Innovative insights into the reproductive morphology of amphibians using synchrotron based high resolution X-ray computed microtomography

S. Kühnel[1], J. Herzen[2], F. Beckmann[2], T. Kleinteich[3,4] and A. Kupfer[1,5]

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

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

[3] Biozentrum Grindel und Zoologisches Museum Universität Hamburg, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany

[4] University of Washington, Friday Harbor Laboratories, 620 University Road, Friday Harbor, WA, 98250, USA

[5] Department Chemie & Biologie, Fachgruppe Integrative Zoologie & Biodiversität, Universität Siegen, Adolf-Reichwein-Str 2, D-57068 Siegen

In order to understand the evolutionary biology of complex and novel structures within organisms a comparative approach is needed. The use of synchrotron based high resolution X-ray computed microtomography (µCT) in the field of comparative evolutionary biology are virtually infinite (for examples see [1], [2], [3]). Synchrotron imaging of a variety of both invertebrates and vertebrates recently gave novel insights into complex structure and function [4]. We employ µCT imaging to elucidate the role genital morphology in the evolution of reproductive modes in a particular group of amphibians; the caecilians or Gymnophiona (but see also previous DESY-projects on caecilian head and genital morphology [4], [5]).

Caecilian amphibians are limbless, fossorial vertebrates inhabiting a diverse array of tropical habitats. Although caecilians only comprise of around 190 species, they show an extraordinary diversity of reproductive modes and parental care strategies (e.g. skin feeding [6]) including oviparity (aka egglaying) and viviparity (aka life-bearing). In comparison to most frogs and salamanders male caecilians possess an evertible copulatory organ, the phallodeum, to ensure internal fertilisation.

The current project is strongly focussed on the morphology of genital structures (see Fig. 1A-B) in various caecilian amphibians representing diverse reproductive modes. Our main objective is to describe complex structures and to reveal any correlations between specific morphologies and reproductive modes in an evolutionary framework.

We aim to include various caecilian species representing all currently recognised reproductive modes in our study (e.g. oviparity with aquatic larva, direct development and viviparity). So far we have gathered µCT imaging-data on the cloacal morphology of both sexes of oviparous *Ichthyophis* cf. *kohtaoensis*, and everted male phalli of viviparous *Geotryptes seraphini* and *Typhlonectes natans* (Fig. 1A-B). The data has been yielded at beamline BW2 in a 48 hrs timeframe.

The resulting datasets of genitalia 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 produced for any vertebrate (see µCT scans of skeletons and references in [7]).

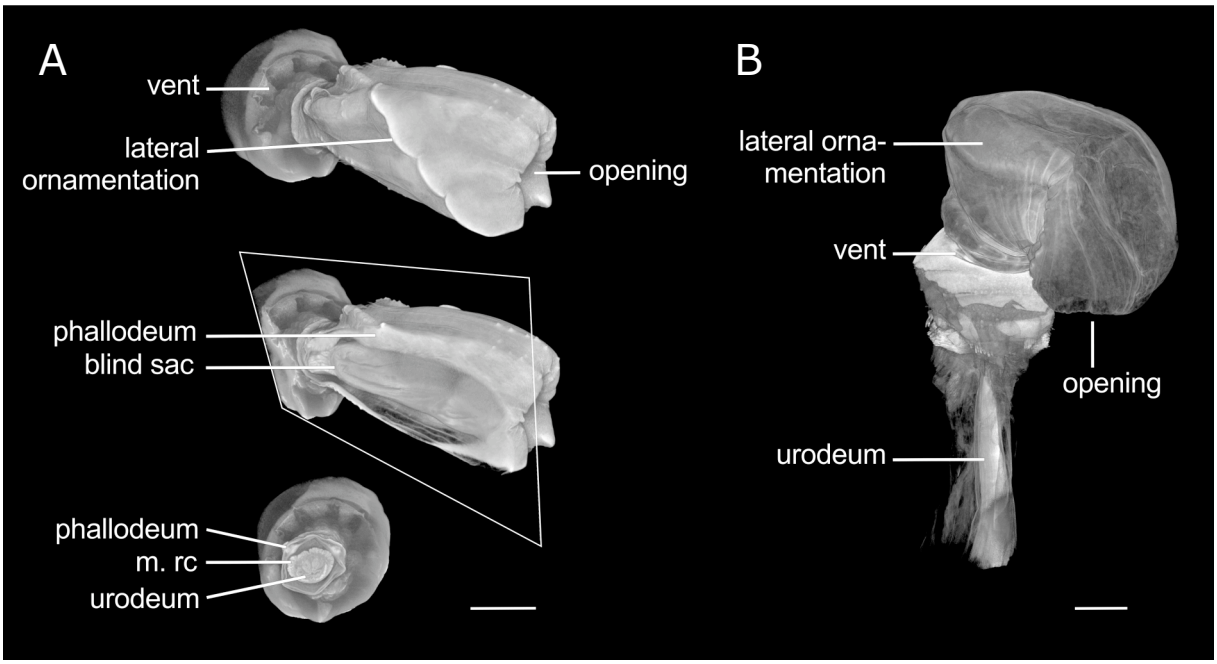

Figure 1: SRµCT data on the reproductive morphology of caecilian amphibians. A: *Typhlonectes natans* (Typhlonectidae), everted male cloaca illustrating layers, B: *Geotrypetes seraphini* (Caeciliidae), everted male cloaca. 3D modelling based on data gathered via non-invasive SRµCT scanning. Scale bar 2 mm.

Another surplus of SRµCT imaging is that the proportions of the complex-build male cloaca are revealed in full detail (Fig.1 A-B) and specific tissues could be easily visualised separately using colour coding. For interspecific comparisons and better understanding of the evolutionary diversity of amphibian genitalia µCT data on further caecilian species and the intromittent organ of the anuran *Ascaphus truei* will be explored in future experiments at beamline BW2.

Results based on µCT imaging produced at DESY were recently presented orally at international scientific meetings such as the International Congress of Vertebrate Morphology (ICVM-9) in Uruguay 2010, the Biosystematics meeting in Berlin 2011 and future presentations are planned for the biannual meeting of the European Society for Evolutionary Biology. In addition further contributions were made at national meetings such as the Annual meeting of the German Zoological Society. First results on the genital morphology of caecilian amphibians featuring SRµCT have been published [6, 9].

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] T. Kleinteich, A. Summers, A. Haas, J. R. Soc. Interface (2008)
[6] S. Kühnel, J. Herzen, T. Kleinteich, et al., Acta Zool. (2011)
[7] A. Kupfer, H. Müller, M.M. Antoniazzi, et al., Nature 440, 926–929 (2006)
[8] Digital Morphology Group, University of Texas, http://www.digimorph.org (2007)
[9] S. Kühnel, S. Reinhard, A. Kupfer, Bonn Zool. Bull. 57, 119-126 (2010)