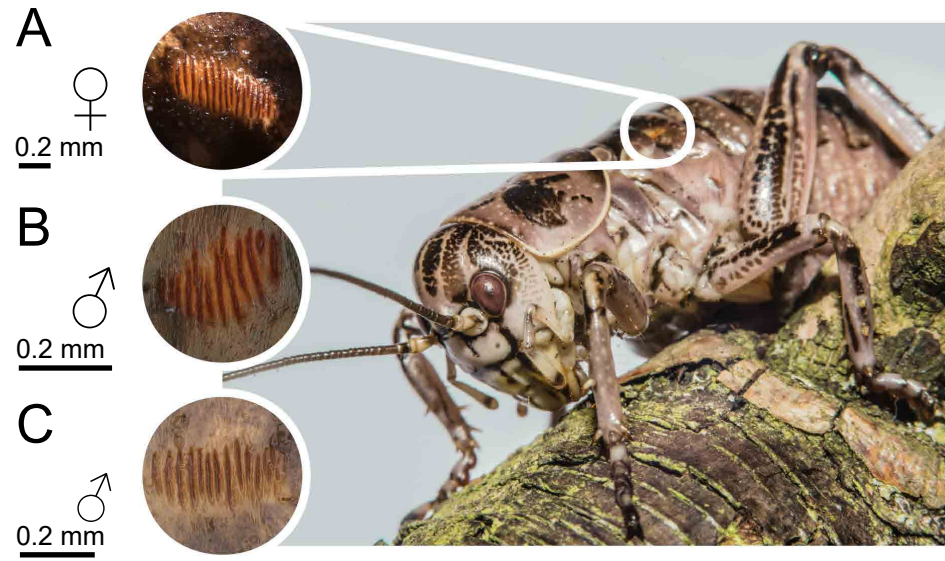


Fig. 1

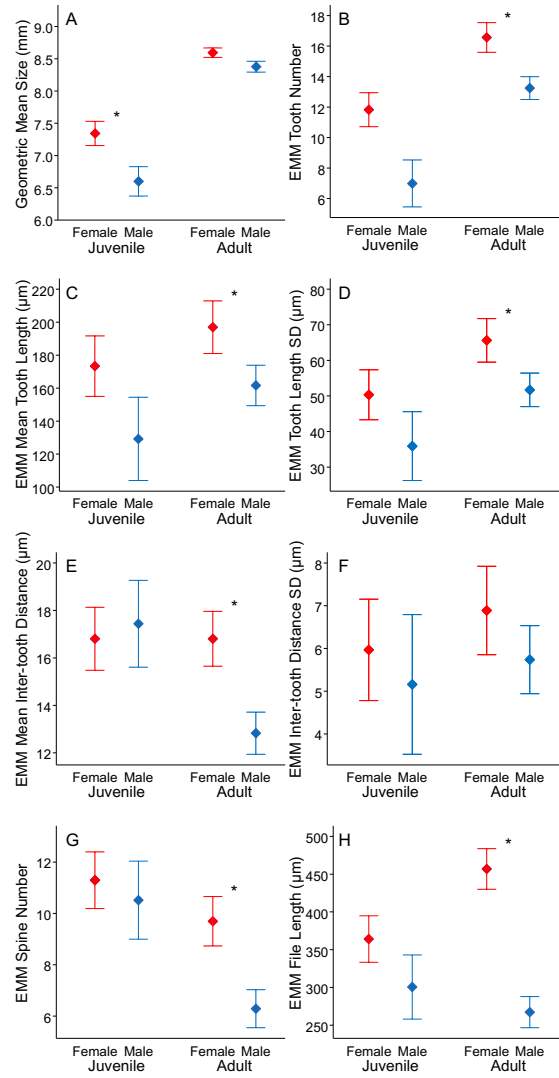


Fig. 2

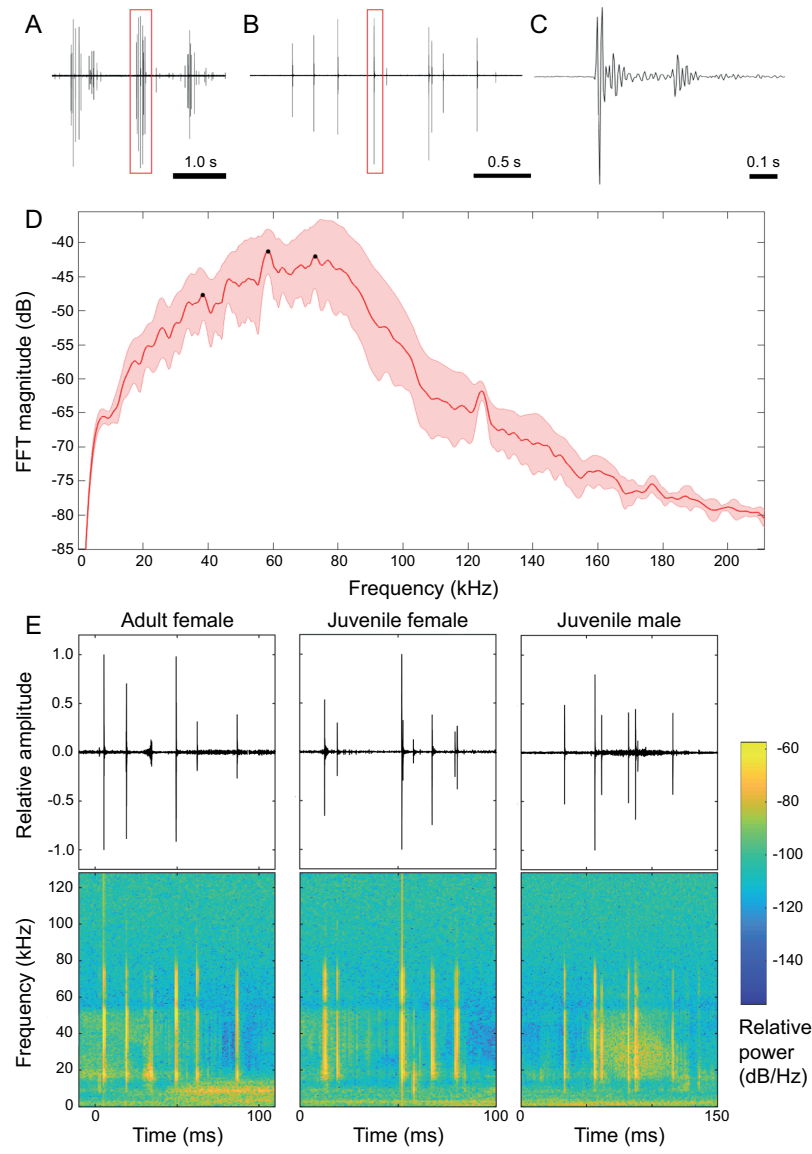


Fig.3

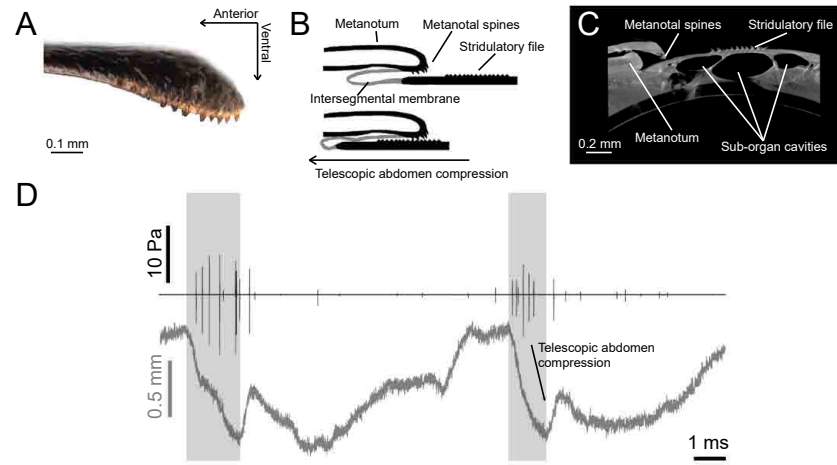


Fig. 4

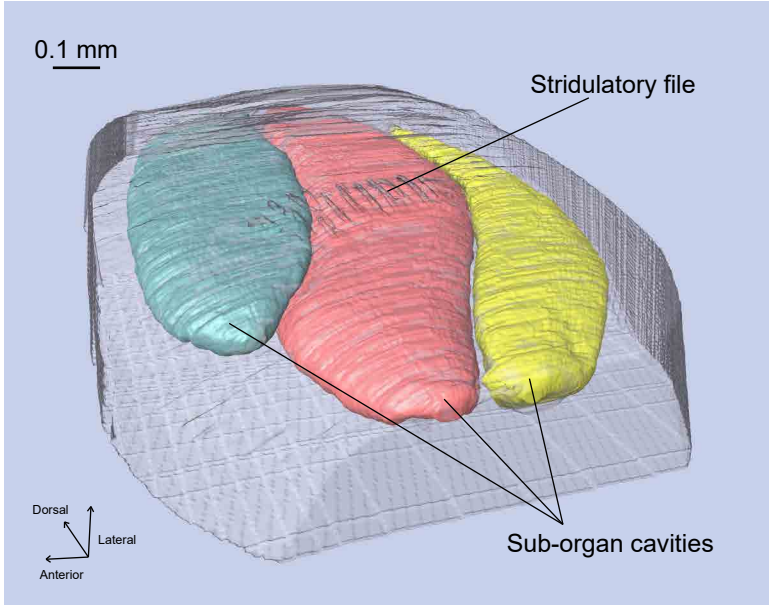


Fig. 5