	Experiment title: **Evolutionary development of metal reinforced cutting tools in insects**	**Experiment number**: MA-416
Beamline: ID19	**Date of experiment**: from: 04-16-2008 to: 04-21-2008	**Date of report**: 08-14-2009
Shifts: 15	**Local contact(s)**: Dr. Peter Cloetens	*Received at ESRF:*

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Report:

1. Element-sensitive microtomography in the mouthparts of arthropods:

Element-sensitive dual energy microtomography was performed on 31 different arthropod species (2-3 scans per species) that comprised millipedes, crustaceans, and insects. The goal of the experiments was to determine and visualize the 3D distribution of both zinc and manganese within the mandibles (jaws) which are thought to play an important role in their function, and calcium in mineralized tissues of crawfish and diplopods. From abrasion tests it is known that wear resistance is increased in the metal-containing regions, so that this feature largely helps to increase the durability of the mouthparts in these animals. Calcium and zinc were well detectable by *k*-edge subtraction. While manganese detection (at 6.530 keV and 6.560 keV, respectively) failed, calcium could clearly be detected in the european crawfish *Astacus astacus* and the diplopod *Tachypodoiulus* sp. Large amounts of zinc ($1.0\ 10^{-2}$ g/cm³ - $2.5\ 10^{-2}$ g/cm³) were found in the incisivi of the cutting edges of the mandibles of almost all investigated ants (Hymenoptera, Formicidae). Zinc was also detected in the incisivi of the drywood termite (Kalotermitidae) *Incisitermes marginipennis*, a caddisfly larva of the genus *Rhyacophila*, and in the incisivi of the caterpillar of *Aglais urticae* (Lepidoptera). Complementing earlier findings in adult locusts (*Schistocerca gregaria*), also the 3rd and 4th instar larva of this species was found to contain zinc inclusions in the incisivi of their mandibels. All other investigated samples (e.g. *Machilis* sp., *Ecdyonourus venosus*, *Dorcus parallelipipedus*, *Strophosoma* sp., *Vespa crabo*, *Polistes dominulus*) did not contain in their cuticle any of the metals of this study.

Our 3D reconstructions and visualizations of the zinc revealed that the zinc-enriched material of the investigated insects, particularly ants, frequently forms cap-like structures covering zinc-free areas of the mandibular teeth (exemplified in Fig.1). Such a covering of "softer" with a wear resistant material is in analogy to the enamel crown in mammals in which it covers the softer dentine. The observed caps structure in ants are in contrast to the zinc distribution found in the locust *Schistocerca gregaria*, where the Zn-enriched material forms an asymmetrical surface layer, i.e. the inner surface of the left mandible and the outer surface of the right mandible are Zn-free. This results in a self-sharpening mechanism that ensures reliable cutting tool performance and a constant cutting blade angle. After we had revealed this mechanism in a previous beamtime session (cf. Fig. 2 of our proposal) for adult locusts we could confirm these conditions in the present experiment for a 3rd or 4th instar larva (Fig.2).

In addition to the cap-like Zn inclusions we detected clasp-like inclusions at only the apical side, at only the basal side, or at both sides of the mandible (cf. Fig.1) that laterally extend toward the mandibular articulation. Such inclusions have not as yet been described in insects. They might add to the mechanical stabilization of the mandibles during stress and strain. Alternatively, they might form an additional cutting edge at this region of the mandible.

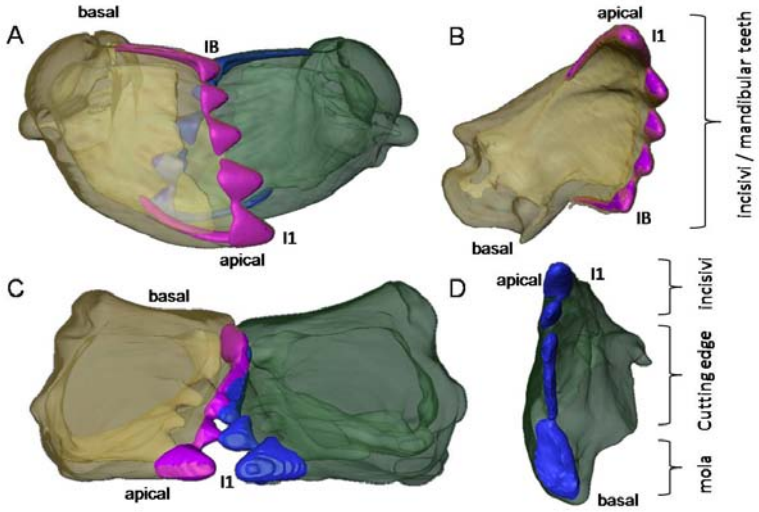

Fig.1: 3D reconstruction of the mandible of the ant *Camponotus herculeanus* (A, B) and the termite *Incisitermes marginipennis* (C, D). A, C Dorsofrontal aspects. B, D: Medial aspect of the cutting edges. The left mandible is shown in green (zinc contents blue), the right one in brown (zinc contents pink). I1 first incisivus (most apical), IB = last incisivus (most basal). The length of the entire cutting edges in B and D amount to ca. 500 µm.

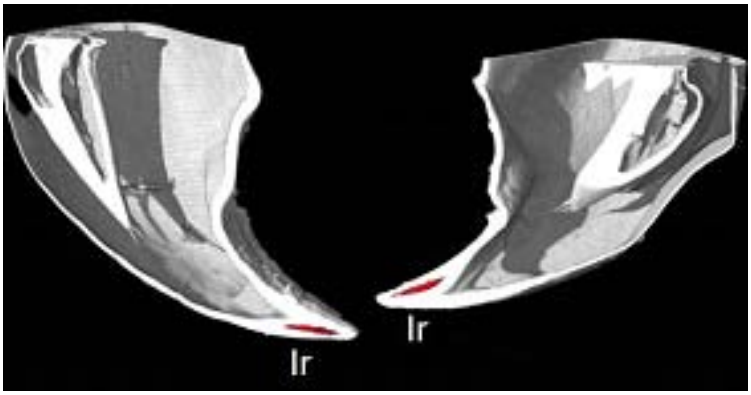

Fig.2: *Schistocerca gregaria*. Larval instar 3 or 4. Virtual section through the mandible in the region of the incisivi of the cutting edge. The zinc contents are shown in red.Ir = Incisivus region. Notice that the Zn inclusions does not form a cap-like outer structure but that it is restricted to the outer (left) and inner (right) side of a mandible.

Element sensitive dual energy synchrotron X-ray microtomography was also used to quantify the Zn content within the Zn enriched regions. The detected zinc-concentrations amounted from 1.0×10^{-2} g/cm³ to 2.5×10^{-2} g/cm³ (corresponding to about 0.8-2.0wt.% when assuming an insect cuticle density of 1.3×10^{-2} g/cm³). We found especially high concentrations in species that feed on hard food (e.g. dry wood or leafes) material such as the termite *Incisitermes marginipennis* (1.66×10^{-2}g/cm³) and the wood ant *Camponotus herculeanus* (2.54×10^{-2} g/cm³). In the plant feeding *Schistocerca* larva we revealed concentrations of about

0.9×10^{-2} g/cm³, which is about 25% of the concentration previously found in adults locusts (Wegst *et al*, in preparation).

Our comparative study of experiment ME-1231 revealed valuable, new results on both zinc concentration and 3D zinc distribution within the mandibles of several arthropods. The dual energy microtomography technique allowed us to visuzalize and analyse what had never been reported before: the three-dimensional shape and overall structure of metal-enriched regions in the otherwise purely polymeric cutting tools found in nature.

2. 3D-reconstruction of the calcium containing embryonic shell in snails

Platinum is used in automobile catalytic converters and is, as a result, increasingly introduced into the environment. Contact to platinum during the early development of *Marisa cornuarietis*—a prosobranch freshwater snail belonging to the Ampullariidae family—has been shown to prevent the formation of an outer shell. In the presence of platinum, the mantle does not overgrow the visceral sac during the embryonic development but remains at the aboral end of the snail and invaginates into the body. As a result, the calcification processes leads to an inner rather than an outer shell, because the visceral sac overgrows the mantle edge. Due to the formation of this inner shell, also the organisation of the organs differs from that found in outer shell-wearing snails. Synchrotron X-ray phase-contrast microtomographs of *M. cornuarietis* have led to a better understanding of the position of the inner calcium shell and their formation during the snails´ early development. The results contributed to a manuscript (Osterauer et al.2010) that has recently been submitted.

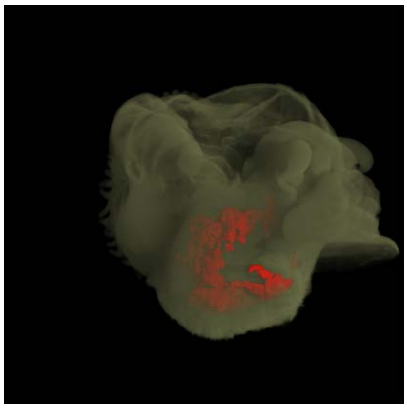

Fig. 3: Synchrotron X-ray phase-contrast microtomograph of *Marisa cornuarietis* exposured to Pt, 26 days old and about1.5 mm in size. The picture displays a semi-transparent view of the snail (grey) and the internal shell (red) made of calcium carbonate.

References that arose from this beamtime :

Dieterich, A. (2009): Zinkeinlagerungen zur mechanischen Verbesserung der Mandibel ausgewählter Insekten, Diploma thesis, 110 pp.

Dieterich, A. & Betz, O. (in press): Elementsensitive Synchrotron-Mikrotomographie zur Darstellung von Zinkeinlagerungen in den Mandibeln ausgewählter Insekten, Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie

Osterauer, R., Marschner, L, Betz, O., Gerberding, M., Sawasdee, B., Cloetens, P., Sures, B., Triebskorn, R., Köhler, H.-R. (in review): From snail to slug: Induced body plan changes and formation of an internal shell

Wegst, U.G.K., Betz, O. & Cloetens, P. (in review): Locusts feed with self-sharpening scissors.