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:** Resolving the structure and composition of weevils cuticular scales and diamond photonic crystals	**Experiment number**: SC-5328
Beamline: ID13	**Date of experiment**: from: 30/10/2022 to: 04/11/2022	**Date of report**:
Shifts: 12	Local contact(s): **Dr. Aicha Asma MEDJAHED** **Dr. Manfred Burghammer**	*Received at ESRF:*

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Report:

The aim of the project is to investigate the hierarchical architecture and contribution of compositions in the cuticular photonic crystals within each weevil's scale using XRF-XRD on the micron and sub-micron length-scale. We expect the detection of XRD and XRF data will be high-quality correlative data for structure and composition of the samples investigated.

The experiment SC-5328 was performed on the microbranch of ID13 with a beamsize of 2.5x2.5 μm² at an energy of 13 keV with multi-bunch mode. The intend of this experiment is to perform XRF-XRD mapping from different rotation angles (1) on differently coloured micron-sized cuticular scales (collected from different weevil species), (2) on several cross-sections of differently coloured scales. Based on the micro-FTIR and confocal fluorescent measurements conducted in our lab, those differently coloured scales are made of similar cuticular materials: mainly proteins and some locally distributed chitin. Weak chitin signals are detected only in the thin skin (1 μm in thickness) of each weevil's scale but not in the core photonic crystals within the scale. By conducting the high-resolution XRF-XRD in ESRF, we aim to understand (a) of differently coloured scales from different species whether there is any difference of their components at molecular length-scale; (b) the variations of compositions at different regions of individual scale.

We sucessfully got XRF and XRD signals of individual scale from different rotation angles (0^{o} -30^{o}) and cross-sections (10 μm in thickness) with very good signal-to-noise with the resolution of 1- 2 μm. The results from SC-5328 help us gain more insight into the system, for example, the XRD generated from each scale is sensitive to the orientations of the photonic crystals, height of the scales, and the directions of scales. Such angle-dependency differs from species.

However, in this experiment, our rotation angle of the stage is limited on only counterclock-wise direction to 30^{o} due to the big frame size of the Si_3N_4 membranes (1x1 cm²). On the other hand, the step size of 1μm which is also the size of the skin of each scale did not provide enough resolution for us to locate precisely the locally distributed components. We therefore apply our next beamtime (proposal: **Continuation Experiment: Resolving the structure and composition of weevils cuticular scales and diamond photonic crystals with intermediate-focused X-ray nanobeam**) for the "***intermediate beam***" available in the nanobranch at ID13.

Results

We took the scales from differently coloured adult Pachyrhynchus weevils as well as from pupa of *P.orbifer* at the day 6 and the day 13 of pupation (Figure 1a). Our work has shown that each scale is plate-like (diameter of 50 μm and thickness of 2-5 μm) and attached to the elytra *via* a stalk, and photonic crystals are enwrapped by a thin cuticular skin with 1 μm in thickness (Figure 1b). Using FIB/SEM (conducted in our lab) we reconstructed the 3D structures of the photonic crystals and scales of adult weevils' scales and of the pupa at different growth stage. The 3D diamond photonic crystals within the adult weevils' scales are composed of cuticular nanofibres and air pores. Such structures and the refractive index (RI) of cuticles and air generates brilliant structural colours while the scales of pupa are transparent because the cuticular nanofibres are randomly oriented and the pores are filled with liquid in a scale (Figure 1c).

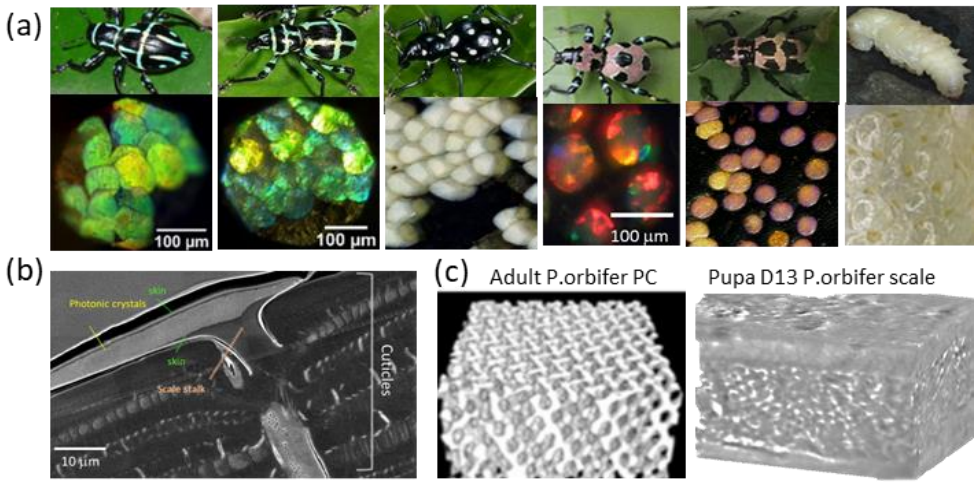

Figure 1. (a) The five selected Pachyrhynchus weevils in this project and the optical microscope images of their scales. From left to right: *P. nobilis, P. sonani, P.insularius, P.orbifer* (Luzon Is.), *P.orbifer* (Fuga Is.), and the pupa of *P.orbifer* (Fuga Is.) at the day 6. (b) SEM of a cross-section of a scale and the related structures in an elyton of a P. orbifer. (c) FIB/SEM of a scale of an adult *P.orbifer* (Fuga Is.) reveals the 3D diamond photonic crystal structures while in the transparent scale of the day 13 pupa the photonic crystals are not well-formed yet.

Taking into account the variations in the orientation of the photonic crystals within a single scale, we mounted those scales individually on several 1x1 cm^2 Si_3N_4 membranes and placed on the rotational stage. To study the compositions in cuticles, we scaned the samples with a broad q range from 0.17- 44 nm^{-1}. We tested different exposure time from 10 ms to 25 ms with the step size of 1 μm or 2 μm on several intact scales of different species to gain good WAXS/SAXS signal-to-noise ratio (Figure 2). We can collect good WAXS/SAXS signals without seeing severe radiation damages on the intact scales of different weevils with current setups of exposure time and resolution.

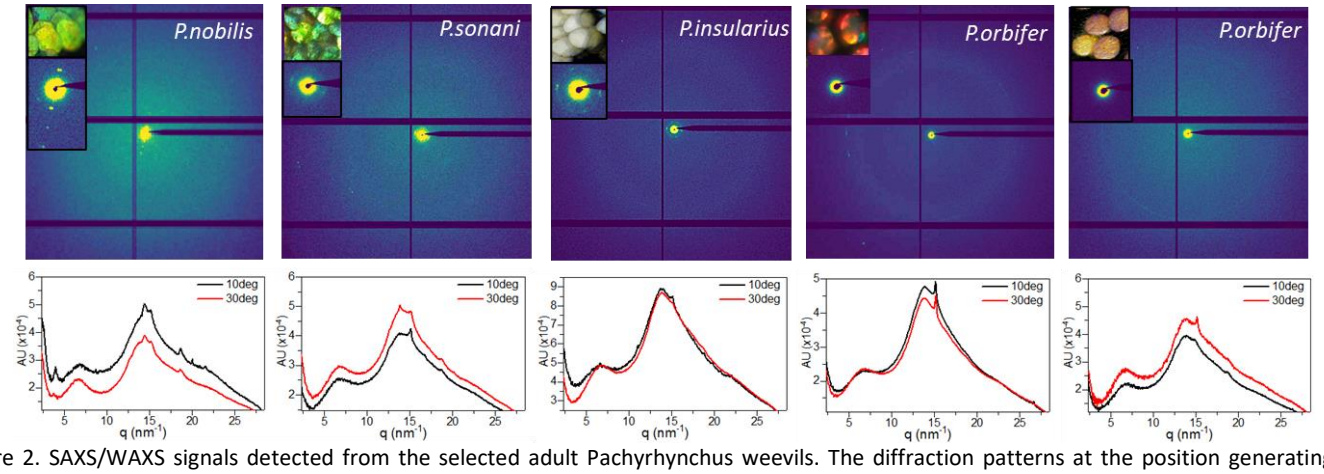

Figure 2. SAXS/WAXS signals detected from the selected adult Pachyrhynchus weevils. The diffraction patterns at the position generating the strongest SAXS showing signals of chitin and protein crystals. Representative 1D XRD profile of the scales detected at rotation angle of 10 degree (black line) and at 30 degree (red line).

From the 1D XRD profiles (Figure 2) we observe diffraction peaks of proteins and chitins generated from scales of all five different species which fits to our micro-FTIR results. We observe the scales of the five different species generates very different fingerprints of XRD and different variations of the XRD peaks over rotational

angles from 10º- 30 º in the q range of around 0.1- 30. We therefore confirm that the SAXS/WAXS generated from each scale are sensitive to the orientations of the scales. The good XRD signals over all rotation angles will allow us to do further statistic analyses and provide reliable results which might answer the question of the correlation of the molecular components with the orientations of photonic crystals. However, due to the size of the Si_3N_4 membrane and the limited spaces between the sample stage and the XRF detector, we could only rotate the stage counterclock-wise from 0 º to 30º during this beamtime. It will be ideal to have a broader range of the rotation angles as well as rotation directions clockwise and counter-clockwise.

Figure 3 demonstrates that we can get good XRF signals from the scales of all the five selected species in this project as well as the pupa of *P.orbifer* (Fuga) at early pupation stage (Day 6 of pupation) and late stage (Day 13 of pupation). The specific variation of traced elements Ca, Zn, Cu, Mn are different between species which might be associated with their different structures of the photonic crystals and the structural colours. In addition, we observe marginal increases of Ca in the scales of pupa from early pupation stage (Day 6) to late pupation stage (Day 13) and dramatical amounts of Ca deposited in the mature scales of adult weevils. Such variations of Ca with development time of weevils corresponds in time of the formation of the diamond photonic crystals as well as the occurrence of structural colours. The result implies that the morphogenesis of photonic crystal structures might relate to the Ca depositions and cellular controls.

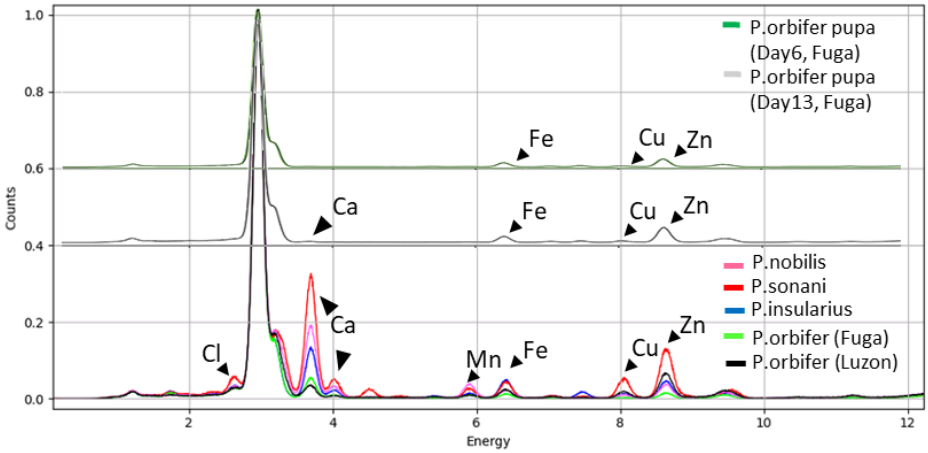

Figure 3. Normalized XRF profiles of scales collected from the five different adult species as well as the pupa of P.orbifer (Fuga) at different growth stage (Day 6 and Day 13 of pupation).

We also conducted few preliminary XRF-XRD tests on the cross-sections of *P.nobilis*'s scales (with cross-section thickness of about 10 μm). XRF as well as XRD analyses show compositional variation between the skin and the core photonic crystals (Figure 4). Representative 2D XRD maps obtained using microbeam suggest that oriented chitin fibres form the skin but not the photonic crystal. However, in order to confirm this finding, we need to improve the spatial resolution that submicron-focused beam size is necessary.

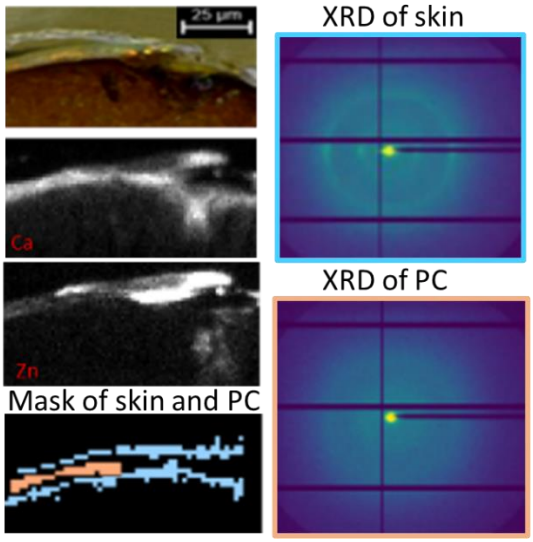

Figure 4. Cross-sections of individual scale measured via the micro-focused beam. XRF maps reveal the skin is rich in traced elements such as Ca and Zn which allows us to mask and extract the XRD of the skin and photonic crystals (PC) separately. Oriented chitin is detected only in the skin not in the core photonic crystals.

In general, from this beamtime SC-5328 using micro-focused beam and the rotation stage, we successfully detected good XRF and XRD signals of individual weevils' scales at various angles. Additionally, we are able to sucessfully distinguish the differences of XRD signals from scales collected from the five selected species. We also confirm that their WAXS/SAXS signals detected with such transmission mode are very sensitive to different positions and thickness of a scale, orientations of the cuticular photonic crystals as well as to the orientation of each scale. We observe the variations of XRF and XRD cross different regions of scales, but to resolve our question about the correlations of components with the hierarchical structures and the formation of the photonic crystals, we will need to improve our spatial resolution to sub-micrometer length-scale.

Therefore, with the pre-discussion with beamline scientists Dr. Manfred Burghammer and Dr.Aicha Asma Medjahed at ID 13, for our next beamtime we will request "***intermediate beam***", with beam cross-section in the order of 200 nm. In this set-up we benefit from sufficient spatial resolution that will allow us to resolve the skin and photonic-crystal core of the scale, at the same time leverage higher photon-flux and increased scattering volumes optimizing the conditions for measuring the cross-sections of weevils' scales with excellent signal to noise ratio of SAXS/WAXS. We will also use a rotational stage to sample the cross-sections at different angles, to account for the orientations of the photonic crystals. We will also mount our samples on small Si_3N_4 membrane (frame size: 4x4 mm^2) in order to rotate our sample stage with broader rotational angles from -40^o to 40^o.