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:** Identification and description of fossil embryos preserved in ovo from the Early Jurassic dinosaur Massospondylus carinatus	**Experiment number**: ES-275
Beamline: ID19	**Date of experiment**: from: 11 June 2015 to: 16 June 2015	**Date of report**:
Shifts: 15	**Local contact(s)**: Vincent Fernandez Paul Tafforeau	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Jonah Choiniere, University of the Witwatersrand
Kimberley Chapelle, University of the Witwatersrand

Report:

Details on the experiment are presented in the previous report. This update documents the research outputs of the proposal.

The data collected in this experiment formed part of the PhD thesis of Kimberley Chapelle, which was successfully defended at the University of the Witwatersrand in October 2019.

The following four peer-reviewed papers have been published using the data collected in this research:

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 relation-ship 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 reten- tion 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.

Chapelle, K.E.J., Fernandez, V. & Choiniere, J.N. Conserved in-ovo cranial ossification sequences of extant saurians allow estimation of embryonic dinosaur developmental stages. *Sci Rep* **10,** 4224 (2020). https://doi.org/10.1038/s41598-020-60292-z

Dinosaur embryos are among the rarest of fossils, yet they provide a unique window into the palaeobiology of these animals. Estimating the developmental stage of dinosaur embryos is hindered by the lack of a quantitative method for age determination, by the scarcity of material, and by the difficulty in visualizing that material. Here we present the results of a broad inquiry, using 3D reconstructions from X-ray computed tomography data, into cranial ossification sequences in extant saurian taxa and in well-preserved embryos of the early branching sauropodomorph dinosaur *Massospondylus carinatus*. Our findings support deep-time conservation of cranial ossification sequences in saurians including dinosaurs, allowing us to develop a new method for estimating the relative developmental percentage of embryos from that clade. We also observe null-generation teeth in the *Massospondylus carinatus* embryos which get resorbed or shed before hatching, similar to those of geckos. These lines of evidence allow us to confidently estimate that the *Massospondylus carinatus* embryos are only approximately 60% through their incubation period, much younger than previously hypothesized. The overall consistency of our results with those of living saurians indicates that they can be generalized to other extinct members of that lineage, and therefore our method provides an independent means of assessing the developmental stage of extinct, in-ovo saurians.

Chapelle, K.E.J., Benson, R.B.J., Stiegler, J., Otero, A., Zhao, Q. and Choiniere, J.N. (2020), A quantitative method for inferring locomotory shifts in amniotes during ontogeny, its application to dinosaurs and its bearing on the evolution of posture. Palaeontology, 63: 229-242. https://doi.org/10.1111/pala.12451

Evolutionary transitions between quadrupedal and bipedal postures are pivotal to the diversification of amniotes on land, including in our own lineage (Hominini). Heterochrony is suggested as a macroevolutionary mechanism for postural transitions but understanding postural evolution in deep time is hindered by a lack of methods for inferring posture in extinct species. Dinosaurs are an excellent natural laboratory for understanding postural transitions because they demonstrate at least four instances of quadrupedality evolving from bipedality, and heterochronic processes have been put forward as an explanatory model for these transitions. We extend a quantitative method for reliably inferring posture in tetrapods to the study of ontogenetic postural transitions using measurements of proportional limb robusticity. We apply this to ontogenetic series of living and extinct amniotes, focusing on dinosaurs. Our method correctly predicts the general pattern of ontogenetic conservation of quadrupedal and bipedal postures in many living amniote species and infers the same pattern in some dinosaurs. Furthermore, it correctly predicts the ontogenetic postural shift from quadrupedal crawling to bipedal walking in humans. We also infer a transition from early ontogenetic quadrupedality to late-ontogenetic bipedality in the transitional sauropodomorph dinosaur *Mussaurus patagonicus* and possibly in the early branching ceratopsian *Psittacosaurus lujiatunensis* but not in the sauropodomorph *Massospondylus carinatus*. The phylogenetic positions of these ontogenetic shifts suggest that heterochrony may play a role in the macroevolution of posture, at least in dinosaurs. Our method has substantial potential for testing evolutionary transitions between locomotor modes, especially in elucidating the role of evolutionary mechanisms like heterochrony.

Neenan, J. M., Chapelle, K. E., Fernandez, V., & Choiniere, J. N. (2018). Ontogeny of the Massospondylus labyrinth: implications for locomotory shifts in a basal sauropodomorph dinosaur. *Palaeontology*. **https://doi.org/10.1111/pala.12400**

Ontogeny is a vital aspect of life history sometimes overlooked in palaeontological studies. However, the changing geometry of anatomical structures during growth can be informative regarding ecological and functional reconstructions. The inner ear, or labyrinth, is an ideal ontogenetic study system because it has a

strong functional signal in its morphology that is linked to locomotor mode. Yet almost nothing is known about labyrinth development in dinosaurs. We quantified labyrinth scale and geometry through ontogeny in the Early Jurassic dinosaur *Massospondylus carinatus*, which has an exceptional fossil record and is hypothesized to have undergone a gait change, from quadrupedal juvenile to bipedal adult. To test whether this putative locomotor shift is reflected in labyrinth morphology, computed microtomography (μCT) and propagation phase-contrast synchrotron radiation microtomography (PPC-SRμCT) were used to obtain labyrinths from eight specimens, ranging from near-hatchling to adult. Labyrinths grow substantially but scale with slight negative allometry compared to skull length throughout ontogeny, the first time this has been documented in dinosaurs. Geometric morphometric analysis of the labyrinth using a sliding semilandmark approach shows some morphological change through ontogeny, but little evidence supporting a locomotor shift. These results have implications for our understanding of sauropodomorph development and provide a better understanding of dinosaur locomotory evolution.

Several other papers using these data are still under preparation and will be added to the report in due course.