Scaling and morphological analysis of the lizards vestibular system, using micro CT scanning

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Aims

The detection of body motion and orientation by the vestibular system of the inner ear forms one of the basic senses shared by all vertebrates. Because the vestibular system is crucial for maintaining balance, studying its anatomical characteristics could lead to a better understanding of balance control in fast moving animals, such as lizards. Lacertid lizards (small to medium sized lizards) use a variety of habitats, that may differ greatly in structural complexity (high or low vegetation, rocky or sandy areas, trees and bushes) (*Figure 1*). As such, we examine whether and how structural habitat complexity selects for changes in the size and shape of the vestibular system. In addition, studying its anatomical characteristics and the way they scale with the animal's size could give us information on the existence of any size constraints and as a result, on the ability of very small or large animals to maintain their balance and stability.

Figure 1. Lizard of the species Phoenicolacerta kulzeri (Muller&Wettstein,1932) . Photo taken by S. Baeckens

Method

Sample preparation for micro CT scanning:

24 male preserved individuals of 24 different lizard species of the family Lacertidae were obtained from the collections of the Functional Morphology laboratory at the University of Antwerp and from a private collection of dr. A. Herrel (Muséum National d'Histoire Naturelle, Paris). Because in this study, we focus on the bony labyrinth, staining of the samples was not necessary. Nevertheless, we stained the samples to enable imaging of the soft tissue (and possibly the membranous labyrinth) for future research. The samples were dehydrated in a graded ethanol series and fixated in 70% ethanol. Next, all animals were decapitated and the heads were placed in a staining solution of 5% phosphomolybdic acid (PMA; Sigma-Aldrich, St Louis, MO, USA) and 70% EtOH for a minimum time of 14 days.

Micro CT scanning:
The samples were scanned with a SkyScan 1172 high resolution micro CT scanner (Bruker micro CT, Kontich, Belgium), which is managed by the BiostrμCT Hercules consortium (https://sites.google.com/view/biostruct). For the scanning of the specimens we used an average voltage of 80kV and a current of 124μA with an Aluminum-Copper filter of 1mm. The pixel size varied from 4.17μm to 13.45μm due to the size difference between species. The rotation angle of the scans was 0.40˚ and the exposure time 1300ms.

Segmentation:
The bony labyrinth was clearly visible on the reconstructed scans as a network of connected tunnels at the posterior end of the skull bone. We imported the reconstructed slice images in the specialized 3D image processing software Amira (Amira 5.4.3 VSG systems, Mérignac, France). We selected the voxels belonging to the bony labyrinth, by combining automatic grey-scale thresholding and manual corrections in the three orthogonal views. The result was a 3D model of the vestibular system (*Figures 2,3,5*).

Measurements
The linear dimensions of the 3D models were measured using the built-in measurement tools of Amira. Some of them were used directly for the scaling analysis whereas others were used to calculate more complicated variables that describe the vestibular system anatomy, such as the length and width of the semi-circular canals, the ampullae surface area and the canal orientations. External head width, length and height were measured for each individual (*Figures 2&3*).

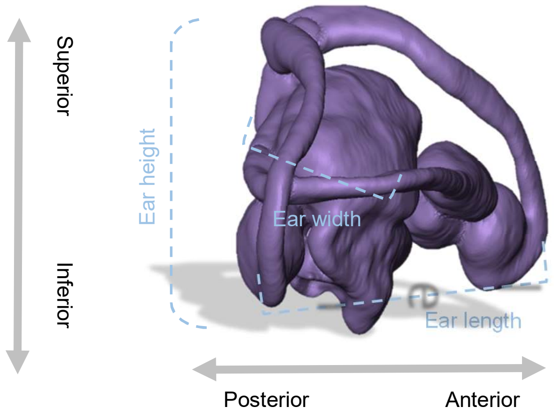

Figure 2. 3D model of the vestibular system of the lizard species Iberolacerta monticola and indication of the ear width, length and height.

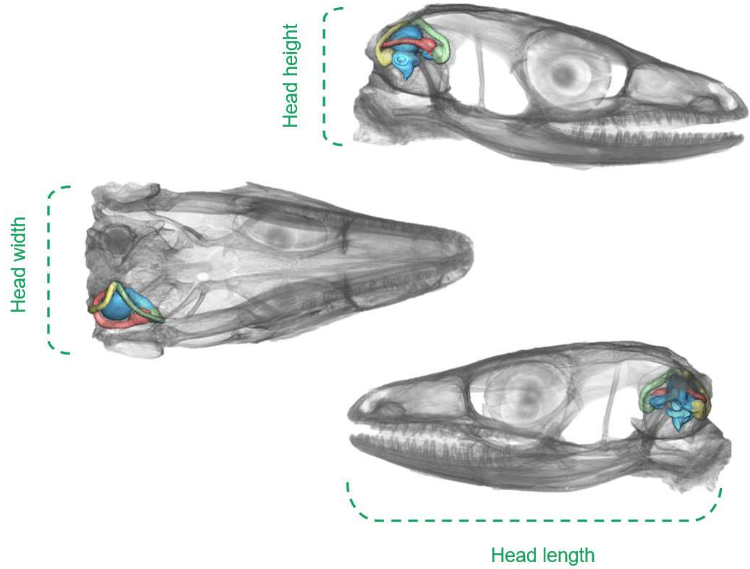

Figure 3. Shadow image of a Takydromus sexlineatus head from a dorsal and two lateral views and indication of the overall size measurements of the head. The 3D model of the vestibular system is illustrated in color.

Results

3D model of the vestibular system

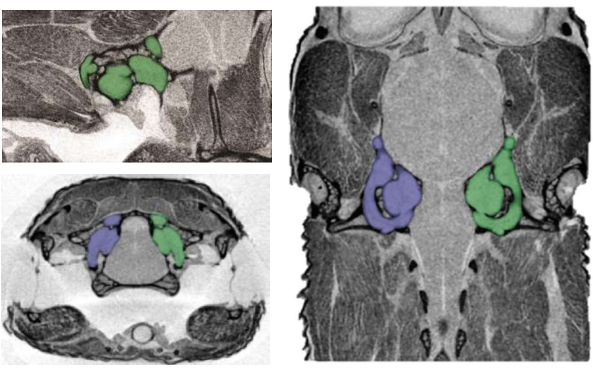

Figure 4. Sagittal (top left), transversal (bottom left) and frontal (right) slices of an Ichnotropis capensis head. The vestibular systems are highlighted in purple and green.

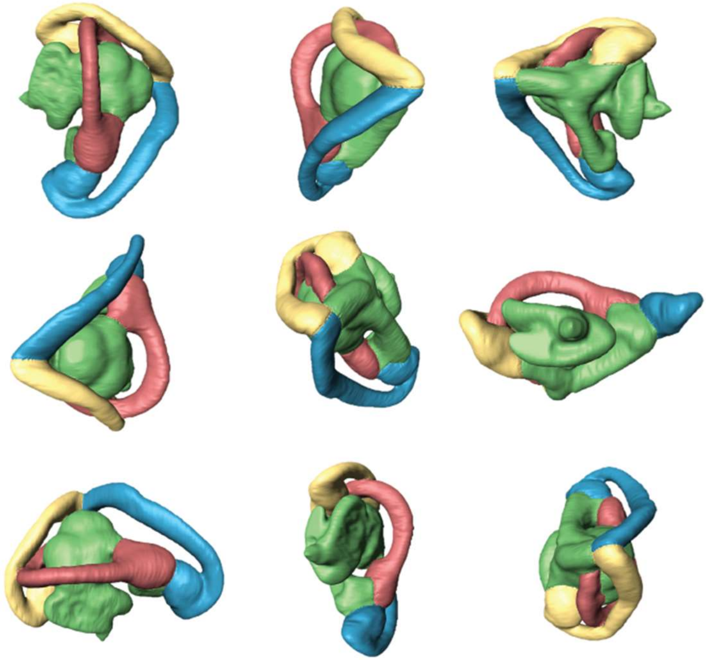

Figure 5. 3D model of the vestibular system of the species Pedioplanis lineoocellata from different views. The different colors represent the three semi-circular canals and their ampullae: anterior (blue), posterior (yellow) and lateral (pink) semi-circular canal.

Segmentation of the images acquired from the scanning procedure (*Figure 4*) gave us the 3D models of the 24 inner ears. *Figure 5* illustrates an example of those results. The bony labyrinth of the inner ear contains a series of interconnecting ducts and sacs, known as the membranous labyrinth. These ducts form three semicircular canals: the anterior, posterior and lateral canal, which can be easily distinguished when looking at the 3D models of the bony labyrinth.

Scaling:

The inter- specific scaling analysis showed a negative allometric relationship between head size of the individuals and most of the inner ear anatomical characteristics. Hence, larger species appear to have disproportionally smaller vestibular system's traits for their given size. However, some of the traits appear to change isometrically with head size.

Conclusion

Using the 3D models acquired from the micro CT scanning, we were able to compare the morphology of the inner ear between the species of our interest. The scaling analysis results suggest that there is a constraint in size for the inner ear which could lead to the assumption that bigger animals do not need an inner ear proportionally as large as their size in order to perform as well as smaller animals. Moreover we could suggest that having a non-proportionally bigger inner ear for a given size is morphogenetically less costly, leaving enough free space in the cranial cavity for the other structures.

Further research on the variability between species but also including phylogeny in our analysis could give us more information on the function of the vestibular system and its variation among species.

Finally, the 3D models and their measurements will be combined with the lizards different ecologies so that we can discover if there is a connection between habitat use, balance maintenance and inner ear morphology.

References:

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