A narrow ear canal reduces sound velocity to create additional acoustic inputs in a microscale insect ear

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Located in the forelegs, katydid ears are unique among arthropods in having outer, middle, and inner components, analogous to the mammalian ear. Unlike mammals, sound is received externally via two tympanic membranes in each ear and internally via a narrow ear canal (EC) derived from the respiratory tracheal system. Inside the EC, sound travels slower than in free air, causing temporal and pressure differences between external and internal inputs. The delay was suspected to arise as a consequence of the narrowing EC geometry. If true, a reduction in sound velocity should persist independently of the gas composition in the EC (e.g., air, CO2). Integrating laser Doppler vibrometry, microcomputed tomography, and numerical analysis on precise three-dimensional geometries of each experimental animal EC, we demonstrate that the narrowing radius of the EC is the main factor reducing sound velocity. Both experimental and numerical data also show that sound velocity is reduced further when excess CO2 fills the EC. Likewise, the EC bifurcates at the tympanal level (one branch for each tympanic membrane), creating two additional narrow internal sound paths and imposing different sound velocities for each tympanic membrane. Therefore, external and internal inputs total to four sound paths for each ear (only one for the human ear). Research paths and implication of findings in avian directional hearing are discussed.

bioacoustics | katydid hearing | sound propagation | finite element analysis

Tetrapod auditory perception is based on sound capturing, transformation of airborne vibrations into fluid vibrations, and mechanical frequency decomposition followed by the transduction of the mechanical energy into electrochemical signals by the auditory receptor neurons (1). Adaptations for the first step—the efficient capture of the sound waves—vary across taxa and have evolved independently in mammals, reptiles, and birds (2–3). In terrestrial mammals, for instance, sound capture involves the pinna and the ear canal (EC), which vary in morphology across the circa 7,000 living species of katydids (4). These insects use hearing for social communication (e.g., mating calls, alarm signals, predator detection). Both experimental and numerical data also show that the major factor causing the internal delay is the EC geometry. The EC bifurcates asymmetrically, producing two additional internal paths that impose different sound velocities for each tympanum. Therefore, various versions of the same signal reach the ears at various times, increasing the chance to pinpoint the source sound. Findings could inspire algorithms for accurate acoustic triangulation in detection sensors.

Significance

The katydid tympanal ears have outer, middle, and inner ear components analogous to mammalian ears. Unlike mammals, each ear has two tympana exposed to sound both externally and internally, with a delayed internal version arriving via the gas-filled ear canal (EC). The two combined inputs in each ear play a significant role in directional hearing. Here, we demonstrate that the major factor causing the internal delay is the EC geometry. The EC bifurcates asymmetrically, producing two additional internal paths that impose different sound velocities for each tympanum. Therefore, various versions of the same signal reach the ears at various times, increasing the chance to pinpoint the source sound. Findings could inspire algorithms for accurate acoustic triangulation in detection sensors.

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The reduction in sound velocity was first reported and inferred in Michelsen et al. (16) for crickets and Larsen (22) for crickets and katydids. This was later experimentally quantified (15). However, the underlying assumptions of the interpretation of results, such as the effect of the respiratory gases in the EC and propagation of sound in highly narrowing tubes being the cause of reduction of sound velocity (15, 23), have not been analyzed using experimental and numerical approaches.

Here, we experimentally determine the velocity of sound propagation inside the EC of the katydid *Copiphora gorgonensis* (Tettigoniidae, Copiphorini) and test the hypothesis that the gradually narrowing EC is the primary cause of any observed changes in sound velocity. Furthermore, to corroborate results, we investigate sound propagation velocity when the EC is filled with different gases, under the expectation that sound velocity will decrease in a narrow tube independently of the gas. Adding to the experimental results, we applied finite element analysis to simulate the propagation of sound in the precise geometry of all experimental ECs (Fig. 1C), obtained through microcomputed tomography (μ-CT). Both the experimental and numerical results demonstrate that EC geometry is the primary cause of sound velocity reduction within the katydid middle ear.

**Results**

We investigated factors leading to the reduction of sound velocity in the EC of *C. gorgonensis* in comparison with sound velocity in free air conditions (15), both experimentally and numerically. In particular, we considered the effect of the gas composition within both the left and right ECs of the specimen on the velocity reduction in three different categories: live insects, dead insects, and numerical models. The results from live and dead specimens were considered separately in order to observe the effect of the metabolic CO$_2$ reduction in the EC, which ensues after the specimen is dead. For each insect status, in addition to the gas composition inside the EC, we also analyzed whether the reduction of sound propagation velocity was affected by the length of the EC and the location of recording on the ATM or PTM. The EC bifurcates near the TMs, so that ATM and PTM lie on two separate branches: the anterior and posterior branches, respectively. The cross-sectional area of the anterior branch is smaller than that of the posterior branch by 2.28% in males and 5.67% in females (SI Appendix, Table S3); hence, the branches are asymmetrical.

We calculated the velocity of sound propagation through the EC by dividing the length of the EC with the time taken for a sound signal to transmit through it to the TM. To measure the time of sound transmission, the insect was mounted on a special platform that separates the internal and external sound inputs (Fig. 2). Sound was produced externally and passed into a probe loudspeaker, which delivered the signal into the EC, while the TMs were monitored with a microscanning laser Doppler vibrometer (LDV). The gas composition of the EC was manipulated by dispensing carbon dioxide gas (CO$_2$) into the acoustic spiralica via a microcapillary needle (Materials and Methods; SI Appendix, section 1 has details). CO$_2$ was used because the EC is derived from the respiratory system, and therefore, the gas could be present in naturally high concentrations; it is also known that sounds travel slower in CO$_2$ (SI Appendix, section 2 has details). Experiments were also carried out with air-filled EC. For these experiments, the natural gas composition of the specimen was flushed out, and it was left to refill by itself, so that by air, we refer to the gas composition inside the room (SI Appendix, section 3 has details). When compared with free-field sound velocity in air, sound velocity within the EC across live, dead, and numerically modeled data was significantly slower [$F (2,45.16) = 68.19$, $P < 0.001$] (Fig. 3 and Table 1). Both live and dead insects did not differ in their velocity reduction of 17.05% (live vs. dead: $t$ ratio = 1.88, $P = 0.158$), but numerical data predicted a significantly greater velocity reduction than was found in live or dead insects, in air (vs. live: $t$ ratio = 10.76, $P < 0.001$; vs. dead: $t$ ratio = 9.09, $P < 0.001$) (Fig. 34 and Table 1). EC length did not affect the sound velocity reduction [$F (1,8.13) = 1.60$, $P = 0.241$], but the reduction of velocity was greater at the ATM [$F (1,41.25) = 4.14$, $P = 0.048$]. In live insects, with the EC filled with air, sound propagated to the ATM and PTM at mean velocities of 284 and 296 m/s, respectively. A numerical investigation on one specimen model showed sound propagation velocities of 224.84 and 228.59 m/s at the ATM and PTM, respectively, of the air-filled left EC; similar results were also obtained in the right EC (Fig. 4B).

Sound velocity data obtained at the TMs after the incident wave traveled through the CO$_2$-filled EC were significantly slower for all three insect statuses in comparison with free-field CO$_2$ sound velocity, but there was no significant difference among the different statuses [$F (1,37.854) = 0.37$, $P = 0.696$] (Fig. 3B and Table 1). More precisely, sound velocity of dead and live insects was 31.56% slower than free-field CO$_2$ sound velocity. Neither EC length [$F (1,6.43) = 2.61$, $P = 0.154$] nor sound velocity, at either tympanum, were significantly related to sound velocity. EC length did not differ in their velocity reduction of $17.05\%$ (live vs. dead: $t$ ratio = 1.88, $P = 0.158$), but numerical data predicted a significantly greater velocity reduction than was found in live or dead insects, in air (vs. live: $t$ ratio = 10.76, $P < 0.001$; vs. dead: $t$ ratio = 9.09, $P < 0.001$) (Fig. 34 and Table 1). EC length did not affect the sound velocity reduction [$F (1,8.13) = 1.60$, $P = 0.241$], but the reduction of velocity was greater at the ATM [$F (1,41.25) = 4.14$, $P = 0.048$]. In live insects, with the EC filled with air, sound propagated to the ATM and PTM at mean velocities of 284 and 296 m/s, respectively. A numerical investigation on one specimen model showed sound propagation velocities of 224.84 and 228.59 m/s at the ATM and PTM, respectively, of the air-filled left EC; similar results were also obtained in the right EC (Fig. 4B).

To explore the effect of the EC radius on the sound propagation velocity inside of the ear in more detail, we also carried out numerical simulations after manipulating the EC geometry by changing its radius. Considering the tube to be filled with air or CO$_2$, we obtained the sound velocity from ECs with median radii 75 to 600 µm. The results are presented in Fig. 4A, from which it is evident that, regardless of the gas composition inside the tube, the ECs with a smaller radius display a greater reduction in sound velocity. In a separate simulation, we have also explored the effect of the asymmetric bifurcation of the EC on sound velocity. The results are presented in Fig. 4B and complement the results in Fig. 4A since sound is distributed asymmetrically, with higher velocity at the tympanum associated with the posterior branch of the EC, which is the wider branch.
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displayed in the sound velocity measurements in CO₂ likely results from random error in gas dispensation.

Differences between the experimental and numerical results can also be attributed to some simplifying assumptions applied in the model. For instance, we assumed that the lumen of the EC is smooth. Instead, the EC is derived from the respiratory trachea built up of taenidia, which generally take the form of spiral thickenings of the tracheal walls joined by soft material, forming an irregular sinusous surface. In the model, the gas is also assumed to be quiescent; hence, it is at rest as the sound stimulus enters the trachea, which in reality, it is not since the tracheal tube is part of the respiratory system of the insect.

As follows from Table 1, the largest discrepancies between the experimental and numerical results are present in the data related to the sound velocity from the air-filled ECs, which suggests some differences of the gas compositions inside the ECs during the experiments and the numerical simulations. The gas content inside the ECs for the experiments with air was ambient during the experiments and the numerical simulations. The gas composition suggests some differences of the gas compositions inside the ECs part of the respiratory system of the insect. The purpose of filling the EC with CO₂ was purely for testing the mechanics of the EC, as the EC containing pure CO₂ is assumed to be under standard conditions and completely free of atmospheric gas composition; hence, the EC might not be completely quiescent inside the tube, the key component causing it is the complex EC geometry.

### The Use of CO₂ as a Potential Mechanism to Hone Sound Localization.

The purpose of filling the EC with CO₂ was for testing the mechanics of the EC, as the EC containing pure CO₂ is an unrealistic situation in nature. However, since the results of these mechanical experiments show that CO₂ causes the sound to propagate even slower than the normal (already reduced) sound velocity in the EC, we would like to discuss a potential effect of metabolic CO₂ on binaural hearing.

In insects where the respiratory trachea are associated with active muscular-controlled spiracle openings, concentration of CO₂ could be significantly higher when those spiracles are closed. When the spiracles are subsequently opened, the levels of CO₂ and O₂ equilibrate with those of air (17). During those open and closed phases, sound propagation velocity would be affected, due to the varying CO₂ concentration inside the EC. The degree of concentration change and the effect on propagation velocity are unknown, but we believe this idea should be further investigated, especially in species that have control of spiracle valves and exhibit tracheal and acoustic connections between the left and the right ECs, like field crickets. To the best of our knowledge, the effect of opened or closed EC spiracles on sound propagation velocity and sound reception at the level of the tympana and the crista acustica has never been investigated [Michelsen et al. (16) and Larsen et al. (22)].

### Dual-Input Ears in the Animal Kingdom.

Other animals, including some insects and tetrapods, utilize pressure difference receivers to localize sound. These include grasshoppers (30), lizards (31), crocodilians (32), and birds. Unlike in katydids, the pressure difference in these animals occurs between the membranes of two internally coupled ears. A signal transmitting internally from one TM to another may suffer attenuation or depending on the interaural distance and frequency of the signal, a time delay. This mechanism can present problems for smaller organisms such as the grasshopper (Acrididae). Grasshopper ears are located at lateral surfaces of the almost cylindrical body, on the first abdominal segment. The tympana are connected by a series of sound-guiding air sacs (30), permitting sound to internally travel from one TM to the other. The vibration of the TM, measured using LDV recordings, shows the occurrence of nulls (i.e., frequencies where eardrum vibration is canceled because its two surfaces receive sounds with an identical amplitude and phase) (23).

The katydid outer ear reported here shows similarities with some bird ears. Avian ears are located at the side of the head, and evidence suggests that some species utilize pressure difference receivers to localize sound (33–35). Across taxa, the documented anatomy of the avian EC varies from the basic interaural canal of the barn owl (Tyto alba) (36) to the complex network of tubes described in the zebra finch (Taeniopygia guttata) (37). The zebra finch overcomes the size limitations of sound localization through a delayed interaural signal (35). The mechanism behind this delay is unknown but could result from a series of narrow tubes that internally connect each ear (37). The TMs of the zebra finch are directly connected by an interaural canal but are also interconnected by several narrow canals [the figure in Larsen et al. (37)]. Perhaps the most significant of the narrow pathways are the superolateral-orbital canals. These begin above the interaural canal, at the sides of the head, arching upward to join together in a sinus on the forehead (superoantero-orbital sinus). The end result is a 27-mm-long canal with a cross-sectional area of 400 $\mu$m² (radius = 11.3 $\mu$m). The superoantero-orbital sinus also associates with other narrow canals, both of which run down the longitudinal plane of the head. Resembling an exponential horn, the 5-mm-long hypomedial-orbital tube connects the sinus to the interaural canal. Near the sinus, at its most narrow point, the cross-sectional area is 400 $\mu$m². The narrowest tube is 7-mm long and has a cross-sectional area of 100 $\mu$m² (radius = 5.6 $\mu$m). This extends from the sinus to a medium-sized tube that runs parallel to the interaural canal. Proposed to be air filled (37), these narrow tubes resemble the geometry of the
katydid EC. If sound does indeed reach these canals, then our models display the potential effect they could have on signal transmission through the internal pathways in birds, and any role in sound localization and potential signal delay warrants investigation.

Another relevant aspect of narrowing tubes and the reduction in sound velocity in katydids (Fig. 4A) is the potential effect of the EC division at the position of the tympanic organ (Fig. 6). The EC divides into two branches prior to reaching the inner ear: an anterior branch leads to the ATM, and a posterior branch leads to the PTM (Fig. 4B). The anterior branch is narrower in width than the posterior branch and is also adjacent to the auditory sensilla (38). Our experimental results show an asymmetrical response of the TMs to sound conduction from the EC, as the sound first arrives to the PTM and then to the ATM. These results are also supported by our numerical simulations in the precise geometry of the EC and the EC branches (Fig. 4B). The sound arrives first at the PTM through the posterior branch, which is wider than the anterior branch (SI Appendix, Table S3). This parallels the results presented in Fig. 4A, which demonstrate that the EC radius and sound velocity have a directly proportional correlation (Fig. 6C and D).

The experimental data from live insects, when the EC is filled with air, showed on average a sound propagation velocity difference of 12 m/s as the sound wave hits the TMs due to a difference in arrival time of 4.95 μs between ATM and PTM. This is equivalent to a phase shift of 40.98° at 23 kHz. The actual effects of the delay on the crista acustica traveling wave are not known. However, calculating the theoretical gain for two superimposed waves of equal frequency, equal amplitude A and the aforementioned phase shift gives the amplitude sum as 1.75A (without the phase shift, this would equal 2A), which could theoretically be a significant amplitude reduction at, for example, the level of the acoustic vesicle, caused by the temporal delay of the arriving signal at the TMs. The numerical results showed a smaller difference of 3.75 m/s (Fig. 4B) in propagation speed, which can be attributed to the small changes in the bifurcation morphology during reconstruction, the flat surface of the EC that contrasts with the irregular surface. Nevertheless, the numerical results are in analogy to the experimental data and demonstrate that the sound arrives at the PTM first before reaching the ATM after a small time delay.

The asymmetrical response of the TMs could also affect the mechanical interaction of the sound waves acting on the external surface of both TMs by, for example, destructive interference, as the flaps or pinna covering the TMs produce an asymmetrical sound pressure effect around the ear (9): the two TMs work in concert as a type 1 cantilever, magnifying the input force to provide a greater output force (39). The ear flaps in C. gorgonensis and many other katydids (e.g., Conocephalinae and Pseudophyllinae) seem to enhance a highly directional response of both TMs (9, 40), and if sound energy entering a spiral is weak [depending on the position of the sound source (41), it can potentially lead to an asymmetric destructive interference between internal and external inputs, in a single ear. When taking into account the two external inputs at each TM, a single ear may face a total of four acoustic inputs (compared with only one for the human ear). Therefore, even though the spiral and the EC system have traditionally been appreciated as the nondirectional component of the auditory receiver (9), our results suggest that the role played by the asymmetrical conduction from the TMs in directional hearing warrants further investigation. Do the bifurcations at the tympanic cavities predominantly contain CO₂? If true, how are the mechanics of the TMs when they are externally exposed to ambient air while internally exposed to concentrated CO₂?

Materials and Methods

Experimental Design. This research combines experimental and numerical approaches to infer the cause of sound velocity reduction inside the acoustic trachea of katydids. The experimental aspect involves measuring the time for an acoustic signal to transmit through the EC of a katydid and determining the length of the EC. This enables sound velocity to be calculated by dividing the length of the EC by the time it takes the sound stimulus to reach the TM. To achieve this, a special platform that isolates the external and internal auditory inputs is utilized (5, 15), along with an array of probe loudspeakers, microphones, function generators, and an LDV. We record the effect of different gas compositions on the sound propagation velocity. Gas is added to the EC through the use of a customized delivery system (SI Appendix, section 1 has more details).

Experimental Animals. This study used specimens of C. gorgonensis that were eighth-generation descendants of a colony collected in November 2015 from the National Natural Park Gorgona, Colombia (latitude = 2°58′7.4208″N; longitude = 78°11′10.4964″W). Insects were maintained in communal vivaria (temperature 22 °C to 27 °C. A total of nine adult individuals (five males and four females) were used in this study (n = 17 ears).

Fig. 4. Finite element analysis of the effect of the EC radius and asymmetric EC bifurcation on sound speed. (A) The speed of sound measured at the end of the ECs with different radii, while filled with air or CO₂, using finite element analysis. (B) The numerical results of the sound velocity as it reaches the ATM and the PTM from the anterior and posterior branches, respectively, of a specimen through air-filled left and right ECs and CO₂-filled left and right ECs of a specimen. The velocity differences between left and right ECs are due to small variations in the μ-CT measurements of the ECs.

Fig. 5. Quantitative relationship between EC (trachea) radius and length. Measured distance displayed from the acoustic spiral to the tympanic organ area in males and females of C. gorgonensis. (A) Female left EC. (B) Female right EC. (C) Male left EC. (D) Male right EC.
Experimental Setup. To observe the effects of the internal pathway only, we isolated the internal and external sound inputs. We did this by mounting the insect on a customized platform (Fig. 2). The platform consists of two Perspex panels that fit together, the dorsal of which encompasses a central notch for the insect’s head along with two adjacent holes for the forelegs. These panels are held together within a metal frame [additional details are in the work by Montalegre-Z et al. (5) and SI Appendix, section 5].

A 23-kHz pure tone is produced by a function generator (SDG1000 Series Function/Arbitrary Waveform Generator, Siglent Technologies Co. Ltd.) and emitted via an MFI-1 S 1 Multi Field Speaker (Tucker Davis Technologies) with a modified probe attachment (SI Appendix, section 6 and Fig. S1).

Prior to each experiment, a standardized reference point was recorded. The probe loudspeaker was mounted onto a micromanipulator (World Precision Instruments, Inc.). A calibrated 1/8-inch (3.2-mm) microphone (Brüel & Kjær) was held in a clamp, and the loudspeaker probe was positioned 2 mm away from the microphone’s protective cap. The time taken for sound to travel from the loudspeaker probe to the microphone was recorded as the reference time.

The mounted insect was positioned in front of the LDV, and the laser point focused on a TM (Figs. 1 and 2). A gas delivery system (enabling the manipulation of the gas composition between experiments) was introduced behind the prothoracic side keel, directed toward the acoustic spiracle (Fig. 1 and SI Appendix, section 1). The probe loudspeaker was then placed 2 mm away from the spiracle (matching the distance of the microphone reference signal), allowing sound to travel through the acoustic trachea and vibrate the tympanum (Fig. 1). For each ear, the LDV was used to record the time for the signal vibrations to arrive at the tympanum, measured as tympanal displacement.

Recordings were first taken without changes to the gas composition within the EC (normal conditions). The time taken for sound to travel from the loudspeaker to the TM was noted and used as a benchmark throughout the experiment, to indicate when the gas composition in the EC was normal. Then, over a period of 5 s, pure CO₂ (Genuine Innovations) was introduced away from the spiracle (matching the distance of the microphone reference signal). The time taken for sound to travel from the loudspeaker probe to the microphone was recorded as the reference time.

External and internal morphology of the EC showing the tracheal division at the tympanal organ location. (A) Lateral view of the body in transparency showing left and right ECs. (B) Internal view inside the EC. (C) Close-up view of the ear section extracted from the dashed box in A. (D) Internal view inside the EC showing the branch division of the trachea at the tympanal organ location. Camera was placed inside the EC lumen approximately at the location of the yellow dashed line in C.

Sound Analysis and Calculation of Velocity. The first local minimum of the sine wave chirp was used to obtain time measurements (SI Appendix, section 7 has more details on the reference signal). The time delay between the reference signal and the amplitude signal of the tympanum was obtained using Matlab (R2014a; The Math-Works, Inc.). This delay was used to calculate the velocity of sound propagation through each EC—dividing the EC lengths obtained from 3D reconstructions by the respective mean time delay.

Mathematical Model and Numerical Simulations. The numerical results are based on the following system of equations.

Let Ω denote the EC of C. gorgonensis, which is the domain of our mathematical model formed by the union of two parts: Ω = the inside of the EC (the fluid domain) and Ωs = the EC wall (solid domain), which intersect at the interface γ = Ω ∩ Ωs. The boundary of Ω consists of γfs, which denotes the acoustic spiracle; γγ, which denotes the TM; and γs, which denotes the outer surface of the wall.

In the fluid domain Ωf, the propagation of the sound wave is modeled with the use of the scalar wave equation, which also takes into account the viscosity of the quiescent fluid. This is represented with the following equation and initial conditions:

\[
\frac{\partial^2 p}{\partial t^2} + \frac{1}{\rho} \left( \frac{\partial}{\partial x} \left( \frac{\partial p}{\partial x} \right) \right) = \frac{1}{\rho} \left( \frac{\partial (\rho u)}{\partial t} \right), \quad t > 0, \quad x \in \Omega_f, \quad t \geq 0.
\]

where \( T > 0 \) is a fixed positive time, \( c = \text{speed of sound}, \ mu = \text{dynamic viscosity}, \rho_f = \text{fluid density}, \nabla = \left( \frac{\partial}{\partial x}, \frac{\partial}{\partial y}, \frac{\partial}{\partial z} \right), \) and \( \Delta = \nabla^2 \). The dependent variable \( p(x, t) \) is the time-dependent sound pressure at the spatial point \( x = (x, y, z) \).

A harmonic incident wave enters into the EC through the spiracle with a frequency of 23 kHz and an amplitude of 1 Pa, traveling at 343 m/s, and \( \rho_f = 1.225 \text{ kg/m}^3, \mu_f = 1.84 \times 10^{-5} \text{ Pa·s} \).\footnote{6}

Taking into account the impedance of the TM, we request the boundary condition (3) given below is satisfied on \( \gamma_s \):

\[
n \cdot \left( \frac{1}{\rho_f} \nabla p \right) + \frac{1}{\rho_f} \left( \frac{\partial p}{\partial t} \right) + \frac{1}{\rho_f} \left( \frac{\partial p}{\partial n} \right) = n \cdot \left( \frac{1}{\rho_f} \nabla p_i \right) + \frac{1}{\rho_f} \left( \frac{\partial p}{\partial t} \right)
\]

\[
\text{on } \gamma_s, \quad \text{where } \rho_i = \sin \left( 2\pi \times 23000 \left( t - \frac{(x - e_i)}{c} \right) \right)
\]

and \( e_i \) is the wave direction.

Next, we consider the displacement in the elastic wall due to the sound propagating inside the EC. This is represented by the elastic wave Eq. 6 given below, which models the mechanical waves in the structure \( \Omega_s \), assuming small deformations (42). We make the simplifying assumption that the EC wall is built of an isotropic, incompressible, and homogeneous material. Eq. 6 also accounts for the damping properties of the EC wall:

\[
\frac{\partial^2 u_i}{\partial t^2} + \rho_s \alpha_{\text{damp}} \frac{\partial u_i}{\partial t} = \nabla \cdot \left( \rho_s \left( u_i + \beta_{\text{damp}} \frac{\partial u_i}{\partial t} \right) \right) \quad \text{in } \Omega_s \times (0, T),
\]

\[
u_i(x, 0) = 0, \quad \frac{\partial u_i}{\partial t}(x, 0) = 0 \quad \text{in } \Omega_s.
\]

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where $\rho_w$ is the density of the wall and the components of the stress tensor $\sigma(u)$ are

$$\sigma_{ij} = \frac{E}{1 + \nu} \epsilon_{ij} + \frac{E \nu}{(1 + \nu)(1 - 2\nu)} \delta_{ij} \sum_{k=1}^{3} \epsilon_{kk}, \quad i, j = 1, 2, 3, \quad [8]$$

with $E$ = Young’s modulus; $\nu$ = Poisson’s ratio; $\epsilon_{ij}$ is the strain tensor; $\delta_{ij}$ is the Kronecker-delta function; and the dependent variable $u_i$ is the displacement vector in $\Omega$. Furthermore, the Rayleigh damping parameters $\alpha_{i,k}$ and $\beta_{i,k}$ satisfy the following relation between the damping ratio $\xi$ and the natural frequencies $\omega_{i}$ of an undamped system:

$$\xi = \frac{\alpha_{i,k}}{2\omega_{i}} + \omega_{i} \beta_{i,k}, \quad [9]$$

where $\alpha_{i,k}$ and $\beta_{i,k}$ are the mass proportional and stiffness proportional Rayleigh damping coefficients, respectively.

On $\gamma_3$, we specify the boundary condition as

$$\sigma_{i} \cdot n = 0, \quad i, j = 1, 2, 3, \quad [10]$$

so that there is no external stress on the wall.

Finally, at the interface $\gamma_2$, the defined fluid and solid systems are coupled. The coupling includes the fluid load on the structure and the structural acceleration as experienced by the fluid:

$$n \frac{1}{\rho} \nabla \rho = -n \cdot (u_i)_{n}, \quad [11]$$

$$F_k = pn, \quad [12]$$

where $n$ is the surface normal and $F_k$ is the force per unit area experienced by the structure.

As well as the pressure distribution in the EC for the time interval $(0, T)$, by solving system (2–12), we are also able to obtain the time taken for the sound stimulus to reach the TM. Using this, we calculate the speed of sound during transmission by the elementary formula

$$\text{speed} = \frac{\text{distance}}{\text{time}},$$

where distance $=$ length of the EC.

For spatial discretization, the numerical solution of the variational form of the introduced equations, along with the defined boundary conditions, is obtained with the finite element method using the commercial software Comsol Multiphysics version 5.5 [43].

The finite element mesh constructed in the EC is demonstrated in Fig. 1. The tetrahedral elements forming the mesh in $\Omega$ have the restriction that the diameter of each element is less than the quantity

$$\lambda = \frac{\text{wavelength of sound wave}}{20},$$

The mesh in $\Omega$ is connected to the mesh on $\Omega_w$, which is formed using shell elements of Mixed Interpolation of Tensorial Components type [44], a common mesh type used for capturing various shell behaviors. The solution of the finite system of finite element equations is based on Quadratic Lagrange elements for all of the dependent variables.

For the discretization of the time derivatives in system (2–12), the Generalized Alpha Method is employed, with a time step of $\tau = 2.174 \times 10^{-3}$ s. Taking into account that the frequency of the sound stimulus is 23 kHz, we took $\tau = 5/(23 kHz) = 2.174$ s, so that we assumed the sound stimulus has five cycles at 23 kHz.

The outlined equations are solved using the 3D Transient Acoustic-Sound interaction module of Comsol Multiphysics (43) (SI Appendix, Table S2) shows the values of the parameters used in Eqs. 2–12.

For the simulations that have been carried out on ECs with different radii, the conditions outlined above have been kept the same. The geometry of the EC model was changed by using the “scale” feature of Comsol Multiphysics version 5.5 (43).

Data Processing and Statistical Analysis. During each experiment, the time taken for sound to propagate through the EC to the TM was recorded using LDV. For each EC sample, the mean of a minimum of three replications was calculated. Postexperiment, recordings were standardized against a reference recording. For some recordings using CO$_2$ gas, the reference signal was invalidated due to too much gas dispersion, and therefore, these recordings were removed from the analysis. The remaining values, along with length data obtained from µ-CT data, were then used to calculate the mean sound propagation velocity for each individual EC. The mean sound propagation velocity and SD were taken and listed in Table 1.

Sound velocity in acoustic trachea was analyzed using Gaussian linear mixed-effects models. Velocity was the dependent variable, with tracheal length as a covariate and tympanum (ATM/PTM) and status (live, dead, numer-ous) as fixed factors. The remaining values, along with length data obtained from µ-CT data, were then used to calculate the mean sound propagation velocity for each individual EC. The mean sound propagation velocity and SD were taken and listed in Table 1.

Data Availability. Numerical simulations, experimental data (laser Doppler vibrometry recordings), Comsol model files, and µ-CT stereolithography files (in .stl format) are available in Dryad (DOI: 10.5061/dryad.2547d7wnn; deposited 8 November 2020).

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