Functional morphology of tegmina-based stridulation in the relict species *Cyphoderris monstrosa* (Orthoptera: Ensifera: Prophalangopsidae)

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Summary statement

Using laser Doppler Vibrometry in a relict species, evolutionary origins of sound generator features are investigated by comparison of functional morphology between distinct lineages of acoustically calling Orthopterans.

Abstract

Male grigs, bush-crickets and field crickets produce mating calls by tegminal stridulation: the scraping together of modified forewings functioning as sound generators. Bush- (Tettigoniidae) and field-crickets (Gryllinae) diverged some 240 million years ago, with each lineage developing unique characteristics in wing morphology and the associated mechanics of stridulation. The grigs (Prophalangopsidae), a relict lineage more closely related to bush-crickets than to field-crickets, are believed to retain plesiomorphic features of wing morphology. The wing cells widely involved in sound production, such as the harp and mirror, are comparatively small, poorly delimited and/or partially filled with cross-veins. Such morphology is similarly observed in the earliest stridulating ensiferans, for which stridulatory mechanics remains poorly understood. The grigs, therefore, are of major importance to investigate the early evolutionary stages of tegminal stridulation, a critical innovation in the evolution of the Orthoptera. The aim of this study is to appreciate the degree of specialisation on grig forewings, through identification of sound radiating area areas and their properties. For well-grounded comparisons, homologies in wing venation (and associated areas) of grigs and bush-crickets are re-evaluated. Then, using direct evidence, this study confirms the mirror cell, in association with two other areas (termed 'neck' and

'pre-mirror'), as the acoustic resonator in the grig *Cyphoderris monstrosa*. Despite the use of largely symmetrical resonators, as found in field-crickets, analogous features of stridulatory mechanics are observed between *C. monstrosa* and bush-crickets. Both morphology and function in grigs represents transitional stages between unspecialised forewings and derived conditions observed in modern species.

Introduction

Within the orthopteran sub-order Ensifera, males of most bush-crickets (Tettigoniidae), field-crickets (Gryllinae), and a few closely-related species, generate acoustic signals through forewing stridulation, primarily to attract females. In all cases, at least one forewing bears a row of teeth, the file, which overlaps the other forewing, itself endowed with a scraper (or plectrum), located along the posterior margin (Pierce, 1948). The plectrum is scraped over the file in sequence, and the resulting vibrations cause particular wing cells (e.g. harp and mirror) to oscillate and radiate sound (Pierce, 1948; Broughton, 1964; Bailey, 1970; Sales and Pye, 1974, Bennet-Clark, 2003). This elaborate system, known from the Triassic (ca. 220 Mya; Béthoux, 2012), has been subject to tremendous evolution, resulting in a wide array of wing morphologies, body sizes, and behaviour. In some cases forewing-based sound radiation is enhanced by particular shapes of the pronotum (Morris and Mason, 1995), holes in plant leaves acting as acoustic baffles (Prozesky-Schulze et al., 1975; Forrest, 1991), particular tegminal inflations (Hemp, 2001; Montealegre-Z and Mason 2005), or burrows purposely shaped for enhancing sound radiation (Bailey et al., 2001; Forrest, 1991).

Field- and bush-crickets represent two main lines of evolution. Field-crickets (sub-family: Gryllinae; ~1100 species according to Eades et al., 2016), produce low frequency calls, ~3-8 kHz (Walker, 1973; Hoy et al., 1982) from visually symmetrical (but functionally asymmetrical) wings with multiple, well delimited, radiating cells (Bennet-Clark, 2003; Montealegre-Z et al., 2011a), the largest of which is the harp. The harp is the primary radiator for the fundamental components of the call, while a secondary area, the mirror, also contributes to sound production for the high frequency components, mostly obvious in the courtship calls (Bennet-clark, 2003; Montealegre-Z et al., 2011a). The resonance of each of these cells is not affected by manipulation of the physical properties of the other membranous areas (Bennet-Clark, 2003). Field crickets predominantly have a 'right-wing-on-top' arrangement (Masaki et al., 1987) which is required for stridulation resulting in tonal purity in the produced signals (Elliott and Koch, 1983). The carrier frequency of these calls is dictated by an escapement mechanism (Elliott and Koch, 1985; Koch et al., 1988; Prestwich et al., 2000) and thus reliant on the natural frequency of the harps. Furthermore, coherent vibration of both wings at this resonant frequency is facilitated by a phase shifting mechanism, which allows oscillatory synchrony between both tegmina (Montealegre-Z et al., 2009).

Most noticeable about most modern bush-crickets (family: Tettigoniidae, ~7000 species according to Eades et al., 2016) is the bilateral asymmetry between the forewings (Dumortier, 1963; Gu et al., 2012; Montealegre-Z and Postles, 2010; Chivers et al., 2014;

Sarria-S et al., 2016; Cole and Chiang, 2016). The major radiating wing cell is named the mirror and is found primarily on the right wing, with the functioning file being on the left wing. The mirror is very well delimited, generally composed of the cross-vein free mirror (purple on Fig. 1. D), sometimes in association with a second cell (light green on Fig. 1. D) (Bailey and Broughton, 1970; Montealegre-Z and Postles, 2010). In many cases, there exists a mirror on the left wing, and a file on the right wing, but the former is usually partially damped and filled with cross-veins, and the latter greatly atrophied (Montealegre-Z and Mason, 2005; Montealegre-Z and Postles, 2010). These morphological wing characteristics result in an obligatory wing arrangement of 'left-over-right' for stridulatory sound production. Specific to bush-crickets is the widespread use of ultrasonic frequencies in the calls, indeed ~70% of species use ultrasounds in the range of 20 – 148 kHz (Morris et al., 1994; Montealegre-Z, 2009; Sarria-S et al., 2014), with some exceptions singing in the sonic range as low as 600 Hz (Heller, 1995). It has been suggested that the production of tonal calls at high ultrasonic frequencies is facilitated by the single radiating structure in this group, whereby there is no need to synchronize radiating cells on two wings for coherent sound generation (Montealegre-Z et al., 2009; Gu et al., 2012).

In summary both lineages exhibit particular modifications with respect to the assumed groundplan. Only a handful of extant species of grigs (family: Prophalangopsidae, eight extant species according to Eades et al., 2016) seem to have retained a more plesiomorphic wing morphology (Gorochov, 2003; Bethoux, 2012). Among these species, *Cyphoderris* spp. are the most easily accessible, and comparatively well-documented. Recent molecular-based phylogenies of the Orthoptera concluded that *Cyphoderris* spp. are more closely related to bush-crickets than to field-crickets (Zhou et al., 2014; Song et al., 2015; Cole and Chiang, 2016). However, similarly to field-crickets, tegminal stridulation in *Cyphoderris* spp. occurs from largely symmetrical forewings (Morris et al., 2002). In addition, unlike both field- and bush-crickets, stridulation in *Cyphoderris* spp. can be performed with either wing on top, and also the wings lack some of the well-delimited wing cells, largely free of cross-veins (Morris and Gwynne, 1978; Fig. 1A, B). Furthermore, *Cyphoderris* spp. sing at ~12 - 15 kHz (Morris and Gwynne, 1978; Morris et al. 2002), a range higher than that in field-crickets (3 - 8 kHz, Hoy et al., 1982; Otte, 1992) but lower than that in most bush-crickets (> 20 kHz, Montealegre-Z 2009). In summary *Cyphoderris* spp. are of major importance to investigate the early evolutionary stages of a critical innovation, at the root of a tremendous diversity. However, little is known about wing function in *Cyphoderris* spp., and a detailed knowledge on stridulatory mechanics in this group will allow a better understanding of the forewing biophysics and communication capabilities in both extant relatives and related fossil taxa.

Prior to functional analysis we reconsider conjectures of topographic homology (THC) in the wing venation of grigs and bush-crickets. This is the primary step to determine which areas became specialized as sound radiators. Then, we investigate the functionality of the sound generators in the extant prophalangopsid *Cyphoderris monstrosa* Uhler, 1864. Using biomechanical evidence and advanced experimental analysis, this study aims to characterise the radiating cells, and associated veins, on the forewings. This will test the hypothesis that the mirror in *C. monstrosa* is the primary oscillating membrane and contributes to dictating the carrier frequency of the male call. Vibratory function of the wings of *C. monstrosa* will then be considered in the context of the evolution of the stridulatory structures between groups of Ensiferans.

Material and methods

Topographic homology conjectures

Insect wing venation conventions and nomenclature

The debate on the insect wing venation groundplan and/or the actual THC to be applied to Orthoptera (Béthoux, 2007; Béthoux, 2008; Gorochov, 2005; Rasnitsyn, 2007) is only remotely related to the present contribution. Any groundplan (i.e. 'serial' or 'M5') can be applied to address THCs aspects within the Ensifera (Béthoux, 2012). We favour the serial groundplan for reasons stated earlier (Béthoux, 2008).

The terminology and color-coding already developed and used for Grylloptera (a formal taxon encompassing ensiferans possessing a file) in Béthoux (2012) is followed, with some modifications. Béthoux (2012) identified the 'column' (as in grigs and field-crickets) in bush-crickets. Herein we argue that the corresponding structure in bush-crickets is not homologous to the column; therefore we propose to refer to it using a new term, 'string'. This also has consequences regarding the harp area (turquoise on Fig. 1; brown in Béthoux, 2012), which can then be divided into three parts (Fig. 1B): the anterior portion (h1) is posteriorly delimited by the string (or its approximate position if it does not occur, as in field-crickets); the median portion (h2) is delimited anteriorly by the string and posteriorly by the column (or its approximate position if it does not occur, as in bush-crickets); the posterior portion (h3) is anteriorly delimited by the column (or its approximate position if it does not occur, as in bush-crickets). The mirror is closed by a specialized (set of) cross-vein(s) that we propose to refer to as the 'frame'. Finally, we propose to complete the terminology of particular areas as follows: the area in gray in Fig. 1B (same color-coding in Béthoux, 2012) is termed the 'pre-mirror'; the area in light blue (Fig. 1B, D, H) is termed the 'post-mirror' (by definition it is delimited basally by the handle and distally by CuPaα2; therefore it does not

occur in field-crickets, in which CuPaα2 is partially fused with the handle; Fig. 1G, H); the area in orange (Fig. 1B, D, H; delimited anteriorly by (M+)CuA and posteriorly by CuPa/ CuPaα) is termed the 'neck' (cf. terminology of harp pieces). An area of putative importance, as far as function is concerned, is anteriorly delimited by R/RP, posteriorly by M/MA, and distally either by MA or a cross-vein connecting RP and MA. This area is color-coded in brown (see Fig. 10) and will be referred to as the 'Larunda' area (see Discussion).

To ease comparison we indicate in Fig. 1 the CuPaα-Cupaβ fork by a white arrow (delimited in black) and the CuPaα1-Cupα2 by a black arrow (delimited in white); the course of CuPaβ is indicated by a blue arrow following the corresponding vein (also for CuPaα2 in Fig. 1E).

Species sample and specimen preparation

We examined forewings of specimens of the three known species of *Cyphoderris* (three males of *C. monstrosa*; three males of *C. buckelli* Hebard, 1934; and three males of *C. strepitans* Morris & Gwynne, 1978). The forewing venations of the three species show no major differences, but a comparatively high level of intra-specific and intra-individual variability (see Fig. 10). This variability does not affect the validity of our interpretations. As for bush- and field-crickets, we selected a representative species (Tettigoniidae: *Copiphora brevirostris* and Gryllinae*: Gryllus bimaculatus)*, and intact preparations, among a small sample of each group belonging to the private collection of one of us (OB; acronym IWC OB). Wing preparation follows Béthoux and Wieland (2009; and see Béthoux 2012), except for the specimen IWC OB 862 (Fig. 1C-F), mounted in 'white Euparal' (Asco Laboratories, Manchester, UK).

Data production

Photographs were taken using a digital camera Canon EOS 5D Mark III coupled to a Canon MP-E 65 mm macro lens. Resulting photographs were dusted off manually (stamp tool) and optimized using Adobe Photoshop CS6. Venation schemes, areas and associated labels were prepared using Adobe Illustrator CS6.

Optimality criterion

How an optimal set of THCs can be identified among competing ones has been already clarified by Béthoux (2012). In short, an optimal set of THCs is the one implying the lesser amount of transformation to explain the observed patterns. In other words, it maximizes correspondences, given the observed elements.

Functional morphology analysis

Specimens

Male specimens of *C. monstrosa* were collected as adults from Paul Lake Provincial Park outside of Kamloops, British Columbia (50°45'15.6"N 120°07'08.8"W). Specimens were transported to the University of Toronto Scarborough, and subsequently to the University of Lincoln, where they were maintained in individual containers at ~9°C, with a 12:12 light cycle, and fed daily with fresh apple and water-soaked cotton.

Acoustic recording

The calls of the singing males were recorded using a wide-bandwidth response 1/8 inch microphone (Brüel & Kjaer, 4138-A-015, with preamplifier model 2670, Brüel & Kjaer, Nærum, Denmark), and Polytec software (PSV 9.2, Waldbronn, Germany). Recordings were made at a sampling frequency of > 256,000 samples/second. All experiments were performed in a sound attenuating chamber, on an anti-vibration table, at temperatures of 25.5 ± 1.4 °C.

Recording of wing vibrations

This method of micro-scanning Doppler vibrometry follows previous works (Montealegre-Z et al., 2009; Montealegre-Z and Postles, 2010; Montealegre-Z et al., 2011; Sarria-S et al., 2016). Specimens were immobilised by exposure to a triethylamine-based mix (Flynap[®], Carolina Biological Supply Company, Burlington, Carolina, USA) for 3 – 5 minutes. The specimens were placed on a block of Blu-Tack[®] (Bostik, La Défense, Paris, France) and their legs were gently clamped to the block with small staple clamps. The surface of the block was flat except for the front end where the surface angled downwards. The specimen was positioned so that the head and thorax of the animal were on the angled surface, with a clamp over the pronotum to maintain the positioning of the specimen. This position forces the prothorax to bend downwards, allowing free manipulation of the forewings. The forewings were then separated from each other in a raised position and fixed with a mix of bees wax (Fisher Scientific, Loughborough, UK) and Colophonium (Sigma-Aldrich, Dorset, UK) (50:50). The Blu-Tack block was affixed to a brass plate which itself was attached to an articulated aluminium rod allowing the specimen to be maneuvered into the required position.

Vibration compliant areas of grig forewings, and associated frequency characteristics, were measured using a micro-scanning laser Doppler vibrometer (Polytec PSV-500; Waldbronn, Germany) fitted with a close up attachment. The mounted specimens were positioned so that the extended wings were perpendicular to the lens of the laser unit. A loudspeaker was

positioned above the laser unit and facing the animal to broadcast the sound stimulus. The acoustic stimulus used was periodic chirps, generated by the Polytec software (PSV 9.2), passed to an amplifier (A-400, Pioneer, Kawasaki, Japan), and sent to the loudspeaker (Ultrasonic Dynamic Speaker Vifa, Avisoft Bioacoustics, Glienicke, Germany). The periodic chirps spanned frequencies between 2 and 50 kHz, and the stimulus was flattened so all frequencies were represented at 60 dB ±1.5 dB (SPL re 20 µPa) at the position of the wings. A B&K 1/8 inch condenser microphone was placed at the position of the wings to monitor and record the acoustic stimulus at the position of the wings as a reference. The laser system was used in scan mode. A scan of the entire extended wings was performed using 670-1300 scan points. Within the frequency domain setting of the vibrometer, a frequency spectrum was calculated for each point using a FFT with a rectangular window, at a sampling rate of 128 kHz, 128 ms sampling time, and with a frequency resolution of 7.8125 Hz. A high-pass filter of 1 kHz was applied to the both the vibrometer and reference microphone signals during the scanning process, with an average of three samples taken at each point. The data of one specimen is included in which each forewing was dissected in turn at the attachment by cutting the pteralia of the wing base, immediately sealing the cut with wax, and affixing it to a short length of wire by the wax seal. The wire was attached to a clamp arm and clamp stand, damped to vibrations with blue-tac, and the wing was scanned using the same protocol as before, within 20 minutes of dissection. To facilitate comparison of vibrational response between groups, a wing scan from the bush-cricket *Copiphora brevirostris* and the field-cricket *Gryllus bimaculatus* are presented (see discussion). These scans were made with the same experimental set-up.

Data analysis

Vibrometer scan data was analysed in the Polytec software (9.2). Frequency spectrums of the vibrometry data were normalised to those of the reference signal by computing the transfer function of the two (Windmill et al., 2005). The magnitude-squared coherence between the vibrometer and microphone signals was also computed for each data point to estimate the amount of unrelated noise (Windmill et al., 2007). Coherence values can range between zero and one, with a value of one indicating the absence of unrelated and external noise. The parameter Q is a dimensionless index indicating the sharpness of tuning in resonant systems (Bennet-Clark, 1999b). Here, for the acoustic data, Q was calculated as the peak frequency divided by the bandwidth at 3 dB below the peak amplitude. Q was also calculated from the frequency spectra of the vibrometry data by dividing the peak frequency by the bandwidth at values equalling 0.707 times the peak amplitude, corresponding to 3 dB below peak (Fletcher, 1992). Data were tested for normality, and peak frequencies and magnitude of vibrations between left wing and right wing were compared with a paired

sample t-test. Q-values of the wing resonances were compared with a Wilcoxon Signed Rank test. Post analysis of vibration data, acoustic data, and statistical analysis was performed in Matlab (r2015b, The Mathworks Inc., Natick, USA.) and SPSS, Version 21 (IBM Corporation, New York, NY, USA) .

Results

Topographic homology conjecture

Here we present new THCs for the wing venation pattern, and the associated areas, for *Cyphoderris* spp. (Fig. 1A, B) and bush-crickets (Fig. 1C, D). To ease comparison with previous accounts, we also provide the challenged THC for bush-crickets (Fig. 1E, F), and one we consider valid for field-crickets (Fig. 1G, H; according to Béthoux, 2012). The most recent account on the topic (Béthoux, 2012) assumed a partial fusion of $CuPa\alpha2$ and $CuPa\beta$ in bush-crickets (Fig. 1E). Both veins were assumed to run along the handle for some distance, with $CuPa\alpha2$ diverging first. As a consequence, the mirror cell was found to be bordered distally by $CuPa\alpha2$ and basally by $CuPa\beta$,as in field-crickets. Moreover, the 'column', a particular cross-vein bridging $CuPa\beta$ and CuPb, was identified in both field- and bush-crickets (Fig. 1).

The emergent consensus on the position of *Cyphoderris* spp. as more closely related to bush-crickets than to field-crickets (Desutter-Grandcolas, 2003; Zhou et al., 2014; Song et al., 2015), as well as the data from this study, prompted us to revise THCs proposed earlier. Indeed, in *Cyphoderris* spp., the mirror cell is not bordered distally by $CuPa\alpha2$, but instead by a series of curved cross-veins ('frame' in Fig. 1. A). Moreover, although there is not a well-individualized column or string in these species, the positions of the existing cross-veins show that the 'premises' of both co-occur. One can then legitimately challenge the homology of the 'column' as identified by Béthoux (2012) for bush-crickets (Fig. 1E) and field-crickets (Fig. 1G).

The THC we propose herein for *Cyphoderris* spp. is not essentially different from that proposed by Béthoux (2012: pl. 1E, F): the pre-mirror (gray in Fig. 1B) is large, as in other Prophalangopsidae and as in stem-Grylloptera. As for bush-crickets, we propose (1) that $CuPa\alpha$ splits (into $CuPa\alpha1$ and $CuPa\alpha2$ in a more distal position than assumed by Béthoux (2012), (2) that the mirror is bordered distally and posteriorly by a specialized cross-vein ('frame' on Fig. 1. C) homologous to the set of unspecialized cross-veins in *Cyphoderris* spp., and (3) that the cross-vein referred to as 'column' by Béthoux (2012; herein 'string', Fig. 1. C) is not homologous to the 'column' of field-crickets, but instead to the set of weakly specialized cross-veins bridging $CuPa\beta$ and CuPb opposite the handle in *Cyphoderris* spp.

The bush-cricket 'harp' according to Béthoux (2012; Fig. 1F) represents the anterior portion of the (partly differentiated) harp in *Cyphoderris* spp. only. Under the favoured THC presented herein, in bush-crickets, CuPaβ runs backwards for some distance (Fig. 1C), along the handle, an assumption proposed earlier (Béthoux, 2012).

The new THC for bush-crickets supposes fewer transformations than that proposed by Béthoux (2012). Notably, a CuPaα1-CuPaα2 fork located in a distal position, close to the fusion of CuA and CuPa$_\alpha$, maximizes correspondences with the THC for *Cyphoderris* spp. and stem-Grylloptera. In addition, the new THC handles the fact that both 'string' and 'column' co-occur in *Cyphoderris* spp., and therefore cannot be homologous.

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Acoustic analysis, vibration compliant areas, and resonances of the forewings

Acoustic analysis agrees with previously reported results for the calling song of *C. monstrosa* (Fig. 3, Morris and Gwynne, 1978; Spooner, 1973; Morris et al., 2002). Mean peak frequency of the call is 13.08 ± 0.1 kHz (n=5). These calls are highly resonant, with the mean *Q* of the calling song being 57.3 ± 18.39 (Fig. 2). Scanning laser vibrometry experiments to reveal the vibration compliant areas of the forewings of *C. monstrosa* were successfully achieved in five specimens, and we hereby present an initial treatment of wing mechanics in this species. Vibrations in response to sound stimulus (at a band around the calling frequency) are limited to the mirror and also the areas herein termed the neck, the pre-mirror and the anterior portion of the harp (Fig. 3). Vibrations of the mirror and adjacent area occur in phase ('as-one' vibrations, Fig. 4), in a basic mode corresponding to the dominant resonant frequency. Although the mirror and adjacent areas are weakly delimited, the vibrating surface is confined within the surrounding veins. This vibration pattern was observed in both wings of all specimens scanned (n=5) and further symmetry of wing function is exhibited. Peak vibration amplitudes are observed on the mirror area (Fig. 4) with the average vibration amplitude of the mirror (as calculated by averaging all scan points on the mirror in displacement) being 182.4 ± 77.5 nm/Pa for the left wing and 138.4 ± 52.8 nm/Pa for the right wing, and this difference was not significant (paired t test, *t* = 0.874, df= 4, *P* = 0.432). Mean resonant frequency of the mirror was 14.2 ± 1.08 kHz (n=5) from the right wing and 14.6 ± 2.2 kHz (n=5) for the left wing (Fig. 3), and this difference was also not significant (paired t test, *t* = 0.618, df = 4, *P* = 0.569). High coherence of vibration of the mirror shows that the observed response is reliable, with coherence approaching one around the frequencies of resonance (Fig. 3). Thus the mirrors exhibit a natural tuning at frequencies close to the calling song (Fig. 5) and this matching suggests the natural frequency of the wings is functionally tuned to a specific frequency, which is in turn being exploited for efficient, resonant sound production. The areas adjacent to the mirror (pre-

mirror, neck and h1) vibrate at a lower amplitude than the mirror, and exhibit no sharp resonance (Fig. 6). The Q of the mirror's resonance, measured from the peak on the spectra, is much lower than that of the calling song, with Q of the mirror area being 27.8 ± 24.8 for left wing and 12.7 ± 3.7 for the right wing (this difference being not significant: Wilcoxon, Z = -0.674, P = 0.5).

Discussion

Multiple conjectures on the evolution of stridulatory apparatus exist in the literature (Gwynne, 1995; Desutter-Grandcolas, 1997; Desutter-Grandcolas, 2003; Jost and Shaw, 2006; Bethoux, 2012 and references therein) with unequivocal homologies between groups of Orthopterans remaining elusive. This work presents a revised THC of wing venation patterns within the Orthoptera, building on previous efforts (Bethoux, 2012). The new THC is considered superior to that proposed by Béthoux (2012) because it requires fewer transformations to explain the observed patterns. An important implication of the new THC is that, in both *Cyphoderris* spp. and bush-crickets, there is a large area composed of the neck, h1 and the pre-mirror which is essentially cross-vein free. Together with the mirror, laser micro-scanning vibrometry has revealed these cells as the main areas functioning for resonant sound production in *Cyphoderris monstrosa*. The reduction of the pre-mirror, complete in bush-crickets thanks to the migration of the first fork of CuPa (white arrow on Fig. 1C) in a distal position, results in an optimal reduction of the partitioning of the corresponding area. Moreover, it is noticeable that CuPa is weakened in both taxa, a point indicative of the vibrational compliance of the corresponding area (a point demonstrated by the functional comparison – Fig. 7 A, B). Together with the mirror this large area acts as a single vibrating structure for sound production in both groups (Bailey, 1970; Montealegre-Z and Postles et al. 2010; Sarria-S et al., 2016).

This situation contrasts with that observed in the field-cricket lineage (Fig. 7 C). Although the pre-mirror was reduced or lost in this lineage (Béthoux, 2012), and the mirror is maintained, the main radiating structure is composed of an area encompassing h1 and h2 (Bennet-Clark, 2003; Montealegre-Z et al., 2011a; Robillard et al., 2013), which have a minor or no contribution in sound production in *Cyphoderris* spp (Fig. 7A).

The wings of *C. monstrosa* have a natural tuning close to the frequency of the call at ca. 13 kHz, a requirement for resonant sound production, and a feature often reported in resonantly calling crickets and bush-crickets (Montealegre-Z and Postles, 2010; Montealegre-Z et al., 2011a). A mismatch of ~2 kHz between wing resonant frequency and call frequency is apparent in two specimens. This difference is likely a result of changes in the physical properties of the wings, the oscillating areas, and associated delimiting veins during

stridulation when the wings are actively engaged and in motion (Montealegre-Z et al., 2011). A similar mismatch is previously reported in bush-crickets (Montealegre-Z and Postles, 2010; Sarria-S et al., 2016). Vibrating areas and natural tuning at the calling frequency is apparent in both wings of *C. monstrosa*. High *Q* values as seen in *C. monstrosa* forewing vibrational response are similar to those previously reported for the wing vibrations of other ensiferans exploiting resonant sound production (Bennet-Clark, 2003; Montealegre-Z and Postles, 2010).

The mirror alone is responsible for dictating the main spectral energy of the call, with the adjacent neck, pre-mirror and h1 having no intrinsic tuning. These adjacent areas likely form a lightly damped area of wing, oscillating in-phase with the resonant frequency of the nearby mirror during sound production. The innovation of a lightly damped sounding board, which indiscriminately amplifies any input frequencies, has been reported in bush-crickets (Morris and Pipher, 1967; Bailey, 1970) as well as in-phase vibrations of the mirror and adjacent area (Montealegre-Z and Postles, 2010; Sarria-S et al., 2016). In *C. monstrosa*, the areas of vibration are minimally delimited, with narrow veins impinging on the area of the mirror, and the bounding veins being non-specialised (i.e. not thicker than other veins). This study demonstrates that sharply tuned structures for resonant sound production can exist outside the maxims of derived wing morphology exhibited in both modern field- and bush-crickets. Given this non-specialisation of vibrating areas, the question of how such resonant tuning is achieved, through the morphology of the wing, presents itself. The mirror of bush-crickets is usually bounded by a thick frame (Morris and Pipher, 1967; Bailey, 1970; Montealegre-Z and Postles, 2010; Bethoux, 2012; Chivers et al., 2014; Sarria-S et al., 2016; Fig. 1C). This frame is reported to act as a cantilever during oscillation, being clamped either along the file bearing vein (Morris and Pipher, 1967; Bailey, 1970) or at the region on the plectrum (Montealegre-Z and Postles, 2010; Sarria-S et al., 2016). Properties of length, mass, stiffness, as well as membrane structure and attachment are implied as contributing to the resultant resonant frequency of this system (Morris and Pipher, 1967; Bailey, 1970; Keuper et al., 1988; Bennet-Clark, 2003). In *C. monstrosa*, under the cantilever model, the frame of the mirror is clamped along the distal region (where CuPaβ meets the 'frame', Fig. 1). In bush-crickets, clamping occurs by the thickness of the framing vein at a single region (Bailey, 1970; Montealegre-Z and Postles, 2010; Sarria-S et al., 2016). However, the mirror frame in *C. monstrosa* is formed by a vein of standard thickness (Fig. 1A). Clamping of this frame can be attributed to its attachment to multiple small cells of the distal region of the wing and, possibly, a depressed area sub-parallel to the frame and reaching the posterior wing margin, here referred to as the 'gorge'. Areas of wing with such hexagonal structures are associated with increased stiffness (Montealegre-Z et al., 2009; Montealegre-Z et al,

2011a), and indeed do not vibrate in response to acoustic stimuli as seen in this study (Fig. 3). This condition thus may represent a transitional stage between resonantly tuned wing cells on unspecialised fore-wings, and the highly modified systems seen in modern bush-crickets.

One particular area was scrutinized for a putative functional role: being located at the widest of the area bordered anteriorly by R/RP and posteriorly by M/MA (brown color-coded in Fig. 8A), herein named 'Larunda' (Larunda was a nymph whose tongue was cut out by Jupiter for her talkativeness). As far as morphology is concerned, the Larunda area is characterized by cross-veins, locally weakened (Fig. 8B-D). But importantly, variation of these cross-veins occurs, including cases where no weakening can be observed (Fig. 8E). It is tempting to interpret the Larunda as a relictual vibration-compliant area. Indeed, in several ancient Grylloptera the corresponding area is broadened, filled with few and/or narrow cross-veins and distally sealed by a secondary structure (Sharov, 1968: fig. 29; Fig. 8F). The morphological variation observed in *Cyphoderris* spp. could then be compared with that of the mirror on the non-functional forewings of bush-crickets. However our analyses revealed no particular vibrational compliance of the Larunda area in forewings of *C. monstrosa*. It is interesting to note that in certain Phaneropterine bush-crickets, the corresponding area of the here defined Larunda area is thin and transparent, thus being suggestive of having a role in sound radiation (Heller at al. 2015). This area merits further investigation across groups.

The calls of *C. monstrosa* are highly resonant (*Q* of ca. 57), and worth noting is the indication that during stridulation both wings must be excited at their resonant frequency by the appropriate tooth strike rate, and that the vibrating areas of both wings must oscillate in phase (Bennet-Clark, 1999; Montealegre-Z et al., 2011). In field-crickets, during stridulation, the different areas of both wings (mirror, harp) all oscillate coherently for resonant sound production (Montealegre et al. 2011). The use of a single oscillating area in the grigs, and the derived extreme of a single and well-delimited mirror in bush-crickets, would reduce the effect of destructive interference of multiple cells, and facilitate resonant sound production at higher frequencies (Montealegre-Z, 2005). Furthermore, coherent sound production from both wings has been shown to rely on a phase shift mechanism, as seen in field-crickets (Bennet-Clark, 2003; Montealegre-Z at al., 2009), due to the varying spatial position of energy input during each closing stroke. A similar phase-shifting mechanism has been observed on the right wing of certain bush-crickets (Bailey, 1970; Montealegre-Z and Postles, 2010), however, the function of such a mechanism in a system in which there is no need to synchronise two wings is unknown. No such mechanism is apparent in *C. monstrosa* from our data (e.g. out of phase scraper and mirror vibration at the resonant frequency) and yet such a system is conceivably required for coherent oscillations of both wings during

stridulation under an 'either-wing-on-top' arrangement. This question deserves further attention among both the grigs and the bush-crickets, with focused investigations into the transmission, and relative phase of, vibrations across areas of each wing in relation to the position of energy input i.e. at the scraper or along the length of the file (after Montealegre-Z et al., 2009). Finally, this raises question the question of how *Cyphoderris* spp. are regulating the frequency of energy input to excite the forewings at their natural frequency. Field crickets, which largely produce resonant calls, achieve this by an escapement mechanism of the file and scraper, with the frequency of energy input regulated by the natural resonant frequency of the wings (Bennet-Clark and Bailey, 2002; Elliott and Koch, 1985; Koch et al., 1988, Prestwich et al., 2000). This escapement is associated with the above-mentioned phase-shift mechanism for pure-tone sound production (Bennet-Clark, 2003; Montealegre-Z et al., 2009). In contrast to the escapement system of frequency regulation, excitation of the natural frequency of the wings in bush-crickets relies on an association between wing closure velocity and arrangement of teeth on the file (Montealegre-Z et al., 2006). In this way a scraper passed over consistently distanced teeth at a stable velocity produces a consistent TSR (Montealegre-Z et al., 2011). Alternatively, an increase of tooth spacing can be associated with an associated increase in wing closure velocity, thus keeping the TSR consistent during each wing closure (Montealegre-Z and Mason, 2005). The lack of a phase-shift mechanism in the forewings of *Cypoderris* spp. suggests that frequency regulation is similar to that of bush-crickets, possibly due to the requirement for tonal signals at higher frequencies (Montealegre-Z et al., 2011). A proper treatment of the mechanism of frequency regulation during stridulation in grigs is without the scope of this study, but certainly deserves further attention.

Conclusion

This study highlights the importance of considering the relationship between the functional and evolutionary aspects of morphology. Consideration of just one of these aspects, while being robust in the chosen field, may lead to unreliable interpretation of data (e.g. Ower et al., 2016). Some attempts have been made to infer basic singing parameters from Jurassic cricket species (Gu et al., 2012), but inferences are currently limited by a lack of knowledge on the characteristics of weakly delimited vibration-compliant areas. Our comparative analysis demonstrates that radiating structures observed in extant groups relate to a single 'ancestral' pattern. The mirror area is the morphological and functional feature shared by grigs, bush- and field-crickets, although its contribution to sound production, in the latter, is more modest. Yet, as the field- and bush-cricket lineages strongly diverged, through similar trends such as cross-vein specialization, the progressive reduction of selected areas occurred, becoming less and less functional and hence more prone to reduction. However, it

must be acknowledged that grigs, in which this reduction and specialisation is nascent, are only partly relevant for inferences on the functional morphology of the earliest stem-Grylloptera. In the former, the radiating area is composed of several cells, but they already are relatively large and cross-vein-free areas compared to the same areas in the latter (Sharov, 1968, 1971; Fig. 8F). Therefore attempts to infer basic singing parameters from Jurassic stem-Grylloptera (Gu et al., 2012) must be regarded as a preliminary effort, given the yet limited knowledge on the vibrational characteristics of areas with a higher density of uniform cross-veins. Other approaches, such as computer-assisted models, will allow a refinement of our understanding of the early stages of tegmina-based stridulation in Grylloptera. The current data on grigs will allow a validation of the models before application to fossil species.

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Competing interests

The authors declare no competing interests.

Author contributions

FMZ led the project. BC, OB, TJ, and FMZ conceived the work. ACM collected and provided the live specimens. BC, OB and FSS collected the data. BC and OB analysed the data. BC, OB and FMZ wrote the paper.

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References

495   **Bailey, W. J.** (1970). The mechanics of stridulation in bush crickets (Tettigonioidea,
496   Orthoptera) I. Tegminal Generator. *J. Exp.Biol.* **52**, 495-505.
497   **Bailey, W. J. and Broughton, W. B.** (1970). The mechanics of stridulation in bush
498   crickets (Tettigonioidea, Orthoptera) II. Conditions for resonance in tegminal generator. *J.*
499   *Exp.Biol.* **52**,507-517.

500   **Bailey, W. J., Bennet-Clark, H. C. and Fletcher, N. H.** (2001). Acoustics of a small
501   Australian burrowing cricket: The control of low-frequency pure-tone songs. *J. Exp.Biol.* **204**,
502   2827-2841.

503   **Bennet-Clark, H. C.** (1999a). Resonators in insect sound production: How insects
504   produce loud pure-tone songs. *J. Exp.Biol.* **202**, 3347-3357.

505   **Bennet-Clark, H. C.** (1999b). Which Qs to choose: questions of quality in
506   bioacoustics? *Bioacoustics* **9**, 351-359.

507   **Bennet-Clark, H. C. and Bailey, W. J.** (2002). Ticking of the clockwork cricket: the
508   role of the escapement mechanism. *J. Exp.Biol.* **205**, 613-625.

509   **Bennet-Clark, H. C.** (2003). Wing resonances in the Australian field cricket
510   Teleogryllus oceanicus. *J. Exp.Biol.* **206**, 1479-1496.

511   **Béthoux, O.** (2008). Groundplan, nomenclature, homology, phylogeny, and the
512   question of the insect wing venation pattern. *Alavesia.* **2**, 219-232.

513   **Béthoux, O. and Wieland, F.** (2009). Evidence for Carboniferous origin of the order
514   Mantodea (Insecta: Dictyoptera) gained from forewing morphology. *Zool. J. Linn. Soc.* **156**,
515   79–113.

516   **Bethoux, O.** (2012). Grylloptera–a unique origin of the stridulatory file in katydids,
517   crickets, and their kin (Archaeorthoptera). *Arthropod. Syst. Phylogeny.* **70**, 43-68.

518   **Broughton, W. B.** (1964). Function of the mirror in Tettigoniidae Orthoptera. *Nature*
519   **201**, 949-950.

520   **Chamorro-Rengifo, J., Braun, H. and Lopes-Andrade, C.** (2014). The secret
521   stridulatory file under the right tegmen in katydids (Orthoptera, Ensifera, Tettigonioidea).
522   *Zootaxa* **3821**, 590-596.

523   **Chivers, B., Jonsson, T., Cadena-Castaneda, O. J. and Montealegre-Z, F.** (2014).
524   Ultrasonic reverse stridulation in the spider-like katydid Arachnoscelis (Orthoptera:
525   Listroscelidinae). *Bioacoustics* **23**, 67-77.

526   **Cole, J. A. and Chiang, B. H.** (2016). The Nearctic Nedubini: The Most Basal
527   Lineage of Katydids Is Resolved Among the Paraphyletic "Tettigoniinae"(Orthoptera:
528   Tettigoniidae). *Ann. Entomol. Soc. Am.* **109**, 652–662.

529   **Desutter Grandcolas, L.** (1997). A phylogenetic analysis of the evolution of the
530   stridulatory apparatus in true crickets (Orthoptera, Grylloidea). *Cladistics.* **13**, 101-108.

531   **Desutter-Grandcolas, L.** (1995). Functional forewing morphology and stridulation in
532   crickets (Orthoptera, Grylloidea). *J. Zool (Lond).* **236**, 243-252.

533   **Desutter-Grandcolas, L.** (2003). Phylogeny and the evolution of acoustic
534   communication in extant Ensifera (Insecta, Orthoptera). *Zool. Scr.* **32**, 525-561.

535   **Dumortier, B.** (1963). The physical characteristics of sound emission in Arthropoda.
536   In *Acoustic Behaviour of Animals*, (ed. R.-G. Busnel), pp. 346-373. Amsterdan: Elsevier.

537   **Eades, D. C., Otte, D., Cigliano, M. M. and Braun, H.** (2016). Orthoptera Species
538   File Online. Version 5.0/5.0. [07/12/2016]. http://Orthoptera.SpeciesFile.org.

539   **Elliott, C. J. H. and Koch, U. T.** (1983). Sensory feedback stabilizing reliable
540   stridulation in the field cricket *Glyllus campestris* L. *Anim. Behav.* **31**, 887-901.

541   **Elliott, C. J. H. and Koch, U. T.** (1985). The clockwork cricket. *Naturwissenschaften*
542   **72**, 150-153.

543   **Forrest, T. G.** (1991). Power output and efficiency of sound production by crickets.
544   *Behav. Ecol.* **2**, 327-338.

545   **Fletcher, N. H.** (1992). Acoustic Systems in Biology. Oxford: Oxford University
546   Press.

547   **Gorochov, A. V.** (2003). New data on taxonomy and evolution of fossil and Recent
548   Prophalangopsidae (Orthoptera: Hagloidea). *Acta. Zool. cracoviensia* **46**, 117-127.

549   **Gorochov, A. V.** (2005). Review of Triassic Orthoptera with descriptions of new and
550   little known taxa. Part 1. *Paleontol. J.* **39**, 178-186.

551   **Gu, J.-j., Montealegre-Z, F., Robert, D., Engel, M. S., Qiao, G.-X. and Ren, D.**
552   (2012). Wing stridulation in a Jurassic katydid (Insecta, Orthoptera) produced low-pitched
553   musical calls to attract females. *Proc. Natl. Acad. Sci. USA.* **109**, 3868-3873.

554   **Gwynne, D. T.** (1995) Phylogeny of the Ensifera (Orthoptera): a hypothesis
555   supporting multiple origins of acoustical signalling, complex spermatophores and maternal
556   care in crickets, katydids and weta. *J. Orthoptera Res.* **4**, 203-218.

557   **Heller, K.-G., Hemp, C., Ingrisch, S. and Liu, C.** (2015). Acoustic Communication in
558   Phaneropterinae (Tettigonioidea)-A Global Review with Some New Data. *J. Orthoptera Res.*
559   **24**, 7-18.

560   **Hemp, C.** (2001). Aerotegmina, a new genus of African Listroscelidinae (Orthoptera:
561   Tettigoniidae, Listroscelidinae, Hexacentrini). *J. Orthoptera Res.* **10**, 121-128.

562   **Hoy, R. R., Pollack, G. S. and Moiseff, A.** (1982). Species-recognition in the field
563   cricket, Teleogryllus oceanicus: behavioral and neural mechanisms. *Am. Zool.* **22**, 597-607.

564   **Heller, K.-G.** (1995). Acoustic signalling in Palaeotropical bush-crickets (Orthoptera,
565   Tettigonioidea, Pseudophyllidae): does predation pressure by eavesdropping enemies differ
566   in the Palaeotropics and Neotropics. *J. Zool. (Lond).* **237**, 469–485.

567   **Jost, M. C. and Shaw, K. L.** (2006). Phylogeny of Ensifera (Hexapoda : Orthoptera)
568   using three ribosomal loci, with implications for the evolution of acoustic communication.
569   Molecular Phylogenetics and Evolution **38**, 510-530.

570   **Keuper, A., Weidemann, S., Kalmring, K. and Kaminski, D.** (1988). Sound
571   production and sound emission in seven species of European Tettigoniids. Part II. Wing
572   morphology and the frequency content of the song. *Bioacoustics* **1**, 171-186.

573   **Koch, U. T., Elliott, C. J. H., Schäffner, K.-H. and Kleindienst, H.-U.** (1988). The

574   mechanics of stridulation of the cricketGryllus campestris. *J. Comp. Physiol. A.* **162**, 213-

575   223.

576   **Masaki, S., Kataoka, M., Shirato, K. and Nakagahara, M.** (1987). Evolutionary

577   differentiation of right and left tegmina in crickets. In *Evolutionary biology of Orthopteroid*

578   *Insects*, (ed. B. Baccetti), pp. 347-357. England: Ellis Horwood Limited.

579   **Montealegre-Z, F.** (2005) Biomechanics of musical stridulation in katydids

580   (Orthoptera: Ensifera: Tettigoniidae): an evolutionary approach, PhD thesis, University of

581   Toronto, Canada.

582   **Montealegre-Z, F.** (2009). Scale effects and constraints for sound production in

583   katydids (orthoptera: tettigoniidae): generator morphology constrains signal parameters. *J.*

584   *Exp. Biol.* **22**, 355–366.

585   **Montealegre-Z, F., Jonsson, T. and Robert, D.** (2011a). Sound radiation and wing

586   mechanics in stridulating field crickets (Orthoptera: Gryllidae). *J. Exp. Biol.* **214**, 2105-2117.

587   **Montealegre-Z, F. and Mason, A. C.** (2005). The mechanics of sound production in

588   *Panacanthus pallicornis* (Orthoptera : Tettigoniidae : Conocephalinae): the stridulatory motor

589   patterns. *J. Exp. Biol.* **208**, 1219-1237.

590   **Montealegre-Z, F., Morris, G. K., Sarria-S, F. A. and Mason, A. C.** (2011b). Quality

591   calls: phylogeny and biogeography of a new genus of neotropical katydid (Orthoptera:

592   Tettigoniidae) with ultra pure-tone ultrasonics. *System. Biodivers.* **9**, 77-94.

593   **Montealegre-Z, F. and Postles, M.** (2010). Resonant sound production in *Copiphora*

594   *gorgonensis* (Tettigoniidae: Copiphorini), an endemic species from Parque Nacional Natural

595   Gorgona, Colombia. *J. Orthoptera Res.* **19**, 347-355.

596   **Montealegre-Z, F., Windmill, J. F. C., Morris, G. K. and Robert, D.** (2009).

597   Mechanical phase shifters for coherent acoustic radiation in the stridulating wings of crickets:

598   the plectrum mechanism. *J. Exp. Biol.* **212**, 257-269.

599   **Morris, G. K. and Gwynne, D. T.** (1978). Geographical distribution and biological 600

        observations of *Cyphoderris* (Orthoptera: Haglidae) with a description of a new species.

601   *Psyche* **85**, 147-167.

602   **Morris, G. K. and Mason, A. C.** (1995). Covert Stridulation - Novel sound generation

603   by a South-American Katydid. *Naturwissenschaften* **82**, 96-98.

604   **Morris, G. K., Mason, A. C., Wall, P. and Belwood, J. J.** (1994). High ultrasonic

605   and tremulation signals in neotropical katydids (Orthoptera, Tettigoniidae). *J. Zool (Lond)*

606   **233**, 129-163.

607   **Morris, G. K. and Pipher, R. E.** (1967). Tegminal amplifiers and spectrum

608   consistencies in *Conocephalus nigropleurum* (Bruner), Tettigoniidae. *J. Insect Physiol.* **13**,

609   1075-1085.

610   **Morris, G. K., DeLuca, P. A., Norton, M. and Mason, A. C.** (2002). Calling-song
611   function in male haglids (Orthoptera : Haglidae, *Cyphoderris*). *Can. J. Zool.* **80**, 271-285.

612   **Robillard, T., Montealegre-Z, F., Desutter-Grandcolas, L., Grandcolas, P. and**
613   **Robert, D.** (2013). Mechanisms of high-frequency song generation in brachypterous crickets
614   and the role of ghost frequencies. *J. Exp. Biol.* **216**, 2001-2011.

615   **Pierce, G. W.** (1948). The songs of insects: with related material on the production,
616   propagation, detection, and measurement of sonic and supersonic vibrations. Cambridge,
617   Massachusetts, USA: Harvard University Press.

618   **Prestwich, K. N., Lenihan, K. M., & Martin, D. M.** (2000). The control of carrier
619   frequency in cricket calls: a refutation of the subalar-tegminal resonance/auditory feedback
620   model. *J. Exp. Biol.* **203**, 585-596.

621   **Prozesky-Schulze, L., Prozesky, O. P. M., Anderson, F. and Van der Merwe, G.**
622   **J. J.** (1975). Use of a self-made sound baffle by a tree cricket. *Nature.* **255**, 142 - 143

623   **Rasnitsyn, A.** (2007). On the discussion of the wing venation of (Archae) Orthoptera
624   (Insecta). *Paleontol. J.* **41**, 341-344.

625   **Otte, D.** (1992). Evolution of cricket songs. J. Ortho. Res. **1**, 25-49.

626   **Ower, G. D., Hunt, J. and Sakaluk, S. K.** (2016). Multivariate sexual selection on
627   male tegmina in wild populations of sagebrush crickets, *Cyphoderris strepitans* (Orthoptera:
628   Haglidae). *J. Evol. Biol.*doi:10.1111/jeb.13008

629   **Sales, G. D. and Pye, J. D.** (1974). Ultrasonic communication in animals. London:
630   Chapman and Hall.

631   **Sarria-S, F. A., Morris, G. K., Windmill, J. F. C., Jackson, J. and Montealegre-Z,**
632   **F.** (2014). Shrinking ings for ultrasonic pitch production: Hyperintense ultra-short-wavelength
633   calls in a new genus of Neotropical katydids (Orthoptera: Tettigoniidae). *PloS ONE.* **9**,
634   e98708.

635   **Sarria-S, F. A., Buxton, K., Jonsson, T. and Montealegre-Z, F.** (2016). Wing
636   mechanics, vibrational and acoustic communication in a new bush-cricket species of the
637   genus Copiphora (Orthoptera: Tettigoniidae) from Colombia. *Zool. Anz.* **263**, 55-65.

638   **Song, H., Amédégnato, C., Cigliano, M. M., Desutter Grandcolas, L., Heads, S.**
639   **W., Huang, Y., Otte, D. and Whiting, M. F.** (2015). 300 million years of diversification:
640   elucidating the patterns of orthopteran evolution based on comprehensive taxon and gene
641   sampling. *Cladistics* **31**, 621-651.

642   **Spooner, J. D.** (1973). Sound production in *Cyphoderris monstrosa* (Orthoptera:
643   Prophalangopsidae). *Ann. Entomol. Soc. Am.* **66**, 4-5.

644   **Walker, T. J.** (1973). Systematics and acoustic behavior of United-States and
645   Caribbean short-tailed crickets "(Orthoptera-Gryllidae-Anurogryllus). *Ann. Entomol. Soc. Am.*
646   **66**, 1269-1277.

647   **Windmill, J. F. C., Gopfert, M. C. and Robert, D.** (2005). Tympanal travelling waves

648   in migratory locusts. *J. Exp. Biol.* **208**, 157-168.

649   **Windmill, J. F. C., Fullard, J. H. and Robert, D.** (2007). Mechanics of a 'simple' ear:

650   tympanal vibrations in noctuid moths. *J. Exp. Biol.* **210**, 2637-2648.

651   **Zhou, Z., Shi, F. and Zhao, L.** (2014). The first mitochondrial genome for the

652   superfamily Hagloidea and implications for its systematic status in Ensifera. *PloS ONE.* **9**,

653   e86027.

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Figure legends

Fig. 1. Revised topographic homology conjectures (THCs) for the grigs (Prophalangopsidae) and the bush-crickets (Tettigoniidae), compared to the field-crickets (Gryllinae). (A,B) *Cyphoderris monstrosa* (grig; specimen IWC OB 530, left forewing, ventral view), wing venation (A) and areas (B). (C–F) *Tettigonia cantans* (bush-cricket; specimen IWC OB 862, right forewing, dorsal view). (C,D) THC newly proposed herein, wing venation (C) and areas (D). (E,F) Topographic homology conjecture according to Béthoux (2012), wing venation (E) and areas (F). (G,H) *Teleogryllus oceanicus* (field-cricket; specimen IWC OB 645, right forewing, ventral view flipped horizontally; THC according to Béthoux, 2012), wing venation (G) and areas (H). See text for details.

Fig. 2. Acoustic analysis of the call of *C. monstrosa*. (A) Typical presentation of the calling song. (B) Details of the call in A. (C) A single phonatome, resulting from one closing stroke of the wings during stridulation. (D) Relative intensity of the signal in C. Inset: male *Cyphoderris monstrosa* habitus.

Fig. 3. Displacement maps and frequency response of mirror vibration of both forewings of *C. monstrosa*. (A,B) Left (A) and right (B) wings showing vibrational response of the entire wing in response to sound stimulus; displacement gain taken from a band at the calling frequency of ~13 kHz. (C,D) Left (C) and right (D) wings from the laser Doppler Vibrometer video feed with vibrating areas highlighted in blue. (E) Mean of left wing mirror vibration velocities ± s.d. (n=5). (F) Mean of right wing mirror vibration velocities ± s.d (n=5). Shaded areas in A and B are the s.d. (G) Coherence of vibrometer response from the mirror area of both wings from all specimens (n= 5); black trace is the average; note all wings exhibit high coherence around the resonant frequency. LW: Left wing, RW: right wing.

Fig. 4. Vibration compliant areas on *C. monstrosa* wings. (A) Displacement map of vibrating area. (B) Deflection pattern of the profile line in A; red-shaded area is the mirror and blue shaded area is the adjacent area; note the mirror vibrates with the highest amplitude. (C) Laterally angled views of wing deflections at ~13 kHz. Vibration and deflection patterns are bilaterally symmetrical between both wings.

687

688

689   **Fig.5. Resonant tuning of the mirror area of both forewings.** (A) Frequency spectrum of

690   the calling song of *C. monstrosa*. (B) Vibrational response of the wings of the same

691   specimen, showing resonant tuning matching the calling frequency; amplitude of wing

692   vibration was normalised to the highest for comparison of frequency characteristics; red

693   trace, right wing, black trace, left wing.

694

695   **Fig. 6. Averaged frequency response of the mirror and adjacent area as vibrational**

696   **displacement in response to broadband acoustic stimuli for all specimens (spectrums**

697   **for each specimen were averaged from all points on the mirror and the adjacent area**

698   **(e.g. centre panels).** (A) Mean of left wing mirror displacement ± s.d. (n=5). (B) Mean of

699   right wing displacement ± s.d. (n=5). Note the mirror area vibrates with higher amplitude

700   than the adjacent area and exhibits resonant tuning around the calling frequency at ~13 kHz.
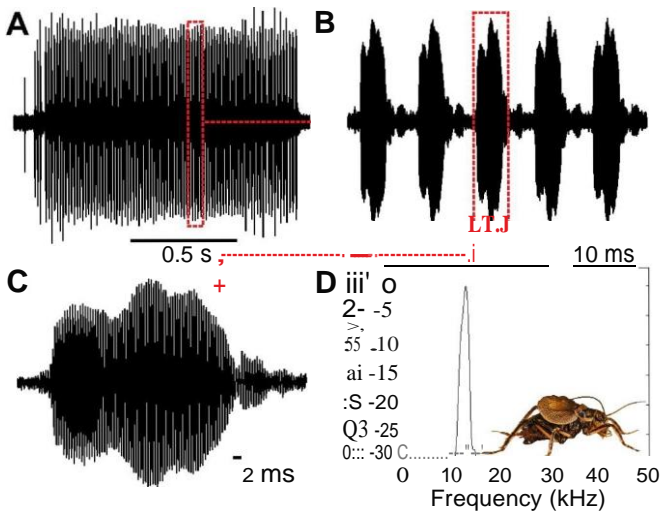
701

702   **Fig. 7. Comparison of vibrational response from laser Doppler vibrometry across**

703   **forewings of various Ensifera (Orthoptera) exhibiting tegmina-based stridulation.** (A)

704   *C. monstrosa* (this study). (B) *Copiphora brevirostris* (C) *Gryllus bimaculatus*.
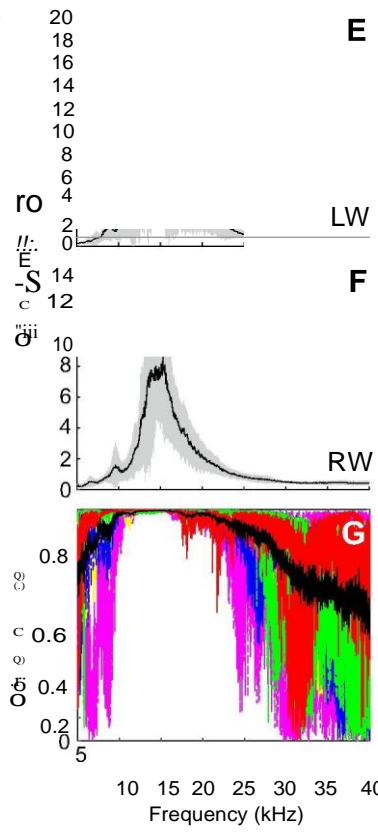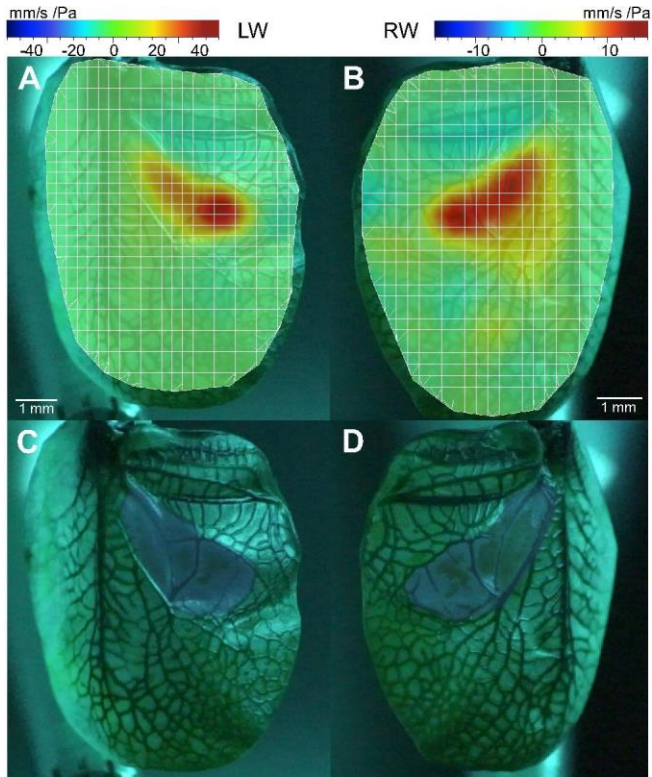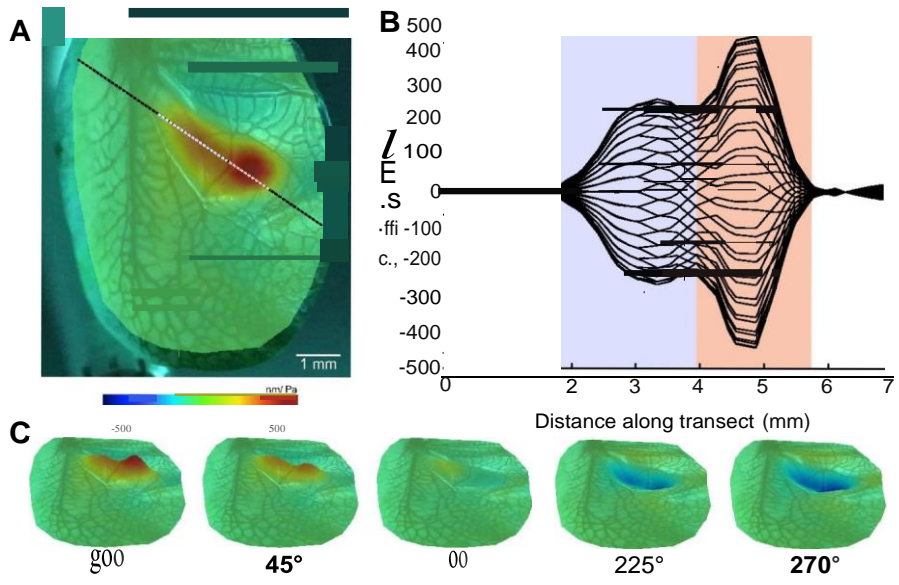
705

706   **Fig. 8. Morphology of the Larunda area in *Cyphoderris* spp. and fossil taxa.** (A) Wing

707   venation of *C. monstrosa* (specimen IWC OB 530, left forewing); Larunda area brown-

708   shaded, other color-coded areas correspond to the actual, main radiating area (mirror and

709   adjacent area). (B–E) Morphological details of the Larunda area in *Cyphoderris* spp. (B) *C.*

710   *monstrosa* (IWC OB 532, right forewing, dorsal view); the Larunda area is delimited by a

711   brown dashed line. (C–E) *C. buckelli* (C: IWC OB 529, left forewing, ventral view; D: IWC OB

712   528, right forewing, dorsal view; E: left forewing, dorsal view). (F) Reconstruction of

713   †*Gryllavus madygenicus* (Lower Triassic; Madygen, Kirgizstan; inspired from Sharov (1968)

714   and Béthoux (2012); all areas recognized in extant species are color-coded but the post-

715   mirror, which is absent (as a consequence of lack of a well-defined frame); note that color-

716   coding does not indicate a putative functional role of the corresponding areas.
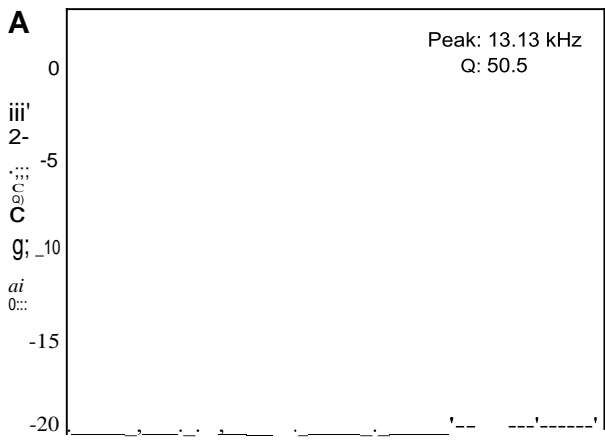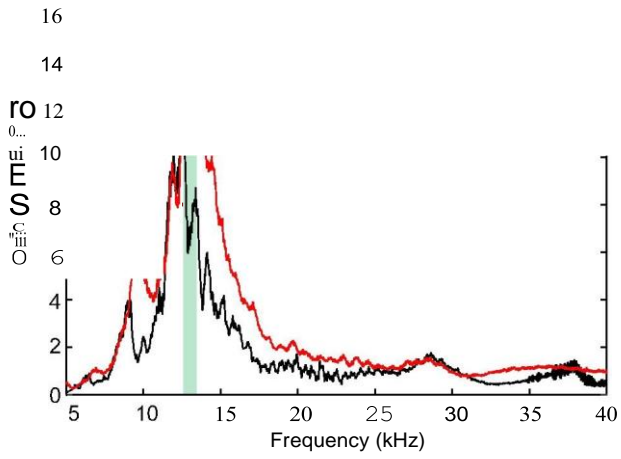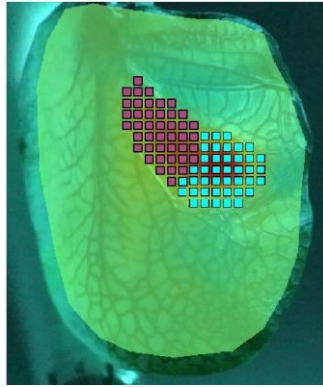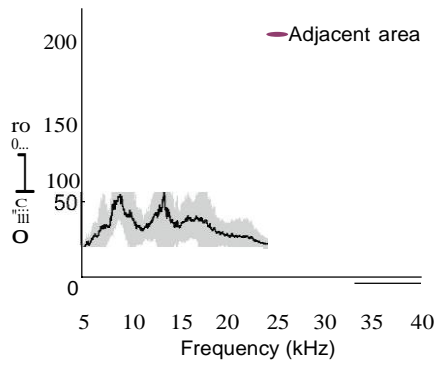
717

A

B

LT.J

0.5 s

10 ms

C

+

2 ms

D iii' o

2- -5
>.
55 -10
ai -15
:S -20
Q3 -25
0::: -30 C

0   10   20   30   40   50
Frequency (kHz)

LW

RW

mm/s /Pa
-40 -20 0 20 40

mm/s /Pa
-10 0 10

A

B

C

D

1 mm

1 mm

E

F

G

LW

RW

Frequency (kHz)

20
18
16
14
12
10
8
6
4
2
0

14
12
10
8
6
4
2
0

0.8
0.6
0.4
0.2
0

5 10 15 20 25 30 35 40

A

1 mm

nm/ Pa

B

l
E
.s
·ffi
c.,

500
400
300
200
100
0
-100
-200
-300
-400
-500

0   2   3   4   5   6   7

Distance along transect (mm)

C

-500   500

g00   **45°**   00   225°   **270°**

A

Peak: 13.13 kHz
Q: 50.5

Frequency (kHz)

A

Adjacent area

D Mirror area

B

Adjacent ar

D Mirror area

i

A — Prophalangopsidae

1 mm

nm /Pa
-100 0 100

B — Tettigoniidae

1 mm

nm /Pa
-40 -20 0 20 40

C — Gryllidae

1 mm

nm /Pa
-400 -200 0 200 400

A

2 mm

B R
M
CuA
CuPaa
2 mm

C R
M
CuA
CuPaa
2 mm

D R
M
CuA
CuPaa
2 mm

E R
M
CuA
CuPaa
2 mm

F h1&2 h3 ?