Journal of Comparative Physiology A

Biomechanics of hearing in katydids

--Manuscript Draft--

Manuscript Number:	JCPA-D-14-00131R2
Full Title:	Biomechanics of hearing in katydids
Article Type:	S.I. : Insect Hearing - from Physics to Ecology
Section/Category:	Review
Abstract:	Animals have evolved a vast diversity of mechanisms to detect sounds. Auditory organs are used to detect intraspecific communicative signals and environmental sounds relevant to survival. To hear, terrestrial animals must convert the acoustic energy contained in the airborne sound pressure waves into neural signals. In mammals, spectral quality is assessed by the decomposition of incoming sound waves into elementary frequency components using a sophisticated cochlear system. Some neotropical insects like katydids (bushcrickets) have evolved biophysical mechanisms for auditory processing that are remarkably equivalent to those of mammals. Located on their front legs, katydid ears are small, yet are capable of performing several of the tasks usually associated with mammalian hearing. These tasks include air-to-liquid impedance conversion, signal amplification, and frequency analysis. Impedance conversion is achieved by a lever system, a mechanism functionally analogous to the mammalian middle ear ossicles, yet morphologically distinct. In katydids, the exact mechanisms supporting frequency analysis seem diverse, yet are seen to result in dispersive wave propagation phenomenologically similar to that of cochlear systems. Phylogenetically unrelated, katydids and tetrapods have evolved remarkably different structural solutions to common biophysical problems. Here, we discuss the biophysics of hearing in katydids and the variations observed across different species.
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Author Comments:	Dear Prof. Römer, Here is the last revised version of our ms after last editorial comments. We accepted the changes suggested by the editor, and also found a few other typos to sentences that needed improvement. Please note that the ms parts that have been newly edited or modified are shown in red fonts. Kind regards fer
Response to Reviewers:	See comments.

Biomechanics of hearing in katydids

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Abstract: Animals have evolved a vast diversity of mechanisms to detect sounds. Auditory organs are thus used to detect intraspecific communicative signals and environmental sounds relevant to survival. To hear, terrestrial animals must convert the acoustic energy contained in the airborne sound pressure waves into neural signals. In mammals, spectral quality is assessed by the decomposition of incoming sound waves into elementary frequency components using a sophisticated cochlear system. Some neotropical insects like katydids (or bushcrickets) have evolved biophysical mechanisms for auditory processing that are remarkably equivalent to those of mammals. Located on their front legs, katydid ears are small, yet are capable of performing several of the tasks usually associated with mammalian hearing. These tasks include air-to-liquid impedance conversion, signal amplification, and frequency analysis. Impedance conversion is achieved by a lever system, a mechanism functionally analogous to the mammalian middle ear ossicles, yet morphologically distinct. In katydids, the exact mechanisms supporting frequency analysis seem diverse, yet are seen to result in dispersive wave propagation phenomenologically similar to that of cochlear systems. Phylogenetically unrelated, katydids and tetrapods have evolved remarkably different structural solutions to common biophysical problems. Here, we discuss the biophysics of hearing in katydids and the variations observed across different species.

Keywords: Cochlea. Insect hearing. Auditory mechanics. Impedance. *Crista acustica*.

In the animal kingdom many species must identify environmental sounds to increase their chance of survival. Acoustic communication occurs in many groups of animals, spanning the phylogeny from invertebrates to vertebrates, and well-studied cases pertain to mammals, birds, amphibians and arthropods (crustaceans and insects, Bradbury and Vehrencamp 1998). Yet, due to their biological diversity, insect species constitute a large percentage of the acoustic community that pervades many terrestrial habitats. Concrete examples of bona fide communication are found in the cicadas, crickets, katydids and grasshoppers. In these groups, males sing to attract conspecific females, a process that opens up numerous possibilities for sexual selection (Robinson and Hall 2002). As in field crickets, with few exceptions, katydid males produce calls by tegminal stridulation: the scraping together of one wing which possesses a vein modified with a series of small teeth against the other wing which bears an edge in the anal margin that works as a scraper (Morris 1999). However, unlike field crickets, which communicate at around 5 kHz, the sound frequencies exploited by katydids vary from 5 kHz to 150 kHz, depending on the species (Montealegre-Z 2009; Sarria-S et al. 2014).

Besides sounds used for intraspecific communication, katydids are also exposed to many other sounds that may require their attention. A large number of tropical species are nocturnal and most Tettigoniidae species are a good example of adaptation to nocturnal life. Ambient noise in typical katydid habitats, tropical rainforests, increases considerably after sunset by about 20 decibels relative to daytime ambient noise (Lang et al. 2006). This environmental noise consists of many sounds, contributing different frequencies altogether. Some of these frequencies are permanent, occurring day and night, such as the sounds of rivers (rapids and waterfalls), while other sounds are temporary, transient and changeable, such as the rustlings and songs of other nocturnal animals, rain, wind, etc. Thus, in addition to conspecific sounds, many species can detect a wide range of frequencies. This should encompass broadband sounds that are identified as potential threats, such as ultrasound produced by insectivorous bats to hunt and navigate at night in the clutter of forested environments, as well as the sound produced by other nocturnal insectivorous mammals (Belwood and Morris

1987; Faure and Hoy 2000; Ramsier et al. 2012; Ratcliffe et al. 2011). The Tettigoniidae ear has evolved in the context of intraspecific communication and predator detection (e.g., Belwood and Morris 1987; Faure and Hoy 2000). The aim of this paper is to present in detail the functional mechanics of katydid hearing, drawing a parallel between the ear of the Tettigoniidae and Tetrapods.

The basics of hearing

In order to detect sounds from the environment, an animal must be able to convert the acoustical energy contained in sound pressure waves into neuronal signals. Generally, this transduction process can be summarized as follows: 1) Transformation of sound into mechanical vibration, using an acousto-mechanical receiver structure like a tympanal membrane or an appendage such an antenna or a long hair. 2) Coupling of this mechanical energy to mechanosensory structures, and finally 3) mechanical and neural analysis of the waveform in terms of signal frequency, amplitude and temporal structure. There are several possibilities of signal amplification and filtering along the hearing chain, for instance during sound capture the ear canal acts as an exponential horn, boosting the sound pressure 30 to 100 fold for frequencies around 3 kHz in humans. Amplification also occurs during translocation of the mechanical energy via lever action of the ossicles in the middle ear, or by motility of the mechanosensory cells (Purves et al. 2013; Mhatre, this volume).

The auditory systems of many animals are capable of performing frequency decomposition of complex waveforms. Such frequency analysis relies on individual mechanosensory receptors to be responsive to a narrow range of frequencies, or ideally, one frequency. Such tuning arises because of the location of receptors on a physical substrate, the basilar membrane in mammals, the *crista acustica* (CA) in katydids. In addition, tuning may also result from intrinsic properties of the molecular machinery responsible for signal transduction in the mechanosensory cell. Mechanically, place-specific tuning has long been described and studied, a process called tonotopy (von Bekesy 1960; Palgath Udajashankar 2012). The best known example is the tonotopic organization of frequency sensitivity along the basilar membrane in the mammalian cochlea (von Bekesy 1960; Ashmore 2008). Frequency tuning within the inner ear results in part from the geometry of the basilar membrane, which is wider and more flexible at the apex and narrower and stiffer at the base. von Békésy (1960) showed that the basilar membrane vibrates maximally at different positions as a function of the stimulus frequency. The points responding to high frequencies are at the base of the basilar membrane, and the points responding to low frequencies are at the apex, giving rise to a topographical mapping of frequency, also known as tonotopy (Robles and Ruggero 2001). In mammals, the sensory hair cells are distributed in an orderly linear array along the length of the basilar membrane. As a result, high frequency receptors are located at the base of the cochlea, where basilar membrane stiffness is high, while low frequency receptors are found at the apex, where basilar membrane stiffness is low. The mechanisms giving rise to such mechanical frequency decomposition are still debated in the details. However, it is believed that the ear's sensitivity arises from an active biomechanical process, as well as from its passive resonant properties (Purves et al. 2013; Mhatre, this volume).

Frequencies are thus represented along a stiffness gradient that is generally regarded as smooth, but is not necessarily homogenous (Bruns and Schmieszek 1980; Schnitzler and Denzinger 2011; Schuller and Pollak 1979). In such non-smooth tonotopic systems, some frequency ranges can be represented in more detail than others, a remarkable adaption often referred to as the “acoustic fovea” by analogy to the visual system (Isobe and Motokawa 1955). Such adaptation was unknown in invertebrate hearing, but past and recent evidence suggest that an acoustic fovea might occur in the katydid ear of some species (Oldfield 1982, Montealegre-Z et al. 2012).

Auditory systems performing frequency analysis using dedicated impedance conversion and a fluid-filled dispersive medium were known only in higher vertebrates like tetrapods. It was recently shown that katydids use a tetrapod-like mechanism of hearing, involving the three canonical steps of hearing. The presence of such mechanisms in insects constitutes a remarkable case of convergent evolution between tetrapods and katydids (Montealegre-Z et al.

2012). Such convergence demonstrates that auditory sensitivity and frequency analysis are possible for microscale auditory systems, using analogous operating principles yet alternative morphological architecture.

In insects, the mechanisms that determine frequency selectivity of individual auditory receptors are diverse. While many auditory insect species are known to have some form of frequency selectivity, for most of them, the biophysical mechanisms are little understood.

Generalized katydid ear anatomy

Katydids have their ear in the basal part of the fore tibia (Fig. 1). Each ear presents paired eardrums; an anterior tympanal membrane (ATM) and a posterior tympanal membrane (PTM), located on the proximal part of the tibia in each foreleg (Fig. 1a). The tympana are partially backed by an air-filled tube, the acoustic trachea (AcT), which extends forwards from the acoustic spiracle in the prothorax through the femoral cavity of the foreleg, enters the tibia (Fig. 1b), and divides into anterior and posterior branches (Bangert et al. 1998; Lewis 1974; Fig. 2c, d). In cross section, the katydid's ear is asymmetrical: the anterior branch occupies a large portion of the dorsal ear surface (Fig. 2c, d; Rössler et al. 1994). The mechanoreceptors, comprised in a long and thin structure, the CA, lie on the dorsal wall of this anterior tracheal division, and are contained within the auditory vesicle (AV), a fluid-filled, partially blind cavity (Stumpner and Nowotny 2014; Montealegre-Z et al. 2012; Fig. 2c-e). Mechanoreceptors on the CA are tonotopically organized (Oldfield 1982), but the sensory cells are not directly in contact with the tympana as it is often the case in other acoustic insects (e.g. locusts, flies and moths; Stephen and Bennet-Clark 1982; Robert 2005; Yack 2004).

The mechanisms that together enable acute hearing and frequency selection in katydid ears are presented here, step by step and in more detail.

Sound capture

In most vertebrates, the ear has one main input, whereby sound pressure acts on the external surface of the tympanal membrane. In katydids, each ear has three possible acoustic inputs: acoustic spiracle, and the two tympanal membranes (Michelsen and Larsen 1978). In these insects sound can reach the external surface of the tympanal membrane and/or reach the internal surface of the tympanal membrane through specialised tracheal pipes. Such tracheal ducts establish a sound passage to the acoustic (or auditory) spiracle that is usually followed by a tracheal expansion known as the auditory bulla (Hill and Oldfield 1981; Bailey 1991). The size of the auditory spiracle and bullae vary across species, and influences sound capture and acoustic energy gain. Different from gryllids (Michelsen et al. 1994b), the acoustic trachea starting at the acoustic spiracle is not connected in the middle by a septum, but the bullae are usually separated (Bailey 1990, Fig. 1a). Most Tettigoniidae species exhibit vestiges of the ventilatory system as a form of a filament that connects the two bullae. But in some species the connection between the two acoustic bullae is open, and consists of a series of narrow channels from one bulla to the other (Bailey 1990). In field crickets the two trachea are clearly connected by a thin membrane, and this design is the anatomical basis for a pressure difference receiver ear (Michelsen et al. 1994b; Hirtenlehner et al. 2014), however, in katydids the filament or narrow channel connections have apparently no acoustic function. Some katydid species exhibit large bullae in complete contact through a large surface area (Bailey 1990); the acoustic adaptation of this morphology is unknown.

In species with large acoustic spiracles and large auditory bullae adjacent to the spiracle (Fig. 1), the AcT represents the main input for sound capture (Heinrich et al. 1993). In these species (e.g., *Decticus albifrons*, *D. verrucivorus* (Decticinae); *Tettigonia viridissima*, *T. cantans* (Tettigoniinae); *Ephippiger ephippiger*, *Ephippigerida taeniata* (Ephippigerinae); *Mygalopsis marki* (Conocephalinae), *Poecilimon thessalicus*, *P. laevissimus*), and in some others, both internal and external inputs are functional and both produce different gains and time delays that enhance directional hearing (Hill and Oldfield 1981; Michelsen et al. 1994a). For example, in these species the AcT acts as an exponential horn promoting a gain of ca. 10-30 dB depending on the species. Nevertheless, it has also been found that sound propagates with lower speed inside the trachea than it does in air. Hence, the signal reaches the external surface of the tympanum travelling at normal sound speed in air (ca. 340 m/s), while the same signal travels inside the AcT a lower speed and arrives to the internal tympanal surface a few microseconds later and with different vibrational phase (Schiolten et al 1981; Michelsen et al. 1994a, Michelsen and Larsen 2008). Each tympanic membrane will therefore experience two events to capture a single signal: the first event (external input) occurs when the tympanum collects the signal travelling at normal sound speed and arriving at its external surface (with low amplitude as no amplification occurs to the airborne signal prior to its arrival at the external tympanal surface), the second event (tracheal input) is experienced when the same signal travels inside the trachea at lower sound speed than the external input. This delayed signal reaches the tympanal internal surface a few micro-seconds later than the external input, but would exhibit high amplitude as the signal has been amplified in the trachea. Both events can be observed recording tympanal vibration using Laser Doppler Vibrometry (LDV- Schiolten et al 1981; Michelsen et al. 1994a, Montealegre-Z and Robert unpublished data). The AcT therefore has a vital function in directional hearing, involving remarkable pressure difference mechanisms (Autrum 1940; Hill and Oldfield 1981; Michelsen and Larsen 2008). Although these mechanisms have been studied in crickets (Michelsen et al. 1994b) and katydids (Schiolten et al. 1981), the neural processing of this pressure-gradient-receiver system is poorly understood.

The benefits of a tracheal sound input in katydids may be multiple, first producing sound amplification (Hill and Oldfield 1981; Heinrich et al. 1993; Hoffmann and Jatho 1995; Michelsen et al. 1994a; Shen 1993) prior to its capture by the tympanal membrane. This would be analogous to the role of the mammalian pinna and ear canal. As explained above, tracheal input might also enhance directional hearing in a pressure difference mechanism. However, besides this sound transmission role, the AcT also serves to equilibrate atmospheric pressure in both sides of the tympana, just as in the Eustachian tubes in terrestrial tetrapods.

In species with small thoracic spiracles, such as most Pseupdophyllinae, the situation appears to be different. In Pseudophyllinae the external input to the TMs seems to dominate the total driving force to the eardrum, at least at some frequencies (Mason et al. 1991), raising the question of how directional hearing is achieved using a pressure receiver only. Interestingly, earlier studies on the ultrasonic neotropical species *Myopophyllum speciosum, Haenschiella ecuadorica and Typophyllum* sp. (Mason et al. 1991; Morris et al. 1994) suggest that the tympanal flaps act as resonating chambers which affect the acoustic pressure reaching the external face of the tympanum. Potentially, pressure could be altered through diffractive effects, with some possible consequences for the timing at which acoustic pressure imparts force on the eardrums. In the palaeotropical species *Onomarchus uninotatus*, a katydid exhibiting a narrow bandwidth call with unusual low carrier frequency of 3.2 kHz, acoustic partitioning between the two tympanal membranes has been documented (Rajaraman et al. 2013). While the ATM acts as a low-pass filter, attenuating sounds at frequencies above 3.5kHz, the PTM acts as a high-pass filter. The PTM which shows maximal sensitivity at several broad frequency ranges, peaking at 3.1, 7.4 and 14.4kHz. This unusual feature of peripheral auditory processing is poorly understood.

The role of the AcT in the Pseudophyllinae is not clear. The results of Mason and Morris (1991) suggest that the acoustic spiracle and narrow trachea of species using very high carriers is linked with predominant sound access via the tympanal slits. But similar spiracle anatomy and narrow acoustic bullae also occur in other Pseudophyllinae using low frequencies (e.g., Heller 1995; Rajaraman et al. 2013). Thus it is not clear what effect the AcT may have on auditory sensitivity and directionality. If not acoustically functional, the narrow acoustic bullae in Pseudophyllinae might serve only to equilibrate atmospheric pressure at both sides of the tympana. This could have been the initial function of the tracheal system associated with the ear before adaptations to collect, conduct and amplify sounds evolved.

The tettigoniidae also exhibit variation in the external morphology of the tympanal organ. As mentioned above in some species the tympanal organ exhibits cuticular folds surrounding the tympanum (Figs. 1 and 2c, d). Other species present a cuticular fold surrounding the anterior tympanum only, while the opposite tympanum is completely or partially exposed. In many other species (e.g., most Phaneropterinae katydids) both tympanic membranes are exposed. The presence of cuticular folds around the tympanam has received attention by some researchers. Autrum (1940, 1963) implied that cuticular folds and tympanal slits help the insect to detect the direction of the sound. Subsequently Bailey and Stephen (1978), Stephen and Bailey (1982), Bailey et al. (1988) supported Autrum's and demonstrated that the tympanal slits and cuticular folds cold function as sound guides to enhance directional hearing in some species.

Transmission of acoustic energy from air to fluid: the katydid middle ear

Once acoustic energy has been converted into tympanal mechanical vibrations, the important process of impedance conversion is required to enable the efficient transmission of mechanical energy from the air to the fluid medium where the mechanosensory cells reside. The morphological solution to this process is the hallmark of the evolution of hearing in mammals, as middle ear ossicles are highly specialised, differ from species to species, and are the key to efficient hearing. In insects, the importance of impedance conversion has received little attention, as often the tympanal membrane is directly connected to the chordotonal mechanosensory organ (e.g., moths, locust; Field and Matheson 1998). For insect species for which signal frequency composition is relevant, such as katydids, this process has been recognised to be important (Bangert et al. 1998,) as it holds the key to efficient and frequency selective hearing.

For decades the role of the tympanal membranes in the hearing process has been a topic of interest for some researchers (e.g. Michelsen and Larsen 1978; Oldfield 1985, Mhatre et al 2009; Notwotny et al 2010; Montealegre-Z et al. 2012). While in the locust the tympanum exhibits different resonant frequencies and vibrates with complex modes that code for travelling waves and frequency selectivity (Michelsen 1971; Windmill et al. 2005), the tympanal membranes of the katydid ear vibrate in a single mode (Michelsen et al. 1994a; Bangert et al. 1998; Nowotny et al. 2010).

Two models have been proposed to explain the transmission of mechanical energy from the TMs to the CA in the katydid ear. Both models are based on the law of levers. A lever is a movable bar that pivots on a fulcrum attached to a fixed point. The ratio of the output force to the input force is the mechanical amplification of the lever (Vogel 2013). Levers are relevant in biological systems because they help to amplify an input force to provide a greater output force.

The first model was proposed by Bangert et al. (1998), described as hinged-flap system. Bangert and co-workers proposed an impedance conversion mechanism of airborne acoustic energy to a dispersive medium, which predicts a force conversion between tympanum and the elastic surface of the tracheal wall bearing the CA. Such conversion changes the fluid space above the CA. This model however does not use TP motion. Bangert et al. (1998) hinge model is therefore based on a class 2 lever (a lever with the fulcrum in one end, the applied force in the other end, and the resulting force in the middle, Vogel 2013), in which a hinge is meant to move a load between the fulcrum and the force. The force gain will be lower close to the hinge, but effective at the location of the load (in the tympanal organ the load should be an area of contact between the tympanum and the elastic dorsal wall of the trachea; Fig. 2f). A similar model was supported by Nowotny et al. (2010) in their studies of tympanal motion in *M. elongata*. In their study of *M. elongata*, in which no middle ear was observed, Palghat Udayashankar et al. (2012) conjectured that pressure waves travelling in the trachea activate vibrations of the CA internally before activating the tympanic membranes, i.e., sound enters the hearing organ at the proximal part of the leg, where low frequencies are represented. Their findings imply that slow waves were transmitted first to the proximal part of the CA, and from there, vibrations travelled distally, and then proximally again as travelling waves. This contrasts with the out-of-phase response between TMs and TPs observed in *Copiphora gorgonensis* (Montealegre-Z et al. 2012), revealing a type 1 lever action (a lever with the applied force in one end, the resulting force in the other, and the fulcrum in the middle, Vogel 2013).

A second model of impedance conversion in the tympanal organ of the katydid *C. gorgonensis* was proposed by Montealegre-Z et al. (2012). These authors state that impedance conversion is a functional part of the katydid ear, and is analogous to the mammalian middle-ear process. This part of mechanical auditory processing is carried out by a lever-like structure, the TP (Fig. 2a, b, e). The TP enables the coupling of sound-induced vibrations from the TMs to the AV and CA. Montealegre-Z et al. (2012) showed that the TM and TP are linked but are distinct structures in *C. gorgonensis* (Fig. 2a, b). The TM is a thin membrane (6-16 µm), which presents both sides to air, while the TP is thicker (20-30µm), and has one side facing air and the other applied to the fluid of AV (Fig. 2c-e). Montealegre-Z et al. (2012) suggested that these two structures operate like a type 1 lever model, a type of seesaw with an eccentric fulcrum (Fig. 2e). Given the proportions of the TM and TP in *C. gorgonensis,* such a lever system should produce a conversion ratio of 1:10 between effort (TM) and load (TP and fluid).

The contour of the TM is distinctly kidney shaped in the katydids studied by Montealegre-Z et al. (2012), with the TP located near the dorsal curvature of the shape (Fig. 2a, b). Without empirical evidence Montealegre-Z et al. (2012) believe this specific and unusual tympanal shape serves to channel vibrations to the distal part of the AV and CA via the TP. Such kidney shaped TMs in some but not all katydid species may be the hallmark of impedance conversion and frequency analysis. More research is needed in this area.

Bangert et al. (1998), Hummel et al. (2011), Palghat Udayashankar et al. (2012), and Montealegre-Z et al. (2012) showed that part of the tympanum, the TP, was in contact with the hemolymphatic fluid in the species studied by them. A key difference between the *P. denticauda*, the *Mecopoda* and the *C. gorgonensis* studies resides in the type of lever mechanism used to help vibrations enter the fluid environment of the sensory organ proper. For *P. denticauda* and *M. elongata*, the vibrations of both TM and TP are in phase and the pivot point seems to lay on the dorsal edge of the TP. The dorsal edge of the TP is hinged to the cuticle of the leg and its displacement amplitudes are lower than those of the ventral part of the TM (Fig. 2f, Bangert et al. 1998; Nowotny et al. 2010; Palghat Udayashankar et al. 2012).

A class 2 lever has been documented in more primitive forms of ensifera, like Anoatostomatidae (e.g., weta, Lomas et al. 2011), and occur in Prophalangopsidae (e.g., *Cyphoderris* spp. FMZ and DR unpublished data), and perhaps in other ensifera with large and centralised TPs. Weta do not have tracheal inputs, thus the tympanal organ seems to function as a normal pressure receiver. The weta TMs are oval or nearly rounded, non-taut, and bear a large sclerotized oval TP. This TP is not isolated from the membrane contour like the TP in *C. gorgonensis* but is embedded within the membrane. Both TMs deflect like a hinge, with the same phase as the TPs (Lomas et al. 2011). Different from the weta's ear, the TP of *C. gorgonensis* vibrates in antiphase with the TMs. Large tympanal displacements produced with low force exert a large force of the small area of the TP, which deflects with small displacement (Montealegre-Z et al. 2012). This is known as mechanical advantage.

The weta's mechanism of impedance conversion is unknown. It is also unknown if these insects need to analyse frequencies, however in cross section the tympanal organ is asymmetrical and shows a CA (Ball and Field 1981; Lomas et al. 2012). Wetas usually live in galleries, which suggests they are not often exposed to insectivorous bats and perhaps that they do not need to resolve a broad range of frequencies. In fact, the audiograms show their frequency sensitivity is very limited and low (2.0-2.5 kHz; Field et al. 1980).

Spectral decomposition of the system and travelling waves: the katydid inner ear

Oldfield (1985) established that the tympanic membranes do not contribute to frequency selectivity in the katydid *Mygalopsis marki*. More recently Hummel et al. (2011) demonstrated that tympanum motion and neuronal response are not coupled directly. Thus frequency decomposition happens somewhere else.

The katydid inner ear is composed of the CA (bearing the fluid-immersed mechano-receptors), and the AV (containing the fluid bathing the mechanoreceptor, Fig. 3). The coupling of the AV and CA was only recently shown, and previous researchers thought of the AV as a simple continuation of the hemolymph channel (HC - e.g., Schumacher 1973, 1975; Rossler et al

1994). In the species documented by Montealegre-Z et al. (2012), and some others studied by the authors using the proposed methods, the AV connects with the leg haemolymph supply through a narrow constriction (Fig. 3). However in *Metrioptera sphagnorum,* such a connection was not observed, and the AV seems to be isolated (Fig. 3a-e). In the conocephaloid katydids *Pancanthus pallicornis* and *C. gorgonensis* a plug of unknown colloidal material is observed in the proximal and distal ends of the AV (Fig. 3e). In a similar way, the fluid contained in the AV appears not to be pure hemolymph. Apolar extraction and comparison of this fluid with hemolymph taken from different body regions suggests that the fluid might contains lipids (Montealegre-Z et al. 2012). In wetas, a plesiomorphic group related to modern katydids (Mugleston et al. 2013), the hemolymphatic fluid bathing the mechanoreceptors is rich in lipids (Lomas et al. 2012). Although the chemical composition of the AV fluid in the katydid species studied so far by the authors is unknown, it is known that the AV is an important component of the katydid tympanal organ as it provides a medium for wave propagation. The AV fluid could also enhance frequency decomposition and produce an additional step for signal amplification or energy localisation. In the mammalian cochlea for example, the passive vibrations of the basilar membrane are the product of different factors, which include not only the flexibility and mass of the basilar membrane and organ of Corti, but also the physical properties of the adjacent fluid (Robles and Ruggero 2001).

Travelling waves and mechanical tonotopy in the tympanal organ of the katydid *M. elongata* were shown for the first time by Palghat Udayashankar et al. (2012). They exposed the anterior tracheal branch containing the CA by removing the dorsal cuticle and AV fluid (regarded as hemolymph), replaced the AV fluid by insect ringer solution to avoid desiccation and stimulated the ear by sound. They isolated the tympanal organ from the tracheal input using a special platform, and monitored CA vibrations using LDV. Input isolation is necessary here because if the dissected tympanal organ with exposed CA is presented with sound in an open field, it is impossible to control for air-borne sound reaching and stimulating the CA surface directly, producing unreliable results. Palghat Udayashankar and co-workers clearly observed travelling waves and frequency decomposition on a CA surface covered with a gentle layer of insect ringer. Vibrations travel from the narrowest part (distal part) of the CA to the broader proximal region; high frequencies are represented at the narrow end, and low frequencies cells at the broader proximal end. Intermediate frequencies were observed between both ends. Such gradient and direction corresponds with the tonotopically ordered mechanoreceptors (Oldfield 1982; Römer 1983; Stolting and Stumpner 1998). Remarkably, this mechanical behaviour can be measured in the absence of the dorsal cuticle, an indication that the decomposition of frequencies results from an intrinsic mechanical property of the CA.

In the study of *C. gorgonensis* (Montealegre-Z et al. 2012), travelling waves and mechanical tonotopy were recorded through the dorsal cuticle (see discussion below). However this study showed that removal of the dorsal cuticle, or simply the removal of the liquid in the vesicle subjacent to the dorsal cuticle, is sufficient to obliterate the build-up of travelling waves and, therefore the resulting tonotopic response (as measured through the dorsal tibial cuticle). The differences between these lines of evidence call for more studies of the tympanal organ across species of different subfamilies.

Montealegre-Z et al. (2012) designed an experiment to obtain vibrations of the CA surface and of both TMs simultaneously to investigate the effect of TM deflection on CA motion. They used a special isolating platform that isolated the spiracular input from the tympanal input. In some of their experiments the CA was exposed following the protocols used by Palghat Udayashankar et al. (2012), and its vibration in response to acoustic stimulation recorded using LDV. Tympanal vibrations were then stimulated by delivering sound uniquely at the acoustic spiracle. In those experiments when the CA was exposed, the isolating platform setup ensured that vibrations recorded from the CA surface were the sole product of AcT input to the auditory system, and not of airborne sound reaching the exposed dorsal surface of the CA or other adjacent cuticular structures. Using this setup, three tests were performed; 1) leave the AV intact and record AV and presumed CA activity through the dorsal cuticle using LDV, 2) drain the AV of its hemolymphatic fluid through a small lateral perforation using a sharp glass micro-pipette and record from the dorsal cuticle using LDV; and 3) remove the dorsal cuticle and AV entirely and gain direct optical access to the thin tracheal wall that bears the CA, following the dissection procedure of Palghat Udayashankar et al. (2012). Vibrometric measurements of the AV and CA recorded through the dorsal cuticle show that dispersive wave propagation, and therefore frequency decomposition, only occurs in the presence of an intact AV (test 1). Removing the AV fluid through a lateral perforation (test 2) and recording activity though the dorsal cuticle eliminates travelling waves. Removing the dorsal cuticle evacuates the AV fluid by default (test 3). In *C. gorgonensis* this procedure eliminates travelling waves as measured from the exposed CA surface; contrary to that observed by Palghat Udayashankar et al. (2012, 2014) in *M. elongata*. With this procedure the measurements of Montealegre-Z et al. (2012) on the vibrational activity of the CA surface is irregular and unclear in most cases. However, vibrational responses were observed in the middle region of the CA surface (Montealegre-Z et al. 2012: fig S7) which was very sensitive to 23 kHz (the frequency of the calling song, Montealegre-Z and Postles 2010), but no obvious travelling waves were observed. Consequently, in *C. gorgonensis* the integrity of the AV is necessary to enhance the appropriate propagation of waves.

Palghat Udayashankar et al. (2012) suggest that travelling waves result from the smooth gradient in the mechanical properties and the geometry of the tracheal wall containing the CA. This conjecture is reasonable, in fact Fig. 2 (c, d) show that the tracheal branch holding the CA is thicker in its narrowest end (distal end or high frequency region) than proximally (the low frequency region). In the basilar membrane of the mammals similar gradient or mechanical anisotropy causes the observed tonotopy (Vater and Kössl 2011).

The fact that in *C. gorgonensis* travelling waves are detected through the cuticle when the AV is intact, suggests the AV fluid is important as a dispersive medium, just like the cochlear fluids in tetrapods. In mammals for instance, cochlear tonotopy can be demonstrated *in vitro* and by replacing the cochlear fluids. The spatial frequency analysis in the cochlea arises from the passive mechanical properties of cochlear fluids and tissues (Robles and Ruggero 2001).

Recording CA and AV vibrations through the dorsal cuticle

Montealegre-Z et al. (2012) first reported that travelling waves could be recorded through the dorsal cuticle using LDV. The recordings of cuticular vibration of Montealegre-Z et al. (2012) also show an area that is highly sensitive to frequencies in the range of the calling song of *C. gorgonensis* (around 23 kHz, Montealegre-Z et al. 2012: fig. S5). These experiments have been repeated in other katydid species of different subfamilies, and with variable calling carrier frequencies, showing similar results (Fig. 4). One wonders how and why the cuticle shielding the hearing organ vibrates. Using the methods established by Montealegre-Z et al. (2012) and Montealegre-Z (2014), the authors went further and conducted the following recordings in 18 females of *C. gorgonensis*: a specimen mounted in a free sound field was presented with a 23 kHz pure-tone of variable sound pressures between 5 and 40 Pa. The amplitude responses of the TMs and dorsal cuticle were recorded using LDV and compared. Then the tracheal input of the specimen was occluded using the sound isolating platform proposed by Montealegre-Z et al. (2012). In both sets of data we observed that the amplitude of the vibrations recorded ‘on’ the dorsal cuticle exceeded those of the tympanic membrane (Fig. 5a, b). These observations came as a surprise as the dorsal cuticle in *C. gorgonensis* is 10-20 times thicker than the TMs themselves (Fig. 2c, d). The thickness of that dorsal wall would not allow for mechanical deflections of such amplitudes.

After studying the tympanal organ of different katydid species from different subfamilies using the same LDV protocols established by Montealegre-Z et al (2012) we noticed that CA activity cannot be recorded by dorsal cuticle measurements in a large number of them (Table 1). This brought us to compare the cuticle structure across the species studied and found that those species that allow the recording of travelling waves on the dorsal cuticle have a very transparent cuticle; while those in which cuticle vibration cannot be recorded using LDV exhibit a more sclerotized dorsal cuticle (Table 1).

In other words, the cuticle of the leg covering the CA and mechanoreceptors in *C. gorgonensis* and a few other species is not completely sclerotized, and therefore highly transparent to visible light. In many other species (Table 1) the dorsal cuticle is opaque and/or pigmented, therefore impeding the laser light to enter. One of the properties of insect cuticle is transparency and this is particularly useful in light detection and vision. The compound eyes and ocelli in all insects, and the infrared receptor organs of fire beetles are examples of transparent cuticles, usually associated with the absence of exocuticle (Vondran et al. 1995; Klowden 2008; Schmit 1995; Mark Klowden 2013). It is unclear why in some katydids the ear dorsal cuticle is transparent. This property should be further studied in katydids as it offers a unique opportunity to access the CA and AV in a non-invasive manner. The techniques available so far for accessing the CA require removal of the dorsal cuticle and AV.

One could also think that the dorsal cuticle is thin enough to allow the experimenter to measure the vibrations of the AV surface, as originally believed (Montealegre-Z et al. 2012). However, when measurements of the thickness of both dorsal cuticle and tympanal membrane in *C. gorgonensis* are compared, the dorsal cuticle is approximately 10-20x thicker than the TMs in this species. Such a thick wall of small area would tend to resonate at high frequencies and oscillate at low amplitudes. The hypothesis of thin walls permeable to vibrations can be discarded because vibration recorded from the dorsal cuticle could be obtained from several species with variable cuticle width and transparent cuticle, but not in species with dull cuticles and variable thickness (Tabe 1). Figure 3 (c, f, i) shows variable thicknesses of the ear's dorsal cuticle and TMs in three species of katydid belonging to three different subfamilies, in which CA vibrations can be accessed through a transparent cuticle using LDV as shown in Fig. 4.

These observations imply that the vibrations obtained via the dorsal cuticle shown by Montealegre-Z et al. (2012) and those presented in Fig. 4 of this document, might come from the katydid inner ear. The transparent cuticle and translucent AV fluid seem to allow the laser light to cross through and reflect from the actual CA surface. Evidence for this is that the TPs vibration is in phase with vibration of the exposed CA, as recorded in the most sensitive region at 23 kHz (which still responds to vibration after ablation of the dorsal cuticle, see Montealegre-Z et al. 2012 supplementary information), and with the vibrations obtained through the dorsal cuticle (Fig. 6a). The phase of the ATM and PTM is always opposite to the TP and CA (Fig. 6b, c). These results were presented by Montealege-Z et al. (2012, supplementary information).

The pressure release system in the ears of katydids

The katydid and tetrapod ears both have a mechanism of pressure release. In the mammalian cochlea, the vibrations transmitted by the ossicles through the oval window cause a change in pressure of the cochlear fluid. Since the fluid is incompressible, pressure changes are released though a little membranous opening, the round window, which vibrates with opposite phase to vibrations entering the inner ear through the oval window. The AV in *C. gorgonensis,* and in some other katydid species studied, is blind in the distal end, while the proximal ends usually exhibits a narrow connection with the HC (Fig. 3). Since the fluid is incompressible, changes in pressure in the AV cavity should be released somewhere. Montealegre-Z et al. (2012) originally associated the high resolution of travelling waves with the vibrations recorded through the dorsal cuticle with a pressure release mechanism. They believed that the dorsal cuticle served as a dispersive medium to release the acoustic energy in the inner ear of the katydid, and that it played the role of the round window in mammals. As mentioned in the previous section, the vibrations recorded on the dorsal cuticle were likely obtained directly from the katydid ‘inner ear’, perhaps from the CA surface. So the ear’s dorsal cuticle seems not to function as a dispersive pressure release medium.

The ‘round window role’ in the katydid ear seems to be played by the connection between AV and HC. The anatomy of the AV varies across species and also its connection with the HC. In some species this constriction is narrow but in others it is broader, but in both situations the connection seems to be mediated by a soft plug of colloidal material. One would expect excess vibration to be scattered towards the HC and less detected through the cuticle. Among the 7000 species of katydids, it is very likely to find a range of variability.

Acknowledgements

This work was sponsored by the Human Frontier Science Program (Cross Disciplinary Fellowship LT00024/2008-C to F.M.-Z.). The authors are currently sponsored by the Royal Society, and by the Leverhulme Trust (grant No. RPG-2014-284). The Colombian Ministry of Environment granted a permit for fieldwork at Gorgona National Park (decree DTS0-G-31 11/07). We would like to thank two anonymous referees for discussions and comments on earlier versions of the manuscript. We also thank Ben Chivers for proof-reading the final version on English grammar. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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Table 1. List of katydid species and ear attributes that facilitate non-invasive access to the inner ear using laser vibrometry. (*) indicates those species in which tonotopy and/or travelling waves have been recorded. Dorsal cuticle transparency was observed in the ear living individuals under light microscope but not physically measured.

Species	**Subfamily**	**Locality**	**Fc (kHz)**	**Dorsal wall thickness**	**CA vibration via cuticle**	**Comments on cuticle**
*Copiphora gorgonensis**	Copiphorinae	COL	22.9	135	Yes	Very transparent
*Panacanthus pallicornis**	Copiphorinae	COL	4.7	200	Yes	Very transparent
*Panacanthus lacrimans**	Copiphorinae	COL	7.0	189	Yes	Moderately transparent
*Panacanthus varius**	Copiphorinae	COL/ECU	9.0	211	Yes	Very transparent
*Artiotonus artius**	Copiphorinae	COL	41.2	74	Yes	Very transparent
*Artiotonus tinae**	Copiphorinae	ECU	36.0	60	Yes	Very transparent
*Supersonus aequoreus**	Listroscelidinae	COL	148.7	32	Yes	Moderately transparent
Supersonus undulus	Listroscelidinae	ECU	117.0	40	Yes	Moderately transparent
Mecopoda elongata	Phaneropteriane	INDIA	6-80	116	No	Opaque
Gnathoclita sodalis	Pseudophyllinae	COL	15.6	177	No	Very opaque
Nastonotus foreli	Pseudophyllinae	COL	22.8	183	No	Very opaque
Parascopioricus cordillericus	Pseudophyllinae	COL	28.0	150	No	Opaque
Onomarchus uninotatus	Pseudophyllinae	INDIA	3.2	140	No	Opaque
*Metrioptera sphagnorum**	Decticinae	CAN	18 & 33	55	Yes	Moderately transparent

Figure legends

Fig. 1. General anatomy of the katydid ear. **a** 3D reconstruction of the body based on Micro-CT scanning techniques, showing internal and external structures of the ears of an insect in dorsal view. **b** Lateral view of the same reconstruction showing the acoustic spiracle. Act= acoustic trachea; ATM= anterior tympanic membrane; PTM=posterior tympanic membrane.

Fig. 2. The impedance conversion mechanism. **a** Photograph of the external ear showing the tympanal membrane (TM) and tympanal plate (TP) in *Copiphora gorgonensis*. **b** Vibration map of the TM and TP amplitude as monitored by LDV. **c, d** µCT cross sections taken at the two different regions in the fore tibia, distal and proximal, as indicated by the dashed lines. **e** Model of impedance converter, using schematic cross section (c) and lever type 1 analogy, as proposed by Montealegre-Z et al. (2012). **f** Hinge model for vibration transmission, based on a 2nd class lever, as proposed by Bangert et al. (1998).

Fig. 3. Internal anatomy of the ear of three unrelated katydid species, reconstructed with micro-CT scanning techniques. **a, b** 3D reconstruction of the tympanal organ of *Supersonus aequoreus*; a katydid species using a calling song of ca. 150 kHz. C. µCT cross section of the ear shown in a. **d, e** 3D reconstruction of the tympanal organ of *Panacanthus pallicornis*, a katydid communicating with a broadband spectrum between 5 and 25 kHz. **f** µCT cross section of the ear shown in d. **g, h** *Metrioptera sphagnorum*, a katydid using two modes of stridulation, with dominant frequencies of 17 and 33 kHz, respectively. **i** µCT cross section of the ear shown in g.

Fig. 4. Tonotopic organization of frequency response as recorded on the dorsal cuticle in three species of Tettigoniidae incorporated in three different subfamiles. The top part of each panel shows the magnitude of the response (recorded with Laser Doppler Vibrometry) to acoustic broadband stimuli (produced as periodic chirps) of variable frequency ranges depending on the species. Specimens were mounted in a special holder and exposed to a free acoustic field. Magnitude response spectra on the right side of the picture were obtained from the regions indicated by the blue points, and labelled with

a number corresponding to respective spectrum on the right side. The lower part of each panel shows the analysis of a representative calling song for each species. **a** *Panacanthus pallicornis*: ear stimulated by periodic chirps between 1-40 kHz. Spectra of magnitude response averaged from 12 specimens (seven males and five females). Calling song recordings and analysis obtained from Montealegre-Z and Morris (2004). **b** *Supersonus aequoreous*: ear stimulated by periodic chirps between 1-200 kHz). Spectra of magnitude response recorded from a single female specimen. Calling song recordings and analysis obtained from Sarria-S et al. (2014). **c** *Metrioptera sphagnorum*: ear stimulated by periodic chirps between 1-40 kHz). Spectra of magnitude response averaged from five specimens 3 females and two males. Calling song recordings and analysis obtained from Morris (2008). For details of experimental protocols see Montealegre-Z et al. (2012).

Fig. 5. The gain of the AV measured through the dorsal cuticle vs. the tympanal gain in *Copiphora gorgonensis*. **a** Gain of the ATM and AV measured with the specimen exposed to a free acoustic field to variable sound pressures of pure tones at 23 kHz (the carrier frequency of the species call, Montealegre-Z and Postles 2010). In this natural condition, both sides of the tympanum are exposed to sound. **b** Similar experiment as above but the tracheal input has been occluded using the isolating platform described by Montealegre-Z et al. (2012) and Montealegre-Z (2014). Note that in both situations the AV gain is considerably larger than the ATM and PTM gains. The normal ranges of sound pressures used by a singing male as recorded with the microphone placed at 10 cm is nearly 94dB (1 Pa). N= 21 individuals (10 males and 11 females). Error bars show standard deviation.

Fig. 6. Mechanical response of tympanal system and sensory organ (*crista acustica*). **a** Time domain and phase transfer function of the vibrations recorded from the dorsal cuticle (DC) and from an exposed CA after removal of the hemolymphatic fluid and exposing the CA surface. Broadband acoustic stimulus was delivered using the preparation described by Montealegre-Z et al. (2012). DC and CA mechanical responses are similar in phase by differ in amplitude, revealing the loss of amplification after the dorsal cuticle has been removed and the hemolymphatic fluid vacated. Lower panel shows the phase

of the oscillations determined by Hilbert transform. The phase spectra indicate no phase difference between recordings obtained from the DC and those obtained on the CA surface. **b** Time-resolved responses of ATM, PTM and exposed CA to a pure tone stimulus (a 23kHz, 4-cycle sound pulse). CA exposure results from dorsal cuticle removal. Lower panel shows the phase of the oscillations determined by Hilbert transform. ATM and PTM oscillate in phase, while CA response is clearly 180° out of phase. **c** Time-resolved oscillations of ATM and PTM plates and the CA to 23kHz. All elements oscillate in phase, confirming the coupling between tympanal plates and CA. Data obtained from Montealegre-Z et al. (2012).

Figure 1

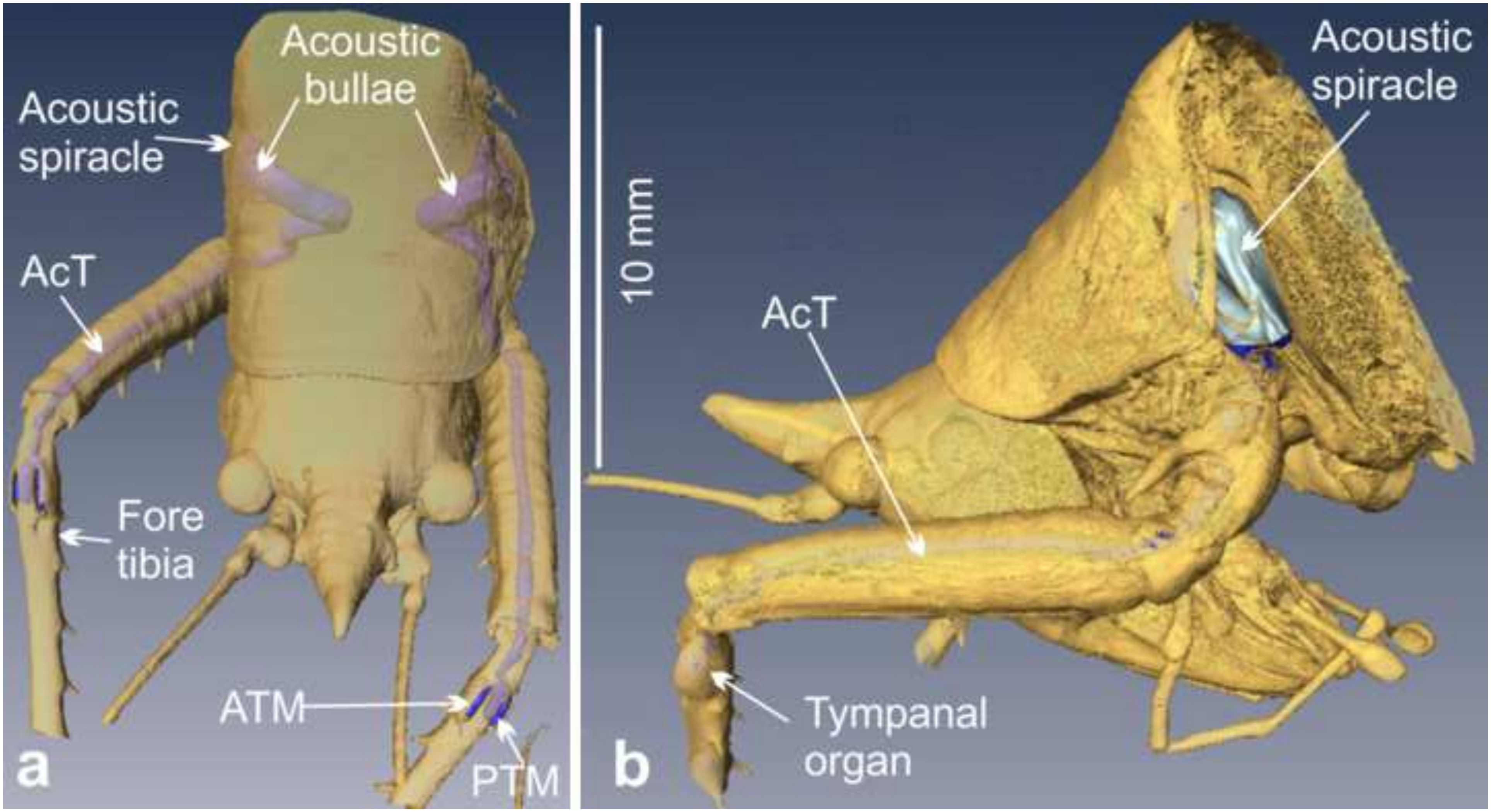

Acoustic
bullae
Acoustic
spiracle
AcT
Fore
tibia
ATM
PTM
a
10 mm
AcT
Acoustic
spiracle
Tympanal
organ
b

Figure 2

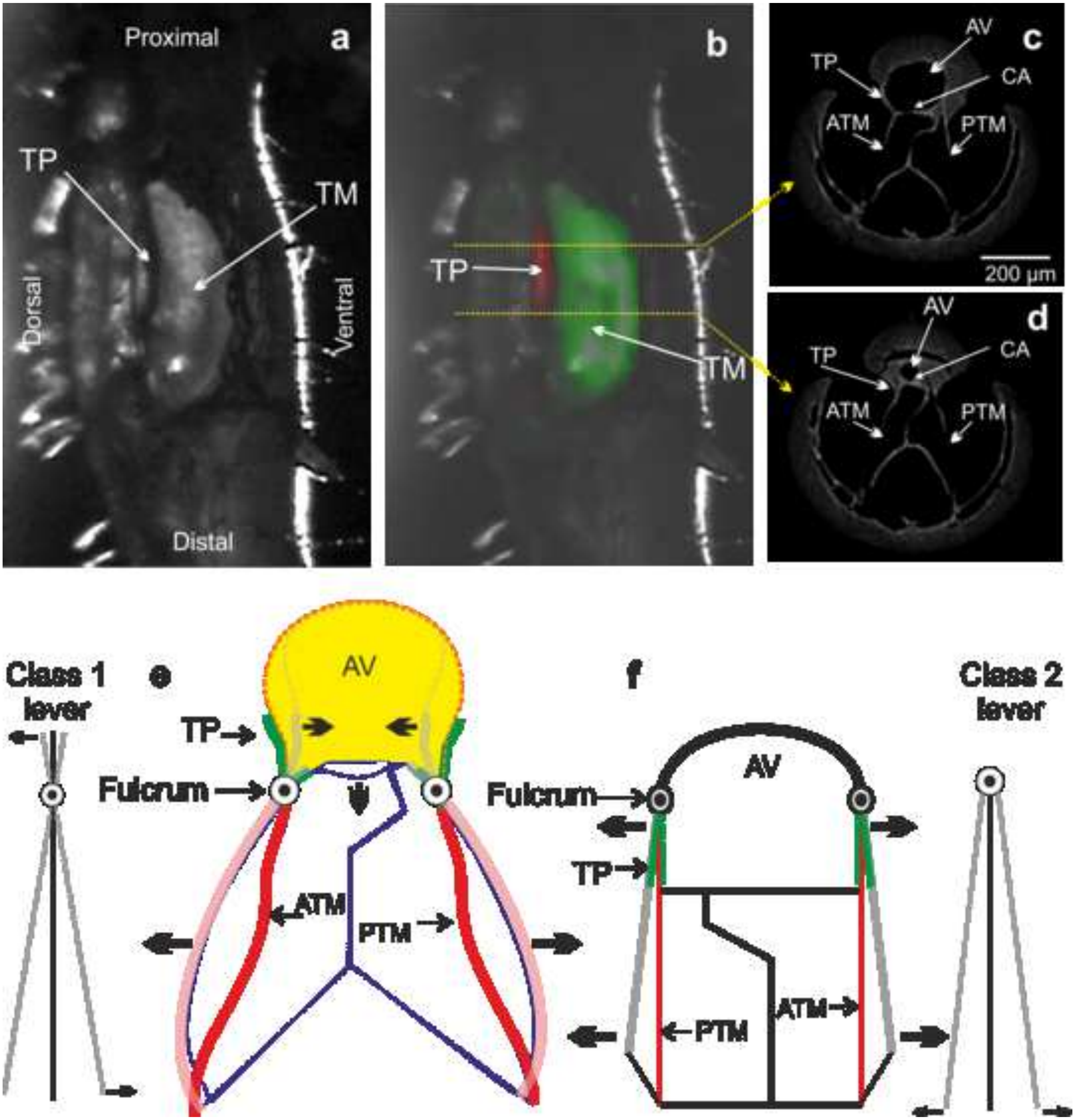

Proximal
a
TP
TM
Dorsal
Ventral
Distal
b
TP
TM
c
AV
TP
CA
ATM
PTM
200 µm
d
AV
TP
CA
ATM
PTM
Class 1 lever
e
AV
TP
Fulcrum
ATM
PTM
f
AV
Fulcrum
TP
ATM
PTM
Class 2 lever

Figure 3

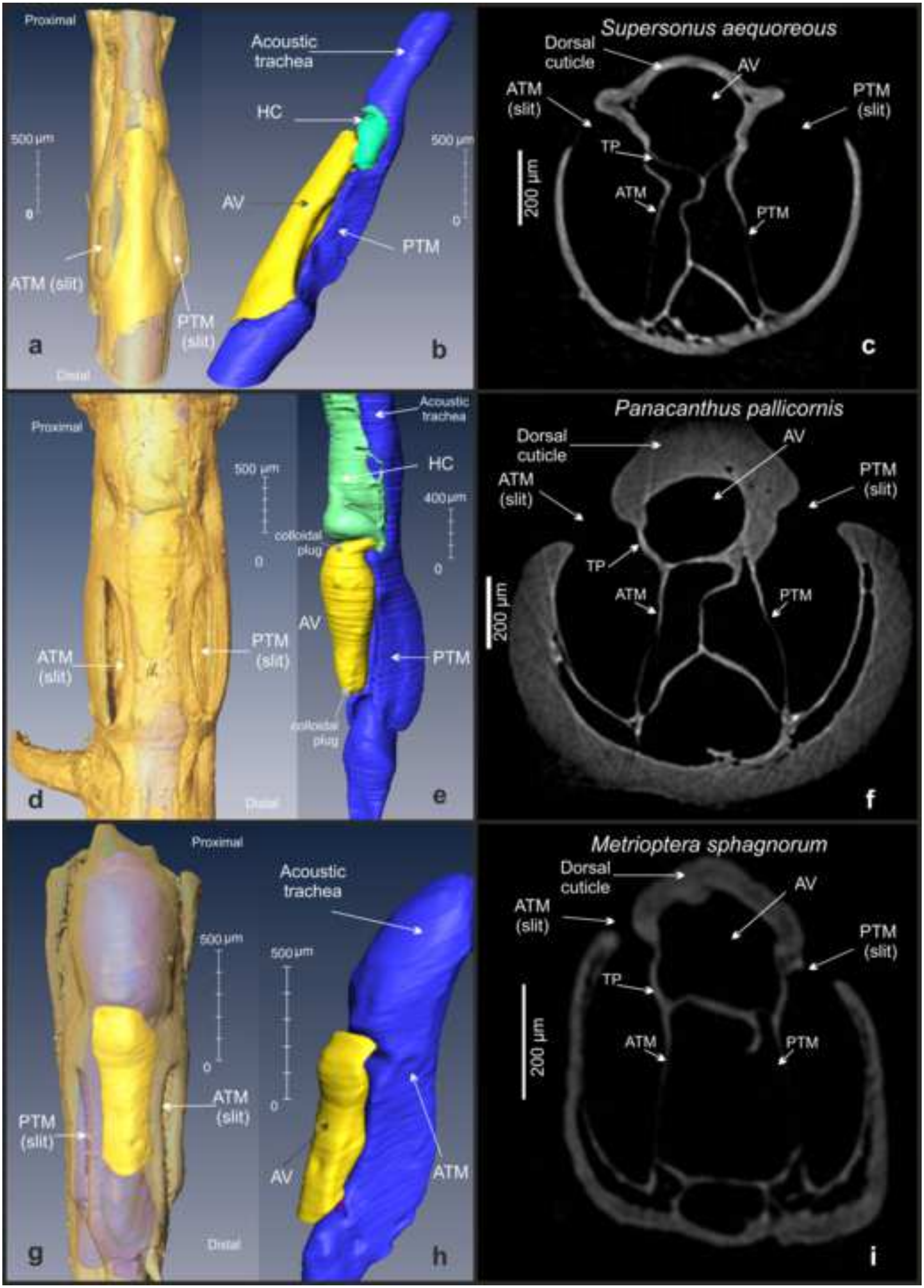

Proximal
500 µm
0
ATM (slit)
PTM (slit)
a
Distal
Acoustic trachea
HC
AV
PTM
500 µm
0
b
Supersonus aequoreous
Dorsal cuticle
AV
ATM (slit)
PTM (slit)
TP
ATM
PTM
200 µm
c
Proximal
500 µm
0
ATM (slit)
PTM (slit)
d
Distal
Acoustic trachea
HC
400 µm
0
colloidal plug
AV
PTM
colloidal plug
e
Panacanthus pallicornis
Dorsal cuticle
AV
ATM (slit)
PTM (slit)
TP
ATM
PTM
200 µm
f
Proximal
500 µm
0
ATM (slit)
PTM (slit)
g
Distal
Acoustic trachea
500 µm
0
AV
ATM
h
Metrioptera sphagnorum
Dorsal cuticle
AV
ATM (slit)
PTM (slit)
TP
ATM
PTM
200 µm
i

Figure 4

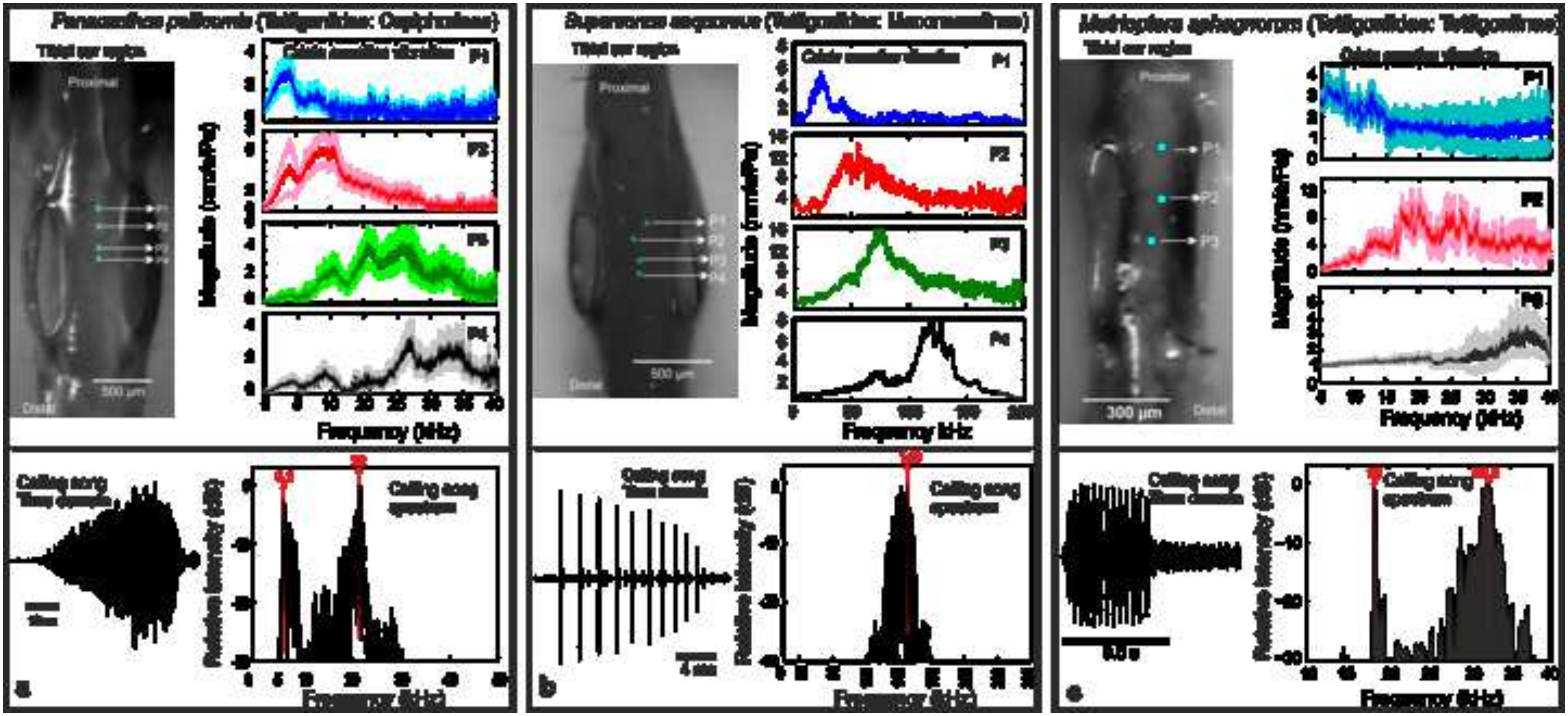
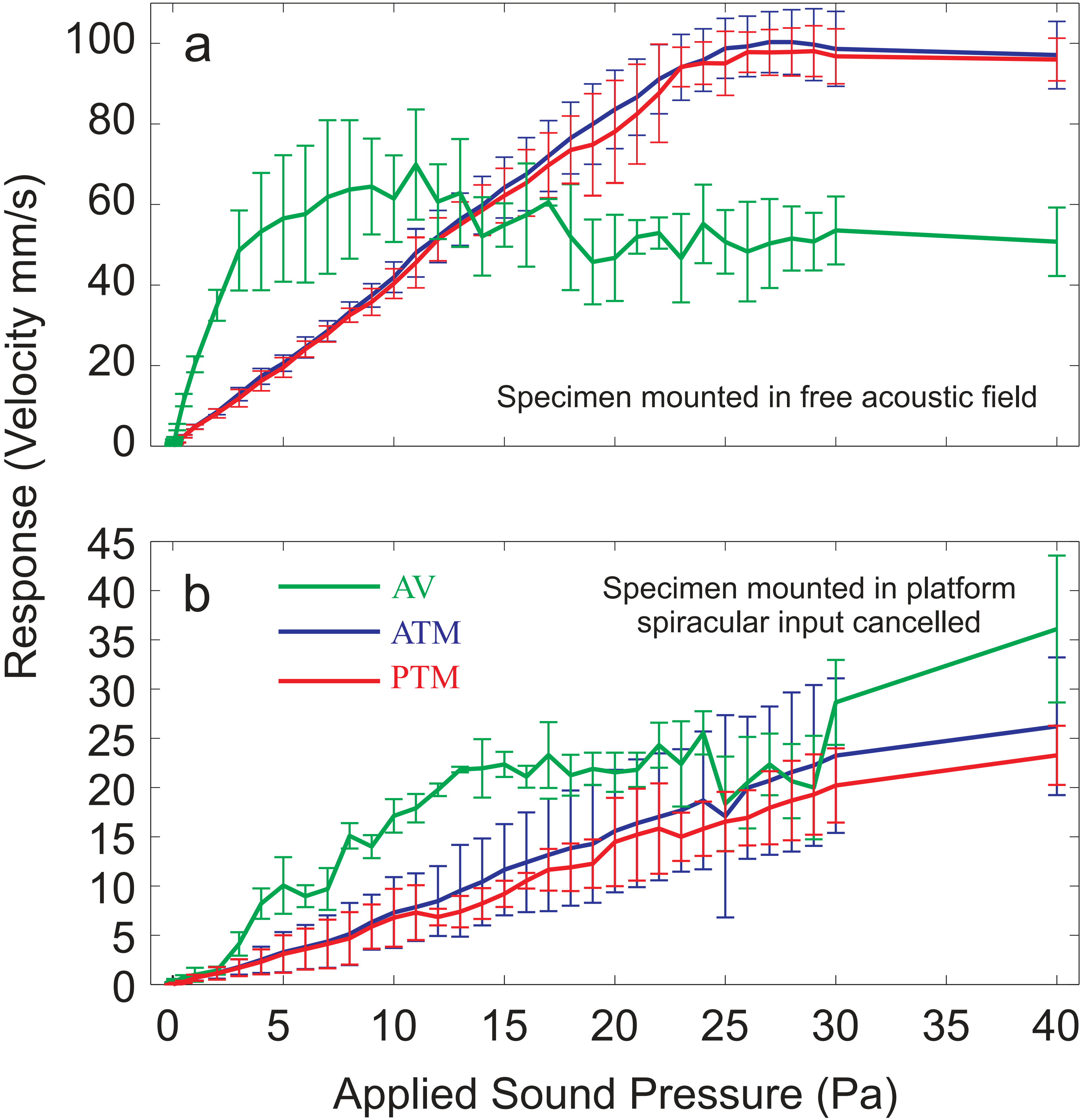
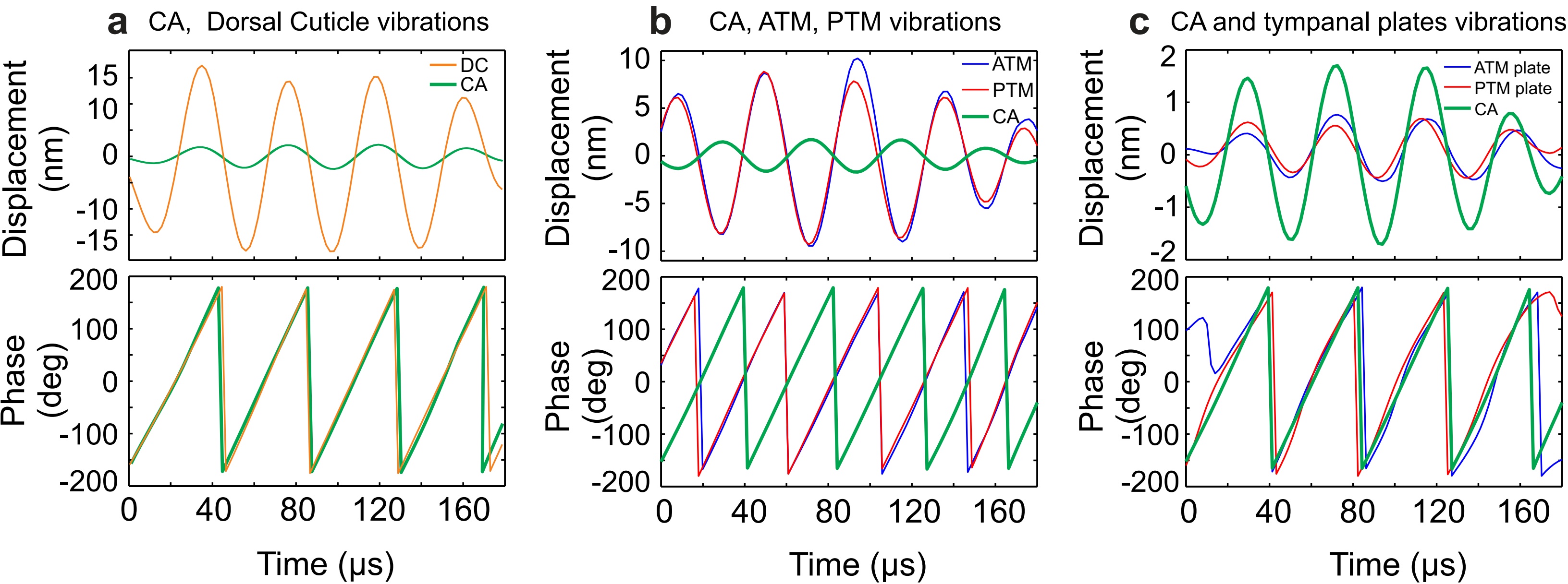

Panacanthus pallicornis (Tettigoniidae: Copiphorinae)
Supersonus aequoreus (Tettigoniidae: Meconematinae)
Metrioptera sphagnorum (Tettigoniidae: Tettigoniinae)
Tibial ear region
Proximal
Distal
P1
P2
P3
P4
500 µm
300 µm
Crista acustica vibration
Magnitude (nm/mPa)
Frequency (kHz)
Frequency kHz
Calling song
Time domain
Calling song
spectrum
Relative intensity (dB)
4 ms
0.5 s
a
b
c

Figure 5

Click here to download Figure: Fig5.eps




a
CA, Dorsal Cuticle vibrations
DC
CA
Displacement (nm)
15
10
0
-10
-15
Phase (deg)
200
100
0
-100
-200
0
40
80
120
160
Time (μs)
b
CA, ATM, PTM vibrations
ATM
PTM
CA
Displacement (nm)
10
5
0
-5
-10
Phase (deg)
200
100
0
-100
-200
0
40
80
120
160
Time (μs)
c
CA and tympanal plates vibrations
ATM plate
PTM plate
CA
Displacement (nm)
2
1
0
-1
-2
Phase (deg)
200
100
0
-100
-200
0
40
80
120
160
Time (μs)

Dear Prof. Römer,
Here is the last revised version of our ms after last editorial comments. We accepted the changes suggested by the editor, and also found a few other typos to sentences that needed improvement. Please note that the ms parts that have been newly edited or modified are shown in red fonts.

Kind regards

fer