ESRF	**Experiment title:** Functional head morphology of the assassin bug *Rhodnius prolixus* (Heteroptera, Reduviidae)	**Experiment number**: LS 2219
Beamline: ID 19	**Date of experiment**: from: 24.7.2013 to: 27.7.2013	**Date of report**: 4.3.2014
Shifts: 9	**Local contact(s)**: Dr. Alexander Rack	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*Prof. Oliver Betz, Professur für Evolutionsbiologie der Invertebraten, Institut für Evolution und Ökologie, Universität Tübingen, Auf der Morgenstelle 28E, D-72076 Tübingen, Germany
Prof. Dr. Oliver Röhrle, Institut für Mechanik (Bauwesen), Lehrstuhl für Kontinuumsmechanik und Exzellenzcluster für Simulation Technology, Abteilung „Continuum Biomechanics and Mechanobiology", Universität Stuttgart, Pfaffenwaldring 7, D-70569 Stuttgart, Germany

Report:
The aim of our experiment was twofold. (1) First, we produced tomographic data sets in high quality (Fig. 1c) that will make possible the identification of the head anatomy (muscles, mouthparts) of blood-sucking assassin bugs (Hemiptera: Reduviidae, Triatominae). (2) Secondly, we performed cineradiographies of live animals during their feeding on fluids (Fig. 2).
The attained results will make it possible to functionally understand how these insects move their stylet-shaped mouthparts forth and back during the blood feeding process and at the same time perform remote-controlled lateral and rotational searching movements although there are no articulations that might facilitate such movements. It is thus assumed that the obeserved joint-free movements result from the combination of the proteinaceous elastic matrix and the pattern (course and packing density) of the chitin fibres embedded into this matrix. The elongated rod-like mouthparts can be either lengthwise interlocked or released during the feeding process. After penetration into the host, the normally interlocked stylets are shifted lengthwise, thereby being partly released from their interlocking counterpart to perform target-oriented lateral and rotational bending movements.
The poduced tomographic data sets will now be used in an engineer driven modelling process to test wether the described functional model assuming the combination of (elastic) material properties of the cuticle and the alternating interlocking and release of the stylets is true and how it needs to be modified, respectively. In a **first step** of further analyis, VG Studio Max will be used to produce virtual serial sections through the head for further analysis (Fig. 1c). From a biological perspective, his will make it possible to describe all the head muscles and identify those muscles that are directly involved in moving the maxillae. In a **second step** of

analyis, the segmentation software tool of AMIRA will be used for 3D reconstructing the mouthpart stylets (labium, maxillae, mandibles) over their entire length. This 3D model will form the basis for the **third step** of analyis, in which the 3D data shall be adopted into small-scale FE models that ascribe cuticular material properties (elastic modulus) to each voxel. Computational modelling on the micro-scale will involve (i) geometrical and (ii) material modelling aspects and (iii) homogenization techniques providing the link between detailed sub-models and the overall structure. To identify the key features for the macro-scale, a sensitivity analysis with respect to the material parameters will be carried out by the co-proposer (Oliver Röhrle: cf. [2-4]).
Depending on the progress, the overall insect model will be finally extended to include muscle descriptions that are key for actuating the rod-like needle structures in a joint-free structure. In order first to investigate the role of the various muscles, the line of action (centreline of the muscles) of each muscle will be determined from our tomographic 3D data (see first step). Thereby, the activation of single muscles will initially be modelled by using a force vector in the direction of the line of action with variable magnitude.
In a **fourth step** of analyis, it will be ecaluated how the attained data can be transferred into biomimetically inspired technical solutions. To this aim, reduced large-scale FE models will be used within simulation-based analyses to transfer and adapt the structures and material behaviour to bio-inspired technical structures.

Experiment 1: We produced about 10 tomographic data sets (2000 projections, energy set to 18.1 keV) of two species of assassin bugs, one bigger species (*Dipetalogatser maxima*) (Fig. 1), and, for comparaive purposes, one smaller species (*Rhodnius prolixus*). According to the length of the mouthpart stylets, each data set consists of several subsets that need to be combined subsequent to the segmenation process to attain the structures along their entire length. Depending on the size of the sample, the data sets were produced with voxel resolutions of 0.7 µm and 1.4 µm, respectively.

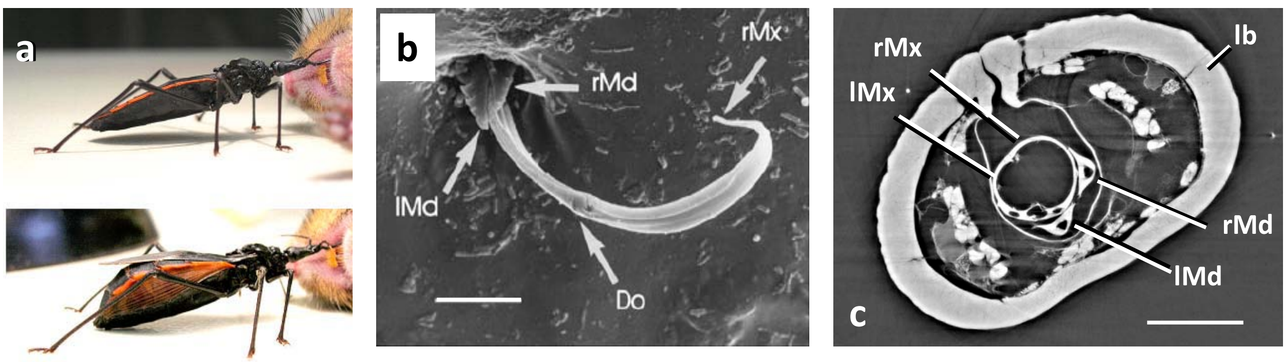

Fig. 1: The piercing and sucking mouthparts of the blood-feeding assassin bug *Dipetalogaster maxima* as a biological concept generator for the joint-free movement of fibre-like interlocking structures concomitantly exhibiting adaptive stiffness. a: Assassin bug feeding on an anaesthetized mouse (above: prior to feeding; below: after several minutes of feeding (with swollen abdomen)). b: SEM image of the lateral searching movements of the maxillae (rMx) within the host tissue after the mandibles (rMd, lMd) have penetrated the host's tissue. Scale bar = 10 µm. From [1].c: Transverse section through the mouthparts bundle as produced by Synchrotron microtomography (SR-µCT) in this project. Such CT datasets will form the basis for the 3D geometric modelling of these structures as performed by the co-proposing engineer involved in this project (Oliver Röhrle). Scale bar = 20 µm.

Abbreviations: Do thorn, lb labium, lMd left mandible, rMd right mandible, lMx left maxilla, rMx right maxilla.

In order to reveal the course of the stylets at different modes of action, we scanned both animals whose stylets were entirly streched forward (as done during blood feeding; cf. Fig. 1a) and specimes whose mouthparts were kept in the resting position, i.e. jackknifed under the head.

Experiment 2: Both *Rhodnius prolixus* and *Dipetalogster maxima* were observed during the fluid feeding process. The animals were placed on top of little vials that were filled with warmed nutritional fluid and covered by a thin Parafilm membrane. Once the bugs had started to penetrate the foil with their stylet-formed mandibles to suck in the fluid (to which contrast agent was added), the beam was switched on. This way, we obtined several seconds of footage showing the influx of the fluid via the maxillae into the gut (Fig. 2). Interestingly enough, the bugs were able to notice the beam and quickly escaped from the vials after a few seconds. This was probably due to the increase of temperature caused by the beam, which can be sensed by the infrared receptors located at the mouthparts of assassin bugs to find their vertebrate hosts. Altghough the attained footage does not show any movement pattern of the mouthpart stylets (which is due to the restricted plane of focus), it will make it possible to quantify the velocity of the fluid stream, which is indicative of the performace of the cibarial sucking pump. Thew following technical setup realed the best results with respect to image quality: camera: pco.dimax, 2016 x 2016 pixels, energy: 36 keV (pink = broadband, u17.6 undulator), effective pixel size: 4 µm (nominal, not measured) via indirect detector, recording speed: 200 FPS (200 images/s, 5 ms exposure time), 1.1 m propagations distance.

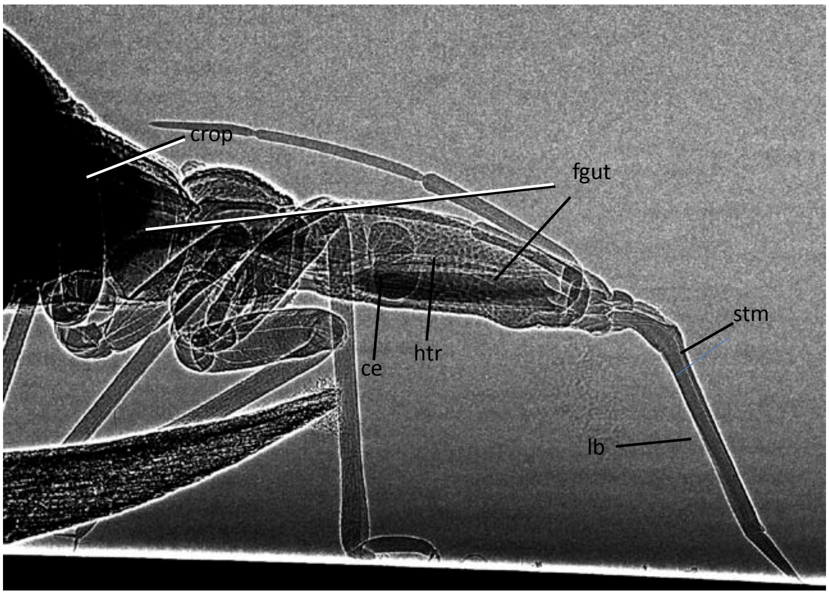

Fig. 2: Lateral view of the fluid feeding assassin bug *Rhodnius prolixus* as recorded in experiment 2. Single frame from attained footage material. The stylets (stm) and the gut (fgut) in which the fluid (recognizable by its dark contrast wihich is due to its enrichment wth contrast agent) is transported are well visible. From the attained footage it will be possible to quantify the velocity of the fluid stream being indicative of the performance of the cibarial sucking pump. Abbreviations: ce: compound eye, crop: crop = ingluvies, fgut: fore gut, htr: head trachea, lb: labium, stm: stylet-shaped mouthparts (= maxillae / mandibles).

Literature cited:

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