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

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

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.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal 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:** Investigation of 251 Million year old burrow casts containing fossorial ancestral mammals	**Experiment number**: EC 847
Beamline: ID17	**Date of experiment**: from: 9/07/2011 to: 12/07/2011	**Date of report**:
Shifts: 9	**Local contact(s)**: Paul Tafforeau	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. Kristian Carlson

Dr. Vincent Fernandez *

Dr. Paul Tafforeau *

Dr. Nestor Abdala

Report:

1. Aim of the proposal

In March 2011, my colleagues and I submitted a proposal to image the content of two burrow casts from the Early Triassic of South Africa (ca. 250 Million years old). The first aim of the experiment was to produce accurate 3 dimensional (3D) data to validate the identity of the enclosed skeleton. We then aimed to use these data in various projects related to the biology of the occupant of the burrows.

2. Current status of the project

In July 2011, we successfully scanned the two burrow casts described from the proposal. The characteristic of the synchrotron beam allowed a much better visualisation of the enclosed skeletal remains compared to the previous attempts with conventional and medical tomographs. The scan of one of the specimen (referred to as BP/1/5558) unexpectedly revealed the presence of a second skeleton in the burrow cast that modified the research plan as taxa aggregated inside burrows from the fossil records are extremely rare.

2.1 Description of the association of two skeletons in the burrow BP/1/5558.

After analyse of the images, we identified the two specimens: the first one is a complete skeleton of a mammal-like reptile (mammal forerunners) Thrinaxodon liorhinus; the second one is a complete skeleton of a temnospondyle amphibian Broomistega putterilli. We focussed our research on the association of these two completely unrelated animals, which

was the first occurrence of this kind from the fossil record. The results of this study have been presented during a conference (Fernandez *et al.*, 2012) and have been published in PLOS ONE (Fernandez *et al.*, 2013).

Figure 13D rendering of the scanned burrow in transparent and the two discovered skeletons Thrinaxodon (in brown) and Broomistega (in white).

2.2 Ontogeny of Thrinaxodon liorhinus

The data from both scans were incorporated in a long term project aiming to describe in details the morphological modification occurring during the development of Thrinaxodon. About 50 specimens representing different developmental stages were studied; 5 of them were scanned using conventional laboratory X-ray microtomography; 3 of them were scanned using synchrotron based microtomography (two during this experiment, one from a previous experiment). The specimens scanned using synchrotron based microtomography represented key individuals of the ontogenetic series with the smallest and largest and a mid-sized individual. The first part of the project focused on the morphology and replacement pattern of teeth. The results were presented during a conference (Abdala *et al.*, 2012) and have recently been approved for publication in the Journal of Vertebrate Paleontology (Abdala *et al.*, In press).

The second part of the project focuses on the variation in morphology of skull bones during ontogeny. A manuscript presenting the result on this study is currently in preparation and should be submitted to the Journal of Vertebrate Paleontology by the end of the year.

2.3 Digging adaptation in Thrinaxodon liorhnius.

Thrinaxodon liorhinus was discovered twice enclosed in a burrow. Additionally several burrow cast presenting similar dimensions have been discovered in Southern Africa and Antarctica, from the same time period. While it seems that Thrinaxodon was an active digger, an adaptation that was important to escape the harsh climatic condition following the Permo-Triassic mass extinction event, little is known about how this animal was digging burrows. The scan from the specimen BP/1/5558 offer the first complete limb of this animal as well as the opportunity to virtually separate each bones of the paws to finally understand their structures. Preliminary results of this study were presented during a conference (Carlson

et al., 2012). Kristian Carlson and Fernando Abdala are currently supervising an honour student at the University of the Witwatersrand who is studying the digging abilities of this animal. The results will result in a honour thesis first and should also be presented to a peer review journal.

2.4 Anatomy of the Temnospondyl Amphibian Broomistega Putterilli.

The specimen of Broomistega that was discovered in the burrow BP/1/5558 is the first complete individual from the fossil record. In fact other specimens are only known from partial portion of the skull. A complete description of the anatomy of Broomistega has started and a manuscript is currently being prepared. This manuscript should provide the most complete description of a temnospondyle as internal structures are usually not visible.

3. Future work plan

3.1 Ontogeny of Thrinaxodon liorhinus

A third project using the synchrotron data will start in 2014, focussing on the morphological modification of the mandible during the development of Thrinaxodon. This project was originally planned to be included in the study of the skull but as the amount of data collected on the scans is more important than expected, it was decided to separate information into two projects. The writing of the manuscript will start once the article focusing on the skull will be finished and submitted for publication.

Current list of publications in journals and conferences

Abdala, F., Jasinoski, S. C., & Fernandez, V. (**2012**). *Ontogeny of the Early Triassic Thrinaxodon liorhinus (Therapsida, Cynodontia). Dental morphology and replacement*. Paper presented at the 72nd Annual Meeting Society of Vertebrate Paleontology, Raleigh, NC, USA.

Abdala, F., Jasinoski, S. C., & Fernandez, V. (**In press**). Ontogeny of the Early Triassic *Thrinaxodon liorhinus* (Therapsida, Cynodontia). Dental morphology and replacement. *Journal of Vertebrate Paleontology*.

Carlson, K. J., Fernandez, V., Abdala, F., Rubidge, B. S., & Tafforeau, P. (**2012**). *Was Thrinaxodon liorhinus a digger?* Paper presented at the Biennial Conference Palaeontological Society of Southern Africa (PSSA), Cape Town, South Africa.

Fernandez, V., Abdala, F., Carlson, K. J., Collins Cook, D., Rubidge, B. S., Yates, A., & Tafforeau, P. (**2012**). *Synchrotron Radiation Sheds Light On Mammal Forerunners Entombed In Their Burrow*. Paper presented at the Biennial Conference Palaeontological Society of Southern Africa (PSSA), Cape Town, South Africa.

Fernandez, V., Abdala, F., Carlson, K. J., Collins Cook, D., Rubidge, B. S., Yates, A., & Tafforeau, P. (**2013**). Synchrotron Reveals Early Triassic Odd Couple: Injured Amphibian and Aestivating Therapsid Share Burrow. *PLoS ONE, 8*(6), e64978. doi: 10.1371/journal.pone.0064978

Abstract of publications

Fernandez, V., Abdala, F., Carlson, K. J., Collins Cook, D., Rubidge, B. S., Yates, A., & Tafforeau, P. (**2013**). Synchrotron Reveals Early Triassic Odd Couple: Injured Amphibian and Aestivating Therapsid Share Burrow. *PLoS ONE, 8*(6), e64978. doi: 10.1371/journal.pone.0064978

Fossorialism is a beneficial adaptation for brooding, predator avoidance and protection from extreme climate. The abundance of fossilised burrow casts from the Early Triassic of

southern Africa is viewed as a behavioural response by many tetrapods to the harsh conditions following the Permo-Triassic mass-extinction event. However, scarcity of vertebrate remains associated with these burrows leaves many ecological questions unanswered. Synchrotron scanning of a lithified burrow cast from the Early Triassic of the Karoo unveiled a unique mixed-species association: an injured temnospondyl amphibian (*Broomistega*) that sheltered in a burrow occupied by an aestivating therapsid (*Thrinaxodon*). The discovery of this rare rhinesuchid represents the first occurrence in the fossil record of a temnospondyl in a burrow. The amphibian skeleton shows signs of a crushing trauma with partially healed fractures on several consecutive ribs. The presence of a relatively large intruder in what is interpreted to be a*Thrinaxodon* burrow implies that the therapsid tolerated the amphibian's presence. Among possible explanations for such unlikely cohabitation, *Thrinaxodon* aestivation is most plausible, an interpretation supported by the numerous *Thrinaxodon* specimens fossilised in curled-up postures. Recent advances in synchrotron imaging have enabled visualization of the contents of burrow casts, thus providing a novel tool to elucidate not only anatomy but also ecology and biology of ancient tetrapods.

Abdala, F., Jasinoski, S. C., & Fernandez, V. (**In press**). Ontogeny of the Early Triassic *Thrinaxodon liorhinus* (Therapsida, Cynodontia). Dental morphology and replacement. *Journal of Vertebrate Paleontology*.

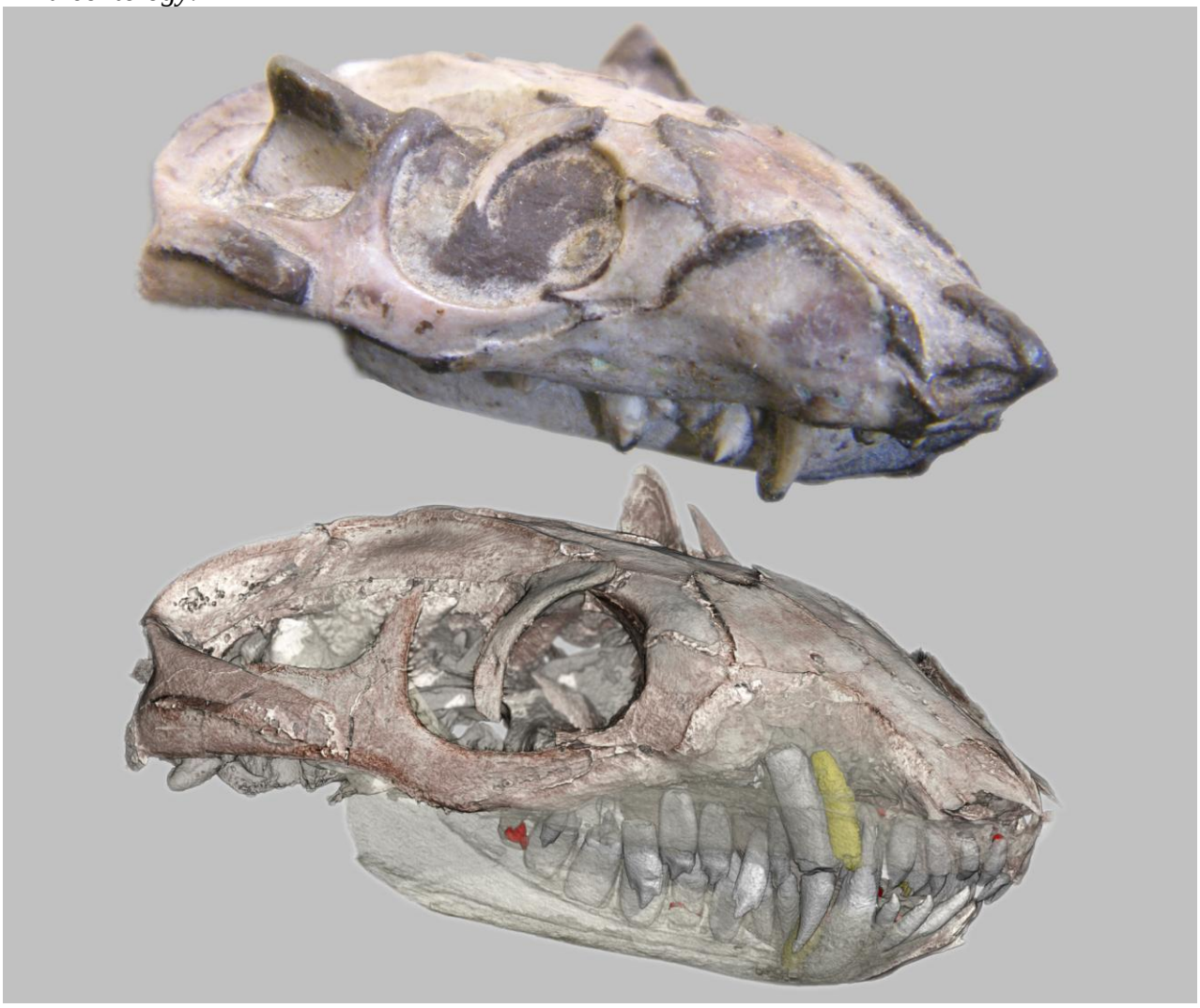

Figure 2- photograph and 3D rendering of a skull of Thrinaxodon showing the information on teeth replacement and morphology provided by synchrotron microtomography.

We present a detailed study on variation of the dental morphology and replacement in the Early Triassic cynodont *Thrinaxodon liorhinus*. For this study we analysed five specimens ranging from 37–87 mm in skull length using micro computed tomography (μCT) scanning techniques, which were supplemented by detailed anatomical analysis of 48 specimens with a basal skull length of 30–96 mm. Our results indicate that lower postcanines are more numerous and present a more complex morphology than the upper postcanines, even in the same individual; only the lower postcanines have more than three sectorial cusps and a cingular collar on the lingual margin. Complexity of the postcanines increases from the smallest individual to specimens of 75 mm of skull length, but complexity decreases in larger specimens. Our results confirm the alternate replacement of the postcanines and the posterior migration of the postcanine series (including the loss without replacement of the anteriormost postcanines). Observations point to a posterior-to-anterior replacement wave in lower postcanines, but the evidence is not clear-cut for the upper series. The virtual extraction of functional and replacement teeth permitted us to conclude that in most of the cases the upper canines were replaced anteriorly while lower canines were replaced posteriorly. The presence of two simultaneous replacements of the upper canine tooth was observed in two small juveniles, suggesting a higher rate of canine replacement at a younger age. Incisors also had a sequential replacement pattern, and more replacement teeth were present in medium-sized individuals.