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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

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:** **Comparative functional morphology and ecomechanics of the jumping apparatus of beetles (Insecta: Coleoptera)**	**Experiment number**: LS 2342
Beamline: ID19	**Date of experiment**: from: 09.11.2014 to: 10.11.2014	**Date of report**: 24.01.2018
Shifts: 3	**Local contact(s)**: Dr. Alexander Rack	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Main proposer

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Co-proposers

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Report:

The goal of the experiment aimed to discover the internal structure of the jumping apparatus of recent and fossil beetles (JA) by the use of 3D microtomography. We have focused on identification both sclerotized internal structures and muscles are involved in the jumping mechanism. In total, full 3D scans of 16 species of recent beetles from the families Chrysomelidae, Curculionidae, Scirtidae, Buprestidae and Anthribidae and three samples of fossil beetles (family Chrysomelidae) in amber have been performed.

The freshly caught specimens of beetles were preserved in 70% alcohol; the hind legs removed and stepwise dehydrated in increasing ethanol concentrations, and critical-point; dry specimens from museum collections and amber samples were left intact. The hind legs and ambers were glued onto the tips of plastic stubs. We used the ID19 beamline at 19 keV (wavelength of $8 \cdot 10^{-11}$ m) and an effective detector pixel size of 0.65 µm with a corresponding field of view of 1.43 mm by 1.43 mm; 6000 projections were recorded over the 180 deg rotation. The detector-to-sample distance was 12 mm. For the selected species the 3D reconstruction of the hind jumping legs and JA correspondingly we used the graphic segmentation tool software Amira® 6.0 (FEI Company, Visage Imaging, Germany).
Based on the obtained 3D reconstructions and following laboratory observations functional models of jumping apparatus and the mechanism of jumping for the beetles families Chrysomelidae (subfamily Galerucinae), Curculionidae (subfamily Curculioninae) and Scirtidae have been proposed.

Family Chrysomelidae, leaf beetles (exemplified by Sphaeroderma testacea).
The jumping apparatus of leaf beetles according to the 3D microtomography-based reconstructions (Nadein & Betz, 2016) is localized in the hind legs (Fig. 1) and formed by femur, tibia, femoro-tibial joint, modified metafemoral extensor tendon (MET), extensor ligament, tibial flexor sclerite (TFS), extensor and flexor muscles. The primary role of the metafemoral extensor tendon is seen in the formation of an increased attachment site for the extensor muscles. The rubber-like protein resilin was detected in the extensor ligament, i.e. a short, elastic element connecting the extensor tendon with the tibial base. The calculated specific joint power (max. 0.714 W g^{-1}) of the femoro-tibial joint during the jumping movement and the fast full extension of the hind tibia (1-3 ms) suggest that jumping is performed via a catapult mechanism releasing energy that has beforehand been stored in the extensor ligament during its stretching by the extensor muscles. In addition, the morphology of the femoro-tibial joint suggests that the co-contraction of the flexor and the extensor muscles in the femur of jumping leg is involved in this process.

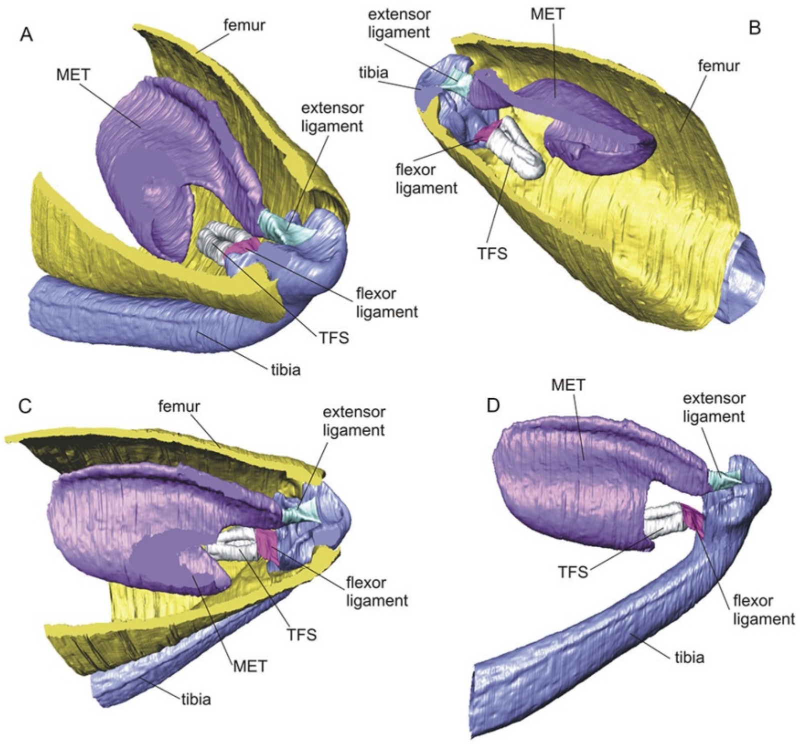

Fig. 1. Femoro-tibial joint of the hind leg of *Sphaeroderma testacea* and the primary structural elements of the jumping apparatus based on the SR-µCT data reconstruction. (A) Sagittal plane section of the femur, anterolateral view. (B) Transverse plane section of the femur, posterodorsal view. (C) Sagittal plane section of the femur, lateral view. (D) Femoro-tibial joint with removed femoral wall. Abbreviations: MET, metafemoral extensor tendon; TFS, tibial flexor sclerite.

Family Curculionidae, weevils (exemplified by Orchestes fagi).

Principal structural elements of the jumping apparatus in weevils (Fig. 2) revealed by the 3D microtomography are (Nadein & Betz, submitted): (1) the femoro-tibial joint, (2) the metafemoral extensor tendon (MET), (3) the extensor ligament (e.l.), (4) the flexor ligament (f.l.), (5) the tibial flexor sclerite (TFS), and (6) the extensor and flexor muscles. The kinematic parameters, the specific joint power and the time for the full extension of the hind tibia suggest that the jump is performed via a catapult mechanism with attribution of the additional kinetic energy. A resilin-bearing elastic extensor ligament that connects the extensor tendon and the tibial base is considered the structure for the accumulation of the elastic strain energy for the jump. According to our functional model, the extensor ligament is loaded by the contraction of the extensor muscles, while the co-contraction of the antagonistic extensor and flexor muscles prevent the early extension of the tibia. This is due to the leverage factors of the femoro-tibial joint that provide a mechanical advantage of the flexor muscles over the extensor muscles in the fully flexed position.

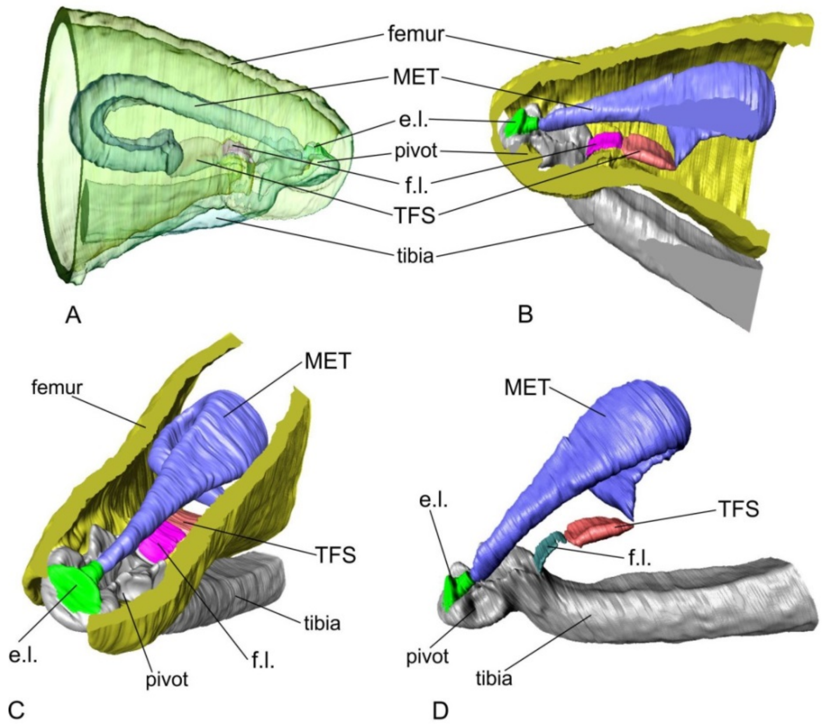

Fig. 2. Femoro-tibial joint in the jumping hind leg of weevil *Orchestes fagi* and the primary structural elements of the jumping apparatus based on synchrotron micro-tomography data reconstruction. (A) Joint structure inside the femur. (B) Sagittal plane section of the femoro-tibial joint. (C) Transverse plane section of the femoro-tibial joint. (D) Tibia and internal structures of the femur with femoral wall removed. Abbreviations: e.l., extensor ligament; f.l., flexor ligament; MET, metafemoral extensor tendon; TFS, tibial flexor sclerite.

Jumping apparatus of fossil beetles in amber.
Due to the peculiar conditions of the fixation, preservation and further fossilization the internal anatomical elements of arthropods in ambers are usually poorly presented. Two specimens of Late Eocene amber (Danish and Rovno ambers, both of the age 37.2-33.9 Ma) with leaf beetle inclusions (family Chrysomelidae) mophologically belonging to the group of jumping leaf beetles Galerucinae have been scanned. Reconstruction of the 3D microtomography data revealed the sclerotized structure inside the swollen hind femur identified as modified extensor tendon (MET) that is characteristic to the most of jumping beetles. MET could be considered as a reliable marker of the presence of jumping apparatus and jumping ability correspondingly. Morphology of the reconstructed MET of the fossil leaf beetles is characterised by the complex structure corresponding to that of the recent relatives of the fossil species.
3D microtomography data for the three specimens of ambers with inclusions of fossil leaf beetles have been obtained in our experiments at the ESRF. The chosen specimens of amber are characterised by the poor visibility of the inclusions, low transparency and the presence of the internal objects making examination hard. The obtained microtomography data of these amber specimens allowed us to reconstruct the 3D virtual volume depictions of the beetle inclusions with the high detalisation. Based on these data two new genera and three new species have been described (Nadein et al. 2016): *Psyllototus viking* Nadein, 2016; *Archealtica convexa* Nadein, 2016; *Paleomolpus hirtus* Nadein, 2016.

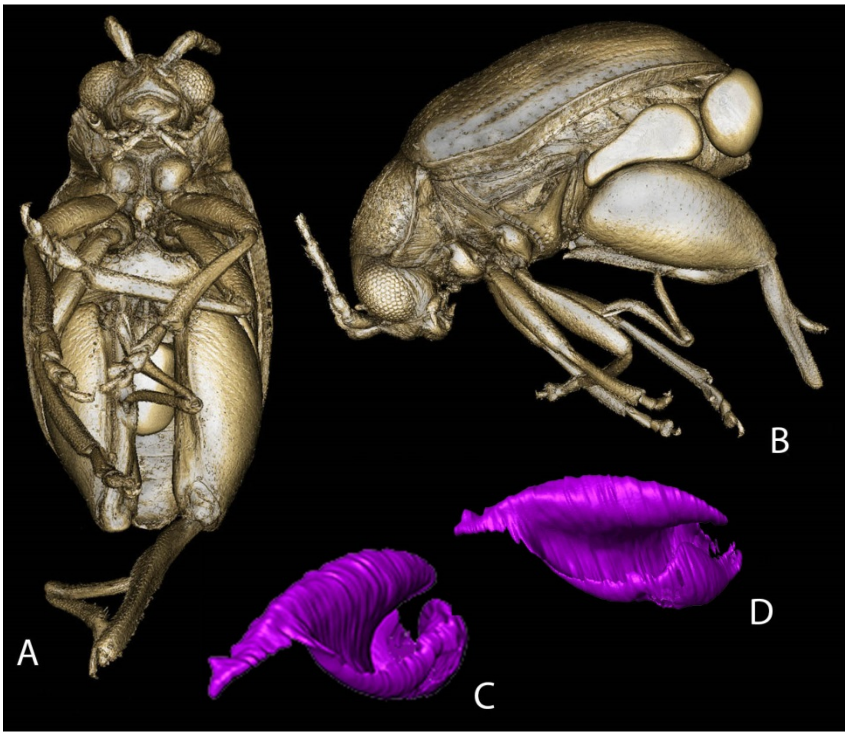

Fig. 3. 3D volume reconstruction of the fossil leaf beetles *Psyllototus viking* Nadein, 2016 (Late Eocene, Danish amber, 37.2-33.9 Ma) and metafemoral extensor tendon localized in the hind femur. (A) Ventral view. (B) Lateral left view. (C) Anterolateral view. (D) Lateral view.

Publications

1. Nadein, K., Betz, O. 2018. Jumping mechanism and performance in beetles. II. Weevils (Insecta: Coleoptera: Curculionidae). *Arthropod Structure and Development* (submitted).
2. Nadein, K., Betz, O., 2016. Jumping mechanisms and performance in beetles. I. Flea beetles (Coleoptera: Chrysomelidae: Alticini). *The Journal of Experimental Biology* 219: 2015–2027.
3. Nadein K.S., Perkovsky E.E., Moseyko A.G. 2016. New Late Eocene Chrysomelidae (Insecta: Coleoptera) from Baltic, Rovno and Danish ambers. *Papers in Paleontology* 2(1): 171–137.