	Experiment title: Development of *Archegozetes longisetosus* (Acari, Oribatida), a model organism for chelicerate evolution	**Experiment number**: SC-2127
Beamline: ID 19	**Date of experiment**: from: 19. March 2007 to: 21. March 2007	**Date of report**: 28. Jan. 2008
Shifts: 6	**Local contact(s)**: Dr. Peter Cloetens	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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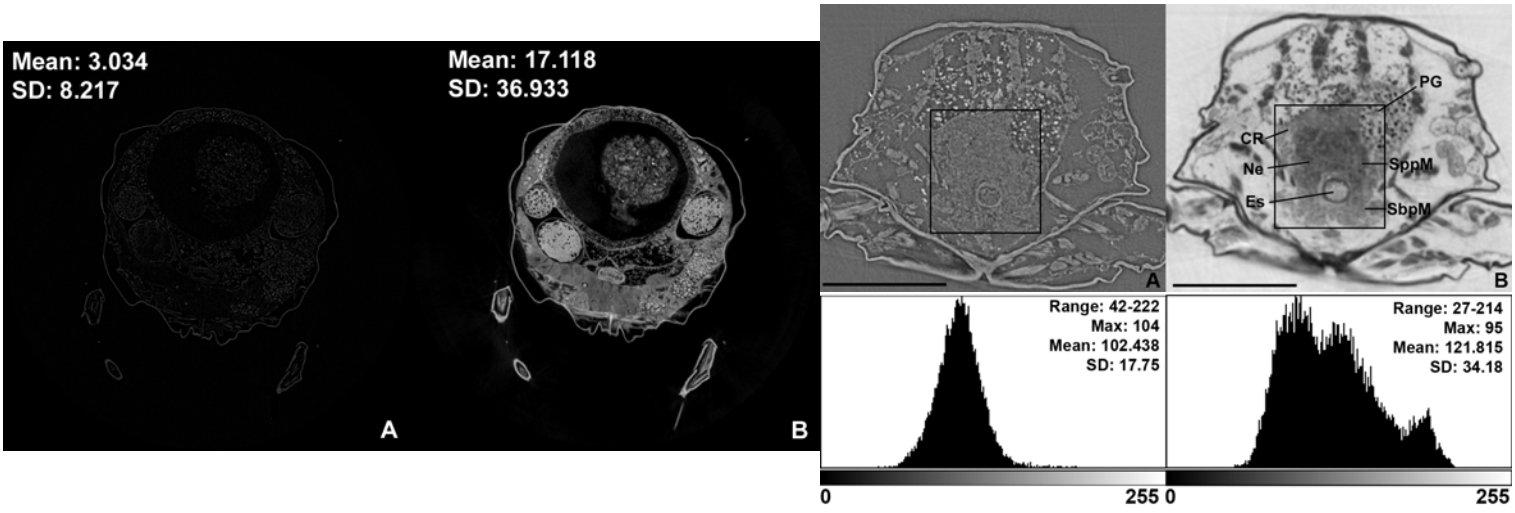
Report:

The experiment SC-2127, initially planned and approved with 9 shifts, was splitted in two separate experimental sessions with each 6 shifts. The first session was held in December, 2006, and used phase-enhanced tomography for the analyses of the functional morphology (muscles and cuticle) of different oribatid mite species and a fossil, 40 million year old specimen in Dominican amber (separate report). The second session (this report) was held in March 2007 and used quantitative phase tomography (holotomography) for the analyses of the developmental biology and differential imaging of soft tissues of *Archegozetes longisetosus*. X-ray tomography was performed at beamline ID19 with an energy of 20.5 keV. The radiographs were recorded with an effective pixel size of 0.7 µm. 1500 projections were recorded over the 180° sample rotation with an exposure time of 0.35 s for each projection. The detector-to-sample distances were 10 mm, 20 mm and 45 mm.

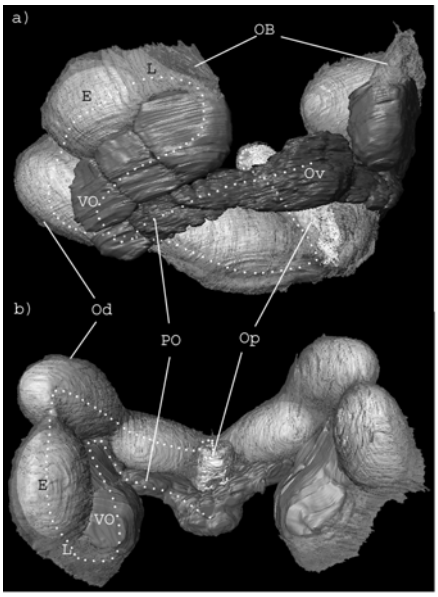
Comparison of phase contrast tomography (PCT) and holotomography (HT)

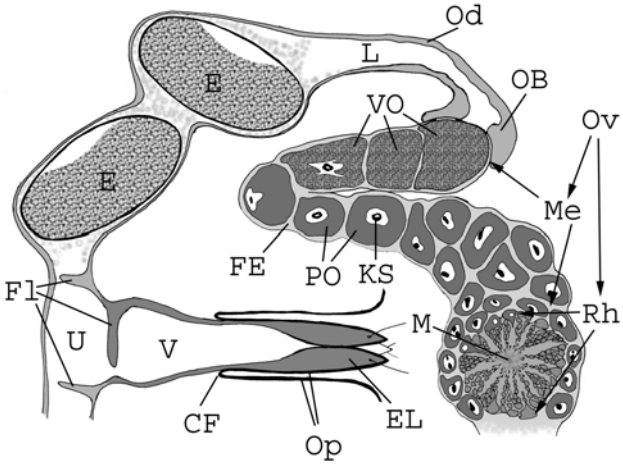
We were the first to study the internal anatomy of whole microarthropods in the December session using phase contrast tomography. In March, we used holotomography to achieve a better differential grey value distribution within soft tissues with low X-ray attenuation coefficients. We scanned a developmental series of *Archegozetes longisetosus* (eggs, larvae, protonymphs, deutonymphs, tritonymphs and adults). The aim was to follow the development of different organs of these microarthropods. A comparison of virtual slices of PCT and HT showed that the grey value distribution had a much higher standard deviation in HT images and that the grey values of the sample can be substracted from the background with a reduced loss of information when compared to PCT (Heethoff, submitted). Regarding the differential mapping of soft tissues, the virtual

HT slices are comparable to histological sections with traditional single staining. The figure below left shows a comparison of a PCT slice (left) and an HT slice (right) after the grey values of the background (a slice above the sample) were subtracted. While the PCT slice lost many of its information, the HT slice shows a differential representation of organs and tissues. Obviously, holotomography is a powerful tool for the non-destructive analysis of soft tissues in microarthropods.
In the next figure (right below), a comparison of the two methods is given for the nervous system. Mites have a single synganglion which consists, like in all arthropods, of a cortical region and a neuropile. The PCT slice in the left part shows no clear differentiation of the two parts of the synganglion. This can also be seen in the histogram underneath (histogram of the boxed region). The HT slice shows nicely the two functional parts of the brain with different local maxima of grey values (CR: cortical region, Es: esophagus, Ne: neuropile, PG: preventicular gland, SbpM: subesophageal mass, SppM: supraesophageal mass. Bar: 100 µm; Heethoff, submitted.). The advantages became obvious by just inspecting the virtual slices, but they can also be nicely quantified based on their grey value distributions.



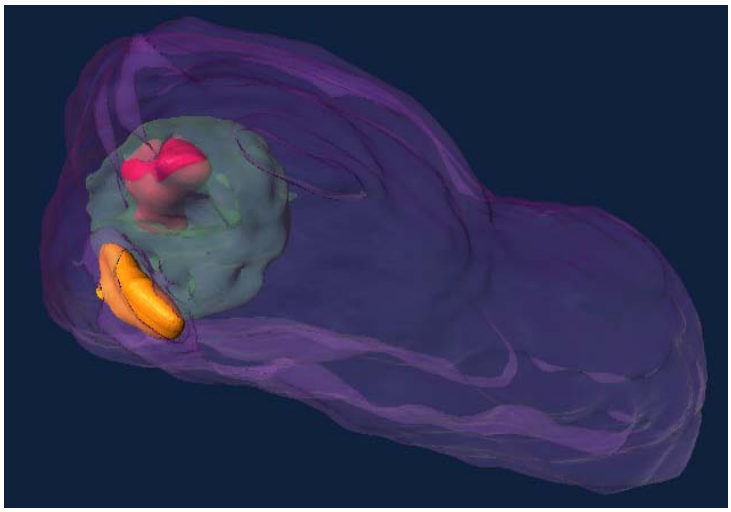
Morphology of the reproductive organs
The reproductive organs of *A. longisetosus* consist of an unpaired ovary from which two oviducts originate and run in a S-shaped loop through the opisthosoma and join at an unpaired uterus and finally into the unpaired vagina (Heethoff et al., 2007, Bergmann et al., submitted). Based on the holotomographic data, we were able to give a detailed description of the complete reproductive system and its functionally different parts (Bergmann et al., submitted). The figure below on the left shows a threedimensional reconstruction after segmentation of the reproductive organs. The figure below on the right shows a scheme with the different functional parts of the reproductive organs of *A. longisetosus*. Using the holotomographic data in combination with histological and TEM data, we could describe for the first time a functional differentiation of some parts of the reproductive organs and their development from tha larva to the adult state (CF: circular fold of the ovipositor, E: egg, EL: Eugenital lobes of the ovipositor, FE: follicle epithelium, Fl: flaps (described here for the first time), KS: karyosphere, L: free lumen inside the oviducts, M: meros of the ovary (described here for the first time), OB: ovarial bulb, Od: oviduct, Op: ovipositor, PO: pre-votellogenetic oocytes, Rh: rhodoid of the ovary (described here for the first time), U: uterus, V. vagina; Bergmann et al., submitted).

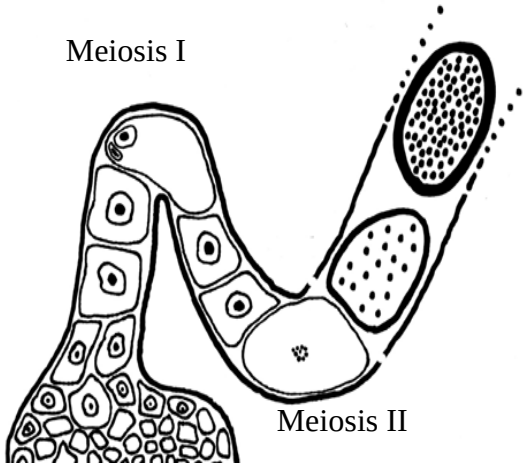




Oogenesis and meiosis in *Archegozetes longisetosus*

After describing the development and the functional morphology of the reproductive system we were able to use the holotomographic data and combine it with histological data to follow the gametogenesis/oogenesis of this parthenogenetic species. Theoretical considerations from the past 20 years suggested an inverted meiotic sequence in combination with holokinetic chromosomes for parthenogenetic oribatid mites (cf. Heethoff et al., 2006). However, empirical data are still scarce. The figure below on the left side shows a segmentation/reconstruction of an oocyte after meiosis I where the small polar body lies at the distal side of the oocyte (green: nucleus of oocyte, red: nucleolus of oocyte, yellow: nucleus of polar body, Laumann et al., submitted). The right figure shows a schematic drawing of the ovarial meros (see also above) and the positions of meiotic cleavages. Meiosis I occurs in the first curve of the meros and meiosis II in the second. It remains now to be uncovered if the chromosomes are indeed holokinetic and if the diploid chromosome number is reduced to a haploid number in meiosis I or meiosis II. Now, that we know when and where in the animal both meiotic cleavages occur, it becomes feasible to analyse the chromosomal kinetics with high-resolution methods such as transmission electron microscopy.





Publications

Results from the second session are submitted for publication as follows:

Bergmann, P., Laumann, M., Heethoff, M. (). Organisation and morphology of the internal reproductive organs of *Archegozetes longisetosus* Aoki (Avari, Oribatida). Submitted

Heethoff, M. ():Synchrotron X-ray phase contrast tomography and holotomography – A comparison for non-invasive investigations of the internal anatomy of extant and fossil microarthropods. Submitted.

Laumann, M., Bergmann, P., Heethoff, M. (). Some remarks on the cytogenetics of oribatid mites (Acari, Oribatida). Submitted.

References

Heethoff, M., Bergmann, P., Norton, R. A. (2006). Karyology and sex determination of oribatid mites. Acarologia 46: 127-131

Heethoff, M., Laumann, M., P. Bergmann (2007): Adding to the reproductive biology of the parthenogenetic oribatid mite *Archegozetes longisetosus* (Acari, Oribatida, Trhypochthoniidae). – Turkish Journal of Zoology 31: 151-159.