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

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:** Anatomy of cranial cartilages of Palaeospondylus gunni: resolving taxonomy and phylogenetic relationships within the Vertebrata, both are disputed and unresolved.	**Experiment number**: EC 690
Beamline: ID 19	**Date of experiment**: from: 2010 to: 2010	**Date of report**: 12/09/2017
Shifts: 6	**Local contact(s)**: Paul Tafforeau and Sophie Sanchez	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Zerina Johanson, Department of Earth Sciences, Natural History Museum, London, UK
Moya Meredith Smith, Tissue Engineering and Biophotonics, Dental Institute, King's College London, London, UK
Sophie Sanchez, postdoc at the ESRF and Uppsala University, Subdept of Evolutionary Organismal Biology, Dept of Physiology & Developmental Biology, Evolutionary Biology Centre, Norbyvägen 18A, 75 236 Uppsala, SWEDEN

Report:

Experiment:

Palaeospondylus gunni was imaged using the powerful X-ray beam at the beamline ID19 from the ESRF [1]. A monochromatic beam was produced through the wiggler opening of 35mm and reflected by the double Si111 Bragg monochromator. The resulting energy was of 40 keV. A FreLON 2k14 CCD detector [2] with a 10 µm thick Gadox scintillator were used to scan the sample with a voxel size of 7.46 µm. In order to use phase contrast, the sample was fixed at the distance of 1000 mm from the detector. 1999 projections were taken over 360°. The time of exposure was of 1 s. A single phase retrieval approach [3] (modified from the algorithm of Paganin et al. [4]) was used to reconstruct the data.

We decided to image an overall view of the *Palaeospondylus* cranium and anterior vertebral column for the purpose of the experiment EC690. In addition, we did a test at higher magnification (voxel size: 0.678µm) of a selected region of the cranium to check out if we could better characterize skeletal tissue composition (in the framework of a future beamtime application).

Results:

Thanks to this scan session we were able to:
1) Gather the most comprehensive scanning data on the morphology of the *Palaeospondylus* cranium, including the position of nerve foramina, never before visualized.
2) Describe the otic region (inner ear) of *Palaeospondylus* for the first time, definitively identifying *Palaeospondylus* as a jawed vertebrate, rather than as a jawless vertebrate related to hagfish.
3) The higher magnification scan allowed us to initially measure cell volumes of the unusual cartilage of the *Palaeospondylus* skeleton, the focus of a new application for beamtime to the ESRF.

Publication abstract:

Zerina Johanson, Moya Smith, Sophie Sanchez, Tim Senden, Kate Trinajstic, Cathrin Pfaff
2017. Questioning hagfish affinities of the enigmatic Devonian vertebrate *Palaeospondylus*. Royal Society Open Science DOI https://doi.org/10.1098/rsos.170214

Palaeospondylus gunni Traquair, 1890 is an enigmatic Devonian vertebrate whose taxonomic affinities have been debated since it was first described. Most recently, *Palaeospondylus* has been identified as a stem-group hagfish (Myxinoidea). However, one character questioning this assignment is the presence of three semicircular canals in the otic region of the cartilaginous skull, a feature of jawed vertebrates (Fig. 1). Additionally, new tomographic data reveal that the following characters of crown-group gnathostomes (chondrichthyans + osteichthyans) are present in *Palaeospondylus*: a longer telencephalic region of the braincase, separation of otic and occipital regions by the otico-occipital fissure, and vertebral centra. As well, a precerebral fontanelle and postorbital articulation of the palatoquadrate are characteristic of certain chondrichthyans. Similarities in the structure of the postorbital process to taxa such as *Pucapampella*, and possible presence of the ventral cranial fissure, both support a resolution of *Pa. gunni* as a stem chondrichthyan. The internally mineralized cartilaginous skeleton in *Palaeospondylus* may represent a stage in the loss of bone characteristic of the Chondrichthyes.

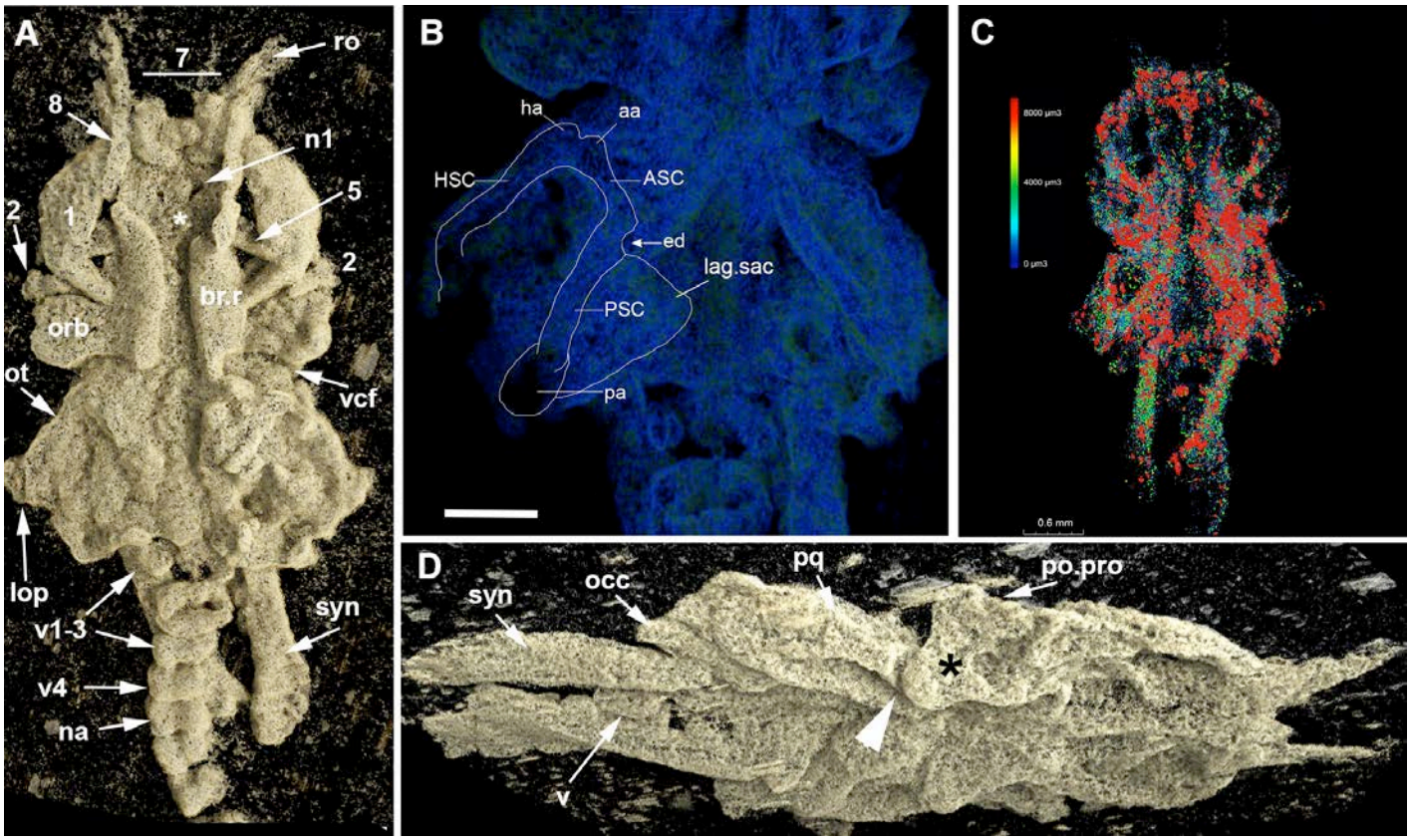

Figure 1: A, dorsal view of *Palaeospondylus* cranium and anterior vertebral column, showing various anatomical features, including orbits, dorsal roof of the braincase (br.r) and anterior vestibular fontanelle (*), the latter a characteristic of chondrichthyans (sharks, rays). B, otic region of *Palaeospondylus*, showing the presence of three semicircular canals, a jawed vertebrate feature. C, Measurement of the cell volumes in the hard tissue of the skull of *Palaeospondylus*. The red regions show cell gathering which can be resolved at high resolution (voxel size: 0.678µm). D, lateral view of *Palaeospondylus* cranium, showing the articulation of the palatoquadrate to the postorbital process of the braincase, another chondrichthyan characteristic.

References:

[1] Tafforeau P, et al. 2006. Applications of X-ray synchrotron microtomography for non-destructive 3D studies of paleontological specimens. *Applied Physics A - Materials Science & Processing* **83**, 195-202.

[2] Labiche J-C, Mathon O, Pascarelli S, Newton MA, Guilera Ferre G, Curfs C, Vaughan G, Homs A, Fernandez Carreiras D. 2007. The fast readout low noise camera as a versatile X-ray detector for time resolved dispersive extended X-ray absorption fine structure and diffraction studies of dynamic problems in materials science, chemistry, and catalysis. *Review of Scientific Instruments* **78**, 091301-091311.

[3] Sanchez S, Ahlberg PE, Trinajstic K, Mirone A, Tafforeau P. 2012. Three dimensional synchrotron virtual paleohistology: a new insight into the world of fossil bone microstructures. *Microscopy and Microanalysis* **18**, 1095-1105.

[4] Paganin D, Mayo SC, Gureyev TE, Miller PR, Wilkins SW. 2002. Simultaneous phase and amplitude extraction from a single defocused image of a homogeneous object. *Journal of Microscopy* **206**, 33-40.