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:** Three-dimensional investigation of muscle attachment architecture on dermal bones in primitive recent fishes	**Experiment number**: EC 519
Beamline: ID 19	**Date of experiment**: From: 24/10/2009 to: 26/10/2009	**Date of report**: 01/03/2012
Shifts: 6	**Local contact(s)**: Paul Tafforeau	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Experiment:

During this second scan session, we did a series of scans at high resolution in different individuals of extant and fossil species, i.e. with a voxel size of 0.678µm to visualize the regions of muscle attachments at the histological scale. We decided to focus on understanding the disturbance created by the muscle insertions on the bone microstructural organization in extant vertebrates. The analysis of the fossil material could then be interpreted thanks to the extrapolation from these previous analyses on extant bones.

Preliminary results:

We were able to:

1) visualize and analyse the histological disorganization at the location of muscle insertions.
2) visualize anchorage fibres where no muscle insertions could be suspected from the observation of the surface of the bone (Fig. 1).
3) distinguish the different kinds of entheses.
4) reconstruct the muscle orientation in a restricted region (1.5mm^2) at mid-shaft in the humerus of *Eusthenopteron* and in the branchial area of *Compagopiscis*.

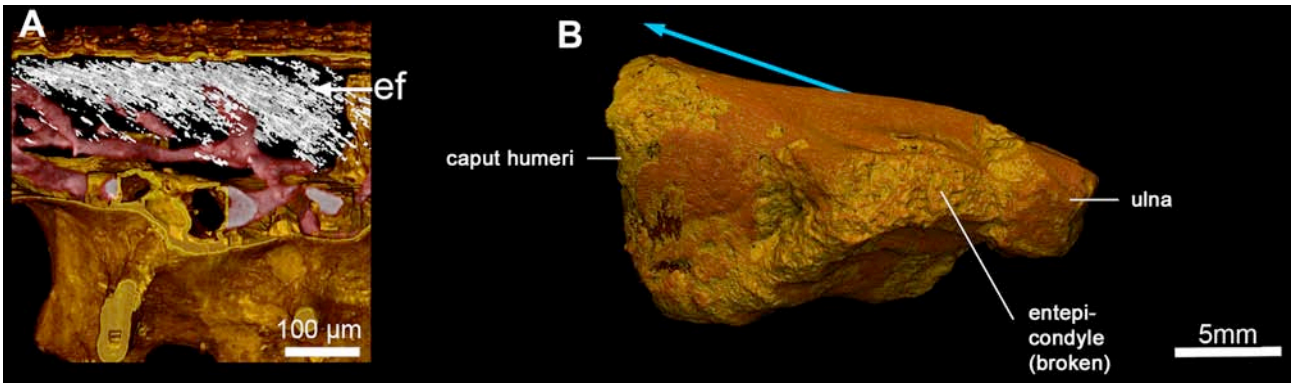

Figure 1: A/ Virtual thin section in the humerus of *Eusthenopteron* showing extrinsinc fibres (ef) in the cortical bone where no muscle insertion was suspected; B/ The blue arrow shows the direction of the muscle reconstructed from the 3D orientation of the anchorage fibres.

A paper is in the final stages of preparation for submission to *Proceedings of the Royal Society - B*.

Congress abstract:

Sanchez S., Dupret V., Ryll B., Trinajstic K., Wretman L., Zylberberg L., Tafforeau P. & P. E. Ahlberg. **2011.** Synchrotron virtual palaeohistology: a new tool for studying the evolution of bone microstructures in 3D. *1st International Symposium on Paleohistology, Barcelona (Spain)*.

Current models of bone growth are based on observations made from 2D thin sections of extant taxa (mainly mammals). Comparative investigations in three dimensions have never yet been carried out on fossil and extant taxa to check and complement these histological models. Up to now, non-destructive submicron synchrotron virtual palaeohistology has been used principally to study tooth microstructure. Here, we present the potential of this technique for the study of bone tissue of extant and extinct species of gnathostomes (jawed vertebrates). This innovative approach provides a wealth of new information about the vascular organization in limb and dermal bones (establishment, integration, extension, and metabolic function). Tracing the 3D rearrangement of the vascular canals during ontogeny permits us to understand the growth modes of the bones, and the microstructural and functional changes that they have undergone. Integrated into the phylogenetic context of gnathostomes, these discoveries complement our current knowledge of endoskeletal and dermal bone growth.

The synchrotron approach also allows a better identification of the nature of soft tissue attachments (*e.g.,* direct muscle attachment, tendon) and the orientation of their anchorage. Based on such 3D data in the branchial area of the thoracic armour of a Devonian placoderm (an armoured member of the gnathostome stem group), *Compagopiscis croucheri*, we have been able to confirm the location of at least two muscle insertions (basibranchial complex, hypobranchials or hypohyals; Fig. 2).

Based on these preliminary results, synchrotron virtual palaeohistology appears as a powerful tool for a better understanding of bone histology and palaeobiological reconstructions of soft tissues.

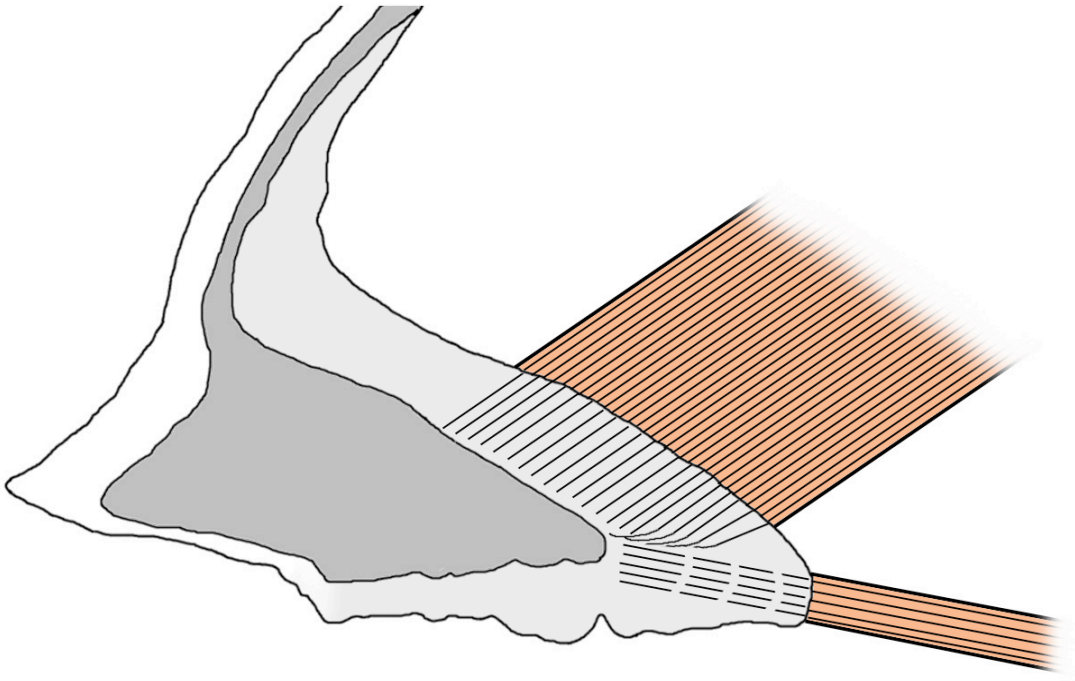

Figure 2: Reconstruction of muscle attachment. Diagrammatic representation of fibre arrangement in IL of *Compagopiscis croucheri*, with inferred orientation of two muscles. Pale grey, outer layer of interolateral; dark grey, middle layer; white, inner layer; pale brown, muscles.