Associated tympanic bullar and cochlear hypertrophy define adaptations to true deserts in African gerbils and laminate-toothed rats (Muridae: Gerbillinae and Murinae)

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Abstract

Hearing capabilities in desert rodents such as gerbils and heteromyids have been inferred from both anatomical and ecological aspects and tested with experiments and theoretical models. However, very few studies have focused on other desert-adapted species. In this study, a refined three-dimensional morphometric approach was used on three African rodent tribes (Otomyini, Taterillini and Gerbillini) to describe the cochlear and tympanic bullar morphology, and to explore the role of phylogeny, allometry and ecology to better understand the underlying mechanism of any observed trends of hypertrophy in the bulla and associated changes in the cochlea. As a result, desert-adapted species could be distinguished from mesic and semi-arid taxa by the gross cochlear dimensions, particularly the oval window, which is larger in desert species. Bullar and cochlear modifications between species could be explained by environment (bulla and oval window), phylogeny (cochlear curvature gradient) and/or allometry (cochlear relative length, oval window and bulla) with some exceptions. Based on their ear anatomy, we predict that *Desmodillus auricularis* and *Parotomys brantsii* should be sensitive to low-frequency sounds, with *D. auricularis* sensitive to high-frequency sounds, too. This study concludes that in both arid and semi-arid adapted laminate-toothed rats and gerbils there is bulla and associated cochlea hypertrophy, particularly in true desert species. Gerbils also show tightly coiled cochlea but the significance of this is debatable and may have nothing to do with adaptations to any specific acoustics in the desert environment.

Key words: adaptation; cochlea; CT scans; hearing capabilities; Muridae; semi-landmarks; three-dimensional morphometrics.

Introduction

Rodents are the most diverse living mammals, colonising a vast array of habitats (Wilson et al. 2016, 2017), with Muridae (or murids) being the most diverse family (Musser & Carleton, 2005). The family consists of species with a wide range of specialisation and morphological adaptations (Happold, 2013). Rodents occupying desert habitats/environment are characterised by numerous common features such as inflated tympanic bullae (Prakash, 1959; Lay, 1972; Nikolai & Bramble, 1983; Alhajeri et al. 2015; Tabatabaei-Yazdi et al. 2015; Mason, 2016), long hind feet and bipedal locomotion (Nikolai & Bramble, 1983), with certain subterranean species showing relatively larger bulla (Stein, 2000). The inflated bulla is not only associated with desert

environments but also with open micro-habitats (Kotler, 1984) and high elevation (Zhao et al. 2013; Tabatabaei-Yazdi et al. 2015). This hypertrophy is said to improve low-frequency hearing, which is hypothesised to help in both prey localisation and predator avoidance (Lay, 1972), and interspecific communication (e.g. vocalisation, tapping, foot drumming, stomping) in burrows and open, mostly desert habitats/environment (Bridelance, 1986; Randall, 1993, 1994, 2014). However, besides the hypertrophied bulla, other aspects of the middle ear morphology as well as the inner ear are also important in improving low-frequency hearing (Dallos, 1970; Lay, 1972; Ravicz et al. 1992; Ravicz & Rosowski, 1997; Salt et al. 2013). Although a multitude of hearing studies (including those inferred from bulla and ear morphology) in desert rodents have been conducted, these have mainly focused on Gerbillinae (Lay, 1972; Plassmann et al. 1987; Plassmann & Kadel, 1991; Zhao et al. 2013; Mason, 2016; Tolnai et al. 2017), including Meriones (Ryan, 1976; Tabatabaei-Yazdi et al. 2014, 2015), Dipodomyinae (Lay, 1972; Webster & Webster, 1975; Heffner & Masterton, 1980; West, 1985; Shaffer & Long, 2004; Randall, 2014) and various subterranean rodents (Heffner & Heffner, 1992; Lange et al. 2004, 2007; Francescoli et al. 2012; Mason et al. 2016) to name a few.

However, no studies have looked at the hearing capabilities of Otomyini in detail despite their adaptation to life in deserts or high altitude. Vocalisation has been studied in two desert-dwelling Parotomys (whistling rats) species, brantsii and littledalei (Le Roux et al. 2001a,b, 2002; García-Navas & Blumstein, 2016) and in gerbils (Dempster & Perrin, 1991, 1994; Dempster et al. 1991; Dempster, 2018). Otomys (unknown species, Cockerell et al. 1914) malleus and incus and Parotomys middle ears (Oaks, 1967, unpublished cited in Mason, 2015) have been described.

The inner ear shows extensive variations across taxa in mammals (Vater & Kössl, 2011), with cochlear function potentially limiting the hearing bandwidth (Ruggero & Temchin, 2002) and semicircular canals responsible for locomotion and balance (Spoor & Zonneveld, 1998; Pfaff et al. 2015; Grohé et al. 2016). The geometry of cochlear features has been said to play a role in determining the limits of high- and low-frequency hearing and octaval ranges in vertebrates (West, 1985; Manoussaki et al. 2008; Ekdale & Racicot, 2015). However, these functional relationships had been inferred from correlations but not linked to causation.

As ear specialisations are said to be more pronounced in the inner ear because of differing lifestyles (Crumpton et al. 2015), it is of importance to test this on other taxa that have not been studied before to see whether this is a general/universal or taxon-specific adaptive trend. Studies relating the bulla size to cochlear parameters and the pattern of their covariance in response to environmental variables are very scarce, especially those looking at rodents. The present study used a three-dimensional (3D) morphometric approach to describe and compare in detail the cochlear

and bullar morphology of African rodent members of three Muridae tribes belonging to two subfamilies (Otomyini in the Murinae, and Taterillini and Gerbillini in the Gerbillinae). These taxa are characterised by a wide variation of different life histories and environmental niches, including both desert and non-desert species (see Table 1 and Fig. 1). Our tribe classification follows Lecompte et al. (2008), Pavlinov (2008) and Wilson et al. (2017). We included the three tribes from two distant murid subfamilies to explore the role of phylogeny, allometry and ecology in an attempt to better understand the underlying mechanism of any observed trends of hypertrophy in the bulla and associated changes in the cochlea. Gerbils are thought to have originated from a desert-adapted ancestor in North Africa (Pavlinov, 2008), and laminate-toothed rats (Murinae: Otomyini) originated 5 MYA from Euryotomys, a murid occupying the presumably mesic temperate central plateau of South Africa (Pocock, 1976; Sénégas, 2001; Denys, 2003; Monadjem et al. 2015). Whereas gerbils are mostly confined to arid or semi-arid habitats, most Otomyini species are associated with temperate or montane or sub-montane habitats, with the exception of Otomys unisulcatus, which is from the semi-arid shrub habitats in the Karoo desert of South Africa, and two species of whistling rats (Parotomys brantsii and Parotomys littledalei) from the Karoo, Kalahari and Namib deserts of southern Africa. The bullae of both desert-dwelling Parotomys species are hypertrophic but not that of O. unisulcatus (Pocock, 1976; Taylor et al. 2004). The very wide habitat range within Otomyini offers an excellent opportunity to understand evolutionary responses of the bulla and cochlea to the transition from mesic to desert environments within a closely related clade of species.

Within Gerbillinae, species differ not only in the size of the bulla but also in the degree and type of pneumatisation (expansion) of the posteriorly located mastoid chamber of the bulla. Extreme desert species (e.g. Desmodillus auricularis) are characterised by larger bulla and a two-chambered pneumatised mastoid cavity; Gerbilliscus, a more semi-arid savannah-associated genus, includes species that typically display a smaller bulla with the mastoid portion hardly expanded; and an intermediate condition of the bulla is found in the genus Gerbillus (Lay, 1972; Pavlinov, 2001, 2008; Mason, 2016). Our study included Gerbillus nigeriae, a species that occupies semi-arid Sahel habitats but has also expanded its range into anthropogenic habitats and therefore is less of a desert specialist than a species such as D. auricularis. Our choice of three gerbil taxa for this study therefore includes a range of species representing various bullar morphologies ranging from more generalised (un-inflated mastoid portions of bulla) to more specialised (highly inflated mastoid) adaptations to arid habitats.

The main objectives of this study are (1) to investigate cochlear morpho-anatomical variation among Otomyini and Gerbillinae, focusing on five cochlea features (Braga et al. 2015) that play a role in hearing capabilities in

Table 1 Brief life history summary of all 10 species.

Species name	Diet	Detailed habitat	Simplified habitat	Mean body mass (g)	Life style	Elevation (m)
Otomys auratus	Herbivorous*[†]	Montane or highveld plateau grassland[†‡]	Mesic	127[‡]	Epigeic[§]	Mid-elevation[‡§]
Otomys angoniensis	Herbivorous*[†]	Mesic savannah[†‡]	Mesic	114[‡]	Epigeic*[†]	Low elevation < 1000*[†]
Otomys unisulcatus	Herbivorous*[†]	Semi-arid[†‡]	Arid	96[‡]	Epigeic[†]	Low elevation[†]
Otomys helleri	Herbivorous*[†]	Alpine[‡]	Mesic	90[‡]	Epigeic[‡]	> 3000 High elevation[‡]
Otomys sloggetti	Herbivorous*[†]	Alpine[†‡]	Mesic	84[‡]	Epigeic[†]	> 2000 Mid-elevation[†]
Otomys barbouri	Herbivorous*[†]	Alpine[†‡]	Mesic	112[‡]	Epigeic[†]	> 3200 High elevation[‡]
Parotomys brantsii	Herbivorous*[†]	Arid[†‡]	Arid	95[‡]	Fossorial[†]	< 1000 Low elevation[†]
Gerbilliscus leucogaster	Omnivorous*[†]	Semi-Arid (Arid savannah)*[†]	Arid	72[‡]	Fossorial[†]	< 1600 Low elevation[†]
Gerbillus nigeriae	Omnivorous*[†]	Semi-arid Sahel[†]	Arid	24[‡]	Fossorial[†]	Low elevation < 1000
Desmodillus auricularis	Omnivorous*[†]	Arid*[†‡]	Arid	46.1[‡]	Fossorial[†]	< 1600 Low elevation[†]

*Skinner & Chimimba (2005).
[†]Happold (2013).
[‡]Monadjem et al. (2015).
[§]Baxter et al. (2017).

mammals (West, 1985; Manoussaki et al. 2008; Vater & Kössl, 2011); (2) to test the correlation between tympanic bulla size vs. cochlear parameters (see Material and methods) in three gerbils and seven Otomyini species; and (3) to test the association of bullar and cochlear parameters with environmental variables (Alhajeri et al. 2015). Following on from these objectives, we predict that the cochlea should scale with body size and bulla size (i.e. hypertrophy of the bulla means hypertrophy (i.e. enlargement) of the cochlea, or some aspects of it). In addition, we predict that both the bullar and cochlear morphology should vary because of phylogeny [i.e. a significant phylogenetic signal between the two subfamilies (Gerbillinae and Murinae) and the two tribes within Gerbillinae], body size (i.e. an increase in cochlea and bulla size with body mass) and environmental factors (i.e. a significant inverse correlation between bullar and cochlear measurements with mean annual rainfall due to hypertrophy in species from low rainfall areas associated with deserts and a significant positive correlation of the same measurements with elevational range).

Materials and methods

Study samples

The present study is based on micro-focal X-ray computed tomography (micro-CT) data obtained for 110 dry skulls of known sex (see Table 2) representing Otomyini and Gerbillinae curated at the Ditsong National Museum of Natural History (Pretoria) and Muséum National d'Histoire Naturelle (Paris). Most of the specimens were adults, as estimated by their molar lamina wear (Otomyini: Taylor & Kumirai, 2001; Gerbillinae: Bates, 1985). On rare occasions where no other specimens were available, specimens from younger adult age classes [e.g. Class 3 = subadult (see Taylor & Kumirai, 2001) in Otomyini] were also used. All the specimens housed in the Ditsong National Museum of Natural History in Pretoria were scanned at the South African Nuclear Energy Corporation (NECSA) tomography centre, using the X-Tek (Metris) H225L industrial micro-XCT scanner. The specimens housed at the Muséum National d'Histoire Naturelle, Paris, France (MNHN), were scanned with the Phoenix Nanotom 180 scanner from the FERMAT Federation at the Centre Inter-Universitaire de Recherche et d'Ingénierie des Matériaux (CIRIMAT) in the University of Toulouse Paul Sabatier. Their isometric voxel size ranged from 19 to 23 μm. Scanner settings of 100–130 kV and 100–180 μA were used.

Phylogenetic sampling and analysis

For the seven Otomyini and three Gerbillinae species included in this analysis (see Table 2) the taxonomy followed Wilson et al. (2017) and Pavlinov (2008). Cytochrome b sequences used here were downloaded from GenBank and one sequence per species was used. The sequences were first aligned with CLUSTALW and a phylogram tree based on the maximum composite likelihood method (Tamura et al. 2004) was generated using MEGA7 (Kumar et al. 2016) software, with an assumption that all the species have been identified correctly (Alhajeri et al. 2015). A neighbour-joining phylogenetic tree (Saitou & Nei, 1987) was generated to test for
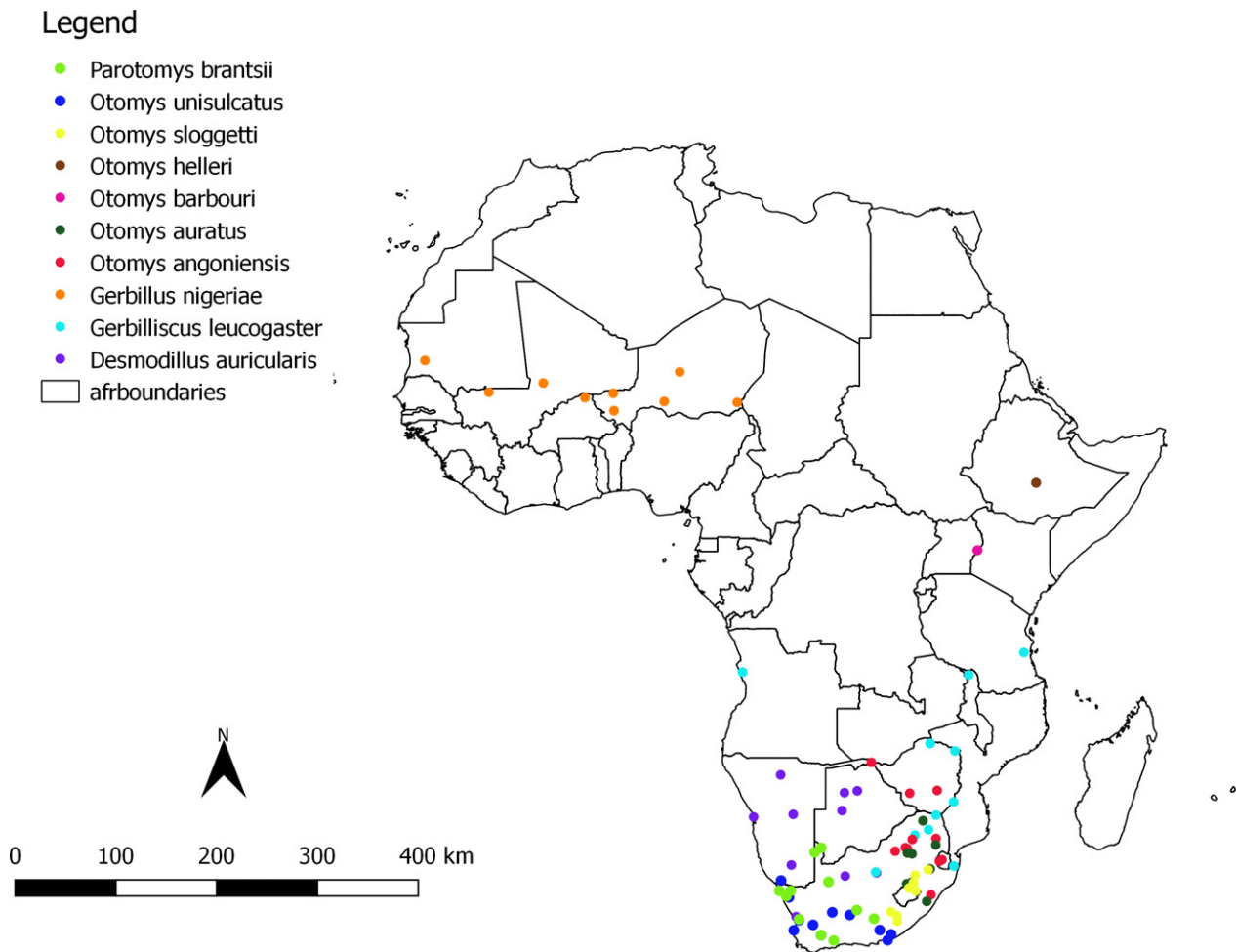
Legend

- Parotomys brantsii
- Otomys unisulcatus
- Otomys sloggetti
- Otomys helleri
- Otomys barbouri
- Otomys auratus
- Otomys angoniensis
- Gerbillus nigeriae
- Gerbilliscus leucogaster
- Desmodillus auricularis
- afrboundaries

N

0   100   200   300   400 km

Fig. 1 Geographical distribution map of all 10 species based on locality coordinates of specimens included in this study and another related study employing linear cranial measurements (A. Nengovhela unpubl. data). Coordinates were obtained from the specimen labels; where there none, locality names were used to search for one using Google Earth/Maps and Geonet gazetteer Southern Africa.

phylogenetic signal among our samples (Supporting Information Fig. S1).

The phylogenetic signal (i.e. the tendency for related species to resemble one another) was computed by calculating the parameter lambda (λ; Pagel, 1999) under a Brownian model for each trait [cochlear parameters, body mass and bulla centroid size (bullaCS)] separately on the entire sample using the 'Geiger' package in R (R Development Core Team, 2015). Pagel's λ varies between 0 (trait values independent of phylogeny) and 1.0 (trait values proportional to time of shared evolution under a Brownian model). The phylogenetic signal test is performed under two possible null hypotheses that $\lambda = 0$, or that $\lambda = 1$ (Supporting Information Table S2). The entire sample was selected because it can be difficult to carry out these calculations on small samples (Braga et al. 2015). For a detailed description of the procedure followed in this analysis, see http://www.anthrotree.info/wiki/projects/pica/The_AnthroTree_Web site.html and Braga et al. (2015).

Bulla data collection

The tympanic bulla semi-landmarks, defined as landmarks on smooth curves or surfaces positioned along the curvature that cannot be identified and that are thus estimated (Mitteroecker & Gunz, 2009), were digitised from the ventral outer edge of the cranial in 3D with AVIZO 8.0 (Visualization Sciences Group, www.vsg3d.c om). Semi-landmarks were used, as the auditory bulla lacks clear homologous landmarks. As most of the skulls were slightly damaged, the whole bulla was then considered, including the bony external auditory meatus. Therefore, we assumed that the relative contribution of bony external meatus to middle ear components in the bulla is the same in each species. The semi-landmarks were placed in a clockwise order, starting from the anterior junction between the bulla and the internal pterygoid process (Alhajeri et al. 2015; Fig. 2). A varying number of points were used with the 'B-Spline' module in AVIZO. These points were then saved in 'ASCII' format and imported into R 3.2.1 (R Development Core Team, 2015) in order to transform them into a fixed number of equally spaced points (200). Generalised Procrustes analysis (GPA) was conducted to obtain bulla centroid sizes (CS, defined as the sum of the squared distances from the centroid to each individual semi-landmark of the bulla; Rohlf & Slice, 1990) and shape coordinates were discarded. CS obtained from GPA on each specimen was averaged to estimate the average species size in R (R Development Core Team, 2015) (Claude, 2008).

Table 2 Composition of samples used in this study.

Species	n	Country (locality)	Collections	Sex (M, F)
Otomyini				
Otomys angoniensis	11	South Africa, Zimbabwe, Swaziland, Zambia	Ditsong*	(6, 5)
Otomys auratus	11	South Africa	Ditsong*	(5, 6)
Otomys unisulcatus	11	South Africa	Ditsong*	(6, 5)
Otomys helleri	09	Ethiopia	MNHN[†]	(4, 5)
Otomys sloggettii	11	South Africa, Lesotho	Ditsong*	(6, 5)
Otomys barbouri	07	Uganda	MNHN[†]	(5, 2)
Parotomys brantsii	11	South Africa	Ditsong*	(5, 6)
Gerbillinae				
Gerbilliscus leucogaster	16	Zimbabwe, South Africa, Malawi, Tanzania, Angola	Ditsong*, MNHN[†]	(8, 8)
Desmodillus auricularus	11	South Africa, Botswana, Namibia	Ditsong*	(5, 6)
Gerbillus nigeriae	12	Mauritania, Burkina Faso, Niger, Mali	MNHN[†]	(6, 6)

n, number of individuals; M, male; F, female.
*Ditsong National Museum of Natural History, Pretoria.
[†]Muséum National d'Histoire Naturelle, Paris.

Cochlea and oval window data processing

Avizo 8.0 was also used to segment and reconstruct in 3D the cochlea and oval window fossa. The left bony labyrinth was used; in cases where it was damaged, the right one was used instead. The modelisation of the cochlea and oval window followed methods used in Braga et al. (2013, 2015) and Gunz et al. (2012) (Fig. 3). To document the cochlea variation within our samples, five cochlea parameters were calculated from the semi-landmark coordinates placed on the external length of the cochlea (Fig. 3) using MATLAB R2013a v8.1 (Mathworks) software. These parameters included the external cochlea length (ECL), number of turns (TUR), relative cochlea length (RECL), the curvature gradient (CUR) and oval window area (OWA); see Braga et al. (2013, 2015) for an illustration. For species values means of these parameters, see Table 3 and for raw data see Table S1.

Environmental data extraction

Each species habitat was categorised based on its distribution and preferred habitat history (see Table 1). To calculate aridity index, we first downloaded bioclimatic variables from WORLDCLIM (Hijmans et al. 2005) using ARCGIS based on the species geographical distribution. This was done by cross-referencing geographical localities from the range distribution map (see Fig. 1) of each species with the WORLDCLIM database. We downloaded Bio 1 (Annual Mean Temperature), Bio 9 (Mean Temperature of Driest Quarter), Bio 12 (Annual Precipitation), Bio 17 (Precipitation of Driest Quarter) and elevation. Bio 1, 9, 12 and 17 were used to calculate the aridity index using the following equation:

$$AI = \frac{\frac{P}{T+10} + \frac{12p}{t+10}}{2}$$

where *P* is the mean annual rainfall in millimetres, *T* is the annual mean temperature in degrees Celsius, *p* is the average rainfall of the driest month in millimetres, and t is the average
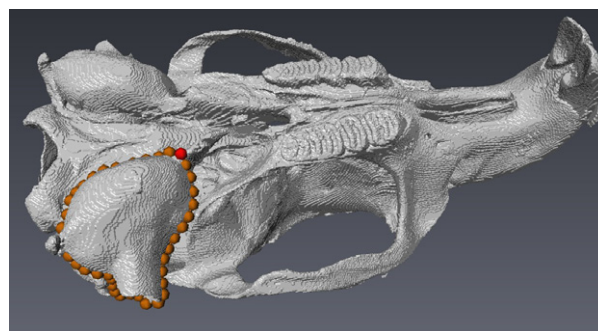

Fig. 2 Position of bulla semi-landmarks used in the geometrical morphometric analyses, to obtain centroid size of the bulla.
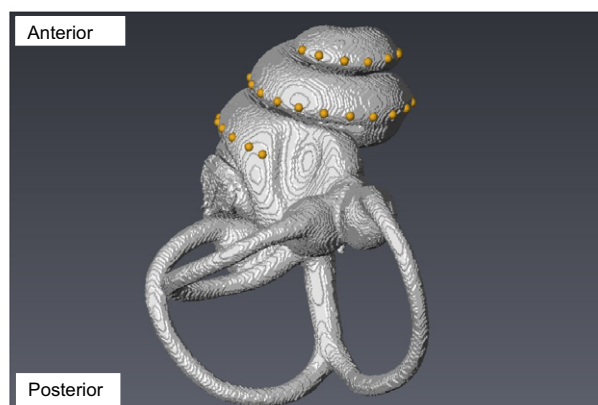

Fig. 3 Position of cochlea semi-landmarks used to calculate five cochlear parameters with the information on the orientation of the inner ear in relation to the position of the skull.

Table 3 Species mean and standard deviations for each cochlear parameter and bulla centroid size.

Species names	RECL (mm)	OWA (mm²)	CUR	ECL (mm)	TUR	bullaCS
O. angoniensis						
Mean	6.08	0.38	1.38	11.78	1.94	50.02414
SD	0.27	0.03	0.06	0.81	0.06	3.04
O. auratus						
Mean	6.48	0.39	1.42	13.23	2.05	51.18687
SD	0.38	0.03	0.09	0.64	0.10	1.63
O. unisulcatus						
Mean	6.3	0.47	1.43	13.32	2.12	56.40981
SD	0.25	0.07	0.1	1.05	0.21	6.96
O. helleri						
Mean	6.03	0.4	1.32	10.66	1.77	52.62486
SD	0.25	0.03	0.06	0.86	0.11	4.62
O. sloggettii						
Mean	6.21	0.47	1.29	11.89	1.91	52.43497
SD	0.37	0.44	0.07	1.07	0.08	2.81
O. barbouri						
Mean	6.00	0.37	1.32	10.96	1.83	48.8853
SD	0.14	0.04	0.06	0.9	0.15	3.74
P. brantsii						
Mean	6.76	0.69	1.29	15.27	2.26	75.10032
SD	0.24	0.05	0.06	0.72	0.1	2.39
G. leucogaster						
Mean	5.36	0.49	1.5	13.13	2.45	60.67969
SD	0.25	0.03	0.11	1.14	0.17	3.24
G. nigeriae						
Mean	5.12	0.37	1.28	10.64	2.08	50.40225
SD	0.16	0.02	0.05	0.79	0.13	2.16
D. auricularis						
Mean	6.02	0.83	1.53	13.75	2.29	75.11491
SD	0.18	0.05	0.14	0.76	0.13	2.53

temperature of the driest month in degrees Celsius (Alhajeri et al. 2015). The aridity index is unitless, with low aridity index value equating to a drier environment (Baltas, 2007).

Statistical analyses

Principal component analysis (PCA) was used to explore the relationship of cochlear parameters among our samples with R (R Development Core Team, 2012). We used the R 'Geiger' and 'phytools' packages for phylogenetic bivariate linear regressions (PGLS) and non-phylogenetic (GLS) regressions of log-transformed mean species values for cochlea parameters and body mass to account for phylogeny and allometry. The correlation between the bullaCS and cochlear parameters was also tested with non-phylogenetic (GLS) general least-squares regression models in R. The relationship of bullaCS and cochlear parameters with aridity index, elevation and habitat was also tested with GLS.

Results

Cochlear variation among our samples (Otomyini and Gerbillinae)

For the variability of the cochlear variables among the species, one-way multivariate analysis of variance (MANOVA)

revealed a significant difference between the species (Wilks' $\lambda = 0.0008$, $F_{45,432.5} = 37.36$, $P < 0.00001$). The interspecies pairwise differences using Tukey's and Mann–Whitney pairwise tests are reported in Supporting Information Table S4, Bonferroni-corrected at $P = 0.001$.

The PCA patterns between species with five (Supporting Information Fig. S2) and three variables (Fig. 4) were similar, and to prevent redundancies (as RECL = ECL/TUR), PCA with only three cochlear variables (OWA, RECL and CUR) was performed (Fig. 4). In this case, the first two principal components (PC1 and PC2) explained 46.31 and 37.08% of the variation, respectively. The three arid species are separated/isolated from the other species when PC1 and PC2 are considered together. Although there is overlap of the *Otomys* species on PC1; *O. unisulcatus* and *O. barbouri* show no overlap (Fig. 4). The PC loadings show that OWA, RECL and CUR are negatively correlated with PC1, with OWA having the highest correlation (Supporting Information Table S3). Though OWA shows higher loadings, other parameter loadings are still high, meaning it is the general cochlear size that is reflected in PC1. PC2 is a 'shape vector' contrasting CUR (positive high loading) with RECL (negative high loading; Table S3). Individuals grouping at higher
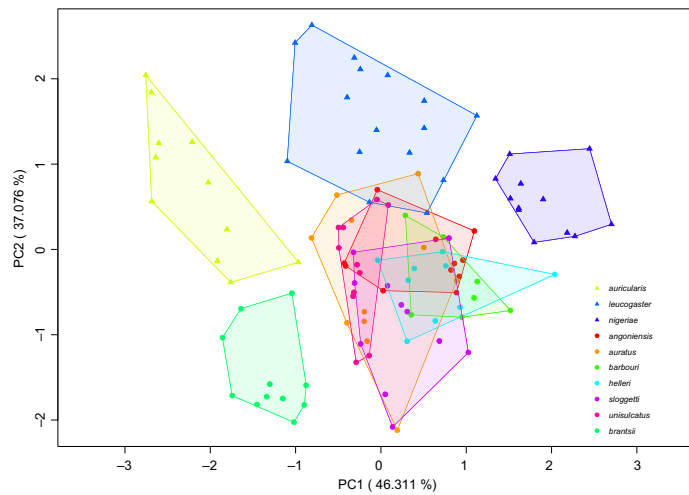
Fig. 4 Principal component analysis of only CUR, OWA and RECL measured among our studied species. Triangles = gerbils; circles = laminate-toothed rats.

positive scores of PC2 therefore have relatively tightly coiled cochlea, i.e. a steeper gradient of curvature. Although there is considerable overlap on PC2, the gerbils (*D. auricularis*, *G. leucogaster* and *G. nigeriae*) group towards the top of the PC plot compared with Otomyini, showing more tightly coiled cochlea (Fig. 4).

Phylogenetic signal and allometry

Only for body mass did we obtain a high phylogenetic signal that was significantly different from 0 at 5% level ($P = 0.0203$, Table S2); however, this is not surprising as body mass is well known for its high phylogenetic signal in mammals (Blomberg et al. 2003; Kamilar & Cooper, 2013). In our study, the two main clades (Gerbillinae and Murinae) were highly divergent in body mass, as gerbils are generally much smaller than Otomyini rodents.

Phylogenetically controlled regressions indicated the absence of any significant allometric associations between cochlea (OWA, CUR and RECL) or bullaCS and body mass ($P > 0.05$, Table 4, Fig. S3). For non-phylogenetic GLS regressions, OWA, CUR and bullaCS showed no significant associations with body mass. However, RECL scaled to body mass with a negative allometry ($r^2 = 0.53$, $P = 0.017$, Table 4), larger species having relatively shorter cochleae. However, looking at Fig. S3 linear regressions, *G. nigeriae* seems to be an outlier; therefore, we also ran an analysis without this species. After removing *G. nigeriae*, RECL and CUR were not significantly correlated with body mass ($P > 0.05$). However, OWA and bullaCS were scaled to body mass with a negative allometry ($r^2 = 0.61$, $P = 0.013$; $r^2 = 0.48$, $P = 0.038$, respectively; Table 4), larger species having relatively smaller OWA and bulla. Gerbils are distinctly smaller than laminate-toothed rats but have a proportionally larger RECL (Fig. S3A), bigger OWA and bulla,

resulting in significant results when inter-family comparisons were included.

In the case of the remaining variable CUR, phylogenetic correction considerably improves the association and slope between this variable and body mass (although still non-significant). Within each of the two subfamilies, CUR shows an association with body mass, although in both cases, these were non-significant based on separate PGLS analyses (results not shown; Fig. S3C). As both families display similar ranges of values for CUR, when data from both families are combined there is an almost horizontal regression line (Fig. S3C). This is the complete opposite of the situation with respect to RECL, where distinct inter-family differences in both body mass and RECL exist (Fig. S3A).

Associations between bullaCS, cochlear parameters and environmental variables

Although maximum likelihood (ML) tests of lambda coefficients showed lack of phylogenetic signal in cochlea and bulla variables, we noted the above differences in the body mass regressions of some bullar/cochlear variables between the Gerbillinae and Murinae (Otomyini). For this reason, thereafter we conducted separate analyses for combined data and for each subfamily. GLS showed non-significant correlations of RECL and CUR with bullaCS (Table 5, Fig. S4AC). However, the GLS of RECL vs. bullaCS in Otomyini showed a significant correlation (Otomyini, $r^2 = 0.67$, $y = 0.870 + 0.241x$, $P = 0.024$; Supporting Information Fig. S4A). For all species and for Otomyini only, there were significant correlations between OWA and bullaCS (all species, $r^2 = 0.94$, $y = -7.386 + 1.642x$, $P < 0.0001$; Otomyini, $r^2 = 0.94$, $y = -6.476 + 1.416x$, $P = 0.0003$; Fig. S4B).

BullaCS was not significantly correlated with either the aridity index ($P > 0.05$) or elevation (Table 5, Supporting

Table 4 Results for the non-phylogenetic and phylogenetic bivariate linear regressions to investigate the relationship between log-transformed mean species values for cochlear parameters, body mass (BodyM) and bulla centroid size (bullaCS). *G.n.* = *Gerbillus nigeriae*.

Non-phylogenetic

Dep/Indep	r^2	Adj r^2	P	Intercept	Slope	Equations
OWA/BodyM	0.03	−0.09	0.64	−0.363	−0.09	$y = -0.363 - 0.09x$
OWA/BodyM_minus *G.n*	0.61	0.55	0.013*	2.483	−0.715	$y = 2.483 - 0.715x$
RECL/BodyM	0.53	0.47	0.017*	1.275	0.119	$y = 1.275 + 0.119x$
CUR/BodyM	0.001	−0.12	0.93	0.297	0.004	$y = 0.297 + 0.004x$
bullaCS/BodyM	0.03	−0.09	0.6	4.279	−0.056	$y = 4.279 - 0.056x$
bullaCS/BodyM_minus *G.n*	0.48	0.41	0.038*	5.769	−0.383	$y = 5.769 - 0.383x$

Phylogenetic

Dep/Indep	P	Intercept	Slope	Equations	AIC
OWA/BodyM	0.34	−0.625	−0.001	$y = -0.625 - 0.001x$	7.423
RECL/BodyM	0.11	1.362	0.096	$y = 1.362 + 0.096x$	−23.283
CUR/BodyM	0.29	0.083	0.06	$y = 0.083 + 0.06x$	−24.013
bullaCS/BodyM	0.89	4.156	−0.022	$y = 4.423 - 0.022x$	−2.733

*Significant correlations (at the 5% level).

Table 5 Results for the non-phylogenetic bivariate linear regressions to investigate the relationship between cochlear parameters and bulla centroid size (bullaCS); bullaCS and cochlear parameters with environmental variables.

Dep/Indep	r^2	Adj r^2	P	Intercept	Slope	Equations
OWA/bullaCS	0.94	0.93	<.0001*	−7.386	1.642	$y = -7.386 + 1.642x$
RECL/bullaCS	0.07	−0.04	0.5	1.239	0.138	$y = 1.239 + 0.138x$
CUR/bullaCS	0.16	0.06	0.3	−0.321	0.158	$y = -0.321 + 0.158x$
bullaCS/Arid	0.27	0.18	0.12	4.117	−0.072	$y = 4.117 - 0.072x$
bullaCS/Elev	0.12	0.01	0.3	4.491	−0.065	$y = 4.491 - 0.065x$
OWA/Arid	0.20	0.10	0.2	−0.643	−0.105	$y = -0.643 - 0.105x$
RECL/Arid	0.08	−0.03	0.4	1.772	0.020	$y = 1.772 + 0.020x$
CUR/Arid	0.05	−0.07	0.5	0.33	−0.012	$y = 0.331 - 0.012x$
OWA/Elev	0.05	−0.07	0.5	−0.238	−0.075	$y = -0.238 - 0.075x$
RECL/Elev	0.19	0.09	0.2	1.497	0.043	$y = 1.497 + 0.043x$
CUR/Elev	0.06	−0.06	0.5	0.441	−0.018	$y = 0.441 - 0.018x$

*Significant correlations (at the 5% level).

Information Fig. S5AB), indicating that among our samples neither elevation nor aridity index influences the size of the bulla. However, after removing *G. nigeriae* the correlation between bullaCS and aridity index becomes significantly inversely correlated ($r^2 = 0.604$, $y = 4.207 - 0.120x$, $P = 0.01$), indicating that bullae were larger in more arid environments (Fig. S5A). Analysis of variance (ANOVA) indicated that desert species (in this case both arid and semi-arid, see Table 1) had a significantly greater bullaCS compared with mesic species ($F = 6.652$, $P = 0.033$; Table 6; Fig. S5C).

RECL, OWA and CUR were not significantly correlated with aridity index when all species were considered

Table 6 Results for the ANOVA investigating the relationship between cochlear parameters, bullaCS and habitat. *G.n.* = *Gerbillus nigeriae*.

Dep/Inde	F	P
OWA/habitat	3.821	0.09
OWA/habitat_minus *G.n.*	9.85	0.02*
RECL/habitat	0.77	0.41
CUR/habitat	0.86	0.38
bullaCS/habitat_with *G.n.*	6.652	0.033*

*Significant correlations (at the 5% level).

(Table 5, Supporting Information Fig. S6). Again removing *G. nigeriae* from all the analyses, we only found a significant inverse correlation of OWA with aridity index ($r^2 = 0.56$, $y = -0.476 - 0.193x$, $P = 0.02$), indicating that OWA was larger in more arid environments (Fig. S6B). A non-significant correlation of RECL, OWA and CUR with elevation (Table 6, Supporting Information Fig. S7) was also observed. The ANOVA of OWA, RECL and CUR indicated a non-significant association with habitat ($P > 0.05$, Table 6; Supporting Information Fig. S8); however, a separate ANOVA of all species minus *G. nigeriae* indicated that arid species had significantly greater OWA compared with mesic species ($P < 0.05$, Table 6).

Discussion

Bullar and cochlear covariation in desert-adapted gerbils and laminate-toothed rats

Morphological variations in the cochlea have been documented in a number of species, with different cochlear features being used to make predictions about hearing capabilities (Braga et al. 2015). West (1985) demonstrated that basilar membrane length (effectively ECL) can be used to predict the values of upper and lower limits of hearing (being inversely correlated with both), and the number of cochlear turns (TUR) can be used to predict octave range (positive correlation). Similar observations were confirmed by Vater & Kössl (2011), and Manoussaki et al. (2008) showed a strong correlation of CUR with low-frequency hearing. Rosowski & Graybeal (1991) and Moggi-Cecchi & Collard (2002) demonstrated that OWA (proxy of stapedial foot plate) is correlated with the range of audible frequencies, with larger OWA correlated with low-frequency and smaller OWA with high-frequency sound detection.

In this study, we assessed the variation of the cochlea in relation to the tympanic bulla of Otomyini and Gerbillinae based on the above-mentioned features, and assessed the effect of environment, phylogeny and body size on the cochlea and bulla. Our analyses show some differences in the morphometry of the cochlea that can shed some light on the hearing capabilities of the investigated species. Two specialist arid-adapted species (*D. auricularis* and *P. brantsii*) from diverse murid subfamilies are clearly distinguishable from mesic and semi-arid taxa of both subfamilies both in their gross cochlea dimensions on PC1 (mostly particularly oval window area) and in general bulla size (extreme hypertrophy). Our findings are in line with previous studies that documented the distinctness of the auditory organ (be it tympanic bulla, middle ear and inner ear) of desert, subterranean, fossorial and non-desert rodents (Lay, 1972; Webster & Webster, 1975; Lange et al. 2004; Shaffer & Long, 2004; Alhajeri et al. 2015; Mason, 2015, 2016; Tabatabaei-Yazdi et al. 2015). As a large oval window is strongly correlated with sensitivity to lower frequencies (see above), the

strong correlation we obtained between oval window and bullaCS ($r^2 = 0.94$) irrespective of phylogeny/subfamily provides indirect evidence that bullar hypertrophy in desert rodents is indeed linked to detection of low-frequency sounds.

Evolution of tightly coiled cochlea in gerbils (CUR)

Even though our results show no phylogenetic signal in the cochlea and bulla based on ML tests of the lambda coefficient (see Table S2), the phylogenetic effect on the cochlea of the two subfamilies cannot be entirely excluded. For example, from PCA, we found that all three species of gerbils have a more tightly coiled cochlea (higher CUR) than the laminate-toothed rats, suggesting that not only is the gross cochlea size important in gerbils, but the cochlear curvature gradient may also be important. As mentioned above, the cochlear curvature gradient (CUR) is thought to relate to low-frequency hearing (Manoussaki et al. 2008). Even though both *P. brantsii* and *D. auricularis* are adapted to the same desert environment through bullar and cochlear 'hypertrophy', gerbils may be further adapted through possessing high CUR. These results suggest that *D. auricularis* and other gerbils may use different mechanisms to cope with environmental challenges (including aridity). Gerbillinae is largely characterised by having a hypertrophied bulla (although the degree of hypertrophy varies between species) and occupies mostly arid, open and semi-arid environments (Happold, 2013; Alhajeri et al. 2015), in contrast to Otomyini, whose species are mostly adapted to mesic montane environments (Happold, 2013; Monadjem et al. 2015; Wilson et al. 2017). The possession of tightly coiled cochlea in gerbils could be a legacy of their evolutionary history, being derived from a desert-adapted ancestor, as compared with *Otomys*, whose ancestor was from the Highveld of South Africa (Denys, 2003).

However, even though the cochlea of gerbils is more coiled than that of the laminate-toothed rats, it is not clear whether our findings support Manoussaki et al.'s (2008) argument, as no cochlear coiling had been studied in closely related outgroups of gerbils to see whether the coiling has increased or decreased evolutionarily.

Evolution of relatively long RECL, enlarged OWA and bulla in gerbils and desert-adapted whistling rats

RECL, OWA and tympanic bulla are said to be related to lower and higher frequency thresholds, with mammals with longer RECL, enlarged OWA and bulla being sensitive to lower frequencies and species with smaller sizes of these attributes being sensitive to higher frequencies (Lay, 1972; West, 1985; Moggi-Cecchi & Collard, 2002; Braga et al. 2015). Our results show that distinctly smaller gerbils have proportionately longer RECL, enlarged OWA and bulla compared with larger-sized laminate-toothed

rats (negative allometry with body mass). This would suggest that gerbils have a higher sensitivity to lower frequencies than laminate-toothed rats do. In spite of this, extreme desert-adapted individuals (*D. auricularis* and *P. brantsii*) in both tribes deviate from the predicted relationship by having even longer than expected RECL (Fig. S3A) and larger OWA and bullaCS. Our results therefore suggest that gerbils have a greater ability to hear lower frequencies than laminate-toothed rats and, over and above this, extreme desert-adapted gerbils, as well as desert-adapted whistling rats (*Parotomys*), have enhanced auditory sensitivity to low frequencies compared with other members of their respective subfamilies.

Association of cochlear morphology and hearing sensitivity

Gerbils have been reported to use high-frequency ultrasonic calls in shorter distance vocalisations (Holman, 1980; Dempster & Perrin, 1991; Dempster et al. 1991; Dempster, 2018) during encounters between individuals. This follows from the observation that, to accurately localise sound, smaller mammals having short inter-aural distance need to use higher frequency sounds for communication (Heffner & Heffner, 1992; Heffner et al. 2001). We posit then that gerbils (in particular extreme desert-adapted gerbils) have good hearing sensitivity in both low-frequency sounds (e.g. vibrations and low-frequency sounds known to be generated by potential predators such as snakes and owls; Dempster, 2018), as suggested by our morphological data, and also in high-frequency sounds (i.e. ultrasonic sounds used in conspecific communication). This kind of auditory sensitivity has also been detected in the gerbil *Meriones unguiculatus* (Ryan, 1976) and in *Dipodomys merriami* from the family Heteromyidae (Heffner & Masterton, 1980). However, it is not clear whether the gerbils in our study follow the *M. unguiculatus* and *D. merriami* frequency trends (i.e. including a broad range of good sensitivity in the middle), as no audiograms are available for our species. The two Heteromyidae rodents are known to use low-frequency foot drumming (Randall, 2001); however, whether this is solely for communication with conspecifics (warning in the presence of predators) or is used to deter predators or both is still debatable.

All southern African gerbil species investigated so far use high-frequency ultrasonic vocalisations for short-range conspecific communication; however, while occasional foot drumming was observed in captivity, their functionality has not yet been discussed. Foot drumming was not observed specifically in *D. auricularis* colonies that have been observed in captivity, even though it was considered likely for this species to use foot drumming to communicate over long distances (Dempster & Perrin, 1994; Dempster, 2018). In the case of *P. brantsii*, vocal alarm calls are in relatively low audible frequencies (Le Roux et al. 2002) and no

information about foot drumming in this species has been found. Thus, as foot drumming is known for gerbils (even though not observed in *D. auricularis*) and not for *Parotomys* species, this could explain the cochlear adaptation of gerbils to lower frequencies as compared with *Parotomys*.

In conclusion, our data cannot decisively support either the communication or the predator avoidance hypothesis as no conclusive data exist pertaining to low-frequency long-distance communication of *D. auricularis* and *P. brantsii*. Even though increased CUR is generally associated with lower frequency hearing, it may be that in gerbils, increased CUR is not adapted to hearing *per se* but could be a structural constraint of the smaller head size of gerbils compared with laminate-toothed rats, allowing more efficient 'packing' of the basilar membrane (West, 1985).

Environmental correlates of cochlear and bullar characters

After correcting for phylogeny and body size, we are left with environmental factors that we predict would impact the morphology of bulla and, in turn, cochlea. The impacts of aridity, elevation and habitat type on the bullar morphology of rodents has long been recognised, with bullar inflation correlated with deserts, high elevation and open microhabitats (Lay, 1972; Webster & Webster, 1980; Kotler, 1984; Zhao et al. 2013; Tabatabaei-Yazdi et al. 2014; Alhajeri et al. 2015). Analysing the above-mentioned relations with regard to bulla and cochlea; aridity, elevation and habitat, we can see that there are some conforming data and some exceptions. BullaCS and OWA are shown to be correlated with aridity index and desert habitats. Interestingly, the variability in parameter values is shown to be much higher among arid-region species than among mesic ones in Figs S4C and S7. The plots seem to strengthen our argument for a degree of specialisation of cochlea and bulla in arid-region adapted species. To speculate slightly, it is as if selection is acting on these structures in arid-region species by affecting hearing, and hearing has been shown to be important in arid environments as compared with mesic ones whose vegetation is closed. This may be explained by diversifying/disruptive selection.

Inflated bullae, the middle ear, and cochlear modification are believed to aid in communication with conspecifics or predators through foot drumming, hitting and stomping; however, this observation doesn't seem to apply to our study animals, as no detailed indication of vibrations being produced by the two subfamilies has been documented. However, occasional and unpredictable bouts of foot drumming in captivity have been observed in gerbils (Dempster & Perrin, 1990; Dempster, 2018).

We did not find any correlation between cochlear and bullaCS and elevation, contrary to what has been reported in Zhao et al. (2013; increase of bulla size with altitude in gerbils); Tabatabaei-Yazdi et al. (2015; positive correlation

of bulla size with elevation in jirds) and Liao et al. (2007; who instead found a decrease in bulla size with increasing altitude in Daurian pikas, Ochotonidae). Our observed trend of no change refutes our prediction that if openness of the environment is a feature of high elevation, then alpine environments should show some response in terms of bullar size and cochlear modification, as high elevations are mostly composed of open habitats similar to desert environments. The increase in bullar size is also said to compensate for a reduction in oxygen partial pressure and strong winds, which are known to impair auditory acuity at high elevations (Zhao et al. 2013). Zhao et al. (2013) believed that a reduction in oxygen partial pressure (hence hypoxia) and strong winds at high elevation may cause a decrease in auditory sensitivity and sound localisation accuracy, therefore the increase in auditory bullar size with elevation is a compensatory adaptation against this selective pressure. We included three Otomyini species known to be mostly confined to very high alpine habitats (> 3000 m) in Ethiopia (*Otomys helleri*), Mt Elgon (*Otomys barbouri*) and the South African high Drakensberg (*Otomys sloggetti*) but none of these species showed any departure in measured cochlear features and bulla from their congeners found at lower elevations (*Otomys angoniensis*, *Otomys auratus*, *Otomys unisulcatus*). It is possible that the highest elevations experienced by these Afroalpine species may still not be sufficient to induce critical levels of hypoxia or hypothermia in these species. However, the elevational range of the species reported above to have inflated bulla at high elevations is lower and their habitats is mostly in arid regions when compared to that of our *Otomys* species. Alternatively, these species may exhibit behavioral and/or physiological adaptations to hypothermia. Since *Otomys* species are typically relatively large and highly sedentary rodents, their metabolic needs may be reduced such that hypoxia does not pose a significant challenge necessitating morphological evolutionary adaptation. An investigation into the extent of possible behavioural or physiological responses to hypothermia in these Afroalpine rodents would be an interesting future study.

Among the gerbils examined here, it seems like *D. auricularis* is the only species that is showing marked bullar and cochlear hypertrophy. Although occurring in sandy semi-arid Sahel habitats in West Africa, *G. nigeriae* does not show a predictable response in either bullar or cochlear dimensions. Similarly, with laminate-toothed rats, only *P. brantsii* shows a marked response, while the semi-arid Karoo bush rat *O. unisulcatus* overlaps completely with other mesic species in the tribe. There could be a threshold between arid and semi-arid habitats, whereby only truly arid habitats evoke an adaptive response. This might be related to cover, as in the case of *O. unisulcatus*, where it was suggested to have relative small bulla size due to it occupying elaborate protective stick-nests (Taylor et al. 2004), as true deserts offer no cover compared with more

semi-arid habitats where shrubs and ground cover are usually present. However, *P. brantsii* does occupy semi-arid thickets in the Karoo, where it coexists with *O. unisucatus*, suggesting that vegetation cover may not be such a critical factor. A wider sample of species of both subfamilies including more arid and semi-arid species could be instructive. Our sample sizes of specialised desert species were relatively small.

Concluding remarks

This study concludes that desert-adapted laminate-toothed rats and gerbils both use bullar and associated cochlear hypertrophy, particularly extreme desert species. Gerbils further show tightly coiled cochlea but the significance of this is debatable and may have nothing to do with adaptations to aridity. More work with a large sample size that covers all habitat types of the three tribes (Taterillini, Gerbillini and Otomyini) still needs to be done. Much attention should be focused on the possibility of foot drumming in southern African gerbils, as our results predict that gerbils should have heightened sensitivity to low-frequency sounds.

Acknowledgements

Author contributions

A.N. designed research, collected measurements from the μCT data, performed data analysis/interpretation and drafting of the manuscript. J.B. designed research and provided critical revisions of the manuscript. C.D. provided critical revision of the manuscript and approval of the article. F.d.B. and C.T. helped with the scanning of CT data. P.J.T. designed the research, assisted in data analysis/interpretation and provided critical revisions of the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Raw data of the studied specimens with individual sex, cochlear micro-CT measurements, body mass (BodyM) and bulla centroid size (bullaCS).

Table S2 Phylogenetic signal calculated for each cochlear trait and body mass. * Phylogenetic signal not significantly different from $\lambda = 1$ but significantly different from $\lambda = 0$ (at 5%).

Table S3 Variable loadings from PCA of three cochlear parameters for our data.

Table S4 Interspecies RECL and OWA; CUR; TUR and ECL differences using a pairwise *t*-tests applied to each pair of rodent species. Da*, Desmodillus auricularis;* Gl*, Gerbilliscus leucogaster;* Gn*, Gerbillus nigeriae;* Oa*, Otomys angoniensis;* Oau*, Otomys auratus;* Ob*, Otomys barbouri;* Oh*, Otomys helleri;* Os*, Otomys sloggetti;* Ou*, Otomys unisulcatus;* Pb*, Parotomys brantsii.* *Significant Bonferroni-corrected *P*-values at *P* = 0.001.

Fig. S1 Phylogram tree produced by neighbour-joining method with branch length.

Fig. S2 Principal component analysis of all five cochlear features (OWA, RECL, CUR, TUR, ECL) investigated in this study, gerbils (triangles) and laminate-toothed rats (circles).

Fig. S3 Non-phylogenetic and phylogenetic controlled linear regressions between RECL, OWA, CUR, bulla and body mass. (A) RECL for gerbils (black triangles) and Otomyini (red dots) with dashed line (non-phylogenetic) and solid line (phylogenetic) regressions. (B) OWA for gerbils (black triangles) and Otomyini (red dots) with dashed line (non-phylogenetic) and solid line (phylogenetic) regressions. (C) CUR for gerbils (black triangles) and Otomyini (red dots) with dashed line (non-phylogenetic) and solid line (phylogenetic) regressions. (D) Bulla for gerbils (black triangles) and Otomyini (red dots) with dashed line (non-phylogenetic) and solid line (phylogenetic) regressions. * Significant level of 5%.

Fig. S4 Association between bulla CS and (A) RECL; with solid line (all species included) and dashed line (Otomyini only), (B) OWA; with solid line (all species included) and dashed line (Otomyini only), (C) CUR. Gerbils (black triangle) and laminate-toothed rats (red dots). * Significant level of 5%.

Fig. S5 Association between bulla CS and environmental variables (A) aridity index; with solid line (all species included) and dashed line (*nigeriae* removed) (B) elevation, (C) habitat. Gerbils (black triangle) and laminate-toothed rats (red dots). * Significant level of 5%.

Fig. S6 Association between aridity index and cochlear parameters (A) RECL, (B) OWA; with solid line (all species included) and dashed line (*nigeriae* removed), (C) CUR. Gerbils (black triangle) and laminate-toothed rats (red dots). * Significant level of 5%.

Fig. S7 Association between elevation and cochlear parameters for all species (A) RECL, (B) OWA, (C) CUR. Gerbils (black triangle) and laminate-toothed rats (red dots).

Fig. S8 Association between habitat and cochlea parameters for all species (A) RECL, (B) OWA, (C) CUR.