Three-dimensional evaluation of structures in small bones by Micro- CT: tail fracture planes of autotomizing lizards (Scincidae and Gecconidae families)

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INTRODUCTION

The evaluation of three dimensional structures in small bones in biological research is often difficult. Histology can provide serial slices, but the preparation is time consuming. Confocal laser scanning microscopy gives 3D images but the penetration depth of the laser is limited. Conventional X-ray produces only 2D images where structures are superimposed. Computed tomography (CT) can produce 3D images, but the resolution is too low to visualize small structures. A relatively new method of 3D imaging is micro-computed tomography. This method allows the 3D imaging of bone with resolutions up to less than 1 µm (Rüegsegger et al. 1996; Ritman 2004). Especially small samples (e.g. bones of small animals) are suitable for the qualitative and quantitative assessment of highest resolution. Micro-CT generates a 3D dataset that can be then viewed in 2D slices as well as 3D reconstructions. A number of image processing operations are available such as filters and segmentation procedures that make it possible to emphasise the most interesting features of the image. Morphometric evaluation of trabecular and cortical structures can be done automatically, providing for example trabecular thickness, length and spacing and cortical thickness. In recent years, µCT was applied frequently to bones of mice, rats and humans (e.g. Müller et al. 1998; Abe et al. 2000; Tamada et al. 2005; Arrington et al. 2006; Bagi et al. 2006). For other species not much data is available.

In this study we used tail vertebra of autotomizing lizards as an example of small bones with extraordinary internal 3D structures. Autotomy (self-amputation), coined by Frédéricq (1883), is the ability of animals to loose anatomically well defined body parts. In reptiles, foremost lizards and salamanders, autotomy of the tail takes place at predicted break points (autotomy plane) to escape from enemies. Until now, these autotomy planes have been described with histological and conventional radiographic methods only. Micro-CT produced high resolution (15 µm voxel size) images clearly showing the autotomy plane in the 3D reconstructions as well as in 2D slices. Micro-CT proved to be of high value for the examination of the 3D position of autotomy planes in tail vertebra of different reptile species. Therefore, µCT will also be useful for the evaluation of 3D bone microarchitecture in other biological research.
µCT for the assessment of bone microarchitecture.

**MATERIAL AND METHODS**

We examined the tails of two Australian reptiles, Bassiana duperrey and Christinus marmoratus. Both animals were found dead and stored as whole in 100% ethanol. Each reptile was put as a whole in a sample holder (30.7 mm diameter and 8 cm length) and placed in the µCT scanner (µCT 40, Scanco Medical, Bassersdorf, Switzerland). The scanning was performed at the high resolution mode (50 kVp, 300 ms integration time, 5-fold frame averaging) resulting in an isotropic voxel size of 15 µm. After reconstruction image pre-processing was performed as described by Rüegsegger et al. (1996). In short, a 3D Gaussian filter (filter width 1.2, support 1) was applied to suppress the noise. Afterwards a global threshold of 22.4% of maximal grey value, the standard threshold for the segmentation of bone, was applied to the images. According to the scout view, the scan was performed at the 3rd to 5th postpygal vertebra.

**RESULTS**

The segmented µCT images show not only the bony vertebral column but also parts of the osteoderm (Fig. 1a). Improved visualisation of the osteoderm is possible by adapting the segmentation threshold.

After cutting away the outer layers of the image containing the osteoderm, the surface of the vertebral column is clearly visible. As it is possible to turn the image on the screen freely, the vertebrae can be inspected from all sides. The autotomy planes show up as thin lines (Fig 1b, 2a). A virtual longitudinal cut through the vertebrae allows e.g. looking at the autotomy planes from the inside (Fig. 1c, 2b).

The vertebral column of Bassiana duperrey shows transverse processes that are split at the base (Fig. 1b). The autotomy planes run through the transverse processes, splitting them in a small proximal and a larger distal part. On the lateral side of the vertebral arch the autotomy planes widen to an aperture. This is easiest to uncover, when viewed from the inside of the neural channel (Fig. 1c, arrows). The autotomy plane cannot be detected in the internal trabecular structure of the vertebral body (Fig 1c, circle).

The transverse processes of Christinus marmoratus are also split, but the autotomy planes run completely distal of them (Fig. 1a). The neural arch also shows lateral openings. Viewed from the inside (Fig. 2b), the autotomy planes are much more prominent than in Bassiana duperrey.

The autotomy planes are also clearly visible in the 2D images (Fig. 2c, arrows). Figure 2c is an oblique slice, so that different areas are visualized in the proximal and distal vertebra. The grey value images also show that the bone next to the dark line of the autotomy plane is of lower density.

**Figure 1**

Figure 1a: 3D µCT reconstruction of Bassiana duperrey tail. Not only the vertebral column is visible, but also part of the osteoderm. The black bar indicates 1 mm.
Figure 2
Figure 1b: 3D μCT reconstruction of Bassina duperrey caudal vertebral column. The transverse processus is split at its base (large arrow). These splits vanish distally. Clearly visible are the autotomy planes (small arrows). The autotomy plane devide the transverse process in a small proximal and a large distal part. The white arrows point at horizontal lines that are caused by the measurement in stacks (μCT imaging artefact). The black bar indicates 1 mm.
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Figure 3
Figure 1c: Transversal cut through Bassina duperrey caudal vertebral column. Note the lateral apertures of the vertebral channel which is part of the fracture plane (arrows). The vertebral bodies show no autotomy planes in their inner structure (circles). The black bar indicates 1 mm.

Figure 4
Figure 2a: 3D µCT reconstruction of Christinus marmoratus vertebral column. The arrow points at the autotomy plane that runs distal of the transverse process. The black bar indicates 1 mm.
Figure 5
Figure 2b: 3D µCT reconstruction of dorsal half of Christinus marmoratus caudal vertebrae, view from inside. At the autotomy plane gaps in the bone are visible (arrows). The black bar indicates 1 mm.
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DISCUSSION

In the present study we used tail vertebra of autotomizing lizards to test the applicability of µCT for the 3D microarchitectural evaluation of very small bones. Histological examinations of such bones are very time consuming and it is difficult to evaluate three-dimensional structures. Further the specimen is destroyed during the preparation and can not be used for further examinations afterwards. This might be critical, when only single specimens of a rare species are available. Compared to X-ray examinations a big advantage of CT is that overlaying structures, such as the osteoderm in our sample (Fig. 1a) would blur X-ray images (Etheridge 1967), while with µCT they are imaged as separate structures that do no superimpose the bone of interest and can be cut away virtually (Fig 1b).

Of both species we examined, no data are available concerning the autotomy planes. Our results obtained by µCT are comparable to those described in earlier studies about different species of the families our specimens belong to. In Scincidae the autotomy planes typically split the transverse processes (Etheridge 1967; Bellairs and Bryant 1985). In Gecconidae the autotomy planes lie distal of the transverse processes (Etheridge 1967; Bellairs and Bryant 1985). Micro-CT proved to be of high value for the 3D evaluation of autotomy planes in reptile tales. The high resolution of the images (15 µm voxel size in our study) and the 3D representations make it easy to illustrate the autotomy plane from different angles. Further, it is possible to get information about the adaptation of the blood vessel to autotomy when combining µCT with casting methods (Heinzer et al. 2006; Krucker et al. 2006).

In conclusion, µCT proved to be of high value for the 2D and 3D microarchitectural examination of small bones. It allows the fast high resolution imaging of the internal structures of such bones and provides tools for the qualitative and quantitative examination. As the examination is not destructive, the specimen is available for further investigations afterwards. This might be of high relevance for the investigation of rare fossil and historic specimens.

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References


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