EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

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:** Were some Mesozoic ray-finned fishes warm-blooded? A 3D histological approach	**Experiment number**: LS2758
Beamline: ID19	**Date of experiment**: from: 14/06/2018 to: 17/06/2018	**Date of report**:
Shifts: 12	**Local contact(s)**: Vincent FERNANDEZ	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Donald DAVESNE* – Department of Earth Sciences, University of Oxford, UK

Roger BENSON* – Department of Earth Sciences, University of Oxford, UK

Matt FRIEDMAN – Museum of Paleontology, University of Michigan, USA

Sophie SANCHEZ* – Evolutionary Biology Centre, Uppsala University, Sweden

Armin SCHMITT* – Department of Earth Sciences, University of Oxford, UK (experimentalist only)

Report:

During this experiment at the ESRF, we succesfully obtained a large amount of histological and microstructural bone data. We employed a multiscale approach, imaging bone at different resolutions: at 4.24 to 4.33 µm voxel size for histological and microstructural data at the scale of an entire bone, and at 0.72 µm voxel size for histological data at the scale of the bone cells (osteocytes). In addition to the main project on the evolution of endothermy, due to time availability and the diverse set of informations that could be obtained from each scan, we also able used the data for other, then ongoing projects, that have since been published: (1) confirm the presence or absence of osteocytes in a range of ray-finned fishes (results published in *Biological Reviews*), (2) measure osteocyte lacuna volumes in a range of fossil and modern ray-finned fishes to evaluate their multi-scale variability (results published in *Journal of Evolutionary Ecology*) and (3) reconstruct the evolution of their genome size (results recently published in *PNAS*). First and foremost, we collected an unprecedented dataset of microstructural and histological data in modern ray-finned fishes allowing to test – amongs other – the main hypotheses laid down by this proposal (study of the results still ongoing).

Publications:

(1) *Davesne D., Meunier F.J., Schmitt A.D., Friedman M., Otero O., Benson R.B.J. (2019): The phylogenetic origin and evolution of acellular bone in teleost fishes: insights into osteocyte function in bone metabolism. Biological Reviews, 94(4): 1338-1363.*

The ESRF data were used to test whether osteocytes are present in various modern ray-finned fishes, closing a knowledge-gap for many key taxa. These data were instrumental in laying down our review of the evolution of acellular bone in teleost fishes, demonstrating that osteocytes were lost in a third of modern vertebrates.

(2) *Davesne D., Schmitt A.D., Fernandez V., Benson R.B.J., Sanchez S. (2020): Three-dimensional characterization of osteocyte volumes at multiple scales, and its relationship with bone biology and genome*

evolution in ray-finned fishes. Journal of Evolutionary Biology, 33(6): 808-830.

The variation in osteocyte volume across different scales is poorly characterized and mostly relies on incomplete, two-dimensional information. In this study, we characterize the variation of osteocyte volumes in ray-finned fishes (Actinopterygii), a clade including more than half of modern vertebrate species in which osteocyte biology is poorly known. We use X-ray synchrotron micro-computed tomography (SRμCT) to achieve a three-dimensional visualization of osteocyte lacunae and direct measurement of their size (volumes). Our specimen sample is designed to characterize variation in osteocyte lacuna morphology at three scales: within a bone **(Fig. 1)**, among the bones of one individual and among species.

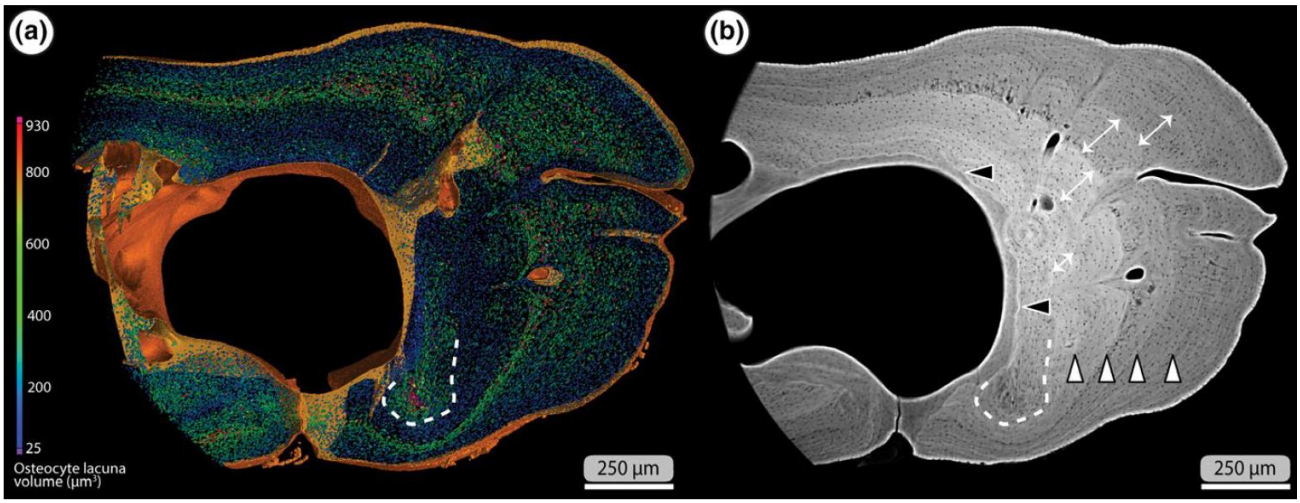

Fig. 1: Example of a specimen in which alternating layers of osteocyte lacunae of varying volumes are well-visible within the periosteal bone deposit. (a) 3D reconstruction of a rib from a carp *Cyprinus carpio* (MNHN.ICOS.00610) and (b) the associated tomogram.

(3) *Davesne D., Friedman M., Schmitt A.D., Fernandez V., Carnevale G., Ahlberg P.E., Sanchez S., Benson R.B.J. (2021): Fossilized cell structures identify an ancient origin for the teleost whole-genome duplication. Proceedings of the National Academy of Sciences, 118(30): e2101780118.*

We infer patterns of genome size evolution in fossil stem-group teleosts using SRμCT to measure the bone cell volumes **(Fig. 2)**, which correlate with genome size in living species. Our findings indicate that WGD occurred very early on the teleost stem lineage and that all extinct stem-group teleosts known so far possessed duplicated genomes.

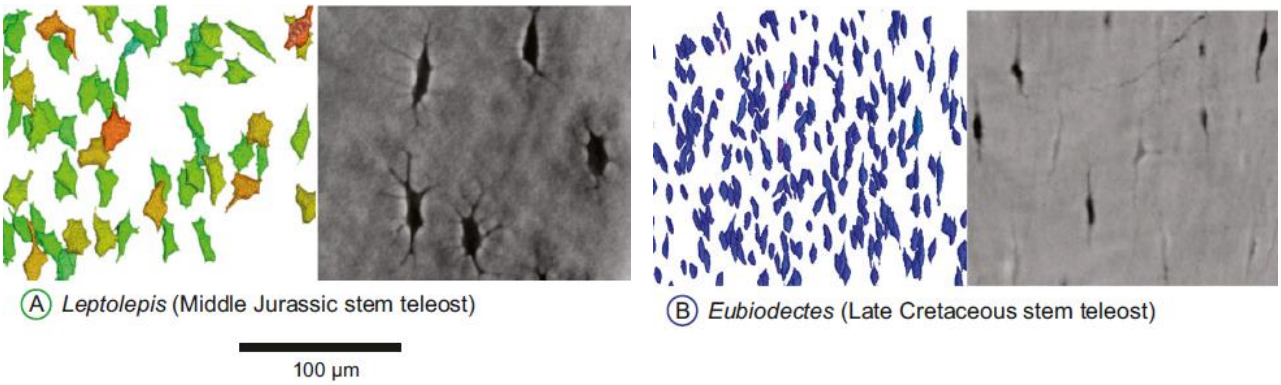

Fig. 2: Examples of fossil stem-teleosts for which we measured osteocyte lacuna volumes. The latter are considerably larger in an early fossil representative (A) than in a more recent one (B), indicative of a genome duplication occuring in the earliest steps of teleost evolution.