EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:
https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report"),*
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Early diversification of Sauria	**Experiment number**:
Beamline: ID19/BM05	**Date of experiment**: from: 29 September 2018 to: 01 October 2018	**Date of report**: August 25, 2021
Shifts: 6	**Local contact(s)**: Paul Tafforeau Vincent Fernandez	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Jonah N Choiniere, University of the Witwatersrand

David M Ford, University of Oxford

Vincent Fernandez, Natural History Museum, London

Report:

In this experiment, we scanned eight total skulls of members of a variety of extinct South African parareptile genera, including: *Owenetta*, *Milleretta*, *Milleropsis*, *Youngina*, *Prolacerta*, and *Paliguana*. Our main objectives were to use the anatomical information from these scans to develop comparative anatomical descriptions and character hypotheses for phylogenetic analysis. The overarching goal of these objectives was to better understand the early evolution and diversification of the Sauria, the group that includes all living reptiles and their extinct relatives.

The transport, mounting, and scanning of the specimens proceeded without incident, and we obtained excellent results in the time alloted, imaging all of the specimens we brought to the ESRF. Many of the specimens contained dense metallic oxide inclusions, as is typical for South African palaeontological specimens, and the reconstruction of the data were time-consuming because of the challenges of reducing artifacts produced by the inclusions. However, reconstructed data were completely delivered to the lead and other experiment participants approximately 4 months from the date of scanning.

As of this writing, this experiment has produced two research papers, a third-year student project, part of a PhD thesis, and research for a postdoctoral fellow at the ESRF. I expand on these results below.

Paliguana whitei: the skull of this specimen was fully segmented by participant David P Ford and analyzed as part of his postdoctoral fellowship at the University of the Witwatersrand. The outcome of the study definitively shows that *Paliguana* is one of the earliest-branching lepidosauromorphs (the group that gives rise to living lizards and snakes) and gives clarity to

previously poorly known, or completely unknown anatomy of the species. These findings were published in 2021 in *Proceedings of the Royal Society, Series B*.

Ford, D., Evans, S., Choiniere, J., Fernandez, V., & Benson, R. B. J. (2021). A reassessment of the enigmatic diapsid Paliguana whitei and the early history of Lepidosauromorpha. Proceedings of the Royal Society B: Biological Sciences.

Lepidosaurs include lizards, snakes, amphisbaenians and the tuatara, comprising a highly speciose evolutionary radiation with widely varying anatomical traits. Their stem-lineage originated by the late middle Permian 259 million years ago, but its early fossil record is poorly documented, obscuring the origins of key anatomical and functional traits of the group. Paliguana whitei, from the Early Triassic of South Africa, is an enigmatic fossil species with potential to provide information on this. However, its anatomy and phylogenetic affinities remain highly uncertain, and have been debated since its discovery more than 100 years ago. We present microtomographic 3D imaging of the cranial anatomy of Paliguana whitei that clarifies these uncertainties, providing strong evidence for lepidosauromorph affinities based on the structure of the temporal region and the implantation of marginal dentition. Phylogenetic analysis including these new data recovers Paliguana as the earliest known stem-lepidosaur, within a long-lived group of early-diverging lepidosauromorphs that persisted to at least the Middle Jurassic. Our results provide insights into cranial evolution on the lepidosaur stem-lineage, confirming that characteristics of pleurodont dental implantation evolved early on the lepidosaur stem-lineage. In contrast, key functional traits related to hearing (quadrate conch) and feeding (streptostyly) evolved later in the lepidosaur crown-group.

Milleretta and *Milleropsis*: skulls of the holotype of *Milleropsis* and referred *Milleretta* specimens are currently under study by PhD candidate Xavier Jenkins of Idaho State University under the supervision of Jonah Choiniere, Roger Benson and Brandon Peecook. Two of the four skulls have been fully segmented.

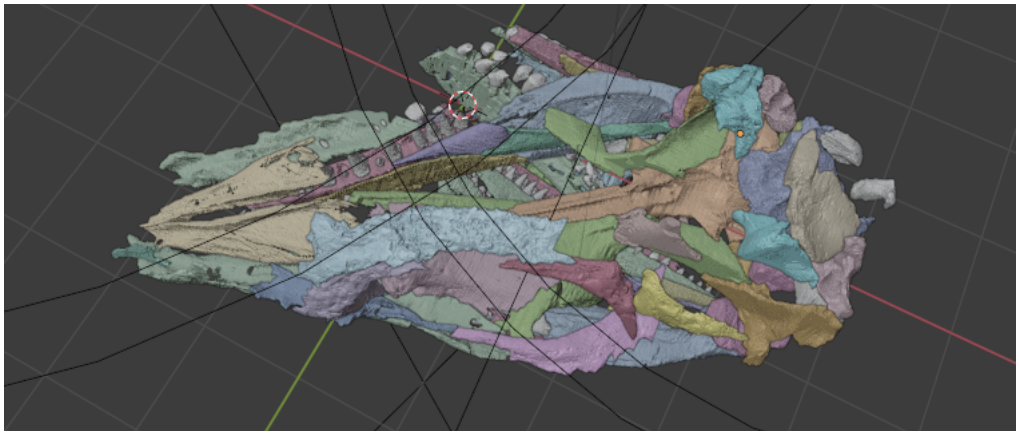

Fig. 1. Screen shot showing segmentation progress on BP-1-720

Youngina: a single skull of this taxon was fully segmented by 3rd-year student Annabel Hunt, who produced a research report for the University of Oxford entitled "**A description of the mandible and palate of *Youngina capensis* (Sauropsida, Diapsida) and the phylogenetic implications".** Ms Hunt, along with Roger Benson and Jonah Choiniere are working on developing this research project into a peer-reviewed publication.

Prolacerta: Information from the skull of *Prolacerta* , mainly comprising shape and size data from the endosseous labyrinth of the inner ear, were used as part of the following collaborative paper.

Bronzati, M., Benson, R. B. J., Evers, S. W., Ezcurra, M. D., Cabreira, S. F., Choiniere, J., . . . Nesbitt, S. J. (2021). Deep evolutionary diversification of semicircular canals in archosaurs. *Current Biology, 31*(12), 2520-2529.e2526. doi:https://doi.org/10.1016/j.cub.2021.03.086

Living archosaurs (birds and crocodylians) have disparate locomotor strategies that evolved since their divergence ~250 mya. Little is known about the early evolution of the sensory structures that are coupled with these changes, mostly due to limited sampling of early fossils on key stem lineages. In particular, the morphology of the semicircular canals (SCCs) of the endosseous labyrinth has a long-hypothesized relationship with locomotion. Here, we analyze SCC shapes and sizes of living and extinct archosaurs encompassing diverse locomotor habits, including bipedal, semi-aquatic, and flying taxa. We test form-function hypotheses of the SCCs and chronicle their evolution during deep archosaurian divergences. We find that SCC shape is statistically associated with both flight and bipedalism. However, this shape variation is small and is more likely explained by changes in braincase geometry than by locomotor changes. We demonstrate high disparity of both shape and size among stem-archosaurs and a deep divergence of SCC morphologies at the bird–crocodylian split. Stem-crocodylians exhibit diverse morphologies, including aspects also present in birds and distinct from other reptiles. Therefore, extant crocodylian SCC morphologies do not reflect retention of a "primitive" reptilian condition. Key aspects of bird SCC morphology that hitherto were interpreted as flight related, including large SCC size and enhanced sensitivity, appeared early on the bird stem-lineage in non-flying dinosaur precursors. Taken together, our results indicate a deep divergence of SCC traits at the bird–crocodylian split and that living archosaurs evolved from an early radiation with high sensory diversity.

Owenetta: Scans of the skulls of this taxon are currently under study by Kathleen Dollman, ESRF Palaeosciences Postdoctoral Fellow.

In total, I anticipate that the research on *Youngina* will be complete by early 2022, with publication by the end of that year. Research on *Milleretta* and *Milleropsis* will continue until 2025 and the completion of Xavier Jenkins' PhD, but we will publish results before that time. Research on *Owenetta* will be completed by end-2022, with a 2023 target publication date.