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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 using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now 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: 3D microtomography for the study of comparative head anatomy and functional leg morphology in Coleoptera (Staphylinidae, Chrysomelidae, Curculionidae)**	**Experiment number**: ME-565
Beamline: ID 19	**Date of experiment**: from: 18.6.2003 to: 20.6.2003	**Date of report**: 25.2.2004 *Received at ESRF:*
Shifts: 6	**Local contact(s)**: Dr. Peter Cloetens	

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Report:

Part 1 (O.Betz): Head morphology. This experiment aimed at analysing the head anatomy of selected groups of staphylinoid beetles (Coleoptera, Staphylinoidea) by the use of 3D microtomography. In our studies we focussed on the subfamily Aleocharinae, in which several taxa have evolved from predaceous ancestors into specialized spore feeders (i.e., feeding on fungal spores). In order to evaluate the structural consequenses of this shift in feeding preference in terms of the internal head anatomy, we produced comparative microtomographic data sets (resolution: 0.7 µm; camera distance: 12 mm) of representatives of nine selected genera (*Polylobus*, *Pagla*, *Eumicrota*, *Pontomalota*, *Aleochara*, *Stictalia*, *Homalota*, *Oxypoda*, and *Gyrophaena*) within the Aleocharinae. Specimens of the genera *Polylobus*, *Pagla*, *Eumicrota*, and *Gyrophaena* are considered obligately sporophagous, whereas *Stictalia* and *Homalota* include facultative spore-feeders. Other genera such as *Pontomalota* were included as non-sporophagous outgroup for comparative purposes. We analysed two con-specific specimens per genus.

Our preliminary analyses of these data sets confirmed the general feasibility of this technique for our type of objects. We attained complete series of virtual sections of good quality through the head, which can be used to obtain 3D reconstructions of the entire head. Based upon the data we obtained at the ESRF, we were able to submit a proposal to the German Science Foundation DFG, which, among others, would allow us to (i) hire an experienced (doctoral) student to perform the anatomical analyses and (ii) acquire a double processor Opteron computer together with the 3D volume rendering software VGStudio Max. The latter components are necessary to comparatively analyze the data in great detail, including the 3D reconstruction of entire head capsules. In a first step, our proposal got acknowledged by the DFG in terms of the requested budget for the student (under the DFG administration number BE-2233/6-1). The requested support for the hard and software is still pending but will probably be acknowledged within the next two months. For the time being, the results of our preliminary survey of the series of virtual sections can be summarized as follows:

In obligate spore feeders striking modifications have taken place in the labium-hypopharynx, which has experienced considerable modification in several taxa in connection with the shift of the mandibular grinding surface from the mesal to the ventral side. In most aleocharines a well-developed molar lobe with a mesal grinding surface has been completely reduced (Ashe 1993) in connection with their primarily predaceous feeding habit. Consequently, the mesal molar face merely forms a narrow strip. However, after the shift of the feeding preference towards mycophagy in several groups, secondary trituration surfaces (“pseudomolae) as substitutes for true molae have evolved *de novo* at various places on the mandible. Most often, secondary grinding structures are established on the ventral inner side of the mandibles in the form of an array of prominent pointed grinding teeth (psm in Fig.1). As confirmed with X-ray microtomography, these structures are correlated with a more or less extensive reconfiguration of the hypopharynx, which is elevated and displaced anteriorly, forming a “second layer” on top of the prementum. The hypopharynx is thus moved adjacent to the ventral mandibular surface, where it can function as an abutment (cab in Fig.1) for the ventral trituration surface of the mandibles (Fig.1), so that the spores can be ground between both these layers. The conditions described so far represent a relatively advanced state, which almost certainly has evolved in the context of obligate mycophagy such as sporophagy. This view is supported by the fact that the observed specific combination of co-adapted features is clearly not developed in non-spore-feeders such as *Pontomalota.* However, in the latter group as well as in several other taxa investigated there might be possible intermediate stages preserved: (1) *Polylobus* (hypopharynx elevated and displaced anteriorly, but ventral mandibular surface without any grinding structures); (2) *Stictalia*, *Pagla*, and *Pontomalota*: hypopharynx displaced to a lesser degree plus ventral mandibular surface bare (*Pagla*), provided with a uniform patch of minute spinules (*Pontomalota*), or with an advanced pseudomolar array of grinding teeth (*Stictalia*). However, only a more comprehensive comparative study including many more lineages of aleocharines can reveal a clear idea of the concurrent evolution of aleocharine hypopharyngeal and mandibular structure in the context of a shift from predation to mycophagy. In order to be able to include more taxa into our comparative analysis, we are going to apply for additional beamtime later this year.

Once we have acquired the VGStudio Max Softare together with a powerful dual processor computer, we will be able to analyse the head anatomy of this group of beetles in much more detail. These analyses will include the description of internal cuticular skeleton structures and the internal organizaition of the major head musculature. We finally intend to map these features on a phylogeny to evaluate their changes from an evolutionary background.

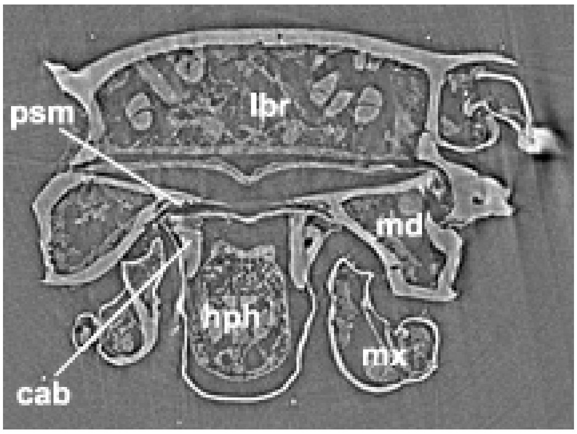

Fig.1: Transverse section through the anterior head region of *Gyrophaena* sp. as obtained with X-ray microtomography. Note the approximation of the hypopharynx (hph) to the ventral side of the mandibular pseudomola (psm). The spores are ground between both the pseudomola and the hypopharyngeal abutment. For further explanations see text. Abbreviations: *cab* cuticular abutment, *hph* hypopharynx, *lbr* labrum, *md* mandible, *mx* maxilla, *psm* pseudomola.

Part 2 (O. Betz): Leg morphology. Leaf-beetles (Chrysomelidae) of the subfamily Halticinae and snout beetles of the genus *Rhynchaenus* are characterized by their jumping ability, which is made possible by a spring-like endoskeletal structure in the hind femur, which is connected with powerful flexor and extensor muscles. In this experiment we aimed at analysing the 3D structure of this complex using the segmentation tool in VGStudio Max after 3D reconstructing the entire leg articulation. We investigated specimens of two genera within the Halticinae (*Phyllotreta* and *Halicta;* 2 specimens per species) and one species of the snout beetle *Rhynchaenus* (*R. fagi;* one specimen) (resolution: 0.7 µm; camera distance: 12 mm). Our preliminary analyses of the virtual serial sections we obtained at the ESRF revealed that it will be easily possible to distinguish between muscle tissue and the endoskeletal tendon. Hence, it will be possible to perform a separate 3D reconstruction of the internal endoskelatal structure (using the segmentation tool in VGStudio Max) to evaluate its possible spring-like nature. We will apply for another set of beamtime to invetigate the structure of this cuticle-muscle complex at different (pre-defined) angles of rotation between femur and tibia.

Part 3 (G. Pass, N. Szucsich): General body anatomy of small arthropods. Fixed and dried specimens of primitive insects and mites were scanned to examine whether microtomography at the ESRF is suitable for the elucidation of comparative general body anatomy. High resolution (about 1μm) is needed because of the very complicated anatomy of these animals - the smallest animals have a body-length below 1 mm. Classical methods, like histological serial sectios are highly time-consuming from both preparation and interpretation, making comparative studies very expensive. Additionally, it is presumed that 3-D-reconstruction can be easier applied to tomographical data, enhancing the quality of visualization.
Five different species of primitive insects (Diplura, Collembola, Archaeognatha) and two species of mites (Acari) were scanned at the beamline (see Table 1), all of them were exposed in an X-ray-beam of 15.5 keV with an exposure time of 0.5-1 sec.

Table1: List of examined species

species	***scanned parts***	***pix size***	***energy***	***duration***	***Ct***
		microns	keV	min	sec
Caloglyphus berlesei (Acari)	entire animal	0,7	15,5	38	1
Austroglyphus sp. (Acari)	entire animal	0,7	15,5	37,5	1
Campodea augens (Diplura)	abdomen	0,7	15,5	37,9	1
	thorax	1,4	15,5	25,2	0,5
	head and thorax	1,4	15,5	25,5	0,5
	head	1,4	15,5	25,4	0,5
	abdomen	0,7	15,5	37,9	1
Catajapyx aquilonaris (Diplura)	head	0,7	15,5	37,9	1
	thorax and abdomen	0,7	15,5	37,9	1
	abdomen	1,4	15,5	25,3	0,5
Tomocerus sp. (Collembola)	head	1,4	15,5	21,1	0,5
Heteromurus nitidus (Collembola)	head and thorax	1,4	15,5	25,5	0,5
	abdomen	0,7	15,5	38	1
Dilta hibernica (Archaeognatha)	abdominal end	0,7	15,5	37,9	1

A detailed analysis of the data is still pending. Although the corresponding proposal has already been acknowledged by the Austrian Science Foundation (FWF) since January 2004, the project could not as yet be started, since the FWF is still waiting for its budget from the Ministry of Science.
From the first results however it can be definitely concluded, that tomography is highly valuable for comparative anatomical studies of small insects and other arthropods. We are very interested to contribute to the improvement of the method in this respect in a following session this year (application for beam-time in preparation).