EUROPEAN SYNCHROTRON RADIATION FACILITY

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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.

| **Experiment title:** Nano-imaging of Lizard Osteoderms- Preliminary Report | **Experiment number**: LS3071 |
|---|---|

| **Beamline**: ID16A | **Date of experiment**: from: 31/08/2022 to: 05/09/2022 | **Date of report**: 08/09/2022 |
|---|---|---|
| **Shifts:** 15 | **Local contact(s)**: Dr Dmitry Karpov | *Received at ESRF:* |

**Names and affiliations of applicants** (* indicates experimentalists):

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**Report:**

Scans were successfully acquired from osteoderms (small bones within the skin) of 5 species of lizards separated by more than 200 million years of evolution. Scans were taken at 200 µm overview at, 100nm resolution, and 100 µm (fine scale field of view, 50 nm resolution) using phase-contrast holo-tomography. This beam time allowed confirmation of the presence of a cell-poor capping tissue of unknown composition (*e.g.,* Fig1, **i**c for *Broadleysaurus major*). This capping tissue is distinct from the underlying bone (Fig1, **i**d), and is overlain by dermis (Fig1, **i**b) and epidermis. Previous descriptions of the capping tissue relied on microCT and light microscopy, and focused primarily on 2 species (*Tarentola* and *Heloderma*) (Williams et al., 2021). As a result of our investigation using the current beam time, we have generated, for the first time, high-resolution images of this enigmatic tissue (Fig 1,2 &3), and documented its presence and distribution both within and between species, and extended the diversity of species that are known to develop it. Fine scans were used to interrogate the cellular, organic and matrix contents of the capping tissue (Fig1, **ii**) as well as to investigate its interface with the underlying bone (Fig1, **iii**).

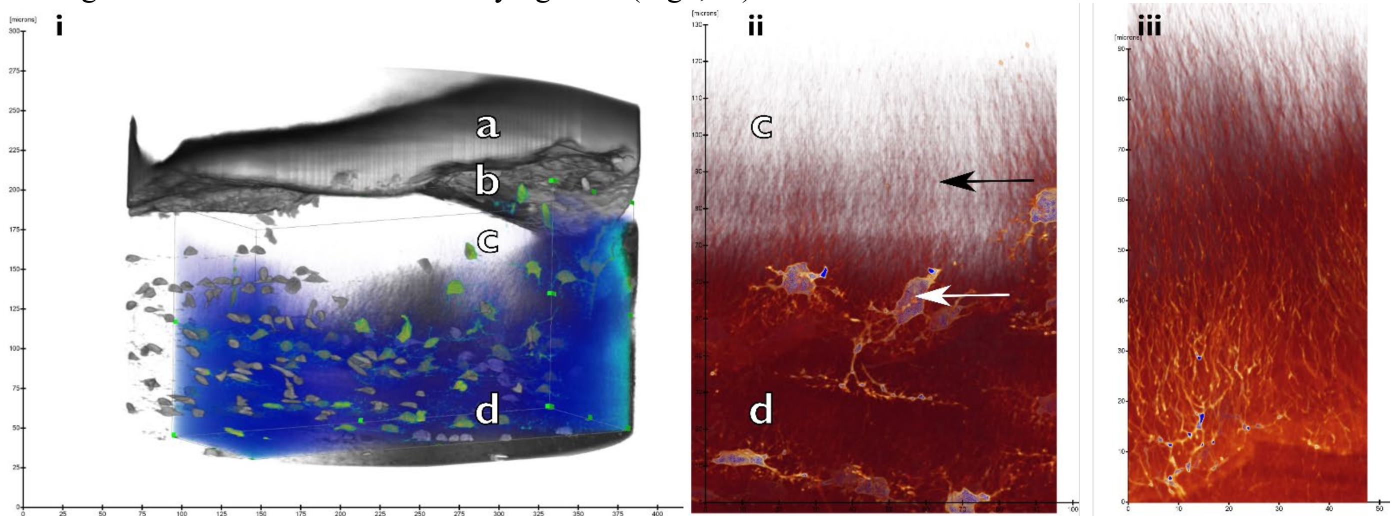


*Figure 1 Broadleysaurus major,* osteoderm **i**, 200µm field of view with (a) vacuum/resin (b) dermis/capping tissue (c) capping tissue and (d) underlying bone, greyscale 3D phase contrast image of whole field with overlain 3D colour visualisation of electron density within the bone and capping tissue. **ii** 100 µm field of view osteocytes (white arrow) at capping tissue and bone boundary, projecting canaliculi (black arrow) vertically into the capping tissue matrix, which appears here as clear **iii** high magnification view of the density of protruding canaliculi.

The samples (sample size: 800 µm diameter, max 3mm depth) studied include osteoderms that vary in scale from cm (*Tiliqua*) to mm (*Heloderma*). All samples were stable within the beam line.

Lizard capping tissue demonstrates species-specific differences in thickness and organization. In *Heloderma* the capping tissue is approximately 5-10% of the thickness of the osteoderm (~30-100 µm). Compared with the underlying bone, it is modestly cellular (Fig2 **i** c-d). In contrast, the capping tissue of *Broadleysaurus* (Fig1) (>50 µm) is virtually acellular and is characterized by numerous vertically directed canaliculi. All species demonstrate an extensive lacuno-canalicular network. Osteocytes of approximately 5 µm cell body diameter (Fig 2 **ii** white arrow) in *Heloderma* were visualised with extensive canaliculi networks (Fig2 **ii** & **iii**), each canaliculi measuring at most 0.5 µm in diameter, similar to human canaliculi (Yu et al., 2020), in a complex network primarily spreading horizontally within the bone and penetrating the capping tissue. The capping tissue also contained multiple collagen bundles, potentially mineralised, of approximately 2.5 µm diameter that tethered it to the superficial dermis. In contrast to the broad spreading horizontal canaliculi of *Heloderma*, the canaliculi of the cells in *Broadleysaurus* (Fig1 **iii**) were exceptionally fine and arranged vertically, from a border of bone.

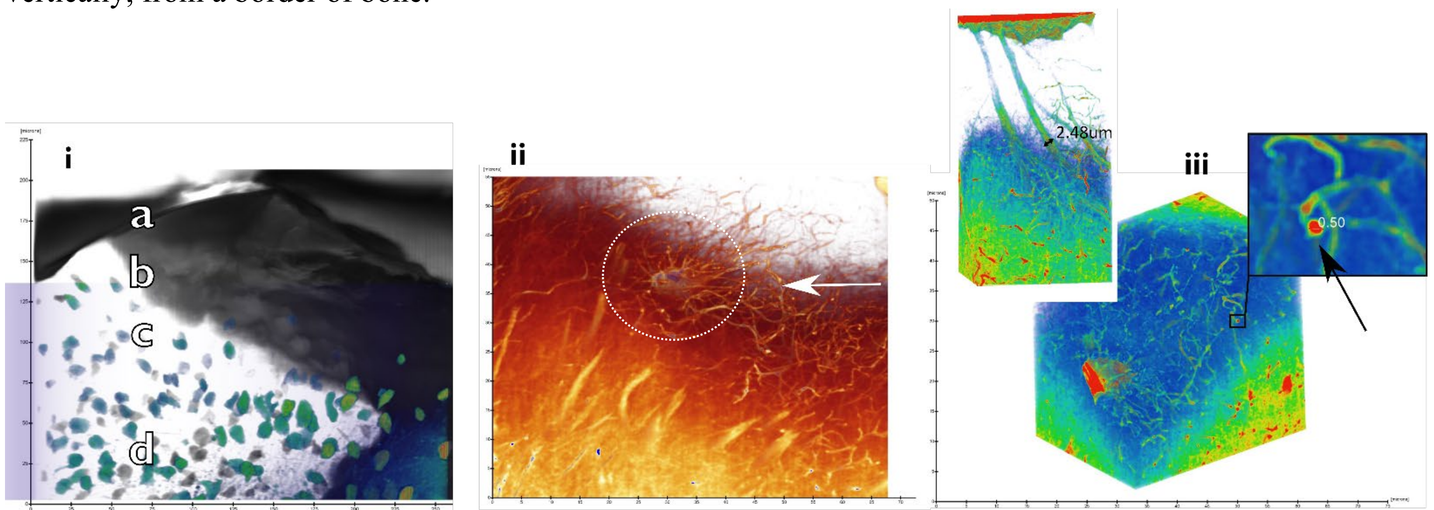


*Figure 2 Heloderma suspectum,* osteoderm at **i**, 200µm field of view with (a) vacuum/resin (b) dermis/capping tissue (c) capping tissue and (d) underlying bone, osteocytes overlain in colour. **ii** osteocyte (white arrow, and circled) at the boundary between capping tissue (clear are dorsal border) and bone (denser phase contrast highlighted structure), projecting canaliculi horizontally, **iii** high magnification view of the density of protruding canaliculi, with *(inserts)* diameter of largest at 0.5 µm, and 2.5µm presumed collagen bundles projecting dorsally.

The capping tissue was composed of a matrix that varied within the sample in its apparent electron density (most visible in the *Tiliqua* sample, where its thickness was much greater >200 µm, and where horizons of electron density were visible at a scale of 1.2 µm (Fig 3 **ii**)), as well as layers within the capping tissue of greater and lesser cellular density at the 10s of µm scale.

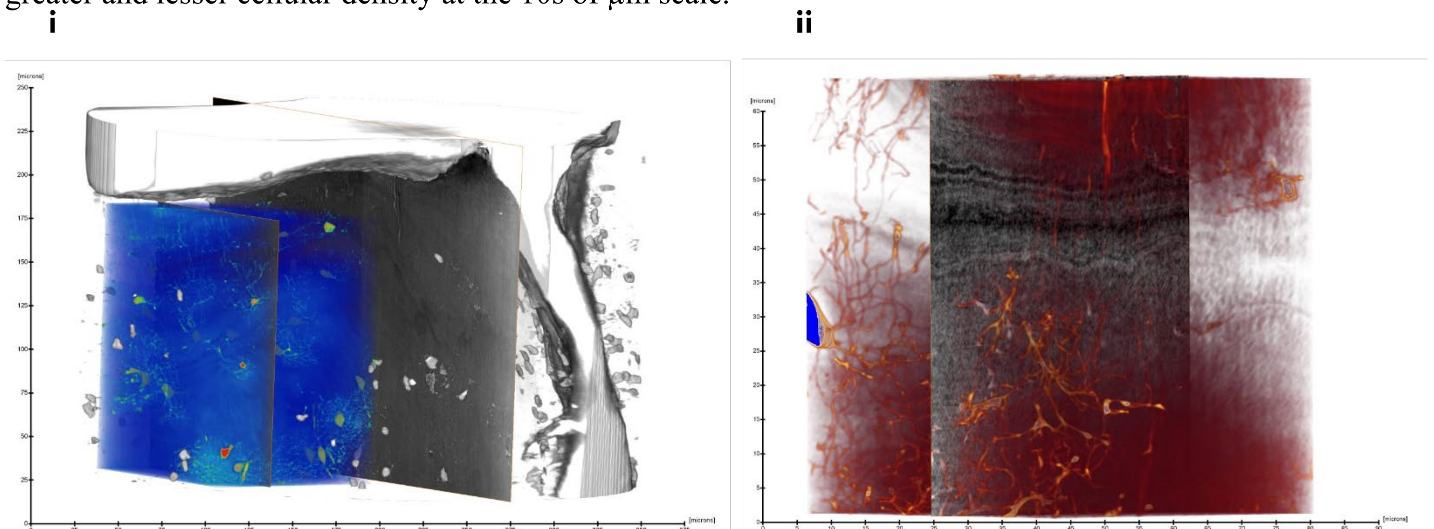


*Figure 3 Tiliqua rugosa,* osteoderm at **i**, 200µm field of view with thick capping tissue extending to the base of the field of view with planes of cell bodies - two layers at approx. 10º from the horizontal centred at 100 and 25 µm on the y axis are visible in this field of view **ii** 60 µm field of view osteocyte canaliculi in red (3D colour image) overly a matrix possessing distinct layers of contrasting electron density in phase contrast imaging (black and white 2D section).

This preliminary report prepared using initial data from three species summarises the qualitative evaluation of the samples. These data will further allow a quantitative comparison of osteoderm capping tissue cellularity, to be combined with material characteristics from nanoindentation, and histological appearance. For example: the capping tissue is harder than bone but softer than enamel (Marghoub et al., 2022), this is consistent with our new findings on its nanoscale structure, where the capping tissue combines matrix and cellular contents, while bone has a higher cellular fraction, and mature enamel is limited to a crystalline matrix with no cellular contents. The data acquired raise new questions concerning the chemical composition of this capping layer – i) is it consistent between species, ii) does it change with depth from the underlying bone, ii) is it mainly inorganic, and where there is a component of cellular processes, e.g., *Broadleysaurus,* how do they contribute to the chemical and material characteristics of this still enigmatic tissue.

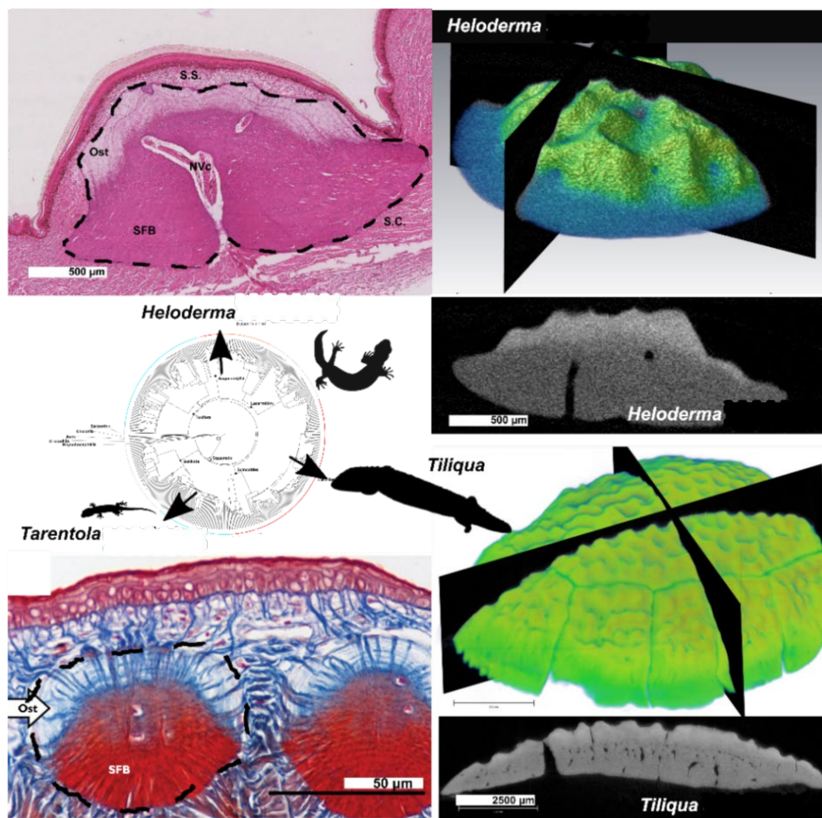


*Figure 4:* The state of knowledge prior to this beam time – It is impossible to see the differences between the capping layers of genera (e.g., *Heloderma and Tiliqua)* from the µCT and histological images.

*Clockwise from left:*
Phylogeny of lizards showing diverse representatives with capping tissue, here highlighting three genera in silhouette scanned in this beam time.

Histological section of *Heloderma* osteoderm (osteoderm highlighted in dashed lines)

3D overview and 2D section from µCT *Heloderma* osteoderm

3D overview and 2D section from µCT *Tiliqua* osteoderm

Histological section of *Tarentola* osteoderm

We have moved in this beam time substantially beyond what was previous possible - where the difference between species could not be clearly seen on µCT or light microscopy (Fig 4).

The central remaining question is: **are the difference we see in holotomography dictated by a chemical shift in composition within the capping layer, or a change in the density of the same material?** This question will be best addressed by a 2D Xray-fluorescence investigation of the same samples. Resulting data will also shed light on large scale evolutionary and ecological questions regarding the relationship of the capping tissue characteristics to global environmental distribution (the species in question are native to N. America, Europe, Australia and Africa, and separated by 200 million years of evolution) diet, seasonality, and function.