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Phase-Contrast Nano-tomographic Imaging in a Commercial X-ray Microscope to Understand the Defensive Behavior of *Suocerathrips linguis*

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Among insects, the order Thysanoptera has about 90 species which are considered agricultural pests, due to their feeding on crops and injuries to various plant tissues caused by ovipositing (laying of eggs). Moreover, out of these 90 species, about 10 are propagative vectors and transmitters of tospoviruses, and gram-negative bacteria [1]. The Thysanoptera are subdivided into two suborders: Tubulifera and Terebrantia, which are very small in size (1–3 mm). A special characteristic to distinguish between the two suborders is the shape of the Xth abdominal tergite, and the Tubulifera bear a conical-elongated last abdominal segment (Fig. 1A, enlarged in B).

When being attacked by an enemy, most species of Tubulifera, such as *Suocerathrips linguis*, raise and lower their abdomen with a drop of fluid. The fluid droplet is retained between setae at the tip of the abdomen (Fig. 1C) and then discharged from the apex of their tube on predators as a defense. If the application of the droplet to the predator fails, the fluid can be resorbed by the insect. Studies suggest that the secretory reservoir is the hindgut [2] and some components of the secretions have been found to be ant repellents, contact irritants, alarm pheromones, or fumigants [3, 4]. Until now, the mechanism involved in holding the drop with the setae, especially during the overhead abdominal movement, is yet not completely understood. As three-dimensional imaging would capture the relative position of all setae and therefore might contribute to understanding this defense behavior, in this work we tested the ability of a commercial 3D X-ray microscope (Xradia Ultra 810) for imaging *S. linguis* in phase-contrast mode. We expect that the findings will be useful for further studies of functional morphology of insects.

S. linguis was reared on *Sansevieria trifasciata* under a constant light regime of 16:8 h light/dark (light on at 6:00 am), at 23° C and a relative humidity of 60 %. For the imaging experiment, the insect was sacrificed by being left at -18° C in a freezer for one day, and then it was glued to a metallic pin with the abdomen towards the top. The imaging experiment was performed in a Carl Zeiss Xradia 810 Ultra (Cr source, 5.4 keV) using phase-contrast imaging mode at the Institute of Physics, Martin-Luther-University Halle-Wittenberg. For that, a Zernike phase ring positioned near the back focal plane of the zone plate lens phase shifts the X-rays not scattered by the sample by $3\pi/2$. Images were acquired with an exposure time of 15 s, and 901 projections over 180°, detector binning of 1 and voxel size of 64 nm, resulting in a total scan time of less than 4 hours. We chose the voxel size according to the dimensions of the setae, which might reach less than 300 nm at the tip. Two datasets were obtained by moving the sample vertically through the X-ray beam. Image reconstruction was performed by filtered back-projection algorithm using the software integrated into the Xradia 810 Ultra. ImageJ was used for image correction and stitching of the datasets. The 3D rendering presented here was created using the commercial software Arivis Vision4D.

We imaged *S. linguis* without the need for extensive sample manipulation and there is no indication of sample damage or dehydration during the experiment. The image contrast observed in the projections (exemplified in Fig. 2 A) produces a detailed visualization of the dorsal longitudinal muscles of the tergite X and the apical setae (Fig. 2 A, respectively black and white arrows). Due to the high-contrast obtained in the images, we could reconstruct the volume of a part of the abdominal segment. Moreover, we could observe the typical setae arrangement with six long and thick setae in a radial arrangement around the tip of the tube (Fig. 2 B). Terminally, they show spoon-shaped structures (Fig. 2 B, grey arrows). Half long and thinner setae are located between the long setae, whereas one medium-length seta is arranged between two long ones [5].

Studies on the chemical properties of the droplet suggest a high-wettability. Indeed, small droplets of fluid are observed on the setae of the *S. linguis* imaged by us (Fig. 2 B, white arrows). Our results suggest the possibility of imaging a *S. linguis* specimen holding a droplet, that we aim to perform in the future [6].

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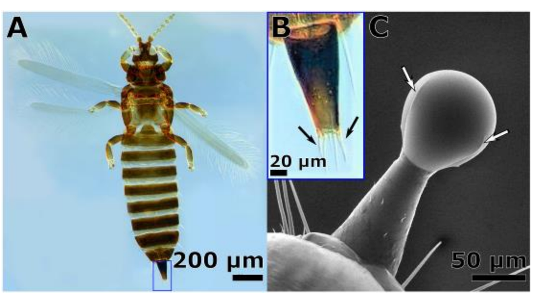

Figure. 1.(A) Optical microscopy of *S. linguis*, with the abdominal segment marked inside the rectangle, enlarged in (B). Electron microscopy showing fluid droplet retained between setae. Setae are indicated by the arrows in (B) and (C).

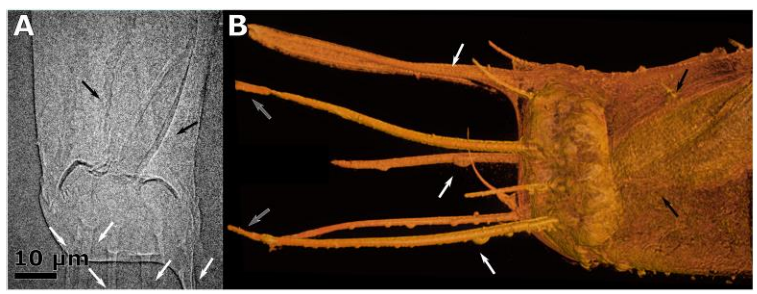

Figure. 2. (A) Projection of *S. linguis* (phase-contrast) and (B) volumetric reconstruction of the insect. Arrows: white indicate the setae, black show the Musculus longitudinalis abdominis dorsalis and grey indicate spoon-shaped structures at the end of two setae.