The ecological signal on the shape of the lacertid vestibular system: simple versus complex microhabitats

M. VASILOPOULOU-KAMPITSI[1,*,©], J. GOYENS[1], R. VAN DAMME[1] and P. AERTS[1,2]

[1]*Laboratory of Functional Morphology, Department of Biology, University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium*
[2]*Department of Movement and Sports Sciences, Ghent University, Watersportlaan 2, 9000 Ghent, Belgium*

Shape variation in the vestibular system is often linked to microhabitat structure and locomotor performance. Highly circular and orthogonal semicircular canal pairs are linked to higher motion sensitivity. Here, we use 3D geometric morphometrics to investigate shape variation in the vestibular system within lacertid lizards and its relationship to balance control. We found that lacertids living in complex microhabitats possess narrow but longer vestibular systems, an S-shaped anterior canal, a straightened lateral canal and a short common crus. However, lacertids specialized for simple microhabitats (open areas) possess wider but shorter vestibular systems, more circular anterior and lateral canals, and a longer common crus. Contrary to our expectations, species living in simple microhabitats possess more anatomical adaptations that enhance the sensitivity of their vestibular system. This suggests that species inhabiting open areas may benefit from increased sensitivity given that they are potentially more visibile to predators and have lower shelter availability. Finally, the wider shape of the vestibular system of the open area species may be linked to a wider and potentially flattened skull, which may be related to sand-diving or prey hardness.

ADDITIONAL KEYWORDS: balance – biomechanics – bony labyrinth – geometric morphometrics – lizards – locomotion – semicircular canals.

INTRODUCTION

The vertebrate inner ear is a complex structure involved in sound detection (cochlear system) and balance control (vestibular system) (Angelaki & Cullen, 2008; Cullen, 2012; Carriot *et al.*, 2013). It is situated in the petrosal bone of the skull. The lower part of the inner ear consists of the cochlea, which is connected to the middle ear at the oval window, and therefore plays a significant role in sound stimulation. The vestibular system is situated in the upper part of the inner ear and forms three interconnected membranous semicircular ducts and a vestibule composed of two otolith organs (sacculus and utricle) (Fig. 1). The membranous ducts are embedded in an osseous canal structure reflecting their shape, called the bony labyrinth (Spoor *et al.*, 2007). The inner ear is capable of sensing linear accelerations (due to the vestibule)

and angular accelerations of the head (semicircular canals) in three dimensions during locomotion, and it provides spatial orientation (Angelaki & Cullen, 2008; David *et al.*, 2016; Goyens & Aerts, 2018). The detection of angular accelerations depends on the fluid dynamics of the endolymph, a fluid flowing within the membranous ducts during head motion. Following the laws of fluid mechanics (Angelaki & Cullen, 2008; Squire *et al.*, 2013), it is likely that the function of the balance apparatus is affected by the morphology (size and shape) of these interconnected semicircular ducts.

The dimensions of the vestibular system and of the semicircular canals have been studied extensively in a wide range of taxa, and have been linked to locomotor performance, agility (i.e. a more agile animal has a locomotor repertoire with higher accelerations and decelerations and is able to cope with unexpected deviations of its body centre of mass path) and habitat use (Spoor *et al.*, 1994; Spoor, 2003; Boistel *et al.*, 2011; Georgi *et al.*, 2013; Pfaff *et al.*, 2015; Perier *et al.*, 2016; Benson *et al.*, 2017; Dickson *et al.*, 2017;

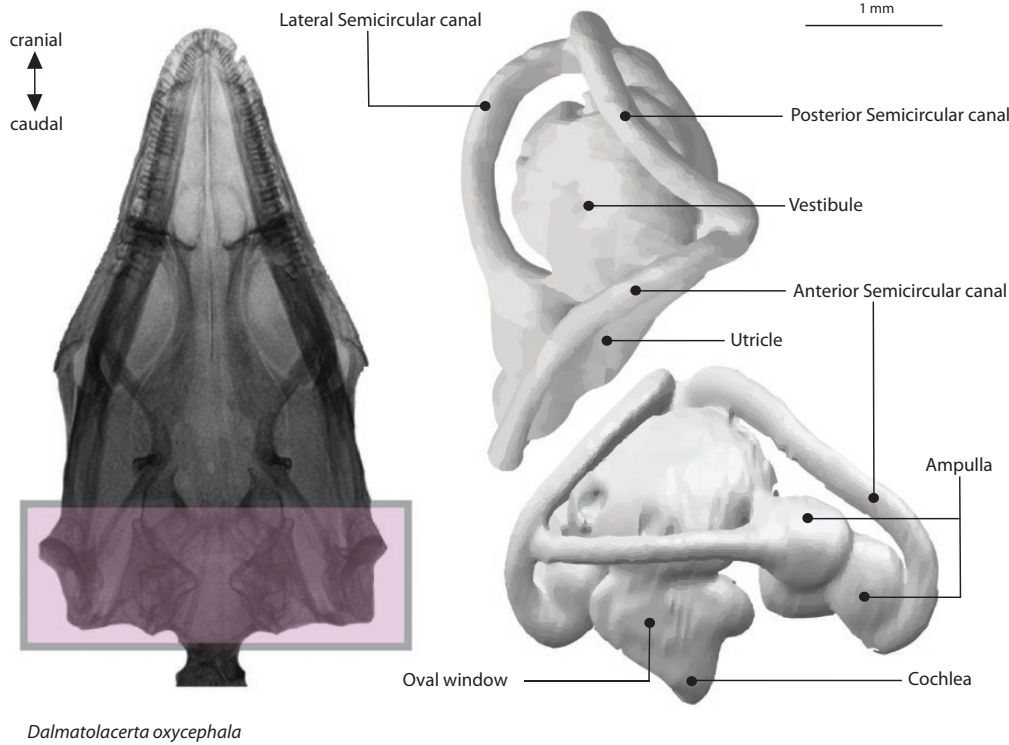*Corresponding author. E-mail: Menelia.Vasilopoulou-Kampitsi@uantwerpen.be

Dalmatolacerta oxycephala

Figure 1. Illustration of a lacertid skull (*Dalmatolacerta oxycephala*). The left and right inner ear are highlighted in the lower part of the skull. Two 3D models of the right vestibular system are used to illustrate the different anatomical parts.

Le Maitre *et al*., 2017; Costeur *et al.*, 2018; Vasilopoulou-Kampitsi *et al*., 2019), as well as to sensitivity (Muller, 1999; Hullar, 2006; Lambert *et al.*, 2008). In a more sensitive vestibular system, the threshold of motion detection is reached for smaller head accelerations. Longer semicircular canals are generally associated with higher sensitivity during motion, offering the advantage of high manoeuvrability during challenged locomotion (Muller, 1999; Rabbitt *et al.*, 2004). Moreover, the negative allometry observed in many taxa between semicircular canal size and body size suggests that there is a minimum absolute size below which the semicircular canals cease to function properly (Jones & Spells, 1963; Muller, 1999; Spoor *et al.*, 2002; Vasilopoulou-Kampitsi *et al*., 2019).

Considering the above, and bearing in mind the anatomical complexity of the vestibular system, clearly not only the size of the semicircular canals, but also their shape can influence the system's sensitivity and response time to head motion. The shape of the vestibular system has been studied in several taxa, both extant and extinct, and was often linked to microhabitat structure, locomotor style (terrestrial vs. arboreal, swimming vs. walking, bipedal vs. quadrupedal) and phylogeny (Spoor *et al.*, 2002; Maddin & Sherratt, 2014; Billet *et al.*, 2015; Pfaff *et al.*, 2015; Grohé *et al.*, 2016; Mennecart *et al.*, 2017; Dickson

et al., 2017; Le Maitre *et al.*, 2017; Loza *et al.*, 2017; Palci *et al.*, 2017; Boyle & Herel, 2018; Grohé *et al.*, 2018). It is generally suggested by previous studies that species which are more agile and more sensitive to motion possess longer and relatively circular canals. For instance, lizards possess less shallow and more rounded semicircular canals than snakes (Boistel *et al.*, 2011; Palci *et al.*, 2017) as an adaptation to the higher rotational accelerations of their heads (Boistel *et al.*, 2011). Furthermore, although the three semicircular canals are usually assumed to be more or less orthogonal, large deviations from orthogonality were linked to slow head rotations (Billet *et al.*, 2012; Malinzak *et al.*, 2012; Berlin *et al.*, 2013; Racicot *et al.*, 2018). As such, Malinzak *et al.* (2012) documented that Strepsirrhini species with more orthogonal canals show higher angular head accelerations during motion than species with less orthogonal canals, and an interspecific comparison by Berlin *et al.* (2013) indicated a negative relationship between vestibular sensitivity and deviation from orthogonality in a wide range of mammals.

However, the effect of an animal's ecology on the shape of the vestibular system is not always so clear-cut. In toothed whales (Odontoceti), the shape of the semicircular canals is similar in marine, coastal and freshwater species (Costeur *et al.*, 2018), and in

birds, differences in flying style are also not reflected in vestibular morphology (Benson *et al.*, 2017). The reason for these discordant findings among studies on the effect of ecology on vestibular morphology is still unclear. One possible confounding factor could be the lack of distinct differences between the locomotor types or ecologies of the groups under study. For instance, Palci *et al.* (2017) showed that determining the ecology of the fossil stem snake *Dinilysia patagonica* based on vestibular morphology of extant snake species specialized for different ecologies is not evident due to the shape resemblance of the ecological groups (similar vestibular systems are found between semi-fossorial and semi-aquatic species) (Palci *et al.*, 2017). Additionally, the degree of phylogenetic relatedness of the study group might affect the interpretation of an ecological signal detected on the vestibular shape. As a result, ecomorphological relationships are often readily visible when examined over a taxonomically broad range of species, but become more difficult to discern at lower taxonomic levels. Therefore, studies of closely related species that differ in ecology (and in particular microhabitat use) may provide the most convincing cases for an ecological signature in vestibular morphology. This is precisely the aim of the present study.

Aiming to investigate whether and how vestibular functioning is related to ecology, we use 3D geometric morphometrics to examine the shape variation of the vestibular systems of 23 lacertid lizard species. Lacertids (334 species) are small, manoeuvrable lizards distributed over most parts of Europe, Asia and Africa. Despite considerable interspecific variation in body size (Arnold, 1989; Meiri, 2008), they share a very similar body build (Arnold, 1989). Interestingly, they have successfully adapted to a variety of habitats and microhabitats [e.g. from open deserts to densely vegetated grasslands, from sandy substrates to rocks and arboreal surfaces (Arnold, 1998; Vanhooydonck & Van Damme, 1999)] and are capable of reaching high speeds for their body size. Based on the concept that microhabitat use determines locomotor behaviour, we predict that lacertid lizards inhabiting dissimilar environments will vary in the shape of the bony labyrinth. One the one hand, we expect lizards living in areas with a complex microhabitat structure (e.g. dense/high vegetation, trees, rocky and uneven substrate) to possess a relatively large vestibular system (e.g. longer canals, larger vestibular system width, length and depth) and more circular and/or orthogonal semicircular canals as a specialization for manoeuvring (facing obstacles, climbing, running on an uneven surface). On the other hand, we anticipate that lizards which are specialized runners on even surfaces and open areas (e.g. deserts) will have smaller vestibular systems with less circular semicircular canals and/or larger deviations from orthogonality. Maintaining balance while running on open and even substrates is thus assumed here to be less challenging (lack of obstacles, straight on flat surface, relying on speed to find shelter) than in a more 'complex' terrain.

MATERIAL AND METHODS

ANIMALS AND MICROHABITAT GROUPS

In this study, we used 23 different lacertid species (32 individuals) from the private collections of the Functional Morphology laboratory at the University of Antwerp (Antwerp, Belgium), and donated by the private collections of Dr A. Herrel (Muséum National d'Histoire Naturelle, Paris), Dr J. Martín (Museo Nacional de Ciencias Naturales in Madrid, Spain) and Dr S. Meiri (University of Stellenbosch and Zoological Museum of Tel Aviv University, Tel Aviv). We used one individual per species except for *Takydromus sexlineatus*, for which we had 10 individuals. The latter were used for the assessment of intraspecific shape variation (see Statistical analyses) and only one of them for interspecific analysis. These samples are the same as those used in an upcoming publication by us.

We assigned the species to two microhabitat groups (m.g.) (Table 1; microhabitat grouping 1) characterized by different microhabitat structure complexity, based on reports in the literature (Martín & Salvador, 1992; Vanhooydonck & Van Damme, 1999b; Van Damme & Vanhooydonck, 2002; Arnold & Ovenden, 2004; Tadevosyan, 2007; Bar & Haimovitch, 2011; Bates *et al.*, 2014; Baeckens *et al.*, 2015). The first group (labelled as 'simple' m.g., Table 1) consists of lizards living in areas with a simple microhabitat structure (e.g. open areas, even surfaces, thin and/or scattered vegetation) and are considered to be specialized in fast sprinting (high speeds and accelerations) on flat surfaces. Their microhabitat structure provides enough space for running, and obstacles, if any, can be anticipated in advance from a distance. The second group (labelled as 'complex' m.g., Table 1) consists of species living in more composite environments with a complex microhabitat structure, including dense vegetation, rocks, trees or uneven substrate, where obstacles are unexpected. Considering the microhabitat complexity, we expect the demands in manoeuvrability to be higher for lacertids of the 'complex' m.g. (i.e. structural aspects of the habitat at the lizard scale). To investigate how the different microhabitat structures of a complex microhabitat affect the shape of the vestibular system (manoeuvring between dense vegetation or climbing), we regrouped the species attributed to the 'complex' m.g. into two subgroups: species inhabiting (1) 'densely vegetated areas' and (2) 'areas including vertical elements'. Considering speed

Table 1. Morphological and ecological information for all 23 species examined. One specimen per species was used for the interspecific shape analysis. Microhabitat grouping 1: 'complex' vs. 'simple' microhabitats. Microhabitat grouping 2: 'simple' microhabitat, 'densely vegetated areas' and 'areas including vertical elements'. Sprint speed data acquired from the literature: a: Vanhooydonck *et al.* (2001); b: Bauwens *et al.* (1995); c: Verwaijen (2007); d: Brecko *et al.* (2008); e: Huey *et al.* (1984); f: unpublished data (personal observations by A. Herrel and B. Vanhooydonck); g: Vanhooydonck *et al.* (2007). Microhabitat type data acquired from the literature: 1: Vanhooydonck & Van Damme (1999b); 2: Tadevosyan (2007); 3: Van Damme & Vanhooydonck (2002); 4: Baeckens *et al.* (2015); 5: Bates *et al.* (2014); 6: Mayer & Beyerlein (1999); 7: van der Meer *et al.* (2010)

Species	Centroid size (mm)	Head width (mm)	Sprint speed (m/s)	Microhabitat grouping 1	Microhabitat grouping 2
Acanthodactylus boskianus	5.10	7.97	2.98 [c]	Simple	Simple [1]
Ichnotropis capensis	7.42	6.40	2.48 [f]	Simple	Simple [1]
Latastia longicaudata	5.16	6.05	3.34 [g]	Simple	Simple [1]
Messalina guttulata	7.80	8.12	—	Simple	Simple [1]
Meroles knoxii	5.76	11	2.36 [f]	Simple	Simple [3]
Nucras tesselata	6.03	3.62	2.05 [e]	Simple	Simple [7]
Ophisops elegans	7.11	5.99	—	Simple	Simple [4]
Australolacerta australis	6.59	5.5	2.14 [f]	Complex	Vertical elements [5]
Dalmatolacerta oxycephala	6.45	9.78	2.02 [a]	Complex	Vertical elements [1]
Eremias acutirostris	7.73	6.81	—	Complex	Vertical elements [2]
Holaspis guentheri	9.92	3.81	—	Complex	Vertical elements [3]
Iberolacerta monticola	4.88	7.85	2.76 [c]	Complex	Vertical elements [3]
Phoenicolacerta laevis	6.35	7.14	—	Complex	Vertical elements [3,4]
Podarcis melisellensis	6.83	3.98	1.81 [d]	Complex	Vertical elements [3]
Podarcis hispanicus	5.67	6.51	1.85 [b]	Complex	Vertical elements [3]
Podarcis peloponnesiacus	6.16	5.55	2.67 [c]	Complex	Vertical elements [6]
Gallotia galloti	7.69	7.08	1.93 [g]	Complex	Densely vegetated areas [1]
Lacerta agilis	5.34	7.98	1.68 [b]	Complex	Densely vegetated areas [3]
Pedioplanis lineoocellata	6.77	7.57	2.63 [e]	Complex	Densely vegetated areas [3]
Psammodromus algirus	5.89	6.77	2.53 [g]	Complex	Densely vegetated areas [3,4]
Takydromus sexlineatus	5.31	5.93	1.33 [a]	Complex	Densely vegetated areas [3]
Tropidosaura gularis	6.56	5.93	1.89 [f]	Complex	Densely vegetated areas [5]
Zootoca vivipara	7.13	5.22	0.87 [a]	Complex	Densely vegetated areas [1,4]

as a determinant variable for running performance, an estimate of maximal sprint speed for each species was acquired from the literature (Vanhooydonck *et al.*, 2001, 2007; Verwaijen, 2007; Brecko *et al.*, 2008), from our unpublished data and from personal observations of A. Herrel & B. Vanhooydonck (Table 1) to investigate whether running speed has an effect on the shape of the vestibular system.

3D SURFACE MODELS

The specimens were stained in a solution of 5% phosphomolybdic acid (Sigma-Aldrich, St Louis, MO, USA) and 70% EtOH for a minimum of 14 days as part of a different study. They were then placed in a micro-computed tomography (μCT) scanner to obtain high-resolution image stacks of the inner ears of all 32 specimens. The *Takydromus sexlineatus* specimens were scanned at the SYREMP beamline of the Elettra synchrotron facility in Bazovizza (Trieste, Italy). The *Gallotia galloti* specimen was scanned with a conventional μCT scanner at the same facility (Tomolab scanner, by Dr L. Marcini). All other specimens were scanned with a SkyScan 1172 high-resolution μCT scanner [Bruker micro CT, Kontich, Belgium, managed by the BiostrμCT Hercules consortium (https://sites.google.com/view/biostruct)] at the Vrije Universiteit Brussel (VUB, Belgium). The *T. sexlineatus* and *Holaspis guentheri* specimens were preserved in formalhyde and the other specimens in 70% EtOH.

We used an average voltage of 80 kV and a current of 124 μA with an Al–Cu filter of 1 mm. Pixel size varied from 4.17 μm to 13.45 μm, due to the size difference between species (rotation angle = 0.40° and exposure time = 1300 ms, frame averaging = 4). All parameters were adjusted to optimize the image resolution and the contrast of the vestibular systems. We reconstructed the projection images using NRecon (Bruker micro CT), focusing on the posterior end of the skull where the vestibular system is located. Next, we imported the reconstructed slice images in the specialized 3D image processing software Amira (Amira 5.4.3 VSG systems, Mérignac, France) and selected those voxels belonging to the bony labyrinth using automatic greyscale thresholding and manual corrections. We then acquired 3D surface models of the right inner ears of all specimens.

3D GEOMETRIC MORPHOMETRIC ANALYSES

The shape of the bony labyrinth of the lizards was described using 3D geometric morphometric analyses. We used the software ISE-Mesh Tools 1.3.3 (http://morphomuseum.com/meshtools; Lebrun, 2014) to digitize homologous landmarks and semi-landmarks

on the 3D surface models of the vestibular systems (following Billet *et al.*, 2015; Grohé *et al.*, 2016). Five homologous landmarks and 80 equidistant semi-landmarks were placed on each 3D surface model. The homologous landmarks where positioned as follows: one in the middle of the anterior ampulla, one in the middle of the posterior ampulla, one in the middle of the lateral ampulla, one at the connection point of the anterior with the posterior semicircular canal (which is also the middle of the starting point of the common crus) and one at the connection point between the lateral semicircular canal and the common crus (Fig. 2; Table 2). The semi-landmarks were placed manually in middle points of each canal (anterior, ASC; posterior, PSC; lateral, LSC) and of the common crus following the curve created by the utricle and ending in the middle of the anterior ampulla. Using the positions of these landmarks in 3D space, and with the same software, we created curves that connect the landmarks and semi-landmarks semi-automatically with respect to the curvatures and the shape of the semicircular canals. Figure 2 and Table 2 illustrate the four curves and indicate the position of the semi-landmarks and landmarks.

We used the interactive software package Morphotools (Lebrun *et al.*, 2010) to slide the semi-landmarks by minimizing the Procrustes distance between each specimen and the mean shape of the entire data set (Bookstein, 1991; Gunz *et al.*, 2012; Perier *et al.*, 2016). Next, all semi-landmarks were superimposed using a Procrustes superimposition (Gower, 1975). Analysis and visualization of the shape variation were performed with Morphotools (Lebrun *et al.*, 2010). Shape variability was analysed by principal component analysis (PCA) using the same software.

PAIR ANGLES AND ORTHOGONALITY OF THE SEMICIRCULAR CANALS

We fitted the 3D landmarks and semi-landmarks of the curves in three different planes using Matlab (Matlab R2016a, Natick, MA, USA, Mathworks documentation) and then calculated the paired angles between the three planes formed by the semicircular canals. More specifically we calculated: (1) the angle between the anterior and posterior semicircular canal (ASC/PSC), (2) the angle between the anterior and lateral semicircular canal (ASC/LSC) and (3) the angle between the posterior and lateral semicircular canal (PSC/LSC). The mean and the variance of the angles for the 23 species were calculated (Table 3). We then tested the deviation from orthogonality of each semicircular canal (SC) pair by calculating the 90var (deviation from orthogonality) (according to Ekdale & Racicot, 2015) for each m.g. separately (Table 4). Lower
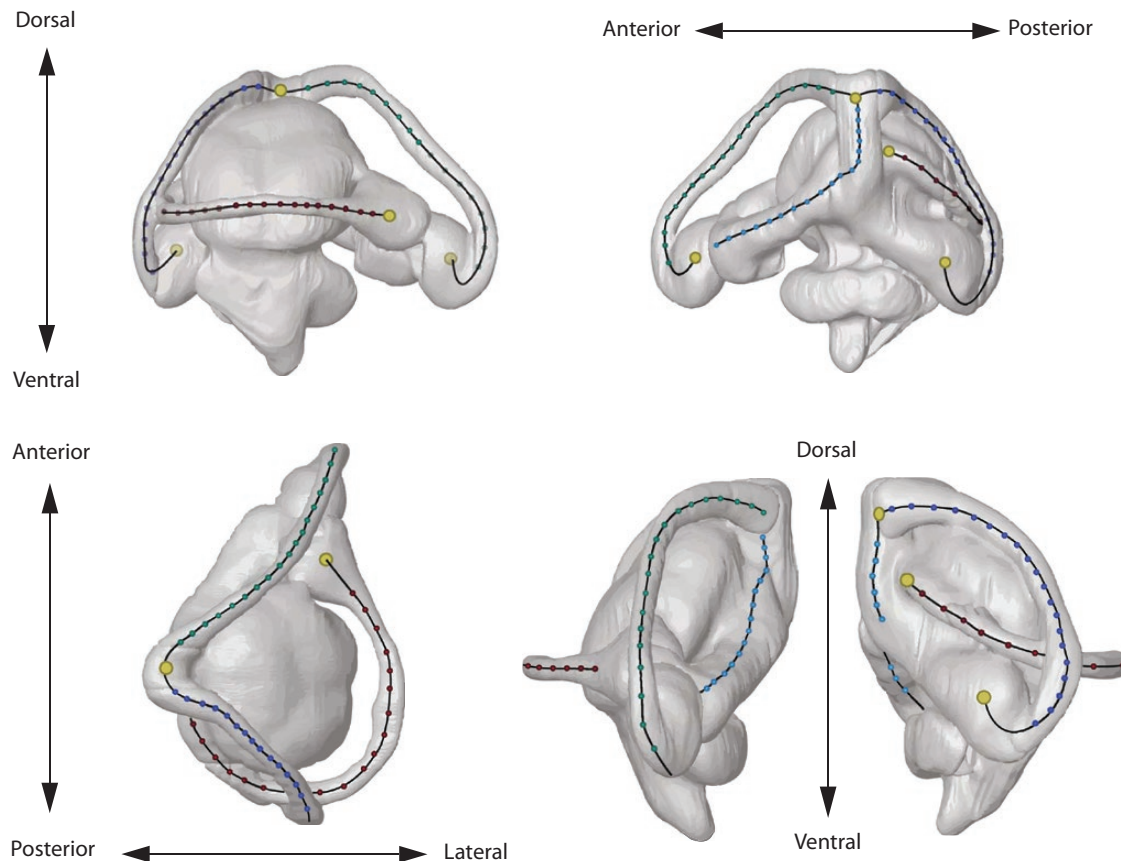
Figure 2. Representation of the curvatures describing the shape of the vestibular system. The homologous landmarks are indicated with larger yellow points. There are 20 semi-landmarks used for the description of each curvature. Five views of the same inner ear with the four curvatures superimposed are illustrated (ASC: green, PSC: dark blue, LSC: red, common duct: light blue).

values correlate with higher sensitivity to rotations (Malinzak *et al.*, 2012).

STATISTICAL ANALYSES

As we possessed multiple specimens for one of our species (*T. sexlineatus*, $N = 10$), we were able to assess the importance of intraspecific (relative to overall) variation in vestibular system shape. To do so, we ran a PCA on the shape data of all specimens available, including the 10 *T. sexlineatus* specimens, using Morphotools. We then used the 'varcomp' function in the R-package 'ape' (Paradis *et al.*, 2004) to obtain estimates of the relative contributions of between- and within-species variation to the total shape variation. We found that between-species variation accounted for 87% of the shape variation in the PC1 scores (i.e. including the 10 *T. sexlineatus*) and for 75% of the variation in PC2 scores, whereas 13% and 25% of the total variation, respectively, was explained by the among-individuals variation. This shows that although vestibular shape may differ to some extent among individuals within a species, more important differences are found at a higher taxonomical level. In subsequent analyses, we used the data of only one (randomly picked) specimen of *T. sexlineatus*.

Next, we performed a PCA based on the Procrustes coordinates of the 23 species using one specimen per species. The PC scores were imported in R Studio (version 1.0.136) (R Studio, 2012; R Core Team, 2015) for further analyses. First we performed a multivariate ANOVA (MANOVA) on the PC scores to test whether species from different microhabitat groups, i.e. 'simple' and 'complex' m.g, possess significantly different vestibular shapes. We then performed the same test, this time after dividing the 'complex' m.g. into two subgroups (microhabitat grouping 2; Table 1): 'densely vegetated areas' m.g. and 'areas including vertical elements' m.g.

To investigate the association between the interspecific variation of the vestibular system shape and the different angles between the planes of the semicircular canals, we run linear regressions between the PC scores of the interspecific PCA and the angles

Table 2. Definition of the 3D landmarks and semi-landmarks used for the Geometric Morphometric analyses

Number	Category	Description
1	Landmark	Centre of the anterior ampulla
2	Landmark	Bifurcation point of the anterior and posterior canals
3	Landmark	Centre of the posterior ampulla
4	Landmark	Centre of the lateral ampulla
5	Landmark	Bifurcation point of the lateral canal and the common crus
6 to 25	Semi-landmark	Curve placed at the midline of the anterior semicircular canal (ASC)
26 to 45	Semi-landmark	Curve placed at the midline of the posterior semicircular canal (PSC)
46 to 65	Semi-landmark	Curve placed at the midline of the lateral semicircular canal (LSC)
66 to 85	Semi-landmark	Curve placed at the midline of the common crus and the utricle, until the anterior ampulla

measured (angle values shown in Table 3). We performed linear regressions between the same PC scores and sprint speed to assess whether the vestibular shape variation is related to the sprinting capacity of the species. Further, we performed an analysis of covariance (ANCOVA) between sprint speed, head width (taking size differences between species into account; see Table 1 for head width measurements) and microhabitat groups (covariate) to investigate if lacertids belonging to different microhabitats differ in their sprinting capacity. Finally, to explore the potential allometric relationship between vestibular shape and inner ear size, we performed a regression of the Procrustes shape coordinates against the log-centroid size using Morphotools (Lebrun *et al.*, 2010). As not all the variables followed a normal distribution (Shapiro–Wilk test; $P < 0.05$) we $\log_{10}$-transformed the angular and sprint speed measurements before the statistical analyses to fulfil assumptions of normality and homoscedascity.

PHYLOGENETIC ANALYSIS

As the species under study share a phylogenetic history, we considered it necessary to implement phylogenetic information in our analyses. To do so, we pruned the Bayesian tree constructed by Baeckens *et al.* (2015) based on interspecific variation at two nuclear and three mitochondrial gene regions. We calculated the multivariate K-statistic for the Procrustes coordinates of each species to test for a phylogenetic signal using the R-package 'geomorph' (Adams, 2014). This method tests the phylogenetic signal of the variables under a Brownian motion model, but would not detect evolution that follows other models. K-values close to 1 or higher indicate a stronger phylogenetic signal whereas K-values close to zero indicate that the traits under study are not conserved within the phylogeny (weak phylogenetic signal) (Blomberg *et al.*, 2003).

To investigate whether there is an effect of microhabitat on vestibular shape variation, whilst taking into account the phylogenetic relationship between species, we performed a phylogenetic MANOVA on the PC scores of the interspecific PCA performed before, using microhabitat as factor. For all phylogenetic analyses the R packages 'nlme' (Pinheiro *et al.*, 2018) 'geiger' (Revell *et al.*, 2007) (function 'aov.phylo') and 'phytools' (Revell, 2012) (function 'pgls') were used.

RESULTS

3D SHAPE

The first four principal components of the interspecific PCA together explained almost 65% of the shape variation that was observed in the 3D surfaces of the bony labyrinth (PC1: 28.2%, PC2: 15.16%, PC3: 11.63%, PC4: 7.6%) (Supporting Information, Fig. S1). Negative PC1 values are associated with: a laterally narrower and anterio-posteriorly longer vestibular system, an S-shaped ASC, a straightened and laterally compressed LSC, a higher (dorsally extended) PSC, a shorter common crus and a longer utricle. Positive PC1 values, by contrast, are associated with a wider, and anterio-posteriorly shorter vestibular system, more circular and wider ASC and LSC, a shorter (ventrally extended) PSC, a longer common crus and a shorter utricle. Finally, the ampullas of the ASC and PSC appear to rotate ventrally and towards the cochlea, transforming the S-shaped ASC to a more circular canal (Table 6; Fig. 3). Despite the changes in overall shape, the length of the three bony canals does not seem to change accordingly. Negative PC2 values are associated with a straightened and anteriorly wider ASC, a laterally narrower LSC, a higher (dorsally extended) and more circular PSC, a shorter common crus and a longer utricle. By contrast, positive PC2 scores are linked to a more circular and higher (dorsally) ASC, a more circular and laterally wider LSC, a lower and anterio-posteriorly compressed PSC, a longer common crus and a shorter utricle (Table 6; Figs 3, 4). The shape differences associated with the third and fourth PC axis are indicated in Table 6 and Figures 4 and 5.

Table 3. Angles (degrees) between the semicircular canals and their variance. ASC/PSC: angle between anterior and posterior SC; ASC/LSC: angle between anterior and lateral SC; PSC/LSC: angle between posterior and lateral SC

Species	ASC/PSC	ASC/LSC	PSC/LSC
Acanthodactylus boskianus	84.39	100.07	92.46
Ichnotropis capensis	81.12	102.39	81.30
Latastia longicaudata	85.68	95.88	83.80
Messalina guttulata	86.94	100.49	86.36
Meroles knoxii	83.00	98.58	92.17
Nucras tesselata	80.41	92.07	77.39
Ophisops elegans	86.75	101.97	80.10
Australolacerta australis	85.36	98.20	83.85
Dalmatolacerta oxycephala	87.66	94.55	78.99
Eremias acutirostris	80.79	93.67	89.60
Holaspis guentheri	86.63	100.03	91.46
Iberolacerta monticola	86.10	92.35	83.62
Phoenicolacerta laevis	88.02	96.59	85.19
Podarcis melisellensis	86.89	98.19	88.64
Podarcis hispanicus	85.31	94.58	87.11
Podarcis peloponnesiacus	83.14	97.64	84.24
Gallotia galloti	78.71	105.09	82.29
Lacerta agilis	85.35	100.11	87.96
Pedioplanis lineoocellata	86.45	97.50	80.65
Psammodromus algirus	86.12	98.82	95.18
Takydromus sexlineatus	85.54	98.96	87.91
Tropidosaura gularis	88.16	92.41	90.46
Zootoca vivipara	76.50	95.00	68.64
Variance	**10.06**	**11.79**	**35.01**
Mean angle	**84.55 ± 3.10**	**97.62 ± 3.36**	**85.19 ± 5.80**

3D SHAPE, MICROHABITAT COMPLEXITY AND PERFORMANCE

The results of the MANOVAs showed significant differences in vestibular shape between species attributed to the 'simple' m.g. and those belonging to the 'complex' m.g. for the shape variation explained by the first three PC axes of the interspecific PCA ($F_{1,21} = 3.5$, $P = 0.035$) (Table 5) but not when we also considered the other PC axes ($F_{1,21} = 2.52$, $P = 0.065$) (Table 5). The shape differences remained significant when we performed an ANOVA using PC1 as a dependent variable ($F_{1,21} = 8.78$, $P = 0.007$) but not for PC2 or PC3 ($F_{1,21} = 0.28$, $P = 0.597$ and $F_{1,21} = 1.05$, $P = 0.315$, respectively). Therefore, we consider that the ecological signal observed in the MANOVA test is driven by the first principal component axis. When we assigned the species to three microhabitat groups, 'simple', 'densely vegetated areas' and 'areas with vertical elements', a significant ecological effect on vestibular shape was found for the shape variation explained only by PC1 [$F_{2,20} = 4.80$, $P = 0.016$; corrected P-value using Tukey's honest significant difference test, R package 'agricolae' (Mendiburu, 2019)] (Table 5), indicating that species living in simple microhabitats ('simple' m.g.) differ in vestibular shape from those living in areas

where vegetation is very dense ($P = 0.04$) ('densely vegetated' m.g.).

We found no significant correlation between the angle pairs of the three semicircular canals and the shape of the vestibular system (all $P > 0.76$), with the exception of the angle between ASC and LSC, which was significantly correlated with PC1 ($P = 0.04$). Calculation of the angular variances indicated the highest variability for the PSC/LSC angle (var = 35.01°), then for ASC/LSC (var = 11.79°) and finally for ASC/PSC (var = 10.06°) (Table 3). We found lower 90var values for species living in open ('simple' m.g.) and densely vegetated areas (90var = 4.56° and 5.81°, respectively) than for species inhabiting areas with vertical elements (90var = 7.3°), indicating a higher deviation from orthogonality for the latter (Table 4). However, there was only a trend towards a significant difference for the 90var values between open ('simple' m.g.) and vertical areas ($P = 0.05$). Note that for species in 'densely vegetated areas' and areas with 'vertical elements', the 90var variation was very high, ranging from 1.57° to 13.29° and from 5.47° to 11.36° respectively, as compared with those of species in the 'simple' microhabitat group (from 4.21° to 6.33°) (Table 4). Sprint capacities (maximal sprint speed) of the lacertids under study did not show any significant relationship

Table 4. Angles (degrees) between the semicircular canals, their variance and deviation from orthogonality (90var) for each microhabitat group

Species	ASC/PSC	ASC/LSC	PSC/LSC	90var	Microhabitat group
Acanthodactylus boskianus	84.39	100.07	92.46	6.05	Simple
Ichnotropis capensis	85.36	98.20	83.85	6.33	
Latastia longicaudata	80.79	93.67	89.60	4.42	
Messalina guttulata	86.63	100.03	91.46	4.95	
Meroles knoxii	86.10	92.35	83.62	4.21	
Nucras tesselata	88.02	96.59	85.19	4.46	
Ophisops elegans	85.31	98.19	88.64	4.74	
				4.56	
Pedioplanis lineoocellata	85.31	94.58	87.11	4.05	Densely vegetated areas
Psammodromus algirus	86.12	98.82	95.18	5.96	
Takydromus sexlineatus	85.54	98.96	87.91	5.17	
Tropidosaura gularis	88.16	92.41	90.46	1.57	
Zootoca vivipara	76.50	95.00	68.64	13.29	
Gallotia galloti	83.00	98.58	92.17	5.92	
Lacerta agilis	87.66	94.55	78.99	5.97	
				5.81	
Holaspis guentheri	80.41	92.07	77.39	8.09	Areas with vertical elements
Phoenicolacerta laevis	83.14	97.64	84.24	6.75	
Podarcis melisellensis	78.71	105.09	82.29	11.36	
Podarcis hispanicus	85.35	100.11	87.96	5.60	
Podarcis peloponnesiacus	86.45	97.50	80.65	6.80	
Australolacerta australis	81.12	102.39	81.30	9.99	
Dalmatolacerta oxycephala	85.68	95.88	83.80	5.47	
Eremias acutirostris	86.94	100.49	86.36	5.73	
Iberolacerta monticola	86.75	101.97	80.10	8.37	
				7.3	

with the curvatures of the SC canals (tested for the first four PC axes; all $P > 0.67$) and they did not differ between microhabitat groups ($P = 0.16$). Finally, the regression of the shape Procrustes coordinates against the log-centroid size showed a significant allometric effect of centroid size upon vestibular shape (slope $= 0.26$, $P < 0.05$), suggesting that the size of the vestibular system affects the shape of the semicircular canals.

PHYLOGENETIC ANALYSES

The results of the multivariate *K*-statistic calculated on the four curvatures show a significant phylogenetic signal (Kmult $= 0.80$, $P = 0.02$, 1000 permutations), indicating an effect of shared ancestry on the shape of the lacertid vestibular system. As such, we repeated the statistical analyses, but with the inclusion of phylogenetic information. For all phylogenetic analyses of variance, the maximum-likelihood (ML) model explained trait evolution best and its results are reported here. The results of the phylogenetic MANOVAs (Table 5) show no significant relationship between shape and microhabitat groups ('simple' vs. 'complex' m.g.) (all $P > 0.215$). However, there was a

significant difference on the shape variation explained by PC1 ($F_{1,21} = 4.32$, $P = 0.049$) (Table 5). Therefore, it is clear that there remains a significant effect of microhabitat complexity on the vestibular system shape variation even after taking into account the phylogeny. We did not find a significant relationship between vestibular shape and microhabitat when we considered three ('simple', 'densely vegetated areas', 'areas with vertical elements'; microhabitat grouping 2; Table 1; all $P > 0.115$; Table 5) instead of two microhabitat groups. Finally, no significant relationship was found between the three SC pair angles and vestibular shape when we took the phylogenetic relationship of the species under study into account (all $P > 0.119$).

DISCUSSION

THE EFFECT OF MICROHABITAT

Our study reveals considerable interspecific variation in the shape of the vestibular system of lacertid lizards. Moreover, it indicates that this variation is related to microhabitat use. This confirms our general prediction that the animals' locomotor style and required motion
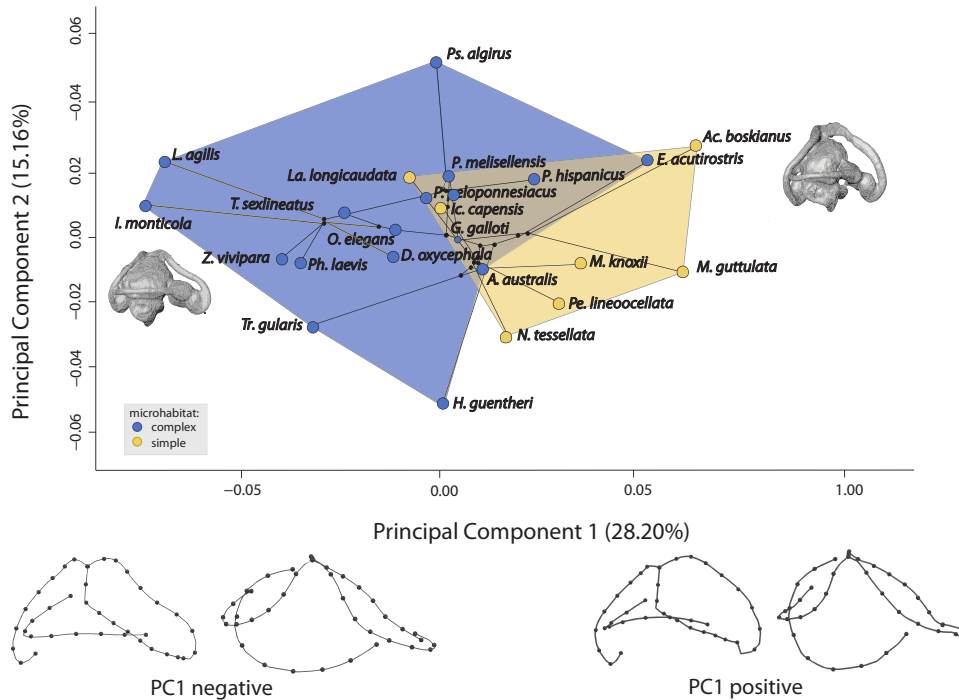
Figure 3. Illustration of the interspecific Procrustes PCA (PC1 vs. PC2). The first and the second principal components are indicated (PC1: 28.2% vs. PC2: 15.16%) and the Bayesian tree by Baeckens *et al.* (2015) is superimposed. Yellow points and 3D models represent species from the 'simple' microhabitat group and are shown on the left side of the graph while blue points represent species from the 'complex' microhabitat group and are shown on the right side of the graph. The curvatures of the SCs (black lines under the PC1 axis) as well as the 3D surface models of the vestibular systems for *Iberolacerta monticola* and *Acanthodactylus boskianus* are illustrated representing the extreme negative and positive shapes explained by PC1 respectively (see Table 6 for detailed shape differences).

sensitivity are reflected in the shape of the vestibular system, which in turn has an impact on its functional characteristics.

Previous studies have related vestibular system enlargement and SC elongation to higher sensitivity and differences in lifestyle. For instance, Grohé *et al.* (2018) found that the vestibular systems of cheetahs (*Acinonyx jubatus*) differ strongly in shape and proportions (larger vestibular systems, longer SCs, ASC less extended anteriorly, PSC and LSC extended dorsally) from those of other cat species (including a closely related extinct cheetah species). They consider this to be an adaptation to the species' specialized high-speed hunting strategy. In addition, Spoor *et al.* (2007) reported gibbons having relatively large SCs for their body size compared to koala lemurs (*Palaeopropithecus*) and they suggested that this is linked to their acrobatic brachiating locomotion. Here, the results of our Procrustes PCA comparing the shape deformation between species show that lacertids inhabiting 'complex' microhabitats possess narrower but longer (anterior–posteriorly) vestibular systems, a quite curved/S-shaped ASC, a straightened and laterally compressed LSC, a shorter common crus and longer utricle than species living in microhabitats of low complexity ('simple' m.g.) (Figs 3–5; Table 6). This is rather surprising, as a shorter common crus (interconnection between ASC and PSC) implies shorter anterior and posterior semicircular membranous ducts (inside the bony canals), decreasing the maximal endolymph volume and therefore the canal's sensitivity. In lacertids attributed to the 'simple' microhabitat group, by contrast, we found anatomical traits of the vestibular system linked to high sensitivity (see Circularity and orthogonality below): a longer common crus and more circular anterior and lateral semicircular canals.

Bearing in mind that only a small percentage of the shape variation is related to the ecological groups in our study (PC1; 28.2%) and the fact that there is a significant relationship between vestibular shape and vestibular size, it is possible that there are ecological–behavioural reasons other than balance and sensitivity affecting the vestibular anatomy and forming the above-mentioned shape. Considering that the two inner ears occupy a large space inside the skull, it is possible that skull shape affects the shape of the bony labyrinth. Moving on the PC1 axis
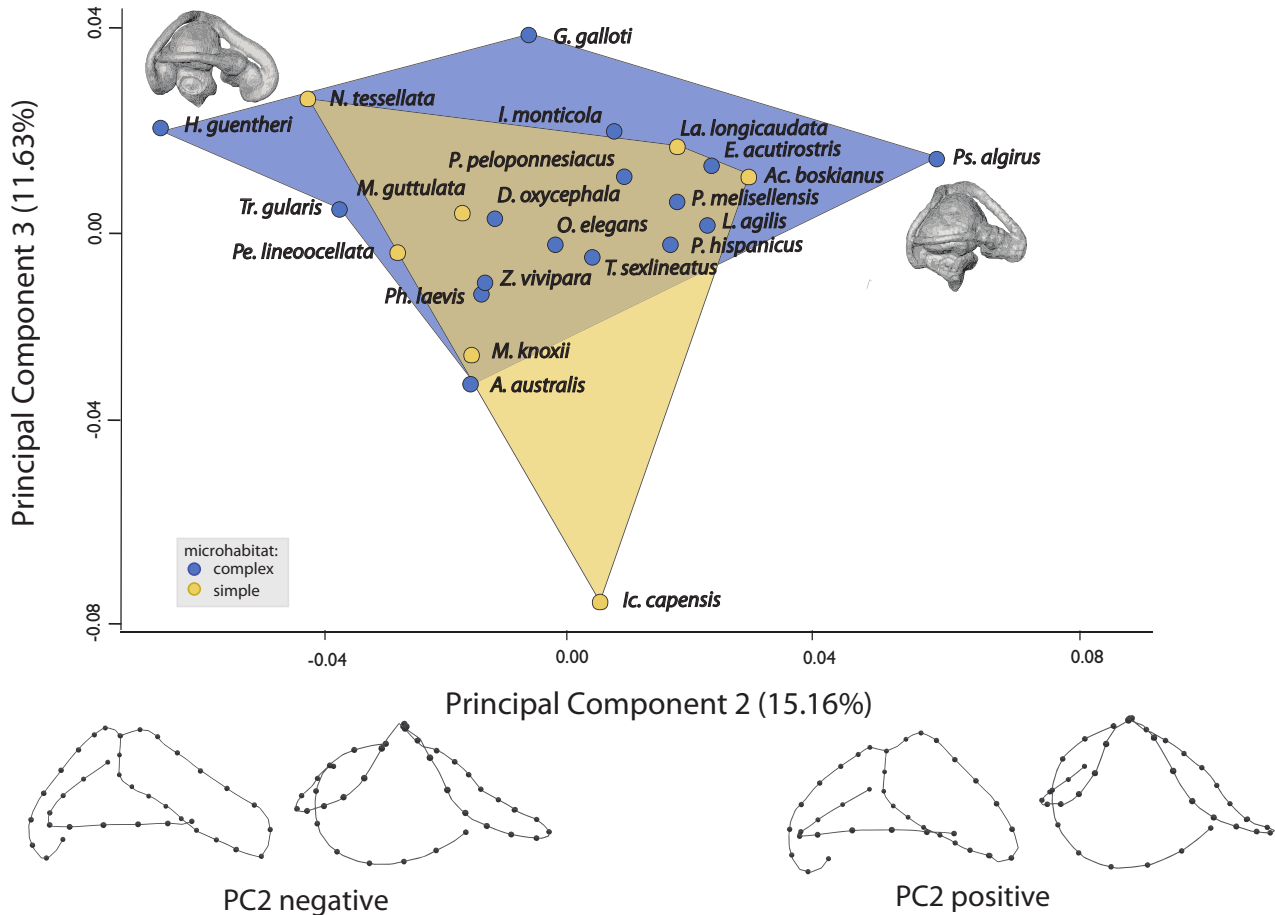
Figure 4. Illustration of the interspecific Procrustes PCA (PC2 vs. PC3). The second and the third principal components (PC2: 15.16% vs. PC3: 11.63%) are indicated. Species belonging to the 'simple' microhabitat group are indicated in yellow, while species inhabiting the 'complex' microhabitat group are indicated in blue. The curvatures of the SCs (black lines under PC2 axis) as well as the 3D surface models of the vestibular systems for *Holaspis guentheri* and *Psammodromus algirus* are illustrated representing the extreme negative and positive shapes explained by PC2 respectively (see Table 6 for detailed shape differences).

of the interspecific PCA morphospace (the only PC axis showing a significant ecological signal), from the 'complex' to the 'simple' microhabitat group, we observe that the lacertid vestibular system becomes wider (Fig. 3). This could potentially be related to a wider head. For a similar head height, a wider head could potentially mean a relatively flatter head, and it is possible that wide, flat heads are beneficial for lacertids living in simple microhabitats. For instance, Edwards *et al.* (2016) observed that sand-diving lizards possess dorsoventrally flatter heads compared to non-diving species. The ability to sand-dive may be crucial for these lizard species to find shelter from their predators in deserts. Apart from sand-diving, the skull shape of open-habitat lizards may also be linked to their diet, as skull anatomy is often adapted to food hardness and size (Herrel *et al.*, 2001, 2004; Sagonas *et al.*, 2014; Edwards *et al.*, 2016). Because we

can assume that (for example) less food may be less readily available in deserts, or more diverse prey may be present in complex microhabitats, skull differences and, as a result, vestibular systems shape differences may be due to the different level of food availability between the two microhabitats.

We found no correlation between sprint speed, microhabitat and vestibular shape variation. This does not necessarily mean that the speed of these lizards does not have an impact on balance control, but it may well be that vestibular shape is more strongly correlated with other aspects of balance control, such as manoeuvrability and turning rate. Given the complex anatomy of the vestibular system, a detailed study of the effect of the membranous duct anatomy on the endolymph flow and hence on the functioning of the system may shed light on the exact relationship that exists between sensitivity, circularity and morphology of the canals.
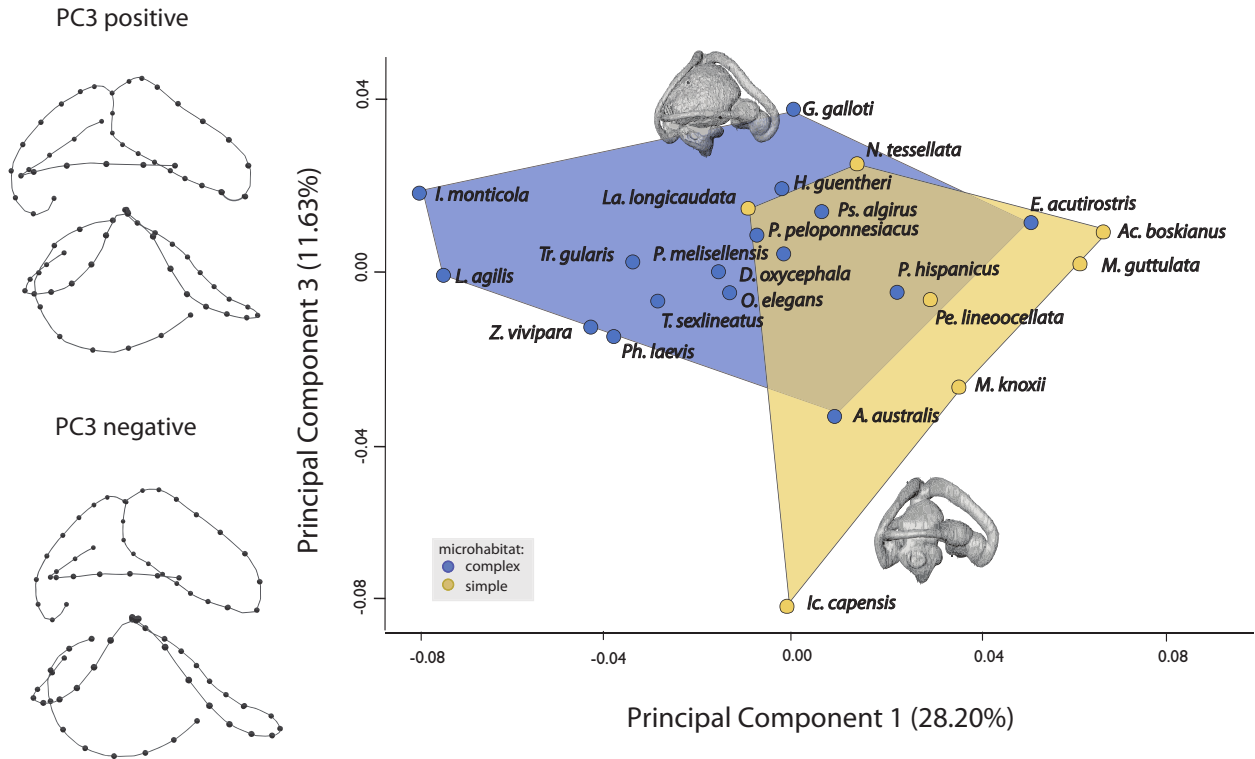
Figure 5. Illustration of the interspecific Procrustes PCA (PC1 vs. PC3). The first and third principal components are indicated (PC1: 28.2% vs. PC3: 11.63%). Species belonging to the 'simple' microhabitat group are indicated in yellow, while species inhabiting the 'complex' microhabitat group are indicated in blue. The curvatures of the SCs (black lines next to PC3 axis) as well as the 3D surface models of the vestibular systems for the species *Ichnotropis capensis* and *Gallotia galloti* are illustrated representing the extreme negative and positive shapes explained by PC3 respectively (see Table 6 for detailed shape differences).

Furthermore, to relate the functional and anatomical aspects to behaviour and ecology, information on the 3D angular accelerations of the head and the body centre of mass during locomotion for the species under study is necessary (Goyens & Aerts, 2018).

Focusing on the lacertids inhabiting complex microhabitats ('densely vegetated', areas with 'vertical elements'; microhabitat grouping 2; Table 1), our results show a significant ecological signal in the anatomy of the vestibular system between species living in open areas and those inhabiting areas where vegetation is very dense ('simple' vs. 'densely vegetated' microhabitat groups). By contrast, species inhabiting areas with vertical elements do not show any significant ecological signal (Table 5).

The results of this comparison do, however, change slightly after implementing phylogeny in our analyses. One explanation for this difference is methodological, and relates to the statistical power of our analyses: by increasing the number of variables (from two to three), the degrees of freedom decrease, which ultimately may be too low to detect an effect of microhabitat, especially in phylogenetic statistical analyses with a sample size of approximately 20 species (Garamszegi & Møller, 2010). Including more lacertid species in each microhabitat group or more individuals per species (and thus increasing the sample size) would be elucidative (Gonzales *et al.*, 2019). Another explanation as to why phylogenetic analyses do not find an effect of complex microhabitat type (densely vegetated vs. vertical) on vestibular system shape, while traditional analyses do, might be attributed to shared ancestry. Species living in similar complex habitats appear to resemble each other in vestibular system morphology only because they are more closely related than species living in dissimilar complex microhabitats. This outcome underlines the importance of including phylogenetic information in interspecific comparisons when studying convergent evolution.

CIRCULARITY AND ORTHOGONALITY

It has been observed that the size of the semicircular canals influences the deviation from circularity (Davies *et al.*, 2013; Maddin & Sherratt, 2014; Grohé *et al.*, 2016; Perier *et al.*, 2016; Le Maitre *et al.*, 2017; Racicot

Table 5. Results of the traditional and phylogenetic MANOVAs and ANOVAs. The PC scores of the interspecific PCA on the Procrustes coordinates used for each test are indicated together with the percentage of the shape variation explained in each test. For all these statistical tests, microhabitat group was used as a factor and the PC scores as dependent variables. Significant *P*-values of the statistical tests are highlighted in bold. Corrected *P*-values refer to the *P*-values after application of Tukey's HSD test

PC axes	Variation explained (%)	Microhabitat grouping 1				
		MANOVA			Phylogenetic MANOVA	
		$F_{1,21}$	*P*-value		$F_{1,21}$	*P*-value
PC1 to PC10	86.9	2.52	0.065		2.52	0.274
PC1 to PC4	62.59	2.75	0.060		2.75	0.254
PC1 to PC3	54.99	3.50	**0.035***		3.50	0.215
		Microhabitat grouping 1				
		ANOVA			Phylogenetic ANOVA	
		$F_{1,21}$	*P*-value		$F_{1,21}$	*P*-value
PC1	28.20	8.78	**0.007***		4.32	**0.049***
PC2	15.16	0.28	0.597		0.15	0.697
PC3	11.63	1.05	0.315		0.12	0.722
		Microhabitat grouping 2				
		(M)ANOVA			Phylogenetic (M) ANOVA	
		$F_{2,20}$	*P*-value		$F_{2,20}$	*P*-value
PC1 to PC3	54.99	1.59	0.151		1.69	0.470
PC1	28.20	4.80	**0.016*** (corrected) – 'simple' vs. 'densely vegetated areas'		4.22	0.115
PC2	15.16	0.18	0.842		0.62	0.546
PC3	11.63	0.64	0.536		0.65	0.532

et al., 2018). In turn, these deviations will alter the fluid dynamics of the endolymph and it is possible that sensitivity to motion is affected. Here we hypothesized that species living in complex microhabitats ('complex' m.g.) would be adapted for higher sensitivity to cope with challenged locomotion and high manoeuvrability demands, and hence we expected them to have more circular and/or longer canals than lacertids living in less complex microhabitats ('simple' m.g.).

Here, except for the overall longer vestibular system and the higher, so potentially longer, PSC we do not find anatomical traits known to be linked to sensitivity for lacertids belonging to the 'complex' microhabitat group (see previous section). Instead, lacertids negotiating open and more simple microhabitats ('simple' m.g.) appear to be more sensitive during locomotion as they possess both a more circular ASC and LSC, suggesting

their microhabitat is more challenging in terms of manoeuvrability. Similarly, Grohé *et al.* (2016) reported that otter and mink species (semi-aquatic) possess a more oval than circular ASC and a more sinuous than straight LSC compared to non-aquatic musteloids, where manoeuvrability demands are higher (lower neck mobility for aquatic and semi-aquatic species, and hence less sensitivity to head motion). Furthermore, previous studies have commonly expressed that the role of SC shape on balance control differs between SC canals. Several studies report that among the three canals, the lateral canal is the most closely related to locomotor agility and navigation (Spoor *et al.*, 2007; Cox & Jeffery, 2010). Interestingly, we observe a more circular (and therefore more sensitive) LSC in lacertids living in open areas ('simple' m.g.). Because of the structure of the open habitat, these animals are largely confined to

Table 6. Description of the interspecific shape differences between the 3D surface models of the vestibular systems. The results of the interspecific Procrustes PCA are indicated. The shape description for the positive and negative values of the four first principal components are listed

Negative values of PCA	Positive values of PCA
PC1: 28.2%	
More S-shaped ASC	More circular/wider ASC
More straightened/compressed (laterally) LSC	More circular/wider LSC
Higher (dorsally extended) PSC	Shorter (extended ventrally) PSC
Narrower (laterally) vestibular system	Wider (laterally) vestibular system
Longer (anterior–posterior) vestibular system	Shorter (anterior–posterior) vestibular system
Shorter common crus	Longer common crus
Longer utricle	Shorter utricle
	Ventrally extended ASC & PSC ampullas
PC2: 15.16%	
More straightened/wider (anteriorly) ASC	More circular/higher (dorsally) ASC
Narrower (laterally) LSC	More circular/wider (laterally) LSC
Higher (dorsally extended) and more circular PSC	Posteriorly compressed and lower (ventrally) PSC
Shorter common crus	Longer common crus
Longer utricle	Shorter utricle
PC3: 11.63%	
More circular/wider (anteriorly)/higher (dorsally) ASC	More S-shaped/narrower ASC
Narrower LSC	More circular/wider LSC
More straightened/higher (dorsally extended) PSC	Narrower posteriorly PSC
Higher (dorsoventrally) vestibular system	Shorter (dorsoventrally) vestibular system
PC4: 7.6%	
More circular/higher (dorsally) ASC	More S-shaped/shorter (dorsally) ASC
Narrower laterally/more circular LSC	Wider latterally/less circular LSC
Wider (laterally) vestibular system	Narrower (laterally) vestibular system

movements in the lateral (frontal) plane. Furthermore, they may need to manoeuvre and turn more than expected purely based on the microhabitat structure, being more susceptible to predation than species living in more complex environments where they are protected by dense vegetation (high shelter availability). Predation risk has been previously correlated with shelter availability and the evolution of defensive morphological traits in fish, lizards and mammal species (Leinonen *et al.*, 2011; Stankowich & Campbell, 2016; Broeckhoven *et al.*, 2018). Therefore, swift and fast responses seem to be needed in the horizontal plane for species inhabiting open areas whereas more careful and slow movements may be used by lacertids using the complexity of their microhabitat to survive.

However, contradictory results are present in the literature: Dickson *et al.* (2017) showed that arboreal *Anolis* lizard species, living on high reaches of trees or on narrow branches in the forest canopy where 3D complexity is high, possess more circular LSCs than species living on the ground or on the trunks of trees. Hence, the shape of the bony labyrinth seems to be specialized for sensitivity when the microhabitat 3D complexity or the risk of falling is higher. *In situ* observations of locomotor behaviour and head

movements would facilitate future studies, by shedding light on the demands in manoeuvrability imposed by the different microhabitats and the link to each of the morphological traits of the vestibular system.

In addition to circularity, and as mentioned before, an orthogonal SC pair is more likely to possess a higher sensitivity to motion. Here, we found that species living in areas including vertical elements, such as rocks or trees, show stronger deviations from orthogonality than species inhabiting open areas. According to previous research (Malinzak *et al.*, 2012; Berlin *et al.*, 2013) this could mean that species living in open areas possess more sensitive vestibular systems supporting the above-mentioned findings. However, we should note that these groups did not differ significantly in SC shape in our interspecific PCA. Furthermore, the high variability in the angular variance between the SCs and the SC pairs should be taken into account when interpreting the results.

CONCLUSIONS

We find that lacertid lizards inhabiting different microhabitats have significantly different SC shapes.

The adaptations differ between the three SCs, indicating the anatomical and functional complexity of the vestibular system. Contrary to our expectations, lacertids inhabiting areas with a simple microhabitat structure (e.g. even surfaces and/or open areas, scarce vegetation) appear more sensitive to motion than lacertids inhabiting more complex environments. As such, we observe more orthogonal SCs and more circular AS and LS canals. Interestingly, for lizards, high sensitivity during locomotion is even more beneficial in open areas when there is an elevated mortality risk due to predation in an exposed and unprotected niche. On the other hand, our results suggest that lacertids inhabiting areas with a more complex microhabitat structure (e.g. dense or high vegetation, rocks, trees) may be less agile and sensitive to motion and possibly rely more on finding shelter as a defence mechanism rather than using manoeuvrability to escape.

ACKNOWLEDGEMENTS

AUTHOR CONTRIBUTIONS

M.V-K., P.A., J.G. and R.V.D. conceived and designed the study, participated in the interpretation of the results and helped draft the manuscript; M.V-K. made the 3D reconstructions, carried out the analyses and drafted the manuscript; M.V-K. designed the illustrations and revised the manuscript. All authors gave final approval for publication. M.V-K. is a research assistant funded by the University of Antwerp. J.G. is an FWO postdoctoral fellow. R.V.D. and P.A. are professors at the University of Antwerp. The authors declare that they have no conflicting interests.

REFERENCES

Adams DC. 2014. A generalized K statistic for estimating phylogenetic signal from shape and other high-dimensional multivariate data. *Systematic Biology* **63:** 685–697.

Angelaki DE, **Cullen KE. 2008.** Vestibular system: the many facets of a multimodal sense. *Annual Review of Neuroscience* **31:** 125–150.

Arnold EN. 1989. Towards a phylogeny and biogeography of the Lacertidae: relationships within an Old-World family of lizards derived from morphology. *Bulletin of the British Museum (Natural History)* **55:** 209–257.

Arnold EN. 1998. Structural niche, limb morphology and locomotion in lacertid lizards (Squamata, Lacertidae); a preliminary survey. *Bulletin of the Natural History Museum. Zoology Series* **64:** 63–89.

Arnold N, **Ovenden WD. 2004.** *Collins field guide to the reptiles and amphibians of Britain & Europe.* New York: HarperCollins.

Baeckens S, **Edwards S**, **Huyghe K**, **Van Damme R. 2015.** Chemical signalling in lizards: An interspecific comparison of femoral pore numbers in Lacertidae. *Biological Journal of the Linnean Society* **114:** 44–57.

Bar A, **Haimovitch G. 2011.** *A field guide to reptiles and amphibians of Israel*. Herzlya: Pazbar Ltd.

Bates MF, **Branch WR**, **Bauer AM**, **Burger M**, **Marais J**, **Alexander GJ**, **de Villiers MS. 2014.** *Atlas and Red List of the Reptiles of south Africa, Lesotho and Swaziland.* Pretoria: South African National Biodiversity Institute.

Bauwens D, **Garland T Jr**, **Castilla AM**, **Van Damme R. 1995.** Evolution of sprint speed in lacertid lizards. *Evolution* **49:** 848–863.

Benson RBJ, **Starmer-Jones E**, **Close RA**, **Walsh SA. 2017.** Comparative analysis of vestibular ecomorphology in birds. *Journal of Anatomy* **231:** 990–1018.

Berlin JC, **Kirk EC**, **Rowe TB. 2013.** Functional implications of ubiquitous semicircular canal non-orthogonality in mammals. *PLoS One* **8:** e79585.

Billet G, **Hautier L**, **Asher RJ**, **Schwarz C**, **Crumpton N**, **Martin T**, **Ruf I. 2012.** High morphological variation of vestibular system accompanies slow and infrequent locomotion in three-toed sloths. *Proceedings of the Royal Society B: Biological Sciences* **279:** 3932–3939.

Billet G, **Hautier L**, **Lebrun R. 2015.** Morphological diversity of the bony labyrinth (inner ear) in extant xenarthrans and its relation to phylogeny. *Journal of Mammalogy* **96:** 658–672.

Blomberg SP, **Garland T**, **Ives AR. 2003.** Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57:** 717–745.

Boistel R, **Herrel A**, **Lebrun R**, **Daghfous G**, **Tafforeau P**, **Losos JB**, **Vanhooydonck B. 2011.** Shake rattle and roll: the bony labyrinth and aerial descent in squamates. *Integrative and Comparative Biology* **51:** 957–968.

Bookstein FL. 1991. *Morphometric tools for landmark data: geometry and biology*. Cambridge: Cambridge University Press.

Boyle KS, **Herrel A. 2018**. Neurocranium shape variation of piranhas and pacus (Characiformes : Serrasalmidae) in association with ecology and phylogeny. *Biological Journal of the Linnean Society* **125:** 93–114.

Brecko J, **Huyghe K**, **Vanhooydonck B**, **Herrel A**, **Grbac I**, **Van Damme R. 2008.** Functional and ecological significance of intraspecific variation in body size and shape in the lizard *Podarcis melisellensis* (Lacertidae). *Biological Journal of the Linnean Society* **94:** 251–264.

Broeckhoven C, **El Adak Y**, **Hui C**, **Van Damme R**, **Stankowich T. 2018.** On dangerous ground: the evolution of body armour in cordyline lizards. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **285**: 20180513.

Carriot J, **Brooks JX**, **Cullen KE. 2013.** Multimodal integration of self-motion cues in the vestibular system: active versus passive translations. *The Journal of Neuroscience* **33:** 19555–19566.

Costeur L, **Grohé C**, **Aguirre-Fernández G**, **Ekdale E**, **Schulz G**, **Müller B**, **Mennecart B. 2018.** The bony labyrinth of toothed whales reflects both phylogeny and habitat preferences. *Scientific Reports* **8:** 7841.

Cox PG, **Jeffery N. 2010.** Semicircular canals and agility: the influence of size and shape measures. *Journal of Anatomy* **216:** 37–47.

Cullen KE. 2012. The vestibular system: multimodal integration and encoding of self-motion for motor control. *Trends in Neurosciences* **35:** 185–196.

David R, **Stoessel A**, **Berthoz A**, **Spoor F**, **Bennequin D. 2016.** Assessing morphology and function of the semicircular duct system: introducing new in-situ visualization and software toolbox. *Scientific Reports* **6:** 32772.

Davies KTJ, **Bates PJJ**, **Maryanto I**, **Cotton JA**, **Rossiter SJ. 2013.** The evolution of bat vestibular systems in the face of potential antagonistic selection pressures for flight and echolocation. *PLoS One* **8:** 8–10.

Dickson BV, **Sherratt E**, **Losos JB**, **Pierce SE. 2017.** Semicircular canals in *Anolis* lizards: ecomorphological convergence and ecomorph affinities of fossil species. *Royal Society Open Science* **4:** 170058.

Edwards S, **Herrel A**, **Vanhooydonck B**, **Measey GJ**, **Tolley KA. 2016.** Diving in head first: investigations into trade-offs between phenotypic traits and sand-diving predator escape strategy in desert lizards (Meroles, Lacertidae, Squamata). *Biological Journal of the Linnean Society*. **119**: 919–931.

Ekdale EG, **Racicot RA. 2015.** Anatomical evidence for low frequency sensitivity in an archaeocete whale: comparison of the inner ear of *Zygorhiza kochii* with that of crown Mysticeti. *Journal of Anatomy* **226:** 22–39.

Garamszegi LZ, **Møller AP. 2010.** Analytical archaeology. *Biological Reviews* **85:** 797–805.

Georgi JA, **Sipla JS**, **Forster CA. 2013.** Turning semicircular canal function on its head: dinosaurs and a novel vestibular analysis. *PLOS ONE* **8:** e58517.

Gonzales LA, **Malinzak MD**, **Kay RF. 2019.** Intraspecific variation in semicircular canal morphology – A missing element in adaptive scenarios? *American Journal of Physical Anthropology* **168:** 10–24.

Gower J. 1975. Generalized Procrustes analysis. *Psychometrika* **40:** 33–51.

Goyens J, **Aerts P. 2018.** Head stabilisation in fast running lizards. *Zoology (Jena, Germany)* **127:** 114–120.

Grohé C, **Lee B**, **Flynn JJ. 2018.** Recent inner ear specialization for high-speed hunting in cheetahs. *Scientific Reports* **8:** 23012308.

Grohé C, **Tseng ZJ**, **Lebrun R**, **Boistel R**, **Flynn JJ. 2016.** Bony labyrinth shape variation in extant Carnivora: a case study of Musteloidea. *Journal of Anatomy* **228:** 366–383.

Gunz P, **Ramsier M**, **Kuhrig M**, **Hublin JJ**, **Spoor F. 2012.** The mammalian bony labyrinth reconsidered, introducing a comprehensive geometric morphometric approach. *Journal of Anatomy* **220:** 529–543.

Herrel A, **De Grauw E**, **Lemos-Espinal JA. 2001.** Head shape and bite performance in xenosaurid lizards. *The Journal of Experimental Zoology* **290:** 101–107.

Herrel A, **Vanhooydonck B**, **Van Damme R. 2004.** Omnivory in lacertid lizards: adaptive evolution or constraint? *Journal of Evolutionary Biology* **17:** 974–984.

Huey RB, **Bennett AF**, **John-Alder H**, **Nagy KA. 1984.** Locomotor capacity and foraging behaviour of Kalahari lacertid lizards. *Animal Behaviour* **32:** 41–50.

Hullar TE. 2006. Semicircular canal geometry, afferent sensitivity, and animal behavior. *The Anatomical Record. Part A, Discoveries in Molecular, Cellular, and Evolutionary Biology* **288:** 466–472.

Jones GM, **Spells KE. 1963.** A theoretical and comparative study of the functional dependence of the semicircular canal upon its physical dimensions. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **157:** 403–419.

Lambert FM, **Beck JC**, **Baker R**, **Straka H. 2008.** Semicircular canal size determines the developmental onset of angular vestibuloocular reflexes in larval *Xenopus*. *The Journal of Neuroscience* **28:** 8086–8095.

Lebrun R. 2014. ISE-MeshTools, a 3D interactive fossil reconstruction freeware. 12th Annual Meeting of EAVP, Torino, Italy; 06/2014.

Lebrun R, **de León MP**, **Tafforeau P**, **Zollikofer C. 2010.** Deep evolutionary roots of strepsirrhine primate labyrinthine morphology. *Journal of Anatomy* **216:** 368–380.

Leinonen T, **Herczeg G**, **Cano JM**, **Merilä J. 2011.** Predation-imposed selection on threespine stickleback (*Gasterosteus aculeatus*) morphology: a test of the refuge use hypothesis. *Evolution* **65:** 2916–2926.

Loza CM, **Latimer AE**, **Sa MR**, **Carlini AA. 2017.** Sensory anatomy of the most aquatic of carnivorans: the Antarctic Ross seal, and convergences with other mammals. *Biology Letters* **13**.

Maddin HC, **Sherratt E. 2014.** Influence of fossoriality on inner ear morphology: insights from caecilian amphibians. *Journal of Anatomy* **225:** 83–93.

Le Maître A, **Schuetz P**, **Vignaud P**, **Brunet M. 2017.** New data about semicircular canal morphology and locomotion in modern hominoids. *Journal of Anatomy* **231:** 95–109.

Malinzak MD, **Kay RF**, **Hullar TE. 2012.** Locomotor head movements and semicircular canal morphology in primates. *Proceedings of the National Academy of Sciences of the United States of America* **109:** 17914–17919.

Martín J, **Salvador A. 1992.** Tail loss consequences on habitat use by the Iberian rock lizard, *Lacerta monticola*. *Oikos* **65:** 328–333.

Mayer W, **Beyerlein P. 1999.** Ecological niche segregation of seven sympatric lacertid lizards in the Peloponnese highlands. *Croatian National History Museum* **8:** 339–344.

Meiri S. 2008. Evolution and ecology of lizard body sizes. *Global Ecology and Biogeography* **17:** 724–734.

Mennecart B, **DeMiguel D**, **Bibi F**, **Rössner GE**, **Métais G**, **Neenan JM**, **Wang S**, **Schulz G**, **Müller B**, **Costeur L. 2017.** Bony labyrinth morphology clarifies the origin and evolution of deer. *Scientific Reports* **7:** 13176.

Mendiburu FD. 2019. *Agricolae: Statistical Procedures for Agricultural Research.* R Package Version 1.3-0. Available at: https://tarwi.lamolina.edu.pe/~fmendiburu/

Muller M. 1999. Size limitations in semicircular duct systems. *Journal of Theoretical Biology* **198:** 405–437.

Palci A, **Hutchinson MN**, **Caldwell MW**, **Lee MSY. 2017.** The morphology of the inner ear of squamate reptiles and its bearing on the origin of snakes. *Royal Society Open Science* **4:** 170685.

Paradis E, **Claude J**, **Strimmer K. 2004.** APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics (Oxford, England)* **20:** 289–290.

Perier A, **Lebrun R**, **Marivaux L. 2016.** Different level of intraspecific variation of the bony labyrinth morphology in slow- versus fast-moving primates. *Journal of Mammalian Evolution* **23:** 353–368.

Pfaff C, **Martin T**, **Ruf I. 2015.** Bony labyrinth morphometry indicates locomotor adaptations in the squirrel-related clade (Rodentia, Mammalia). *Proceedings of the Royal Society B: Biological Sciences* **282:** 20150744.

Pinheiro J, **Bates D**, **DebRoy S**, **Sarker D**; R Development Core Team. **2018.** *nlme: linear and nonlinear mixed effects models. R package version 3.1–111.* Available at: https://CRAN.R-project.org/package=nlme

R Core Team. **2012.** *A language and environment for statistical computing.* Vienna: R Foundation for Statistical Computing.

R Studio. **2012.** *R Studio: integrated development environment for R, Version 0.97.390.* Boston: R Studio.

Rabbitt R, **Damiano E**, **Grant JW. 2004.** Biomechanics of the vestibular semicircular canals and otolith organs. In: Highstein S, Fay R, Popper A, eds. *The vestibular system.* New York: Springer, 153–201.

Racicot RA, **Gearty W**, **Kohno N**, **Flynn JJ. 2018.** Comparative anatomy of the bony labyrinth of extant and extinct porpoises (Cetacea : Phocoenidae). *Biological Journal of the Linnean Society* **119:** 831–846.

Revell LJ. 2012. phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* **3:** 217–223.

Revell LJ, **Harmon LJ**, **Langerhans RB**, **Kolbe JJ. 2007.** A phylogenetic approach to determining the importance of constraint on phenotypic evolution in the neotropical lizard *Anolis cristatellus*. *Evolutionary Ecology Research* **9:** 261–282.

Sagonas K, **Pafilis P**, **Lymberakis P**, **Donihue CM**, **Herrel A**, **Valakos ED. 2014.** Insularity affects head morphology, bite force and diet in a Mediterranean lizard. *Biological Journal of the Linnean Society* **112:** 469–484.

Spoor F. 2003. The semicircular canal system and locomotor behaviour, with special reference to hominid evolution. *Courier Forschungsinstitut Senckenberg* **243:** 93–104.

Spoor F, **Bajpai S**, **Hussain ST**, **Kumar K**, **Thewissen JG. 2002.** Vestibular evidence for the evolution of aquatic behaviour in early cetaceans. *Nature* **417:** 163–166.

Spoor F, **Garland T Jr**, **Krovitz G**, **Ryan TM**, **Silcox MT**, **Walker A. 2007.** The primate semicircular canal system and locomotion. *Proceedings of the National Academy of Sciences of the United States of America* **104:** 10808–10812.

Spoor F, **Wood B**, **Zonneveld F. 1994.** Implications of early hominid labyrinthine morphology for evolution of human bipedal locomotion. *Nature* **369:** 645–648.

Squire L, **Berg D**, **Bloom FE**, **du Lac S**, **Ghosh A**, **Spitzer NC. 2013.** *Fundamental neuroscience, 4th edn.* New York: Academic Press.

Stankowich T, **Campbell LA. 2016.** Living in the danger zone: exposure to predators and the evolution of spines and body armor in mammals. *Evolution* **70:** 1501–1511.

Tadevosyan T. 2007. The role of vegetation in microhabitat selection of syntopic lizards, *Phrynocephalus persicus*, *Eremias pleskei*, and *Eremias strauchi* from Armenia. *Amphibia-Reptilia* **28:** 444–448.

Van Damme R, **Vanhooydonck B. 2002.** Speed versus manoeuvrability: association between vertebral number and habitat structure in lacertid lizards. *Journal of Zoology* **258:** 327–334.

Van der Meer MH, **Whiting MJ**, **Branch WR. 2010.** Ecology of Southern African Sandveld lizards (Lacertidae, *Nucras*). *Copeia* **2010:** 568–577.

Vanhooydonck B, **Van Damme R. 1999.** Evolutionary relationships between body shape and habitat use in lacertid lizards. *Evolutionary Ecology Research* **1:** 785–803.

Vanhooydonck B, **Van Damme R**, **Aerts P. 2001.** Speed and stamina trade-off in lacertid lizards. *Evolution* **55:** 1040–1048.

Vanhooydonck B, **Herrel A**, **van Damme R. 2007.** Interactions between habitat use, behavior, and the trophic niche of lacertid lizards. In: Reilly S, McBrayer L, Miles D, eds. *Lizard Ecology*. Cambridge: Cambridge University Press, 427–449.

Vasilopoulou-Kampitsi M, **Goyens J**, **Baeckens S, van Damme R**, **Aerts P. 2019.** Habitat use and vestibular system's dimensions in lacertid lizards. *Journal of Anatomy* (accepted 5 March 2019).

Verwaijen D. 2007. *Foraging of (lacertid) lizards: a test of the Syndrome Hypothesis*. Ph.D. dissertation. University of Antwerp.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Figure S1. Percentages of shape variation explained by each principal component of the Procrustes interspecific PCA. The first four principal component axes represent 62.59% of the total variation (PC1: 28.2%, PC2:15.16%, PC3: 11.63%, PC4: 7.6%).