Funtional and evolutionary morphology of amphibians: state of art insights using synchrotron based high resolution X-ray computed microtomography

S. Kühnel[1], T. Kleinteich[2], S. Reinhard[1], S. Schweiger[1], F. Beckmann[3], H. Müller[1] and A. Kupfer[1,4]

[1] *Institut für Spezielle Zoologie und Evolutionsbiologie mit Phyletischem Museum, Friedrich-Schiller-Universität Jena, Erbertstr. 1, 07743 Jena, Germany*

[2] *Department of Zoology – Functional Morphology and Biomechanics, Christian-Albrechts Universität Kiel, Am Botanischen Garten 1-9, 24098 Kiel, Germany*

[3] *GKSS Research Center, Max-Planck-Str. 121502 Geesthacht, Germany*

[4] *Institut für Biochemie und Biologie, Universität Potsdam, Karl-Liebknecht-Str. 24-25, Haus 26. 14476 Potsdam, Germany*

A comparative approach facilitates our understanding of the evolutionary morphology and history of complex and novel structures within organisms. By using synchrotron based high resolution X-ray computed microtomography (μCT) in the field of comparative evolutionary biology, insights into complex novel structures and function and their evolutionary history are virtually unlimited (for recent examples see [1, 2, 3, 4, 5]).

We employ μCT imaging to elucidate mainly (1) the role of genital morphology in the evolution of reproductive modes and (2) the functional morphology of the skull in a particular group of amphibians, the caecilians or Gymnophiona (but see also previous DESY-projects on caecilian head and genital morphology [6, 7, 8]). Caecilian amphibians are limbless, fossorial vertebrates inhabiting a diverse array of tropical habitats.

In a project on the evolution of the solid skull in caecilians (Gymnophiona), we used synchrotron based x-ray μCT imaging to produce detailed 3D computer surfaces of caecilian skulls (Fig. 1A-D). The shape of the resulting surfaces was then interactively modified to simulate different scenarios in the evolution of a solid skull roof [9]. Original and modified 3D computer surfaces were subject to a finite element analysis in which a load was applied to the rostral region of the skulls to simulate frontal forces that caecilians encounter during digging (Fig. 1C,D). Surprisingly, the effects of temporal fenestration on the stress distribution were negligible, suggesting that frontal loading may not have been the driving force in the evolution of a solid skull.

One project is focussed on the morphology of genital structures in caecilian amphibians to reveal potential correlations between specific morphologies and reproductive modes in an evolutionary framework. The resulting datasets of genitalia generated at beamline BW2 (see Fig 1E) show highest possible detailing in soft tissues. The resolution of the μCT data (voxel-sizes) ranges from 2 to 9 μm, depending on sample dimensions. Single muscle fibers, nerves, and connective tissues can be easily identified in the images. Such richness in detail of vertebrate genital structures, especially within soft tissues, has never been seen before for any vertebrate. For interspecific comparisons and better understanding of the evolutionary diversity of caecilian genitalia, μCT data on additional caecilian species will be explored in future experiments at beamline BW2.

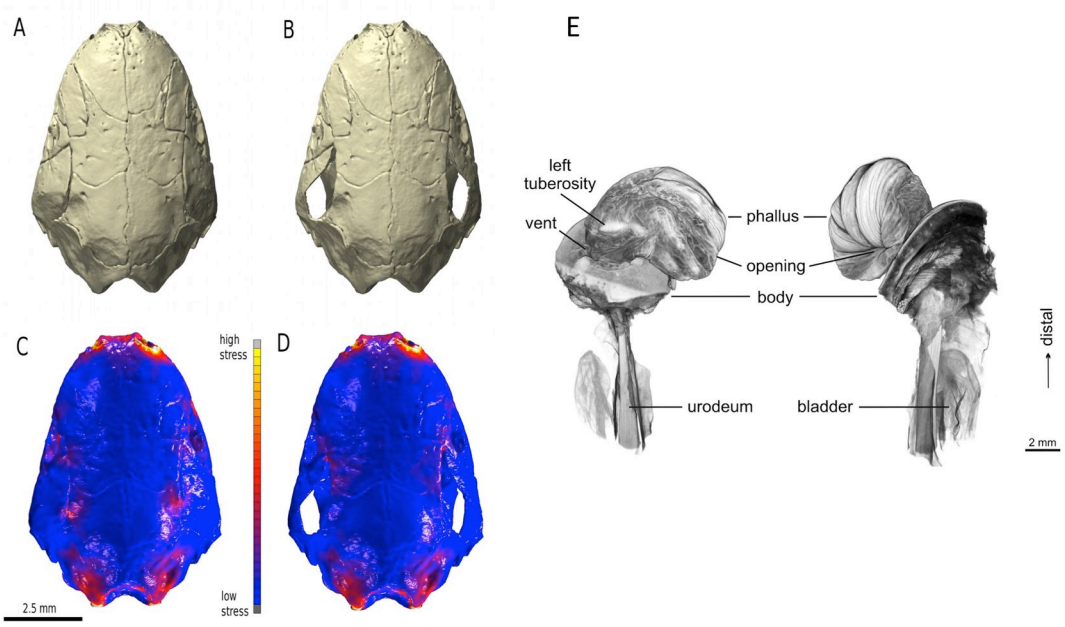

Fig. 1: SRμCT imaging of the functional analysis of the skull and aspects of genital morphology of caecilian amphibians. A: surface rendering of the skull in *Ichthyophis* cf. *kohtaoensis* (Gymnophiona) in dorsal view. The surface is based on synchrotron μCT data gathered at W2. B: to explore the functional significance of the solid skull, we prepared a modified 3D computer surface by fenestrating the temporal region. D, E: results of finite element analysis of the original and modified skull shapes. F: Intromittent organ of *Boulengerula uluguruensis* illustrating frontal and lateral views of the everted male cloaca (lower part represents the urodeum and enlarged upper part the ornamented phallodeum) scanned at BW2. The phallus is inserted into the female vent during copulation.

Several University of Jena graduate students (Susan Schweiger, Nadine Pömpner and Constanze Sänger) gathered μCT imaging data of vertebrates at beamline BW2 and W2 to study the functional morphology of salamander and frog skulls. Results based on μCT imaging of vertebrates produced at DESY were recently presented orally on various international scientific meetings such as the biannual meeting of the European Society for Evolutionary Biology (ESEB) in Tübingen 2011 or the Biosystematics meeting in Berlin 2011. Future presentations in 2012 are planned for the World Congress of Herpetology (WCH7) in Vancouver, Canada and at national meetings such as the annual meeting of the German Zoological Society (DZG).

References

[1] O. Betz, U. Wegst, D. Weide, et al., J. Microsc. **227**, 51–71 (2007)
[2] M. Nickel, T. Donath, M. Schweikert, et al., Zoomorph. **125**, 209-223 (2006)
[3] J. Hammel, J. Herzen, F. Beckmann, et al., Frontiers Zool. **6**, 19 (2009)
[4] M.W. Westneat, J.J. Socha, W.-K. Lee, Ann. Rev. Physiol. **70**, 119-142 (2008)
[5] A. Pradel, M. Langer, J.G. Maisey et al, Proc. Natl. Acad. Sci. USA **106**, 5224-5228 (2009)
[6] T. Kleinteich, A. Haas, A.P. Summers, J. R. Soc. Interface **5**, 1491-1504 (2008)
[7] S. Kuehnel, S. Reinhard, A. Kupfer, Bonn Zool. Bull. **57**, 119-126 (2010)
[8] S. Kuehnel, J. Herzen, T. Kleinteich et al, Acta Zool. **93**, 208-221 (2012)
[9] T. Kleinteich, H.C. Maddin, J. Herzen et al, J. Exp. Biol. **215**, 833-844 (2012)