X-ray microtomography in herpetological research: a review

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Abstract. Herpetological research, like any other (palaeo)biological science, relies heavily on accurate data collection, particularly visualisation and quantification of anatomical features. While several high-resolution imaging methods are currently available, one technique in particular, x-ray microtomography or micro-computed tomography, is on the verge of revolutionising our understanding of the morphology of amphibians and reptiles. Here, we present a review on the prevalence and trends of x-ray microtomography in herpetological studies carried out over the last two decades. We describe its current use, provide practical guidelines for future research that focusses on the morphological study of reptiles and amphibians, and highlight emerging trends including soft-tissue and *in vivo* scanning. Furthermore, while x-ray microtomography is a rapidly evolving field with great potential, various important drawbacks are associated with its use, including sample size effect and measurement errors resulting from differences in spatial resolution and preparation techniques. By providing recommendations to overcome these hurdles, we ultimately aim to maximise the benefits of x-ray microtomography to herpetological research.

Keywords: μCT, anatomy, imaging technique, micro-computed tomography, micro-CT scanning, morphology, staining.

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Supplementary material

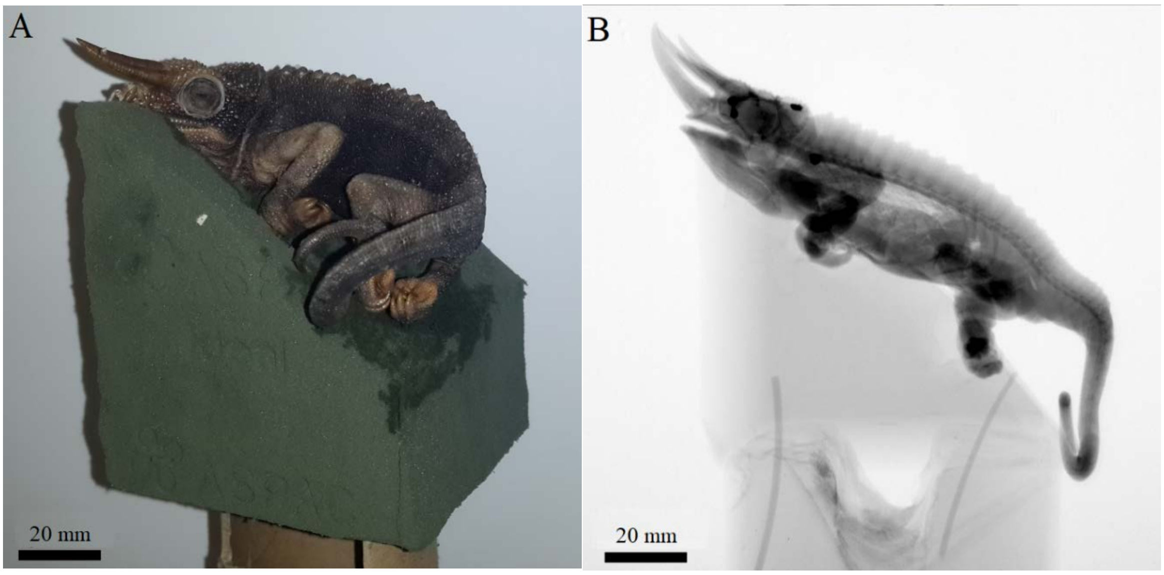

Figure S1. Mounting of a formalin-fixed, ethanol-preserved *Trioceros jacksonii* specimen. The sample was air-dried until the skin was dry-to-touch and mounted on floral foam at slight angle. The two-dimensional x-ray projection image on the right shows the low-density nature of the mounting material. From du Plessis et al. (2017b) under a CC BY 4.0 licence.

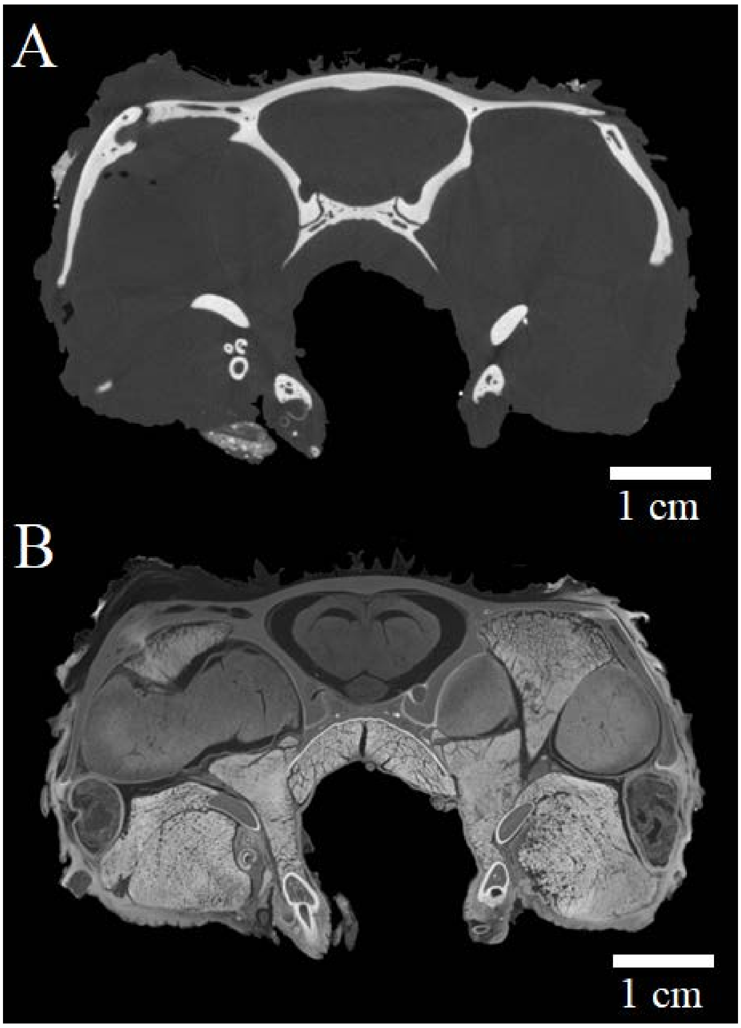

Figure S2. Image illustrating the differences between an unstained section (A) and section stained with PTA (B) through the head of *Bitis nasicornis*.

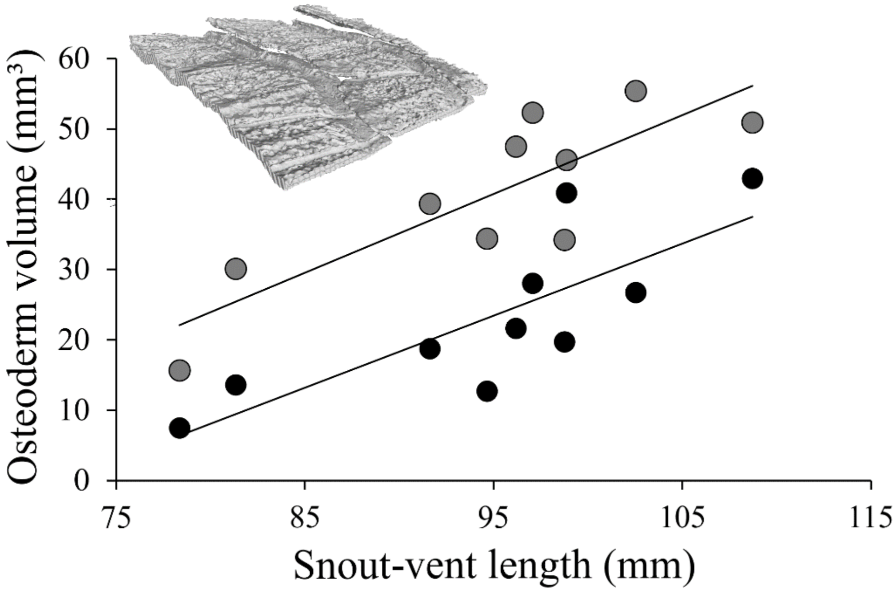

Figure S3. Graph showing the relationship between osteoderm volume and body size in *Ouroborus cataphractus* and demonstrate the effect of spatial resolution on measurement error. The osteoderm volumes of 1 cm^3 osteoderm region-of-interests (illustrated in the top left corner) extracted from scans obtained at 100 μm resolution (grey circles) are nearly double those from scans obtained at 35 μm resolution (black circles), indicating that low-resolution scans can greatly overestimate actual structure sizes.

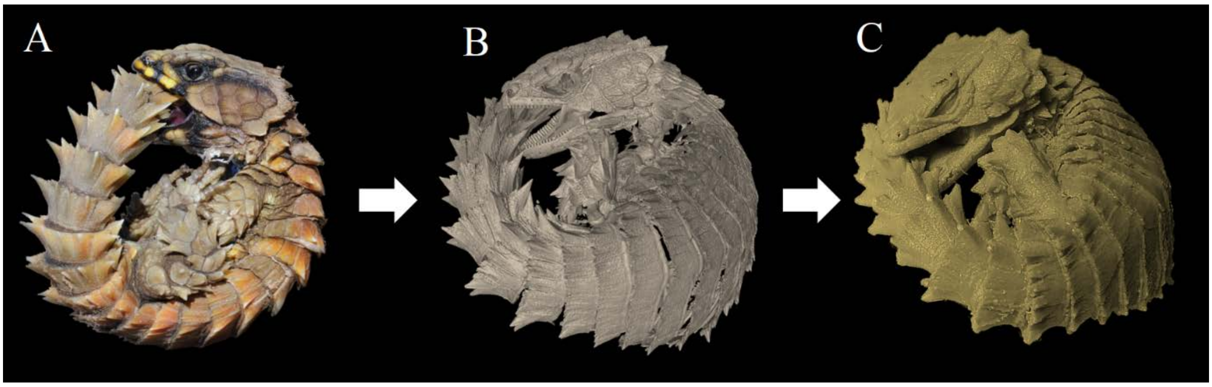

Figure S4. Flow diagram illustrating a potential use of microtomography scans for educational purposes. A. Photograph of *Ouroborus cataphractus* displaying defensive tail-biting behaviour. B. Three-dimensionally rendered image of a similar individual displaying the same behaviour and scanned using *in vivo* x-ray microtomography following Broeckhoven et al. (2017c). C. Three-dimensional print of the virtual model obtained from (B). Photograph (A) Copyright © le Fras Mouton.