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:** Palaeohistological implications in the origin of jawed vertebrates and the fish/tetrapod transition revealed by the ESRF Synchrotron	**Experiment number**: EC 203
Beamline: ID 19	**Date of experiment**: From: 31/08/2007 to: 02/09/2007	**Date of report**: 01/03/2012
Shifts: 6	**Local contact(s)**: Paul Tafforeau	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Sophie Sanchez*, UMR 5143 CNRS, Département Histoire de la Terre, Muse´um national d'Histoire naturelle, CP 38, 57 rue Cuvier, 75005 Paris, France

Paul Tafforeau*, European Synchrotron Radiation Facility, BP220, 6 rue Jules Horowitz, 38043 Grenoble Cedex, France

Per E. Ahlberg*, Department of Organismal Biology, Uppsala University, Norbyvägen 18A, 752 36 Uppsala, Sweden;

Georgy Koentges*, Systems Biology Centre, Coventry House, University of Warwick, Coventry CV4 7AL, United Kingdom

Gaël Clement*, UMR 5143 CNRS, Département Histoire de la Terre, Muse´um national d'Histoire naturelle, CP 38, 57 rue Cuvier, 75005 Paris, France

Didier Geffard Kuriyama*, UMR 5143 CNRS, Département Histoire de la Terre, Muse´um national d'Histoire naturelle, CP 38, 57 rue Cuvier, 75005 Paris, France

J.-Sébastien Steyer, UMR 5143 CNRS, Département Histoire de la Terre, Muse´um national d'Histoire naturelle, CP 38, 57 rue Cuvier, 75005 Paris, France

Jennifer Clack, University Museum of Zoology, Downing Street, Cambridge CB2 3EJ, United Kingdom

Report:

Experiment:

During this experiment, we applied a multiscale approach to identify regions of interest that we could then focus on at higher resolution. We therefore performed a series of scans at 20.24µm, a series at 5.05µm and a last one at 0.678µm.

We decided to image specimens related to the two topics set out in the proposal (the 'fish-tetrapod transition' and the 'origin of early gnathostomes') in order to gain an idea of the best preserved specimens we should focus on during the second scan session.

Thanks to this first scan session we were able to:

1) Gather ontogenetic and life-history data on Devonian tetrapods and their relatives, from the distribution of recurrent growth-arrest surfaces and associated features in their bones. Models of three specimens have already been made and ontogenetic analyses have been initiated. Two more scan data sets will be modelled this year and a paper should be submitted by the end of the year 2012.

2) Map changing patterns of trabecular architecture of the limb bones across the fish-tetrapod transition. Models have been finished but biomechanical analyses still have to be done. This is planned for the end of the year 2012.

3) Assess the utility of phase contrast SR-mCT as a technique for imaging muscle attachments and other histological bone features in fossils. A paper has been submitted to *Microscopy and Microanalysis* and is currently under review.

Publication abstract:

Sophie Sanchez, Per E. Ahlberg, Katherine M. Trinajstic, Alessandro Mirone and Paul Tafforeau. **Submitted**. Three Dimensional Synchrotron Virtual Paleohistology: A New Insight into the World of Fossil Bone Microstructures. *Microscopy and Microanalysis*.

The recent developments of phase-contrast synchrotron imaging techniques have been of great interest for paleontologists, providing three-dimensional (3D) tomographic images of anatomical structures, thereby leading to new palaeobiological insights and the discovery of new species. However, until now, it was never used on features smaller than 5-7µm voxel size in fossil bones. Because much information is contained within the 3D histological architecture of bone, including an ontogenetic record, crucial for understanding the paleobiology of fossil species, the application of phase-contrast synchrotron tomography to bone at higher resolutions is potentially of great interest. Here we use this technique to provide new 3D insights into the submicron-scale histology of fossil and recent bone, based on the development of new pink-beam configurations, data acquisition strategies, and improved processing tools. Not only do the scans reveal by non-destructive means all the major features of the histology at a resolution comparable to that of optical microscopy, they provide 3D information that cannot be obtained by any other method (Fig. 1).

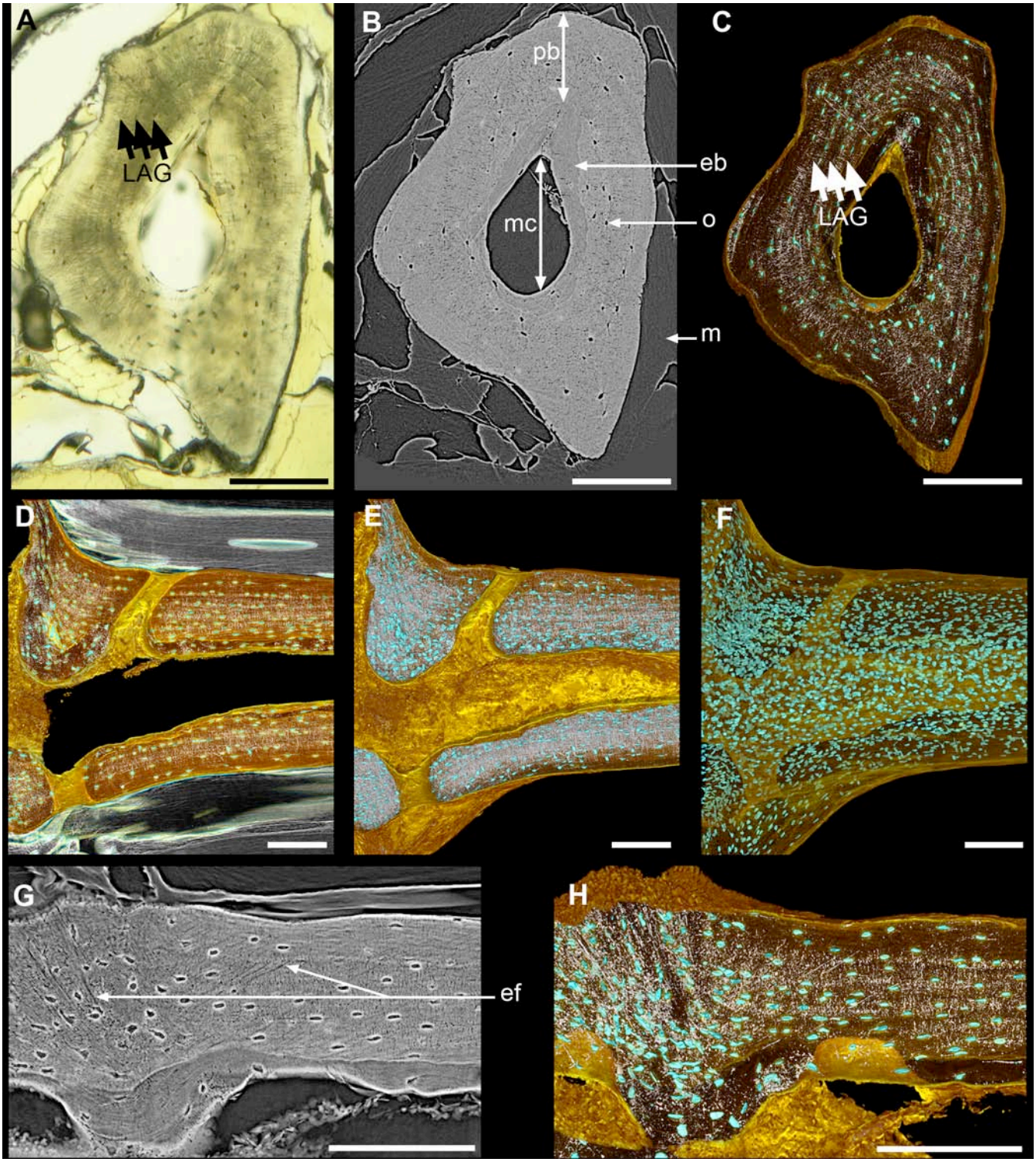

Figure 1: Comparative series of virtual and actual sections through a humerus of the salamander Desmognathus. A/ Real transverse thin section made after the sample had been scanned. B/ Single tomographic slice matching the focal plane of the thin section. The osteocytes (o) can be recognized; the endosteal bone (eb) surrounding the medullar cavity (mc) can be distinguished from the rest of the periosteal bone (pb); and soft tissues such as muscles (m) are visible. C/ Virtual thin section (42µm thick, voxel size = 0.7µm) made from the scan data and modeled a posteriori to match perfectly with the real thin section. The cell lacunae are modeled in blue, the canaliculi in white, the surface of the bone in brownish gold. The alignment of the bone cells and their dendritic organization reveal the lines of arrested growth (LAG) as observed in the real thin section in A. D/ Visualization of microstructures in a longitudinal plane. E/ Longitudinal visualization in 3D of the microstructures in half bone. F/ Longitudinal visualization in 3D of the microstructures in the whole bone. G/ Virtual thin section made in a plane permitting the visualization of extrinsic fibers (ef) related to muscle or tendon insertions. H/ 3D model rendering the same virtual thin section as in G. Scale bars: 250µm.