ESRF	**Experiment title:** **Origins and dynamics of evolutionary modules in the mammalian skull: a deep time perspective from non-mammalian therapsids**	**Experiment number**: HG-23
Beamline: ID17	**Date of experiment**: from: 12.06.2014 to: 14.06.2014	**Date of report**: 27.02.2017
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Report:

Synapsida, the clade that ultimately led to the evolution of mammals, is traditionally subdivided into a basal grade also known as "pelycosaurs" and the more mammal-like clade called Therapsida. By understanding organismal design, with modularity as a hypothesis, we also understand the evolution of a group that experienced profound anatomical transformations throughout the past 270 Ma, eventually leading to the evolution of the mammalian bauplan. Defects on skull phenotypic modularity are known to cause lethal dysmorphologies in humans, such as craniosynostosis (premature development of cranial sutures). This defect has been proposed to have its origins in the early stages of therapsid evolution.

It is aimed to access with exceptional detail the anatomy of selected major therapsid groups. Therefore, we are using synchrotron radiation-based micro-computed tomography (SRµCT) to reveal the morphological traits encumbered by the fossils rock matrix. Anatomical description provides the primary data for subsequent interpretation and inferences. Thus, a thorough description of the non-mammalian therapsid osteology provides the essential data for understanding evolutionary relationships among groups, potentiates serendipitous discoveries of key anatomical novelties (e.g., turbinals) and is the primary source of information for understanding evolutionary modules.

EXPERIMENTAL

The fossils (Tab. 1) scanned at the ID17 beamline using Propagation Phase Contrast SRµCT (Fig. 1) can be consulted at the Institut und Museum für Geologie und Paläontologie, Eberhard Karls Universität, Tübingen, Germany.

Fossil designation:	Attributed to:	Bonv structure:	Publication	Recovered from:
GPIT/RE/7139	*Silphoictoides ruhuhuensis*	Skull	Huene 1950 fig.43	L Perm, Kingori, Tanzania
GPIT/RE/7125	*Galerhinus rubigdei*	Skull	Huene 1950 S.99	L Perm, Kingori, Tanzania
GPIT/RE/7124	*Aloposaurus gracilis*	Skull	Huene 1938 Tab.22	Late Perm, Karroo, RSA
GPIT/RE/9272	*Cistocephalus planiceps*	Skull	Huene 1942 S.168	Late Perm, Tanzania
GPIT/RE/9272	*Cistocephalus planiceps*	Mandible	Orig. v. Huene 1942 S.168	Late Perm, Tanzania
GPIT/RE/7119	*Dixeia nasuta*	Skull	Huene 1950	L Perm, Kingori, Tanzania
GPIT/RE/9275	*Dicynodon megalorhinus*	Skull	Huene 1931, fig.24	Middle Perm, Karroo, RSA

Table 1 – Fossil specimens studied at ID17.

X-RAY TOMOGRAPHY: propagation phase contrast microtomography (11 m sample-detector distance); 150 keV double bent Laue monochromator.

OPTIC: tapered scintillating fiber optic; 0.3x lens; FReLoN-2k camera (46.5 microns isotropic voxel size).

ACQUISITION: 4998 projections of 0.2 sec each; 30% overlap of consecutive scans along the Z-axis to correct for vertical beam profile.

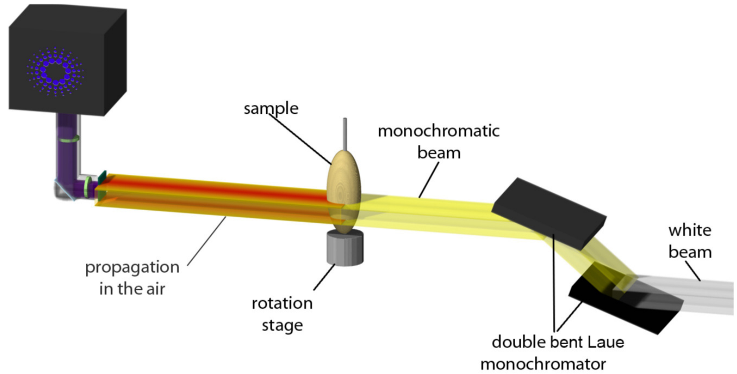

Figure 1 – ID17 beamline X-ray tomography setup.

RECONSTRUCTION: filtered back projection with single distance phase retrieval Paganin approach [1,2].

POST-PROCESSING: stack of 32 bits EDF converted to 16 bits TIF; vertical stitching; ring correction.

RESULTS

Tomographic data collected at ID17 allow us to investigate, within a phylogenetic context, the origins and dynamics of cranial modules during a pivotal evolutionary transition that shaped vertebrate life onwards: the therapsid-mammalian transition. Anatomical descriptions and interpretations of the specimens have been written as segmentation proceeds. This provides a basis for discussion with colleagues about dubious aspects of the anatomy. Preliminary phylogenetic analysis is also being performed, because it allows independent assessment of characters to avoid errors and provides a preliminary anatomical investigation. A final phylogenetic analysis will be performed once all segmentation work is finished and the data matrix is completely coded.

Each segmented specimen is a relatively independent project, thus individual manuscripts can be submitted along the overarching project on the evolutionary modules [3-5]. An example is the manuscript in press titled [3]: "Aspects of gorgonopsian paleobiology and evolution: insights from the basicranium, occiput, osseous labyrinth, vasculature, and neuroanatomy". In this manuscript, we explored the braincase anatomy and rendered various skull cavities of two gorgonopsian specimens, GPIT/RE/7119 and GPIT/RE/7124 (Fig. 2), but we also used other comparative material (e.g. GPIT/RE/7139). Notably, we found that there is a separate ossification between what was previously referred to as the "parasphenoid" and the basioccipital. We showed that the previously called "parasphenoid" is in fact the co-ossification of the dermal parasphenoid and the endochondral basipresphenoid. The orientation of the horizontal semicircular canal suggests that gorgonopsians had an anteriorly tilted alert head posture. The morphology of the brain endocast is in accordance with the more reptilian endocast shape of other non-mammaliaform neotherapsids.

Data will be available in the ESRF once the study is fully published.

The highly visual content that are produced (e.g., 3D reconstructions, 3D pdf, video animations) offer an introductory platform for deeper scientific topics such as pre-mammalian evolution, anatomy, skull modularity, taxonomy, physiology, or phylogeny.

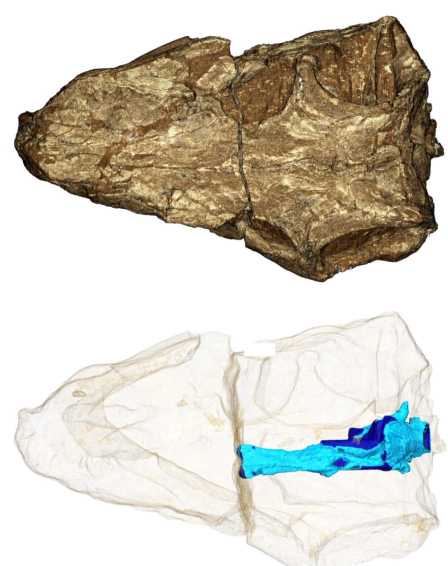

Figure 2 - 3D rendering of GPIT/RE/7124 skull in dorsal (top), and semi-transparent rendering of the skull with endocast (bottom).

References

[1] Mirone et al. (2014) *Nucl. Instr. Meth. Phys. Res. B 324, 41-48.*
[2] Paganin et al. (2002) *J. Microsc.* 206, 33-40.
[3] Araújo et al. (2017) *PeerJ* (https://doi.org/10.7287/peerj.preprints.2313v2)
[4] Araújo et al. (2017) *Plos One* (submitted).
[5] Araújo et al. (2017) *Nat. Commun.* (in preparation).