

# Variation in head and pinna morphology of preserved *Peromyscus* spp. specimens and implications for auditory function

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## Funding information

National Science Foundation, Grant/  
Award Number: DBI 2216648

## Abstract

The physical characteristics of an animal's head and pinna mark the beginning of auditory communication. Auditory communication is broadly achieved by receiving sounds from the environment and plays a vital role in an animal's ability to perceive and localize sounds. Natural history museums and collections, along with their vast repositories of specimens, provide a unique resource for examining how the variability in both the size and shape of the head and pinna causes variability in the detection of acoustic signals across species. Using this approach, we measured morphological features of the head and pinna on over 1200 preserved specimens of *Peromyscus boylii*, *P. californicus*, *P. gossypinus*, *P. leucopus*, *P. maniculatus*, and *P. truei*, followed by a series of head-related transfer functions (HRTFs) on several individuals to study the relationship between morphology and available auditory information. Our morphological results show significant variation in pinna length and width, as well as in the distance between the two ears across the six species. Interaural time differences and interaural level differences were calculated and demonstrated consistent results across species, suggesting the differences in head and pinna size do not significantly modify these cues. Not only does this study contribute to existing research on external morphology and auditory function, but it also provides valuable insight into the use of preserved zoological specimens in auditory research, an area that is currently understudied.

## KEY WORDS

head-related transfer functions, interaural level difference (ILD), interaural time difference (ITD), pinna morphology, spectral notches, zoological specimens

## 1 | INTRODUCTION

Morphological features give great insight into the function of the structures that comprise an animal. For example, the skeleton, dentition, and body size/composition tell us much about an

animal's locomotion, habitat, diet, and even behavior (Schwab et al., 2023). Similarly, the head and pinna are the first contact points for sound waves before reaching the inner ear. The pinna functions primarily to localize and direct sounds into the middle and inner ear, where they can then be interpreted as electrical

signals in the brain. It is also thought that pinnae play a role in thermoregulation in many species, an idea first proposed by biologist Joseph Asaph Allen in 1877, which predicts that animals living at higher latitudes (generally colder environments) should have smaller appendages (i.e., limbs, pinna, or tail) than animals living in lower latitudes based on the properties of heat conservation (Alhajeri et al., 2020; Allen, 1877). Tracking these different pressures on morphology requires ample samples covering a broad geographic and temporal range, such as those present in zoological collections.

Natural history museums and zoological collections are permanent repositories of biodiversity throughout history and provide immense value to scientific research. Collections often work directly in collaboration with zoos and research institutions to allow their specimens to be used for studying evolution, morphology, conservation, genetics, and much more across a variety of scientific disciplines (Nakahama, 2020; Poo et al., 2022). They are often overlooked in auditory research as many of the structures of the auditory system are small, fragile, located within the skull, and are therefore not easily preserved (Stoessel et al., 2016). Limited research has been conducted, however, on the overall pinna size and shape (such as here), the tympanic membrane, ossicle sizes, and middle ear cavities of zoological specimens (Mason et al., 2017; Stephens et al., 2014). Thus, to expand our understanding of the usefulness of museum collections in auditory research, we need to compare specimens from a group of species that are highly variable in both their morphology and ecology, such as deer mice from the genus *Peromyscus* (Cricetidae; Rodentia).

Deer mice are among North America's most abundant groups of animals (Bedford & Hoekstra, 2015). Many species, such as *P. leucopus* and *P. maniculatus* are colloquially regarded as habitat generalists, occupying diverse habitats, including grasslands, mountains, and forests (Kamler et al., 1998). In contrast, some species, such as *P. californicus* and *P. truei*, occupy more specialized regions, such as damp oak-woodlands and pinyon-juniper woodlands, respectively (Fellers, 1994; Kobrina et al., 2021). Here, we focused on six *Peromyscus* species—*P. boylii*, *P. californicus*, *P. gossypinus*, *P. leucopus*, *P. maniculatus*, and *P. truei* to assess morphological and functional differences in head and pinna size. These species were selected due to the habitats in which they occur, giving insight into whether geographic location plays a role in terms of either form or function. *P. leucopus* and *P. maniculatus* were chosen because they are known for being some of the most widespread *Peromyscus* species, and there are only a few areas of North America that they do not inhabit. *P. truei*, on the other hand, are primarily found in western North America and are known for thriving within pinyon-juniper woodlands and rocky slopes (Hoffmeister, 1981). *P. boylii* thrive at higher elevations (above 2000m), especially throughout mountainous regions in the Western US (Ribble et al., 2002). Lastly, *P. gossypinus* and *P. californicus* were selected based on their intermediate environmental preferences, providing a comprehensive representation

across habitats. If there are significant morphological differences pertaining to the auditory system within *Peromyscus*, we expect to find them within this group of species.

The biomechanics of hearing and sound localization encompasses the highly complex interplay between the ear and brain structures. The ability to accurately locate and process complex acoustic stimuli from the environment has been linked to the pinna in multiple mammalian species, including bats (Aytekin et al., 2004), guinea pigs (Greene et al., 2014), cats (Benichoux et al., 2016; Rébillat et al., 2014; Xu & Middlebrooks, 2000; Young et al., 1996), and chinchillas (Heffner et al., 1996; Koka et al., 2011; Osmanski & Wang, 2011). Particularly, the presence of the pinna plays a major role in the front/back and vertical discrimination of acoustic signals (Alves-Pinto et al., 2014; Heffner et al., 1996; Jones et al., 2011). This is due to the folds and convolutions of the pinna which filter sounds to create unique, generally high-frequency spectral patterns (notches and peaks) that the brain then associates with specific locations in elevation (Anbuhl et al., 2017; Middlebrooks, 2015; Rice et al., 1992). After the pinna receives a sound, the brain can then assess the approximate location in azimuth, relying on the computation of two cues: interaural time differences (ITDs) and interaural level differences (ILDs) or the shift in time and sound level of a particular cue at each of the pinnae (Hartmann, 2021; Wightman & Kistler, 1993). To resolve front/back ambiguity, the position and orientation of the head, body, and pinna must be used due to the similarity in ITD and ILD of sounds arriving from the front and back. Head-related transfer functions (HRTFs) are one unique way of measuring ITD and ILD that accounts for the size and shape of the head and pinna to determine how a specific sound stimulus is both received and impacted by the morphology of an individual at a set distance and elevation in space in the ear canal (Li & Peissig, 2020). They are distinctive to every individual and lend perspective into whether morphological differences are correlated with functional differences in signal detection (Lange et al., 2004; Pec et al., 2007). Notably, as the acoustics of the HRTF rely only on anatomical/morphological features of the head and pinna, these measurements can easily be performed on preserved zoological specimens (Benichoux et al., 2016; Rébillat et al., 2014).

This research aims to demonstrate the utility of zoological specimens in studying the impacts of head and pinna size differences on available auditory cues across different species. To do this, we will first test the accuracy of using zoological specimens in morphological research by performing statistical analyses on historical measurements taken at the time of collection and preserved measurements taken presently, in which we expect to find shrinkage in the preserved measurements due to the age of the specimen, drying time, and variability in preparation. Next, our work helps to uncover the relationship between pinna morphology, habitat, and auditory function across the six *Peromyscus* species mentioned. We hypothesize that if significant differences are seen in head and pinna

morphology, then sound localization cues may also be affected, reflecting different auditory niches.

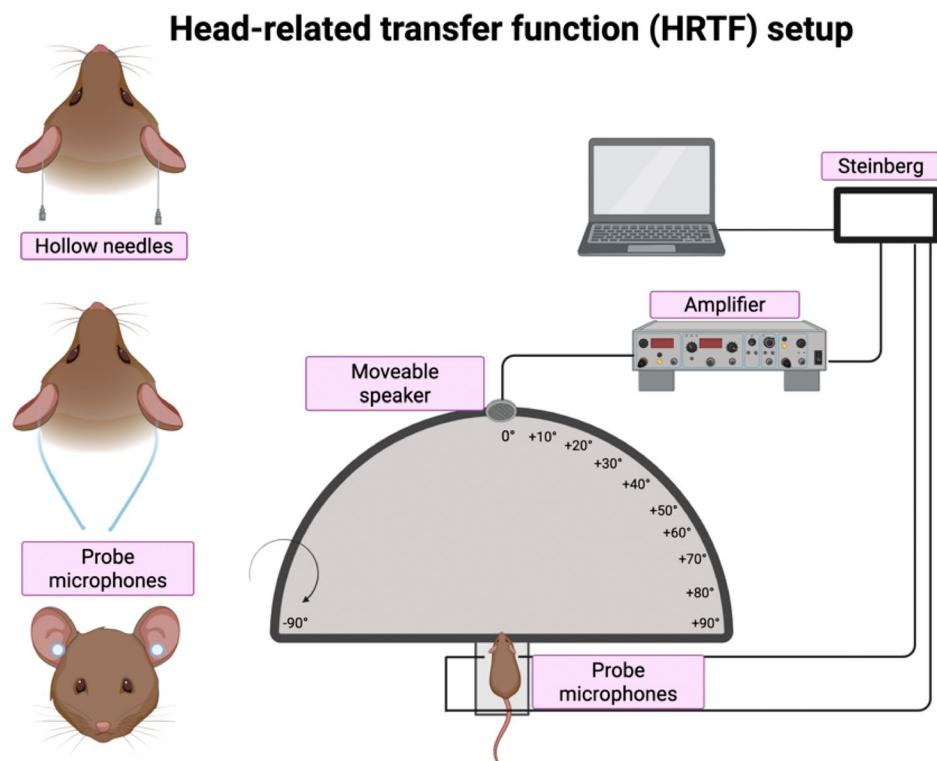
## 2 | MATERIALS AND METHODS

### 2.1 | Animal models

Preserved specimens of *Peromyscus boylii*, *P. californicus*, *P. gossypinus*, *P. leucopus*, *P. maniculatus*, and *P. truei* were provided by the Oklahoma State University's Collection of Vertebrates. Individual specimens were given to the collection through various institutional researchers and zoological donations over the years, with some specimens used dating back to 1903. Each animal in this study had the following data associated with them: genus, species, date of collection, precise location of collection site, name of the collector and specimen preparator, as well as initial measurements of the body, tail, hindfoot, and ear lengths. Initial measurements are generally taken when the animal is recently deceased; however, there is variability due to the differences in specimen collectors and differences outside of the control of the current analysis. Given the variability in specimen preparation, only the best-preserved specimens were chosen for analysis (i.e., pinnae tissue was not folded or visibly damaged, and the head was stuffed to mimic the natural position as best as possible). All specimens used were collected from North America, with the majority being from Oklahoma and Colorado.

### 2.2 | Morphology

We measured the head and pinna of 1274 preserved *Peromyscus* specimens (*P. boylii*,  $n=63$ ; *P. californicus*,  $n=4$ ; *P. gossypinus*,  $n=12$ ; *P. leucopus*,  $n=780$ ; *P. maniculatus*,  $n=382$ ; *P. truei*,  $n=33$ ). Measurements included pinna size (length, width, and effective diameter), distance between the pinnae (abbreviated as IT), and distance from the tip of the nose to the midpoint between the pinnae (abbreviated as NT) (Figure 1). Length measurements of the pinna were taken as the maximum distance from the base to the tip, while width measurements were taken at the widest part of the pinna to obtain the greatest perpendicular distance across the structure. In cases where the pinnae were slightly folded longitudinally, the pinnae were gently flattened for measurement, ensuring minimal disruption to the natural morphology. The effective diameter was calculated using the formula  $ED = \sqrt{\text{Pinna length} \times \text{Pinna width}}$  to draw inferences on sound detection capabilities. IT measurements were taken as the distance between ear canals, while NT measurements were taken as the linear distance from the tip of the nose to the midpoint between the two ears, measured with calipers positioned above the head. Although these measurements lacked distinct anatomical landmarks as anchor points, all measurements were performed at the same relative positions on each specimen by two researchers using the same 6-inch Stainless Steel Electronic Vernier Caliper (Digi-Science Accumatic digital caliper, Gyros Precision Tools,



**FIGURE 1** Head-related transfer function equipment. Figure representation of the custom-made HRTF setup and microphone placement strategy used in this study. The array offers a total of 361 unique possible speaker locations along the horizontal and vertical axes.

Monsey, NY, USA) in each measurement session. Due to the age and nature of zoological specimens, we also tested the accuracy of research collection measurements by comparing our measurements to the historical measurements taken at the time of collection. This limitation was addressed by testing the correlation between the two. All pinna measurements were taken on the right side of the animal. Mean values for each parameter measured were calculated in RStudio (version 4.2.1).

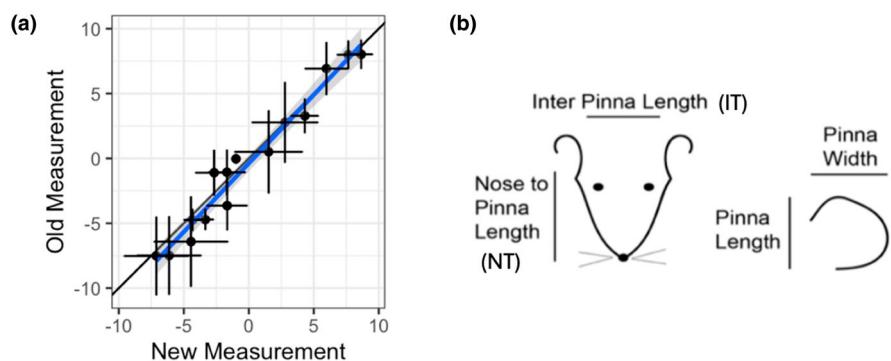
### 2.3 | Live animal morphology

A total of 62 newly captured *Peromyscus* (*P. leucopus*,  $n=38$ ; *P. maniculatus*,  $n=24$ ) were used to compare craniofacial and pinna morphology measurements between live and museum specimens. Animals were live-trapped between June 2021 and December 2024 at various locations across Oklahoma (Stillwater, Arnet, Freedom, and Quinton) using Sherman nonfolding traps (H.B Sherman Traps, Inc., Tallahassee, FL). Traps were baited with oats and peanut butter, set in the evening (around 6:00 PM), and checked ~12 hours later, the following morning. Upon capture, animals were identified as species in the field according to Caire et al. (1989) and transported to the laboratory for physiological (not reported here) and morphological measurements. Morphological features of live animals were measured following similar procedures as outlined above. In brief, measurements of pinna length, pinna width, body length, nose-to-pinna distance, and inter-pinna distance were recorded by a trained researcher using a 6-inch Stainless Steel Electronic Vernier Caliper (DIGI-Science Accumatic digital Caliper Gyros Prediction Tools Monsey, New York, USA). All procedures were approved by the Oklahoma State University Institutional Animal Care and Use Committee and were in accordance with the guidelines and recommendations of the American Society of Mammologists for the use of wild mammals in research. Animals were captured using an Oklahoma Department of Wildlife Conservation scientific collecting permit.

### 2.4 | Head-related transfer functions

To assess auditory function in the six *Peromyscus* species represented here, we conducted a series of HRTFs to determine how sounds are captured at a specific location in the ear canal and whether morphological differences across species lead to functional changes. We performed HRTFs on a total of 18 preserved *Peromyscus* specimens (*P. boylii*,  $n=5$ ; *P. leucopus*,  $n=5$ ; *P. maniculatus*,  $n=5$ ; *P. truei*,  $n=3$ ). These specimens displayed measurements characteristic of the range observed in the means recorded from the museum sample. HRTFs were conducted in a sound-attenuating chamber (86 × 112 × 86 in.; O'Neill Engineered Systems, Hartland, WI) with a custom-made speaker system array that consisted of a single movable speaker (Dayton Audio ND65-4, Springboro, OH), capable of moving 180° around the subject in 10° increments, making for a total of 19 possible locations on the horizontal plane and 19 possible locations on the vertical plane for a total of 361 potential measurements (Figure 2). In azimuth, -90° and +90° were defined as directly on the left and right sides, respectively, and 0° being directly in the middle. In elevation, 0° V was defined as directly in front of the subject, with 180° V being directly behind the subject.

In light of the logistical and technical constraints of the experimental setup, we focused only on capturing ITD and ILD data. We, therefore, took measurements at each location on the horizontal axis (-90° to +90°) both in front of and behind the animal, for a total of 38 measurements per animal. ER-7C probe tube microphones (Etymotic Research Inc., Elk Grove Village, IL) were placed into the preserved specimen's ear canal by threading the tubing through a hollow 14-gauge needle carefully inserted through the base of the pinna, remaining as close to the eardrum as possible, ensuring the microphone tips did not come in contact with any tissue or hair. Due to the fragility of zoological specimens, small perforations were made with the tip of the needle near the insertion point to eliminate tearing and excess space around the microphones during the placement. Measurements were taken with room acoustics software REW



**FIGURE 2** Comparison of preserved and historical pinna length measurements. (a) Comparison of historical measurements taken at the time of collection to present measurements taken on preserved specimens. Each dot represents an average pairwise distance between species, with their respective measurement errors, while the solid black line shows the 1:1 correspondence. See Figure S2 to see species labels. (b) A simplified depiction of the morphological measurements taken on each animal subject.

(Room EQ Wizard, version 5.20.13 Pro Upgrade, Montrose, Angus, Scotland, UK). A Steinberg UR22C (Steinberg Media Technologies GmbH, Hamburg, Germany) was used as the audio interface with a Sony stereo amplifier (STR-DH190, New York, NY) to drive the speaker. The system was calibrated using microphone calibration files provided by Etymotic as well as by performing a manual calibration using a dB meter and sound card to ensure the input and output levels were within 6 dBfs (full scale) of each other. To eliminate any loudspeaker effects, additional calibration measurements were taken with only the microphones positioned on the platform in a similar location to where the microphones would rest with the animal present.

Once the initial calibration was complete, the specimen was placed on an acrylic platform in the center of the array, with the microphones precisely positioned in the ear canal. Acoustic stimuli were presented as 128k point sine sweeps ( $-28$  dBfs), starting at 250 Hz and ending at 20 kHz over 3 s with a sampling rate of 44.1 kHz, using a loopback timing reference. Each sine sweep stimulus was repeated five times at each location on the horizontal axis while the microphones recorded responses from the left and right sides simultaneously. The responses were amplified and digitized with the Steinberg UR22C and displayed by the REW software as transfer function and impulse response from the fast Fourier transform (FFT) of the left, right, and combined signals. HRTFs were calculated as the gain of sound pressure level (SPL) between the response received with the specimen present and the microphone-only response. REW up-samples exported files to 131,072 Hz. Impulse responses were windowed using a Tukey window (taper: 0.25, left: 218 ms, right: 500 ms) to minimize the influence of reflections. ITDs were derived by finding the time shift between the signals received by the left and right ear microphones that maximize the signal cross-correlation. ITD dynamic range was calculated as the mean of the 5th and 95th percentile ITD magnitudes across azimuth angles. ILDs were calculated as the frequency-dependent difference in gain between the right ear microphone relative to the left at each speaker position (in dB).

To further examine the relationship between ITD and head size, we applied Kuhn's ITD model (Kuhn, 1977), which describes ITD as a function of azimuth angles and effective head diameter. This model was fitted to the measured ITD data using nonlinear least squares, allowing us to estimate the fitted ITD range and an effective head diameter for each specimen. Anatomical measurements, including inter-pinna-tip and inter-pinna-base distances, were also measured and compared with the model-fitted effective head diameters.

## 2.5 | Statistical analysis

All figures and statistical analysis were completed in RStudio (version 4.2.1) or Python (version 3.11.7). Because preservation is thought to alter morphology by the drying out of tissues via shrinkage, a thorough analysis was taken to ensure drying time and

preparation variation were accounted for in overall morphological measurements by comparing historical measurements taken at the time of collection to the preserved measurements taken presently. First, we performed a correlation analysis between historical and preserved measurements for each species to assess whether the intraspecific variation had changed. Second, we calculated pairwise species differences to evaluate if interspecific differences were maintained. These two previous analyses were only performed on the pinna length, which is the standardized measurement obtained in preserved specimens in zoological collections. Lastly, we compared our preserved measurements on collection-derived animals to measurements taken on live animals using paired t-tests for each species.

To test for species differences in each measurement type within the preserved measurements, we employed a one-way analysis of variance (ANOVA), using species as fixed effects (R packages ggplot2, dplyr, reshape2, and ggrepel). If significant differences in fixed effects were found through the models, a Tukey's honest significant difference (THSD) test was then performed to show all pairwise comparisons between species. Measurements within and between species are depicted by box and whisker plots.

For HRTF analysis, amplitude spectra were calculated using a 512-point FFT and filtered with a 1/48 octave filter within REW. HRTF traces were adjusted by a factor of 2.5 and were exported as .txt files and analyzed in Python.

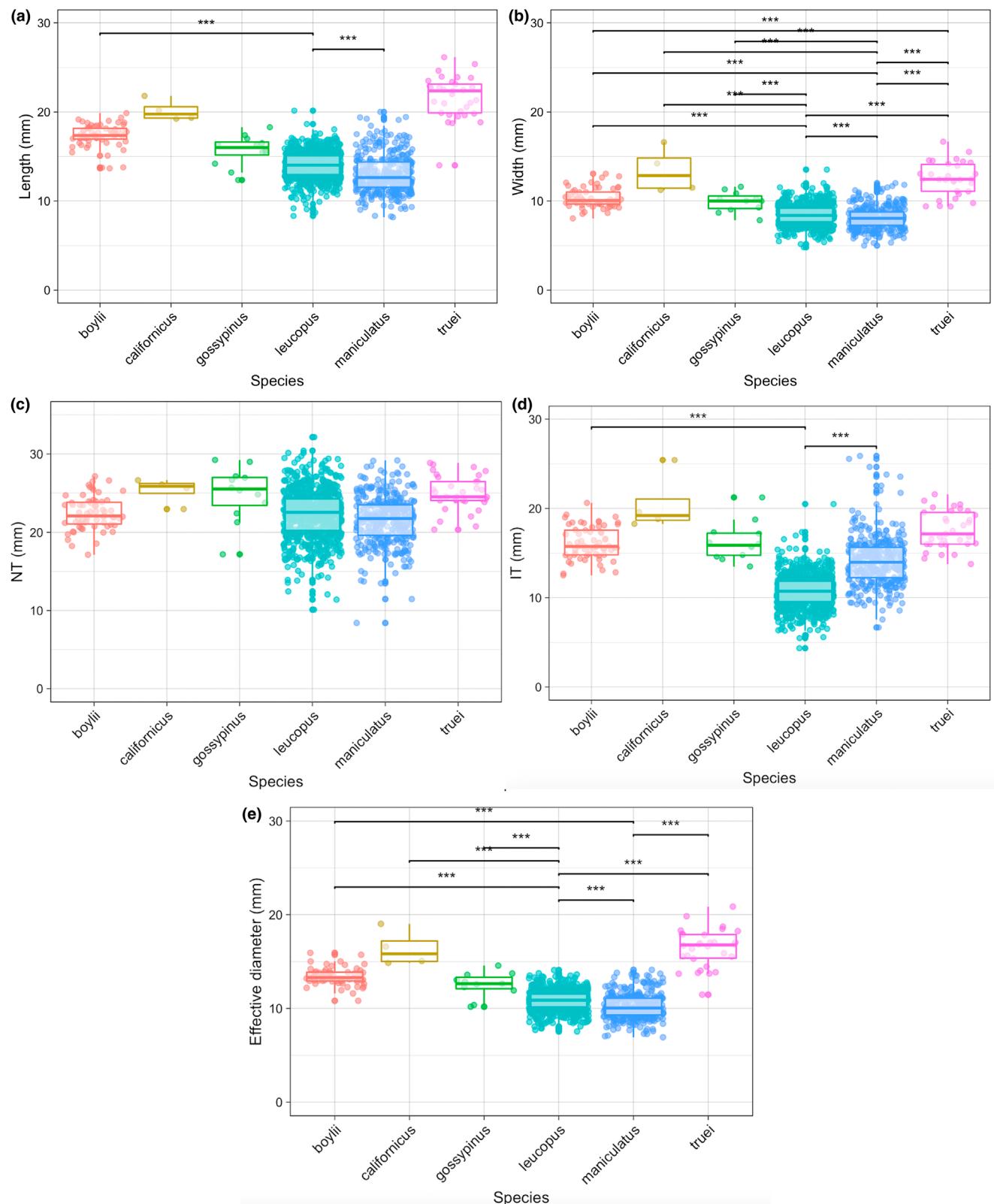
## 3 | RESULTS

### 3.1 | Morphology

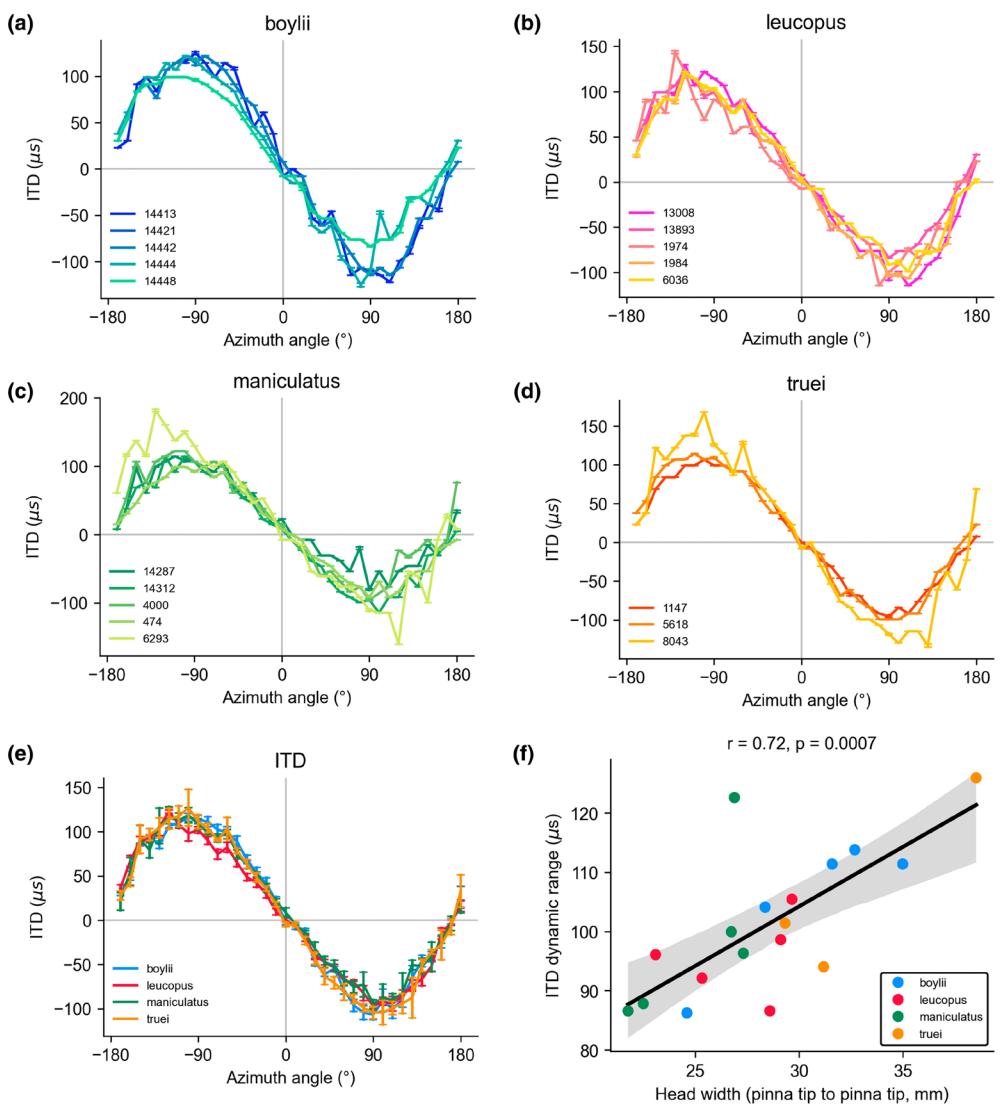
The correlation values between the historical and preserved measurements follow a consistent linear relationship (Figure 3, Figure S2). Given the amount of error in individual measurements, we focused on differences across species and not individuals.

The paired t-test identified statistical differences in three of the four measurements: pinna length, pinna width, and IT (Figure 4). IT showed the greatest difference across species means, while pinna length and width had less pronounced differences. None of the NT measurements showed significant results between species. Pairwise comparisons for each measurement across species can be found in Tables S1–S4. Overall, *P. truei* had the longest and widest pinnae and the greatest IT compared with the other species analyzed (Figure 4, Tables 1 and 2). Notably, *P. leucopus* and *P. maniculatus*, two very closely related species, overlapped in measurements yet were still significantly different in pinna width and IT (Figure 4, Tables 1 and 2). *P. leucopus* had smaller ITs ( $p < 0.001$ ) with larger pinna widths compared with *P. maniculatus* ( $p < 0.001$ ).

Comparisons between live animal measurements and preserved measurements on *P. leucopus* and *P. maniculatus* demonstrate that there are significant differences in pinna length within *P. leucopus*, and in IT within both species (Figure S3A,C). No significant differences were found in pinna width or NT measurements.



**FIGURE 3** Head and pinna measurements. Pinna length (a), pinna width (b), NT (c), and IT (d) measurements in preserved specimens of *P. boylii*, *P. californicus*, *P. gossypinus*, *P. leucopus*, *P. maniculatus*, and *P. truei*. Each point represents an individual measurement. (Significance codes:  $p=0$  (\*\*\*) $, p=0.001$ ).



**FIGURE 4** ITD results for *P. boylii* (a), *P. leucopus* (b), *P. maniculatus* (c), *P. truei* (d), combined species (e), and as a function of head width (f). Measurements were taken in front of and behind the animal in 10° increments in azimuth. -90° to +90° azimuth represents measurements taken in front of the animal, while +/-90° to +/-180° indicates measurements taken behind the animal. Each line represents a separate individual in the species in (a-d). The combined ITD results for each species are shown in (e). The measured ITD dynamic ranges (5th and 95th percentile ITD) and inter-pinna-tip head size are strongly correlated ( $r=0.72, p=0.0007$ ; Pearson correlation) as shown in (f).

**TABLE 1** Mean measurements for pinna length and width by species.

Species	n	Mean length (mm)	SD	Mean width (mm)	SD
<i>leucopus</i>	780	14.01	1.51	8.43	1.17
<i>maniculatus</i>	382	13.03	2.14	8.11	1.24
<i>gossypinus</i>	12	15.70	1.71	9.92	1.13
<i>truei</i>	33	21.67	2.38	12.49	1.92
<i>boylii</i>	63	17.36	1.26	10.32	1.08
<i>californicus</i>	4	20.14	1.18	13.40	2.53

**TABLE 2** Mean measurements for inter-pinna distance and nose-to-pinna distance by species.

Species	n	Mean IT (mm)	SD	Mean NT (mm)	SD
<i>leucopus</i>	780	10.73	1.83	24.94	72.78
<i>maniculatus</i>	382	14.12	2.73	21.55	3.03
<i>gossypinus</i>	12	16.28	2.13	24.96	3.43
<i>truei</i>	33	17.60	2.11	25.02	2.21
<i>boylii</i>	63	17.71	12.89	22.43	2.14
<i>californicus</i>	4	20.53	3.31	25.34	1.64

### 3.2 | Head-related transfer functions

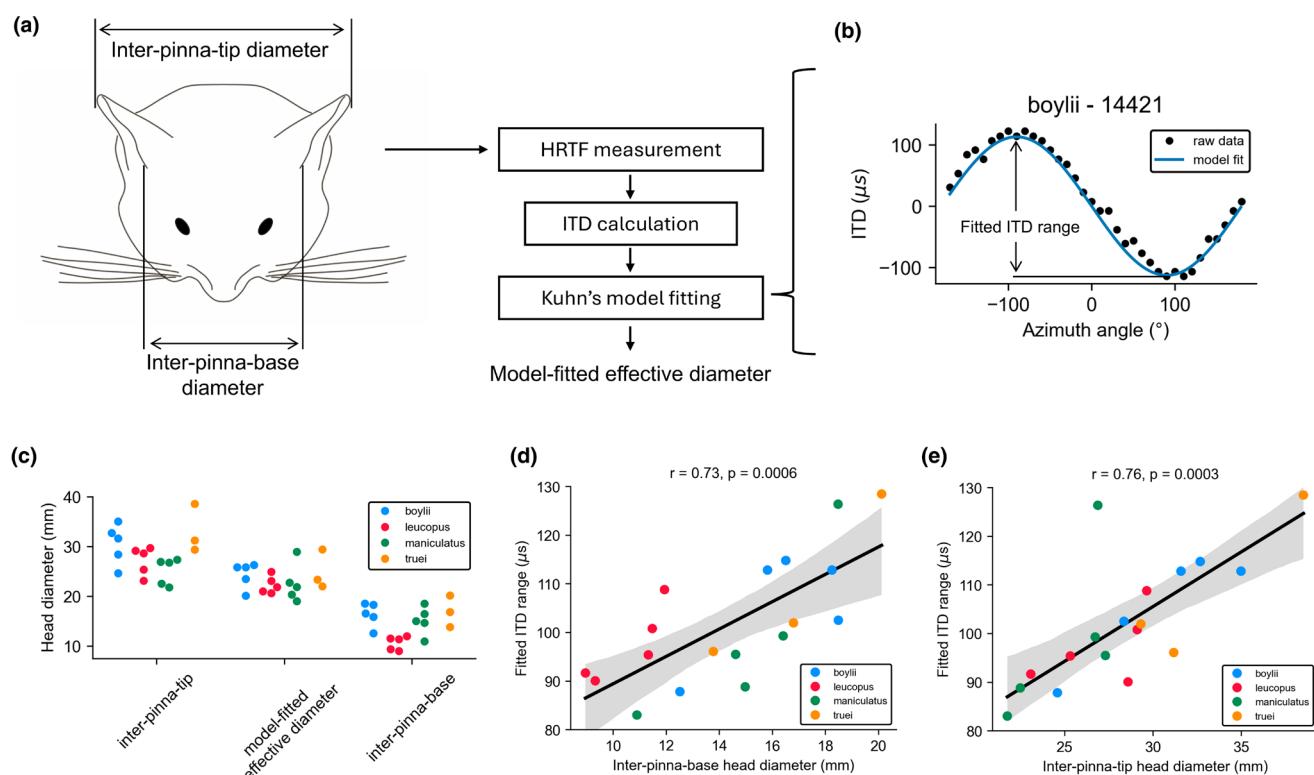
HRTFs were used to explore how morphological features of the head and pinna influence how sound cues are captured in preserved *Peromyscus* specimens. Although six species were initially considered for this study, only four had sufficient specimens available in the research collection utilized for this study. Therefore, the lack of representation led to the exclusion of *P. gossypinus* and *P. californicus* from the HRTF analysis. HRTF gain (characterized by peaks in y-axis values) and spectral notches (characterized by dips in y-axis values) specifically give insight into how different frequencies are amplified (gain) or attenuated (spectral notches) based on the morphological features of the individual.

ITD was similar for all species tested across measured angles (Figure 5). This measurement, along with ILD, provides spatial acoustic information to the animal. ITD values showed consistent patterns of cue availability across all four species, with notably greater variability for locations behind the animal compared with the front. We see the largest difference in maximum ITD between *P. boylii* and *P. truei*, which does not correlate with the differences in head and pinna sizes. *P. leucopus*, despite having the smallest pinna width across species, has the second longest ITD (128.174 µs). The average maximum and minimum ITD values for each species can be found in Table S6.

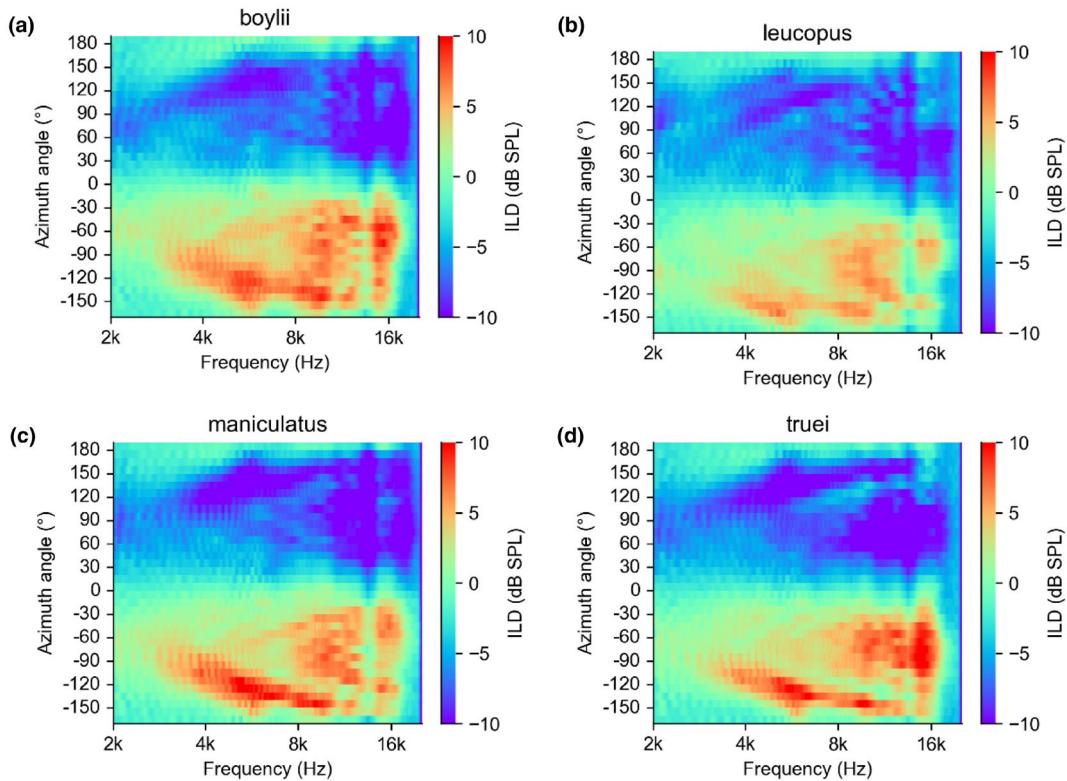
To investigate how head size influences ITD, we compared anatomical head measurements (Figure 6a) with model-fitted estimates

of effective head width to assess their relationship with ITD. Fitting Kuhn's ITD model to each sample yielded individualized estimates of ITD range and effective head diameter (Figure 6a,b). Model-fitted diameters fell between inter-pinna-base and inter-pinna-tip distances (Figure 6c) and were strongly correlated with both (Figure 6d,e): pinna base (D;  $r=0.73$ ,  $p=0.0006$ ; Pearson correlation) and pinna tip (E;  $r=0.76$ ,  $p=0.0003$ ; Pearson correlation), suggesting that the pinnae effectively extend the acoustic path length and extend the ranges of perceived ITDs.

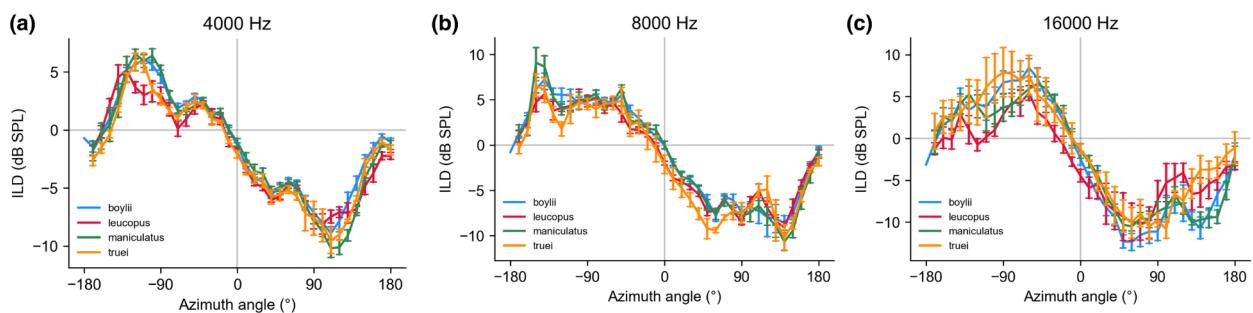
As mentioned above, ILD, calculated as the difference in sound intensity at the two pinnae, is a common cue for sound localization. Our results show that ILD is consistent across all four species and is optimized at higher frequencies, such as at 8 kHz and above, where the greatest differences are seen, and therefore, the most information is derived (Figures 7 and 8). Maximal ILD values were obtained by *P. maniculatus* at 8 kHz (9.94 dB) and by *P. boylii* at 16 kHz (8.75 dB). The presence of a decrease in signal strength around 15 kHz, present across animals, is likely an artifact of the measurement process and is not considered to represent meaningful or biologically relevant data. ILDs in front of the animal at both 8 and 16 kHz were largest at azimuths greater than 60° on either side (Figure 8a,c). The greatest differences were seen in ILDs closer to  $-/+30^\circ$  azimuth, and the widest variability in ILD between species occurred during stimuli presented behind the animal at 16 kHz (Figure 8).



**FIGURE 5** Model-based estimates of ITD range and effective head diameter across species. (a) Schematic of head diameter measurements and model-fitting process. (b) Example model-fitting results using the ITD model proposed by Kuhn (1977). Black dots: Raw ITD data; blue line: Kuhn model fit. (c) Comparison of head diameter measurements across species for inter-pinna-tip, model-fitted effective diameter, and inter-pinna-base. (d, e) Fitted ITD range correlates with head diameter measured at the pinna base (d;  $r=0.73$ ,  $p=0.0006$ ) and pinna tip (e;  $r=0.76$ ,  $p=0.0003$ ) across species (Pearson correlation).



**FIGURE 6** ILD spectrogram results. ILD results for *P. boylii* (a), *P. leucopus* (b), *P. maniculatus* (c), and *P. truei* (d). Measurements were taken directly in front and behind the animal at  $10^\circ$  increments in azimuth.  $-90^\circ$  to  $+90^\circ$  azimuth represents measurements taken in front of the animal while  $+/-90^\circ$  to  $+/-180^\circ$  azimuth represents measurements taken behind the animal.



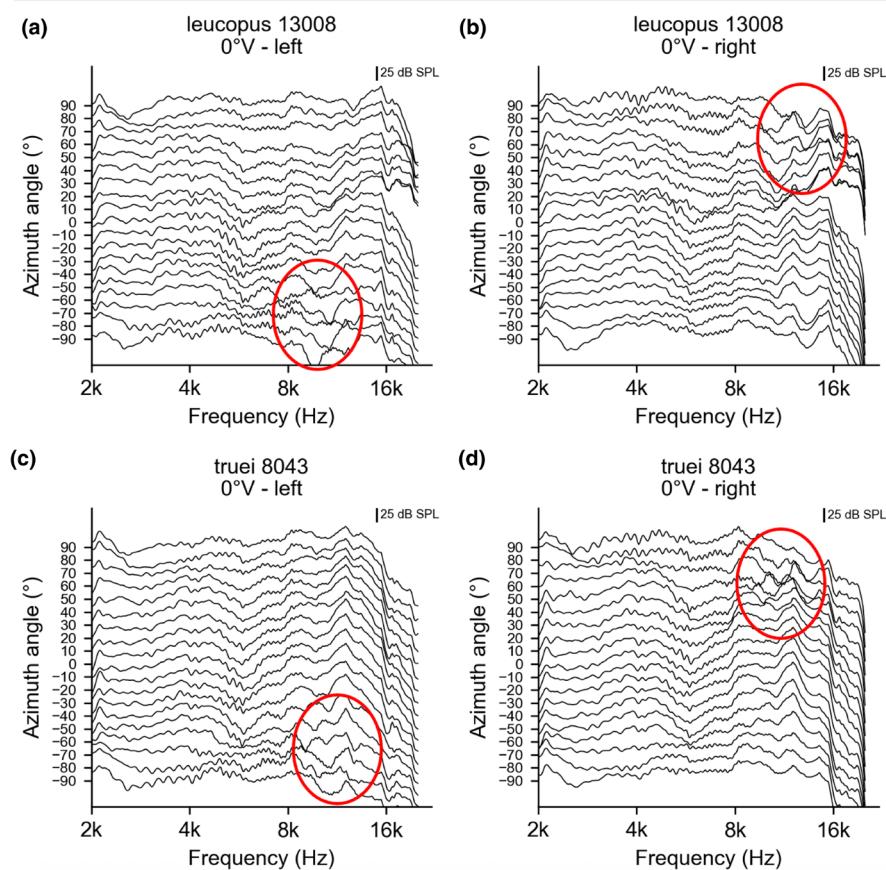
**FIGURE 7** ILD per azimuth angle at 4 (a), 8 (b), and 16 (c) kHz for each species. Species-wide ILD results for each azimuth angle at 4, 8, and 16 kHz frequencies.  $-90^\circ$  to  $+90^\circ$  azimuth represents measurements taken in front of the animal, while  $+/-90^\circ$  to  $+/-180^\circ$  azimuth represents measurements taken behind the animal.

Raw traces of the HRTF data between *P. leucopus* and *P. truei* demonstrate how specific frequencies are altered based on the location of the sound source across different individuals. Specifically, the presence of variability observed around 10 kHz on the same side as the ear measured is indicative of the presence of spectral notches, which are denoted with red circles in Figure 8.

## 4 | DISCUSSION

Previous literature describes the use of museum specimens in auditory research utilizing the skull and middle ear bones, but few

researchers have performed HRTFs on zoological specimens (Rébillat et al., 2014). Works that have done so typically used taxidermy mounts, which understandably provide a more realistic biological shape and size compared with average museum specimens (Benichoux et al., 2016; Rébillat et al., 2014). Our findings align with this rationale, as they indicate that specimen preparation and aging influence the morphology, as pinna measurements taken on preserved specimens do not accurately reflect the measurements taken at the time of capture. Despite this, our results show significant differences in pinna length, pinna width, and IT measurements across species in ways consistent with the described differences among them. Thus, even though preservation might alter details of the



**FIGURE 8** Raw HRTF traces for two individual animals. Panels (a) and (b) show left and right side responses, respectively, for one *P. leucopus* specimen, while panels (c) and (d) show left and right responses for one *P. truei* specimen.  $-90^\circ$  in azimuth represents the left side of the animal while red circles denote variability at higher frequencies, suggesting the presence of spectral notches at ipsilateral positions in comparison to contralateral positions.

relative size of soft tissue structures, these differences might disrupt within-species variation but maintain significant signal, allowing for the use, albeit limited, of these specimens in auditory research.

The results of our HRTF demonstrated consistent results across species, suggesting the differences in head and pinna size identified in our external measurements do not modify auditory cues. Even though we expected that species with the largest head and pinna (i.e., *P. boylii* or *P. truei*) would produce longer ITDs and ILDs, this pattern was not observed at the species level. However, ITD and ILD were indeed positively correlated with head and pinna size, particularly when considered in combination rather than as separate dimensions. The results of the HRTF suggest that all species investigated have their ITD and ILD cues optimized for higher frequencies, which would be consistent with an adaptation to help animals precisely locate sounds of those frequencies in their environment (Heffner & Heffner, 2008; Stitt and Katz, 2021).

This correlates with what we know about the physical properties of sound, as shorter wavelengths (higher frequencies) are more affected by the head and pinna than longer wavelengths (lower frequencies). Mice, along with many other mammals, utilize high-frequency hearing to aid in sound localization, a trait that is robustly correlated with functional head size (Heffner & Heffner, 2008).

Mice rely extensively on the detection of these signals due to the mechanical restraints of their small morphology, as smaller heads do not block low-frequency signals nearly as well as higher frequencies. Thus, to have optimal binaural cues to detect the approximate location of a sound, frequencies must be high enough to be affected by the head and pinna. However, there are exceptions to this, as some small desert mammals hear and detect especially low frequencies (below 3 kHz) to be better adapted to the sounds in their environment, while subterranean mammals have completely lost high-frequency hearing and sound localization abilities (Heffner & Heffner, 2008; Mason, 2016; Mason et al., 2017). It is possible that animals living within habitats that scatter and reflect sound more, such as within canyon slopes, have adapted by having larger pinna to optimize the available cues (Mason et al., 2017). We cannot conclude this with the current study, as HRTF results were consistent across species, but a larger sample size may provide a clearer relationship between habitat and functional implications of morphological differences.

If the results from our HRTF are correct, that is, all species share a similar auditory profile, this could be a case of a “many-to-one mapping,” which explains functional redundancy in varying morphological structures (Thompson et al., 2017; Wainwright et al., 2005).

In the case of *Peromyscus*, this could be a function of their small size, leading to functional degeneration, implying that slight changes in head and pinna sizes would not be enough to cause a functional impact. This could release pinna size to respond to other ecological pressures, such as the ones implied by Allen's rule, which states that animals in colder environments will evolve shorter extremities, including pinna, to preserve temperature (Alhajeri et al., 2020; Allen, 1877). While this morphological trend can be observed among species (*P. truei*, which had the larger pinna are known from warmer and drier regions), we failed to see any clear geographical trend in pinna size within the most widely distributed species in our sample, *P. maniculatus* (either not shown, or [Supporting Information](#)). While this could be evidence of a lack of Allen's rule within this species, it could also be the effect of drying and aging in disturbing within-species variation. Studies with either fresh specimens or a series of preserved specimens collected and measured in a similar way might be necessary to investigate both the presence of Allen's rule and intraspecific local adaptation in pinna size.

## 5 | CONCLUSIONS

While we provide the first evidence for the usefulness of preserved specimens in studying auditory function, our results should be interpreted with caution. While the morphology represented in preserved specimens might not accurately represent the morphology of live animals, size differences among species were observed, and they should have translated into different auditory performances. The fact that they did not suggest that differences of the sort we can observe in these preserved specimens do not impact the auditory cues investigated here and suggest similar performance among the investigated species. These conclusions hinge on various assumptions which should be the focus of future work. On specimen preservation, experiments should be conducted investigating not only the direct and observable effects of time on preserved specimens but also their possible role in affecting HRTF results. On auditory performance, studies including a broader range of species with different morphologies and sizes and from a wider habitat range would allow the assessment of the impact of size and environment. Furthermore, future research should explore spectral notch cues across elevations to better understand how sounds are perceived at any given point in space, rather than horizontal cues, which were studied here. By addressing these considerations, we can bridge knowledge gaps and importantly strengthen the reliability of using preserved specimens in comparative auditory research.

## ACKNOWLEDGMENTS

We sincerely thank the Collection of Vertebrates of Oklahoma State University for loaning us their valuable research specimens and Stillwater Designs (Kicker) of Stillwater, Oklahoma, for the construction of our equipment (especially Steve Irby and Aaron Surratt). A special thanks to Peyton Williams and Cameron Miller for their help in constructing our equipment as well. Finally, thank you to Dr.

Michael Reichert for the use of his sound-attenuating chamber to make HRTF measurements.

## FUNDING INFORMATION

This work was in part supported by the NSF RaMP DBI 2216648, G.A.A.

## CONFLICT OF INTEREST STATEMENT

No competing interests declared.

## DATA AVAILABILITY STATEMENT

No publicly available datasets were used throughout this work.

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**How to cite this article:** Sergott, C.E., Rodman, K., Greene, N.T., Li, B.-Z., Joseph, L., Machado, F.A. et al. (2025) Variation in head and pinna morphology of preserved *Peromyscus* spp. specimens and implications for auditory function. *Journal of Anatomy*, 00, 1–12. Available from: <https://doi.org/10.1111/joa.70011>